

BUKTI KORESPONDENSI
ARTIKEL JURNAL INTERNASIONAL BEREPUTASI

Judul Artikel : *In vitro* Evaluation and Molecular Docking Study of the Antibacterial Potential of *Macaranga hullettii* King ex Hook.f. Leaf Extract

Jurnal : Tropical Journal of Natural Product Research (TJNPR) Vol. 9 No. 7 (2025)

Penulis : **Eva Marlina**, Ade Danova, Rita Hairani, Ritbey Ruga , Elvira Hermawati, Ana Yulvia.

Ringkasan Proses Korespondensi :

No.	Perihal (Link)	Tanggal
1.	Bukti submission 1. Bukti submit di sistim 2. Cover letter 3. Manuscript 4. Potential reviewer	2 April 2025
2.	Bukti email Submission Acknowledgement	2 April 2025
3.	Bukti email Submission Request	3 April 2025, 10.45 pm
4.	Bukti respon submission request pada website TJNPR Lampiran : 4 potential reviewer Author contribution statements Bukti email Response submission request	3 April 2025, 11.15 pm
5.	Bukti email Editorial Decision	26 April 2025
6.	Bukti email Editor and review comment 1 Lampiran : 1. TJNPR-2025-420-Reviewer 1 2. TJNPR-2025-420-Reviewed 1	1 Mei 2025
7.	Bukti email Respon to editor and review comment 1 Lampiran : 1. Response to reviewer 1 2. TJNPR-2025-420 revised 1 3. Turnitin	6 Mei 2025
8.	Bukti email Tambahan respon untuk reviewer 1 Dokumen tracking Cambridge Proofreading 1. Proofreading traked (Cambridge) 2. Proofreading summary 3. Sertificate for english editing Lampiran revisi proofreading TJNPR-2025-420-revised_proofreading	10 Mei 2025

9.	Bukti email Permintaan editor : english editing and proper formating TJNPR Lampiran : TJNPR-2025-420-revised proofreading	11 Mei 2025 5:47 am
10.	Bukti email Permohonan English editing and proper formating TJNPR dengan Dr. Erharuyi	11 Mei 2025 8:58 am
11.	Bukti email Editor dan review comment 2 Lampiran : TJNPR-2025-420 reviewed 2	21 Mei 2025
12.	Bukti email Mengirim revisi dan Permohonan menunggu hasil editing dan formating dari Dr. Erharuyi Lampiran : 1. Response to reviewer 2 2. TJNPR-2025-420 revised Bukti email Respon Editor	25 Mei 2025 11.25 am 10.33 pm
13.	Bukti email Response english editing dari Dr. Erharuyi	25 Mei 2025 10.50 pm
14.	Bukti email Revised english editing Lampiran : TJNPR-2025-420 revised	26 Mei 2025
15.	Bukti email Response English editing and formating Lampiran : Sertificate english editing and formating	27 Mei 2025 07.08 am
16.	Bukti email Response to reviewer 2 Lampiran : 1. Certificate english editing 2. TJNPR-2025-420 revised 2	27 Mei 2025 10.57 am
17.	Bukti email Check Galley Proof Lampiran : TJNPR-2025-420 STA	1 Juni 2025
18.	Bukti email Revised Galley Proof Lampiran : 1. Response to editor 2. TJNPR-2025-420 STA Revised	2 Juni 2025
19.	artikel Published Bukti email : Artikel telah terbit	Vol 9 No 7 (2025) 31 Juli 2025

Dokumen bukti terlampir pada halaman berikut.

LAMPIRAN DOKUMEN KORESPONDENSI



← Back to Submissions

Workflow

Publication

Submission

Review

Copyediting

Production

Submission Files

Search

▶		13765	Four Potential Reviewers.pdf	April 2, 2025	Other
▶		13764	Cover Letter.pdf	April 2, 2025	Other
▶		13763	Manuscript Final.docx	April 2, 2025	Article Text
					Download All Files

Pre-Review Discussions

Add discussion

Organic Chemistry Division, Department of Chemistry
Faculty of Mathematics and Natural Sciences,
Institut Teknologi Bandung,
Bandung, West Java 40132, Indonesia

April 02, 2025

Professor Abiodun Falodun, PhD
Editor-in-Chief
Tropical Journal of Natural Product Research

Dear Professor Abiodun Falodun, PhD

We would like to submit an original research article entitled “***In Vitro* and Molecular Docking Evaluation of New Antibacterial Activity of *Macaranga hulletti* King ex Hook.f. Leaf Extract**” for consideration by Tropical Journal of Natural Product Research. We confirm that **this work is original and has not been published** elsewhere, **nor** is it currently **under consideration for publication** elsewhere.

In this paper, we show that **both methanol extract and ethyl acetate fraction of *Macaranga hulletti* King ex Hook.f. exhibited antibacterial activity** against three bacterial strains (*S. aureus*, *S. mutants*, and *P. acnes*) with a minimum inhibitory concentration (MIC) of < 0.15%, except for the methanol extract with MIC of 0.15-0.31% against *P. acnes*. Additionally, **molecular docking analysis** reveals that flavonoids possessing prenyl, geranyl and hydroxyl groups, namely, 6-isoprenyleriodictyol, nymphaeol A, nymphaeol B, and solophenol D, showed the highest binding affinity and dominant hydrogen bonding interactions with key amino acid residues in the binding site of the three proteins (IJIJ, 3IPK, and 7LBU). Thus, these findings suggest that the methanol extract and ethyl acetate fraction from *Macaranga hulletti* King ex Hook.f. has potential applications as **a new source of antibacterial agents**.

We have no conflict of interest to disclose. Please address all correspondence concerning this manuscript to me at adedanova@itb.ac.id (Telp. +6281357944698).

Thank you very much for your consideration of this manuscript.

Yours sincerely,



Ade Danova, Ph.D.

***In Vitro* and Molecular Docking Evaluation of New Antibacterial Activity of *Macaranga hulletti* King ex Hook.f. Leaf Extract**

Eva Marlina¹, Ade Danova^{2*}, Rita Hairani¹, Ritbey Ruga¹, Elvira Hermawati², and Ana Yulvia³

1. Department of Chemistry, Faculty of Mathematics and Natural Science, Mulawarman University, Samarinda 75123, East Kalimantan, Indonesia.

2. Organic Chemistry Division, Department of Chemistry, Faculty of Mathematics and Natural Sciences, Institut Teknologi Bandung Jl. Ganesha No. 10, Bandung 70132, West Java, Indonesia.

3. Department of Chemistry, Faculty of Mathematics and Natural Science, Universitas Jember, East Java, Indonesia.

*Corresponding author. E-mail: adedanova@itb.ac.id; Tel.: +6281357944698.

Abstract

Bacterial infections are a common occurrence, and with the increasing resistance of bacteria to antibiotics, utilization of plant extracts is gaining prominence. *Macaranga* plants are known to contain secondary metabolites such as flavonoids, which exhibit antibacterial properties. *Macaranga hullettii* King ex Hook.f. is particularly noteworthy because of its widespread presence in East Kalimantan. Therefore, this study aimed to investigate the antibacterial potential of *Macaranga hullettii* leaf extract through *in vitro* and molecular docking studies. The research involved testing the methanol extract, n-hexane fraction, and ethyl acetate fraction from *Macaranga hullettii* King ex Hook.f. leaves against three bacterial strains, *Staphylococcus aureus*, *Streptococcus mutans*, and *Propionibacterium acnes*. The results

demonstrated that both the methanol extract and ethyl acetate fraction exhibited antibacterial activity against three bacterial strains with a minimum inhibitory concentration (MIC) of < 0.15%, except for the methanol extract with MIC of 0.15-0.31% against *Propionibacterium acnes*. Additionally, molecular docking analysis revealed that five flavonoids possessing prenyl or geranyl, namely 6-isoprenyleriodictyol, nymphaeol A, nymphaeol B, and solophenol D, showed the highest binding affinity and dominant hydrogen bonding interactions with key amino acid residues in the binding site of the three proteins (IJJJ, 3IPK, and 7LBU). Thus, these findings suggest that the methanol extract and ethyl acetate fraction from *Macaranga hulletti* King ex Hook.f. has potential applications as a new source of antibacterial agents.

Keywords: Antibacterial, Extract, Flavonoid, *Macaranga hulletti*, Molecular Docking.

Introduction

Despite substantial advancements in medicine and fundamental research over recent decades, infectious diseases caused by transmissible agents, such as bacteria, eukaryotes, and viruses, continue to present significant challenges to scientists and healthcare professionals. These challenges are further intensified by the limitations in the rapid and accurate diagnosis of infections and the escalating issue of antimicrobial resistance.¹ Extracts from medicinal plants, which are abundant in diverse phytochemicals with a range of pharmacological properties, present a promising approach for addressing multi-drug resistant bacteria. Additionally, it may contribute to restoring the efficacy of antibiotics in the treatment of infectious diseases.² Therefore, it is imperative to continue the pursuit of more efficacious antimicrobial agents, particularly those sourced from natural sources, which are both readily available and economically viable.

Macaranga belongs to the Euphorbiaceae family and is known as "Mahang." Based on the literature, it is known that the secondary metabolite compounds contained in this genus are unique flavonoids and stilbenoids with prenylated substituents.³⁻¹³ Nine flavonoids containing either prenyl or geranyl group with antioxidant, antiplasmodial, and anticancer activities were successfully isolated from three *Macaranga* species present in East Kalimantan (*Macaranga hosei*, *Macaranga pearsonii*, and *Macaranga tanarius*): 4'-O-methyl-8-isoprenylnaringenin, lonchocarpol A, 4'-O-methyl-8-isoprenyleriodictyol, 6-isoprenyleriodictyol, solophenol D, nymphaeol A, nymphaeol B, nymphaeol C, including macahuilletin A that was isolated from *Macaranga hullettii* obtained from Central Kalimantan, which have antioxidant, antiplasmodial, and anticancer activities.¹⁴⁻¹⁸ *Macaranga tanarius* leaf extract exhibited antibacterial activity.¹⁹ Moreover, the plant extracts of *Macaranga conglomerata*, *Macaranga kilimandscharica* and *Macaranga capensis* from Kenya showed antibacterial activities against *E. coli*, *E. aerogenes*, *K. pneumoniae*, *P. stuartii*, *P. aeruginosa*, and *S. aureus*.²⁰ The flavonol rhamnetin, isolated from *Macaranga peltata*, also shows antibacterial activity against *Staphylococcus aureus*.²¹ Two flavanone group compounds, 5-hydroxy-7,4'-dimethoxyflavone and 5-hydroxy-6,7,4'-trimethoxyflavone, isolated from *Macaranga hosei*, showed weak antibacterial activity against three pathogenic bacteria.²² In this study, the leaves of *Macaranga hullettii* King ex Hook.f. was used as the object of analysis to identify new sources for the treatment of infectious diseases caused by bacteria. This plant is known to be a plant that can be found in all areas of East Kalimantan, where this species is commonly found in mountain forests and logging forests in the Kalimantan region.^{23, 24} Phytochemical screening and isolated compounds of *Macaranga hulletti* King ex Hook.f. leaf extract contained flavonoid compounds.^{18,25} Extensive research on the antibacterial activity of the *Macaranga* has been performed. However, the antibacterial properties of the methanol extract and its fractions from *Macaranga hulletti* King ex Hook.f. species have not yet been evaluated. Therefore, this study

aimed to evaluate the antibacterial activity of *Macaranga hullettii* King ex Hook.f. leaf extract and its fractions using *in vitro* and molecular docking studies.

Materials and Methods

Materials

The materials used in this study were *Macaranga hullettii* leaves, methanol, n-hexane, ethyl acetate, *Staphylococcus aureus* ATCC 25923, *Streptococcus mutans* ATCC 25175, and *Propionibacterium acnes* KCCM 41747, nutrient agar (NA) solid media, Luria Bertani liquid medium (1% tryptone, 0.5% yeast, and 0.5% NaCl), ampicillin (10 µg/mL), cotton swabs, aluminum foil, gauze, Petri dish, Ose needle, paper discs, autoclave, oven, rotary vacuum evaporator, freezer, Lenovo Yoga 7 with AMD Ryzen AI 7-8840HS w/ Radeon 780M graphics, 3301 Mhz, 8 Core(s), and RAM 16.0 GB.

Methods

Sample preparation

The research samples used in this study were leaves of *Macaranga hullettii* King ex Hook.f. plants obtained from the Lesan River Forest, Kelay District, Berau Regency, and East Kalimantan. Plant samples were identified at the Laboratory of Anatomy and Plant Systematics, FMIPA Mulawarman University (No. 0174/UN.17.8.5.7.16/HA/XI/2017).

Extraction

Macaranga hullettii King ex Hook.f. leaf powder (1 kg) was extracted twice by maceration for 24 h using methanol. The extract was evaporated using a rotary vacuum evaporator to obtain a methanol extract. The methanol extract was fractionated successively using n-hexane and ethyl acetate. Each fraction was evaporated using a rotary vacuum evaporator to obtain n-hexane and

ethyl acetate fractions. Furthermore, methanol extract, n-hexane fraction, and ethyl acetate fraction were prepared to evaluate their antibacterial activity.

Pathogens

Staphylococcus aureus ATCC 25923 was provided by the Department of Microbiology, Faculty of Science, Chulalongkorn University. *Streptococcus mutans* ATCC 25175 was prepared by the Department of Biochemistry, Faculty of Dentistry, Chulalongkorn University. Both strains were purchased from the American Type Culture Collection (ATCC). *Propionibacterium acnes* KCCM 41747 was obtained from the Faculty of Forestry, Mulawarman University, and was purchased from the Korean Culture Center of Microorganisms (KCCM). Furthermore, the tested bacteria were periodically subcultured and maintained in nutrient agar (NA) under suitable conditions, depending on the tested bacteria.

In vitro antibacterial assay

Determination of antibacterial prospects in methanol extract, n-hexane, and ethyl acetate fractions of *Macaranga hullettii* King ex Hook.f. plant leaves were analyzed using disc paper or the Kirby-Bauer method at varying concentrations.^{26,27} In this study, *Staphylococcus aureus* ATCC 25923, *Streptococcus mutans* ATCC 25175, and *Propionibacterium acnes* KCCM 41747 were employed. Ampicillin was used as a positive control. Organic solvents, such as methanol, n-hexane, and ethyl acetate, were used as negative controls. Nutrient Agar (NA) media was prepared by dissolving 3.3 grams in 150 mL of distilled water. Luria Bertani (LB) liquid media as much as 4 grams was dissolved in 500 mL of distilled water, then stirred, and put 5 mL each into a test tube. Subsequently, NA and LB were sterilized using an autoclave at 121 °C with a pressure of 1 atm for 15 minutes. Each tested bacterium was collected using an Ose needle, after which it was diluted into a test tube containing sterile LB media and then

incubated for 24 h at 37 °C. After the incubation period, the tested bacteria were ready for use in the subsequent stages.

Nutrient Agar (25 mL) was poured into a sterilized Petri dish. The NA medium was maintained at room temperature for solidification. The bacterial suspension in the test tube was then collected using a sterile cotton swab. A sterile cotton swab was applied to the entire surface of the agar on a Petri dish. Each 30 µL of the tested *M. hulleitii* leaf samples prepared at various concentrations (in organic solvent) was poured onto sterile adsorbent filter paper discs (6 mm in diameter), and the solvent was removed. Afterward, the paper discs containing the tested sample were placed onto the surface of agar media, closed the lid, and incubated at 37 °C for 18-24 hours. The inhibition zone formed in each well was measured using a ruler. Furthermore, the Minimum Inhibitory Concentration (MIC) value was determined for the methanol extract as well as the n-hexane and ethyl acetate fractions.

Molecular docking

The molecular structures were drawn using ChemOffice Professional 15.0 and retrieved from PubChem (<https://pubchem.ncbi.nlm.nih.gov/>). Structure refinement was carried out using the molecular mechanics energy minimization method with the Merck molecular force field (MMFF94) using Open Babel in PyRx V.1.1. Furthermore, three proteins were download from protein data bank (PDB ID: 1JJJ, 3IPK, 7LBU),²⁸⁻³⁰ the location of active site was calculated using AlphaFold from <https://prankweb.cz/>.³¹ Molecular docking was performed using the AutoDock Vina tool in PyRx V.1.1 software with an exhaustiveness of 32 and a mode value of 9 poses for each docked ligand.^{32,33} The protein binding site was defined as a box with dimensions of 30 × 30 × 30 Å. In the final step, the binding interactions were analyzed, and the docking results were visualized in three dimensions using the BIOVIA Discovery Studio Visualizer.

Results and Discussion

In vitro antibacterial activity

The strength of antibacterial activity was determined based on the diameter of the clear zone to obtain the inhibition zone (mm), which can be classified as ≤ 5 mm (weak), 5-10 mm (moderate), 10-20 mm (strong), and ≥ 20 mm (very strong). Moreover, the smaller the minimum inhibitory concentration of a compound, the greater its ability to inhibit the growth of test bacteria.³⁴ In this study, ampicillin was used as a positive control against three bacteria: *Staphylococcus aureus* ATCC 25923, *Streptococcus mutans* ATCC 25175, and *Propionibacterium acnes* KCCM 41747, as shown in Table 1. The positive control served to determine whether the test bacteria could be inhibited by growth or were resistant to antibiotics. *Staphylococcus aureus* can cause dentoalveolar infections, jaw cysts, paroritis, oral mucosal lesions, and stomatitis. It can also produce exotoxins, lecosidine, enterotoxins, and quagulase compounds. Enterotoxins are compounds that cause food poisoning in humans.³⁵ Antibacterial activity of the methanol extract, hexane fraction, and ethyl acetate fraction of *Macaranga hulettii* King ex Hook. f. leaves showed moderate antibacterial activity against *S. aureus* because of the inhibition zone of 5-10 mm at concentration from 0.15-2.5%, as presented in Table 1. The inhibition zone produced by the methanol extract was 6.67 mm at 0.15%. Meanwhile, the ethyl acetate fraction at 0.15% exhibited an inhibition zone at 6.33 mm. However, the n-hexane fraction exhibited antibacterial activity at 2.5%, with an inhibition zone of 6.50 mm. The results indicated that the methanol extract and ethyl acetate fraction of *Macaranga hulettii* King ex Hook, f leaves had the most potent antibacterial activity against *S. aureus*, with a minimum inhibitory concentration (MIC) of $<0.15\%$. Moreover, *Macaranga tanarius* is known to inhibit the growth of *S. aureus* most effectively in the n-hexane fraction, with an MIC value of 0.125-0.5% and an inhibition zone of approximately 7.25 mm.³⁶

Streptococcus mutans is a prominent bacterial species that is implicated in the pathogenesis of dental caries. This facultative anaerobe primarily ferments carbohydrates, resulting in the production of various organic acids, with lactic acid as the predominant byproduct. The acidification of the dental environment by *S. mutans* is a crucial factor contributing to enamel demineralization and the subsequent formation of carious lesions.^{37,38} These bacteria produce glucosyltransferase (GTF) to produce glucan, which causes dental caries.³⁹ *S. mutans* adheres to the salivary pellicle because of the many receptors for attachment to the salivary pellicle.⁴⁰ Furthermore, the antibacterial activity of *Macaranga hulettii* King ex Hook f. leaf extract and fractions were tested against *S. mutans*, as shown in Table 1. The result revealed that both extract and fraction exhibited moderate antibacterial activity because of the inhibition zone of 5-10 mm at a concentration of 0.15-2.5%. The inhibition zones created by the methanol extract and ethyl acetate fraction were 6.67 and 6.33 mm at 0.15%, respectively. Meanwhile, the n-hexane fraction showed antibacterial activity at 1.25-2.5% with an inhibition zone of 6.33-6.67 mm. These results demonstrated that the methanol extract and ethyl acetate fraction of *Macaranga hulettii* King ex Hook. leaves showed the highest potential antibacterial activity against *S. mutans*, with an MIC <0.15% (Table 1).

Furthermore, *Propionibacterium acnes* is a gram-positive anaerobic bacterium that can induce unusual keratinization within the sebaceous glands of hair follicles and increase sebum production.⁴¹ The enzyme is capable of hydrolyzing triglycerides (TG) present in sebum into free fatty acids (FFA), which may subsequently induce inflammation in and around the hair follicles.⁴² These bacteria also produce hyaluronidase, protease, lecithinase and neuramidase which cause inflammation of the skin, *Macaranga triloba* ethanol extract is known to inhibit the growth of *P. acnes* at 2.5% and the largest inhibition value of 5.54 mm at 20%.⁴³ As shown in Table 1, the methanol extract and ethyl acetate fraction of *Macaranga hulettii* King ex Hook f. showed strong antibacterial activity against *P. acnes* because of the inhibition values of 10.67

and 14.67 mm at 2.5%, respectively. Meanwhile, the n-hexane fraction exhibited moderate antibacterial activity with inhibition value of 5-10 mm at 1.25-2.5%. Furthermore, the methanol extract showed antibacterial activity with an inhibition value of 7.67 at 0.31%. The ethyl acetate fraction showed antibacterial activity of 0.15%, with an inhibition zone of 6.67 mm. The results revealed that the ethyl acetate fraction of *Macaranga hulettii* King ex Hook.f leaves presented the highest potential antibacterial activity against *P. acnes*, with a minimum inhibitory concentration (MIC) of <0.15%, as presented in Table 1.

Based on the data of antibacterial activity, both extracts and their fractions from *Macaranga hulettii*, the methanol extract and ethyl acetate fraction exhibited good antibacterial activity against the three bacterial strains. This finding was consistent with previous studies indicating that the methanol extract and ethyl acetate fraction demonstrated antibacterial activity, attributable to the presence of flavonoids and their prenylated or geranylated forms in *Macaranga* species, such as euchrestaflavanone A, bonanione A, macarangaflavanone A, macarangaflavanone B, macatrichocarpin A, propolin D (nymphaeol B), senegalensin, schweinfurthin B, schweinfurthin O, and isomacarangain.⁴⁴⁻⁵⁴ Flavonoid compounds can damage bacterial cell walls and denature proteases that disrupt bacterial metabolic processes.⁵⁵ In addition, alkaloids can damage the peptidoglycan layer in bacterial cells, causing damage to the bacterial cell wall and inhibiting bacterial growth. Terpenoids also inhibit bacterial growth by damaging bacterial cell membranes. Moreover, the phenolic compounds of secondary metabolites can damage bacterial cells by increasing the permeability of the cytoplasmic membrane, resulting in cytoplasmic coagulation and cell division.³⁴

Molecular docking study

Eight flavonoid derivatives possessing either prenyl or geranyl group were found in the three *Macaranga* species in East Kalimantan, Indonesia (*Macaranga hosei*, *Macaranga pearsonii*,

and *Macaranga tanarius*): 4'-O-methyl-8-isoprenylnaringenin, lonchocarpol A, 4'-O-methyl-8-isoprenyleriodictyol, 6-isoprenyleriodictyol, solophenol D, nymphaeol A, nymphaeol B, and nymphaeol C.¹⁴⁻¹⁷ Macahuilettiin A was prenylated flavonoid found in the *Macaranga huletti* obtained from Central Kalimantan (Indonesia).¹⁸ Furthermore, a comparative analysis of the molecular docking results of prenylated/geranylated flavonoids with methoxylated flavonoids was conducted, focusing on 5-hydroxy-7,4'-dimethoxyflavone and 5-hydroxy-6,7,4'-trimethoxyflavone, which were found in the *Macaranga hosei* from Trengganu, Malaysia,²² as presented in Table 2. Molecular docking was performed to evaluate the interaction of the flavonoids with the amino acid residues of the three proteins. This study used three proteins from *Staphylococcus aureus*, *Streptococcus mutans*, and *Propionibacterium acnes* that were retrieved from the Protein Data Bank with PDB ID 1IJJ, 3IPK, and 7LBU, respectively.²⁷⁻²⁹ As illustrated in Table 2, the molecular docking results revealed that 11 flavonoid derivatives exhibited binding affinities ranging from -7.2 to -10.5 kcal/mol, -7.5 to -8.9 kcal/mol, and -7.1 to -8.9 kcal/mol against *Staphylococcus aureus* tyrosyl-tRNA synthetase (PDB ID: 1IJJ), *Streptococcus mutans* antigen I/II (AgI/II) (PDB ID: 3IPK), and *Propionibacterium acnes* surface sialidase (PDB ID: 7LBU), respectively. Furthermore, the interaction of flavonoids and ampicillin with the protein target is depicted in two dimensions, as presented in Figure 1-3.

Among the compounds against *S. aureus* tyrosyl-tRNA synthetase, nymphaeol A-B and solophenol D had the highest binding affinity compared to ampicillin as a positive control, as shown in Table 2. Furthermore, ampicillin showed hydrogen bonding with LYS84 and GLN174, and amide- π interactions with GLY38, as depicted in Figure 1. Nymphaeol A and nymphaeol B exhibited hydrogen bonding with four amino acid residues, π -anions, and hydrophobic interactions, whereas nymphaeol B and ampicillin showed similar interactions with LYS84. In addition, solophenol D displayed hydrogen bonding with TYR36, THR75, GLN190, ASP177, and GLN174, as well as hydrophobic interactions with HIS50, LEU70,

PRO53, and ALA39 (Figure 1). This result was in line with previous study, where nymphaeol A-B and solophenol D exhibited antibacterial activity.^{56,57}

As presented in Table 2, five compounds exhibited high antibacterial activity against *S. mutans* antigen I/II (AgI/II), including 6-isoprenyleriodictyol, nymphaeol A-B, and solophenol D, with binding affinity from -8.7 to -8.9 kcal/mol. As shown in Figure 2, ampicillin formed hydrogen bonding with four amino acid residues, ASN590, SER591, SER588, and ASP760, as well as π -sulfur and π - π interactions with TRP 816. Furthermore, 6-isoprenyleriodictyol showed only hydrogen bonding with three amino acid residues: SER697, ASN699, and ASN814. Meanwhile, nymphaeol A and nymphaeol B displayed hydrogen bonding with four amino acid residues (Figure 2), but nymphaeol A exhibited a similar π - π interaction with TRP816, as observed with ampicillin. Solophenol D formed hydrogen bonds with SER818, ARG824, and THR586, similar π - π interactions (TRP816) with ampicillin, and π -sigma interactions with SER762. Furthermore, 6-isoprenyleriodictyol is a prenylated flavonoid similar to nymphaeol A. Thus, 6-isoprenyleriodictyol may have antibacterial potency.

Moreover, three compounds displayed stronger binding affinities than ampicillin, with values of -8.0 and -8.4 kcal/mol against *P. acnes* surface sialidases, such as 6-isoprenyleriodictyol and nymphaeol A, respectively, as described in Table 2. As depicted in Figure 3, ampicillin showed hydrogen bonding with four amino acid residues, ARG282, GLN287, ASP146, ASP185, and π - π and π -alkyl interactions with PHE257, ALA147, and VAL202. 6-Isoprenyleriodictyol demonstrated π - π interactions with PHE257, π -anion interactions with ASP146, and alkyl-alkyl interactions with ALA147, PHE209, VAL202, and LEU224. Furthermore, this compound displayed hydrogen bonding with six amino acid residues, including ASP146, ARG329, TYR423, ARG282, and GLU287, which were analogous to those observed with ampicillin. Nymphaeol A showed hydrogen bonding with five amino acid residues (ARG329, TYR423, ASP185, ALA147, and SER184), π -anion interactions with ASP312 and ASP185, alkyl-alkyl

interactions with MET310, ALA331, and ALA147, and Van der Waals interactions with PHE257. Thus, these compounds may have potent antibacterial activity.

Structure-activity relationships were also studied based on the binding affinity results, as presented in Table 2. Flavonoids possessing prenyl, geranyl, and hydroxyl groups showed good potency as antibacterial agents owing to their strong binding affinity with amino acid residues in the binding site of proteins compared with ampicillin. Nevertheless, the absence of hydroxy group on prenylated or geranylated flavonoids decreased binding affinity.^{22,53,58}

Conclusion

The extract and fraction of *Macaranga hulettii* King ex Hook, f, have potential as antibacterial agents against three bacterial strains, *Staphylococcus aureus* ATCC 25923, *Streptococcus mutans* ATCC 25175, and *Propionibacterium acnes* KCCM 41747. The methanol extract exhibited antibacterial activity against *S. aureus* and *S. mutans* with MIC (%) of <0.15 against *S. aureus* and *S. mutans*, and 0.15-0.31% against *P. acnes*. n-Hexane fraction showed antibacterial activity with MIC (%) of 1.25-2.5% against *S. aureus*, and 0.62-1.25% against *S. mutans* and *P. acnes*. Moreover, the ethyl acetate fraction displayed antibacterial activity with an MIC (%) <0.15% against three bacterial strains (*S. aureus*, *S. mutans*, and *P. acnes*). Molecular docking studies have suggested that 6-isoprenyleriodictyol, nymphaeol A, nymphaeol B, and solophenol D are promising antibacterial agents because those compounds exhibit the highest binding affinity and form hydrogen bonding interactions with key amino acid residues in the active site of the protein targets.

Conflict of Interest

The authors declare no conflicts of interest.

Author's Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims related to the content of this article will be borne by them.

Acknowledgement

This research was funded by the Islamic Development Bank (PIU-IsDB) project under contract no. 137/UN17.11/PL/2019.

References

1. Wong F, de la Fuente-Nunez C, Collins JJ. Leveraging artificial intelligence in the fight against infectious diseases. *Science*. 2023;381(6654):164-70.
2. Seukep AJ, Kuete V, Nahar L, Sarker SD, Guo M. Plant-derived secondary metabolites as the main source of efflux pump inhibitors and methods for identification. *Journal of pharmaceutical analysis*. 2020;10(4):277-90.
3. Magadula JJ. Phytochemistry and pharmacology of the genus *Macaranga*: a review. *J.Med. Plants Res*. 2014;8(12):489-503.
4. Vu LT, Ngan TB, Phuong L, Huong DT, Litaudon M, Van Hung N, Thach TD, Van Cuong P. Chemical constituents from fruits of *Macaranga denticulata* (Euphorbiaceae)(Part 2). *Vietnam J. Chem*. 2018;56(4):516-20.
5. Marliana E, Hairani R, Tjahjandarie TS, Tanjung M. Antiplasmodial activity of flavonoids from *Macaranga tanarius* leaves. In *IOP Conference Series: Earth and Environmental Science* 2018;144(1):012011).
6. Tjahjandarie TS, Tanjung M, Saputri RD, Nadar PB, Aldin MF, Marliana E, Permadi A. Flavestins K, An isoprenylated stilbene from the leaves of *Macaranga recurvata* Gage. *Natural Product Sciences*. 2019;25(3):244-7.

7. Huong DT, Linh NT, Van TT, Litaudon M, Roussi F, Van Nam V, Van Cuong P. Stilbenes from *Macaranga tanarius* (Euphorbiaceae) growing in Vietnam. *Vietnam J. Chem.* 2020;58(3):338-42.
8. Aldin MF, Tjahjandarie TS, Saputri RD, Tanjung M. Macasiamenene V, a New Stilbenoid from the Leaves of *Macaranga inermis*. *Nat. Prod. Sci.* 2021;27(1):45-8.
9. Muharram A, Rachmawati DA, Mardhiyyah S, Tjahjandarie TS, Saputri RD, Ahmat N, Tanjung M. Cytotoxic and antioxidant activities of flavonoids and diterpenoids from *Macaranga involucrata* (Roxb.) Baill. *J. Appl. Pharm. Sci.* 2023;13(6):087-92.
10. Tanjung M, Tjahjandarie TS, Aldin MF, Mardhiyyah S, Ahmat N, Saputri RD. Macagigantin A, A New Flavonoid from *Macaranga gigantea* (Rchb. f & Zoll.) Mull. Arg. *Nat. Prod. Sci.* 2023;29(4):287-94.
11. Tjahjandarie TS, Aldin MF, Saputri RD, Tanjung M. Dihydrostilbenes from *Macaranga javanica* (Blume) Müll. Arg. and their antiplasmodial activity. *Nat. Prod. Sci.* 2024:1-7.
12. Tanjung M, Tjahjandarie TS, Aldin MF, Mardhiyyah S, Saputri RD, Syah YM, Ahmat N. Two new flavonols from *Macaranga inermis* pax & K. Hoffm. *Nat. Prod. Sci.* 2025 Feb 1;39(3):498-505.
13. Tanjung M, Tjahjandarie TS, Aldin MF, Mardhiyyah S, Saputri RD, Syah YM, Ahmat N. Two new flavonols from *Macaranga inermis* pax & K. Hoffm. *Nat. Prod. Sci.* 2025;39(3):498-505.
14. Marliana E, Tjahjandarie TS, Tanjung M. Isoprenylated flavanone derivatives from *Macaranga hosei* King ex Hook F. *Der Pharm. Lett.* 2015;7(3):153-6.
15. Marliana E, Tjahjandarie TS, Tanjung M. Aktivitas Antioksidan Senyawa Flavonoid Dari *Macaranga pearsonii* Merr. *J. Kim. Mulawarman.* 2016;13(2):97-100.

16. Marlina E, Astuti W, Kosala K, Hairani R, Tjahjandarie TS, Tanjung M. Chemical composition and anticancer activity of *Macaranga hosei* leaves. *Asian J. Chem.* 2018;30(4):795-8.
17. Marlina E, Ruga R, Hairani R, Tjahjandarie TS, Tanjung M. Antioxidant activity of flavonoid constituents from the leaves of *Macaranga tanarius*. In *IOP Conference Series: Journal of Physics*. 2019;1277(1):012014.
18. Saputri RD, Tukiran T, Wati FA, Purnamasari AP, Wardhana MW, Tjahjandarie TS, Tanjung M. Macahulleitiiin A, a new isoprenylated flavanone from the leaves of *Macaranga hulleitii* King ex Hook and their antiplasmodial activity. *Vietnam J. Chem.* 2024;62(3):394-8.
19. Musdalifah M, Khumaidi A, Suwastika IN. Uji Daya Hambat dan Skrining Fitokimia Ekstrak Daun *Macaranga tanarius* (L.) Mull. Arg Sebagai Antibakteri *Salmonella typhi*. *Nat. Sci: J. Sci. Technol.* 2017;6(3).
20. Ibrahim H, Omosa L, Nchiozem-Ngnitedem V, Onyari J, Maru S, Guefack M. Antibacterial activities and phytochemical screening of crude extracts from Kenyan *macaranga* species toward MDR phenotypes expressing efflux pumps. *Pharmacogn. Commn.* 2021;11(2):119-26.
21. Bijesh K, Sebastian D. Isolation and characterization of antibacterial compounds from *Macaranga peltata* against clinical isolates of *Staphylococcus aureus*. *Int. J. Biol. Pharma Res.* 2013;4:1196-203.
22. Salleh WM, Razak NZ, Ahmad F. Phytochemicals and biological activities of *Macaranga hosei* and *Macaranga constricta* (Euphorbiaceae). *Marmara Pharm. J.* 2017;21(4):881-8.
23. Slik JW, Priyono P, Welzen PV. Key to the *Macaranga* Thou. and *Mallotus* Lour. species (Euphorbiaceae) of East Kalimantan, Indonesia. Singapore, Gardens' Bulletin (Singapore), National Parks Board, Singapore Botanic Gardens; 2000. 11-87 p.

24. Amirta R, Angi EM, Ramadhan R, Kusuma IW, Wiati CB, Haqiqi MT. Potensi pemanfaatan macaranga. Samarinda: Mulawarman University Press; 2017.
25. Rismawati R, Marlina E, Daniel D. Phytochemical Test on Methanol Extract of Leaf of *Macaranga hullettii* King ex Hook. f. J. Atomik. 2018;3(2):91-4.
26. Hudzicki J. Kirby-Bauer disk diffusion susceptibility test protocol. American society for microbiology. 2009;15(1):1-23.
27. Inna M, Astuti W, Saleh C. Aktivitas antibakteri ekstrak metanol daun tanaman sambung urat (*Cayratia carnos*) terhadap *Salmonella thypi* and *Propionibacterium acnes*. J. Atomik. 2022;7(1):1-5.
28. Qiu X, Janson CA, Smith WW, Green SM, McDevitt P, Johanson K, Carter P, Hibbs M, Lewis C, Chalker A, Fosberry A. Crystal structure of *Staphylococcus aureus* tyrosyl-tRNA synthetase in complex with a class of potent and specific inhibitors. Protein Sci. 2001;10(10):2008-16.
29. Larson MR, Rajashankar KR, Patel MH, Robinette RA, Crowley PJ, Michalek S, Brady LJ, Deivanayagam C. Elongated fibrillar structure of a streptococcal adhesin assembled by the high-affinity association of α -and PPII-helices. Proc Natl Acad Sci. 2010;107(13):5983-8.
30. Yu AC, Volkers G, Jongkees SA, Worrall LJ, Withers SG, Strynadka NC. Crystal structure of the *Propionibacterium acnes* surface sialidase, a drug target for *P. acnes*-associated diseases. Glycobiology. 2022;32(2):162-70.
31. Jakubec D, Skoda P, Krivak R, Novotny M, Hoksza D. PrankWeb 3: accelerated ligand-binding site predictions for experimental and modelled protein structures. Nucleic Acids Res. 2022;50(W1):W593-7.

32. Dallakyan S, Olson AJ. Small-molecule library screening by docking with PyRx. In Chemical biology: methods and protocols. 2014: 243-250. New York, NY: Springer New York.
33. Trott O, Olson AJ. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. J. Comput. Chem. 2010;31(2):455-61.
34. Yulianti MF, Amat AL, Hutasoit RM, Pakan PD. Antibacterial activity of jamblang leaf ethanol extract (*Syzygium cumini*) against the growth of *Propionibacterium acnes*. Acta Biochimica Indones. 2023;6(2):1161.
35. Malangu N, editor. Poisoning: From Specific Toxic Agents to Novel Rapid and Simplified Techniques for Analysis. BoD–Books on Demand; 2017.
36. Sari AA, Saleh C. Uji fitokimia, toksisitas dan aktivitas antibakteri ekstrak berbagai fraksi daun mara (*Macaranga tanarius* (L.) MA) terhadap bakteri *Staphylococcus aureus* dan *Escherichia coli*. J. Kim. Mulawarman. 2015;12(2):53-8.
37. Imelda R., Mariam M., Satari M. Effect of cassava (*manihot esculenta* cranzt), rice (*oryza sativa* l.), and potato (*solanum tuberosum*) water extract to decrease ph phase fermentation of *streptococcus mutans* atcc 25175. Padjajaran J. Dent .2019;31(1):14.
38. Alkhaled A., Alsabek L., Al-assaf M. , & Badr F.. Effect of chlorhexidine, honey and propolis on *streptococcus mutans* counts: in vitro study. Dentistry 3000 2021;9(1).
39. Ren Z, Chen L, Li J, Li Y. Inhibition of *Streptococcus mutans* polysaccharide synthesis by molecules targeting glycosyltransferase activity. Journal of oral microbiology. 2016;8(1):31095.
40. Lee BS, Chen YJ, Wei TC, Ma TL, Chang CC. Comparison of antibacterial adhesion when salivary pellicle is coated on both poly (2-hydroxyethyl-methacrylate)-and polyethylene-

glycol-methacrylate-grafted poly (methyl methacrylate). International Journal of Molecular Sciences. 2018;19(9):2764.

41. Chen KC, Yang CH, Li TT, Zouboulis CC, Huang YC. Suppression of *Propionibacterium acnes*-stimulated proinflammatory cytokines by Chinese bayberry extracts and its active constituent myricetin in human sebocytes in vitro. *Phytother. Res.* 2019;33(4):1104-13.
42. Xu J, Chen X, Song J, Wang C, Xu W, Tan H, Suo H. Antibacterial activity and mechanism of cell-free supernatants of *Lactocaseibacillus paracasei* against *Propionibacterium acnes*. *Microb. Pathog.* 2024;189:106598
43. Warnida H, Mustika D, Supomo S, Sukawaty Y. Efektivitas Ekstrak Etanol Daun Mahang (*Macaranga Triloba*) sebagai Obat Anti-acne. *J. Penelit. Sos. Ekon. Kehuta.* 2018;4(1):9-18.
44. Schütz BA, Wright AD, Rali T, Sticher O. Prenylated flavanones from leaves of *Macaranga pleiostemona*. *Phytochemistry.* 1995;40(4):1273-7.
45. Lim TY, Lim YY, Yule CM. Evaluation of antioxidant, antibacterial and anti-tyrosinase activities of four *Macaranga* species. *Food Chem.* 2009;114(2):594-9.
46. Fareza MS, Syah YM, Mujahidin D, Juliawaty LD, Kurniasih I. Antibacterial flavanones and dihydrochalcones from *Macaranga trichocarpa*. *Zeitschrift für Naturforschung C.* 2014;69(9-10):375-80.
47. Hasanat A, Kabir MS, Hossain MM, Hasan M, Al Masum MA, Chowdhury TA, Bhuiyan DI, Mamur A, Kibria AS. Antibacterial activity of methanol extract of *Macaranga denticulata* leaves and in silico PASS prediction for its six secondary metabolites. *World J. Pharm. Pharm. Sci.* 2015:1258-66.
48. O Akanbi B, Anene P, Olayanju S. Preliminary Screening Indicates Promising Antimicrobial Properties of the Stem Bark Extracts of *Macaranga rosea*. *Anti-Infective Agents.* 2015;13(2):123-8.

49. Ogundajo A, Okeleye B, Ashafa AO. Chemical constituents, in vitro antimicrobial and cytotoxic potentials of the extracts from *Macaranga barteri* Mull-Arg. *Asian Pac. J. Trop. Biomed.* 2017;7(7):654-9.
50. Putri R, Hendra R, Teruna HY. Anti-Bacterial and Anti-Fungal Activities from *Macaranga bancana* Leaves Extract. *Pharmacology and Clinical Pharmacy Research.* 2019;4(1):1-4.
51. Lee JH, Kim YG, Khadke SK, Yamano A, Woo JT, Lee J. Antimicrobial and antibiofilm activities of prenylated flavanones from *Macaranga tanarius*. *Phytomedicine.* 2019;63:153033.
52. Pagna JI, Awazi T, Mbarga PE, Mbekou IM, Mkounga P, Fotie J, Frese M, Fabrice FB, Lenta BN, Sewald N, Nkengfack EA. Antibacterial flavonoids from the fruits of *Macaranga hurifolia*. *J. Asian Nat. Prod. Res.* 2022;24(11):1041-51.
53. Kamsi VF, Simo Fotso CC, Kanko Mbekou IM, Toussie BT, Ndjakou Lenta B, Boyom FF, Sewald N, Frese M, Ngadjui BT, Wabo Fotso G. Chemical constituents of *Macaranga occidentalis*, antimicrobial and chemophenetic studies. *Molecules.* 2022;27(24):8820.
54. Rosamah E, Haqiqi MT, Putri AS, Kuspradini H, Kusuma IW, Amirta R, Yuliansyah Y, Suwinarti W, Paramita S, Ramadhan R, Tarmadi D. The potential of *Macaranga* plants as skincare cosmetic ingredients: A review. *J. Appl. Pharm. Sci.* 2023;13(7):001-12.
55. Li AP, He YH, Zhang SY, Shi YP. Antibacterial activity and action mechanism of flavonoids against phytopathogenic bacteria. *Pesticide Biochemistry and Physiology.* 2022;188:105221.
56. Chen YW, Ye SR, Ting C, Yu YH. Antibacterial activity of propolins from Taiwanese green propolis. *J. Food Drug Anal.* 2018;26(2):761-8.
57. Inui S, Hosoya T, Shimamura Y, Masuda S, Ogawa T, Kobayashi H, Shirafuji K, Moli RT, Kozono I, Shin-ya K, Kumazawa S. Solophenols B–D and Solomonin: New Prenylated

Polyphenols Isolated from Propolis Collected from The Solomon Islands and Their Antibacterial Activity. *J. Agri. Food Chem.* 2012;60(47):11765-70.

58. Ruga R, Kingkaew K, Tamsampaoloet K, Chavasiri W. Enhancing antibacterial activity against *Propionibacterium acnes* and *Staphylococcus aureus* by combination of tetracycline with selected compounds. *Chem. Lett.* 2018;47(12):1538-41.

Table 1: Antibacterial Activity of *Macaranga hullettii* King ex Hook Leaf Extracts and Fractions against *Staphylococcus aureus*, *Streptococcus mutans*, and *Propionibacterium acnes*

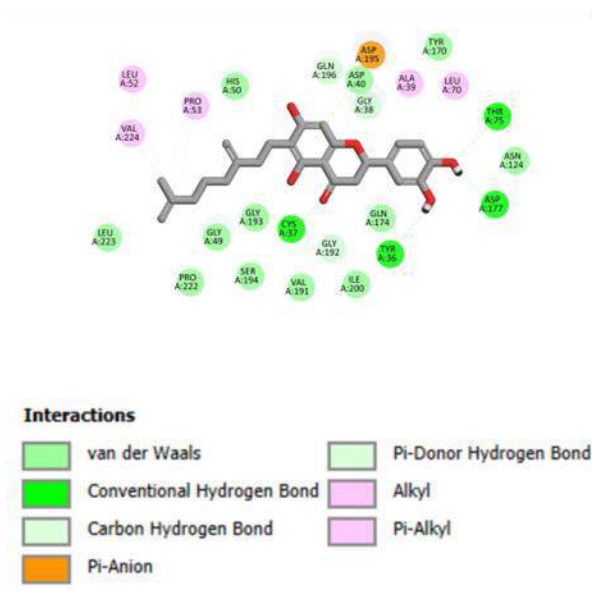
Sample	Concentration (%)	Inhibition Zone (mm)*		
		<i>Staphylococcus</i>	<i>Streptococcus</i>	<i>Propionibacterium</i>
		<i>aureus</i>	<i>mutans</i>	<i>acnes</i>
Methanol Extract	0	0	0	0
	0.15	6.67	6.67	0
	0.31	6.67	6.67	7.67
	0.62	6.67	7	8
	1.25	7.17	7.33	9.67
	2.5	7.5	8	10.67
	MIC (%)	<0.15	<0.15	0.15-0.31
n-Hexane Fraction	0	0	0	0
	0.15	0	0	0
	0.31	0	0	0
	0.62	0	0	0
	1.25	0	6.33	6.33
	2.5	6.5	6.67	6.67
	MIC (%)	1.25-2.5	0.62-1.25	0.62-1.25
Ethyl acetate Fraction	0	0	0	0
	0.15	6.33	6.33	6.67
	0.31	6.5	6.67	8
	0.62	6.5	6.83	9
	1.25	6.67	7	11
	2.5	7	7.67	14.67

	MIC (%)	<0.15	<0.15	<0.15
Ampicillin	0.01	20.33	21	21

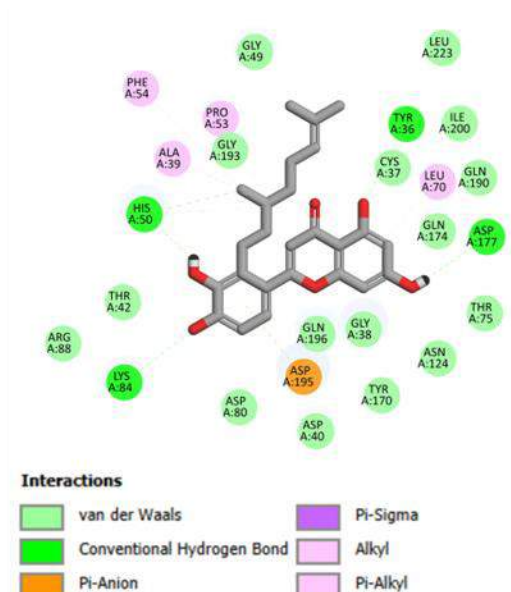
*Data were obtained from triplicate experiments.

Table 2: Binding affinity of flavonoids against three proteins (1JIJ, 3IPK, 7LBU)

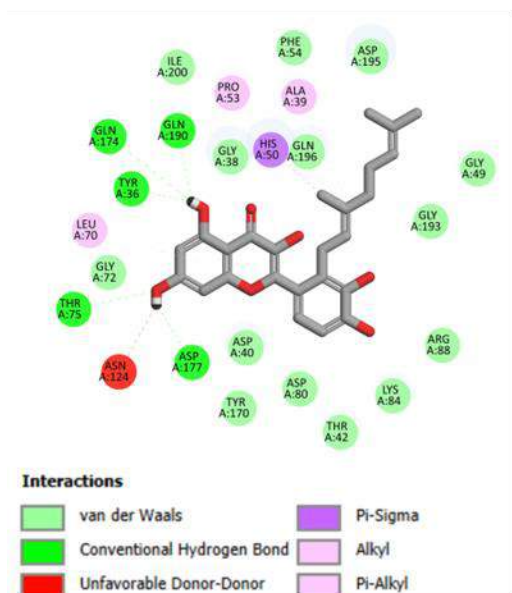
No.	Compound	Binding Affinity (kcal/mol)		
		1JIJ	3IPK	7LBU
1	4'-O-methyl-8-isoprenylnaringenin	-7.4	-8.1	-7.7
2	4'-O-methyl-8-isoprenyleriodictyol	-7.2	-8.1	-7.6
3	6-Isoprenyleriodictyol	-7.7	-8.7	-8.0
4	5-hydroxy-7,4'-dimethoxyflavone	-8.6	-7.8	-7.1
5	5-hydroxy-6,7,4'-trimethoxyflavone	-8.3	-7.5	-7.3
6	Macahuilettiin A	-7.6	-8.4	-7.1
7	Lonchocarpol A	-9.4	-7.8	-7.8
8	Nymphaeol A	-9.7	-8.9	-8.4
9	Nymphaeol B	-10.5	-8.9	-7.7
10	Nymphaeol C	-8.4	-8.3	-7.4
11	Solophenol D	-10.0	-8.7	-7.9
12	Ampicillin	-8.5	-7.7	-7.6



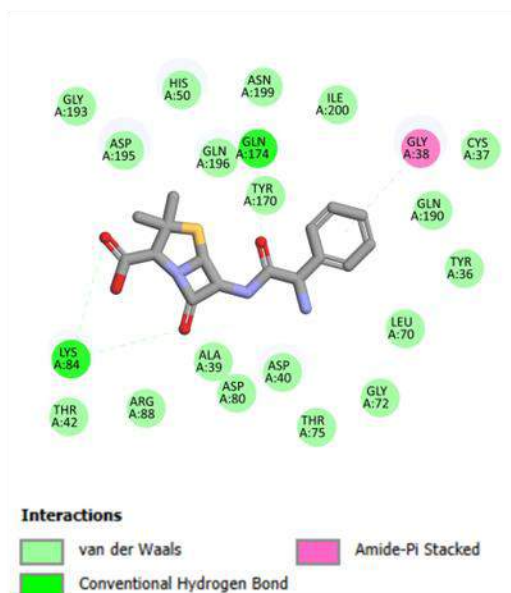
Nymphaeol A



Nymphaeol B



Solophenol D



Ampicillin

Figure 1: 2D interaction of the best docked flavonoids and ampicillin with *Staphylococcus aureus* tyrosyl-tRNA synthetase (PDB ID: 1JIJ)

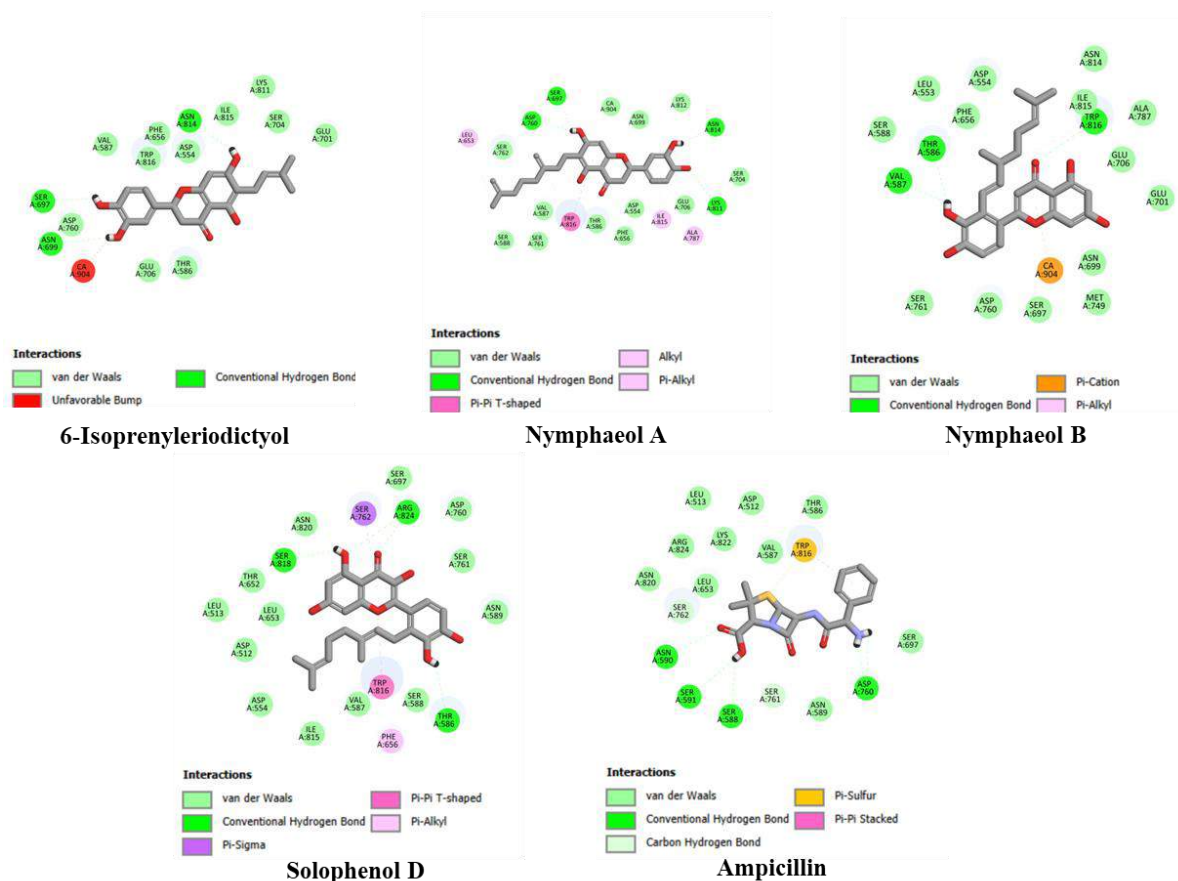


Figure 2: 2D interaction of the best-docked flavonoids and ampicillin with *Streptococcus mutans* antigen I/II (PDB ID: 3IPK).

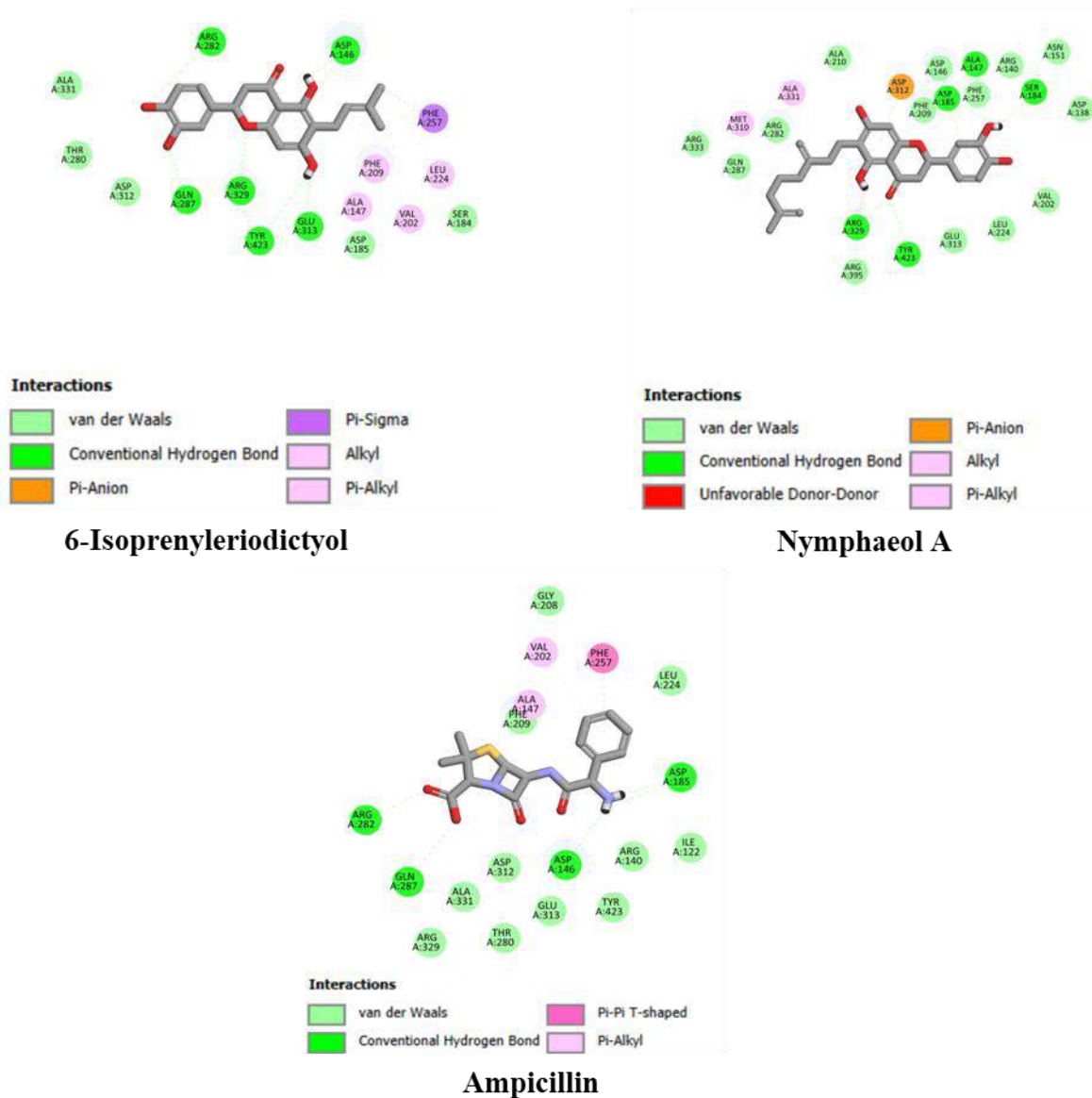


Figure 3: 2D interaction of the best-docked flavonoids and ampicillin with *Propionibacterium acnes* surface sialidase (PDB ID: 7LBU).

Submission Acknowledgement

From Prof Abiodun Falodun <tjnpuwnx@business99.web-hosting.com>

Date Wed 4/2/2025 9:49 PM

To Ade Danova <adedanova@itb.ac.id>

Ade Danova:

Thank you for submitting the manuscript, "In Vitro and Molecular Docking Evaluation of New Antibacterial Activity of Macaranga hulletti King ex Hook.f. Leaf Extract" to Tropical Journal of Natural Product Research (TJNPR). With the online journal management system that we are using, you will be able to track its progress through the editorial process by logging in to the journal web site:

Submission URL: <https://tjnpr.org/index.php/home/authorDashboard/submission/6088>

Username: adedanova

If you have any questions, please contact me. Thank you for considering this journal as a venue for your work.

[Tropical Journal of Natural Product Research \(TJNPR\)](#)

Email: editor.tjnpr@gmail.com | Phone: +2348073184488

Caution: This Message was sent from outside of Institute Technology Bandung (ITB). Please do not click Link or open attachment unless you recognize the source of this email and know the content is safe. Please report all suspicious email to "it-helpdesk@itb.ac.id" as an attachment.



TJNPR Manuscript Submission Request

From Editor-in-Chief Tjnpr <editor.tjnpr@gmail.com>

Date Thu 4/3/2025 10:45 PM

To Ade Danova <adedanova@itb.ac.id>

Thank you for submitting your original manuscript to the Tropical Journal of Natural Product Research (TJNPR) www.tjnpr.org TJNPR is indexed in SCOPUS/SCIMAGO **Q3** Journal Ranking (<https://www.scopus.com/sourceid/21100933230>).

Please, provide the following information:

1. suggest four (4) competent reviewers { two (2) foreign/international, two (2) National/from your country}. Their names, university-based email addresses and affiliations should be submitted.
2. The email addresses of all the authors including their roles/contributions in the manuscript should also be submitted.

The peer-review process will commence immediately, as the manuscript will be passed to an editor for initial assessment as soon as possible. If there are any problems with your submission, we will contact you. Also, note that manuscripts submitted and undergoing peer review will not be accepted for withdrawal or retraction.

Title: ***In Vitro* and Molecular Docking Evaluation of New Antibacterial Activity of *Macaranga hulletti* King ex Hook.f. Leaf Extract**

Best regards

Abiodun

Professor Abiodun Falodun, PhD; FAAS, FISPON

Editor-in-Chief:
Tropical Journal of Natural Product Research (TJNPR)
Head, Natural Product Research Group, University of Benin
Email: editor.tjnpr@gmail.com
www.tjnpr.org SCOPUS, SCImago SJR Q3

Professor of Pharmaceutical Chemistry, FAAS
Fellow, Fulbright (USA)
Deputy Vice-Chancellor (Academic) 2014-2016
Faculty of Pharmacy

University of Benin

[Google Scholar Citations](#)

SCOPUS <https://www.scopus.com/authid/detail.uri?>



www.tjnpr.org

Caution: This Message was sent from outside of Institute Technology Bandung (ITB). Please do not click Link or open attachment unless you recognize the source of this email and know the content is safe. Please report all suspicious email to "it-helpdesk@itb.ac.id" as an attachment.

Recommendation Reviewers and Author Contribution

×

Participants

- Odudu Udocox (dynettech)
- Ade Danova (adedanova)

Messages

Note	From
Dear Professor Professor Abiodun Falodun, PhD	adedanova
I would like to send the list of reviewers and author contribution to complete our submission process. Thank you very much.	2025-04-03
Best regards, Ade Danova, Ph.D.	11:15 PM
📎 Four Potential Reviewers.docx	
📎 Author Contribution Statements.docx	

Add Message

Dear Professor Abiodun Falodun, PhD
Editor-in-Chief
Tropical Journal of Natural Product Research

I would like to recommend four potential reviewers as follows:

1. Dr. Fera Kurniadewi, Universitas Negeri Jakarta, Indonesia (email: fera@unj.ac.id)
2. Dr. Iqbal Musthapa, Universitas Pendidikan Indonesia, Indonesia (email: iqbalm@upi.edu)
3. Dr. Huong Thi Thu Le, Ho Chi Minh City University of Education Vietnam, Vietnam (email: huongltht@hcmue.edu.vn)
4. Noraziah Nordin, Ph.D., Faculty of Medicine and Health Sciences, Universiti Sains Islam Malaysia (email: noraziahnordin@usim.edu.my)

Thank you very much.

Best regards,

A handwritten signature in black ink, appearing to be 'Ade Danova', with a large, stylized initial 'A' and 'D'.

Ade Danova, Ph.D.

Author Contribution Statements

Author	Institution	E-mail	Contribution
Eva Marliana	Mulawarman University	evakia@fmipa.unmul.ac.id	Conceptualization, Designed the Methodology, Collected Sample, Conducted the Experiments, Collected and Analysis Data, Validation, Writing- Original draft Preparation, Writing- Reviewing and Editing, Supervision
Ade Danova	Institut Teknologi Bandung	adedanova@itb.ac.id	Conducted the Experiments, Collected and Analysis Data, Writing- Reviewing and Editing.
Rita Hairani	Mulawarman University	ritahairani@fmipa.unmul.ac.id	Collected Sample, Conducted the Laboratory Experiments, Collected and Analysis Data.
Ritbey Ruga	Mulawarman University	ritbey.r@fmipa.unmul.ac.id	Conducted the Laboratory Experiments, Collected and Analysis Data.
Elvira Hermawati	Institut Teknologi Bandung	elvira.hermawati@itb.ac.id	Analysis Data, Writing- Reviewing and Editing,
Ana Yulvia	Universitas Jember	anayulvia@unej.ac.id	Analysis Data, Writing- Reviewing and Editing



Outlook

Re: TJNPR Manuscript Submission Request

From Ade Danova <adedanova@itb.ac.id>

Date Mon 4/7/2025 5:15 AM

To Editor-in-Chief Tjnpr <editor.tjnpr@gmail.com>

Dear Editor-in-Chief TJNPR
Professor Abiodun Falodun

I would like to inform you that I already have submitted on the website for reviewer suggestion and author contribution list. Thank you very much.

Best Regards,
Ade Danova, Ph.D.

Organic Chemistry Division

Department of Chemistry, Faculty of Mathematics and Natural Sciences

Institut Teknologi Bandung, Indonesia



Outlook

Trop J Nat Prod Res Editorial Decision

From Editor-in-Chief Tjnpr <editor.tjnpr@gmail.com>

Date Sat 4/26/2025 7:47 PM

To Ade Danova <adedanova@itb.ac.id>

 1 attachment (329 KB)

Provisional acceptance 1121.pdf;

Dear Dr. Ade Danova,

The manuscript submitted to the Tropical Journal of Natural Product Research www.tjnpr.org Q3 <https://www.scopus.com/sourceid/21100933230> has been carefully reviewed by competent experts.

Find attached the details of the decision.

Please send your response urgently to the Editor-in-Chief, to enable us to process your manuscript for the next issue **Vol 9 issue 5, 2025**.

Kindly acknowledge the receipt of the mail.

Title: *In Vitro and Molecular Docking Evaluation of New Antibacterial Activity of Macaranga hulletti King ex Hook.f. Leaf Extract*

Authors: Eva Marliana, Ade Danova*, Rita Hairani, Ritbey Ruga, Elvira Hermawati, and Ana Yulvia

TJNPR Editorial Decision: accepts with moderate revisions

Congratulations.



<https://www.scimagojr.com/journalsearch.php?q=21100933230&tip=sid>

Best regards

Abiodun

Professor Abiodun Falodun, PhD; FAAS, FISPN

Editor-in-Chief:

Tropical Journal of Natural Product Research (TJNPR)

Head, Natural Product Research Group, University of Benin

Email: editor.tjnpr@gmail.com

www.tjnpr.org SCOPUS, SCImago SJR Q3

Professor of Pharmaceutical Chemistry, FAAS

Fellow, Fulbright (USA)

Deputy Vice-Chancellor (Academic) 2014-2016

Faculty of Pharmacy

University of Benin

[Google Scholar Citations](#)

SCOPUS <https://www.scopus.com/authid/detail.uri?>



www.tjnpr.org

Caution: This Message was sent from outside of Institute Technology Bandung (ITB). Please do not click Link or open attachment unless you recognize the source of this email and know the content is safe. Please report all suspicious email to "it-helpdesk@itb.ac.id" as an attachment.

Editorial and Review comments

From Editor-in-Chief Tjnpr <editor.tjnpr@gmail.com>

Date Thu 5/1/2025 11:35 PM

To Ade Danova <adedanova@itb.ac.id>

 3 attachments (1,013 KB)

1-Editorial Comment - TJNPR-2025-M420.docx; 2-TJNPR-2025-420 Reviewer 1.pdf; 3-TJNPR-2025-420 Reviewed 1.docx;

Please see the editorial comments (below) and attached copies of the reviewer comments for manuscript title “ **In Vitro and Molecular Docking Evaluation of New Antibacterial Activity of Macaranga hulletti King ex Hook.f. Leaf Extract** ” .

Editorial comments to authors

Title: Names (First and Last name in full, middle name as initials) and affiliations of authors should be written correctly. Correspondence authors' contact address (email and telephone number) should also be stated.

Abstract: format to accommodate to the required word (250) limit. Format to a single paragraph and avoid subheadings. Begin with a brief background, the aim of study should be clearly stated, methodology, results and conclusion.

Move all figures and tables under the reference section.

Graphics under the same figure title should be grouped together as a single figure representation.

All botanical and zoological names should be **italicized including those in reference section**

For non-integers, use periods/decimal point NOT commas.

Write all page number in full. E.g 12345 – 12359 and not 12345 – 59

Remove italics and abbreviate journal title in reference section

In-text references should be in superscript numerals without square brackets, and placed after commas or full-stop.

References: Cite relevant and related references from the published articles of TJNPR www.tjnpr.org

Adhere strictly to the Journal's style for listing references. Abbreviate all journal names, and replace ‘et al’ with names of all contributing authors;

Falodun A, Siraj R, Choudhary MI. GC-MS Insecticidal Leaf essential oil of *P. staudtii* Hutch and Dalz (Icacinaceae). Trop J. Pharm Res. 2009; 82:139-143.

Okolie NP, Falodun A, Oluseyi D. Evaluation of the antioxidant activity of root extract of pepper fruit (*Dennetia tripetala*), and its potential for the inhibition of Lipid peroxidation. Afr J. Trad Compl and Altern Med. 2014; 11(3):221-227. Doi: 10.4314/ajtcam.v11i3.31

Conflict of interest session should be included, and if there is no conflict of interest, this should be stated clearly as follows; **The authors declare no conflict of interest.**

A declaration of the liability of the authors for claims relating to the content of this article should also be included when submitting the revised manuscript. This should be stated as follows;

Authors' Declaration

The authors hereby declare that the work presented in this article are original and that any liability for claims relating to the content of this article will be borne by them.

All comments/corrections made by reviewers should be completely addressed, point by point, and make appropriate changes in the manuscript, or provide a suitable rebuttal to any specific request for change that has not been made. All corrections/changes made in the manuscript should be highlighted in yellow colour when submitting the manuscript in the revised form.

Please keep the revised manuscript code format as “TJNPR-(Year)-(Code). Do not Rename the document.

All corrections/changes made in the manuscript should be highlighted in yellow colour when submitting the manuscript in the revised form.

The authors should carefully revise and correct the manuscript taking into consideration the comments of all the reviewers. 50% of the references cited should be between 2020-2025. The revised and corrected manuscript should be subjected to plagiarism checker (17% allowed in TJNPR) and English language editing. Evidence of the checks should be attached when submitting the revised/corrected manuscript.

During submission of the revised manuscript include another file labelled “Responses to reviewers’ comments” (a matrix) clearly showing your responses to each of the issues raised by the reviewers; mention the section, page and paragraph/lines where and how the changes/corrections have been made.

Strictly adhere to the author guidelines. Make sure that all the facts and information provided in the manuscript are correct. Check grammar, spelling, spacing, other information and facts including scientific names, formulae, symbols, equations, etc.

Ensure that all the references are correctly cited in the text and list. Verify all the references from their original sources. Confirm correctness of the citation info such as authors’ names (surnames, initials, spelling, arrangements, etc), year, title, journal, volume, pages, punctuation, etc. The numbers and units must be presented according to the journal style. Use clearly distinguishable patterns for the illustrations/figures (e.g., graphs and charts) such that they should be legible even for black and white printing or when reduced in size.

Proofread the whole document after effecting all the corrections. The revised version should be approved by all the co-authors before submitting it.

Important Points for authors

A. Authors strongly advised to read and comply with items 1-11.

B. Authors are also required to submit the files (1-4) when submitting the revised manuscript.

C.

1. The revised and edited manuscript
2. The list of responses to reviewers' comments (All the issues raised by the reviewers must be addressed)
3. Evidence of plagiarism check (17% allowed)
4. Evidence of grammar check by a native English speaker
5. All references cited MUST in English Language
6. All references cited should tally with the number of references listed
7. All journal titles must be abbreviated in line with the international citation standard eg Tropical Journal of Natural Product Research is abbreviated as Trop J Nat Prod Res
8. Tables and figures should be at the last page after the list of references
9. The tables must comply with the journal style.
10. Authors advised to download some articles from the TJNPR website to serve as a guide.
11. *et al* is not allowed in the references. The full authors list should be mentioned.

D. For references: Use the references as guide

Falodun A, Siraj R, Choudhary MI. GC-MS Insecticidal Leaf essential oil of *P. staudtii* Hutch and Dalz (Icacinaeae). Trop J. Pharm Res. 2009; 82:139-143.

Okolie NP, Falodun A, Oluseyi D. Evaluation of the antioxidant activity of root extract of pepper fruit (*Denetia tripetala*), and its potential for the inhibition of Lipid peroxidation. Afr J. Trad Compl and Altern Med. 2014; 11(3):221-227. Doi:

Best regards

Abiodun

Professor Abiodun Falodun, PhD; FAAS, FISPON

Editor-in-Chief:

Tropical Journal of Natural Product Research (TJNPR)

Head, Natural Product Research Group, University of Benin

Email: editor.tjnpr@gmail.com

www.tjnpr.org, SCOPUS, SCImago SJR Q3

Professor of Pharmaceutical Chemistry, FAAS

Fellow, Fulbright (USA)

Deputy Vice-Chancellor (Academic) 2014-2016

Faculty of Pharmacy

University of Benin

[Google Scholar Citations](#)

SCOPUS <https://www.scopus.com/authid/detail.uri?>



www.tjnpr.org

Caution: This Message was sent from outside of Institute Technology Bandung (ITB). Please do not click Link or open attachment unless you recognize the source of this email and know the content is safe. Please report all suspicious email to "it-helpdesk@itb.ac.id" as an attachment.

REVIEW FORM

The Editorial Team of the Tropical Journal of Natural Product Research kindly request you to review the enclosed article. Please complete the form and return to the Editor-in-Chief, editor.tjnpr@gmail.com

A. MANUSCRIPT

Journal	Tropical Journal of Natural Product Research
Manuscript Number	TJNPR MH1121AR
Type of paper	Research article
Title of paper	<i>In Vitro</i> and Molecular Docking Evaluation of New Antibacterial Activity of <i>Macaranga hulletti</i> King ex Hook.f. Leaf Extract
Name of Authors	

B. REVIEWER'S SPECIFIC COMMENTS PER SECTION OF MANUSCRIPT

Title	The title is mostly appropriate
Abstract	The abstract word count is < 250. The authors should correct all grammatical, spellings and punctuation errors. The abstract should be revised as recommended
Keywords	Keywords are okay
Introduction	The aim and objectives of the research are obvious. The authors included a statement of research novelty. Authors should briefly discuss the relevance of the research methods to the research. All grammatical, spellings and punctuation errors should be corrected. The literature review should mostly be contextual. Use recent citations. Abbreviated words should be mentioned in full prior to abbreviations The introduction should be improved as recommended
Methodology	Include methods for the statistical analysis. Mention the software used in the statistical/in silico analysis alongside the manufacturer, version and release year. Include the number of replicate measurements. All chemicals and reagents used should be mentioned alongside the manufacturer, % purity, conc., grade and specificities. All equipment and instrument used should be mentioned alongside their model, manufacturer and country. Authors should specify instruments/equipment used for each experiment. All methods used should be validated with relevant citations. All grammatical, spellings and punctuation errors should be corrected. Authors should include the GPS location of the sample collection site. Methods used are mostly appropriate for the research
Results	All results should be presented based on the journal guideline. Images should be of a very high quality/resolution (300 dpi). Authors should forward all supplementary data (input and output files) associated with the research to the journal management for validations and authentications. Use footnotes were necessary to define terms. Crosscheck the methodologies to ensure that results obtained tally with methods used. Since, the binding affinity was not adhered to – what criteria was used to select ligands for molecular docking i.e. for the 2D images presented herein what criteria did the authors use to select them. The results obtained are mostly appropriate for the methods used.

Discussion	The discussions should be mostly comparative. Authors should discuss mainly the significant findings in details. Authors should use recent citations in discussing their findings. All grammatical, spellings and punctuation errors should be corrected. The discussion should be improved
Conclusion	Authors should capture the future prospects of the research.
References	Authors should ensure that all the references follow the journal guideline. The number of cited references must tally with those at the references sections
Figures	All figures should be presented based on the journal guideline. All figures should be of a very high quality/resolution. Figures captured were discussed in the manuscript.
Tables	All tables should be presented based on the journal guideline. Table captured was discussed in the manuscript.

Note: Further corrections are contained in the reviewed manuscript

C. REVIEWER'S GENERAL COMMENTS AND REMARKS

The authors studied "*In Vitro* and Molecular Docking Evaluation of New Antibacterial Activity of *Macaranga hulletti* King ex Hook.f. Leaf Extract". The abstract should be revised as recommended. The introduction should be improved as recommended. Methods used are mostly appropriate for the research. The results obtained are mostly appropriate for the methods used. The discussion should be improved. Authors should capture the future prospects of the research. Authors should ensure that all the references follow the journal guideline. The number of cited references must tally with those at the references sections.

The manuscript should be formatted based on the journal guideline

The manuscript should be grammar edited by a professional English language editor – very important

The manuscript should be checked for plagiarism

Authors should forward all supplementary data (input and output files) associated with the research to the journal management for validations and authentications.

Accept with major corrections

D. REVIEWER'S RECOMMENDATION

Please mark with "**X**" one of the options.

You state the article should:

Publish As is	
Accept with minor revisions (editor will check), specific comments to the editor below	
Accept with moderate revisions as recommended by reviewer	
Accept with major corrections (the article should be thoroughly changed)	X
Article not suitable for publication in Trop J Nat Prod Res	
Reject for reasons noted by the reviewer (please be specific)	

***In Vitro* and Molecular Docking Evaluation of New Antibacterial Activity of *Macaranga hulletti* King ex Hook.f. Leaf Extract**

Abstract

Bacterial infections are a common occurrence, and with the increasing resistance of bacteria to antibiotics, utilization of plant extracts is gaining prominence. *Macaranga* plants are known to contain secondary metabolites such as flavonoids, which exhibit antibacterial properties. *Macaranga hulletti* King ex Hook.f. is particularly noteworthy because of its widespread presence in East Kalimantan. Therefore, this study aimed to investigate the antibacterial potential of *Macaranga hulletti* leaf extract through *in vitro* and molecular docking studies. The research involved testing the methanol extract, n-hexane fraction, and ethyl acetate fraction from *Macaranga hulletti* King ex Hook.f. leaves against three bacterial strains, *Staphylococcus aureus*, *Streptococcus mutans*, and *Propionibacterium acnes*. The results demonstrated that both the methanol extract and ethyl acetate fraction exhibited antibacterial activity against three bacterial strains with a minimum inhibitory concentration (MIC) of < 0.15%, except for the methanol extract with MIC of 0.15-0.31% against *Propionibacterium acnes*. Additionally, molecular docking analysis revealed that five flavonoids possessing prenyl or geranyl, namely 6-isoprenylerydiol, nymphaeol A, nymphaeol B, and solophenol D, showed the highest binding affinity and dominant hydrogen bonding interactions with key amino acid residues in the binding site of the three proteins (IJJ, 3IPK, and 7LBU). Thus, these findings suggest that the methanol extract and ethyl acetate fraction from *Macaranga hulletti* King ex Hook.f. has potential applications as a new source of antibacterial agents.

Keywords: Antibacterial, Extract, Flavonoid, *Macaranga hulletti*, Molecular Docking.

Introduction

Despite substantial advancements in medicine and fundamental research over recent decades, infectious diseases caused by transmissible agents, such as bacteria, eukaryotes, and viruses, continue to present significant challenges to scientists and healthcare professionals. These challenges are further intensified by the limitations in the rapid and accurate diagnosis of infections and the escalating issue of antimicrobial resistance.¹ Extracts from medicinal plants, which are abundant in diverse phytochemicals with a range of pharmacological properties, present a promising approach for addressing multi-drug resistant bacteria. Additionally, it may contribute to restoring the efficacy of antibiotics in the treatment of infectious diseases.² Therefore, it is imperative to continue the pursuit of more efficacious antimicrobial agents, particularly those sourced from natural sources, which are both readily available and economically viable.

Macaranga belongs to the Euphorbiaceae family and is known as "Mahang." Based on the literature, it is known that the secondary metabolite compounds contained in this genus are unique flavonoids and stilbenoids with prenylated substituents.³⁻¹³ Nine flavonoids containing either prenyl or geranyl group with antioxidant, antiplasmodial, and anticancer activities were successfully isolated from three *Macaranga* species present in East Kalimantan (*Macaranga hosei*, *Macaranga pearsonii*, and *Macaranga tanarius*): 4'-O-methyl-8-isoprenylnaringenin, lonchocarpol A, 4'-O-methyl-8-isoprenyleriodictyol, 6-isoprenyleriodictyol, solophenol D, nymphaeol A, nymphaeol B, nymphaeol C, including macahuillettin A that was isolated from *Macaranga hulleitii* obtained from Central Kalimantan, which have antioxidant, antiplasmodial, and anticancer activities.¹⁴⁻¹⁸ *Macaranga tanarius* leaf extract exhibited antibacterial activity.¹⁹ Moreover, the plant extracts of *Macaranga conglomerata*, *Macaranga kilimandscharica* and *Macaranga capensis* from Kenya showed antibacterial activities against

E. coli, *E. aerogenes*, *K. pneumoniae*, *P. stuartii*, *P. aeruginosa*, and *S. aureus*.²⁰ The flavonol rhamnetin, isolated from *Macaranga peltata*, also shows antibacterial activity against *Staphylococcus aureus*.²¹ Two flavanone group compounds, 5-hydroxy-7,4'-dimethoxyflavone and 5-hydroxy-6,7,4'-trimethoxyflavone, isolated from *Macaranga hosei*, showed weak antibacterial activity against three pathogenic bacteria.²² In this study, the leaves of *Macaranga hullettii* King ex Hook.f. was used as the object of analysis to identify new sources for the treatment of infectious diseases caused by bacteria. This plant is known to be a plant that can be found in all areas of East Kalimantan, where this species is commonly found in mountain forests and logging forests in the Kalimantan region.^{23, 24} Phytochemical screening and isolated compounds of *Macaranga hullettii* King ex Hook.f. leaf extract contained flavonoid compounds.^{18,25} Extensive research on the antibacterial activity of the *Macaranga* has been performed. However, the antibacterial properties of the methanol extract and its fractions from *Macaranga hullettii* King ex Hook.f. species have not yet been evaluated. Therefore, this study aimed to evaluate the antibacterial activity of *Macaranga hullettii* King ex Hook.f. leaf extract and its fractions using *in vitro* and molecular docking studies.

Commented [II1]: Rephrase

Materials and Methods

Materials

The materials used in this study were *Macaranga hullettii* leaves, methanol, n-hexane, ethyl acetate, *Staphylococcus aureus* ATCC 25923, *Streptococcus mutans* ATCC 25175, and *Propionibacterium acnes* KCCM 41747, nutrient agar (NA) solid media, Luria Bertani liquid medium (1% tryptone, 0.5% yeast, and 0.5% NaCl), ampicillin (10 µg/mL), cotton swabs, aluminum foil, gauze, Petri dish, Ose needle, paper discs, autoclave, oven, rotary vacuum evaporator, freezer, Lenovo Yoga 7 with AMD Ryzen AI 7-8840HS w/ Radeon 780M graphics, 3301 Mhz, 8 Core(s), and RAM 16.0 GB.

Methods

Sample preparation

The research samples used in this study were leaves of *Macaranga hullettii* King ex Hook.f. plants obtained from the Lesan River Forest, Kelay District, Berau Regency, and East Kalimantan. Plant samples were identified at the Laboratory of Anatomy and Plant Systematics, FMIPA Mulawarman University (No. 0174/UN.17.8.5.7.16/HA/XI/2017).

Commented [112]: Include GPS location

Extraction

Macaranga hullettii King ex Hook.f. leaf powder (1 kg) was extracted twice by maceration for 24 h using methanol. The extract was evaporated using a rotary vacuum evaporator to obtain a methanol extract. The methanol extract was fractionated successively using n-hexane and ethyl acetate. Each fraction was evaporated using a rotary vacuum evaporator to obtain n-hexane and ethyl acetate fractions. Furthermore, methanol extract, n-hexane fraction, and ethyl acetate fraction were prepared to evaluate their antibacterial activity.

Commented [113]: Expunged

Pathogens

Staphylococcus aureus ATCC 25923 was provided by the Department of Microbiology, Faculty of Science, Chulalongkorn University. *Streptococcus mutans* ATCC 25175 was prepared by the Department of Biochemistry, Faculty of Dentistry, Chulalongkorn University. Both strains were purchased from the American Type Culture Collection (ATCC). *Propionibacterium acnes* KCCM 41747 was obtained from the Faculty of Forestry, Mulawarman University, and was purchased from the Korean Culture Center of Microorganisms (KCCM). Furthermore, the tested bacteria were periodically subcultured and maintained in nutrient agar (NA) under suitable conditions, depending on the tested bacteria.

In vitro antibacterial assay

Determination of antibacterial prospects in methanol extract, n-hexane, and ethyl acetate fractions of *Macaranga hullettii* King ex Hook.f. plant leaves were analyzed using disc paper or the Kirby-Bauer method at varying concentrations.^{26,27} In this study, *Staphylococcus aureus* ATCC 25923, *Streptococcus mutans* ATCC 25175, and *Propionibacterium acnes* KCCM 41747 were employed. Ampicillin was used as a positive control. Organic solvents, such as methanol, n-hexane, and ethyl acetate, were used as negative controls. Nutrient Agar (NA) media was prepared by dissolving 3.3 grams in 150 mL of distilled water. Luria Bertani (LB) liquid media as much as 4 grams was dissolved in 500 mL of distilled water, then stirred, and put 5 mL each into a test tube. Subsequently, NA and LB were sterilized using an autoclave at 121 °C with a pressure of 1 atm for 15 minutes. Each tested bacterium was collected using an Ose needle, after which it was diluted into a test tube containing sterile LB media and then incubated for 24 h at 37 °C. After the incubation period, the tested bacteria were ready for use in the subsequent stages.

Nutrient Agar (25 mL) was poured into a sterilized Petri dish. The NA medium was maintained at room temperature for solidification. The bacterial suspension in the test tube was then collected using a sterile cotton swab. A sterile cotton swab was applied to the entire surface of the agar on a Petri dish. Each 30 µL of the tested *M. hullettii* leaf samples prepared at various concentrations (in organic solvent) was poured onto sterile adsorbent filter paper discs (6 mm in diameter), and the solvent was removed. Afterward, the paper discs containing the tested sample were placed onto the surface of agar media, closed the lid, and incubated at 37 °C for 18-24 hours. The inhibition zone formed in each well was measured using a ruler. Furthermore, the Minimum Inhibitory Concentration (MIC) value was determined for the methanol extract as well as the n-hexane and ethyl acetate fractions.

Commented [114]: Citation needed

Molecular docking

The molecular structures were drawn using ChemOffice Professional 15.0 and retrieved from PubChem (<https://pubchem.ncbi.nlm.nih.gov/>). Structure refinement was carried out using the molecular mechanics energy minimization method with the Merck molecular force field (MMFF94) using Open Babel in PyRx V.1.1. Furthermore, three proteins were download from protein data bank (PDB ID: 1JII, 3IPK, 7LBU),²⁸⁻³⁰ the location of active site was calculated using AlphaFold from <https://prankweb.cz/>.³¹ Molecular docking was performed using the AutoDock Vina tool in PyRx V.1.1 software with an exhaustiveness of 32 and a mode value of 9 poses for each docked ligand.^{32,33} The protein binding site was defined as a box with dimensions of $30 \times 30 \times 30$ Å. In the final step, the binding interactions were analyzed, and the docking results were visualized in three dimensions using the BIOVIA Discovery Studio Visualizer.

Results and Discussion

***In vitro* antibacterial activity**

The strength of antibacterial activity was determined based on the diameter of the clear zone to obtain the inhibition zone (mm), which can be classified as ≤ 5 mm (weak), 5-10 mm (moderate), 10-20 mm (strong), and ≥ 20 mm (very strong). Moreover, the smaller the minimum inhibitory concentration of a compound, the greater its ability to inhibit the growth of test bacteria.³⁴ In this study, ampicillin was used as a positive control against three bacteria: *Staphylococcus aureus* ATCC 25923, *Streptococcus mutans* ATCC 25175, and *Propionibacterium acnes* KCCM 41747, as shown in Table 1. The positive control served to determine whether the test bacteria could be inhibited by growth or were resistant to antibiotics. *Staphylococcus aureus* can cause dentoalveolar infections, jaw cysts, paroritis, oral mucosal lesions, and stomatitis. It can also produce exotoxins, lecosidine, enterotoxins, and quagulase

compounds. Enterotoxins are compounds that cause food poisoning in humans.³⁵ Antibacterial activity of the methanol extract, hexane fraction, and ethyl acetate fraction of *Macaranga hulettii* King ex Hook. f. leaves showed moderate antibacterial activity against *S. aureus* because of the inhibition zone of 5-10 mm at concentration from 0.15-2.5%, as presented in Table 1. The inhibition zone produced by the methanol extract was 6.67 mm at 0.15%. Meanwhile, the ethyl acetate fraction at 0.15% exhibited an inhibition zone at 6.33 mm. However, the n-hexane fraction exhibited antibacterial activity at 2.5%, with an inhibition zone of 6.50 mm. The results indicated that the methanol extract and ethyl acetate fraction of *Macaranga hulettii* King ex Hook, f leaves had the most potent antibacterial activity against *S. aureus*, with a minimum inhibitory concentration (MIC) of <0.15%. Moreover, *Macaranga tanarius* is known to inhibit the growth of *S. aureus* most effectively in the n-hexane fraction, with an MIC value of 0.125-0.5% and an inhibition zone of approximately 7.25 mm.³⁶

Streptococcus mutans is a prominent bacterial species that is implicated in the pathogenesis of dental caries. This facultative anaerobe primarily ferments carbohydrates, resulting in the production of various organic acids, with lactic acid as the predominant byproduct. The acidification of the dental environment by *S. mutans* is a crucial factor contributing to enamel demineralization and the subsequent formation of carious lesions.^{37,38} These bacteria produce glucosyltransferase (GTF) to produce glucan, which causes dental caries.³⁹ *S. mutans* adheres to the salivary pellicle because of the many receptors for attachment to the salivary pellicle.⁴⁰ Furthermore, the antibacterial activity of *Macaranga hulettii* King ex Hook f. leaf extract and fractions were tested against *S. mutans*, as shown in Table 1. The result revealed that both extract and fraction exhibited moderate antibacterial activity because of the inhibition zone of 5-10 mm at a concentration of 0.15-2.5%. The inhibition zones created by the methanol extract and ethyl acetate fraction were 6.67 and 6.33 mm at 0.15%, respectively. Meanwhile, the n-hexane fraction showed antibacterial activity at 1.25-2.5% with an inhibition zone of 6.33-6.67

mm. These results demonstrated that the methanol extract and ethyl acetate fraction of *Macaranga hulettii* King ex Hook. leaves showed the highest potential antibacterial activity against *S. mutans*, with an MIC <0.15% (Table 1).

Furthermore, *Propionibacterium acnes* is a gram-positive anaerobic bacterium that can induce unusual keratinization within the sebaceous glands of hair follicles and increase sebum production.⁴¹ The enzyme is capable of hydrolyzing triglycerides (TG) present in sebum into free fatty acids (FFA), which may subsequently induce inflammation in and around the hair follicles.⁴² These bacteria also produce hyaluronidase, protease, lecithinase and neuramidase which cause inflammation of the skin, *Macaranga triloba* ethanol extract is known to inhibit the growth of *P. acnes* at 2.5% and the largest inhibition value of 5.54 mm at 20%.⁴³ As shown in Table 1, the methanol extract and ethyl acetate fraction of *Macaranga hulettii* King ex Hook f. showed strong antibacterial activity against *P. acnes* because of the inhibition values of 10.67 and 14.67 mm at 2.5%, respectively. Meanwhile, the n-hexane fraction exhibited moderate antibacterial activity with inhibition value of 5-10 mm at 1.25-2.5%. Furthermore, the methanol extract showed antibacterial activity with an inhibition value of 7.67 at 0.31%. The ethyl acetate fraction showed antibacterial activity of 0.15%, with an inhibition zone of 6.67 mm. The results revealed that the ethyl acetate fraction of *Macaranga hulettii* King ex Hook.f leaves presented the highest potential antibacterial activity against *P. acnes*, with a minimum inhibitory concentration (MIC) of <0.15%, as presented in Table 1.

Based on the data of antibacterial activity, both extracts and their fractions from *Macaranga hulettii*, the methanol extract and ethyl acetate fraction exhibited good antibacterial activity against the three bacterial strains. This finding was consistent with previous studies indicating that the methanol extract and ethyl acetate fraction demonstrated antibacterial activity, attributable to the presence of flavonoids and their prenylated or geranylated forms in *Macaranga* species, such as euchrestaflavanone A, bonanione A, macarangaflavanone A,

macarangaflavanone B, macatrichocarpin A, propolin D (nymphaeol B), senegalensin, schweinfurthin B, schweinfurthin O, and isomacarangain.⁴⁴⁻⁵⁴ Flavonoid compounds can damage bacterial cell walls and denature proteases that disrupt bacterial metabolic processes.⁵⁵ In addition, alkaloids can damage the peptidoglycan layer in bacterial cells, causing damage to the bacterial cell wall and inhibiting bacterial growth. Terpenoids also inhibit bacterial growth by damaging bacterial cell membranes. Moreover, the phenolic compounds of secondary metabolites can damage bacterial cells by increasing the permeability of the cytoplasmic membrane, resulting in cytoplasmic coagulation and cell division.³⁴

Molecular docking study

Eight flavonoid derivatives possessing either prenyl or geranyl group were found in the three *Macaranga* species in East Kalimantan, Indonesia (*Macaranga hosei*, *Macaranga pearsonii*, and *Macaranga tanarius*): 4'-O-methyl-8-isoprenylnaringenin, lonchocarpol A, 4'-O-methyl-8-isoprenyleriodictyol, 6-isoprenyleriodictyol, solophenol D, nymphaeol A, nymphaeol B, and nymphaeol C.¹⁴⁻¹⁷ Macahuilettiin A was prenylated flavonoid found in the *Macaranga huletti* obtained from Central Kalimantan (Indonesia).¹⁸ Furthermore, a comparative analysis of the molecular docking results of prenylated/geranylated flavonoids with methoxylated flavonoids was conducted, focusing on 5-hydroxy-7,4'-dimethoxyflavone and 5-hydroxy-6,7,4'-trimethoxyflavone, which were found in the *Macaranga hosei* from Trengganu, Malaysia.,²² as presented in Table 2. Molecular docking was performed to evaluate the interaction of the flavonoids with the amino acid residues of the three proteins. This study used three proteins from *Staphylococcus aureus*, *Streptococcus mutans*, and *Propionibacterium acnes* that were retrieved from the Protein Data Bank with PDB ID 1JJJ, 3IPK, and 7LBU, respectively.²⁷⁻²⁹ As illustrated in Table 2, the molecular docking results revealed that 11 flavonoid derivatives exhibited binding affinities ranging from -7.2 to -10.5 kcal/mol, -7.5 to -8.9 kcal/mol, and -7.1

Commented [II5]: Move to molecular docking methodology to should how the various ligands were selected

to -8.9 kcal/mol against *Staphylococcus aureus* tyrosyl-tRNA synthetase (PDB ID: 1JIJ), *Streptococcus mutans* antigen I/II (AgI/II) (PDB ID: 3IPK), and *Propionibacterium acnes* surface sialidase (PDB ID: 7LBU), respectively. Furthermore, the interaction of flavonoids and ampicillin with the protein target is depicted in two dimensions, as presented in Figure 1-3.

Among the compounds against *S. aureus* tyrosyl-tRNA synthetase, nymphaeol A-B and solophenol D had the highest binding affinity compared to ampicillin as a positive control, as shown in Table 2. Furthermore, ampicillin showed hydrogen bonding with LYS84 and GLN174, and amide- π interactions with GLY38, as depicted in Figure 1. Nymphaeol A and nymphaeol B exhibited hydrogen bonding with four amino acid residues, π -anions, and hydrophobic interactions, whereas nymphaeol B and ampicillin showed similar interactions with LYS84. In addition, solophenol D displayed hydrogen bonding with TYR36, THR75, GLN190, ASP177, and GLN174, as well as hydrophobic interactions with HIS50, LEU70, PRO53, and ALA39 (Figure 1). This result was in line with previous study, where nymphaeol A-B and solophenol D exhibited antibacterial activity.^{56,57}

As presented in Table 2, five compounds exhibited high antibacterial activity against *S. mutans* antigen I/II (AgI/II), including 6-isoprenyleriodictyol, nymphaeol A-B, and solophenol D, with binding affinity from -8.7 to -8.9 kcal/mol. As shown in Figure 2, ampicillin formed hydrogen bonding with four amino acid residues, ASN590, SER591, SER588, and ASP760, as well as π -sulfur and π - π interactions with TRP 816. Furthermore, 6-isoprenyleriodictyol showed only hydrogen bonding with three amino acid residues: SER697, ASN699, and ASN814. Meanwhile, nymphaeol A and nymphaeol B displayed hydrogen bonding with four amino acid residues (Figure 2), but nymphaeol A exhibited a similar π - π interaction with TRP816, as observed with ampicillin. Solophenol D formed hydrogen bonds with SER818, ARG824, and THR586, similar π - π interactions (TRP816) with ampicillin, and π -sigma interactions with

SER762. Furthermore, 6-isoprenyleriodictyol is a prenylated flavonoid similar to nymphaeol A. Thus, 6-isoprenyleriodictyol may have antibacterial potency.

Moreover, three compounds displayed stronger binding affinities than ampicillin, with values of -8.0 and -8.4 kcal/mol against *P. acnes* surface sialidases, such as 6-isoprenyleriodictyol and nymphaeol A, respectively, as described in Table 2. As depicted in Figure 3, ampicillin showed hydrogen bonding with four amino acid residues, ARG282, GLN287, ASP146, ASP185, and π - π and π -alkyl interactions with PHE257, ALA147, and VAL202. 6-Isoprenyleriodictyol demonstrated π - π interactions with PHE257, π -anion interactions with ASP146, and alkyl-alkyl interactions with ALA147, PHE209, VAL202, and LEU224. Furthermore, this compound displayed hydrogen bonding with six amino acid residues, including ASP146, ARG329, TYR423, ARG282, and GLU287, which were analogous to those observed with ampicillin. Nymphaeol A showed hydrogen bonding with five amino acid residues (ARG329, TYR423, ASP185, ALA147, and SER184), π -anion interactions with ASP312 and ASP185, alkyl-alkyl interactions with MET310, ALA331, and ALA147, and Van der Waals interactions with PHE257. Thus, these compounds may have potent antibacterial activity.

Structure-activity relationships were also studied based on the binding affinity results, as presented in Table 2. Flavonoids possessing prenyl, geranyl, and hydroxyl groups showed good potency as antibacterial agents owing to their strong binding affinity with amino acid residues in the binding site of proteins compared with ampicillin. Nevertheless, the absence of hydroxy group on prenylated or geranylated flavonoids decreased binding affinity.^{22,53,58}

Conclusion

The extract and fraction of *Macaranga hulettii* King ex Hook, f, have potential as antibacterial agents against three bacterial strains, *Staphylococcus aureus* ATCC 25923, *Streptococcus mutans* ATCC 25175, and *Propionibacterium acnes* KCCM 41747. The methanol extract

exhibited antibacterial activity against *S. aureus* and *S. mutans* with MIC (%) of <0.15 against *S. aureus* and *S. mutans*, and 0.15-0.31% against *P. acnes*. n-Hexane fraction showed antibacterial activity with MIC (%) of 1.25-2.5% against *S. aureus*, and 0.62-1.25% against *S. mutans* and *P. acnes*. Moreover, the ethyl acetate fraction displayed antibacterial activity with an MIC (%) <0.15% against three bacterial strains (*S. aureus*, *S. mutans*, and *P. acnes*). Molecular docking studies have suggested that 6-isoprenyleriodictyol, nymphaeol A, nyphameol B, and solophenol D are promising antibacterial agents because those compounds exhibit the highest binding affinity and form hydrogen bonding interactions with key amino acid residues in the active site of the protein targets.

Conflict of Interest

The authors declare no conflicts of interest.

Author's Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims related to the content of this article will be borne by them.

Acknowledgement

This research was funded by the Islamic Development Bank (PIU-IsDB) project under contract no. 137/UN17.11/PL/2019.

References

1. Wong F, de la Fuente-Nunez C, Collins JJ. Leveraging artificial intelligence in the fight against infectious diseases. *Science*. 2023;381(6654):164-70.

2. Seukep AJ, Kuete V, Nahar L, Sarker SD, Guo M. Plant-derived secondary metabolites as the main source of efflux pump inhibitors and methods for identification. *Journal of pharmaceutical analysis*. 2020;10(4):277-90.
3. Magadula JJ. Phytochemistry and pharmacology of the genus *Macaranga*: a review. *J.Med. Plants Res*. 2014;8(12):489-503.
4. Vu LT, Ngan TB, Phuong L, Huong DT, Litaudon M, Van Hung N, Thach TD, Van Cuong P. Chemical constituents from fruits of *Macaranga denticulata* (Euphorbiaceae)(Part 2). *Vietnam J. Chem*. 2018;56(4):516-20.
5. Marlina E, Hairani R, Tjahjandarie TS, Tanjung M. Antiplasmodial activity of flavonoids from *Macaranga tanarius* leaves. In *IOP Conference Series: Earth and Environmental Science* 2018;144(1):012011).
6. Tjahjandarie TS, Tanjung M, Saputri RD, Nadar PB, Aldin MF, Marlina E, Permadi A. Flavestins K, An isoprenylated stilbene from the leaves of *Macaranga recurvata* Gage. *Natural Product Sciences*. 2019;25(3):244-7.
7. Huong DT, Linh NT, Van TT, Litaudon M, Roussi F, Van Nam V, Van Cuong P. Stilbenes from *Macaranga tanarius* (Euphorbiaceae) growing in Vietnam. *Vietnam J. Chem*. 2020;58(3):338-42.
8. Aldin MF, Tjahjandarie TS, Saputri RD, Tanjung M. Macasiamenene V, a New Stilbenoid from the Leaves of *Macaranga inermis*. *Nat. Prod. Sci*. 2021;27(1):45-8.
9. Muharram A, Rachmawati DA, Mardhiyyah S, Tjahjandarie TS, Saputri RD, Ahmat N, Tanjung M. Cytotoxic and antioxidant activities of flavonoids and diterpenoids from *Macaranga involucreata* (Roxb.) Baill. *J. Appl. Pharm. Sci*. 2023;13(6):087-92.
10. Tanjung M, Tjahjandarie TS, Aldin MF, Mardhiyyah S, Ahmat N, Saputri RD. Macagigantin A, A New Flavonoid from *Macaranga gigantea* (Rchb. f & Zoll.) Mull. Arg. *Nat. Prod. Sci*. 2023;29(4):287-94.

11. Tjahjandarie TS, Aldin MF, Saputri RD, Tanjung M. Dihydrostilbenes from *Macaranga javanica* (Blume) Müll. Arg. and their antiplasmodial activity. *Nat. Prod. Sci.* 2024;1-7.
12. Tanjung M, Tjahjandarie TS, Aldin MF, Mardhiyyah S, Saputri RD, Syah YM, Ahmat N. Two new flavonols from *Macaranga inermis* pax & K. Hoffm. *Nat. Prod. Sci.* 2025 Feb 1;39(3):498-505.
13. Tanjung M, Tjahjandarie TS, Aldin MF, Mardhiyyah S, Saputri RD, Syah YM, Ahmat N. Two new flavonols from *Macaranga inermis* pax & K. Hoffm. *Nat. Prod. Sci.* 2025;39(3):498-505.
14. Marlina E, Tjahjandarie TS, Tanjung M. Isoprenylated flavanone derivatives from *Macaranga hosei* King ex Hook F. *Der Pharm. Lett.* 2015;7(3):153-6.
15. Marlina E, Tjahjandarie TS, Tanjung M. Aktivitas Antioksidan Senyawa Flavonoid Dari *Macaranga pearsonii* Merr. *J. Kim. Mulawarman.* 2016;13(2):97-100.
16. Marlina E, Astuti W, Kosala K, Hairani R, Tjahjandarie TS, Tanjung M. Chemical composition and anticancer activity of *Macaranga hosei* leaves. *Asian J. Chem.* 2018;30(4):795-8.
17. Marlina E, Ruga R, Hairani R, Tjahjandarie TS, Tanjung M. Antioxidant activity of flavonoid constituents from the leaves of *Macaranga tanarius*. In *IOP Conference Series: Journal of Physics.* 2019;1277(1):012014.
18. Saputri RD, Tukiran T, Wati FA, Purnamasari AP, Wardhana MW, Tjahjandarie TS, Tanjung M. Macahuletiiin A, a new isoprenylated flavanone from the leaves of *Macaranga hullettii* King ex Hook and their antiplasmodial activity. *Vietnam J. Chem.* 2024;62(3):394-8.
19. Musdalifah M, Khumaidi A, Suwastika IN. Uji Daya Hambat dan Skrining Fitokimia Ekstrak Daun *Macaranga tanarius* (L.) Mull. Arg Sebagai Antibakteri *Salmonella typhi*. *Nat. Sci: J. Sci. Technol.* 2017;6(3).

20. Ibrahim H, Omosa L, Nchiozem-Ngnitedem V, Onyari J, Maru S, Guefack M. Antibacterial activities and phytochemical screening of crude extracts from Kenyan macaranga species toward MDR phenotypes expressing efflux pumps. *Pharmacogn. Commn.* 2021;11(2):119-26.
21. Bijesh K, Sebastian D. Isolation and characterization of antibacterial compounds from *Macaranga peltata* against clinical isolates of *Staphylococcus aureus*. *Int. J. Biol. Pharma Res.* 2013;4:1196-203.
22. Salleh WM, Razak NZ, Ahmad F. Phytochemicals and biological activities of *Macaranga hosei* and *Macaranga constricta* (Euphorbiaceae). *Marmara Pharm. J.* 2017;21(4):881-8.
23. Slik JW, Priyono P, Welzen PV. Key to the *Macaranga* Thou. and *Mallotus* Lour. species (Euphorbiaceae) of East Kalimantan, Indonesia. Singapore, Gardens' Bulletin (Singapore), National Parks Board, Singapore Botanic Gardens; 2000. 11-87 p.
24. Amirta R, Angi EM, Ramadhan R, Kusuma IW, Wiati CB, Haqiqi MT. Potensi pemanfaatan macaranga. Samarinda: Mulawarman University Press; 2017.
25. Rismawati R, Marlina E, Daniel D. Phytochemical Test on Methanol Extract of Leaf of *Macaranga hullettii* King ex Hook. f. *J. Atomik.* 2018;3(2):91-4.
26. Hudzicki J. Kirby-Bauer disk diffusion susceptibility test protocol. American society for microbiology. 2009;15(1):1-23.
27. Inna M, Astuti W, Saleh C. Aktivitas antibakteri ekstrak metanol daun tanaman sambung urat (*Cayratia carnos*) terhadap *Salmonella thypi* and *Propionibacterium acnes*. *J. Atomik.* 2022;7(1):1-5.
28. Qiu X, Janson CA, Smith WW, Green SM, McDevitt P, Johanson K, Carter P, Hibbs M, Lewis C, Chalker A, Fosberry A. Crystal structure of *Staphylococcus aureus* tyrosyl-tRNA synthetase in complex with a class of potent and specific inhibitors. *Protein Sci.* 2001;10(10):2008-16.

29. Larson MR, Rajashankar KR, Patel MH, Robinette RA, Crowley PJ, Michalek S, Brady LJ, Deivanayagam C. Elongated fibrillar structure of a streptococcal adhesin assembled by the high-affinity association of α - and PPII-helices. *Proc Natl Acad Sci.* 2010;107(13):5983-8.
30. Yu AC, Volkers G, Jongkees SA, Worrall LJ, Withers SG, Strynadka NC. Crystal structure of the *Propionibacterium acnes* surface sialidase, a drug target for *P. acnes*-associated diseases. *Glycobiology.* 2022;32(2):162-70.
31. Jakubec D, Skoda P, Krivak R, Novotny M, Hoksza D. PrankWeb 3: accelerated ligand-binding site predictions for experimental and modelled protein structures. *Nucleic Acids Res.* 2022;50(W1):W593-7.
32. Dallakyan S, Olson AJ. Small-molecule library screening by docking with PyRx. In *Chemical biology: methods and protocols.* 2014: 243-250. New York, NY: Springer New York.
33. Trott O, Olson AJ. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *J. Comput. Chem.* 2010;31(2):455-61.
34. Yulianti MF, Amat AL, Hutasoit RM, Pakan PD. Antibacterial activity of jamblang leaf ethanol extract (*Syzygium cumini*) against the growth of *Propionibacterium acnes*. *Acta Biochimica Indones.* 2023;6(2):1161.
35. Malangu N, editor. *Poisoning: From Specific Toxic Agents to Novel Rapid and Simplified Techniques for Analysis.* BoD—Books on Demand; 2017.
36. Sari AA, Saleh C. Uji fitokimia, toksisitas dan aktivitas antibakteri ekstrak berbagai fraksi daun mara (*Macaranga tanarius* (L.) MA) terhadap bakteri *Staphylococcus aureus* dan *Escherichia coli*. *J. Kim. Mulawarman.* 2015;12(2):53-8.

37. Imelda R., Mariam M., Satari M. Effect of cassava (*manihot esculenta* crantz), rice (*oryza sativa* L.), and potato (*solanum tuberosum*) water extract to decrease pH phase fermentation of *Streptococcus mutans* ATCC 25175. *Padjajaran J. Dent.* 2019;31(1):14.
38. Alkhaled A., Alsabek L., Al-assaf M. , & Badr F.. Effect of chlorhexidine, honey and propolis on *Streptococcus mutans* counts: in vitro study. *Dentistry* 2021;9(1).
39. Ren Z, Chen L, Li J, Li Y. Inhibition of *Streptococcus mutans* polysaccharide synthesis by molecules targeting glycosyltransferase activity. *Journal of oral microbiology.* 2016;8(1):31095.
40. Lee BS, Chen YJ, Wei TC, Ma TL, Chang CC. Comparison of antibacterial adhesion when salivary pellicle is coated on both poly (2-hydroxyethyl-methacrylate)-and polyethylene-glycol-methacrylate-grafted poly (methyl methacrylate). *International Journal of Molecular Sciences.* 2018;19(9):2764.
41. Chen KC, Yang CH, Li TT, Zouboulis CC, Huang YC. Suppression of *Propionibacterium acnes*-stimulated proinflammatory cytokines by Chinese bayberry extracts and its active constituent myricetin in human sebocytes in vitro. *Phytother. Res.* 2019;33(4):1104-13.
42. Xu J, Chen X, Song J, Wang C, Xu W, Tan H, Suo H. Antibacterial activity and mechanism of cell-free supernatants of *Lactobacillus paracasei* against *Propionibacterium acnes*. *Microb. Pathog.* 2024;189:106598
43. Warnida H, Mustika D, Supomo S, Sukawaty Y. Efektivitas Ekstrak Etanol Daun Mahang (*Macaranga triloba*) sebagai Obat Anti-acne. *J. Penelit. Sos. Ekon. Kehuta.* 2018;4(1):9-18.
44. Schütz BA, Wright AD, Rali T, Sticher O. Prenylated flavanones from leaves of *Macaranga pleiostemona*. *Phytochemistry.* 1995;40(4):1273-7.
45. Lim TY, Lim YY, Yule CM. Evaluation of antioxidant, antibacterial and anti-tyrosinase activities of four *Macaranga* species. *Food Chem.* 2009;114(2):594-9.

46. Fareza MS, Syah YM, Mujahidin D, Juliawaty LD, Kurniasih I. Antibacterial flavanones and dihydrochalcones from *Macaranga trichocarpa*. *Zeitschrift für Naturforschung C*. 2014;69(9-10):375-80.
47. Hasanat A, Kabir MS, Hossain MM, Hasan M, Al Masum MA, Chowdhury TA, Bhuiyan DI, Mamur A, Kibria AS. Antibacterial activity of methanol extract of *Macaranga denticulata* leaves and in silico PASS prediction for its six secondary metabolites. *World J. Pharm. Pharm. Sci*. 2015:1258-66.
48. O Akanbi B, Anene P, Olayanju S. Preliminary Screening Indicates Promising Antimicrobial Properties of the Stem Bark Extracts of *Macaranga rosea*. *Anti-Infective Agents*. 2015;13(2):123-8.
49. Ogundajo A, Okeleye B, Ashafa AO. Chemical constituents, in vitro antimicrobial and cytotoxic potentials of the extracts from *Macaranga barteri* Mull-Arg. *Asian Pac. J. Trop. Biomed*. 2017;7(7):654-9.
50. Putri R, Hendra R, Teruna HY. Anti-Bacterial and Anti-Fungal Activities from *Macaranga bancana* Leaves Extract. *Pharmacology and Clinical Pharmacy Research*. 2019;4(1):1-4.
51. Lee JH, Kim YG, Khadke SK, Yamano A, Woo JT, Lee J. Antimicrobial and antibiofilm activities of prenylated flavanones from *Macaranga tanarius*. *Phytomedicine*. 2019;63:153033.
52. Pagna JI, Awazi T, Mbarga PE, Mbekou IM, Mkounga P, Fotie J, Frese M, Fabrice FB, Lenta BN, Sewald N, Nkengfack EA. Antibacterial flavonoids from the fruits of *Macaranga hurifolia*. *J. Asian Nat. Prod. Res*. 2022;24(11):1041-51.
53. Kamso VF, Simo Fotso CC, Kanko Mbekou IM, Toussie BT, Ndjakou Lenta B, Boyom FF, Sewald N, Frese M, Ngadjui BT, Wabo Fotso G. Chemical constituents of *Macaranga occidentalis*, antimicrobial and chemophenetic studies. *Molecules*. 2022;27(24):8820.

54. Rosamah E, Haqiqi MT, Putri AS, Kuspradini H, Kusuma IW, Amirta R, Yuliansyah Y, Suwinarti W, Paramita S, Ramadhan R, Tarmadi D. The potential of Macaranga plants as skincare cosmetic ingredients: A review. *J. Appl. Pharm. Sci.* 2023;13(7):001-12.
55. Li AP, He YH, Zhang SY, Shi YP. Antibacterial activity and action mechanism of flavonoids against phytopathogenic bacteria. *Pesticide Biochemistry and Physiology.* 2022;188:105221.
56. Chen YW, Ye SR, Ting C, Yu YH. Antibacterial activity of propolins from Taiwanese green propolis. *J. Food Drug Anal.* 2018;26(2):761-8.
57. Inui S, Hosoya T, Shimamura Y, Masuda S, Ogawa T, Kobayashi H, Shirafuji K, Moli RT, Kozono I, Shin-ya K, Kumazawa S. Solophenols B–D and Solomonin: New Prenylated Polyphenols Isolated from Propolis Collected from The Solomon Islands and Their Antibacterial Activity. *J. Agri. Food Chem.* 2012;60(47):11765-70.
58. Ruga R, Kingkaew K, Tamsampaoloet K, Chavasiri W. Enhancing antibacterial activity against *Propionibacterium acnes* and *Staphylococcus aureus* by combination of tetracycline with selected compounds. *Chem. Lett.* 2018;47(12):1538-41.

Commented [116]: Authors should ensure that all the references follow the journal guideline. The number of cited references must tally with those at the references sections

Table 1: Antibacterial Activity of *Macaranga hullettii* King ex Hook Leaf Extracts and Fractions against *Staphylococcus aureus*, *Streptococcus mutans*, and *Propionibacterium acnes*

Sample	Concentration (%)	Inhibition Zone (mm)*		
		<i>Staphylococcus</i>	<i>Streptococcus</i>	<i>Propionibacterium</i>
		<i>aureus</i>	<i>mutans</i>	<i>acnes</i>
Methanol Extract	0	0	0	0
	0.15	6.67	6.67	0
	0.31	6.67	6.67	7.67
	0.62	6.67	7	8
	1.25	7.17	7.33	9.67
	2.5	7.5	8	10.67
	MIC (%)	<0.15	<0.15	0.15-0.31
n-Hexane Fraction	0	0	0	0
	0.15	0	0	0
	0.31	0	0	0
	0.62	0	0	0
	1.25	0	6.33	6.33
	2.5	6.5	6.67	6.67
	MIC (%)	1.25-2.5	0.62-1.25	0.62-1.25
	0	0	0	0

Ethyl acetate	0.15	6.33	6.33	6.67
Fraction	0.31	6.5	6.67	8
	0.62	6.5	6.83	9
	1.25	6.67	7	11
	2.5	7	7.67	14.67
	MIC (%)	<0.15	<0.15	<0.15
Ampicillin	0.01	20.33	21	21

*Data were obtained from triplicate experiments.

Commented [117]: Include the Standard error of the mean or the SD

Table 2: Binding affinity of flavonoids against three proteins (1IJJ, 3IPK, 7LBU)

No.	Compound	Binding Affinity (kcal/mol)		
		1IJJ	3IPK	7LBU
1	4'-O-methyl-8-isoprenylnaringenin	-7.4	-8.1	-7.7
2	4'-O-methyl-8-isoprenyleriodictyol	-7.2	-8.1	-7.6
3	6-Isoprenyleriodictyol	-7.7	-8.7	-8.0
4	5-hydroxy-7,4'-dimethoxyflavone	-8.6	-7.8	-7.1
5	5-hydroxy-6,7,4'-trimethoxyflavone	-8.3	-7.5	-7.3
6	Macahulleitin A	-7.6	-8.4	-7.1
7	Lonchocarpol A	-9.4	-7.8	-7.8
8	Nymphacol A	-9.7	-8.9	-8.4

9	Nymphaeol B	-10.5	-8.9	-7.7
10	Nymphaeol C	-8.4	-8.3	-7.4
11	Solophenol D	-10.0	-8.7	-7.9
12	Ampicillin	-8.5	-7.7	-7.6

Commented [118]: Since, the binding affinity was not adhered to – what criteria was used to select ligands for molecular docking i.e. for the 2D images presented herein what criteria did the authors use to select them..

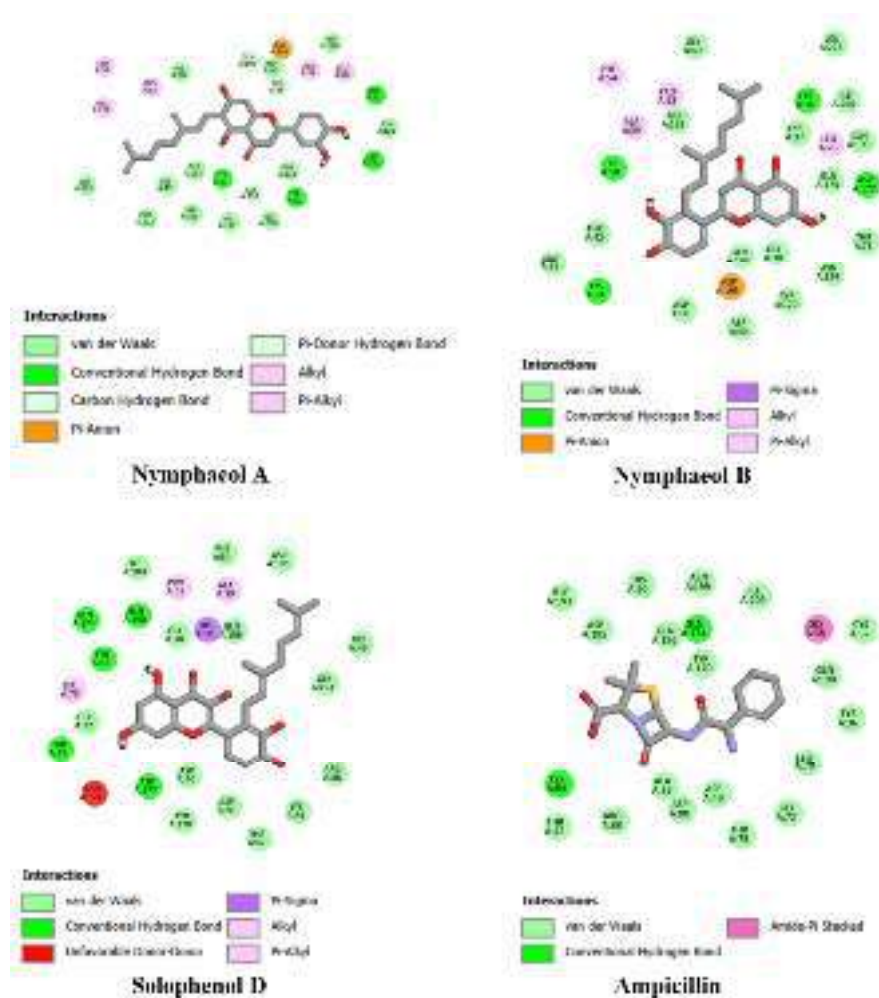


Figure 1: 2D interaction of the best docked flavonoids and ampicillin with *Staphylococcus aureus* tyrosyl-tRNA synthetase (PDB ID: 1JJJ)

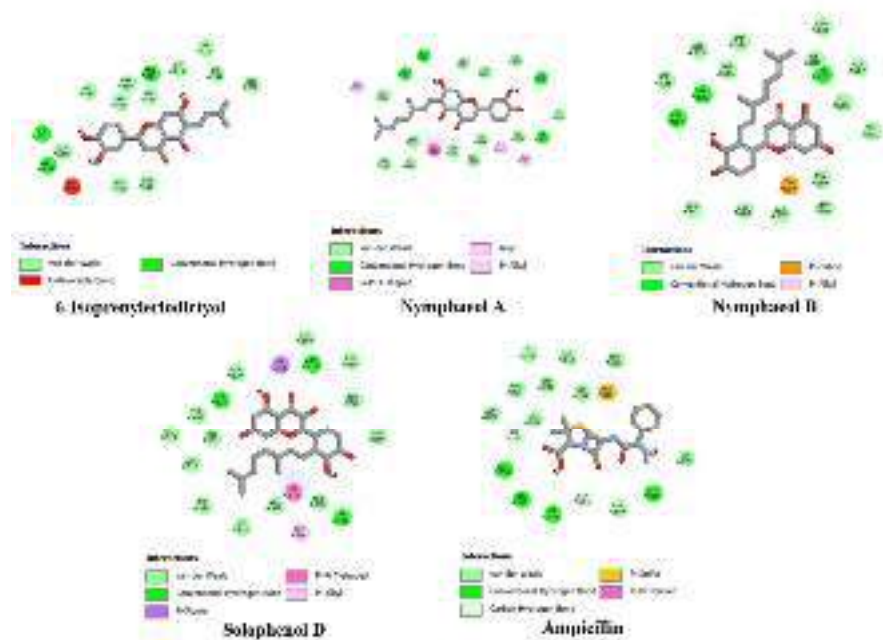


Figure 2: 2D interaction of the best-docked flavonoids and ampicillin with *Streptococcus mutans* antigen I/II (PDB ID: 3IPK).

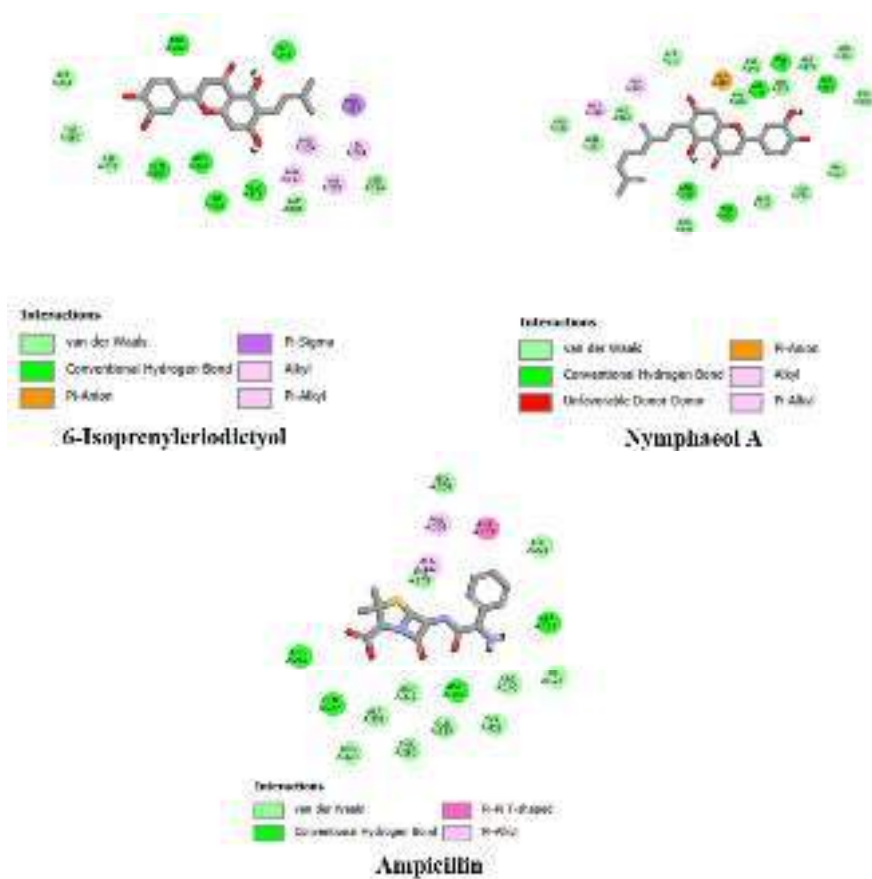


Figure 3: 2D interaction of the best-docked flavonoids and ampicillin with *Propionibacterium acnes* surface sialidase (PDB ID: 7LBU).



Revision

From Ade Danova <adedanova@itb.ac.id>

Date Tue 5/6/2025 8:28 PM

To Editor-in-Chief Tjnpr <editor.tjnpr@gmail.com>

Cc Dr. Elvira Hermawati, S.Si., M.Si. <elvira.hermawati@itb.ac.id>; evakia@fmipa.unmul.ac.id <evakia@fmipa.unmul.ac.id>; ritahairani@fmipa.unmul.ac.id <ritahairani@fmipa.unmul.ac.id>; ritbey.r@fmipa.unmul.ac.id <ritbey.r@fmipa.unmul.ac.id>; anayulvia@unej.ac.id <anayulvia@unej.ac.id>

 3 attachments (32 MB)

Turnitin check.pdf; Responds to the reviewer.docx; 3-TJNPR-2025-420 Revised 1.docx;

Dear Editor-in-Chief
Tropical Journal of Natural Product Research

I would like to submit the revised manuscript. I already highlighted for all change using yellow color based on your suggestion. In this email, you will find three documents, such as revised manuscript, Turnitin check (13%), and responds for reviewer.

Best regards,
Ade Danova

Respond to the reviewer

No	Comment	Respond
1	The abstract word count is < 250. The authors should correct all grammatical, spelling and punctuation errors. • The abstract should be revised as recommended	Thank you very much for your kind comment. I already checked that the abstract word count is less than 250. The English writing, I already checked using Paperpal for grammar, spelling and punctuation. I already highlighted all revision using yellow color.
2	The aim and objectives of the research are obvious. The authors included a statement of research novelty. Authors should briefly discuss the relevance of the research methods to the research. All grammatical, spelling and punctuation errors should be corrected. The literature review should mostly be contextual. Use recent citations. Abbreviated words should be mentioned in full prior to abbreviations • The introduction should be improved as recommended	Thanks for your comments. I think I already explained it in the introduction about previous research and what we did it in our study here. The English writing, I already checked and evaluated using Paperpal premium for grammar, spelling and punctuation. I already highlighted all revision using yellow color.
3	Include methods for statistical analysis. Mention the software used in the statistical/in silico analysis alongside the manufacturer, version and release year. Include the number of replicate measurements. All chemicals and reagents used should be mentioned alongside the manufacturer, % purity, conc., grade and specificities. All equipment and instruments used should be mentioned alongside their model, manufacturer and country. Authors should specify instruments/equipment used for each experiment. All methods used should be validated with relevant citations. All grammatical, spelling and punctuation errors should be corrected. Authors should include the GPS location of the sample collection site. • Methods used are mostly appropriate for the research	Thanks for your comments. I already revised it. The English writing, I already checked and evaluated using Paperpal premium for grammar, spelling and punctuation. I already highlighted all revision using yellow color.

4	All results should be presented based on the journal guideline. Images should be of a very high quality/resolution (300 dpi). Authors should forward all supplementary data (input and output files) associated with the research to the journal management for validations and authentications. Using footnotes was necessary to define terms. Crosscheck the methodologies to ensure that results obtained tally with methods used. Since, the binding affinity was not adhered to – what criteria was used to select ligands for molecular docking i.e. for the 2D images presented herein what criteria did the authors use to select them. • The results obtained are mostly appropriate for the methods used.	Thanks for your comments. I already revised it. I already highlighted all revision using yellow color.
5	The discussions should be mostly comparative. Authors should discuss mainly the significant findings in detail. Authors should use recent citations in discussing their findings. All grammatical, spelling and punctuation errors should be corrected. • The discussion should be improved	Thanks for your comments. I already revised it. The English writing, I already checked and evaluated using Paperpal premium for grammar, spelling and punctuation. I already highlighted all revision using yellow color.
6	Authors should capture the future prospects of the research.	Thanks for your comments. I already added in the abstract and conclusion.
7	Authors should ensure that all the references follow the journal guideline. The number of cited references must tally with those at the references sections	Thanks for your comments. I already revised it. I already highlighted all revision using yellow color.
8	All figures should be presented based on the journal guideline. All figures should be of a very high quality/resolution. Figures captured were discussed in the manuscript.	Thanks for your comments. I already revised it. I already highlighted all revision using yellow color.
9	All tables should be presented based on the journal guideline. Table captured was discussed in the manuscript.	Thanks for your comments. I think I already present it based on the journal guideline. I already highlighted all revision using yellow color.

***In vitro* and Molecular Docking Evaluation of New Antibacterial Activity of *Macaranga hulletti* King ex Hook.f. Leaf Extract**

Eva Marlina^{1,3}, Ade Danova^{2*}, Rita Hairani^{1,3}, Ritbey Ruga^{1,3}, Elvira Hermawati², and Ana Yulvia⁴

1. Department of Chemistry, Faculty of Mathematics and Natural Science, Mulawarman University, Samarinda 75123, East Kalimantan, Indonesia.

2. Organic Chemistry Division, Department of Chemistry, Faculty of Mathematics and Natural Sciences, Institut Teknologi Bandung Jl. Ganesha No. 10, Bandung 70132, West Java, Indonesia.

3. Research Center for Medicine and Cosmetic from Tropical Rainforest Resources, Mulawarman University, Samarinda, Indonesia

4. Department of Chemistry, Faculty of Mathematics and Natural Science, Universitas Jember, East Java, Indonesia.

*Corresponding author. E-mail: adedanova@itb.ac.id; Tel.: +6281357944698.

Abstract

Bacterial infections are a common occurrence, and with the increasing resistance of bacteria to antibiotics, utilization of plant extracts is gaining prominence. *Macaranga* plants are known to contain secondary metabolites such as flavonoids, which exhibit antibacterial properties. *Macaranga hullettii* King ex Hook.f. is particularly noteworthy because of its widespread presence in East Kalimantan. Therefore, the objective of this study was to investigate the antibacterial potential of *Macaranga hullettii* leaf extract through *in vitro* and molecular docking studies. The research involved testing the methanol extract, n-hexane fraction, and

ethyl acetate fraction from *Macaranga hullettii* King ex Hook.f. leaves against three bacterial strains, *Staphylococcus aureus*, *Streptococcus mutans*, and *Propionibacterium acnes*. The results revealed that both the methanol extract and ethyl acetate fraction displayed antibacterial activity against three bacterial strains with a minimum inhibitory concentration (MIC) of < 0.15%, except for the methanol extract with MIC of 0.15-0.31% against *Propionibacterium acnes*. Additionally, molecular docking analysis showed that five flavonoids possessing prenyl or geranyl groups, namely 6-isoprenyleriodictyol, nymphaeol A, nymphaeol B, and solophenol D, showed the highest binding affinity and dominant hydrogen bonding interactions with key amino acid residues in the binding site of the three proteins (IJJJ, 3IPK, and 7LBU). Thus, these findings suggest that the methanol extract and ethyl acetate fraction from *Macaranga hullettii* King ex Hook.f. has potential applications as a new source of antibacterial agents, and further studies are needed to isolate the bioactive compounds and evaluate them by *in vitro* and *in vivo* studies.

Keywords: Antibacterial, Extract, Flavonoid, *Macaranga hullettii*, Molecular Docking.

Introduction

Despite substantial advancements in medicine and fundamental research over recent decades, infectious diseases because of transmissible agents, such as bacteria, eukaryotes, and viruses, continue to present significant challenges to scientists and healthcare professionals. These challenges are further intensified by the limitations in the rapid and accurate diagnosis of infections and the escalating issue of antimicrobial resistance.¹ Extracts from medicinal plants, which are abundant in diverse phytochemicals with a range of pharmacological properties, present a promising approach for addressing multidrug-resistant bacteria. Additionally, it may contribute to restoring the efficacy of antibiotics to treat infectious diseases.² Therefore, it is

imperative to continue the pursuit of more efficacious antimicrobial agents, particularly those sourced from natural sources, which are both readily available and economically viable.

Macaranga belongs to the Euphorbiaceae family and is known as "Mahang." Based on the literature, the secondary metabolites contained in this genus are unique flavonoids and stilbenoids with prenylated substituents.³⁻¹³ Nine flavonoids containing either prenyl or geranyl group with antioxidant, antiplasmodial, and anticancer activities were successfully isolated from three *Macaranga* species present in East Kalimantan (*Macaranga hosei*, *Macaranga pearsonii*, and *Macaranga tanarius*): 4'-O-methyl-8-isoprenylharingenin, lonchocarpol A, 4'-O-methyl-8-isoprenyleriodictyol, 6-isoprenyleriodictyol, solophenol D, nymphaeol A, nymphaeol B, nymphaeol C, including macahuilletin A that was isolated from *Macaranga hullettii* obtained from Central Kalimantan, which have antioxidant, antiplasmodial, and anticancer activities.¹⁴⁻¹⁸ *Macaranga tanarius* leaf extract exhibited antibacterial activity.¹⁹ Moreover, the plant extracts of *Macaranga conglomerata*, *Macaranga kilimandscharica* and *Macaranga capensis* from Kenya showed antibacterial activities against *E. coli*, *E. aerogenes*, *K. pneumoniae*, *P. stuartii*, *P. aeruginosa*, and *S. aureus*.²⁰ The flavonol rhamnetin, isolated from *Macaranga peltata*, also showed antibacterial activity against *Staphylococcus aureus*.²¹ Two flavanone groups, 5-hydroxy-7,4'-dimethoxyflavone and 5-hydroxy-6,7,4'-trimethoxyflavone, obtained from *Macaranga hosei*, showed weak antibacterial activity against three pathogenic bacteria.²² In this study, the leaves of *Macaranga hullettii* King ex Hook.f. was used as the object of analysis to identify new sources to treat infectious diseases triggered by bacteria. This plant is known to be a plant that can be found in all areas of East Kalimantan, where this species is commonly found in mountain forests and logging forests in the Kalimantan region.^{23, 24} Phytochemical screening and isolated compounds of *Macaranga hulletti* King ex Hook.f. leaf extract contained flavonoid compounds.^{18,25} Extensive research on the antibacterial activity of the *Macaranga* has been performed. Nonetheless, the methanol

extract and its fractions were derived from *Macaranga hulletti* King ex Hook.f. species are yet to be assessed for their antibacterial effects. Therefore, this study was to assess the antibacterial activity of *Macaranga hulletti* King ex Hook.f. leaf extract and its fractions using *in vitro*. Moreover, all flavonoids isolated from *Macaranga* species in the East Kalimantan, Indonesia, were evaluated their antibacterial activity using molecular docking study.

Materials and Methods

Materials

The solvent was purified by distillation before use (methanol, n-hexane, and ethyl acetate). The materials used in this study were *Macaranga hulletti* leaves, *Staphylococcus aureus* ATCC 25923 from Chulalongkorn University, *Streptococcus mutans* ATCC 25175 from Chulalongkorn University, *Propionibacterium acnes* KCCM 41747 from Mulawarman University, nutrient agar (NA) from Merck, Luria Bertani liquid medium (1% tryptone, 0.5% yeast, and 0.5% NaCl), Yeast extract (Oxoid 100% (w/w)), Tryptone (Oxoid 100% (w/w)), ampicillin 100 mg/ml (Sigma Aldrich), NaCl (Sigma Aldrich), cotton swabs, aluminum foil, gauze, Petri dish, Ose needle, paper discs, autoclave (Autoclave TOMY, Model SX-700), oven (SHARP EO-28 WH), rotary vacuum evaporator (EYELA Rotary evaporator N-1300E V.S.Series), and freezer (Sanyo Medicoool). Lenovo Yoga 7 with AMD Ryzen AI 7-8840HS w/ Radeon 780M graphics, 3301 Mhz, 8 Core(s), and RAM 16.0 GB.

Methods

Sample preparation

The research samples used in this study were leaves of *Macaranga hulletti* King ex Hook.f. plants obtained from the Lesan River Forest, Kelay District, Berau Regency, East Kalimantan, Indonesia (1°32'2.5919"N - 117°3'11.950"E). Plant samples were acknowledged at the

Laboratory of Anatomy and Plant Systematics, FMIPA Mulawarman University (No. 0174/UN.17.8.5.7.16/HA/XI/2017).

Extraction

Macaranga hullettii King ex Hook.f. leaf powder (1 kg) was obtained by maceration for 24 h using methanol. The extract was evaporated using a rotary vacuum evaporator to get a methanol extract. The methanol extract was fractionated successively using n-hexane and ethyl acetate. Each fraction was evaporated using a rotary vacuum evaporator to obtain n-hexane and ethyl acetate fractions. Furthermore, methanol extract, n-hexane fraction, and ethyl acetate fraction were prepared to evaluate their antibacterial activity.

Pathogens

Staphylococcus aureus ATCC 25923 was required by the Department of Microbiology, Faculty of Science, Chulalongkorn University. *Streptococcus mutans* ATCC 25175 was prepared by the Department of Biochemistry, Faculty of Dentistry, Chulalongkorn University. Both strains were purchased from the American Type Culture Collection (ATCC). *Propionibacterium acnes* KCCM 41747 was obtained from the Faculty of Forestry, Mulawarman University, and was gained from the Korean Culture Center of Microorganisms (KCCM). Furthermore, the tested bacteria were periodically subcultured and maintained in nutrient agar (NA) under suitable conditions, depending on the tested bacteria.

In vitro antibacterial assay

Determination of antibacterial prospects in methanol extract, n-hexane, and ethyl acetate fractions of *Macaranga hullettii* King ex Hook.f. plant leaves were analyzed using disc paper or the Kirby-Bauer method at varying concentrations.^{26,27} In this study, *Staphylococcus aureus*

ATCC 25923, *Streptococcus mutans* ATCC 25175, and *Propionibacterium acnes* KCCM 41747 were employed. Ampicillin was used as a positive control. Organic solvents, such as methanol, n-hexane, and ethyl acetate, were used as negative controls. Nutrient Agar (NA) media was prepared by dissolving 3.3 grams in 150 mL of distilled water. Luria Bertani (LB) liquid media as much as 4 grams was dissolved in 500 mL of distilled water, then stirred, and put 5 mL each into a test tube. Subsequently, NA and LB were sterilized using an autoclave at 121 °C with a pressure of 1 atm for 15 minutes. Each tested bacterium was collected using an Ose needle, after which it was diluted into a test tube containing sterile LB media and then incubated for 24 h at 37 °C. After the incubation period, the tested bacteria were ready for use in the subsequent stages.

Nutrient Agar (25 mL) was poured into a sterilized Petri dish. The NA medium was maintained at room temperature for solidification. The bacterial suspension in the test tube was then collected using a sterile cotton swab. A sterile cotton swab was applied to the entire surface of the agar on a Petri dish. Each 30 µL of the tested *M. hullettii* leaf samples prepared at various concentrations (in organic solvent) was poured onto sterile adsorbent filter paper discs (6 mm in diameter), and the solvent was removed. Afterward, the paper discs containing the tested sample were placed onto the surface of agar media, closed the lid, and incubated at 37 °C for 18-24 hours. The inhibition zone formed in each well was measured using a ruler.^{28,29} Furthermore, the Minimum Inhibitory Concentration (MIC) was determined for the methanol extract as well as the n-hexane and ethyl acetate fractions. MIC was the minimum concentration that halted this alteration and completely stopped bacterial growth.³⁰

Molecular docking

Eight flavonoid derivatives possessing either prenyl or geranyl group were found in the three *Macaranga* species in East Kalimantan, Indonesia (*Macaranga hosei*, *Macaranga pearsonii*,

and *Macaranga tanarius*): 4'-O-methyl-8-isoprenylnaringenin, lonchocarpol A, 4'-O-methyl-8-isoprenyleriodictyol, 6-isoprenyleriodictyol, solophenol D, nymphaeol A, nymphaeol B, and nymphaeol C.¹⁴⁻¹⁷ Macahuilettiin A was prenylated flavonoid found in the *Macaranga huletti* obtained from Central Kalimantan (Indonesia).¹⁸ Furthermore, a comparative analysis of the molecular docking results of prenylated/geranylated flavonoids with methoxylated flavonoids was conducted, focusing on 5-hydroxy-7,4'-dimethoxyflavone and 5-hydroxy-6,7,4'-trimethoxyflavone, which were found in the *Macaranga hosei* from Trengganu, Malaysia,²² as presented in Table 2.

Furthermore, the molecular structures of the flavonoids isolated from *Macaranga* species in East Kalimantan, Indonesia, were drawn using ChemOffice Professional 15.0 and obtained from PubChem (<https://pubchem.ncbi.nlm.nih.gov/>). Structure refinement was performed using the molecular mechanics energy minimization method with the Merck molecular force field (MMFF94) using Open Babel in PyRx V.1.1. Furthermore, three proteins were download from protein data bank (PDB ID: 1JIJ, 3IPK, 7LBU),³¹⁻³³ the location of active site was calculated using AlphaFold from <https://prankweb.cz/>.³⁴ Molecular docking was performed using the AutoDock Vina tool in PyRx V.1.1 software with an exhaustiveness of 32 and a mode value of 9 poses for each docked ligand.^{35,36} The protein binding site was defined as a box with dimensions of 30 × 30 × 30 Å. In the final step, the binding interactions were analyzed, and the docking results were visualized in 2D using the BIOVIA Discovery Studio Visualizer.

Statistical analysis

Data from the experiments were investigated using Microsoft Excel.

Results and Discussion

***In vitro* antibacterial activity**

The strength of antibacterial activity was determined based on the diameter of the clear zone to obtain the inhibition zone (mm), which can be classified as ≤ 5 mm (weak), 5-10 mm (moderate), 10-20 mm (strong), and ≥ 20 mm (very strong). Moreover, the smaller the minimum inhibitory concentration of a compound, the greater its ability to inhibit the growth of test bacteria.²⁸ In this study, ampicillin was used as a positive control against three bacteria: *Staphylococcus aureus* ATCC 25923, *Streptococcus mutans* ATCC 25175, and *Propionibacterium acnes* KCCM 41747, as shown in Table 1. The positive control served to determine whether the test bacteria could be inhibited by growth or were resistant to antibiotics.

Staphylococcus aureus can cause dentoalveolar infections, jaw cysts, paroritis, oral mucosal lesions, and stomatitis. It can also produce exotoxins, lecosidine, enterotoxins, and quagulase compounds. Enterotoxins are compounds that cause food poisoning in humans.³⁷ Antibacterial activity of the methanol extract, hexane fraction, and ethyl acetate fraction of *Macaranga hulettii* King ex Hook. f. leaves showed moderate antibacterial activity against *S. aureus* because of the inhibition zone of 5-10 mm at concentration from 0.15-2.5%, as presented in Table 1. The inhibition zone produced by the methanol extract was 6.67 mm at 0.15%. Meanwhile, the ethyl acetate fraction at 0.15% exhibited an inhibition zone at 6.33 mm. However, the n-hexane fraction exhibited antibacterial activity at 2.5%, with an inhibition zone of 6.50 mm. The results indicated that the methanol extract and ethyl acetate fraction of *Macaranga hulettii* King ex Hook,f leaves had the most potent antibacterial activity against *S. aureus*, with a minimum inhibitory concentration (MIC) of $<0.15\%$. Moreover, *Macaranga tanarius* is known to prevent the growth of *S. aureus* most effectively in the n-hexane fraction, with an MIC value of 0.125-0.5% and an inhibition zone of approximately 7.25 mm.³⁸

Streptococcus mutans is a prominent bacterial species that is implicated in the pathogenesis of dental caries. This facultative anaerobe primarily ferments carbohydrates, resulting in the production of various organic acids, with lactic acid as the predominant byproduct. The

acidification of the dental environment by *S. mutans* is a crucial factor contributing to enamel demineralization and the subsequent formation of carious lesions.^{39,40} These bacteria produce glucosyltransferase (GTF) to produce glucan, which causes dental caries.⁴¹ *S. mutans* adheres to the salivary pellicle because of the many receptors for attachment to the salivary pellicle.⁴² Furthermore, the antibacterial activity of *Macaranga hulettii* King ex Hook f. leaf extract and fractions was tested against *S. mutans*, as presented in Table 1. The result revealed that both extract and fraction displayed moderate antibacterial activity because of the inhibition zone of 5-10 mm at a concentration of 0.15-2.5%. The inhibition zones created by the methanol extract and ethyl acetate fraction were 6.67 and 6.33 mm at 0.15%, respectively. Meanwhile, the n-hexane fraction showed antibacterial activity at 1.25-2.5% with an inhibition zone of 6.33-6.67 mm. These results demonstrated that the methanol extract and ethyl acetate fraction of *Macaranga hulettii* King ex Hook. leaves presented the highest potential antibacterial activity against *S. mutans*, with an MIC <0.15% (Table 1).

Furthermore, *Propionibacterium acnes* is a gram-positive anaerobic bacterium that can induce unusual keratinization within the sebaceous glands of hair follicles and increase sebum production.⁴³ The enzyme is capable of hydrolyzing triglycerides (TG) present in sebum into free fatty acids (FFA), which may subsequently induce inflammation in and around the hair follicles.⁴⁴ These bacteria also produce hyaluronidase, protease, lecithinase, and neurimidase, which cause inflammation of the skin; *Macaranga triloba* ethanol extract is known to prevent the growth of *P. acnes* at 2.5% and the largest inhibition value of 5.54 mm at 20%.⁴⁵ As shown in Table 1, the methanol extract and ethyl acetate fraction of *Macaranga hulettii* King ex Hook f. showed strong antibacterial activity against *P. acnes* because of the inhibition values of 10.67 and 14.67 mm at 2.5%, respectively. Meanwhile, the n-hexane fraction exhibited moderate antibacterial activity with inhibition value of 5-10 mm at 1.25-2.5%. Furthermore, the methanol extract showed antibacterial activity with an inhibition value of 7.67 at 0.31%. The ethyl acetate

fraction showed antibacterial activity of 0.15%, with an inhibition zone of 6.67 mm. The results revealed that the ethyl acetate fraction of *Macaranga hulettii* King ex Hook.f leaves presented the highest potential antibacterial activity against *P. acnes*, with a minimum inhibitory concentration (MIC) of <0.15%, as presented in Table 1.

Based on the data of antibacterial activity, both extracts and their fractions from *Macaranga hulettii*, the methanol extract and ethyl acetate fraction exhibited good antibacterial activity against the three bacterial strains. This finding was consistent with previous studies indicating that the methanol extract and ethyl acetate fraction demonstrated antibacterial activity, attributable to the presence of flavonoids and their prenylated or geranylated forms in *Macaranga* species, such as euchrestaflavanone A, bonanione A, macarangaflavanone A, macarangaflavanone B, macatrichocarpin A, propolin D (nymphaeol B), senegalensin, schweinfurthin B, schweinfurthin O, and isomacarangain.⁴⁶⁻⁵⁶ Flavonoid compounds can damage bacterial cell walls and denature proteases that disrupt bacterial metabolic processes.⁵⁷ In addition, alkaloids can damage the peptidoglycan layer in bacterial cells, causing damage to the bacterial cell wall and inhibiting bacterial growth. Terpenoids also inhibit bacterial growth by damaging bacterial cell membranes. Moreover, the phenolic compounds of secondary metabolites can damage bacterial cells by increasing the permeability of the cytoplasmic membrane, resulting in cytoplasmic coagulation and cell division.²⁸

Molecular docking study

Molecular docking was performed to predict the interaction of the flavonoids with the amino acid residues of the three proteins. This study used three proteins from *Staphylococcus aureus*, *Streptococcus mutans*, and *Propionibacterium acnes* that were retrieved from the Protein Data Bank with PDB ID 1JJJ, 3IPK, and 7LBU, respectively.³¹⁻³³ As illustrated in Table 2, the molecular docking results revealed that 11 flavonoid derivatives revealed binding affinities

ranging from -7.2 to -10.5 kcal/mol, -7.5 to -8.9 kcal/mol, and -7.1 to -8.9 kcal/mol against *Staphylococcus aureus* tyrosyl-tRNA synthetase (PDB ID: 1JII), *Streptococcus mutans* antigen I/II (AgI/II) (PDB ID: 3IPK), and *Propionibacterium acnes* surface sialidase (PDB ID: 7LBU), respectively. Furthermore, the interaction of flavonoids and ampicillin with the protein target is depicted in two dimensions, as presented in Figure 1-3.

Among the compounds against *S. aureus* tyrosyl-tRNA synthetase, nymphaeol A-B and solophenol D had the highest binding affinity compared to ampicillin as a positive control, as shown in Table 2. Thus, the interaction of these compounds with the active site of the enzyme was conducted in a 2D structure (Figure 1). Furthermore, ampicillin showed hydrogen bonding with LYS84 and GLN174, and amide- π interactions with GLY38, as depicted in Figure 1. Nymphaeol A and nymphaeol B exhibited hydrogen bonding with four amino acid residues, π -anions, and hydrophobic interactions, whereas nymphaeol B and ampicillin showed similar interactions with LYS84. In addition, solophenol D displayed hydrogen bonding with TYR36, THR75, GLN190, ASP177, and GLN174, as well as hydrophobic interactions with HIS50, LEU70, PRO53, and ALA39 (Figure 1). This result was in line with previous study, where nymphaeol A-B and solophenol D exhibited antibacterial activity.^{58,59}

As presented in Table 2, five compounds exhibited high antibacterial activity against *S. mutans* antigen I/II (AgI/II), including 6-isoprenyleriodictyol, nymphaeol A-B, and solophenol D, with binding affinity from -8.7 to -8.9 kcal/mol. Furthermore, the interaction between the ligand and amino acid residues in the binding site of these compounds is shown in a 2D structure (Figure 2). As shown in Figure 2, ampicillin formed hydrogen bonding with four amino acid residues, ASN590, SER591, SER588, and ASP760, as well as π -sulfur and π - π interactions with TRP 816. Furthermore, 6-isoprenyleriodictyol showed only hydrogen bonding with three amino acid residues: SER697, ASN699, and ASN814. Meanwhile, nymphaeol A and nymphaeol B displayed hydrogen bonding with four amino acid residues (Figure 2), but nymphaeol A

exhibited a similar π - π interaction with TRP816, as observed with ampicillin. Solophenol D formed hydrogen bonds with SER818, ARG824, and THR586, similar π - π interactions (TRP816) with ampicillin, and π -sigma interactions with SER762. Furthermore, 6-isoprenyleriodictyol is a prenylated flavonoid similar to nymphaeol A. Thus, 6-isoprenyleriodictyol may have antibacterial potency.

Moreover, three compounds displayed stronger binding affinities than ampicillin, with values of -8.0 and -8.4 kcal/mol against *P. acnes* surface sialidases, such as 6-isoprenyleriodictyol and nymphaeol A, respectively, as described in Table 2. Therefore, both compounds were imaged in 2D to investigate the interaction between the ligand and the amino acid residues in the binding site (Figure 3). As depicted in Figure 3, ampicillin showed hydrogen bonding with four amino acid residues, ARG282, GLN287, ASP146, ASP185, and π - π and π -alkyl interactions with PHE257, ALA147, and VAL202. 6-Isoprenyleriodictyol demonstrated π - π interactions with PHE257, π -anion interactions with ASP146, and alkyl-alkyl interactions with ALA147, PHE209, VAL202, and LEU224. Furthermore, this compound displayed hydrogen bonding with six amino acid residues, including ASP146, ARG329, TYR423, ARG282, and GLU287, which were analogous to those observed with ampicillin. Nymphaeol A showed hydrogen bonding with five amino acid residues (ARG329, TYR423, ASP185, ALA147, and SER184), π -anion interactions with ASP312 and ASP185, alkyl-alkyl interactions with MET310, ALA331, and ALA147, and Van der Waals interactions with PHE257. Thus, these compounds may have potent as antibacterial.

Structure-activity relationships were also studied based on the binding affinity results, as presented in Table 2. Flavonoids possessing prenyl, geranyl, and hydroxyl groups showed good potency as antibacterial agents owing to their strong binding affinity with amino acid residues in the binding site of proteins compared with ampicillin. Nevertheless, the absence of hydroxy group on prenylated or geranylated flavonoids decreased binding affinity.^{22,56,60}

Conclusion

The extract and fraction of *Macaranga hulettii* King ex Hook, f, have potential as antibacterial agents against three bacterial strains, *Staphylococcus aureus* ATCC 25923, *Streptococcus mutans* ATCC 25175, and *Propionibacterium acnes* KCCM 41747. The methanol extract demonstrated antibacterial activity against *S. aureus* and *S. mutans* with MIC (%) of <0.15 against *S. aureus* and *S. mutans*, and 0.15-0.31% against *P. acnes*. n-Hexane fraction showed antibacterial activity with MIC (%) of 1.25-2.5% against *S. aureus*, and 0.62-1.25% against *S. mutans* and *P. acnes*. Moreover, the ethyl acetate fraction displayed antibacterial activity with an MIC (%) <0.15% against three bacterial strains (*S. aureus*, *S. mutans*, and *P. acnes*). Molecular docking studies have suggested that 6-isoprenyleriodictyol, nymphaeol A, nymphameol B, and solophenol D are promising antibacterial agents because those compounds exhibit the highest binding affinity and form hydrogen bonding interactions with key amino acid residues in the active site of the protein targets. Therefore, the development of this plant extract is required to further study the isolation of secondary metabolites *in vitro* and *in vivo*.

Conflict of Interest

The authors declare no conflicts of interest.

Author's Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims related to the content of this article will be borne by them.

Acknowledgement

This research was funded by the Islamic Development Bank (PIU-IsDB) project under contract no. 137/UN17.11/PL/2019.

References

1. Wong F, de la Fuente-Nunez C, Collins JJ. Leveraging artificial intelligence in the fight against infectious diseases. *Science*. 2023; 381(6654):164-70.
2. Seukep AJ, Kuete V, Nahar L, Sarker SD, Guo M. Plant-derived secondary metabolites as the main source of efflux pump inhibitors and methods for identification. *J. Pharm. Anal.* 2020; 10(4):277-90.
3. Magadula JJ. Phytochemistry and pharmacology of the genus *Macaranga*: a review. *J. Med. Plants Res.* 2014; 8(12):489-503.
4. Vu LT, Ngan TB, Phuong L, Huong DT, Litaudon M, Van Hung N, Thach TD, Van Cuong P. Chemical constituents from fruits of *Macaranga denticulata* (Euphorbiaceae)(Part 2). *Vietnam J. Chem.* 2018; 56(4):516-20.
5. Marliana E, Hairani R, Tjahjandarie TS, Tanjung M. Antiplasmodial activity of flavonoids from *Macaranga tanarius* leaves. *IOP Conf. Ser.: Earth Environ. Sci.* 2018; 144(1):012011).
6. Tjahjandarie TS, Tanjung M, Saputri RD, Nadar PB, Aldin MF, Marliana E, Permadi A. Flavestins K, An isoprenylated stilbene from the leaves of *Macaranga recurvata* Gage. *Nat. Prod. Sci.* 2019; 25(3):244-7.
7. Huong DT, Linh NT, Van TT, Litaudon M, Roussi F, Van Nam V, Van Cuong P. Stilbenes from *Macaranga tanarius* (Euphorbiaceae) growing in Vietnam. *Vietnam J. Chem.* 2020; 58(3):338-42.

8. Aldin MF, Tjahjandarie TS, Saputri RD, Tanjung M. Macasiamenene V, a New Stilbenoid from the Leaves of *Macaranga inermis*. Nat. Prod. Sci. 2021; 27(1):45-8.
9. Muharram A, Rachmawati DA, Mardhiyyah S, Tjahjandarie TS, Saputri RD, Ahmat N, Tanjung M. Cytotoxic and antioxidant activities of flavonoids and diterpenoids from *Macaranga involucrata* (Roxb.) Baill. J. Appl. Pharm. Sci. 2023; 13(6):087-92.
10. Tanjung M, Tjahjandarie TS, Aldin MF, Mardhiyyah S, Ahmat N, Saputri RD. Macagigantin A, A New Flavonoid from *Macaranga gigantea* (Rchb. f & Zoll.) Mull. Arg. Nat. Prod. Sci. 2023; 29(4):287-94.
11. Tjahjandarie TS, Aldin MF, Saputri RD, Tanjung M. Dihydrostilbenes from *Macaranga javanica* (Blume) Müll. Arg. and their antiplasmodial activity. Nat. Prod. Sci. 2024:1-7.
12. Tanjung M, Tjahjandarie TS, Aldin MF, Mardhiyyah S, Saputri RD, Syah YM, Ahmat N. Two new flavonols from *Macaranga inermis* pax & K. Hoffm. Nat. Prod. Sci. 2025; 39(3):498-505.
13. Tanjung M, Tjahjandarie TS, Aldin MF, Mardhiyyah S, Saputri RD, Syah YM, Ahmat N. Two new flavonols from *Macaranga inermis* pax & K. Hoffm. Nat. Prod. Sci. 2025; 39(3):498-505.
14. Marlina E, Tjahjandarie TS, Tanjung M. Isoprenylated flavanone derivatives from *Macaranga hosei* King ex Hook F. Der Pharm. Lett. 2015; 7(3):153-6.
15. Marlina E, Tjahjandarie TS, Tanjung M. Aktivitas Antioksidan Senyawa Flavonoid Dari *Macaranga pearsonii* Merr. J. Kim. Mulawarman. 2016; 13(2):97-100.
16. Marlina E, Astuti W, Kosala K, Hairani R, Tjahjandarie TS, Tanjung M. Chemical composition and anticancer activity of *Macaranga hosei* leaves. Asian J. Chem. 2018; 30(4):795-8.

17. Marliana E, Ruga R, Hairani R, Tjahjandarie TS, Tanjung M. Antioxidant activity of flavonoid constituents from the leaves of *Macaranga tanarius*. IOP Conf. Ser.: J. Phys. 2019; 1277(1):012014.
18. Saputri RD, Tukiran T, Wati FA, Purnamasari AP, Wardhana MW, Tjahjandarie TS, Tanjung M. Macahuilettiin A, a new isoprenylated flavanone from the leaves of *Macaranga hullettii* King ex Hook and their antiplasmodial activity. Vietnam J. Chem. 2024; 62(3):394-8.
19. Musdalifah M, Khumaidi A, Suwastika IN. Uji Daya Hambat dan Skrining Fitokimia Ekstrak Daun *Macaranga tanarius* (L.) Mull. Arg Sebagai Antibakteri Salmonella typhi. Nat. Sci: J. Sci. Technol. 2017; 6(3).
20. Ibrahim H, Omosa L, Nchiozem-Ngnitedem V, Onyari J, Maru S, Guefack M. Antibacterial activities and phytochemical screening of crude extracts from Kenyan macaranga species toward MDR phenotypes expressing efflux pumps. Pharmacogn. Commn. 2021; 11(2):119-26.
21. Bijesh K, Sebastian D. Isolation and characterization of antibacterial compounds from *Macaranga peltata* against clinical isolates of *Staphylococcus aureus*. Int. J. Biol. Pharma Res. 2013; 4:1196-203.
22. Salleh WM, Razak NZ, Ahmad F. Phytochemicals and biological activities of *Macaranga hosei* and *Macaranga constricta* (Euphorbiaceae). Marmara Pharm. J. 2017;21(4):881-8.
23. Slik JW, Priyono P, Welzen PV. Key to the *Macaranga Thou.* and *Mallotus Lour.* species (Euphorbiaceae) of East Kalimantan, Indonesia. Singapore, Gardens' Bulletin (Singapore), National Parks Board, Singapore Botanic Gardens; 2000. 11-87 p.
24. Amirta R, Angi EM, Ramadhan R, Kusuma IW, Wiati CB, Haqiqi MT. Potensi pemanfaatan macaranga. Samarinda: Mulawarman University Press; 2017.

25. Rismawati R, Marlina E, Daniel D. Phytochemical Test on Methanol Extract of Leaf of *Macaranga hullettii* King ex Hook. f. J. Atomik. 2018; 3(2):91-4.
26. Hudzicki J. Kirby-Bauer disk diffusion susceptibility test protocol. American society for microbiology. 2009; 15(1):1-23.
27. Inna M, Astuti W, Saleh C. Aktivitas antibakteri ekstrak metanol daun tanaman sambung urat (*Cayratia carnosa*) terhadap *Salmonella thypi* and *Propionibacterium acnes*. J. Atomik. 2022; 7(1):1-5.
28. Yulianti MF, Amat AL, Hutasoit RM, Pakan PD. Antibacterial activity of jamblang leaf ethanol extract (*Syzygium cumini*) against the growth of *Propionibacterium acnes*. Acta Biochimica Indones. 2023; 6(2):1161.
29. Hossain MR, Biplob AI, Sharif SR, Bhuiya AM, Sayem AS. Antibacterial Activity of Green Synthesized Silver Nanoparticles of *Lablab purpureus* Flowers Extract against Human Pathogenic Bacteria. Trop. J. Nat. Prod. Res. 2023;7(8).
30. Yanda L, Tatsimo SJ, Tamokou JD, Matsute-Takongmo G, Meffo-Dongmo SC, Meli Lannang A, Sewald N. Antibacterial and antioxidant activities of isolated compounds from *Prosopis africana* leaves. Int. J. Anal. Chem. 2022; 4205823.
31. Qiu X, Janson CA, Smith WW, Green SM, McDevitt P, Johanson K, Carter P, Hibbs M, Lewis C, Chalker A, Fosberry A. Crystal structure of *Staphylococcus aureus* tyrosyl-tRNA synthetase in complex with a class of potent and specific inhibitors. Protein Sci. 2001; 10(10):2008-16.
32. Larson MR, Rajashankar KR, Patel MH, Robinette RA, Crowley PJ, Michalek S, Brady LJ, Deivanayagam C. Elongated fibrillar structure of a *streptococcal adhesin* assembled by the high-affinity association of α -and PPII-helices. Proc. Natl. Acad. Sci. 2010; 107(13):5983-8.

33. Yu AC, Volkers G, Jongkees SA, Worrall LJ, Withers SG, Strynadka NC. Crystal structure of the *Propionibacterium acnes* surface sialidase, a drug target for *P. acnes*-associated diseases. *Glycobiology*. 2022; 32(2):162-70.
34. Jakubec D, Skoda P, Krivak R, Novotny M, Hoksza D. PrankWeb 3: accelerated ligand-binding site predictions for experimental and modelled protein structures. *Nucleic Acids Res*. 2022; 50:W593-7.
35. Dallakyan S, Olson AJ. Small-molecule library screening by docking with PyRx. In *Chemical biology: methods and protocols*. 2014: 243-250. New York, NY: Springer New York.
36. Trott O, Olson AJ. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *J. Comput. Chem*. 2010; 31(2):455-61.
37. Malangu N, editor. *Poisoning: From Specific Toxic Agents to Novel Rapid and Simplified Techniques for Analysis*. BoD–Books on Demand; 2017.
38. Sari AA, Saleh C. Uji fitokimia, toksisitas dan aktivitas antibakteri ekstrak berbagai fraksi daun mara (*Macaranga tanarius* (L.) MA) terhadap bakteri *Staphylococcus aureus* dan *Escherichia coli*. *J. Kim. Mulawarman*. 2015; 12(2):53-8.
39. Imelda R., Mariam M., Satari M. Effect of cassava (*manihot esculenta* cranzt), rice (*oryza sativa* l.), and potato (*solanum tuberosum*) water extract to decrease pH phase fermentation of *streptococcus mutans* atcc 25175. *Padjajaran J. Dent*. 2019; 31(1):14.
40. Alkhaled A., Alsabek L., Al-assaf M. , & Badr F.. Effect of chlorhexidine, honey and propolis on *streptococcus mutans* counts: *in vitro* study. *Dentistry*. 2021; 9(1).
41. Ren Z, Chen L, Li J, Li Y. Inhibition of *Streptococcus mutans* polysaccharide synthesis by molecules targeting glycosyltransferase activity. *J. Oral Microbiol*. 2016; 8(1):31095.

42. Lee BS, Chen YJ, Wei TC, Ma TL, Chang CC. Comparison of antibacterial adhesion when salivary pellicle is coated on both poly (2-hydroxyethyl-methacrylate)-and polyethylene-glycol-methacrylate-grafted poly (methyl methacrylate). *Int. J. Mol. Sci.* 2018; 19(9):2764.
43. Chen KC, Yang CH, Li TT, Zouboulis CC, Huang YC. Suppression of *Propionibacterium acnes*-stimulated proinflammatory cytokines by Chinese bayberry extracts and its active constituent myricetin in human sebocytes *in vitro*. *Phytother. Res.* 2019; 33(4):1104-13.
44. Xu J, Chen X, Song J, Wang C, Xu W, Tan H, Suo H. Antibacterial activity and mechanism of cell-free supernatants of *Lacticaseibacillus paracasei* against *Propionibacterium acnes*. *Microb. Pathog.* 2024; 189:106598
45. Warnida H, Mustika D, Supomo S, Sukawaty Y. Efektivitas Ekstrak Etanol Daun Mahang (*Macaranga Triloba*) sebagai Obat Anti-acne. *J. Penelit. Sos. Ekon. Kehuta.* 2018; 4(1):9-18.
46. Schütz BA, Wright AD, Rali T, Sticher O. Prenylated flavanones from leaves of *Macaranga pleiostemona*. *Phytochemistry.* 1995; 40(4):1273-7.
47. Lim TY, Lim YY, Yule CM. Evaluation of antioxidant, antibacterial and anti-tyrosinase activities of four *Macaranga* species. *Food Chem.* 2009; 114(2):594-9.
48. Fareza MS, Syah YM, Mujahidin D, Juliawaty LD, Kurniasih I. Antibacterial flavanones and dihydrochalcones from *Macaranga trichocarpa*. *Z. Naturforsch C J. Biosci.* 2014; 69(9-10):375-80.
49. Hasanat A, Kabir MS, Hossain MM, Hasan M, Al Masum MA, Chowdhury TA, Bhuiyan DI, Mamur A, Kibria AS. Antibacterial activity of methanol extract of *Macaranga denticulata* leaves and in silico PASS prediction for its six secondary metabolites. *World J. Pharm. Pharm. Sci.* 2015:1258-66.

50. O Akanbi B, Anene P, Olayanju S. Preliminary Screening Indicates Promising Antimicrobial Properties of the Stem Bark Extracts of *Macaranga rosea*. *Anti-Infective Agents*. 2015; 13(2):123-8.
51. Ogundajo A, Okeleye B, Ashafa AO. Chemical constituents, *in vitro* antimicrobial and cytotoxic potentials of the extracts from *Macaranga barteri* Mull-Arg. *Asian Pac. J. Trop. Biomed*. 2017; 7(7):654-9.
52. Putri R, Hendra R, Teruna HY. Anti-Bacterial and Anti-Fungal Activities from *Macaranga bancana* Leaves Extract. *Pharmacol. Clin. Pharm. Res*. 2019; 4(1):1-4.
53. Lee JH, Kim YG, Khadke SK, Yamano A, Woo JT, Lee J. Antimicrobial and antibiofilm activities of prenylated flavanones from *Macaranga tanarius*. *Phytomedicine*. 2019; 63:153033.
54. Pagna JI, Awazi T, Mbarga PE, Mbekou IM, Mkounga P, Fotie J, Frese M, Fabrice FB, Lenta BN, Sewald N, Nkengfack EA. Antibacterial flavonoids from the fruits of *Macaranga hurifolia*. *J. Asian Nat. Prod. Res*. 2022; 24(11):1041-51.
55. Kamso VF, Simo Fotso CC, Kanko Mbekou IM, Toussie BT, Ndjakou Lenta B, Boyom FF, Sewald N, Frese M, Ngadjui BT, Wabo Fotso G. Chemical constituents of *Macaranga occidentalis*, antimicrobial and chemophenetic studies. *Molecules*. 2022; 27(24):8820.
56. Rosamah E, Haqiqi MT, Putri AS, Kuspradini H, Kusuma IW, Amirta R, Yuliansyah Y, Suwinarti W, Paramita S, Ramadhan R, Tarmadi D. The potential of Macaranga plants as skincare cosmetic ingredients: A review. *J. Appl. Pharm. Sci*. 2023; 13(7):001-12.
57. Li AP, He YH, Zhang SY, Shi YP. Antibacterial activity and action mechanism of flavonoids against phytopathogenic bacteria. *Pesticide Biochemistry and Physiology*. 2022; 188:105221.
58. Chen YW, Ye SR, Ting C, Yu YH. Antibacterial activity of propolins from Taiwanese green propolis. *J. Food Drug Anal*. 2018; 26(2):761-8.

59. Inui S, Hosoya T, Shimamura Y, Masuda S, Ogawa T, Kobayashi H, Shirafuji K, Moli RT, Kozono I, Shin-ya K, Kumazawa S. Solophenols B–D and Solomonin: New Prenylated Polyphenols Isolated from Propolis Collected from The Solomon Islands and Their Antibacterial Activity. *J. Agri. Food Chem.* 2012; 60(47):11765-70.
60. Ruga R, Kingkaew K, Tamsampaolot K, Chavasiri W. Enhancing antibacterial activity against *Propionibacterium acnes* and *Staphylococcus aureus* by combination of tetracycline with selected compounds. *Chem. Lett.* 2018; 47(12):1538-41.

Table 1: Antibacterial Activity of *Macaranga hullettii* King ex Hook Leaf Extracts and Fractions against *Staphylococcus aureus*, *Streptococcus mutans*, and *Propionibacterium acnes*

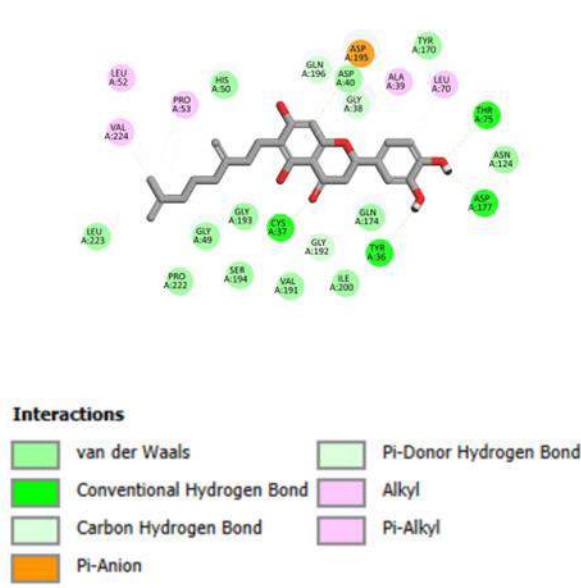
Sample	Concentration (%)	Inhibition Zone (mm)*		
		<i>Staphylococcus</i>	<i>Streptococcus</i>	<i>Propionibacterium</i>
		<i>aureus</i>	<i>mutans</i>	<i>acnes</i>
Methanol Extract	0	0	0	0
	0.15	6.67	6.67	0
	0.31	6.67	6.67	7.67
	0.62	6.67	7	8
	1.25	7.17	7.33	9.67
	2.5	7.5	8	10.67
	MIC (%)	<0.15	<0.15	0.15-0.31
n-Hexane Fraction	0	0	0	0
	0.15	0	0	0
	0.31	0	0	0
	0.62	0	0	0
	1.25	0	6.33	6.33
	2.5	6.5	6.67	6.67
	MIC (%)	1.25-2.5	0.62-1.25	0.62-1.25
Ethyl acetate Fraction	0	0	0	0
	0.15	6.33	6.33	6.67
	0.31	6.5	6.67	8
	0.62	6.5	6.83	9
	1.25	6.67	7	11
	2.5	7	7.67	14.67

	MIC (%)	<0.15	<0.15	<0.15
Ampicillin	0.01	20.33	21	21

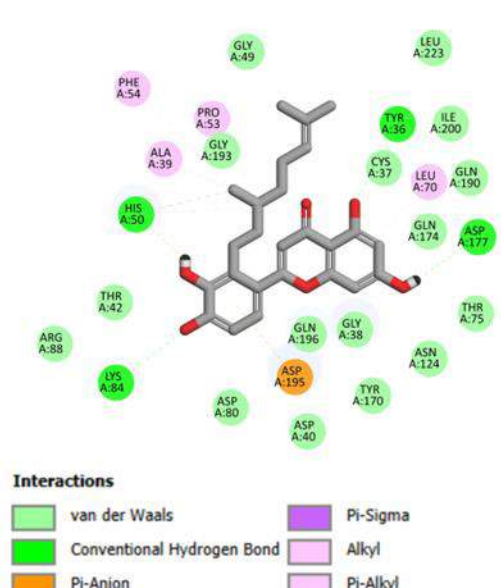
*Data were obtained from triplicate experiments.

Table 2: Binding affinity of flavonoids against three proteins (1JJJ, 3IPK, and 7LBU)

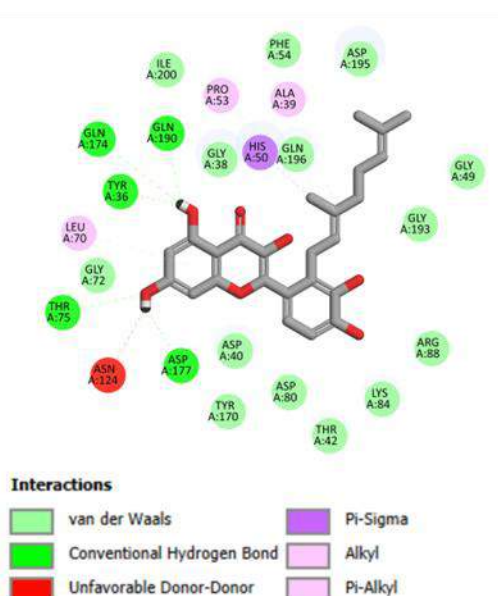
No.	Compound	Binding Affinity (kcal/mol)		
		1JJJ	3IPK	7LBU
1	4'-O-methyl-8-isoprenylnaringenin	-7.4	-8.1	-7.7
2	4'-O-methyl-8-isoprenyleryiodictyol	-7.2	-8.1	-7.6
3	6-Isoprenyleryiodictyol	-7.7	-8.7	-8.0
4	5-hydroxy-7,4'-dimethoxyflavone	-8.6	-7.8	-7.1
5	5-hydroxy-6,7,4'-trimethoxyflavone	-8.3	-7.5	-7.3
6	Macahuiltiin A	-7.6	-8.4	-7.1
7	Lonchocarpol A	-9.4	-7.8	-7.8
8	Nymphaeol A	-9.7	-8.9	-8.4
9	Nymphaeol B	-10.5	-8.9	-7.7
10	Nymphaeol C	-8.4	-8.3	-7.4
11	Solophenol D	-10.0	-8.7	-7.9
12	Ampicillin	-8.5	-7.7	-7.6



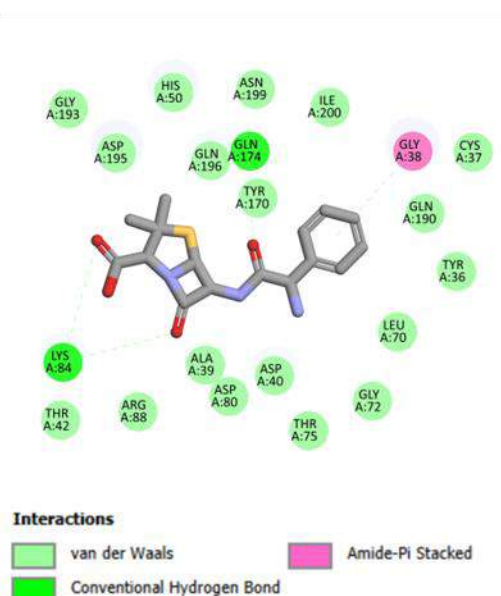
Nymphaeol A



Nymphaeol B

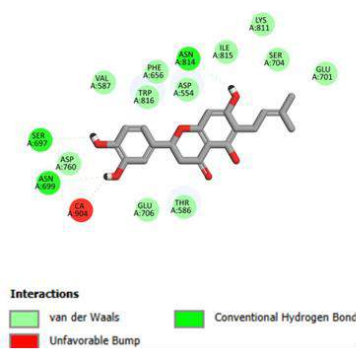


Solophenol D

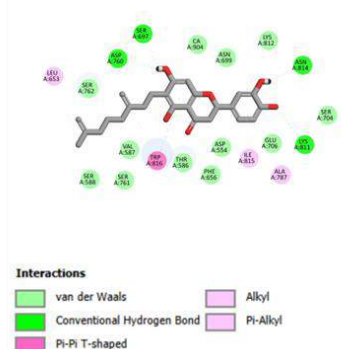


Ampicillin

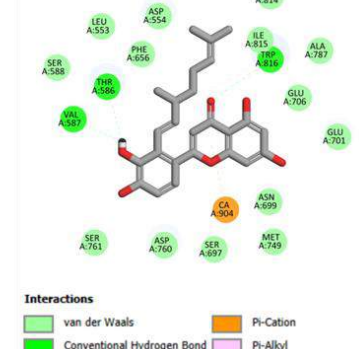
Figure 1: 2D interaction of the best docked flavonoids and ampicillin with *Staphylococcus aureus* tyrosyl-tRNA synthetase (PDB ID: 1JIJ)



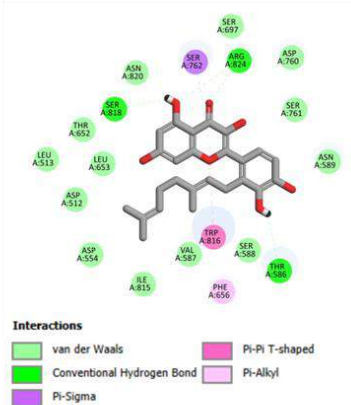
6-Isoprenylioridictyol



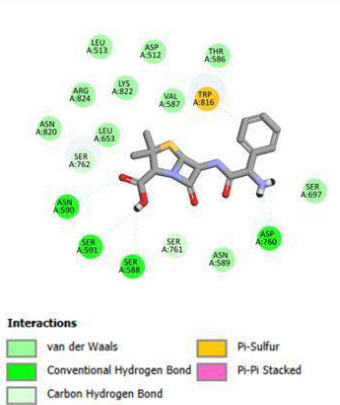
Nymphaeol A



Nymphaeol B



Solophenol D



Ampicillin

Figure 2: 2D interaction of the best-docked flavonoids and ampicillin with *Streptococcus mutans* antigen I/II (PDB ID: 3IPK).

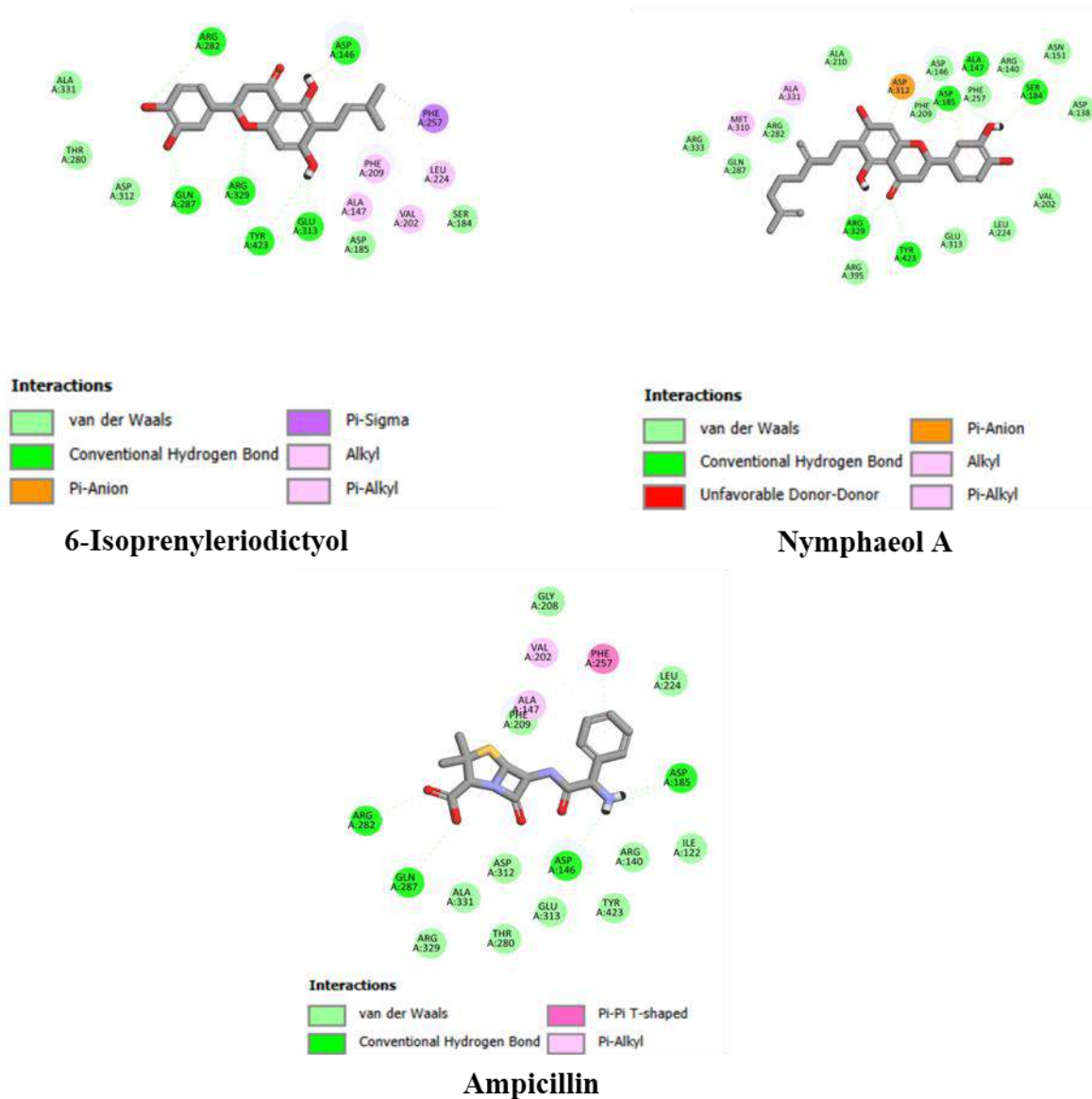


Figure 3: 2D interaction of the best-docked flavonoids and ampicillin with *Propionibacterium acnes* surface sialidase (PDB ID: 7LBU).

turnitin check

by Ade Danova

Submission date: 06-May-2025 08:04PM (UTC+0700)

Submission ID: 2668096045

File name: Turnitin_Check-3-TJNPR-2025-420_Revised_1.docx (27.96M)

Word count: 5451

Character count: 32750

***In vitro* and Molecular Docking Evaluation of New Antibacterial Activity of *Macaranga hulletii* King ex Hook.f. Leaf Extract**

Abstract

Bacterial infections are a common occurrence, and with the increasing resistance of bacteria to antibiotics, utilization of plant extracts is gaining prominence. *Macaranga* plants are known to contain secondary metabolites such as flavonoids, which exhibit antibacterial properties. *Macaranga hulletii* King ex Hook.f. is particularly noteworthy because of its widespread presence in East Kalimantan. Therefore, the objective of this study was to investigate the antibacterial potential of *Macaranga hulletii* leaf extract through *in vitro* and molecular docking studies. The research involved testing the methanol extract, n-hexane fraction, and ethyl acetate fraction from *Macaranga hulletii* King ex Hook.f. leaves against three bacterial strains, *Staphylococcus aureus*, *Streptococcus mutans*, and *Propionibacterium acnes*. The results revealed that both the methanol extract and ethyl acetate fraction displayed antibacterial activity against three bacterial strains with a minimum inhibitory concentration (MIC) of < 0.15%, except for the methanol extract with MIC of 0.15-0.31% against *Propionibacterium acnes*. Additionally, molecular docking analysis showed that five flavonoids possessing prenyl or geranyl groups, namely 6-isoprenylerythrodictyol, nymphaeol A, nymphaeol B, and solophenol D, showed the highest binding affinity and dominant hydrogen bonding interactions with key amino acid residues in the binding site of the three proteins (UU, 3IPK, and 7LBU). Thus, these findings suggest that the methanol extract and ethyl acetate fraction from *Macaranga hulletii* King ex Hook.f. has potential applications as a new source of antibacterial agents, and further studies are needed to isolate the bioactive compounds and evaluate them by *in vitro* and *in vivo* studies.

Keywords: Antibacterial, Extract, Flavonoid, *Macaranga hollettii*, Molecular Docking.

Introduction

Despite substantial advancements in medicine and fundamental research over recent decades, infectious diseases because of transmissible agents, such as bacteria, eukaryotes, and viruses, continue to present significant challenges to scientists and healthcare professionals. These challenges are further intensified by the limitations in the rapid and accurate diagnosis of infections and the escalating issue of antimicrobial resistance.¹ Extracts from medicinal plants, which are abundant in diverse phytochemicals with a range of pharmacological properties, present a promising approach for addressing multidrug-resistant bacteria. Additionally, it may contribute to restoring the efficacy of antibiotics to treat infectious diseases.² Therefore, it is imperative to continue the pursuit of more efficacious antimicrobial agents, particularly those sourced from natural sources, which are both readily available and economically viable.

Macaranga belongs to the Euphorbiaceae family and is known as "Mahang." Based on the literature, the secondary metabolites contained in this genus are unique flavonoids and stilbenoids with prenylated substituents.³⁻¹² Nine flavonoids containing either prenyl or geranyl group with antioxidant, antiparasmodial, and anticancer activities were successfully isolated from three *Macaranga* species present in East Kalimantan (*Macaranga hasei*, *Macaranga pearsonii*, and *Macaranga tanarius*): 4'-O-methyl-8-isoprenylnaringenin, lonchocarpol A, 4'-O-methyl-8-isoprenyleriodytyol, 6-isoprenyleriodytyol, solophenol D, nymphacol A, nymphacol B, nymphacol C, including macanulletin A that was isolated from *Macaranga hollettii* obtained from Central Kalimantan, which have antioxidant, antiparasmodial, and anticancer activities.¹⁴⁻¹⁸ *Macaranga tanarius* leaf extract exhibited antibacterial activity.¹⁹ Moreover, the plant extracts of *Macaranga conglomerata*, *Macaranga kilimandscharica* and

Macaranga capensis from Kenya showed antibacterial activities against *E. coli*, *E. aerogenes*, *K. pneumoniae*, *P. stuartii*, *P. aeruginosa*, and *S. aureus*.²⁸ The flavonol rhamnetin, isolated from *Macaranga peltata*, also showed antibacterial activity against *Staphylococcus aureus*.²³ Two flavanone groups, 5-hydroxy-7,4'-dimethoxyflavone and 5-hydroxy-6,7,4'-trimethoxyflavone, obtained from *Macaranga hovei*, showed weak antibacterial activity against three pathogenic bacteria.¹² In this study, the leaves of *Macaranga hulletii* King ex Hook.f. was used as the object of analysis to identify new sources to treat infectious diseases triggered by bacteria. This plant is known to be a plant that can be found in all areas of East Kalimantan, where this species is commonly found in mountain forests and logging forests in the Kalimantan region.^{23,24} Phytochemical screening and isolated compounds of *Macaranga hulletii* King ex Hook.f. leaf extract contained flavonoid compounds.^{18,25} Extensive research on the antibacterial activity of the *Macaranga* has been performed. Nonetheless, the methanol extract and its fractions were derived from *Macaranga hulletii* King ex Hook.f. species are yet to be assessed for their antibacterial effects. Therefore, this study was to assess the antibacterial activity of *Macaranga hulletii* King ex Hook.f. leaf extract and its fractions using *in vitro* and molecular docking studies.

Materials and Methods

Materials

The solvent was purified by distillation before use (methanol, n-hexane, and ethyl acetate). The materials used in this study were *Macaranga hulletii* leaves, *Staphylococcus aureus* ATCC 25923 from Chulalongkorn University, *Staphylococcus mutans* ATCC 25175 from Chulalongkorn University, *Propionibacterium acnes* KCCM 41747 from Malawarian University, nutrient agar (NA) from Merck, Luria Bertani liquid medium (1% tryptone, 0.5% yeast, and 0.5% NaCl), Yeast extract (Oxoid 100% (w/w)), Tryptone (Oxoid 100% (w/w)),

ampicillin 100 mg/ml (Sigma Aldrich), NaCl (Sigma Aldrich), cotton swabs, aluminum foil, gauze, Petri dish, Ose needle, paper discs, autoclave (Autoclave TOMY, Model SX-700), oven (SHARP EO-28 WH), rotary vacuum evaporator (EYELA Rotary evaporator N-1300E V.S.Series), and freezer (Sanyo Mediceol). Lenovo Yoga 7 with AMD Ryzen AI 7-8840HS w/ Radeon 780M graphics, 3301 Mhz, 8 Core(s), and RAM 16.0 GB.

Methods

Sample preparation

The research samples used in this study were leaves of *Macaranga hulletii* King ex Hook.f. plants obtained from the Lesan River Forest, Kelsy District, Berau Regency, East Kalimantan, Indonesia (1°32'2.5919"N - 117°3'11.950"E). Plant samples were acknowledged at the Laboratory of Anatomy and Plant Systematics, FMIPA Mulawarman University (No. 0174/UN.17.8.5.7.16/HA/XI/2017).

Extraction

Macaranga hulletii King ex Hook.f. leaf powder (1 kg) was obtained by maceration for 24 h using methanol. The extract was evaporated using a rotary vacuum evaporator to get a methanol extract. The methanol extract was fractionated successively using n-hexane and ethyl acetate. Each fraction was evaporated using a rotary vacuum evaporator to obtain n-hexane and ethyl acetate fractions. Furthermore, methanol extract, n-hexane fraction, and ethyl acetate fraction were prepared to evaluate their antibacterial activity.

Pathogens

Staphylococcus aureus ATCC 25923 was required by the Department of Microbiology, Faculty of Science, Chulalongkorn University. *Streptococcus mutans* ATCC 25175 was prepared by the

Department of Biochemistry, Faculty of Dentistry, Chulalongkorn University. Both strains were purchased from the American Type Culture Collection (ATCC). *Propionibacterium acnes* KCCM 41747 was obtained from the Faculty of Forestry, Mulawarman University, and was gained from the Korean Culture Center of Microorganisms (KCCM). Furthermore, the tested bacteria were periodically subcultured and maintained in nutrient agar (NA) under suitable conditions, depending on the tested bacteria.

In vitro antibacterial assay

Determination of antibacterial prospects in methanol extract, n-hexane, and ethyl acetate fractions of *Macaranga hulletii* King ex Hook f. plant leaves were analyzed using disc paper or the Kirby-Bauer method at varying concentrations.^{26,27} In this study, *Staphylococcus aureus* ATCC 25923, *Streptococcus mutans* ATCC 25175, and *Propionibacterium acnes* KCCM 41747 were employed. Ampicillin was used as a positive control. Organic solvents, such as methanol, n-hexane, and ethyl acetate, were used as negative controls. Nutrient Agar (NA) media was prepared by dissolving 3.3 grams in 150 mL of distilled water. Luria Bertani (LB) liquid media as much as 4 grams was dissolved in 500 mL of distilled water, then stirred, and put 5 mL each into a test tube. Subsequently, NA and LB were sterilized using an autoclave at 121 °C with a pressure of 1 atm for 15 minutes. Each tested bacterium was collected using an Ose needle, after which it was diluted into a test tube containing sterile LB media and then incubated for 24 h at 37 °C. After the incubation period, the tested bacteria were ready for use in the subsequent stages.

Nutrient Agar (25 mL) was poured into a sterilized Petri dish. The NA medium was maintained at room temperature for solidification. The bacterial suspension in the test tube was then collected using a sterile cotton swab. A sterile cotton swab was applied to the entire surface of the agar on a Petri dish. Each 30 µL of the tested *M. hulletii* leaf samples prepared at various

concentrations (in organic solvent) was poured onto sterile adsorbent filter paper discs (6 mm in diameter), and the solvent was removed. Afterward, the paper discs containing the tested sample were placed onto the surface of agar media, closed the lid, and incubated at 37 °C for 18-24 hours. The inhibition zone formed in each well was measured using a ruler.^{20,29} Furthermore, the Minimum Inhibitory Concentration (MIC) was determined for the methanol extract as well as the n-hexane and ethyl acetate fractions. MIC was the minimum concentration that halted this alteration and completely stopped bacterial growth.³⁰

Molecular docking

Eight flavonoid derivatives possessing either prenyl or geranyl group were found in the three *Macaranga* species in East Kalimantan, Indonesia (*Macaranga hasei*, *Macaranga pearsonii*, and *Macaranga tosarum*): 4'-O-methyl-8-isoprenylaringenin, lonchocarpol A, 4'-O-methyl-8-isoprenylerioidictyol, 6-isoprenylerioidictyol, islophenol D, nymphaccol A, nymphaccol B, and nymphaccol C.¹⁴⁻¹⁷ Macahuilettin A was prenylated flavonoid found in the *Macaranga hasei* obtained from Central Kalimantan (Indonesia).¹⁶ Furthermore, a comparative analysis of the molecular docking results of prenylated/geranylated flavonoids with methoxylated flavonoids was conducted, focusing on 5-hydroxy-7,4'-dimethoxyflavone and 5-hydroxy-6,7,4'-trimethoxyflavone, which were found in the *Macaranga hasei* from Trengganu, Malaysia,²² as presented in Table 2.

Furthermore, the molecular structures of the flavonoids isolated from *Macaranga* species in East Kalimantan, Indonesia, were drawn using ChemOffice Professional 15.0 and obtained from PubChem (<https://pubchem.ncbi.nlm.nih.gov/>). Structure refinement was performed using the molecular mechanics energy minimization method with the Merck molecular force field (MMFF94) using Open Babel in PyRx V.1.1. Furthermore, three proteins were downloaded from protein data bank (PDB ID: 1JJJ, 3IPK, 7LBU),³¹⁻³³ the location of active site was

calculated using AlphaFold from <https://prankweb.cz/>.²⁴ Molecular docking was performed using the AutoDock Vina tool in PyRx V.1.1 software with an exhaustiveness of 32 and a mode value of 9 poses for each docked ligand.^{25,26} The protein binding site was defined as a box with dimensions of 30 × 30 × 30 Å. In the final step, the binding interactions were analyzed, and the docking results were visualized in 2D using the BIOVIA Discovery Studio Visualizer.

Statistical analysis

Data from the experiments were investigated using Microsoft Excel.

Results and Discussion

In vitro antibacterial activity

The strength of antibacterial activity was determined based on the diameter of the clear zone to obtain the inhibition zone (mm), which can be classified as ≤ 5 mm (weak), 5-10 mm (moderate), 10-20 mm (strong), and ≥ 20 mm (very strong). Moreover, the smaller the minimum inhibitory concentration of a compound, the greater its ability to inhibit the growth of test bacteria.²⁸ In this study, ampicillin was used as a positive control against three bacteria: *Staphylococcus aureus* ATCC 25923, *Streptococcus mutans* ATCC 25175, and *Propionibacterium acnes* KCCM 41747, as shown in Table 1. The positive control served to determine whether the test bacteria could be inhibited by growth or were resistant to antibiotics. *Staphylococcus aureus* can cause dentoalveolar infections, jaw cysts, parotitis, oral mucosal lesions, and stomatitis. It can also produce exotoxins, leucosidine, enterotoxins, and quagulase compounds. Enterotoxins are compounds that cause food poisoning in humans.²⁷ Antibacterial activity of the methanol extract, hexane fraction, and ethyl acetate fraction of *Macaranga kulanii* King ex Hook. f. leaves showed moderate antibacterial activity against *S. aureus* because of the inhibition zone of 5-10 mm at concentration from 0.15-2.5%, as presented in

Table 1. The inhibition zone produced by the methanol extract was 6.67 mm at 0.15%. Meanwhile, the ethyl acetate fraction at 0.15% exhibited an inhibition zone of 6.33 mm. However, the n-hexane fraction exhibited antibacterial activity at 2.5%, with an inhibition zone of 6.50 mm. The results indicated that the methanol extract and ethyl acetate fraction of *Macaranga huletti* King ex Hook.f leaves had the most potent antibacterial activity against *S. aureus*, with a minimum inhibitory concentration (MIC) of <0.15%. Moreover, *Macaranga tinctoria* is known to prevent the growth of *S. aureus* most effectively in the n-hexane fraction, with an MIC value of 0.125–0.5% and an inhibition zone of approximately 7.25 mm.³⁸

Streptococcus mutans is a prominent bacterial species that is implicated in the pathogenesis of dental caries. This facultative anaerobe primarily ferments carbohydrates, resulting in the production of various organic acids, with lactic acid as the predominant byproduct. The acidification of the dental environment by *S. mutans* is a crucial factor contributing to enamel demineralization and the subsequent formation of carious lesions.^{39,40} These bacteria produce glucosyltransferase (GTF) to produce glucan, which causes dental caries.⁴¹ *S. mutans* adheres to the salivary pellicle because of the many receptors for attachment to the salivary pellicle.⁴² Furthermore, the antibacterial activity of *Macaranga huletti* King ex Hook.f. leaf extract and fractions was tested against *S. mutans*, as presented in Table 1. The result revealed that both extract and fraction displayed moderate antibacterial activity because of the inhibition zone of 5–10 mm at a concentration of 0.15–2.5%. The inhibition zones created by the methanol extract and ethyl acetate fraction were 6.67 and 6.33 mm at 0.15%, respectively. Meanwhile, the n-hexane fraction showed antibacterial activity at 1.25–2.5% with an inhibition zone of 6.33–6.67 mm. These results demonstrated that the methanol extract and ethyl acetate fraction of *Macaranga huletti* King ex Hook.f. leaves presented the highest potential antibacterial activity against *S. mutans*, with an MIC <0.15% (Table 1).

Furthermore, *Propionibacterium acnes* is a gram-positive anaerobic bacterium that can induce unusual keratinization within the sebaceous glands of hair follicles and increase sebum production.⁴² The enzyme is capable of hydrolyzing triglycerides (TG) present in sebum into free fatty acids (FFA), which may subsequently induce inflammation in and around the hair follicles.⁴⁴ These bacteria also produce hyaluronidase, protease, lecithinase, and neuramidase, which cause inflammation of the skin; *Macaranga triloba* ethanol extract is known to prevent the growth of *P. acnes* at 2.5% and the largest inhibition value of 5.54 mm at 20%.⁴⁵ As shown in Table 1, the methanol extract and ethyl acetate fraction of *Macaranga holstii* King ex Hook f. showed strong antibacterial activity against *P. acnes* because of the inhibition values of 10.67 and 14.67 mm at 2.5%, respectively. Meanwhile, the n-hexane fraction exhibited moderate antibacterial activity with inhibition value of 5–10 mm at 1.25–2.5%. Furthermore, the methanol extract showed antibacterial activity with an inhibition value of 7.67 at 0.31%. The ethyl acetate fraction showed antibacterial activity of 0.15%, with an inhibition zone of 6.67 mm. The results revealed that the ethyl acetate fraction of *Macaranga holstii* King ex Hook f. leaves presented the highest potential antibacterial activity against *P. acnes*, with a minimum inhibitory concentration (MIC) of <0.15%, as presented in Table 1.

Based on the data of antibacterial activity, both extracts and their fractions from *Macaranga holstii*, the methanol extract and ethyl acetate fraction exhibited good antibacterial activity against the three bacterial strains. This finding was consistent with previous studies indicating that the methanol extract and ethyl acetate fraction demonstrated antibacterial activity, attributable to the presence of flavonoids and their prenylated or geranylated forms in *Macaranga* species, such as eucrestaffavanone A, bonanione A, macarangaffavanone A, macarangaffavanone B, macatrichocarpin A, propolin D (nymphaeol B), senegalensin, schweinfurthin B, schweinfurthin O, and isomacarangain.^{46–48} Flavonoid compounds can damage bacterial cell walls and denature proteases that disrupt bacterial metabolic processes.⁴⁹

In addition, alkaloids can damage the peptidoglycan layer in bacterial cells, causing damage to the bacterial cell wall and inhibiting bacterial growth. Terpenoids also inhibit bacterial growth by damaging bacterial cell membranes. Moreover, the phenolic compounds of secondary metabolites can damage bacterial cells by increasing the permeability of the cytoplasmic membrane, resulting in cytoplasmic coagulation and cell division.²⁸

Molecular docking study

Molecular docking was performed to predict the interaction of the flavonoids with the amino acid residues of the three proteins. This study used three proteins from *Staphylococcus aureus*, *Streptococcus mutans*, and *Propionibacterium acnes* that were retrieved from the Protein Data Bank with PDB ID 1JJJ, 3IPK, and 7LBU, respectively.³¹⁻³³ As illustrated in Table 2, the molecular docking results revealed that 11 flavonoid derivatives revealed binding affinities ranging from -7.2 to -10.5 kcal/mol, -7.5 to -8.9 kcal/mol, and -7.1 to -8.9 kcal/mol against *Staphylococcus aureus* tyrosyl-tRNA synthetase (PDB ID: 1JJJ), *Streptococcus mutans* antigen I/II (AgI/II) (PDB ID: 3IPK), and *Propionibacterium acnes* surface sialidase (PDB ID: 7LBU), respectively. Furthermore, the interaction of flavonoids and ampicillin with the protein target is depicted in two dimensions, as presented in Figure 1-5.

Among the compounds against *S. aureus* tyrosyl-tRNA synthetase, nymphaeol A-B and solophenol D had the highest binding affinity compared to ampicillin as a positive control, as shown in Table 2. Thus, the interaction of these compounds with the active site of the enzyme was conducted in a 2D structure (Figure 1). Furthermore, ampicillin showed hydrogen bonding with LYS84 and GLN174, and amide- π interactions with GLY38, as depicted in Figure 1. Nymphaeol A and nymphaeol B exhibited hydrogen bonding with four amino acid residues, π -anions, and hydrophobic interactions, whereas nymphaeol B and ampicillin showed similar interactions with LYS84. In addition, solophenol D displayed hydrogen bonding with TYR36,

THR75, GLN190, ASP177, and GLN174, as well as hydrophobic interactions with HIS50, LEU70, PRO53, and ALA39 (Figure 1). This result was in line with previous study, where nymphaeol A-B and solophenol D exhibited antibacterial activity.^{18,25}

As presented in Table 2, five compounds exhibited high antibacterial activity against *S. mutans* antigen I/II (Agl/II), including 6-isoprenyliodictyol, nymphaeol A-B, and solophenol D, with binding affinity from -8.7 to -8.9 kcal/mol. Furthermore, the interaction between the ligand and amino acid residues in the binding site of these compounds is shown in a 2D structure (Figure 2). As shown in Figure 2, ampicillin formed hydrogen bonding with four amino acid residues, ASN590, SER591, SER588, and ASP760, as well as π -sulfur and π - π interactions with TRP816. Furthermore, 6-isoprenyliodictyol showed only hydrogen bonding with three amino acid residues: SER697, ASN699, and ASN814. Meanwhile, nymphaeol A and nymphaeol B displayed hydrogen bonding with four amino acid residues (Figure 2), but nymphaeol A exhibited a similar π - π interaction with TRP816, as observed with ampicillin. Solophenol D formed hydrogen bonds with SER818, ARG824, and THR586, similar π - π interactions (TRP816) with ampicillin, and π -sigma interactions with SER762. Furthermore, 6-isoprenyliodictyol is a prenylated flavonoid similar to nymphaeol A. Thus, 6-isoprenyliodictyol may have antibacterial potency.

Moreover, three compounds displayed stronger binding affinities than ampicillin, with values of -8.0 and -8.4 kcal/mol against *P. aeruginosa* surface sialidases, such as 6-isoprenyliodictyol and nymphaeol A, respectively, as described in Table 2. Therefore, both compounds were imaged in 2D to investigate the interaction between the ligand and the amino acid residues in the binding site (Figure 3). As depicted in Figure 3, ampicillin showed hydrogen bonding with four amino acid residues, ARG282, GLN287, ASP146, ASP185, and π - π and π -alkyl interactions with PHE257, ALA147, and VAL202. 6-Isoprenyliodictyol demonstrated π - π interactions with PHE257, π -anion interactions with ASP146, and alkyl-alkyl interactions with ALA147,

PHE209, VAL202, and LEU224. Furthermore, this compound displayed hydrogen bonding with six amino acid residues, including ASP146, ARG329, TYR423, ARG282, and GLU287, which were analogous to those observed with ampicillin. Nymphaeol A showed hydrogen bonding with five amino acid residues (ARG329, TYR423, ASP185, ALA147, and SER184), π -anion interactions with ASP312 and ASP185, alkyl-alkyl interactions with MET310, ALA331, and ALA147, and Van der Waals interactions with PHE257. Thus, these compounds may have potent as antibacterial.

Structure-activity relationships were also studied based on the binding affinity results, as presented in Table 2. Flavonoids possessing prenyl, geranyl, and hydroxyl groups showed good potency as antibacterial agents owing to their strong binding affinity with amino acid residues in the binding site of proteins compared with ampicillin. Nevertheless, the absence of hydroxy group on prenylated or geranylated flavonoids decreased binding affinity.^{22,39,40}

Conclusion

The extract and fraction of *Mucunaega huletti* King ex Hook.f. have potential as antibacterial agents against three bacterial strains, *Staphylococcus aureus* ATCC 25923, *Streptococcus mutans* ATCC 25175, and *Propionibacterium acnes* KCCM 41747. The methanol extract demonstrated antibacterial activity against *S. aureus* and *S. mutans* with MIC (%) of <0.15 against *S. aureus* and *S. mutans*, and 0.15-0.31% against *P. acnes*. *n*-Hexane fraction showed antibacterial activity with MIC (%) of 1.25-2.5% against *S. aureus*, and 0.62-1.25% against *S. mutans* and *P. acnes*. Moreover, the ethyl acetate fraction displayed antibacterial activity with an MIC (%) <0.15% against three bacterial strains (*S. aureus*, *S. mutans*, and *P. acnes*). Molecular docking studies have suggested that 6-isoprenylteriodictyol, nymphaeol A,

nyphamcol B, and solophenol D are promising antibacterial agents because those compounds exhibit the highest binding affinity and form hydrogen bonding interactions with key amino acid residues in the active site of the protein targets. Therefore, the development of this plant extract is required to further study the isolation of secondary metabolites *in vitro* and *in vivo*.

References

1. Wong F, de la Fuente-Nunez C, Collins JJ. Leveraging artificial intelligence in the fight against infectious diseases. *Science*. 2023; 381(6654):164-70.
2. Seukep AJ, Kute V, Nahar L, Sarker SD, Guo M. Plant-derived secondary metabolites as the main source of efflux pump inhibitors and methods for identification. *J. Pharm. Anal.* 2020; 10(4):277-90.
3. Magadula JJ. Phytochemistry and pharmacology of the genus *Macaranga*: a review. *J. Med. Plants Res.* 2014; 8(12):489-503.
4. Vu LT, Ngan TB, Phucng L, Huong DT, Litaudon M, Van Hung N, Thach TD, Van Cuong P. Chemical constituents from fruits of *Macaranga denticulata* (Euphorbiaceae) (Part 2). *Vietnam J. Chem.* 2018; 56(4):516-20.
5. Marlana E, Hairani R, Tjahjandarie TS, Tanjung M. Antiplasmodial activity of flavonoids from *Macaranga tamaris* leaves. *IOP Conf. Ser.: Earth Environ. Sci.* 2018; 144(1):012011).
6. Tjahjandarie TS, Tanjung M, Saputri RD, Nadar PB, Aldin MF, Marlana E, Permadi A, Flavestin K. An isoprenylated stilbene from the leaves of *Macaranga recurvata* Gage. *Nat. Prod. Sci.* 2019; 25(3):244-7.
7. Huong DT, Linh NT, Van TT, Litaudon M, Roussi F, Van Nam V, Van Cuong P. Stilbenes from *Macaranga tamaris* (Euphorbiaceae) growing in Vietnam. *Vietnam J. Chem.* 2020; 58(3):338-42.

8. Aldin MF, Tjahjandarie TS, Saputri RD, Tanjung M. Macasiamenene V, a New Stilbenoid from the Leaves of *Macaranga inermis*. *Nat. Prod. Sci.* 2021; 27(1):45-8.
9. Muhiarom A, Rachmawati DA, Mardhiyyah S, Tjahjandarie TS, Saputri RD, Ahmat N, Tanjung M. Cytotoxic and antioxidant activities of flavonoids and diterpenoids from *Macaranga involucreata* (Roxb.) Baill. *J. Appl. Pharm. Sci.* 2023; 13(6):087-92.
10. Tanjung M, Tjahjandarie TS, Aldin MF, Mardhiyyah S, Ahmat N, Saputri RD. Macagigantin A, A New Flavonoid from *Macaranga gigantea* (Rchb. f & Zoll.) Müll. Arg. *Nat. Prod. Sci.* 2023; 29(4):287-94.
11. Tjahjandarie TS, Aldin MF, Saputri RD, Tanjung M. Dihydrostilbenes from *Macaranga javanica* (Blume) Müll. Arg. and their antiparasmodial activity. *Nat. Prod. Sci.* 2024:1-7.
12. Tanjung M, Tjahjandarie TS, Aldin MF, Mardhiyyah S, Saputri RD, Syah YM, Ahmat N. Two new flavonoids from *Macaranga inermis* pax & K. Hoffm. *Nat. Prod. Sci.* 2025; 39(3):498-505.
13. Tanjung M, Tjahjandarie TS, Aldin MF, Mardhiyyah S, Saputri RD, Syah YM, Ahmat N. Two new flavonoids from *Macaranga inermis* pax & K. Hoffm. *Nat. Prod. Sci.* 2025; 39(3):498-505.
14. Marlina E, Tjahjandarie TS, Tanjung M. Isoprenylated flavanone derivatives from *Macaranga busei* King ex Hook F. *Der Pharm. Lett.* 2015; 7(3):153-6.
15. Marlina E, Tjahjandarie TS, Tanjung M. Aktivitas Antioksidan Senyawa Flavonoid Dari *Macaranga pearsonii* Merr. *J. Kim. Mulawaman*. 2016; 13(2):97-100.
16. Marlina E, Astuti W, Kosala K, Hairani R, Tjahjandarie TS, Tanjung M. Chemical composition and anticancer activity of *Macaranga busei* leaves. *Asian J. Chem.* 2018; 30(4):795-8.

17. Marlina E, Ruga R, Hairani R, Tjahjandrie TS, Tanjung M. Antioxidant activity of flavonoid constituents from the leaves of *Macaranga tanarius*. IOP Conf. Ser.: J. Phys. 2019; 1277(1):012014.
18. Saputri RD, Tukiran T, Wati EA, Purnamasari AP, Wardhana MW, Tjahjandrie TS, Tanjung M. Macahuletin A, a new isoprenylated flavanone from the leaves of *Macaranga kalleri* King ex Hook and their antiparasitic activity. Vietnam J. Chem. 2024; 62(3):394-8.
19. Musdalifah M, Khumidi A, Suwastika IN. Uji Daya Hambat dan Skrining Fitokimia Ekstrak Daun *Macaranga tanarius* (L.) Mull. Arg Sebagai Antibakteri *Salmonella typhi*. Nat. Sci: J. Sci. Technol. 2017; 6(3).
20. Ibrahim H, Omosa L, Nchaczem-Ngnitedem V, Onyaei J, Mana S, Guefack M. Antibacterial activities and phytochemical screening of crude extracts from Kenyan *macaranga* species toward MDR phenotypes expressing efflux pumps. Pharmacogn. Comm. 2021; 11(2):119-26.
21. Bijesh K, Sebastian D. Isolation and characterization of antibacterial compounds from *Macaranga peltata* against clinical isolates of *Staphylococcus aureus*. Int. J. Biol. Pharma Res. 2013; 4:1196-203.
22. Salleh WM, Razak NZ, Ahmad F. Phytochemicals and biological activities of *Macaranga huxel* and *Macaranga constricta* (Euphorbiaceae). Mammara Pharm J. 2017;21(4):881-8.
23. Slik JW, Priyono P, Welzen PV. Key to the *Macaranga* Thunb. and *Mallotus* Lour. species (Euphorbiaceae) of East Kalimantan, Indonesia. Singapore, Gardens' Bulletin (Singapore), National Parks Board, Singapore Botanic Gardens; 2000. 11-87 p.
24. Amiria R, Angi EM, Ramadhan R, Kusuma IW, Wati CB, Haqiqi MT. Potensi pemanfaatan macaranga. Samarinda: Mulawarman University Press; 2017.

25. Rismawati R, Maflana E, Daniel D. Phytochemical Test on Methanol Extract of Leaf of *Macaranga kuettingii* King ex Hook. f. *J. Atomik*. 2018; 3(2):91-4.
26. Hutzicki J. Kirby-Bauer disk diffusion susceptibility test protocol. American society for microbiology. 2009; 15(1):1-23.
27. Inna M, Astuti W, Saleh C. Aktivitas antibakteri ekstrak metanol daun tanaman sambung urat (*Cayratia carnosa*) terhadap *Salmonella typhi* and *Propionibacterium acnes*. *J. Atomik*. 2022; 7(1):1-5.
28. Yulianti MF, Amat AL, Hutason RM, Pakan PD. Antibacterial activity of jambang leaf ethanol extract (*Syzgium cumini*) against the growth of *Propionibacterium acnes*. *Acta Biochimica Indones*. 2023; 6(2):1161.
29. Hossain MR, Biplob AI, Sharif SR, Bhuiya AM, Sayem AS. Antibacterial Activity of Green Synthesized Silver Nanoparticles of *Lalbah purpureus* Flowers Extract against Human Pathogenic Bacteria. *Trop. J. Nat. Prod. Res*. 2023;7(8).
30. Yanda L, Tatsimo SJ, Tumokou JD, Matsute-Takongmo G, Meflo-Dongmo SC, Meli Lamang A, Sewald N. Antibacterial and antioxidant activities of isolated compounds from *Prosopis africana* leaves. *Int. J. Anal. Chem*. 2022; 4205823.
31. Qiu X, Janson CA, Smith WW, Green SM, McDevitt P, Johanson K, Carter P, Hibbs M, Lewis C, Chalker A, Fosberry A. Crystal structure of *Staphylococcus aureus* tyrosyl-tRNA synthetase in complex with a class of potent and specific inhibitors. *Protein Sci*. 2001; 10(10):2008-16.
32. Larson MR, Rajashankar KR, Patel MH, Robinette RA, Crowley PJ, Michalek S, Brady LJ, Deivanayagam C. Elongated fibrillar structure of a streptococcal adhesin assembled by the high-affinity association of α - and PP1I-helices. *Proc. Natl. Acad. Sci*. 2010; 107(13):5983-8.

33. Yu AC, Volkers G, Jongkees SA, Worrall LJ, Withers SG, Strynadka NC. Crystal structure of the *Propionibacterium acnes* surface sialidase, a drug target for *P. acnes*-associated diseases. *Glycobiology*. 2022; 32(2):162-70.
34. Jakuber D, Skoda P, Krivak R, Novotny M, Hoksza D. FrankWeb 3: accelerated ligand-binding site predictions for experimental and modelled protein structures. *Nucleic Acids Res*. 2022; 50:W593-7.
35. Dallakyan S, Olson AJ. Small-molecule library screening by docking with PyRx. In *Chemical biology: methods and protocols*. 2014; 243-250. New York, NY: Springer New York.
36. Trott O, Olson AJ. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *J. Comput. Chem*. 2010; 31(2):455-61.
37. Malangu N, editor. *Poisoning: From Specific Toxic Agents to Novel Rapid and Simplified Techniques for Analysis*. BoD-Books on Demand; 2017.
38. Sari AA, Saleh C. Uji fitokimia, toksisitas dan aktivitas antibakteri ekstrak berbagai fraksi daun mara (*Morungga tomaria* (L.) MA) terhadap bakteri *Staphylococcus aureus* dan *Escherichia coli*. *J. Kim. Mulawaman*. 2015; 12(2):53-8.
39. Imelda R., Mariam M., Satari M. Effect of cassava (*manihot esculenta* crantz), rice (*oryza sativa* L.), and potato (*solanum tuberosum*) water extract to decrease pH phase fermentation of *streptococcus mutans* atcc 25175. *Padjajaran J. Dent*. 2019; 31(1):14.
40. Alkhaled A., Alsabek L., Al-assaf M. , & Bade F. Effect of chlorhexidine, honey and propolis on *streptococcus mutans* counts: *in vitro* study. *Dentistry*. 2021; 9(1).
41. Ren Z, Chen L, Li J, Li Y. Inhibition of *Streptococcus mutans* polysaccharide synthesis by molecules targeting glycosyltransferase activity. *J. Oral Microbiol*. 2016; 8(1):31095.

42. Lee BS, Chen YJ, Wei TC, Ma TL, Chang CC. Comparison of antibacterial adhesion when salivary pellicle is coated on both poly (2-hydroxyethyl-methacrylate)-and polyethylene-glycol-methacrylate-grafted poly (methyl methacrylate). *Int. J. Mol. Sci.* 2018; 19(9):2764.
43. Chen KC, Yang CH, Li TT, Zouboulis CC, Huang YC. Suppression of *Propionibacterium* acnes-stimulated proinflammatory cytokines by Chinese bayberry extracts and its active constituent myricetin in human sebocytes *in vitro*. *Phytother. Res.* 2019; 33(4):1104-13.
44. Xu J, Chen X, Song J, Wang C, Xu W, Tan H, Sao H. Antibacterial activity and mechanism of cell-free supernatants of *Lactocaseibacillus paracasei* against *Propionibacterium acnes*. *Microb. Pathog.* 2024; 189:106598.
45. Wamida H, Mustika D, Supomo S, Sukawaty Y. Efektivitas Ekstrak Etanol Daun Mahang (*Macaranga triloba*) sebagai Obat Anti-acne. *J. Penelit. Sos. Ekon. Kehuta*, 2018; 4(1):9-18.
46. Schütz BA, Wright AD, Rali T, Sticher O. Prenylated flavanones from leaves of *Macaranga plicatostemona*. *Phytochemistry*. 1995; 40(4):1273-7.
47. Lim TY, Lim YY, Yule CM. Evaluation of antioxidant, antibacterial and anti-tyrosinase activities of four *Macaranga* species. *Food Chem.* 2009; 114(2):594-9.
48. Fareza MS, Syah YM, Mujahidin D, Juliwaty LD, Kurniasih I. Antibacterial flavanones and dihydrochalcones from *Macaranga trichocarpa*. *Z. Naturforsch C J. Biosci.* 2014; 69(9-10):375-80.
49. Hasanat A, Kabir MS, Hossain MM, Hasan M, Al Masum MA, Chowdhury TA, Bhuiyan DI, Mamur A, Kibria AS. Antibacterial activity of methanol extract of *Macaranga denticulata* leaves and *in silico* PASS prediction for its six secondary metabolites. *World J. Pharm. Pharm. Sci.* 2015; 1258-66.

50. O. Akanbi B, Anene P, Olayanju S. Preliminary Screening Indicates Promising Antimicrobial Properties of the Stem Bark Extracts of *Macaranga rosea*. *Anti-Infective Agents*. 2015; 13(2):123-8.
51. Ogundajo A, Okeleye B, Ashafa AO. Chemical constituents, *in vitro* antimicrobial and cytotoxic potentials of the extracts from *Macaranga hirta* Mull-Arg. *Asian Pac. J. Trop. Biomed*, 2017; 7(7):654-9.
52. Putri R, Hendra R, Terana HY. Anti-Bacterial and Anti-Fungal Activities from *Macaranga boncana* Leaves Extract. *Pharmacol. Clin. Pharm. Res.* 2019; 4(1):1-4.
53. Lee JH, Kim YG, Khadke SK, Yamano A, Woo JT, Lee J. Antimicrobial and antibiofilm activities of prenylated flavanones from *Macaranga tinctoria*. *Phytomedicine*. 2019; 65:153033.
54. Pagna JL, Awazi T, Mhanga PE, Mbekou IM, Mkounga P, Fotie J, Frese M, Fabrice FB, Lenta BN, Sewald N, Nkengfack EA. Antibacterial flavonoids from the fruits of *Macaranga kirkii*. *J. Asian Nat. Prod. Res.* 2022; 24(11):1041-51.
55. Kamso VF, Simo Foto CC, Kanko Mbekou IM, Toussie BT, Ndjakou Lenta B, Boyom FF, Sewald N, Frese M, Ngadjui BT, Wabo Foto G. Chemical constituents of *Macaranga occidentalis*, antimicrobial and chemophenetic studies. *Molecules*. 2022; 27(24):8820.
56. Rosamah E, Haqiqi MT, Putri AS, Kuspradini H, Kusuma IW, Amirta R, Yuliansyah Y, Surwinarti W, Paramita S, Ramadhan R, Tarnadi D. The potential of *Macaranga* plants as skincare cosmetic ingredients: A review. *J. Appl. Pharm. Sci.* 2023; 13(7):001-12.
57. Li AP, He YH, Zhong SY, Shi YP. Antibacterial activity and action mechanism of flavonoids against phytopathogenic bacteria. *Pesticide Biochemistry and Physiology*. 2022; 188:105221.
58. Chen YW, Ye SR, Ting C, Yu YH. Antibacterial activity of propolis from Taiwanese green propolis. *J. Food Drug Anal.* 2018; 26(2):761-8.

59. Inui S, Hosoya T, Shimamura Y, Masuda S, Ogawa T, Kobayashi H, Shirafuji K, Moli RT, Kozono I, Shin-ya K, Kumazawa S. Solophenols B-D and Solomonin: New Prenylated Polyphenols Isolated from Propolis Collected from The Solomon Islands and Their Antibacterial Activity. *J. Agri. Food Chem.* 2012; 60(47):11765-70.
60. Ruga R, Kingkew K, Tansampholot K, Chavasiri W. Enhancing antibacterial activity against *Propionibacterium acnes* and *Staphylococcus aureus* by combination of tetracycline with selected compounds. *Chem. Lett.* 2018; 47(12):1538-41.

Table 1: Antibacterial Activity of *Macaranga hillebrandii* King ex Hook Leaf Extracts and Fractions against *Staphylococcus aureus*, *Streptococcus mutans*, and *Propionibacterium acnes*

Sample	Concentration (%)	Inhibition Zone (mm)*		
		<i>Staphylococcus</i>	<i>Streptococcus</i>	<i>Propionibacterium</i>
		<i>aureus</i>	<i>mutans</i>	<i>acnes</i>
Methanol Extract	0	0	0	0
	0.15	6.67	6.67	0
	0.31	6.67	6.67	7.67
	0.62	6.67	7	8
	1.25	7.17	7.33	9.67
	2.5	7.5	8	10.67
	MIC (%)	<0.15	<0.15	0.15-0.31
n-Hexane Fraction	0	0	0	0
	0.15	0	0	0
	0.31	0	0	0
	0.62	0	0	0
	1.25	0	6.33	6.33
	2.5	6.5	6.67	6.67
	MIC (%)	1.25-2.5	0.62-1.25	0.62-1.25
Ethyl acetate Fraction	0	0	0	0
	0.15	6.33	6.33	6.67
	0.31	6.5	6.67	8
	0.62	6.5	6.83	9
	1.25	6.67	7	11
	2.5	7	7.67	14.67

	MIC (%)	<0.15	<0.15	<0.15
Ampicillin	0.01	20.33	21	21

*Data were obtained from triplicate experiments.

Table 2: Binding affinity of flavonoids against three proteins (1JJJ, 3IPK, and 7LBU)

No.	Compound	Binding Affinity (kcal/mol)		
		1JJJ	3IPK	7LBU
1	4'-O-methyl-8-isoprenylaringenin	-7.4	-8.1	-7.7
2	4'-O-methyl-8-isoprenyleriodytol	-7.2	-8.1	-7.6
3	6-Isoprenyleriodytol	-7.7	-8.7	-8.0
4	5-hydroxy-7,4'-dimethoxyflavone	-8.6	-7.8	-7.1
5	5-hydroxy-6,7,4'-trimethoxyflavone	-8.3	-7.5	-7.3
6	Macabuleitin A	-7.6	-8.4	-7.1
7	Lonchocarpol A	-9.4	-7.8	-7.8
8	Nymphaeol A	-9.7	-8.9	-8.4
9	Nymphaeol B	-10.5	-8.9	-7.7
10	Nymphaeol C	-8.4	-8.3	-7.4
11	Solophenol D	-10.0	-8.7	-7.9
12	Ampicillin	-8.5	-7.7	-7.6

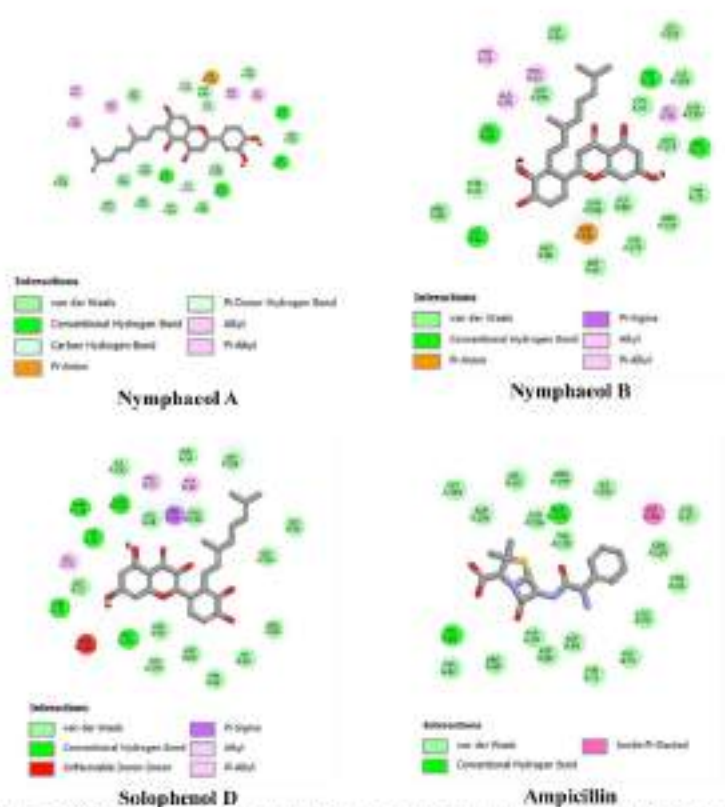


Figure 1: 2D interaction of the best docked flavonoids and ampicillin with *Staphylococcus aureus* tyrosyl-tRNA synthetase (PDB ID: 1JJJ)

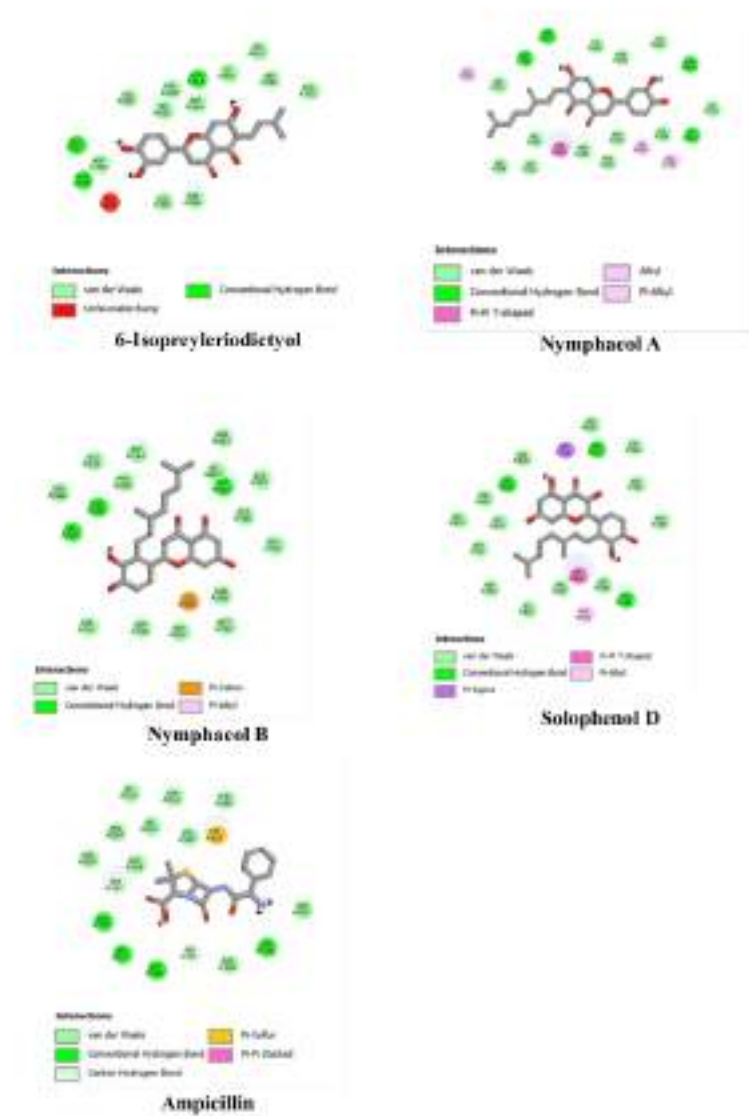


Figure 2: 2D interaction of the best-docked flavonoids and ampicillin with *Streptococcus mutans* antigen I/II (PDB ID: 3BPX).

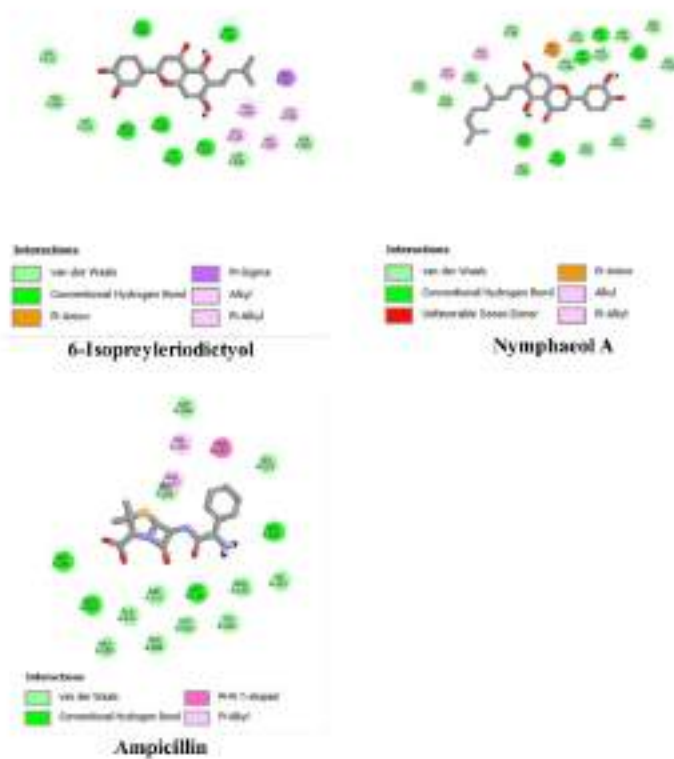


Figure 3: 2D interaction of the best-docked flavonoids and ampicillin with *Propionibacterium acnes* surface sinilidase (PDB ID: 7LBU).

turnitin check

ORIGINALITY REPORT

13%

SIMILARITY INDEX

9%

INTERNET SOURCES

12%

PUBLICATIONS

1%

STUDENT PAPERS

PRIMARY SOURCES

1	cst.kipmi.or.id Internet Source	1%
2	Jiahui Xu, Xiaoyong Chen, Jiajia Song, Chen Wang, Weiping Xu, Han Tan, Huayi Suo. "Antibacterial activity and mechanism of cell-free supernatants of <i>Lactocaseibacillus paracasei</i> against <i>Propionibacterium acnes</i> ", <i>Microbial Pathogenesis</i> , 2024 Publication	1%
3	journals.plos.org Internet Source	1%
4	Hedya Nadhrati Surura, Hairus Abdullah, Sahna Ferdinand Ginting, Erny Tandanu, Refi Ikhtiari. "Green synthesis of silver nanoparticles from papaya seed extracts with alkaloid content for antibacterial application", <i>Jurnal Teknologi Laboratorium</i> , 2021 Publication	1%
5	journal.umpr.ac.id Internet Source	1%
6	www.mdpi.com Internet Source	1%
7	D N Lestari, A Massinai, A Haris. "Antibacterial activity of n-hexane and ethanol extracts of <i>Polycarpa aurata</i> against pathogenic bacteria of shrimp and fish", <i>IOP Conference Series: Earth and Environmental Science</i> , 2022	1%

8	Arti Gupta, Ram Prasad. "Antimicrobials in Food Science and Technology", CRC Press, 2023 Publication	<1 %
9	aunilo.uum.edu.my Internet Source	<1 %
10	repository.uhamka.ac.id Internet Source	<1 %
11	Sri Mulyaningsih, Febriyati Adji Rachmadani. "Determination of the Active Chemical Compounds and the Antibacterial Activity of Various Fractions of Lawsonia inermis L.", Borneo Journal of Pharmacy, 2022 Publication	<1 %
12	ejournal.undip.ac.id Internet Source	<1 %
13	pubs.acs.org Internet Source	<1 %
14	qa1.scielo.br Internet Source	<1 %
15	www.thefreelibrary.com Internet Source	<1 %
16	www.tjnpr.org Internet Source	<1 %
17	Gerald N Teke, Paul K Lunga, Hippolyte K Wabo, Jules-Roger Kuate et al. "Antimicrobial and antioxidant properties of methanol extract, fractions and compounds from the stem bark of Entada abyssinica Stend ex A. Satabie", BMC Complementary and Alternative Medicine, 2011 Publication	<1 %

18 Minarti, Antonius Herry Cahyana, Akhmad Darmawan. "Secondary metabolite compound isolated from the leaves of *Macaranga magna* Turrill", AIP Publishing, 2019

Publication

<1 %

19 japsonline.com
Internet Source

<1 %

20 S D Hardiningtyas, A Sabiliilaika, K Tarman, R F Pari. "Antimicrobial activity of extracellular compounds from endophytic fungus BAR1.5 cultivated with different media", IOP Conference Series: Earth and Environmental Science, 2023

Publication

<1 %

21 Submitted to Universiti Teknologi MARA
Student Paper

<1 %

Exclude quotes On

Exclude matches < 15 words

Exclude bibliography On

turnitin check

GRADEMARK REPORT

FINAL GRADE

GENERAL COMMENTS

/0

PAGE 1

PAGE 2

PAGE 3

PAGE 4

PAGE 5

PAGE 6

PAGE 7

PAGE 8

PAGE 9

PAGE 10

PAGE 11

PAGE 12

PAGE 13

PAGE 14

PAGE 15

PAGE 16

PAGE 17

PAGE 18

PAGE 19

PAGE 20

PAGE 21

PAGE 22

PAGE 23

PAGE 24

PAGE 25



Outlook

Re: Revision

From Ade Danova <adedanova@itb.ac.id>

Date Sat 5/10/2025 1:59 PM

To Editor-in-Chief Tjnpr <editor.tjnpr@gmail.com>

Cc evakia@fmipa.unmul.ac.id <evakia@fmipa.unmul.ac.id>; ritbey.r@fmipa.unmul.ac.id <ritbey.r@fmipa.unmul.ac.id>; ritahairani@fmipa.unmul.ac.id <ritahairani@fmipa.unmul.ac.id>; Dr. Elvira Hermawati, S.Si., M.Si. <elvira.hermawati@itb.ac.id>; anayulvia@unej.ac.id <anayulvia@unej.ac.id>

 2 attachments (1 MB)

3-TJNPR-2025-420 Revised_Proofreading.docx; certificate_TJNPR-2025-420_Proofreading.pdf;

Dear Editor-in-Chief

Tropical Journal of Natural Product Research

I would like to submit the revised manuscript that has been edited by English speaker. Here, I attach the revised manuscript and certificate for English Editing. Thank you very much.

Best regards,
Ade Danova

***In vitro* Evaluation and Molecular Docking StudyEvaluation of New Antibacterial
Activity Compounds of from Macaranga hulletti King ex Hook.f. Leaf Extract**

Eva Marlina^{1,3}, Ade Danova^{2*}, Rita Hairani^{1,3}, Ritbey Ruga^{1,3}, Elvira Hermawati², and Ana
Yulvia⁴

1. Department of Chemistry, Faculty of Mathematics and Natural Science, Mulawarman
University, Samarinda 75123, East Kalimantan, Indonesia.

2. Organic Chemistry Division, Department of Chemistry, Faculty of Mathematics and Natural
Sciences, Institut Teknologi Bandung Jl. Ganesha No. 10, Bandung 70132, West Java,
Indonesia.

3. Research Center for Medicine and ~~Cosmetic-Cosmetics~~ from Tropical Rainforest Resources,
Mulawarman University, Samarinda, Indonesia

4. Department of Chemistry, Faculty of Mathematics and Natural Science, Universitas Jember,
East Java, Indonesia.

*Corresponding author. E-mail: adedanova@itb.ac.id; Tel.: +6281357944698.

Abstract

~~Bacterial infections are a common occurrence, and with~~Due to the ~~increasing~~ resistance of
bacteria to antibiotics, ~~utilization there has been increasing interest in the use~~ of plant extracts
~~to fight infectionsis gaining prominence. Plants in the genus Macaranga plants are known to~~
contain secondary metabolites such as flavonoids ~~that, which exhibit have~~ antibacterial
properties. *Macaranga hulletti* King ex Hook.f. is particularly noteworthy ~~because of~~due to its
widespread ~~presence distribution~~ in East Kalimantan. Therefore, the objective of this study was
to investigate the antibacterial potential of *M. ~~acaranga~~ hulletti* leaf extracts through *in vitro*

Formatted: Font: Italic

and molecular docking studies. The research involved testing the methanol extract, ~~the~~ n-hexane fraction, and ~~the~~ ethyl acetate fraction from ~~*Macaranga hullettii* King ex Hook.f.~~ leaves against three bacterial ~~strains~~species, *Staphylococcus aureus*, *Streptococcus mutans*, and *Propionibacterium acnes*. The results ~~revealed~~showed that both the methanol extract and ethyl acetate fraction displayed antibacterial activity against ~~the~~ three bacteria ~~1-strains~~ with a minimum inhibitory concentrations (MICs) ~~of~~ < 0.15%, except for the methanol extract ~~with~~, which had an MIC of 0.15-0.31% against *Propionibacterium acnes*. ~~Additionally~~In addition, molecular docking analysis showed that five flavonoids possessing prenyl or geranyl groups, ~~namely~~ (6-isoprenylerioidictyol, nymphaeol A, nymphaeol B, and solophenol D), showed the highest binding affinity and dominant hydrogen bonding interactions with key amino acid residues in the binding sites of ~~the~~ three bacterial proteins (IJJJ, 3IPK, and 7LBU). ~~Thus,~~ these findings suggest that the methanol extract and ethyl acetate fraction from *Macaranga hullettii* ~~King ex Hook.f.~~ has have potential applications as ~~a~~ new sources of antibacterial agents, ~~and~~. ~~Thus,~~ further studies are needed to isolate and evaluate the bioactive compounds ~~and evaluate them by in vitro and in vivo studies.~~

Commented [DET1]: Note that one citation of the complete scientific name, including the describing author, is sufficient.

Formatted: Not Highlight

Keywords: Antibacterial, Extract, Flavonoid, *Macaranga hullettii*, Molecular Docking.

Introduction

Despite substantial advancements in medicine and fundamental research ~~over recent decades~~, infectious diseases ~~because of~~caused by transmissible agents, such as bacteria, ~~eukaryotes~~protists, and viruses, continue to present significant challenges to ~~scientists and~~ healthcare professionals. These challenges ~~are further intensified by the limitations in the rapid and accurate diagnosis of infections and the escalating~~include the issue of antimicrobial resistance.¹ Extracts from medicinal plants, ~~which are abundant in diverse phytochemicals with~~

~~a range of pharmacological properties,~~ present a promising approach for addressing multidrug-resistant bacteria. ~~Additionally, it~~In addition, medicinal plants ~~may contribute to~~can help ~~restoring—restore~~ the efficacy of antibiotics to treat infectious diseases.² Therefore, it is imperative to ~~continue the pursuit of~~develop more efficacious antimicrobial agents, particularly those sourced from natural sources, ~~which as these~~ are both readily available and economically viable.

~~Plants in the genus~~ *Macaranga* belongs to the ~~family~~ Euphorbiaceae ~~family and is are~~ commonly known as "Mahang." ~~Based on the literature, the~~The secondary metabolites contained in ~~plants of~~ this genus ~~are unique~~include flavonoids and stilbenoids with prenylated substituents.³⁻¹³ Nine flavonoids containing either prenyl or geranyl groups ~~with and displayng~~ antioxidant, antiplasmodial, and anticancer activities ~~were have been successfully~~isolated from three ~~species of~~ *Macaranga* ~~species~~ present in East Kalimantan (*Macaranga hosei*, *Macaranga pearsonii*, and *Macaranga tanarius*): 4'-O-methyl-8-isoprenylnaringenin, lonchocarpol A, 4'-O-methyl-8-isoprenyleryiodictyol, 6-isoprenyleryiodictyol, solophenol D, nymphaeol A, nymphaeol B, ~~and~~ nymphaeol C, including macahuillettin A that was isolated from *Macaranga hullettii* obtained from Central Kalimantan, ~~which have antioxidant, antiplasmodial, and anticancer activities.~~¹⁴⁻¹⁸ *Macaranga tanarius* leaf extract exhibited antibacterial activity.¹⁹ ~~Moreover, the~~The plant extracts of *Macaranga conglomerata*, *Macaranga kilimandscharica*, and *Macaranga capensis* from Kenya showed antibacterial activities against *E. coli*, *E. aerogenes*, *K. pneumoniae*, *P. stuartii*, *P. aeruginosa*, and *S. aureus*.²⁰ The flavonol rhamnetin, isolated from *Macaranga peltata*, also showed antibacterial activity against *Staphylococcus aureus*.²¹ Two flavanone groups ~~obtained from~~ *Macaranga hosei*, 5-hydroxy-7,4'-dimethoxyflavone and 5-hydroxy-6,7,4'-trimethoxyflavone, ~~obtained from~~ *Macaranga hosei*, showed weak antibacterial activity against three pathogenic bacteria.²² In this study, the leaves of *Macaranga hullettii* King ex Hook.f. ~~were~~was used ~~as the object of analysis~~to identify new

Formatted: Font: Italic

Formatted: Font: Italic

sources of medicinal compounds to treat infectious diseases triggered caused by bacteria. This plant is known to be a plant that can be found in all areas of East Kalimantan, where this the species is commonly found occurs in mountain forests and logging forests areas in the Kalimantan region.^{23, 24} Phytochemical screening and isolated isolation of secondary compounds from the leaf extract of *Macaranga hulletti* King ex Hook.f. leaf extract contained identified several flavonoid compounds.^{18,25} Extensive research on the antibacterial activity of the *Macaranga* has been performed. Nonetheless, the The methanol extract and its fractions were derived from *Macaranga hulletti* King ex Hook.f. species are have not yet to be been assessed analyzed for their antibacterial effects. Therefore, this study was aimed to assess the antibacterial activity of *Macaranga hulettii* King ex Hook.f. leaf extract and its fractions using in vitro studies. Moreover, all The flavonoids isolated from *Macaranga* species in the East Kalimantan, Indonesia, were evaluated for their antibacterial activity using molecular docking study analysis.

Materials and Methods

Materials

The solvents used (methanol, n-hexane, and ethyl acetate) were was purified by distillation before use (methanol, n-hexane, and ethyl acetate). The materials used in this study were *Macaranga hullettii* leaves, *Staphylococcus aureus* ATCC 25923 and from Chulalongkorn University, *Streptococcus mutans* ATCC 25175 obtained from Chulalongkorn University, *Propionibacterium acnes* KCCM 41747 obtained from Mulawarman University, nutrient agar (NA) from Merck, Luria Bertani liquid medium (1% tryptone, 0.5% yeast, and 0.5% NaCl), Yeast yeast extract (Oxoid 100% (w/w)), Tryptone tryptone (Oxoid 100% (w/w)), ampicillin 100 mg/ml (Sigma Aldrich), NaCl (Sigma Aldrich), cotton swabs, aluminum foil, gauze, Petri dishes, Ose needles, paper discs, an autoclave (Autoclave TOMY, Model SX-700), an oven

Formatted: Not Highlight

Formatted: Font: Italic, Not Highlight

Formatted: Not Highlight

Formatted: Not Highlight

Formatted: Not Highlight

Formatted: Not Highlight

Formatted: Not Highlight

Formatted: Not Highlight

Formatted: Not Highlight

Formatted: Not Highlight

Formatted: Font: Italic, Not Highlight

Formatted: Not Highlight

Formatted: Not Highlight

Formatted: Not Highlight

Formatted: Not Highlight

Formatted: Not Highlight

Formatted: Not Highlight

Formatted: Not Highlight

Formatted: Not Highlight

Formatted: Not Highlight

(SHARP EO-28 WH), ~~a rotary vacuum evaporator (EYELA Rotary evaporator N-1300E~~
V.S.Series), and ~~a freezer (Sanyo Medicoool). For data recording and analysis, we used a~~ Lenovo
Yoga 7 ~~computer~~ with AMD Ryzen AI 7-8840HS w/ Radeon 780M graphics, 3301 Mhz, 8
Core(s), and ~~RAM~~ 16.0 GB ~~of RAM~~.

Formatted: Not Highlight

Formatted: Not Highlight

Formatted: Not Highlight

Methods

Sample preparation

The research samples used in this study were leaves of *Macaranga hullettii* ~~King ex Hook.f.~~
plants ~~obtained from the Lesan River Forest, Kelay District, Berau Regency, East Kalimantan,~~
~~Indonesia (1°32'2.5919"N~~ collected from the Lesan River Forest, Kelay District, Berau
~~Regency, East Kalimantan, Indonesia (1°32'2.5919" N, 117°3'11.950" E). The Plant-plant~~
samples were ~~acknowledged-identified~~ at the Laboratory of Anatomy and Plant Systematics,
FMIPA Mulawarman University (No. 0174/UN.17.8.5.7.16/HA/XI/2017).

Formatted: Not Highlight

Extraction

Macaranga hullettii ~~King ex Hook.f.~~ leaf powder (1 kg) was obtained by maceration for 24 h
using methanol. ~~The extract was evaporated using a~~ rotary vacuum evaporator ~~was then used~~
to ~~get-obtain~~ a methanol extract. The methanol extract was ~~successively~~ fractionated
~~successively~~ using n-hexane and ethyl acetate. Each fraction was evaporated using a rotary
vacuum evaporator ~~to obtain n-hexane and ethyl acetate fractions. Furthermore, The~~ methanol
extract, n-hexane fraction, and ethyl acetate fraction were ~~prepared to-evaluated their-for~~
antibacterial activity.

Formatted: Not Highlight

Commented [DET2]: The phrase is redundant and can be omitted.

Pathogens

Staphylococcus aureus ATCC 25923 was ~~required by~~obtained from the Department of Microbiology, Faculty of Science, Chulalongkorn University. *Streptococcus mutans* ATCC 25175 was prepared by the Department of Biochemistry, Faculty of Dentistry, Chulalongkorn University. Both strains were purchased from the American Type Culture Collection (ATCC). *Propionibacterium acnes* KCCM 41747 was obtained from the Faculty of Forestry, Mulawarman University, and ~~was gained~~from the Korean Culture Center of Microorganisms (KCCM). ~~Furthermore, the~~The tested bacteria were periodically subcultured and maintained in nutrient agar (NA) under suitable conditions, ~~depending on the tested bacteria~~.

Formatted: Not Highlight

In vitro antibacterial assay

~~Determination of~~The antibacterial ~~prospects-properties of the in~~-methanol-extract, n-hexane, and ethyl acetate ~~fractions-extracts~~ of *Macaranga hullettii* ~~King ex Hook.f. plant~~ leaves were analyzed using the disc paper or the Kirby-Bauer method ~~at varying concentrations~~.^{26,27} ~~In this study,~~*Staphylococcus aureus* ATCC 25923, *Streptococcus mutans* ATCC 25175, and *Propionibacterium acnes* KCCM 41747 were ~~employed~~assayed. Ampicillin was used as a positive control, ~~and the three: Organic-organic solvents, such as methanol, n-hexane, and ethyl acetate, were~~solvents were used as negative controls. Nutrient Agar (NA) ~~media-medium~~ was prepared by dissolving 3.3 grams in 150 mL of distilled water. Luria Bertani (LB) liquid media ~~as much as was prepared by dissolving~~ 4 grams ~~was dissolved~~ in 500 mL of distilled water, ~~then stirred~~stirring, and ~~put adding~~ 5 mL ~~each into~~to a test tube. ~~Subsequently, The~~ NA and LB ~~media~~ were sterilized using an autoclave at 121 °C with a pressure of 1 atm for 15 minutes. Each tested bacterium was collected using an Ose needle, ~~followed by, after which it was diluted~~ ~~dilution into in~~ a test tube containing sterile LB media and ~~then incubated incubation~~ for 24 h at 37 °C. ~~After the incubation period, the tested bacteria were ready for use in the subsequent stages.~~

Commented [DET3]: The phrase is redundant and can be omitted

Nutrient Agar (25 mL) was poured into a sterilized Petri dishes and. The NA medium was maintained at room temperature for solidification. The A sample of the bacterial suspension in the test tubes was then collected using a sterile cotton swab and. A sterile cotton swab was applied to the entire surface of the agar on a in the Petri dish. Each 30 µL of the tested *M. hulleitii* leaf samples prepared at in various concentrations (in of the organic solvents) was poured onto sterile adsorbent filter paper discs (6 mm in diameter), and the solvent was removed. Afterward, the paper discs containing the tested sample were placed onto the surface of agar media, closed the lid, and incubated at 37 °C on the surface of the agar media and incubated at 37 °C for 18-24 hours. The inhibition zone formed in each well was measured using a ruler.^{28,29} Furthermore, the The Minimum minimum Inhibitory inhibitory Concentration concentration (MIC) was determined for the methanol extract as well as the n-hexane and ethyl acetate fractions. MIC was defined as the minimum concentration that halted this alteration and completely stopped inhibited bacterial growth. bacterial growth.³⁰

Formatted: Font: Italic

Commented [DET4]: Note that the actual terms of acronyms need not be capitalized.

Formatted: Not Highlight

Formatted: Not Highlight

Molecular docking

Eight flavonoid derivatives possessing either prenyl or geranyl group were found occurred in the three *Macaranga* species in East Kalimantan, Indonesia (*Macaranga hosei*, *Macaranga pearsonii*, and *Macaranga tanarius*): 4'-O-methyl-8-isoprenyl naringenin, lonchocarpol A, 4'-O-methyl-8-isoprenyl eriodictyol, 6-isoprenyl eriodictyol, solophenol D, nymphaeol A, nymphaeol B, and nymphaeol C.¹⁴⁻¹⁷ Macahuleitii A was is a prenylated flavonoid found in the *Macaranga huleitii* obtained from Central Kalimantan (Indonesia).¹⁸ Furthermore, a comparative analysis of the molecular docking results of for prenylated/geranylated flavonoids with methoxylated flavonoids was conducted, focusing on 5-hydroxy-7,4'-dimethoxyflavone

Formatted: Not Highlight

Formatted: Not Highlight

Formatted: Font: Italic, Not Highlight

Formatted: Not Highlight

Formatted: Not Highlight

Formatted: Not Highlight

Formatted: Not Highlight

and 5-hydroxy-6,7,4'-trimethoxyflavone ~~that are, which were~~ found in ~~the~~ *Macaranga hosei* from Trengganu, Malaysia,²² ~~as presented in~~ (Table 2).

Formatted: Not Highlight

~~Furthermore, the~~ The molecular structures of the flavonoids ~~isolated from Macaranga species in East Kalimantan, Indonesia,~~ were drawn using ChemOffice Professional 15.0 and obtained

Formatted: Not Highlight

Formatted: Not Highlight

from PubChem (<https://pubchem.ncbi.nlm.nih.gov/>). Structure refinement was performed using the molecular mechanics energy minimization method with the Merck molecular force

field (MMFF94) using Open Babel in PyRx V.1.1. ~~Furthermore,~~ Three proteins were downloaded ~~ed~~ from ~~the~~ protein data bank (PDB ID: 1JIJ, 3IPK, 7LBU),³¹⁻³³ ~~and~~ the locations of

Commented [DET5]: This was a comma splice/run-on sentence. These can be avoided by using a conjunction or a semicolon.

active sites ~~was were~~ calculated using AlphaFold from <https://prankweb.cz/>.³⁴ Molecular

docking was performed using the AutoDock Vina tool in PyRx V.1.1 software with an exhaustiveness of 32 and a mode value of 9 poses for each docked ligand.^{35,36} ~~The~~ A protein

binding site was defined as a box with dimensions of $30 \times 30 \times 30$ Å. In the final step, the binding interactions were analyzed, and the docking results were visualized in 2D using the

BIOVIA Discovery Studio Visualizer.

Statistical analysis

Formatted: Not Highlight

Data from the experiments were ~~investigated analyzed~~ using Microsoft Excel.

Formatted: Not Highlight

Results and Discussion

In vitro antibacterial activity

The strength of antibacterial activity was determined based on the diameter of the clear zone ~~to obtain the inhibition zone (mm), which can be classified as,~~ which was used to measure the

~~inhibition zone (mm). This classification was as follows:~~ ≤ 5 mm (weak), 5-10 mm (moderate),

10-20 mm (strong), and ≥ 20 mm (very strong). Moreover, the ~~smaller lower~~ the minimum inhibitory concentration of a compound, the greater its ability to inhibit the growth of test

bacteria.²⁸ In this study, ampicillin was used as a positive control against three bacteria: *Staphylococcus aureus* ATCC 25923, *Streptococcus mutans* ATCC 25175, and *Propionibacterium acnes* KCCM 41747, as shown in (Table 1). The positive control served to determine whether the test bacteria could be inhibited by ~~growth~~ or were resistant to the antibiotics.

Staphylococcus aureus can cause dentoalveolar infections, jaw cysts, paroritis, oral mucosal lesions, and stomatitis. ~~It~~ The bacteria can also produce exotoxins, lecosidine, enterotoxins,

and quagulase compounds. Enterotoxins are compounds that cause food poisoning in humans.³⁷ ~~Antibacterial activity of the~~ The methanol extract, hexane fraction, and ethyl acetate fraction of *Macaranga hulettii* ~~King ex Hook. f.~~ leaves showed moderate antibacterial activity against *S. aureus*, ~~because of the~~ with an inhibition zone of 5-10 mm at concentrations ~~from of~~ 0.15 ~~to~~ 2.5%, ~~as presented in~~ (Table 1). The inhibition zone ~~produced by of~~ the 0.15% methanol extract ~~was measured~~ 6.67 mm ~~at 0.15%.~~ Meanwhile, ~~the~~ The 0.15% ethyl acetate ~~fraction at~~ 0.15% ~~exhibited fraction produced~~ an inhibition zone ~~at of~~ 6.33 mm, ~~and. However,~~ the 2.5% n-hexane fraction ~~exhibited antibacterial activity at 2.5%, with~~ produced an inhibition zone of 6.50 mm. The results indicated that the methanol extract and ethyl acetate fraction ~~of~~ *Macaranga hulettii* ~~King ex Hook. f. leaves~~ had the most potent antibacterial activity against *S. aureus*, with a minimum inhibitory concentration (MIC) of < 0.15%. Moreover, the hexane fraction of *Macaranga tanarius* ~~is known to prevent the growth of~~ was most effective against *S. aureus*, ~~most effectively in the n-hexane fraction,~~ with ~~an~~ MIC values of 0.125-0.5% and an inhibition zone of approximately 7.25 mm.³⁸

Streptococcus mutans is ~~a prominent bacterial species that is~~ implicated in the pathogenesis of dental caries. This facultative anaerobe primarily ferments carbohydrates, resulting in the production of ~~various-several organic~~ organic acids, with lactic acid as the predominant byproduct. The acidification of the dental environment by *S. mutans* is a crucial factor

Commented [DET6]: The edit was made to avoid a possibly ambiguous antecedent (it may not be clear which noun "It" refers to).

contributing to enamel demineralization and the subsequent formation of carious lesions.^{39,40}

These bacteria produce glucosyltransferase (GTF) to produce glucan, ~~which the compound that~~ causes dental caries.⁴¹ *S. mutans* adheres to the salivary pellicle ~~because of due to the having~~ many receptors for attachment ~~to the salivary pellicle~~.⁴² Furthermore, ~~We tested~~ the antibacterial activity of *Macaranga hulettii* King ~~ex Hook f.~~ leaf extract ~~and fractions was tested~~ against *S. mutans*, ~~as presented in~~ (Table 1). The result revealed that ~~b~~Both extracts ~~and fraction~~ displayed moderate antibacterial activity, ~~because of the with~~ inhibition zones of 5-10 mm at ~~a~~ concentrations of 0.15-2.5%. The ~~widths of the~~ inhibition zones created by the methanol extract and ethyl acetate fraction were 6.67 and 6.33 mm at 0.15%, respectively. Meanwhile, the n-hexane fraction showed antibacterial activity at ~~concentrations of~~ 1.25-2.5%, with ~~an~~ inhibition zones of 6.33-6.67 mm. These results demonstrated that the methanol extract and ethyl acetate fraction of *Macaranga hulettii* ~~King ex Hook.~~ leaves presented the highest potential antibacterial activity against *S. mutans*, with an MIC $< 0.15\%$ (Table 1).

~~Furthermore,~~ *Propionibacterium acnes* is a ~~gram~~Gram-positive anaerobic bacterium that can induce unusual keratinization within the sebaceous glands of hair follicles and increase sebum production.⁴³ The enzyme is capable of hydrolyzing triglycerides (TG) present in sebum into free fatty acids (FFA) ~~that, which~~ may subsequently induce inflammation in and around the hair follicles.⁴⁴ These bacteria also produce hyaluronidase, protease, lecithinase, and ~~neurimidase~~neuraminidase, ~~which that can~~ cause inflammation of the skin.~~;~~ *Macaranga triloba* ethanol ~~extract is known to can~~ prevent the growth of *P. acnes*, ~~at 2.5%~~ and the ~~largest-most~~ significant inhibition value of 5.54 mm at 20%.⁴⁵ As shown in Table 1, the methanol extract and ethyl acetate fraction of *Macaranga hulettii* ~~King ex Hook f.~~ showed ~~strong-potent~~ antibacterial activity against *P. acnes*, ~~because of the with~~ ~~respective~~ inhibition values of 10.67 and 14.67 mm ~~at 2.5%, respectively. Meanwhile, the~~ The n-hexane fraction exhibited moderate antibacterial activity, with inhibition values of 5-10 mm at 1.25-2.5%. ~~Furthermore, the~~ The

Commented [DET7]: I believe the authors mean neuraminidase.

Formatted: Font: Italic

methanol extract showed antibacterial activity with an inhibition value of 7.67 at 0.31%. The ethyl acetate fraction showed antibacterial activity of 0.15%, with an inhibition zone of 6.67 mm. The results ~~revealed~~ demonstrated that the ethyl acetate fraction of *Macaranga hulettii* ~~King ex Hook.f~~ leaves presented the highest potential antibacterial activity against *P. acnes*, with a minimum inhibitory concentration (MIC) of < 0.15%, ~~as presented in~~ (Table 1).

~~Based on the data of antibacterial activity, both extracts and their fractions from Macaranga hulettii, the~~ The methanol extract and ethyl acetate fraction exhibited good antibacterial activity against the three bacterial strains. This finding was consistent with previous studies ~~indicating that the methanol extract and ethyl acetate fraction demonstrated antibacterial activity, that attributable~~ attributed the antibacterial effects to the presence of flavonoids and their prenylated or geranylated forms in ~~species of Macaranga~~ species, such ~~These included as~~

euchrestaflavanone A, bonanione A, macarangaflavanone A, macarangaflavanone B, macatrichocarpin A, propolin D (nymphaeol B), senegalensin, schweinfurthin B, schweinfurthin O, and isomacarangain.⁴⁶⁻⁵⁶ Flavonoid compounds can damage bacterial cell walls and denature proteases, ~~that thereby~~ disrupting bacterial ~~metabolic~~ metabolism ~~processes~~.⁵⁷ In addition, alkaloids can damage the peptidoglycan layer ~~in of~~ bacterial cells, ~~causing damage damaging to the bacterial~~ cell wall and inhibiting bacterial growth. Terpenoids also inhibit bacterial growth by damaging ~~bacterial~~ cell membranes. ~~Moreover, the~~ The phenolic compounds of secondary metabolites can damage bacterial cells by increasing the permeability of the ~~cytoplasmic cell~~ membrane, resulting in cytoplasmic coagulation and cell division.²⁸

Molecular docking study

Molecular docking was performed to predict the interactions of the flavonoids with the amino acid residues of the three proteins. This study used three proteins from *Staphylococcus aureus*,

Formatted: Font: Italic

Streptococcus mutans, and *Propionibacterium acnes* that were retrieved from the Protein Data Bank with PDB ID: 1JII, 3IPK, and 7LBU, respectively.³¹⁻³³ As illustrated in Table 2, the The molecular docking results (Table 2) revealed that 11 flavonoid derivatives revealed had binding affinities ranging from -7.2 to -10.5 kcal/mol, -7.5 to -8.9 kcal/mol, and -7.1 to -8.9 kcal/mol against *Staphylococcus aureus* tyrosyl-tRNA synthetase (PDB ID: 1JII), *Streptococcus mutans* antigen I/II (AgI/II) (PDB ID: 3IPK), and *Propionibacterium acnes* surface sialidase (PDB ID: 7LBU), respectively. Furthermore, the The interactions of flavonoids and ampicillin with the protein targets is depicted are shown in two-dimensional graphs, as presented in Figures 1-3.

Among the compounds against-targeting *S. aureus* tyrosyl-tRNA synthetase, nymphaeol A-B and solophenol D had the highest binding affinity-affinities compared to ampicillin as a positive control, as shown in Table 2. Thus, the interactions of these compounds with the active site of the enzyme was conducted-modeled in as a 2D structure (Figure 1). Furthermore, a Ampicillin showed-formed hydrogen bonding-bonds with LYS84 and GLN174; and amide- π interactions with GLY38, as depicted in (Figure 1). Nymphaeol A and nymphaeol B exhibited-formed hydrogen bonding-bonds with four amino acid residues, π -anions, and hydrophobic interactions, whereas nymphaeol B and ampicillin showed similar interactions with LYS84. In addition, solophenol D displayed-formed hydrogen bonding-bonds with TYR36, THR75, GLN190, ASP177, and GLN174, as well as hydrophobic interactions with HIS50, LEU70, PRO53, and ALA39 (Figure 1). This-These results was in line-agreed with previous studystudies, where nymphaeol A-B and solophenol D exhibited antibacterial activity.^{58,59} As presented in Table 2, fiveFive compounds exhibited high antibacterial activity against *S. mutans* antigen I/II (AgI/II), including 6-isoprenyleriodictyol, nymphaeol A-B, and solophenol D, with binding affinity-affinities from -8.7 to -8.9 kcal/mol. Furthermore, the The interactions between the ligands and amino acid residues in the binding sites of these compounds is-are

Commented [DET8]: This information has been given and is not relevant to a Discussion.

Commented [DET9]: The edit was made to avoid repetitive writing (repetition of 'revealed').

Formatted: Not Highlight

shown in a 2D structure views (Figure 2). As shown in Figure 2, ampicillin formed hydrogen bonding bonds with four amino acid residues, ASN590, SER591, SER588, and ASP760, as well as π -sulfur and π - π interactions with TRP 816, while, Furthermore, 6-isoprenyleriodictyol showed only formed hydrogen bonding bonds with three amino acid residues: SER697, ASN699, and ASN814. Meanwhile, nymphaeol A and nymphaeol B displayed-formed hydrogen bonding bonds with four amino acid residues (Figure 2), but nymphaeol A exhibited a similar π - π interaction with TRP816, as observed with ampicillin. Solophenol D formed hydrogen bonds with SER818, ARG824, and THR586, similar to the π - π interactions (TRP816) with ampicillin, and π -sigma interactions with SER762. Furthermore, As 6-isoprenyleriodictyol is a prenylated flavonoid similar to nymphaeol A, and hence, Thus, 6-isoprenyleriodictyol may have antibacterial potenevactivity.

Moreover, threeThree compounds, including 6-isoprenyleriodictyol and nymphaeol A, displayed stronger binding affinities than ampicillin, with values of -8.0 and -8.4 kcal/mol against *P. acnes* surface sialidases, such as 6-isoprenyleriodictyol and nymphaeol A, respectively, as described in Table 2. Therefore, both compounds were imaged in 2D to investigate the interactions between the ligands and the amino acid residues in the binding sites (Figure 3). As depicted in Figure 3, ampicillin showed-formed hydrogen bonding bonds with four amino acid residues, ARG282, GLN287, ASP146, ASP185, and π - π and π -alkyl interactions with PHE257, ALA147, and VAL202. 6-Isoprenyleriodictyol demonstrated π - π interactions with PHE257, π -anion interactions with ASP146, and alkyl-alkyl interactions with ALA147, PHE209, VAL202, and LEU224. Furthermore, thisThis compound displayed hydrogen-formed bonding bonds with six amino acid residues, including ASP146, ARG329, TYR423, ARG282, and GLU287, that were, which were analogous to those observed with ampicillin. Nymphaeol A showed-formed hydrogen bonding bonds with five amino acid residues (ARG329, TYR423, ASP185, ALA147, and SER184), π -anion interactions with

Formatted: Not Highlight

Commented [DET10]: The phrase is redundant since the figure has already been cited.

Formatted: Not Highlight

ASP312 and ASP185, alkyl-alkyl interactions with MET310, ALA331, and ALA147, and Van der Waals interactions with PHE257. Thus, these compounds may have potent ~~as antibacterial~~antibacterial activity.

Structure-activity relationships were also studied based on the binding affinity results,~~as presented in~~ (Table 2). Flavonoids possessing prenyl, geranyl, and hydroxyl groups showed ~~good-potency~~potential as antibacterial agents ~~owing due~~ to their strong ~~binding~~affinity ~~with amino acid residues in the binding site of proteins~~for amino acid residues in the binding sites of proteins, compared ~~with-to~~ ampicillin. ~~Nevertheless~~However, the absence of a hydroxyl group on prenylated or geranylated flavonoids decreased ~~the~~binding affinity.^{22,56,60}

Conclusion

The extracts and fractions of *Macaranga hulettii* ~~King ex Hook.f.~~ have potential as antibacterial agents against three bacterial strains, *Staphylococcus aureus* ATCC 25923, *Streptococcus mutans* ATCC 25175, and *Propionibacterium acnes* KCCM 41747. The methanol extract demonstrated antibacterial activity against *S. aureus* and *S. mutans* with ~~an~~MIC (%) of ~~<0.15~~ against *S. aureus* and *S. mutans*, and 0.15-0.31% against *P. acnes*. ~~The n-Hexane-hexane~~ fraction ~~showed antibacterial activity with~~had an MIC (%) of 1.25-2.5% against *S. aureus*, and 0.62-1.25% against *S. mutans* and *P. acnes*. ~~Moreover, the~~The ethyl acetate fraction ~~displayed antibacterial activity with~~had an MIC (%) ~~<0.15%~~ against ~~the~~three bacterial strains (~~*S. aureus*, *S. mutans*, and *P. acnes*~~). Molecular docking studies ~~have~~suggested that 6-isoprenylerydiol, nymphaeol A, ~~nymphaeol~~nymphaeol B, and solophenol D are promising antibacterial agents. ~~as because~~those compounds exhibited the highest binding affinity and formed hydrogen bonding ~~bonds~~interactions with key amino acid residues in the active sites of the protein

Commented [DET11]: The re-listing of the bacteria is redundant.

Commented [DET12]: I believe the authors mean nymphaeol.

targets. ~~Therefore, the development of this plant extract is required to further~~ Further *in vitro*,
~~and *in vivo* study studies should be conducted to isolate the active~~ the isolation of secondary
metabolites ~~*in vitro* and *in vivo* from this species.~~

Formatted: Font: Italic, Not Highlight

Formatted: Not Highlight

Formatted: Font: Italic, Not Highlight

Formatted: Not Highlight

Formatted: Font: Not Italic, Not Highlight

Formatted: Not Highlight

Conflict of Interest

The authors declare that they have no conflicts of interest.

Author's Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims related to the content of this article will be borne by them.

Acknowledgement

This research was funded by the Islamic Development Bank (PIU-IsDB) project under contract no. 137/UN17.11/PL/2019.

References

1. Wong F, de la Fuente-Nunez C, Collins JJ. Leveraging artificial intelligence in the fight against infectious diseases. *Science*. 2023; 381(6654):164-70.
2. Seukep AJ, Kuete V, Nahar L, Sarker SD, Guo M. Plant-derived secondary metabolites as the main source of efflux pump inhibitors and methods for identification. *J. Pharm. Anal.* 2020; 10(4):277-90.
3. Magadula JJ. Phytochemistry and pharmacology of the genus *Macaranga*: a review. *J. Med. Plants Res.* 2014; 8(12):489-503.

4. Vu LT, Ngan TB, Phuong L, Huong DT, Litaudon M, Van Hung N, Thach TD, Van Cuong P. Chemical constituents from fruits of *Macaranga denticulata* (Euphorbiaceae)(Part 2). Vietnam J. Chem. 2018; 56(4):516-20.
5. Marliana E, Hairani R, Tjahjandarie TS, Tanjung M. Antiplasmodial activity of flavonoids from *Macaranga tanarius* leaves. IOP Conf. Ser.: Earth Environ. Sci. 2018; 144(1):012011).
6. Tjahjandarie TS, Tanjung M, Saputri RD, Nadar PB, Aldin MF, Marliana E, Permadi A. Flavestins K, An isoprenylated stilbene from the leaves of *Macaranga recurvata* Gage. Nat. Prod. Sci. 2019; 25(3):244-7.
7. Huong DT, Linh NT, Van TT, Litaudon M, Roussi F, Van Nam V, Van Cuong P. Stilbenes from *Macaranga tanarius* (Euphorbiaceae) growing in Vietnam. Vietnam J. Chem. 2020; 58(3):338-42.
8. Aldin MF, Tjahjandarie TS, Saputri RD, Tanjung M. Macasiamenene V, a New Stilbenoid from the Leaves of *Macaranga inermis*. Nat. Prod. Sci. 2021; 27(1):45-8.
9. Muharram A, Rachmawati DA, Mardhiyyah S, Tjahjandarie TS, Saputri RD, Ahmat N, Tanjung M. Cytotoxic and antioxidant activities of flavonoids and diterpenoids from *Macaranga involucrata* (Roxb.) Baill. J. Appl. Pharm. Sci. 2023; 13(6):087-92.
10. Tanjung M, Tjahjandarie TS, Aldin MF, Mardhiyyah S, Ahmat N, Saputri RD. Macagigantin A, A New Flavonoid from *Macaranga gigantea* (Rchb. f & Zoll.) Mull. Arg. Nat. Prod. Sci. 2023; 29(4):287-94.
11. Tjahjandarie TS, Aldin MF, Saputri RD, Tanjung M. Dihydrostilbenes from *Macaranga javanica* (Blume) Müll. Arg. and their antiplasmodial activity. Nat. Prod. Sci. 2024:1-7.
12. Tanjung M, Tjahjandarie TS, Aldin MF, Mardhiyyah S, Saputri RD, Syah YM, Ahmat N. Two new flavonols from *Macaranga inermis* pax & K. Hoffm. Nat. Prod. Sci. 2025; 39(3):498-505.

13. Tanjung M, Tjahjandarie TS, Aldin MF, Mardhiyyah S, Saputri RD, Syah YM, Ahmat N. Two new flavonols from *Macaranga inermis* pax & K. Hoffm. Nat. Prod. Sci. 2025; 39(3):498-505.
14. Marlina E, Tjahjandarie TS, Tanjung M. Isoprenylated flavanone derivatives from *Macaranga hosei* King ex Hook F. Der Pharm. Lett. 2015; 7(3):153-6.
15. Marlina E, Tjahjandarie TS, Tanjung M. Aktivitas Antioksidan Senyawa Flavonoid Dari *Macaranga pearsonii* Merr. J. Kim. Mulawarman. 2016; 13(2):97-100.
16. Marlina E, Astuti W, Kosala K, Hairani R, Tjahjandarie TS, Tanjung M. Chemical composition and anticancer activity of *Macaranga hosei* leaves. Asian J. Chem. 2018; 30(4):795-8.
17. Marlina E, Ruga R, Hairani R, Tjahjandarie TS, Tanjung M. Antioxidant activity of flavonoid constituents from the leaves of *Macaranga tanarius*. IOP Conf. Ser.: J. Phys. 2019; 1277(1):012014.
18. Saputri RD, Tukiran T, Wati FA, Purnamasari AP, Wardhana MW, Tjahjandarie TS, Tanjung M. Macahuletiiin A, a new isoprenylated flavanone from the leaves of *Macaranga hullettii* King ex Hook and their antiplasmodial activity. Vietnam J. Chem. 2024; 62(3):394-8.
19. Musdalifah M, Khumaidi A, Suwastika IN. Uji Daya Hambat dan Skrining Fitokimia Ekstrak Daun *Macaranga tanarius* (L.) Mull. Arg Sebagai Antibakteri Salmonella typhi. Nat. Sci: J. Sci. Technol. 2017; 6(3).
20. Ibrahim H, Omosa L, Nchiozem-Ngnitedem V, Onyari J, Maru S, Guefack M. Antibacterial activities and phytochemical screening of crude extracts from Kenyan macaranga species toward MDR phenotypes expressing efflux pumps. Pharmacogn. Commn. 2021; 11(2):119-26.

21. Bijesh K, Sebastian D. Isolation and characterization of antibacterial compounds from *Macaranga peltata* against clinical isolates of *Staphylococcus aureus*. Int. J. Biol. Pharma Res. 2013; 4:1196-203.
22. Salleh WM, Razak NZ, Ahmad F. Phytochemicals and biological activities of *Macaranga hosei* and *Macaranga constricta* (Euphorbiaceae). Marmara Pharm. J. 2017;21(4):881-8.
23. Slik JW, Priyono P, Welzen PV. Key to the *Macaranga Thou.* and *Mallotus Lour.* species (Euphorbiaceae) of East Kalimantan, Indonesia. Singapore, Gardens' Bulletin (Singapore), National Parks Board, Singapore Botanic Gardens; 2000. 11-87 p.
24. Amirta R, Angi EM, Ramadhan R, Kusuma IW, Wiati CB, Haqiqi MT. Potensi pemanfaatan macaranga. Samarinda: Mulawarman University Press; 2017.
25. Rismawati R, Marlina E, Daniel D. Phytochemical Test on Methanol Extract of Leaf of *Macaranga hullettii* King ex Hook. f. J. Atomik. 2018; 3(2):91-4.
26. Hudzicki J. Kirby-Bauer disk diffusion susceptibility test protocol. American society for microbiology. 2009; 15(1):1-23.
27. Inna M, Astuti W, Saleh C. Aktivitas antibakteri ekstrak metanol daun tanaman sambung urat (*Cayratia carnosa*) terhadap *Salmonella thypi* and *Propionibacterium acnes*. J. Atomik. 2022; 7(1):1-5.
28. Yulianti MF, Amat AL, Hutasoit RM, Pakan PD. Antibacterial activity of jambalang leaf ethanol extract (*Syzygium cumini*) against the growth of *Propionibacterium acnes*. Acta Biochimica Indones. 2023; 6(2):1161.
29. Hossain MR, Biplob AI, Sharif SR, Bhuiya AM, Sayem AS. Antibacterial Activity of Green Synthesized Silver Nanoparticles of *Lablab purpureus* Flowers Extract against Human Pathogenic Bacteria. Trop. J. Nat. Prod. Res. 2023;7(8).

30. Yanda L, Tatsimo SJ, Tamokou JD, Matsute-Takongmo G, Meffo-Dongmo SC, Meli Lannang A, Sewald N. Antibacterial and antioxidant activities of isolated compounds from *Prosopis africana* leaves. *Int. J. Anal. Chem.* 2022; 4205823.
31. Qiu X, Janson CA, Smith WW, Green SM, McDevitt P, Johanson K, Carter P, Hibbs M, Lewis C, Chalker A, Fosberry A. Crystal structure of *Staphylococcus aureus* tyrosyl-tRNA synthetase in complex with a class of potent and specific inhibitors. *Protein Sci.* 2001; 10(10):2008-16.
32. Larson MR, Rajashankar KR, Patel MH, Robinette RA, Crowley PJ, Michalek S, Brady LJ, Deivanayagam C. Elongated fibrillar structure of a *streptococcal adhesin* assembled by the high-affinity association of α - and PPII-helices. *Proc. Natl. Acad. Sci.* 2010; 107(13):5983-8.
33. Yu AC, Volkers G, Jongkees SA, Worrall LJ, Withers SG, Strynadka NC. Crystal structure of the *Propionibacterium acnes* surface sialidase, a drug target for *P. acnes*-associated diseases. *Glycobiology.* 2022; 32(2):162-70.
34. Jakubec D, Skoda P, Krivak R, Novotny M, Hoksza D. PrankWeb 3: accelerated ligand-binding site predictions for experimental and modelled protein structures. *Nucleic Acids Res.* 2022; 50:W593-7.
35. Dallakyan S, Olson AJ. Small-molecule library screening by docking with PyRx. In *Chemical biology: methods and protocols.* 2014: 243-250. New York, NY: Springer New York.
36. Trott O, Olson AJ. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *J. Comput. Chem.* 2010; 31(2):455-61.
37. Malangu N, editor. *Poisoning: From Specific Toxic Agents to Novel Rapid and Simplified Techniques for Analysis.* BoD–Books on Demand; 2017.

38. Sari AA, Saleh C. Uji fitokimia, toksisitas dan aktivitas antibakteri ekstrak berbagai fraksi daun mara (*Macaranga tanarius* (L.) MA) terhadap bakteri *Staphylococcus aureus* dan *Escherichia coli*. J. Kim. Mulawarman. 2015; 12(2):53-8.
39. Imelda R., Mariam M., Satari M. Effect of cassava (*manihot esculenta* cranzt), rice (*oryza sativa* L.), and potato (*solanum tuberosum*) water extract to decrease pH phase fermentation of *streptococcus mutans* atcc 25175. Padjajaran J. Dent. 2019; 31(1):14.
40. Alkhaled A., Alsabek L., Al-assaf M. , & Badr F.. Effect of chlorhexidine, honey and propolis on *streptococcus mutans* counts: *in vitro* study. Dentistry. 2021; 9(1).
41. Ren Z, Chen L, Li J, Li Y. Inhibition of *Streptococcus mutans* polysaccharide synthesis by molecules targeting glycosyltransferase activity. J. Oral Microbiol. 2016; 8(1):31095.
42. Lee BS, Chen YJ, Wei TC, Ma TL, Chang CC. Comparison of antibacterial adhesion when salivary pellicle is coated on both poly (2-hydroxyethyl-methacrylate)-and polyethylene-glycol-methacrylate-grafted poly (methyl methacrylate). Int. J. Mol. Sci. 2018; 19(9):2764.
43. Chen KC, Yang CH, Li TT, Zouboulis CC, Huang YC. Suppression of Propionibacterium acnes-stimulated proinflammatory cytokines by Chinese bayberry extracts and its active constituent myricetin in human sebocytes *in vitro*. Phytother. Res. 2019; 33(4):1104-13.
44. Xu J, Chen X, Song J, Wang C, Xu W, Tan H, Suo H. Antibacterial activity and mechanism of cell-free supernatants of *Lactocaseibacillus paracasei* against *Propionibacterium acnes*. Microb. Pathog. 2024; 189:106598
45. Warnida H, Mustika D, Supomo S, Sukawaty Y. Efektivitas Ekstrak Etanol Daun Mahang (*Macaranga Triloba*) sebagai Obat Anti-acne. J. Penelit. Sos. Ekon. Kehuta. 2018; 4(1):9-18.
46. Schütz BA, Wright AD, Rali T, Sticher O. Prenylated flavanones from leaves of *Macaranga pleiostemona*. Phytochemistry. 1995; 40(4):1273-7.

47. Lim TY, Lim YY, Yule CM. Evaluation of antioxidant, antibacterial and anti-tyrosinase activities of four *Macaranga* species. *Food Chem.* 2009; 114(2):594-9.
48. Fareza MS, Syah YM, Mujahidin D, Juliawaty LD, Kurniasih I. Antibacterial flavanones and dihydrochalcones from *Macaranga trichocarpa*. *Z. Naturforsch C J. Biosci.* 2014; 69(9-10):375-80.
49. Hasanat A, Kabir MS, Hossain MM, Hasan M, Al Masum MA, Chowdhury TA, Bhuiyan DI, Mamur A, Kibria AS. Antibacterial activity of methanol extract of *Macaranga denticulata* leaves and in silico PASS prediction for its six secondary metabolites. *World J. Pharm. Pharm. Sci.* 2015:1258-66.
50. O Akanbi B, Anene P, Olayanju S. Preliminary Screening Indicates Promising Antimicrobial Properties of the Stem Bark Extracts of *Macaranga rosea*. *Anti-Infective Agents.* 2015; 13(2):123-8.
51. Ogundajo A, Okeleye B, Ashafa AO. Chemical constituents, *in vitro* antimicrobial and cytotoxic potentials of the extracts from *Macaranga barteri* Mull-Arg. *Asian Pac. J. Trop. Biomed.* 2017; 7(7):654-9.
52. Putri R, Hendra R, Teruna HY. Anti-Bacterial and Anti-Fungal Activities from *Macaranga bancana* Leaves Extract. *Pharmacol. Clin. Pharm. Res.* 2019; 4(1):1-4.
53. Lee JH, Kim YG, Khadke SK, Yamano A, Woo JT, Lee J. Antimicrobial and antibiofilm activities of prenylated flavanones from *Macaranga tanarius*. *Phytomedicine.* 2019; 63:153033.
54. Pagna JI, Awazi T, Mbarga PE, Mbekou IM, Mkouna P, Fotie J, Frese M, Fabrice FB, Lenta BN, Sewald N, Nkengfack EA. Antibacterial flavonoids from the fruits of *Macaranga hurifolia*. *J. Asian Nat. Prod. Res.* 2022; 24(11):1041-51.

55. Kamso VF, Simo Fotso CC, Kanko Mbekou IM, Toussie BT, Ndjakou Lenta B, Boyom FF, Sewald N, Frese M, Ngadjui BT, Wabo Fotso G. Chemical constituents of *Macaranga occidentalis*, antimicrobial and chemophenetic studies. *Molecules*. 2022; 27(24):8820.
56. Rosamah E, Haqiqi MT, Putri AS, Kuspradini H, Kusuma IW, Amirta R, Yuliansyah Y, Suwinarti W, Paramita S, Ramadhan R, Tarmadi D. The potential of Macaranga plants as skincare cosmetic ingredients: A review. *J. Appl. Pharm. Sci*. 2023; 13(7):001-12.
57. Li AP, He YH, Zhang SY, Shi YP. Antibacterial activity and action mechanism of flavonoids against phytopathogenic bacteria. *Pesticide Biochemistry and Physiology*. 2022; 188:105221.
58. Chen YW, Ye SR, Ting C, Yu YH. Antibacterial activity of propolins from Taiwanese green propolis. *J. Food Drug Anal*. 2018; 26(2):761-8.
59. Inui S, Hosoya T, Shimamura Y, Masuda S, Ogawa T, Kobayashi H, Shirafuji K, Moli RT, Kozono I, Shin-ya K, Kumazawa S. Solophenols B–D and Solomonin: New Prenylated Polyphenols Isolated from Propolis Collected from The Solomon Islands and Their Antibacterial Activity. *J. Agri. Food Chem*. 2012; 60(47):11765-70.
60. Ruga R, Kingkaew K, Tamsampaolot K, Chavasiri W. Enhancing antibacterial activity against *Propionibacterium acnes* and *Staphylococcus aureus* by combination of tetracycline with selected compounds. *Chem. Lett*. 2018; 47(12):1538-41.

Table 1: Antibacterial Activity of *Macaranga hullettii* King ex Hook Leaf Extracts and Fractions against *Staphylococcus aureus*, *Streptococcus mutans*, and *Propionibacterium acnes*

Sample	Concentration (%)	Inhibition Zone (mm)*		
		<i>Staphylococcus</i>	<i>Streptococcus</i>	<i>Propionibacterium</i>
		<i>aureus</i>	<i>mutans</i>	<i>acnes</i>
Methanol Extract	0	0	0	0
	0.15	6.67	6.67	0
	0.31	6.67	6.67	7.67
	0.62	6.67	7	8
	1.25	7.17	7.33	9.67
	2.5	7.5	8	10.67
	MIC (%)	<0.15	<0.15	0.15-0.31
n-Hexane Fraction	0	0	0	0
	0.15	0	0	0
	0.31	0	0	0
	0.62	0	0	0
	1.25	0	6.33	6.33
	2.5	6.5	6.67	6.67
	MIC (%)	1.25-2.5	0.62-1.25	0.62-1.25
Ethyl acetate Fraction	0	0	0	0
	0.15	6.33	6.33	6.67
	0.31	6.5	6.67	8
	0.62	6.5	6.83	9
	1.25	6.67	7	11
	2.5	7	7.67	14.67

	MIC (%)	<0.15	<0.15	<0.15
Ampicillin	0.01	20.33	21	21

*Data were obtained from triplicate experiments.

Table 2: Binding affinity of flavonoids against three proteins (1JIJ, 3IPK, and 7LBU)

No.	Compound	Binding Affinity (kcal/mol)		
		1JIJ	3IPK	7LBU
1	4'-O-methyl-8-isoprenylnaringenin	-7.4	-8.1	-7.7
2	4'-O-methyl-8-isoprenyleriodictyol	-7.2	-8.1	-7.6
3	6-Isoprenyleriodictyol	-7.7	-8.7	-8.0
4	5-hydroxy-7,4'-dimethoxyflavone	-8.6	-7.8	-7.1
5	5-hydroxy-6,7,4'-trimethoxyflavone	-8.3	-7.5	-7.3
6	Macahuiettiin A	-7.6	-8.4	-7.1
7	Lonchocarpol A	-9.4	-7.8	-7.8
8	Nymphaeol A	-9.7	-8.9	-8.4
9	Nymphaeol B	-10.5	-8.9	-7.7
10	Nymphaeol C	-8.4	-8.3	-7.4
11	Solophenol D	-10.0	-8.7	-7.9
12	Ampicillin	-8.5	-7.7	-7.6

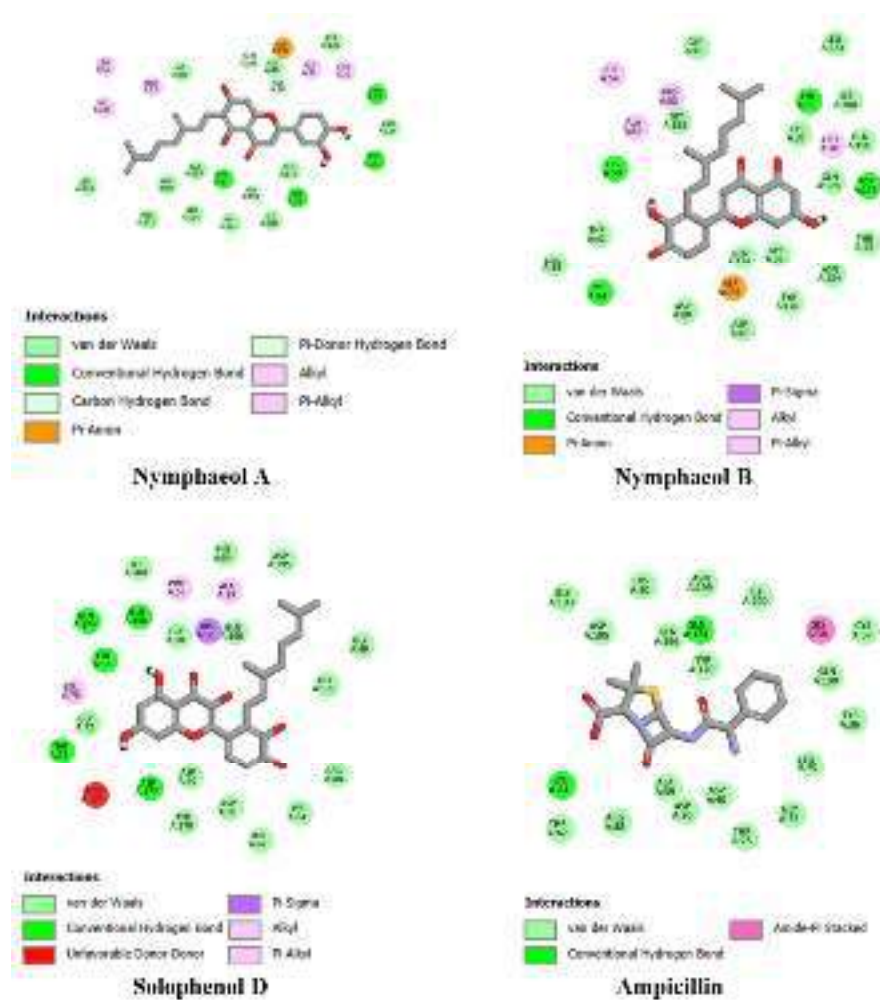


Figure 1: 2D interaction of the best docked flavonoids and ampicillin with *Staphylococcus aureus* tyrosyl-tRNA synthetase (PDB ID: 1JJJ)

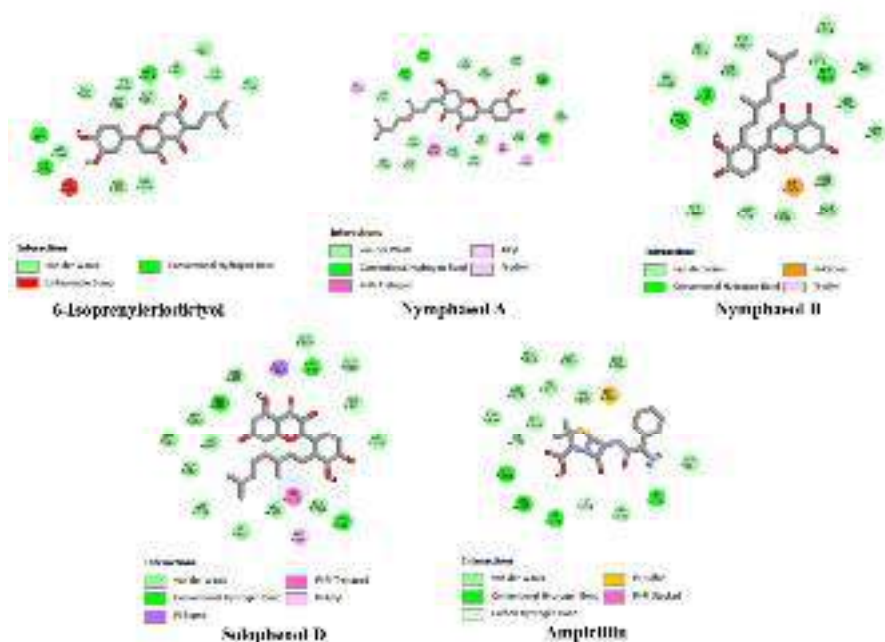


Figure 2: 2D interaction of the best-docked flavonoids and ampicillin with *Streptococcus mutans* antigen I/II (PDB ID: 3IPK).

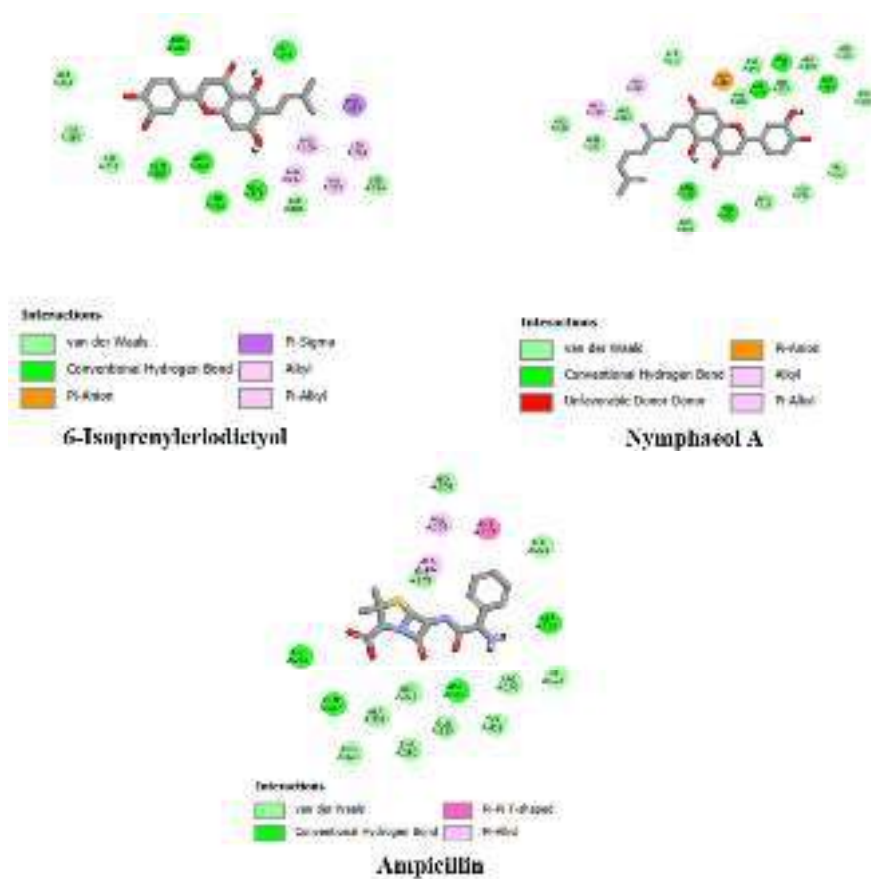


Figure 3: 2D interaction of the best-docked flavonoids and ampicillin with *Propionibacterium acnes* surface sialidase (PDB ID: 7LBU).

Order No 245-81-66

09/05/2025

Editor's Report

Thank you for the opportunity to edit your manuscript. I found the study extremely interesting, as it concerned new research on natural products and their application as antibacterial agents. As the editor for English, I focused on improving the flow of language to give the manuscript an academic tone. Please read carefully through the edits and comments, as these provide further details, before accepting or rejecting any changes. In addition, please review the notes below for brief explanations of several changes.

I wish you the best of luck with your endeavour, and I look forward to working with you again in the future.

Sincerely,

Dr. DE Taneyhill, PhD

Overall Assessment: The manuscript presents an analysis of the antibacterial effects of leaf extracts of the medicinal plant *Macaranga hullettii*. Standard assays indicated that the extracts were effective against common bacteria, including *Staphylococcus aureus* and *Streptococcus mutans*. The authors augmented their study by performing molecular docking analyses using the secondary compounds contained in *M. hullettii* against proteins from the tested bacterial species. The research presented in the manuscript appears sound and should provide a foundation for further studies designed to identify the compounds responsible for the antibacterial effects. The main weakness of the manuscript was the frequent redundancy in the exposition; with the editing for more concise writing, the manuscript should soon be ready for submission.

Comments on The Manuscript

Title: I have suggested a slightly altered title: “*In vitro* Evaluation and Molecular Docking Study of New Antibacterial Compounds from *Macaranga hulletti* King ex Hook.f. Leaf Extract”. The original title may have been a bit confusing as to what was being evaluated.

Abstract: The Abstract did a good job of summarizing the aims and results of the study. This section required editing to eliminate repetition, including the listing of the complete scientific name of the study species, *Macaranga hullettii*. The normal pattern for scientific names is to list the full name (including the describing author) once, then use just the genus and species or the form with the genus abbreviated, e.g., *M. hullettii*. Note that this issue occurred throughout the manuscript.

Introduction: The editing in this section focused on using concise language to avoid overly wordy writing. Thus, for example, a sentence such as “These challenges are further intensified by the limitations in the rapid and accurate diagnosis of infections and the escalating issue of antimicrobial resistance” was edited to the more concise “These challenges include the issue of antimicrobial resistance.” The Introduction also tended to overuse words such as “Moreover” and “Furthermore” to introduce sentences; these should be used sparingly, and only when justified.

Materials and Methods: This section required relatively less editing than other sections. The listing of every reagent and piece of equipment used in the experiments was a bit unusual; normally, equipment and the manufacturer are listed when the procedures are described. The authors might consider using this more standard way of listing materials. The exposition in the molecular docking section was generally clear and required minimal editing.

Results and Discussion: Much of the editing in this section was done to eliminate redundancy (i.e., use of unnecessary words) and repetition. As mentioned above, the complete scientific name of the species (including the describing author) does not need to be given after the first usage. This also applies to the bacterial species tested and the names of the proteins (the PDB indentifications do not need to be repeated at each citation). Editing was performed to avoid overly wordy writing as well; for example phrases such as “displayed hydrogen bonding” were edited to “formed hydrogen bonds”. I thought that this section could be improved by being shortened, as there was a lot of repetition as a result of listing all numerical results in the text, but also referring to those results in a Table. Normally, editors and reviewers prefer to see data listed either in Tables or Figures or in the text, but not in both places; the text should ideally summarize the main findings while leaving the data values for a Table or Figure.

EDITING CERTIFICATE

This document certifies that the manuscript listed below was edited by Cambridge Proofreading LLC for English grammar, punctuation, spelling, and style. We endeavoured to ensure that the authors' intended meaning was not altered during the review. All amendments were tracked with Microsoft Word's 'Track Changes' feature, allowing the authors full control over the changes made. We bear no responsibility for revisions made to the document after our edit on the date listed below.

Document title:

In vitro and Molecular Docking Evaluation of New
Antibacterial Activity of *Macaranga hullei* King ex Hook.f.
Leaf Extract

Document authors:

Eva Marliana

Date edited: 05/09/2025

Order number: 245-81-66

For queries or verification contact info@cambridgeproofreading.com

Cambridge Proofreading LLC is a registered company headquartered in Chicago, Illinois, with a global presence. Our Certificate of Good Standing can be found in the Illinois state business database.



***In vitro* Evaluation and Molecular Docking Study of the Antibacterial Potential of
Macaranga hulletti King ex Hook.f. Leaf Extract**

Eva Marlina^{1,3}, Ade Danova^{2*}, Rita Hairani^{1,3}, Ritbey Ruga^{1,3}, Elvira Hermawati², Ana Yulvia⁴

¹*Department of Chemistry, Faculty of Mathematics and Natural Sciences, Mulawarman University, Samarinda 75123, East Kalimantan, Indonesia*

²*Organic Chemistry Division, Department of Chemistry, Faculty of Mathematics and Natural Sciences, Institut Teknologi Bandung Jl. Ganesha No. 10, Bandung 70132, West Java, Indonesia*

³*Research Center for Medicine and Cosmetics from Tropical Rainforest Resources, Mulawarman University, Samarinda, Indonesia*

⁴*Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Jember, East Java, Indonesia*

***Corresponding author. E-mail: adedanova@itb.ac.id**

Tel.: +6281357944698.

Abstract

Due to the resistance of bacteria to antibiotics, there has been increasing interest in the use of plant extracts to combat infections. Plants in the genus *Macaranga* contain secondary metabolites such as flavonoids with antibacterial properties. *Macaranga hullettii* King ex Hook.f. are particularly noteworthy due to their widespread distribution in East Kalimantan. This study aimed to investigate the antibacterial potential of *M. hullettii* leaf extract through *in vitro* and molecular docking studies. The antibacterial activity of the methanol extract, n-hexane, and ethyl acetate fractions from *M. hullettii* leaves was evaluated against three bacterial species, *Staphylococcus aureus*, *Streptococcus mutans*, and *Propionibacterium acnes* using the disc diffusion assay. Molecular docking of eleven flavonoid derivatives presents in three *Macaranga* species was performed against selected bacterial proteins (PDB ID: IJIJ, 3IPK, and 7LBU). The results showed that both the methanol extract and ethyl acetate fraction displayed antibacterial activity against the three bacteria strains, with minimum inhibitory concentrations (MICs) < 0.15%, except for the methanol extract, which had MIC of 0.15-0.31% against *Propionibacterium acnes*. In addition, molecular docking study showed that four flavonoids possessing prenyl or geranyl groups (6-isoprenyleriodictyol, nymphaeol A, nymphaeol B, and solophenol D) showed the highest binding affinity and dominant hydrogen bonding interactions with key amino acid residues in the binding sites of three bacterial proteins. The findings suggest that the methanol extract and its ethyl acetate fraction from *Macaranga hulletti* leaves could be a potential source of new antibacterial agents. Further studies are needed to isolate and evaluate the bioactive compounds.

Keywords: Antibacterial, Extract, Flavonoid, *Macaranga hulletti*, Molecular Docking.

Introduction

Despite substantial advancements in medicine and fundamental research, infectious diseases caused by transmissible agents such as bacteria, protists, and viruses continue to present significant challenges to healthcare professionals. These challenges include the issue of antimicrobial resistance.¹ Extracts from medicinal plants present a promising approach to addressing multidrug-resistant bacteria. In addition, medicinal plants can augment the efficacy of antibiotics for the treatment of infectious diseases.² Therefore, it is imperative to develop more efficacious antimicrobial agents, particularly those sourced from natural sources, as these are both readily available and economically viable.

Plants in the genus *Macaranga* belong to the family Euphorbiaceae and are commonly known as "Mahang." The secondary metabolites contained in plants of this genus include flavonoids and stilbenoids with prenylated substituents.³⁻¹³ Nine flavonoids containing either prenyl or geranyl groups and displaying antioxidant, antiparasitic, and anticancer activities have been isolated from three species (*Macaranga hosei*, *Macaranga pearsonii*, and *Macaranga tanarius*) of *Macaranga* present in East Kalimantan: 4'-*O*-methyl-8-isoprenylnaringenin, 4'-*O*-methyl-8-isoprenyleriodictyol, 6-isoprenyleriodictyol, solophenol D, nymphaeol A, and nymphaeol B, including lonchocarpol A, nymphaeol C, and macahuillettin A that was isolated from *Macaranga hullettii* obtained from Central Kalimantan.¹⁴⁻¹⁸ *Macaranga tanarius* leaf extract has been shown to exhibit antibacterial activity.¹⁹ The extracts of *Macaranga conglomerata*, *Macaranga kilimandscharica*, and *Macaranga capensis* from Kenya has shown antibacterial activity against *E. coli*, *E. aerogenes*, *K. pneumoniae*, *P. stuartii*, *P. aeruginosa*, and *S. aureus*.²⁰ The flavonol rhamnetin, isolated from *Macaranga peltata*, also showed antibacterial activity against *Staphylococcus aureus*.²¹ Two flavanone groups; 5-hydroxy-7,4'-dimethoxyflavone and 5-hydroxy-6,7,4'-trimethoxyflavone obtained from *Macaranga hosei* showed weak antibacterial activity against three pathogenic bacteria.²² In this study, the leaves of *Macaranga hullettii* King ex Hook.f. were identified as potential new source of medicinal compounds against bacterial infection. This plant is found in all areas of East Kalimantan, where the species commonly occurs in mountain forests and logging areas in the Kalimantan region.^{23,24} Phytochemical screening and isolation of secondary metabolites from the leaf extract of *Macaranga hullettii* identified several flavonoid compounds.^{18,25} The methanol extract and its fractions derived from *Macaranga hullettii* have not yet been analyzed for their antibacterial activity. Therefore, this study aimed to assess the antibacterial activity of *Macaranga hullettii* leaf extract and its fractions using *in vitro* studies. The flavonoids isolated

from *Macaranga* species in East Kalimantan, Indonesia, were evaluated for their antibacterial activity using molecular docking analysis.

Materials and Methods

Chemicals and equipment

The solvents used (methanol, n-hexane, and ethyl acetate) were purified by distillation before use, nutrient agar (NA) from Merck, Luria Bertani liquid medium (1% tryptone, 0.5% yeast, and 0.5% NaCl), yeast extract (Oxoid 100% (w/w)), tryptone (Oxoid 100% (w/w)), ampicillin 100 mg/ml (Sigma Aldrich), NaCl (Sigma Aldrich), cotton swabs, aluminum foil, gauze, Petri dishes, Ose needles, paper discs, an autoclave (Autoclave TOMY, Model SX-700), an oven (SHARP EO-28 WH), a rotary vacuum evaporator (EYELA Rotary evaporator N-1300E V.S.Series), and a freezer (Sanyo Medicoool). For molecular docking analysis, we used a Lenovo Yoga 7 computer with AMD Ryzen AI 7-8840HS w/ Radeon 780M graphics, 3301 Mhz, 8 Core(s), and 16.0 GB RAM.

Plant collection and identification

Fresh leaves of *Macaranga hullettii* were collected from the Lesan River Forest, Kelay District, Berau Regency, East Kalimantan, Indonesia (1°32'2.5919" N, 117°3'11.950" E). The plant samples were identified at the Laboratory of Anatomy and Plant Systematics, FMIPA Mulawarman University where voucher No. 0174/UN.17.8.5.7.16/HA/XI/2017 was assigned.

Extraction and fractionation

Macaranga hullettii leaf powder (1.3 kg) was extracted by maceration in methanol (2 x 7.0 L) at room temperature for 24 h. The extract was filtered, and filtrate was evaporated *in vacuo* using a rotary vacuum evaporator to obtain the crude methanol extract (126 g). The crude methanol extract (100 g) was dissolved in 500 mL of methanol-water mixture (4:1), and then successively fractionated with 5 x 200 mL each of n-hexane and ethyl acetate to obtain n-hexane fraction (28 g) and ethyl acetate fraction (18 g), respectively. Each fraction was evaporated using a rotary vacuum evaporator. The methanol extract, n-hexane fraction, and ethyl acetate fraction were evaluated for antibacterial activity.

Test organisms

Staphylococcus aureus ATCC 25923 was obtained from the Department of Microbiology, Faculty of Science, Chulalongkorn University. *Streptococcus mutans* ATCC 25175 was obtained from the Department of Biochemistry, Faculty of Dentistry, Chulalongkorn University. Both strains were purchased from the American Type Culture Collection (ATCC). *Propionibacterium acnes* KCCM 41747 was obtained from the Faculty of Forestry, Mulawarman University, and from the Korean Culture Center of Microorganisms (KCCM). The bacteria were periodically sub-cultured and maintained in nutrient agar (NA) under suitable conditions.

In vitro antibacterial assay

The antibacterial activity of the methanol extract, n-hexane, and ethyl acetate fractions of *Macaranga hullettii* leaves was evaluated using the disc diffusion or the Kirby-Bauer method.^{26,27} Nutrient Agar (NA) medium was prepared by dissolving 3.3 g in 150 mL of distilled water. Luria Bertani (LB) liquid medium was prepared by dissolving 4 g in 500 mL of distilled water, stirring, and adding 5 mL to a test tube. The NA and LB media were sterilized in an autoclave at 121°C with a pressure of 1 atm for 15 minutes. Each test bacterium was collected using an Ose needle, followed by dilution in a test tube containing sterile LB media and incubated at 37 °C for 24 h.

Nutrient Agar (25 mL) was poured into sterilized Petri dishes and maintained at room temperature for solidification. A sample of the bacterial suspension in the test tubes was then collected using a sterile cotton swab and applied to the surface of the agar in the Petri dish. Each 30 µL of the test *M. hullettii* leaf samples prepared in various concentrations in the organic solvents (methanol, n-hexane, and ethyl acetate) was poured onto sterile adsorbent filter paper discs (6 mm in diameter), and the solvent was removed. Afterward, the paper discs containing the test sample were placed on the surface of the agar media and incubated at 37 °C for 18-24 hours. The inhibition zone formed in each well was measured using a ruler.^{28,29} The minimum inhibitory concentration (MIC) was determined for the methanol extract as well as the n-hexane and ethyl acetate fractions. MIC was defined as the minimum concentration that completely inhibited bacterial growth.³⁰ Ampicillin was used as a positive control, and the three organic solvents were used as negative controls.

Molecular docking

Molecular docking was conducted to eleven (11) flavonoid derivatives possessing either prenyl or geranyl group presented in the three *Macaranga* species in East Kalimantan, Indonesia (*Macaranga hosei*, *Macaranga pearsonii*, and *Macaranga tanarius*), *Macaranga hulettii* in Central Kalimantan (Indonesia), and *Macaranga hosei* in Trengganu (Malaysia): 4'-*O*-methyl-8-isoprenylnaringenin, 4'-*O*-methyl-8-isoprenyleriodictyol, 6-isoprenyleriodictyol, 5-hydroxy-7,4'-dimethoxyflavone, lonchocarpol A, 5-hydroxy-6,7,4'-trimethoxyflavone, Macahuilettiin A, nymphaeol A, nymphaeol B, nymphaeol C, and solophenol D,^{14-18, 22} were used for the study. Macahuilettiin A, together with lonchocarpol A and nymphaeol C, were a prenylated flavonoid found in *Macaranga hulettii* obtained from Central Kalimantan (Indonesia).¹⁸ A comparative analysis of the molecular docking results for prenylated/geranylated flavonoids with methoxylated flavonoids was conducted, focusing on 5-hydroxy-7,4'-dimethoxyflavone and 5-hydroxy-6,7,4'-trimethoxyflavone that are found in *Macaranga hosei* from Trengganu, Malaysia.²²

The molecular structures of the flavonoids were drawn using ChemOffice Professional 15.0 and obtained from PubChem (<https://pubchem.ncbi.nlm.nih.gov/>). Structure refinement was performed using the molecular mechanics energy minimization method with the Merck molecular force field (MMFF94) using Open Babel in PyRx V.1.1. Three bacterial proteins, including *Staphylococcus aureus* tyrosyl-tRNA synthetase (PDB ID: 1JIJ), *Streptococcus mutans* antigen I/II (AgI/II) (PDB ID: 3IPK), and *Propionibacterium acnes* surface sialidase (PDB ID: 7LBU) were downloaded from the protein data bank,³¹⁻³³ and the locations of active sites were calculated using AlphaFold from <https://prankweb.cz/>.³⁴ Molecular docking was performed using the AutoDock Vina tool in PyRx V.1.1 software with an exhaustiveness of 32 and a mode value of 9 poses for each docked ligand.^{35,36} A protein binding site was defined as a box with dimensions of 30 × 30 × 30 Å. In the final step, the binding interactions were analyzed, and the docking results were visualized in 2D using the BIOVIA Discovery Studio Visualizer.

Statistical analysis

Data from the experiments were analyzed using Microsoft Excel.

Results and Discussion

In vitro antibacterial activity

The strength of antibacterial activity of the test samples was determined based on the diameter of the clear zone of inhibition (mm). The antibacterial activity was classified as follows: ≤ 5 mm (weak), 5 - 10 mm (moderate), 10 - 20 mm (strong), and ≥ 20 mm (very strong). Moreover, the lower the minimum inhibitory concentration of a compound, the greater its ability to inhibit the growth of test bacteria.²⁸ In this study, ampicillin was used as a positive control against three bacteria: *Staphylococcus aureus* ATCC 25923, *Streptococcus mutans* ATCC 25175, and *Propionibacterium acnes* KCCM 41747 (Table 1). The positive control served to determine whether the test bacteria could be inhibited by or were resistant to the antibiotics.

Staphylococcus aureus can cause dentoalveolar infections, jaw cysts, paroritis, oral mucosal lesions, and stomatitis. The bacteria can also produce exotoxins, lecosidine, enterotoxins, and coagulase enzyme. Enterotoxins are compounds that cause food poisoning in humans.³⁷ The methanol extract, hexane fraction, and ethyl acetate fraction of *Macaranga hulettii* leaves showed moderate antibacterial activity against *S. aureus*, with an inhibition zone diameter of 5 - 10 mm at concentrations of 0.15 to 2.5% (Table 1). The inhibition zone diameter of the 0.15% methanol extract was 6.67 mm. The 0.15% ethyl acetate fraction produced an inhibition zone diameter of 6.33 mm, and the 2.5% n-hexane fraction produced an inhibition zone diameter of 6.50 mm. The results indicated that the methanol extract and ethyl acetate fraction had the most potent antibacterial activity against *S. aureus*, with a minimum inhibitory concentration (MIC) of $< 0.15\%$. Moreover, the study of Sari and Saleh (2015) showed that ethyl acetate fraction of *Macaranga tanarius* was most effective against *S. aureus*, with MIC values of 0.125-0.5% and an inhibition zone of approximately 7.25 mm.³⁸

Streptococcus mutans is implicated in the pathogenesis of dental caries. This facultative anaerobe primarily ferments carbohydrates, resulting in the production of several organic acids, with lactic acid as the predominant byproduct. The acidification of the dental environment by *S. mutans* is a crucial factor contributing to enamel demineralization and the subsequent formation of carious lesions.^{39,40} The bacteria produce glucosyltransferase (GTF) which produces glucan, the compound that causes dental caries.⁴¹ *S. mutans* adheres to the salivary pellicle via its numerous receptors.⁴² The antibacterial activity of *Macaranga hulettii* King leaf extract and fractions was tested against *S. mutans*. Both extract and fractions displayed moderate antibacterial activity, with inhibition zone diameters of 5 - 10 mm at concentrations of 0.15 - 2.5%. The diameters of the inhibition zones produced by the methanol extract and ethyl acetate fraction were 6.67 and 6.33 mm, respectively at 0.15%. Meanwhile, the n-hexane fraction showed antibacterial activity at concentrations of 1.25 - 2.5%, with inhibition zone diameter ranging from 6.33 - 6.67 mm. These results demonstrated that the methanol extract

and ethyl acetate fraction of *Macaranga hulettii* leaves produced the highest antibacterial activity against *S. mutans*, with an MIC < 0.15% (Table 1).

Propionibacterium acnes is a Gram-positive anaerobic bacterium that can induce unusual keratinization within the sebaceous glands of hair follicles and increase sebum production.⁴³ The enzyme is capable of hydrolyzing triglycerides (TG) present in sebum into free fatty acids (FFA) that may subsequently induce inflammation in and around the hair follicles.⁴⁴ The bacteria also produce hyaluronidase, protease, lecithinase, and neuraminidase, that can cause inflammation of the skin. *Macaranga triloba* ethanol extract has been shown to prevent the growth of *P. acnes*, resulting in an inhibition zone diameter of 5.54 mm at 20%.⁴⁵ As shown in Table 1, the methanol extract and ethyl acetate fraction of *Macaranga hulettii* showed potent antibacterial activity against *P. acnes*, with inhibition zone diameters of 10.67 and 14.67 mm, respectively. The n-hexane fraction exhibited moderate antibacterial activity, with inhibition zone diameter of 5 - 10 mm at 1.25 - 2.5%. At concentration of 0.31%, the methanol extract showed antibacterial activity with an inhibition zone diameter of 7.67 mm, whereas at 0.15% concentration, the ethyl acetate fraction showed antibacterial activity with inhibition zone diameter of 6.67 mm. The results demonstrated that the ethyl acetate fraction of *Macaranga hulettii* leaves presented the highest antibacterial activity against *P. acnes*, with a minimum inhibitory concentration (MIC) of < 0.15% (Table 1).

The methanol extract and ethyl acetate fraction of *Macaranga hulettii* leaves exhibited good antibacterial activity against the three bacterial strains. This finding was consistent with previous studies that attributed the antibacterial effects to the presence of flavonoids and their prenylated or geranylated forms in species of *Macaranga*. These included euchrestaflavanone A, bonanione A, macarangaflavanone A, macarangaflavanone B, macatrichocarpin A, propolin D (nymphaeol B), senegalensin, schweinfurthin B, schweinfurthin O, and isomacarangain.⁴⁶⁻⁵⁶ Flavonoids can damage bacterial cell walls and denature proteases, thereby disrupting bacterial metabolism.⁵⁷

Molecular docking result

Molecular docking was performed to predict the interactions of the flavonoids with the amino acid residues of three bacterial proteins: *Staphylococcus aureus* tyrosyl-tRNA synthetase, *Streptococcus mutans* antigen I/II (AgI/II), and *Propionibacterium acnes* surface sialidase.³¹⁻³³ The molecular docking results (Table 2) revealed that 11 flavonoid derivatives had binding affinities ranging from -7.2 to -10.5 kcal/mol, -7.5 to -8.9 kcal/mol, and -7.1 to -8.9 kcal/mol against *Staphylococcus aureus* tyrosyl-tRNA synthetase, *Streptococcus mutans* antigen I/II

(AgI/II), and *Propionibacterium acnes* surface sialidase, respectively. The interactions of flavonoids and ampicillin with the protein targets are shown in two-dimensional graphs in Figures 1-3.

Among the compounds targeting *S. aureus* tyrosyl-tRNA synthetase, nymphaeol A, nymphaeol B, and solophenol D had the highest binding affinities, higher than that of the positive control ligand (ampicillin). Thus, the interactions of these compounds with the active site of the enzyme were modeled as a 2D structure (Figure 1). Ampicillin formed hydrogen bonds with LYS84 and GLN174 and amide- π interactions with GLY38 (Figure 1). Nymphaeol A and nymphaeol B formed hydrogen bonds as well as π -anions, and hydrophobic interactions with four amino acid residues. Nymphaeol B and ampicillin showed similar interactions with LYS84. In addition, solophenol D formed hydrogen bonds with TYR36, THR75, GLN190, ASP177, and GLN174, as well as hydrophobic interactions with HIS50, LEU70, PRO53, and ALA39 (Figure 1). These results agreed with previous studies where nymphaeol A-B and solophenol D exhibited antibacterial activity.^{58,59}

Four compounds including 6-isoprenyleriodictyol, nymphaeol A, nymphaeol B, and solophenol D exhibited high antibacterial activity against *S. mutans* antigen I/II (AgI/II) with binding affinities ranging from -8.7 to -8.9 kcal/mol. The interactions between the ligands and amino acid residues in the binding sites of these compounds are shown in 2D format (Figure 2). Ampicillin formed hydrogen bonds with four amino acid residues, ASN590, SER591, SER588, and ASP760, as well as π -sulfur and π - π interactions with TRP 816, while 6-isoprenyleriodictyol only formed hydrogen bonds with three amino acid residues: SER697, ASN699, and ASN814. Nymphaeol A and nymphaeol B formed hydrogen bonds with four amino acid residues (Figure 2). Nymphaeol A exhibited a similar π - π interaction with TRP816, as observed with ampicillin. Solophenol D formed hydrogen bonds with SER818, ARG824, and THR586, similar π - π interaction as ampicillin with TRP816), and π -sigma interaction with SER762. The compound 6-isoprenyleriodictyol is a prenylated flavonoid similar to nymphaeol A, and hence may have antibacterial activity.

Two compounds, including 6-isoprenyleriodictyol and nymphaeol A, displayed stronger binding affinities than ampicillin, with values of -8.0 and -8.4 kcal/mol against *P. acnes* surface sialidases. Therefore, both compounds were visualized in 2D format to investigate the interactions between the ligands and the amino acid residues in the protein binding sites (Figure 3). Ampicillin formed hydrogen bonds with four amino acid residues, ARG282, GLN287, ASP146, ASP185, and π - π and π -alkyl interactions with PHE257, ALA147, and VAL202; 6-Isoprenyleriodictyol demonstrated π - π interactions with PHE257, π -anion interactions with

ASP146, and alkyl-alkyl interactions with ALA147, PHE209, VAL202, and LEU224. This compound formed bonds with five amino acid residues, including ASP146, ARG329, TYR423, ARG282, and GLU287, that were analogous to those observed with ampicillin. Nymphaeol A formed hydrogen bonds with five amino acid residues (ARG329, TYR423, ASP185, ALA147, and SER184), π -anion interactions with ASP312 and ASP185, alkyl-alkyl interactions with MET310, ALA331, and ALA147, and Van der Waals interactions with PHE257. Thus, these compounds may have potent antibacterial activity.

Structure-activity relationships were also studied based on the binding affinity results (Table 2). Flavonoids possessing prenyl, geranyl, and hydroxyl groups showed potential as antibacterial agents due to their strong affinity for amino acid residues in the binding sites of the proteins, compared to ampicillin. However, the absence of a hydroxyl group on prenylated or geranylated flavonoids decreased the binding affinity.^{56,60}

Conclusion

The extract and fractions of *Macaranga hulettii* leaves have potential as antibacterial agents against three bacterial strains, *Staphylococcus aureus* ATCC 25923, *Streptococcus mutans* ATCC 25175, and *Propionibacterium acnes* KCCM 41747. The methanol extract demonstrated antibacterial activity against *S. aureus* and *S. mutans* with MIC of < 0.15% against *S. aureus* and *S. mutans*, and 0.15-0.31% against *P. acnes*. The n-hexane fraction had an MIC of 1.25-2.5% against *S. aureus*, and 0.62-1.25% against *S. mutans* and *P. acnes*. The ethyl acetate fraction had an MIC < 0.15% against the three bacterial strains. Molecular docking studies suggested that 6-isoprenyliodictyol, nymphaeol A, nymphaeol B, and solophenol D are promising antibacterial agents, as these compounds exhibited the highest binding affinity and formed hydrogen bonds with key amino acid residues in the active sites of the protein targets. Further *in vitro* and *in vivo* studies should be conducted by isolating the active secondary metabolites from this species.

Conflict of Interest

The authors declare no conflict of interest.

Author's Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims related to the content of this article will be borne by them.

Acknowledgements

This research was funded by the Islamic Development Bank (PIU-IsDB) project under contract no. 137/UN17.11/PL/2019.

References

1. Wong F, de la Fuente-Nunez C, Collins JJ. Leveraging artificial intelligence in the fight against infectious diseases. *Science*. 2023; 381(6654):164-170.
2. Seukep AJ, Kuete V, Nahar L, Sarker SD, Guo M. Plant-derived secondary metabolites as the main source of efflux pump inhibitors and methods for identification. *J Pharm Anal*. 2020; 10(4):277-290.
3. Magadula JJ. Phytochemistry and pharmacology of the genus *Macaranga*: a review. *J Med Plants Res*. 2014; 8(12):489-503.
4. Vu LT, Ngan TB, Phuong L, Huong DT, Litaudon M, Van Hung N, Thach TD, Van Cuong P. Chemical constituents from fruits of *Macaranga denticulata* (Euphorbiaceae) (Part 2). *Vietnam J Chem*. 2018; 56(4):516-520.
5. Marliana E, Hairani R, Tjahjandarie TS, Tanjung M. Antiplasmodial activity of flavonoids from *Macaranga tanarius* leaves. *IOP Conf Ser Earth Environ Sci*. 2018; 144(1):012011
6. Tjahjandarie TS, Tanjung M, Saputri RD, Nadar PB, Aldin MF, Marliana E, Permadi A. Flavestins K, An isoprenylated stilbene from the leaves of *Macaranga recurvata* Gage. *Nat Prod Sci*. 2019; 25(3):244-247.
7. Huong DT, Linh NT, Van TT, Litaudon M, Roussi F, Van Nam V, Van Cuong P. Stilbenes from *Macaranga tanarius* (Euphorbiaceae) growing in Vietnam. *Vietnam J Chem*. 2020; 58(3):338-342.
8. Aldin MF, Tjahjandarie TS, Saputri RD, Tanjung M. Macasiamenene V, a New Stilbenoid from the Leaves of *Macaranga inermis*. *Nat Prod Sci*. 2021; 27(1):45-48.
9. Muharram A, Rachmawati DA, Mardhiyyah S, Tjahjandarie TS, Saputri RD, Ahmat N, Tanjung M. Cytotoxic and antioxidant activities of flavonoids and diterpenoids from *Macaranga involucreata* (Roxb.) Baill. *J Appl Pharm Sci*. 2023; 13(6):087-92.
10. Tanjung M, Tjahjandarie TS, Aldin MF, Mardhiyyah S, Ahmat N, Saputri RD. Macagigantin A, A New Flavonoid from *Macaranga gigantea* (Rchb. f & Zoll.) Mull Arg *Nat Prod Sci*. 2023; 29(4):287-294.
11. Tjahjandarie TS, Aldin MF, Saputri RD, Tanjung M. Dihydrostilbenes from *Macaranga javanica* (Blume) Müll. Arg. and their antiplasmodial activity. *Nat Prod Sci*. 2024:1-7.
12. Toko EG, Tchapo CED, Tsamo AT, Kemzeu R, Wang Y, Fekam FB, Ndinteh DT, Choudhary MI, Nkengfack EA, Mmutlane EM, Mkounga P. Three New Polyphenol Derivatives from the Fruits of *Macaranga Monandra* and their Antioxidant Potential. *Chem. Biodivers*. 2024;21(7):e202301816.

13. Tanjung M, Tjahjandarie TS, Aldin MF, Mardhiyyah S, Saputri RD, Syah YM, Ahmat N. Two new flavonols from *Macaranga inermis* pax & K. Hoffm. Nat Prod Sci. 2025; 39(3):498-505.
14. Marliana E, Tjahjandarie TS, Tanjung M. Isoprenylated flavanone derivatives from *Macaranga hosei* King ex Hook F. Der Pharm Lett. 2015; 7(3):153-156.
15. Marliana E, Tjahjandarie TS, Tanjung M. Antioxidant activity of flavonoids from *Macaranga pearsonii* Merr. J Kim Mulawarman. 2016; 13(2):97-100.
16. Marliana E, Astuti W, Kosala K, Hairani R, Tjahjandarie TS, Tanjung M. Chemical composition and anticancer activity of *Macaranga hosei* leaves. Asian J Chem. 2018; 30(4):795-798.
17. Marliana E, Ruga R, Hairani R, Tjahjandarie TS, Tanjung M. Antioxidant activity of flavonoid constituents from the leaves of *Macaranga tanarius*. IOP Conf Ser J Phys. 2019; 1277(1):012014.
18. Saputri RD, Tukiran T, Wati FA, Purnamasari AP, Wardhana MW, Tjahjandarie TS, Tanjung M. Macahuilettiin A, a new isoprenylated flavanone from the leaves of *Macaranga hullettii* King ex Hook and their antiplasmodial activity. Vietnam J Chem 2024; 62(3):394-398.
19. Musdalifah M, Khumaidi A, Suwastika IN. Inhibition test and phytochemical screening of leaf extracts of *Macaranga tanarius* (L.) Mull. Arg against *Salmonella typhi* as an antibacterial. Nat Sci J Sci Technol. 2017; 6(3):9194.
20. Ibrahim H, Omosa L, Nchiozem-Ngnitedem V, Onyari J, Maru S, Guefack M. Antibacterial activities and phytochemical screening of crude extracts from Kenyan macaranga species toward MDR phenotypes expressing efflux pumps. Pharmacogn Commun. 2021; 11(2):119-126.
21. Bijesh K and Sebastian D. Isolation and characterization of antibacterial compounds from *Macaranga peltata* against clinical isolates of *Staphylococcus aureus*. Int J Biol Pharm Res. 2013; 4(12):1196-1203.
22. Salleh WM, Razak NZ, Ahmad F. Phytochemicals and biological activities of *Macaranga hosei* and *Macaranga constricta* (Euphorbiaceae). Marmara Pharm J. 2017; 21(4):881-888.
23. Slik JW, Priyono P, Welzen PV. Key to the *Macaranga Thou.* and *Mallotus Lour.* species (Euphorbiaceae) of East Kalimantan, Indonesia. Singapore, Gardens' Bulletin (Singapore), National Parks Board, Singapore Botanic Gardens; 2000. 11-87 p.
24. Amirta R, Angi EM, Ramadhan R, Kusuma IW, Wiati CB, Haqiqi MT. Potential utilization of macaranga. Samarinda: Mulawarman University Press; 2017.

25. Rismawati R, Marlina E, Daniel D. Phytochemical Test on Methanol Extract of Leaf of *Macaranga hullettii* King ex Hook. f. J Atomik. 2018; 3(2):91-94.
26. Hudzicki J. Kirby-Bauer disk diffusion susceptibility test protocol. Am Soc Microbiol. 2009; 15(1):1-23.
27. Inna M, Astuti W, Saleh C. Antibacterial activity of methanolic extract of uric patch plant leaves (*Cayratia carnosa*) against *Salmonella thypi* and *Propionibacterium acnes*. J Atomik. 2022; 7(1):1-5.
28. Yulianti MF, Amat AL, Hutasoit RM, Pakan PD. Antibacterial activity of jamblang leaf ethanol extract (*Syzygium cumini*) against the growth of *Propionibacterium acnes*. Acta Biochimica Indones. 2023; 6(2):1161.
29. Hossain MR, Biplob AI, Sharif SR, Bhuiya AM, Sayem AS. Antibacterial Activity of Green Synthesized Silver Nanoparticles of *Lablab purpureus* Flowers Extract against Human Pathogenic Bacteria. Trop J Nat Prod Res. 2023;7(8):3647-3651
30. Yanda L, Tatsimo SJ, Tamokou JD, Matsute-Takongmo G, Meffo-Dongmo SC, Meli Lannang A, Sewald N. Antibacterial and antioxidant activities of isolated compounds from *Prosopis africana* leaves. Int J Anal Chem. 2022; 2022:4205823.
31. Qiu X, Janson CA, Smith WW, Green SM, McDevitt P, Johanson K, Carter P, Hibbs M, Lewis C, Chalker A, Fosberry A. Crystal structure of *Staphylococcus aureus* tyrosyl-tRNA synthetase in complex with a class of potent and specific inhibitors. Protein Sci. 2001; 10(10):2008-2016.
32. Larson MR, Rajashankar KR, Patel MH, Robinette RA, Crowley PJ, Michalek S, Brady LJ, Deivanayagam C. Elongated fibrillar structure of a *streptococcal adhesin* assembled by the high-affinity association of α - and PPII-helices. Proc Natl Acad Sci. 2010; 107(13):5983-5988.
33. Yu AC, Volkers G, Jongkees SA, Worrall LJ, Withers SG, Strynadka NC. Crystal structure of the *Propionibacterium acnes* surface sialidase, a drug target for *P. acnes*-associated diseases. Glycobiol. 2022; 32(2):162-170.
34. Jakubec D, Skoda P, Krivak R, Novotny M, Hoksza D. PrankWeb 3: accelerated ligand-binding site predictions for experimental and modelled protein structures. Nucl Acids Res. 2022; 50:(W1):W593-W597.
35. Dallakyan S and Olson AJ. Small-molecule library screening by docking with PyRx. Methods Mol Biol. 2014; 1263:243-250.
36. Trott O and Olson AJ. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. J Comput Chem. 2010; 31(2):455-461.
37. Malangu N. ed. Poisoning: From Specific Toxic Agents to Novel Rapid and Simplified Techniques for Analysis. BoD–Books on Demand; 2017.

38. Sari AA and Saleh C. Phytochemical test, toxicity and antibacterial activity of extracts of various macaranga leaf fractions (*Macaranga tanarius* (L.) MA) against *Staphylococcus aureus* and *Escherichia coli*. J Kim Mulawarman. 2015; 12(2):53-58.
39. Imelda R, Mariam M, Satari M. Effect of cassava (*Manihot esculenta* cranzt), rice (*oryza sativa* l.), and potato (*solanum tuberosum*) water extract to decrease pH phase fermentation of *streptococcus mutans* atcc 25175. Padjajaran J Dent. 2019; 31(1):14.
40. Alkhaled A, Alsabek L, Al-assaf M, Badr F. Effect of chlorhexidine, honey and propolis on *streptococcus mutans* counts: *in vitro* study. Dentistry. 2021; 9(1):a001.
41. Ren Z, Chen L, Li J, Li Y. Inhibition of *Streptococcus mutans* polysaccharide synthesis by molecules targeting glycosyltransferase activity. J Oral Microbiol. 2016; 8(1):31095.
42. Lee BS, Chen YJ, Wei TC, Ma TL, Chang CC. Comparison of antibacterial adhesion when salivary pellicle is coated on both poly (2-hydroxyethyl-methacrylate)-and polyethylene-glycol-methacrylate-grafted poly (methyl methacrylate). Int J Mol Sci. 2018; 19(9):2764.
43. Chen KC, Yang CH, Li TT, Zouboulis CC, Huang YC. Suppression of Propionibacterium acnes-stimulated proinflammatory cytokines by Chinese bayberry extracts and its active constituent myricetin in human sebocytes *in vitro*. Phytother Res. 2019; 33(4):1104-1113.
44. Xu J, Chen X, Song J, Wang C, Xu W, Tan H, Suo H. Antibacterial activity and mechanism of cell-free supernatants of *Lactocaseibacillus paracasei* against *Propionibacterium acnes*. Microb. Pathog. 2024; 189:106598
45. Warnida H, Mustika D, Supomo S, Sukawaty Y. Effectiveness of Mahang Leaf Ethanol Extract (*Macaranga Triloba*) as an anti-acne. J Penelit. Sos. Ekon. Kehuta. 2018; 4(1):9-18.
46. Schütz BA, Wright AD, Rali T, Sticher O. Prenylated flavanones from leaves of *Macaranga pleiostemona*. Phytochem. 1995; 40(4):1273-1277.
47. Lim TY, Lim YY, Yule CM. Evaluation of antioxidant, antibacterial and anti-tyrosinase activities of four Macaranga species. Food Chem. 2009; 114(2):594-599.
48. Fareza MS, Syah YM, Mujahidin D, Juliawaty LD, Kurniasih I. Antibacterial flavanones and dihydrochalcones from *Macaranga trichocarpa*. Z. Naturforsch C J Biosci. 2014; 69(9-10):375-380.
49. Hasanat A, Kabir MS, Hossain MM, Hasan M, Al Masum MA, Chowdhury TA, Bhuiyan DI, Mamur A, Kibria AS. Antibacterial activity of methanol extract of *Macaranga denticulata* leaves and in silico PASS prediction for its six secondary metabolites. World J Pharm Pharm. Sci. 2015:1258-1266.
50. O Akanbi B, Anene P, Olayanju S. Preliminary Screening Indicates Promising Antimicrobial Properties of the Stem Bark Extracts of *Macaranga rosea*. Anti-Infective Agents. 2015; 13(2):123-128.

51. Ogundajo A, Okeleye B, Ashafa AO. Chemical constituents, *in vitro* antimicrobial and cytotoxic potentials of the extracts from *Macaranga barteri* Mull-Arg. Asian Pac J Trop Biomed. 2017; 7(7):654-659.
52. Putri R, Hendra R, Teruna HY. Anti-Bacterial and Anti-Fungal Activities from *Macaranga bancana* Leaves Extract. Pharmacol Clin Pharm Res. 2019; 4(1):1-4.
53. Lee JH, Kim YG, Khadke SK, Yamano A, Woo JT, Lee J. Antimicrobial and antibiofilm activities of prenylated flavanones from *Macaranga tanarius*. Phytomed. 2019; 63:153033.
54. Pagna JI, Awazi T, Mbarga PE, Mbekou IM, Mkounga P, Fotie J, Frese M, Fabrice FB, Lenta BN, Sewald N, Nkengfack EA. Antibacterial flavonoids from the fruits of *Macaranga hurifolia*. J Asian Nat Prod Res. 2022; 24(11):1041-1051.
55. Kamso VF, Simo Fotso CC, Kanko Mbekou IM, Toussie BT, Ndjakou Lenta B, Boyom FF, Sewald N, Frese M, Ngadjui BT, Wabo Fotso G. Chemical constituents of *Macaranga occidentalis*, antimicrobial and chemophenetic studies. Molecules. 2022; 27(24):8820.
56. Rosamah E, Haqiqi MT, Putri AS, Kuspradini H, Kusuma IW, Amirta R, Yuliansyah Y, Suwinarti W, Paramita S, Ramadhan R, Tarmadi D. The potential of *Macaranga* plants as skincare cosmetic ingredients: A review. J Appl Pharm Sci. 2023; 13(7):001-12.
57. Li AP, He YH, Zhang SY, Shi YP. Antibacterial activity and action mechanism of flavonoids against phytopathogenic bacteria. Pestic Biochem Physiol. 2022; 188:105221.
58. Chen YW, Ye SR, Ting C, Yu YH. Antibacterial activity of propolins from Taiwanese green propolis. J Food Drug Anal. 2018; 26(2):761-768.
59. Inui S, Hosoya T, Shimamura Y, Masuda S, Ogawa T, Kobayashi H, Shirafuji K, Moli RT, Kozono I, Shin-ya K, Kumazawa S. Solophenols B–D and Solomonin: New Prenylated Polyphenols Isolated from Propolis Collected from The Solomon Islands and Their Antibacterial Activity. J Agric Food Chem. 2012; 60(47):11765-11770.
60. Ruga R, Kingkaew K, Tamsampaoloet K, Chavasiri W. Enhancing antibacterial activity against *Propionibacterium acnes* and *Staphylococcus aureus* by combination of tetracycline with selected compounds. Chem Lett. 2018; 47(12):1538-1541.

Table 1: Antibacterial activity of *Macaranga hullettii* King ex Hook leaf extracts and fractions against *Staphylococcus aureus*, *Streptococcus mutans*, and *Propionibacterium acnes*

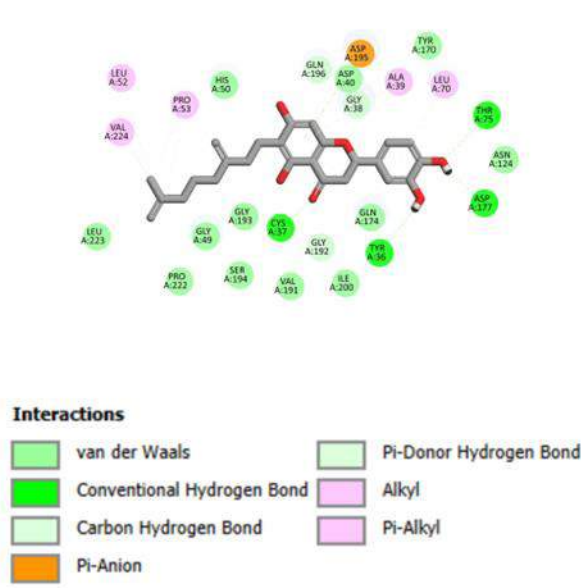
Sample	Concentration (%)	Inhibition Zone Diameter (mm)*		
		<i>Staphylococcus aureus</i>	<i>Streptococcus mutans</i>	<i>Propionibacterium acnes</i>
Methanol Extract	0	0	0	0
	0.15	6.67 ± 0.58	6.67 ± 0.58	0
	0.31	6.67 ± 0.58	6.67 ± 0.58	7.67 ± 0.58
	0.62	6.67 ± 0.58	7.0 ± 0.0	8.0 ± 1.0
	1.25	7.17 ± 0.29	7.33 ± 0.58	9.67 ± 0.58
	2.5	7.5 ± 0.5	8.0 ± 1.0	10.67 ± 0.58
	MIC (%)	<0.15	<0.15	0.15-0.31
n-Hexane Fraction	0	0	0	0
	0.15	0	0	0
	0.31	0	0	0
	0.62	0	0	0
	1.25	0	6.33 ± 0.29	6.33 ± 0.58
	2.5	6.5 ± 0.5	6.67 ± 0.58	6.67 ± 0.58
	MIC (%)	1.25-2.5	0.62-1.25	0.62-1.25
Ethyl acetate Fraction	0	0	0	0
	0.15	6.33 ± 0.58	6.33 ± 0.58	6.67 ± 0.58
	0.31	6.5 ± 0.5	6.67 ± 0.58	8.0 ± 0.0
	0.62	6.5 ± 0.5	6.83 ± 0.29	9.0 ± 0.0
	1.25	6.67 ± 0.29	7.0 ± 0.0	11.0 ± 1.0
	2.5	7.0 ± 0.0	7.67 ± 0.58	14.67 ± 0.58
	MIC (%)	<0.15	<0.15	<0.15
Ampicillin	0.01	20.33 ± 1.15	21.0 ± 1.0	21.0 ± 1.0

*Data were obtained from triplicate experiments.

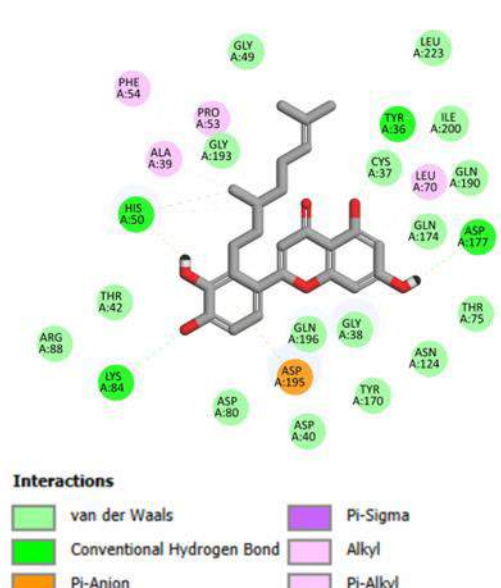
Table 2: Binding affinity of flavonoids against bacterial proteins

No.	Compound	Binding Affinity (kcal/mol)		
		1JIJ	3IPK	7LBU
1	4'-O-methyl-8-isoprenylnaringenin	-7.4	-8.1	-7.7
2	4'-O-methyl-8-isoprenyleriodictyol	-7.2	-8.1	-7.6
3	6-Isoprenyleriodictyol	-7.7	-8.7	-8.0

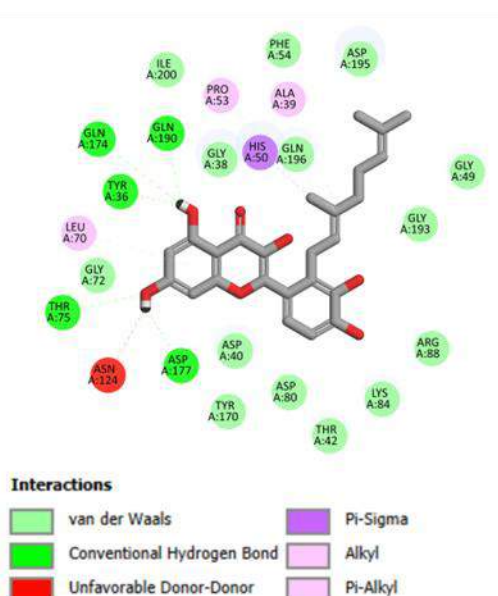
4	5-hydroxy-7,4'-dimethoxyflavone	-8.6	-7.8	-7.1
5	5-hydroxy-6,7,4'-trimethoxyflavone	-8.3	-7.5	-7.3
6	Macahuilettiin A	-7.6	-8.4	-7.1
7	Lonchocarpol A	-9.4	-7.8	-7.8
8	Nymphaeol A	-9.7	-8.9	-8.4
9	Nymphaeol B	-10.5	-8.9	-7.7
10	Nymphaeol C	-8.4	-8.3	-7.4
11	Solophenol D	-10.0	-8.7	-7.9
12	Ampicillin	-8.5	-7.7	-7.6



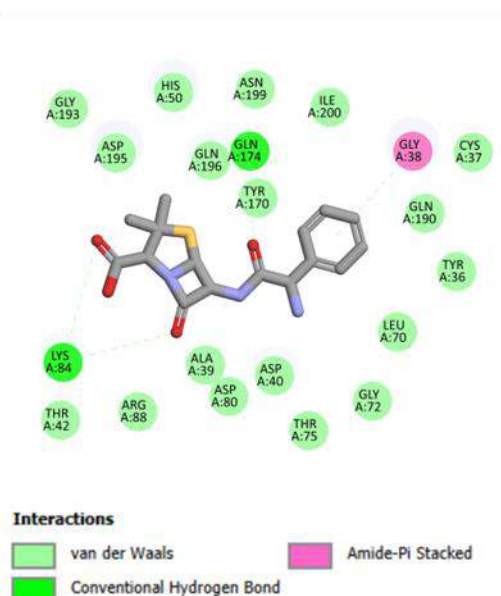
Nymphaeol A



Nymphaeol B



Solophenol D



Ampicillin

Figure 1: 2D visualization of interaction of the best docked flavonoids and ampicillin with *Staphylococcus aureus* tyrosyl-tRNA synthetase (PDB ID: 1JIJ)

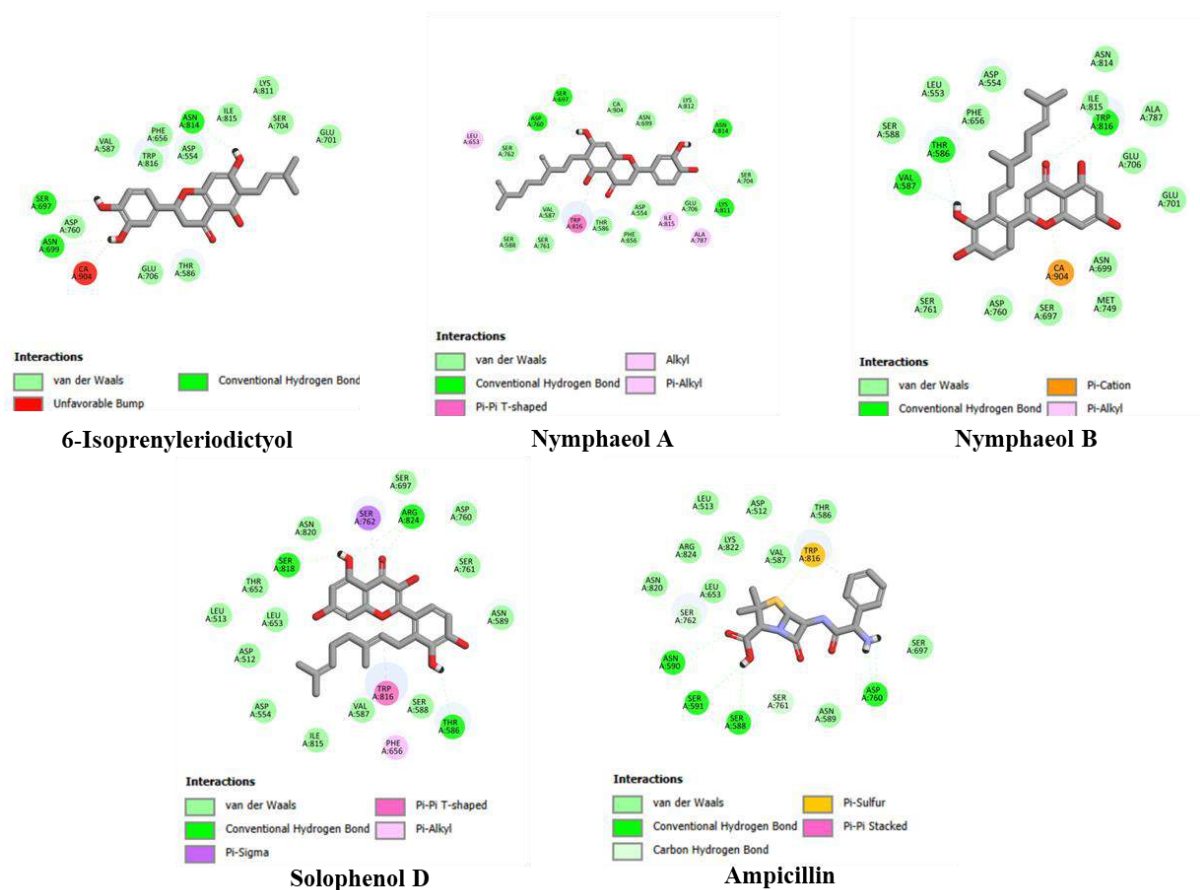


Figure 2: 2D visualization of interaction of the best-docked flavonoids and ampicillin with *Streptococcus mutans* antigen I/II (PDB ID: 3IPK).

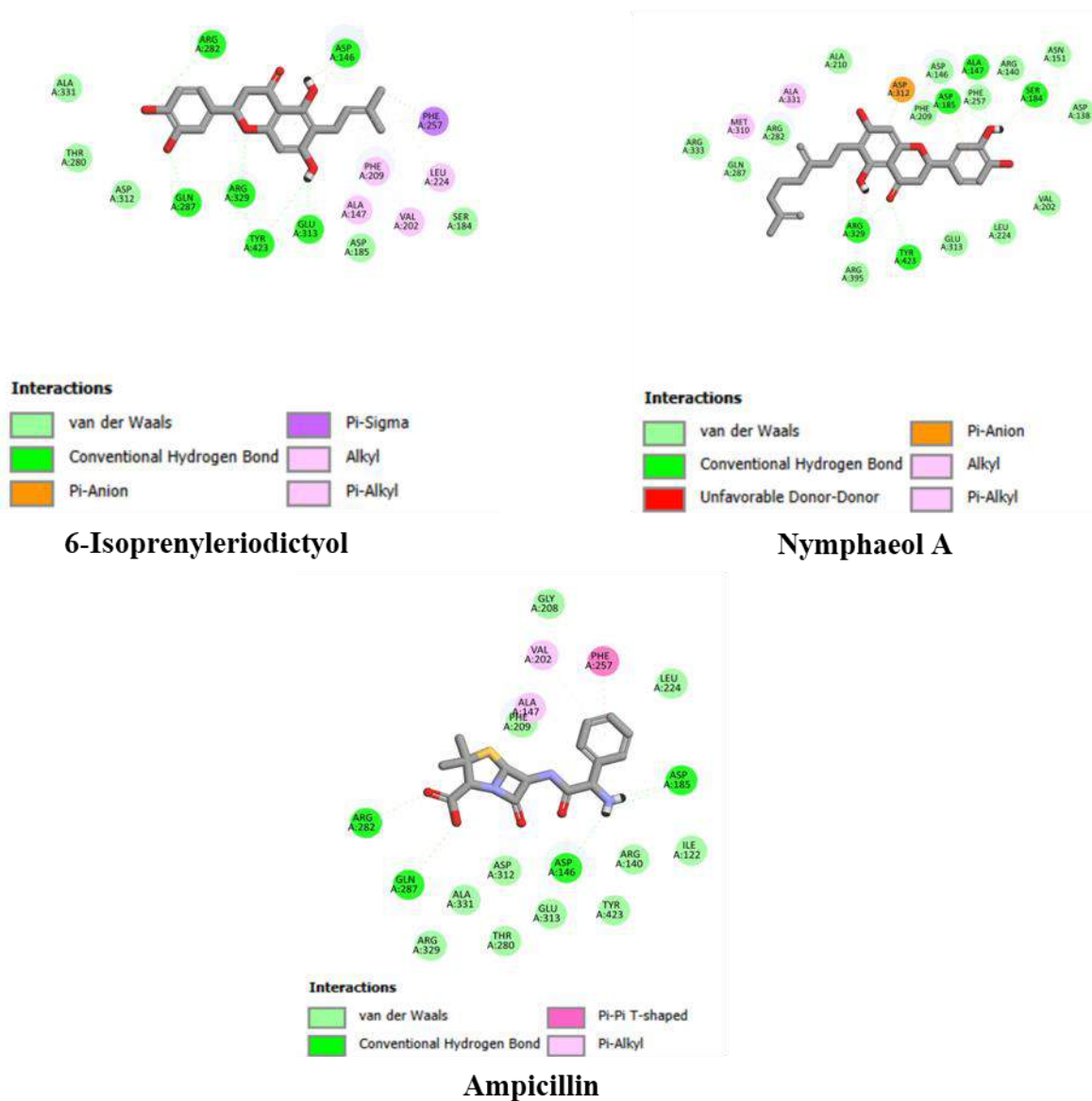


Figure 3: 2D visualization of interaction of the best-docked flavonoids and ampicillin with *Propionibacterium acnes* surface sialidase (PDB ID: 7LBU).



Re: Revision

From Editor-in-Chief Tjnpr <editor.tjnpr@gmail.com>

Date Sun 5/11/2025 5:47 AM

To Ade Danova <adedanova@itb.ac.id>

 1 attachment (881 KB)

3-TJNPR-2025-420 Revised_Proofreading (1).docx;

Please seek the assistance of a native English speaker/ editor for the English editing and proper formatting. Dr Erharuyi (erharuyiosaz@gmail.com); Send the revised copy and the certificate of English editing when submitting the revised version

Best regards

Abiodun

Professor Abiodun Falodun, PhD; FAAS, FISPON

Editor-in-Chief:

Tropical Journal of Natural Product Research (TJNPR)

Head, Natural Product Research Group, University of Benin

Email: editor.tjnpr@gmail.com

www.tjnpr.org SCOPUS, SCImago SJR Q3

Professor of Pharmaceutical Chemistry, FAAS

Fellow, Fulbright (USA)

Deputy Vice-Chancellor (Academic) 2014-2016

Faculty of Pharmacy

University of Benin

[Google Scholar Citations](#)

SCOPUS <https://www.scopus.com/authid/detail.uri?>



www.tjnpr.org

***In vitro* Evaluation and Molecular Docking Study of the Antibacterial Potential of
Macaranga hulletti King ex Hook.f. Leaf Extract**

Eva Marlina^{1,3}, Ade Danova^{2*}, Rita Hairani^{1,3}, Ritbey Ruga^{1,3}, Elvira Hermawati², Ana Yulvia⁴

¹*Department of Chemistry, Faculty of Mathematics and Natural Sciences, Mulawarman University, Samarinda 75123, East Kalimantan, Indonesia*

²*Organic Chemistry Division, Department of Chemistry, Faculty of Mathematics and Natural Sciences, Institut Teknologi Bandung Jl. Ganesha No. 10, Bandung 70132, West Java, Indonesia*

³*Research Center for Medicine and Cosmetics from Tropical Rainforest Resources, Mulawarman University, Samarinda, Indonesia*

⁴*Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Jember, East Java, Indonesia*

***Corresponding author. E-mail: adedanova@itb.ac.id**

Tel.: +6281357944698.

Abstract

Due to the resistance of bacteria to antibiotics, there has been increasing interest in the use of plant extracts to combat infections. Plants in the genus *Macaranga* contain secondary metabolites such as flavonoids with antibacterial properties. *Macaranga hullettii* King ex Hook.f. are particularly noteworthy due to their widespread distribution in East Kalimantan. This study aimed to investigate the antibacterial potential of *M. hullettii* leaf extract through *in vitro* and molecular docking studies. The antibacterial activity of the methanol extract, n-hexane, and ethyl acetate fractions from *M. hullettii* leaves was evaluated against three bacterial species, *Staphylococcus aureus*, *Streptococcus mutans*, and *Propionibacterium acnes* using the disc diffusion assay. Molecular docking of eleven flavonoid derivatives presents in three *Macaranga* species was performed against selected bacterial proteins (PDB ID: IJIJ, 3IPK, and 7LBU). The results showed that both the methanol extract and ethyl acetate fraction displayed antibacterial activity against the three bacteria strains, with minimum inhibitory concentrations (MICs) < 0.15%, except for the methanol extract, which had MIC of 0.15-0.31% against *Propionibacterium acnes*. In addition, molecular docking study showed that four flavonoids possessing prenyl or geranyl groups (6-isoprenyleriodictyol, nymphaeol A, nymphaeol B, and solophenol D) showed the highest binding affinity and dominant hydrogen bonding interactions with key amino acid residues in the binding sites of three bacterial proteins. The findings suggest that the methanol extract and its ethyl acetate fraction from *Macaranga hulletti* leaves could be a potential source of new antibacterial agents. Further studies are needed to isolate and evaluate the bioactive compounds.

Keywords: Antibacterial, Extract, Flavonoid, *Macaranga hulletti*, Molecular Docking.

Introduction

Despite substantial advancements in medicine and fundamental research, infectious diseases caused by transmissible agents such as bacteria, protists, and viruses continue to present significant challenges to healthcare professionals. These challenges include the issue of antimicrobial resistance.¹ Extracts from medicinal plants present a promising approach to addressing multidrug-resistant bacteria. In addition, medicinal plants can augment the efficacy of antibiotics for the treatment of infectious diseases.² Therefore, it is imperative to develop more efficacious antimicrobial agents, particularly those sourced from natural sources, as these are both readily available and economically viable.

Plants in the genus *Macaranga* belong to the family Euphorbiaceae and are commonly known as "Mahang." The secondary metabolites contained in plants of this genus include flavonoids and stilbenoids with prenylated substituents.³⁻¹³ Nine flavonoids containing either prenyl or geranyl groups and displaying antioxidant, antiparasitic, and anticancer activities have been isolated from three species (*Macaranga hosei*, *Macaranga pearsonii*, and *Macaranga tanarius*) of *Macaranga* present in East Kalimantan: 4'-*O*-methyl-8-isoprenylnaringenin, 4'-*O*-methyl-8-isoprenyleriodictyol, 6-isoprenyleriodictyol, solophenol D, nymphaeol A, and nymphaeol B, including lonchocarpol A, nymphaeol C, and macahuillettin A that was isolated from *Macaranga hullettii* obtained from Central Kalimantan.¹⁴⁻¹⁸ *Macaranga tanarius* leaf extract has been shown to exhibit antibacterial activity.¹⁹ The extracts of *Macaranga conglomerata*, *Macaranga kilimandscharica*, and *Macaranga capensis* from Kenya has shown antibacterial activity against *E. coli*, *E. aerogenes*, *K. pneumoniae*, *P. stuartii*, *P. aeruginosa*, and *S. aureus*.²⁰ The flavonol rhamnetin, isolated from *Macaranga peltata*, also showed antibacterial activity against *Staphylococcus aureus*.²¹ Two flavanone groups; 5-hydroxy-7,4'-dimethoxyflavone and 5-hydroxy-6,7,4'-trimethoxyflavone obtained from *Macaranga hosei* showed weak antibacterial activity against three pathogenic bacteria.²² In this study, the leaves of *Macaranga hullettii* King ex Hook.f. were identified as potential new source of medicinal compounds against bacterial infection. This plant is found in all areas of East Kalimantan, where the species commonly occurs in mountain forests and logging areas in the Kalimantan region.^{23,24} Phytochemical screening and isolation of secondary metabolites from the leaf extract of *Macaranga hullettii* identified several flavonoid compounds.^{18,25} The methanol extract and its fractions derived from *Macaranga hullettii* have not yet been analyzed for their antibacterial activity. Therefore, this study aimed to assess the antibacterial activity of *Macaranga hullettii* leaf extract and its fractions using *in vitro* studies. The flavonoids isolated

from *Macaranga* species in East Kalimantan, Indonesia, were evaluated for their antibacterial activity using molecular docking analysis.

Materials and Methods

Chemicals and equipment

The solvents used (methanol, n-hexane, and ethyl acetate) were purified by distillation before use, nutrient agar (NA) from Merck, Luria Bertani liquid medium (1% tryptone, 0.5% yeast, and 0.5% NaCl), yeast extract (Oxoid 100% (w/w)), tryptone (Oxoid 100% (w/w)), ampicillin 100 mg/ml (Sigma Aldrich), NaCl (Sigma Aldrich), cotton swabs, aluminum foil, gauze, Petri dishes, Ose needles, paper discs, an autoclave (Autoclave TOMY, Model SX-700), an oven (SHARP EO-28 WH), a rotary vacuum evaporator (EYELA Rotary evaporator N-1300E V.S.Series), and a freezer (Sanyo Medicoool). For molecular docking analysis, we used a Lenovo Yoga 7 computer with AMD Ryzen AI 7-8840HS w/ Radeon 780M graphics, 3301 Mhz, 8 Core(s), and 16.0 GB RAM.

Plant collection and identification

Fresh leaves of *Macaranga hullettii* were collected from the Lesan River Forest, Kelay District, Berau Regency, East Kalimantan, Indonesia (1°32'2.5919" N, 117°3'11.950" E). The plant samples were identified at the Laboratory of Anatomy and Plant Systematics, FMIPA Mulawarman University where voucher No. 0174/UN.17.8.5.7.16/HA/XI/2017 was assigned.

Extraction and fractionation

Macaranga hullettii leaf powder (1.3 kg) was extracted by maceration in methanol (2 x 7.0 L) at room temperature for 24 h. The extract was filtered, and filtrate was evaporated *in vacuo* using a rotary vacuum evaporator to obtain the crude methanol extract (126 g). The crude methanol extract (100 g) was dissolved in 500 mL of methanol-water mixture (4:1), and then successively fractionated with 5 x 200 mL each of n-hexane and ethyl acetate to obtain n-hexane fraction (28 g) and ethyl acetate fraction (18 g), respectively. Each fraction was evaporated using a rotary vacuum evaporator. The methanol extract, n-hexane fraction, and ethyl acetate fraction were evaluated for antibacterial activity.

Test organisms

Staphylococcus aureus ATCC 25923 was obtained from the Department of Microbiology, Faculty of Science, Chulalongkorn University. *Streptococcus mutans* ATCC 25175 was obtained from the Department of Biochemistry, Faculty of Dentistry, Chulalongkorn University. Both strains were purchased from the American Type Culture Collection (ATCC). *Propionibacterium acnes* KCCM 41747 was obtained from the Faculty of Forestry, Mulawarman University, and from the Korean Culture Center of Microorganisms (KCCM). The bacteria were periodically sub-cultured and maintained in nutrient agar (NA) under suitable conditions.

In vitro antibacterial assay

The antibacterial activity of the methanol extract, n-hexane, and ethyl acetate fractions of *Macaranga hullettii* leaves was evaluated using the disc diffusion or the Kirby-Bauer method.^{26,27} Nutrient Agar (NA) medium was prepared by dissolving 3.3 g in 150 mL of distilled water. Luria Bertani (LB) liquid medium was prepared by dissolving 4 g in 500 mL of distilled water, stirring, and adding 5 mL to a test tube. The NA and LB media were sterilized in an autoclave at 121°C with a pressure of 1 atm for 15 minutes. Each test bacterium was collected using an Ose needle, followed by dilution in a test tube containing sterile LB media and incubated at 37 °C for 24 h.

Nutrient Agar (25 mL) was poured into sterilized Petri dishes and maintained at room temperature for solidification. A sample of the bacterial suspension in the test tubes was then collected using a sterile cotton swab and applied to the surface of the agar in the Petri dish. Each 30 µL of the test *M. hullettii* leaf samples prepared in various concentrations in the organic solvents (methanol, n-hexane, and ethyl acetate) was poured onto sterile adsorbent filter paper discs (6 mm in diameter), and the solvent was removed. Afterward, the paper discs containing the test sample were placed on the surface of the agar media and incubated at 37 °C for 18-24 hours. The inhibition zone formed in each well was measured using a ruler.^{28,29} The minimum inhibitory concentration (MIC) was determined for the methanol extract as well as the n-hexane and ethyl acetate fractions. MIC was defined as the minimum concentration that completely inhibited bacterial growth.³⁰ Ampicillin was used as a positive control, and the three organic solvents were used as negative controls.

Molecular docking

Molecular docking was conducted to eleven (11) flavonoid derivatives possessing either prenyl or geranyl group presented in the three *Macaranga* species in East Kalimantan, Indonesia (*Macaranga hosei*, *Macaranga pearsonii*, and *Macaranga tanarius*), *Macaranga hulettii* in Central Kalimantan (Indonesia), and *Macaranga hosei* in Trengganu (Malaysia): 4'-*O*-methyl-8-isoprenylnaringenin, 4'-*O*-methyl-8-isoprenyleriodictyol, 6-isoprenyleriodictyol, 5-hydroxy-7,4'-dimethoxyflavone, lonchocarpol A, 5-hydroxy-6,7,4'-trimethoxyflavone, Macahuilettiin A, nymphaeol A, nymphaeol B, nymphaeol C, and solophenol D,^{14-18, 22} were used for the study. Macahuilettiin A, together with lonchocarpol A and nymphaeol C, were a prenylated flavonoid found in *Macaranga hulettii* obtained from Central Kalimantan (Indonesia).¹⁸ A comparative analysis of the molecular docking results for prenylated/geranylated flavonoids with methoxylated flavonoids was conducted, focusing on 5-hydroxy-7,4'-dimethoxyflavone and 5-hydroxy-6,7,4'-trimethoxyflavone that are found in *Macaranga hosei* from Trengganu, Malaysia.²²

The molecular structures of the flavonoids were drawn using ChemOffice Professional 15.0 and obtained from PubChem (<https://pubchem.ncbi.nlm.nih.gov/>). Structure refinement was performed using the molecular mechanics energy minimization method with the Merck molecular force field (MMFF94) using Open Babel in PyRx V.1.1. Three bacterial proteins, including *Staphylococcus aureus* tyrosyl-tRNA synthetase (PDB ID: 1JIJ), *Streptococcus mutans* antigen I/II (AgI/II) (PDB ID: 3IPK), and *Propionibacterium acnes* surface sialidase (PDB ID: 7LBU) were downloaded from the protein data bank,³¹⁻³³ and the locations of active sites were calculated using AlphaFold from <https://prankweb.cz/>.³⁴ Molecular docking was performed using the AutoDock Vina tool in PyRx V.1.1 software with an exhaustiveness of 32 and a mode value of 9 poses for each docked ligand.^{35,36} A protein binding site was defined as a box with dimensions of 30 × 30 × 30 Å. In the final step, the binding interactions were analyzed, and the docking results were visualized in 2D using the BIOVIA Discovery Studio Visualizer.

Statistical analysis

Data from the experiments were analyzed using Microsoft Excel.

Results and Discussion

In vitro antibacterial activity

The strength of antibacterial activity of the test samples was determined based on the diameter of the clear zone of inhibition (mm). The antibacterial activity was classified as follows: ≤ 5 mm (weak), 5 - 10 mm (moderate), 10 - 20 mm (strong), and ≥ 20 mm (very strong). Moreover, the lower the minimum inhibitory concentration of a compound, the greater its ability to inhibit the growth of test bacteria.²⁸ In this study, ampicillin was used as a positive control against three bacteria: *Staphylococcus aureus* ATCC 25923, *Streptococcus mutans* ATCC 25175, and *Propionibacterium acnes* KCCM 41747 (Table 1). The positive control served to determine whether the test bacteria could be inhibited by or were resistant to the antibiotics.

Staphylococcus aureus can cause dentoalveolar infections, jaw cysts, paroritis, oral mucosal lesions, and stomatitis. The bacteria can also produce exotoxins, lecosidine, enterotoxins, and coagulase enzyme. Enterotoxins are compounds that cause food poisoning in humans.³⁷ The methanol extract, hexane fraction, and ethyl acetate fraction of *Macaranga hulettii* leaves showed moderate antibacterial activity against *S. aureus*, with an inhibition zone diameter of 5 - 10 mm at concentrations of 0.15 to 2.5% (Table 1). The inhibition zone diameter of the 0.15% methanol extract was 6.67 mm. The 0.15% ethyl acetate fraction produced an inhibition zone diameter of 6.33 mm, and the 2.5% n-hexane fraction produced an inhibition zone diameter of 6.50 mm. The results indicated that the methanol extract and ethyl acetate fraction had the most potent antibacterial activity against *S. aureus*, with a minimum inhibitory concentration (MIC) of $< 0.15\%$. Moreover, the study of Sari and Saleh (2015) showed that ethyl acetate fraction of *Macaranga tanarius* was most effective against *S. aureus*, with MIC values of 0.125-0.5% and an inhibition zone of approximately 7.25 mm.³⁸

Streptococcus mutans is implicated in the pathogenesis of dental caries. This facultative anaerobe primarily ferments carbohydrates, resulting in the production of several organic acids, with lactic acid as the predominant byproduct. The acidification of the dental environment by *S. mutans* is a crucial factor contributing to enamel demineralization and the subsequent formation of carious lesions.^{39,40} The bacteria produce glucosyltransferase (GTF) which produces glucan, the compound that causes dental caries.⁴¹ *S. mutans* adheres to the salivary pellicle via its numerous receptors.⁴² The antibacterial activity of *Macaranga hulettii* King leaf extract and fractions was tested against *S. mutans*. Both extract and fractions displayed moderate antibacterial activity, with inhibition zone diameters of 5 - 10 mm at concentrations of 0.15 - 2.5%. The diameters of the inhibition zones produced by the methanol extract and ethyl acetate fraction were 6.67 and 6.33 mm, respectively at 0.15%. Meanwhile, the n-hexane fraction showed antibacterial activity at concentrations of 1.25 - 2.5%, with inhibition zone diameter ranging from 6.33 - 6.67 mm. These results demonstrated that the methanol extract

and ethyl acetate fraction of *Macaranga hulettii* leaves produced the highest antibacterial activity against *S. mutans*, with an MIC < 0.15% (Table 1).

Propionibacterium acnes is a Gram-positive anaerobic bacterium that can induce unusual keratinization within the sebaceous glands of hair follicles and increase sebum production.⁴³ The enzyme is capable of hydrolyzing triglycerides (TG) present in sebum into free fatty acids (FFA) that may subsequently induce inflammation in and around the hair follicles.⁴⁴ The bacteria also produce hyaluronidase, protease, lecithinase, and neuraminidase, that can cause inflammation of the skin. *Macaranga triloba* ethanol extract has been shown to prevent the growth of *P. acnes*, resulting in an inhibition zone diameter of 5.54 mm at 20%.⁴⁵ As shown in Table 1, the methanol extract and ethyl acetate fraction of *Macaranga hulettii* showed potent antibacterial activity against *P. acnes*, with inhibition zone diameters of 10.67 and 14.67 mm, respectively. The n-hexane fraction exhibited moderate antibacterial activity, with inhibition zone diameter of 5 - 10 mm at 1.25 - 2.5%. At concentration of 0.31%, the methanol extract showed antibacterial activity with an inhibition zone diameter of 7.67 mm, whereas at 0.15% concentration, the ethyl acetate fraction showed antibacterial activity with inhibition zone diameter of 6.67 mm. The results demonstrated that the ethyl acetate fraction of *Macaranga hulettii* leaves presented the highest antibacterial activity against *P. acnes*, with a minimum inhibitory concentration (MIC) of < 0.15% (Table 1).

The methanol extract and ethyl acetate fraction of *Macaranga hulettii* leaves exhibited good antibacterial activity against the three bacterial strains. This finding was consistent with previous studies that attributed the antibacterial effects to the presence of flavonoids and their prenylated or geranylated forms in species of *Macaranga*. These included euchrestaflavanone A, bonanione A, macarangaflavanone A, macarangaflavanone B, macatrichocarpin A, propolin D (nymphaeol B), senegalensin, schweinfurthin B, schweinfurthin O, and isomacarangain.⁴⁶⁻⁵⁶ Flavonoids can damage bacterial cell walls and denature proteases, thereby disrupting bacterial metabolism.⁵⁷

Molecular docking result

Molecular docking was performed to predict the interactions of the flavonoids with the amino acid residues of three bacterial proteins: *Staphylococcus aureus* tyrosyl-tRNA synthetase, *Streptococcus mutans* antigen I/II (AgI/II), and *Propionibacterium acnes* surface sialidase.³¹⁻³³ The molecular docking results (Table 2) revealed that 11 flavonoid derivatives had binding affinities ranging from -7.2 to -10.5 kcal/mol, -7.5 to -8.9 kcal/mol, and -7.1 to -8.9 kcal/mol against *Staphylococcus aureus* tyrosyl-tRNA synthetase, *Streptococcus mutans* antigen I/II

(AgI/II), and *Propionibacterium acnes* surface sialidase, respectively. The interactions of flavonoids and ampicillin with the protein targets are shown in two-dimensional graphs in Figures 1-3.

Among the compounds targeting *S. aureus* tyrosyl-tRNA synthetase, nymphaeol A, nymphaeol B, and solophenol D had the highest binding affinities, higher than that of the positive control ligand (ampicillin). Thus, the interactions of these compounds with the active site of the enzyme were modeled as a 2D structure (Figure 1). Ampicillin formed hydrogen bonds with LYS84 and GLN174 and amide- π interactions with GLY38 (Figure 1). Nymphaeol A and nymphaeol B formed hydrogen bonds as well as π -anions, and hydrophobic interactions with four amino acid residues. Nymphaeol B and ampicillin showed similar interactions with LYS84. In addition, solophenol D formed hydrogen bonds with TYR36, THR75, GLN190, ASP177, and GLN174, as well as hydrophobic interactions with HIS50, LEU70, PRO53, and ALA39 (Figure 1). These results agreed with previous studies where nymphaeol A-B and solophenol D exhibited antibacterial activity.^{58,59}

Four compounds including 6-isoprenyleriodictyol, nymphaeol A, nymphaeol B, and solophenol D exhibited high antibacterial activity against *S. mutans* antigen I/II (AgI/II) with binding affinities ranging from -8.7 to -8.9 kcal/mol. The interactions between the ligands and amino acid residues in the binding sites of these compounds are shown in 2D format (Figure 2). Ampicillin formed hydrogen bonds with four amino acid residues, ASN590, SER591, SER588, and ASP760, as well as π -sulfur and π - π interactions with TRP 816, while 6-isoprenyleriodictyol only formed hydrogen bonds with three amino acid residues: SER697, ASN699, and ASN814. Nymphaeol A and nymphaeol B formed hydrogen bonds with four amino acid residues (Figure 2). Nymphaeol A exhibited a similar π - π interaction with TRP816, as observed with ampicillin. Solophenol D formed hydrogen bonds with SER818, ARG824, and THR586, similar π - π interaction as ampicillin with TRP816), and π -sigma interaction with SER762. The compound 6-isoprenyleriodictyol is a prenylated flavonoid similar to nymphaeol A, and hence may have antibacterial activity.

Two compounds, including 6-isoprenyleriodictyol and nymphaeol A, displayed stronger binding affinities than ampicillin, with values of -8.0 and -8.4 kcal/mol against *P. acnes* surface sialidases. Therefore, both compounds were visualized in 2D format to investigate the interactions between the ligands and the amino acid residues in the protein binding sites (Figure 3). Ampicillin formed hydrogen bonds with four amino acid residues, ARG282, GLN287, ASP146, ASP185, and π - π and π -alkyl interactions with PHE257, ALA147, and VAL202; 6-Isoprenyleriodictyol demonstrated π - π interactions with PHE257, π -anion interactions with

ASP146, and alkyl-alkyl interactions with ALA147, PHE209, VAL202, and LEU224. This compound formed bonds with five amino acid residues, including ASP146, ARG329, TYR423, ARG282, and GLU287, that were analogous to those observed with ampicillin. Nymphaeol A formed hydrogen bonds with five amino acid residues (ARG329, TYR423, ASP185, ALA147, and SER184), π -anion interactions with ASP312 and ASP185, alkyl-alkyl interactions with MET310, ALA331, and ALA147, and Van der Waals interactions with PHE257. Thus, these compounds may have potent antibacterial activity.

Structure-activity relationships were also studied based on the binding affinity results (Table 2). Flavonoids possessing prenyl, geranyl, and hydroxyl groups showed potential as antibacterial agents due to their strong affinity for amino acid residues in the binding sites of the proteins, compared to ampicillin. However, the absence of a hydroxyl group on prenylated or geranylated flavonoids decreased the binding affinity.^{56,60}

Conclusion

The extract and fractions of *Macaranga hulettii* leaves have potential as antibacterial agents against three bacterial strains, *Staphylococcus aureus* ATCC 25923, *Streptococcus mutans* ATCC 25175, and *Propionibacterium acnes* KCCM 41747. The methanol extract demonstrated antibacterial activity against *S. aureus* and *S. mutans* with MIC of < 0.15% against *S. aureus* and *S. mutans*, and 0.15-0.31% against *P. acnes*. The n-hexane fraction had an MIC of 1.25-2.5% against *S. aureus*, and 0.62-1.25% against *S. mutans* and *P. acnes*. The ethyl acetate fraction had an MIC < 0.15% against the three bacterial strains. Molecular docking studies suggested that 6-isoprenyleriodictyol, nymphaeol A, nymphaeol B, and solophenol D are promising antibacterial agents, as these compounds exhibited the highest binding affinity and formed hydrogen bonds with key amino acid residues in the active sites of the protein targets. Further *in vitro* and *in vivo* studies should be conducted by isolating the active secondary metabolites from this species.

Conflict of Interest

The authors declare no conflict of interest.

Author's Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims related to the content of this article will be borne by them.

Acknowledgements

This research was funded by the Islamic Development Bank (PIU-IsDB) project under contract no. 137/UN17.11/PL/2019.

References

1. Wong F, de la Fuente-Nunez C, Collins JJ. Leveraging artificial intelligence in the fight against infectious diseases. *Science*. 2023; 381(6654):164-170.
2. Seukep AJ, Kuete V, Nahar L, Sarker SD, Guo M. Plant-derived secondary metabolites as the main source of efflux pump inhibitors and methods for identification. *J Pharm Anal*. 2020; 10(4):277-290.
3. Magadula JJ. Phytochemistry and pharmacology of the genus *Macaranga*: a review. *J Med Plants Res*. 2014; 8(12):489-503.
4. Vu LT, Ngan TB, Phuong L, Huong DT, Litaudon M, Van Hung N, Thach TD, Van Cuong P. Chemical constituents from fruits of *Macaranga denticulata* (Euphorbiaceae) (Part 2). *Vietnam J Chem*. 2018; 56(4):516-520.
5. Marliana E, Hairani R, Tjahjandarie TS, Tanjung M. Antiplasmodial activity of flavonoids from *Macaranga tanarius* leaves. *IOP Conf Ser Earth Environ Sci*. 2018; 144(1):012011
6. Tjahjandarie TS, Tanjung M, Saputri RD, Nadar PB, Aldin MF, Marliana E, Permadi A. Flavestins K, An isoprenylated stilbene from the leaves of *Macaranga recurvata* Gage. *Nat Prod Sci*. 2019; 25(3):244-247.
7. Huong DT, Linh NT, Van TT, Litaudon M, Roussi F, Van Nam V, Van Cuong P. Stilbenes from *Macaranga tanarius* (Euphorbiaceae) growing in Vietnam. *Vietnam J Chem*. 2020; 58(3):338-342.
8. Aldin MF, Tjahjandarie TS, Saputri RD, Tanjung M. Macasiamenene V, a New Stilbenoid from the Leaves of *Macaranga inermis*. *Nat Prod Sci*. 2021; 27(1):45-48.
9. Muharram A, Rachmawati DA, Mardhiyyah S, Tjahjandarie TS, Saputri RD, Ahmat N, Tanjung M. Cytotoxic and antioxidant activities of flavonoids and diterpenoids from *Macaranga involucreata* (Roxb.) Baill. *J Appl Pharm Sci*. 2023; 13(6):087-92.
10. Tanjung M, Tjahjandarie TS, Aldin MF, Mardhiyyah S, Ahmat N, Saputri RD. Macagigantin A, A New Flavonoid from *Macaranga gigantea* (Rchb. f & Zoll.) Mull Arg *Nat Prod Sci*. 2023; 29(4):287-294.
11. Tjahjandarie TS, Aldin MF, Saputri RD, Tanjung M. Dihydrostilbenes from *Macaranga javanica* (Blume) Müll. Arg. and their antiplasmodial activity. *Nat Prod Sci*. 2024:1-7.
12. Toko EG, Tchapo CED, Tsamo AT, Kemzeu R, Wang Y, Fekam FB, Ndinteh DT, Choudhary MI, Nkengfack EA, Mmutlane EM, Mkounga P. Three New Polyphenol Derivatives from the Fruits of *Macaranga Monandra* and their Antioxidant Potential. *Chem. Biodivers*. 2024;21(7):e202301816.

13. Tanjung M, Tjahjandarie TS, Aldin MF, Mardhiyyah S, Saputri RD, Syah YM, Ahmat N. Two new flavonols from *Macaranga inermis* pax & K. Hoffm. Nat Prod Sci. 2025; 39(3):498-505.
14. Marliana E, Tjahjandarie TS, Tanjung M. Isoprenylated flavanone derivatives from *Macaranga hosei* King ex Hook F. Der Pharm Lett. 2015; 7(3):153-156.
15. Marliana E, Tjahjandarie TS, Tanjung M. Antioxidant activity of flavonoids from *Macaranga pearsonii* Merr. J Kim Mulawarman. 2016; 13(2):97-100.
16. Marliana E, Astuti W, Kosala K, Hairani R, Tjahjandarie TS, Tanjung M. Chemical composition and anticancer activity of *Macaranga hosei* leaves. Asian J Chem. 2018; 30(4):795-798.
17. Marliana E, Ruga R, Hairani R, Tjahjandarie TS, Tanjung M. Antioxidant activity of flavonoid constituents from the leaves of *Macaranga tanarius*. IOP Conf Ser J Phys. 2019; 1277(1):012014.
18. Saputri RD, Tukiran T, Wati FA, Purnamasari AP, Wardhana MW, Tjahjandarie TS, Tanjung M. Macahuilettiin A, a new isoprenylated flavanone from the leaves of *Macaranga hullettii* King ex Hook and their antiplasmodial activity. Vietnam J Chem 2024; 62(3):394-398.
19. Musdalifah M, Khumaidi A, Suwastika IN. Inhibition test and phytochemical screening of leaf extracts of *Macaranga tanarius* (L.) Mull. Arg against *Salmonella typhi* as an antibacterial. Nat Sci J Sci Technol. 2017; 6(3):9194.
20. Ibrahim H, Omosa L, Nchiozem-Ngnitedem V, Onyari J, Maru S, Guefack M. Antibacterial activities and phytochemical screening of crude extracts from Kenyan macaranga species toward MDR phenotypes expressing efflux pumps. Pharmacogn Commun. 2021; 11(2):119-126.
21. Bijesh K and Sebastian D. Isolation and characterization of antibacterial compounds from *Macaranga peltata* against clinical isolates of *Staphylococcus aureus*. Int J Biol Pharm Res. 2013; 4(12):1196-1203.
22. Salleh WM, Razak NZ, Ahmad F. Phytochemicals and biological activities of *Macaranga hosei* and *Macaranga constricta* (Euphorbiaceae). Marmara Pharm J. 2017; 21(4):881-888.
23. Slik JW, Priyono P, Welzen PV. Key to the *Macaranga Thou.* and *Mallotus Lour.* species (Euphorbiaceae) of East Kalimantan, Indonesia. Singapore, Gardens' Bulletin (Singapore), National Parks Board, Singapore Botanic Gardens; 2000. 11-87 p.
24. Amirta R, Angi EM, Ramadhan R, Kusuma IW, Wiati CB, Haqiqi MT. Potential utilization of macaranga. Samarinda: Mulawarman University Press; 2017.

25. Rismawati R, Marlina E, Daniel D. Phytochemical Test on Methanol Extract of Leaf of *Macaranga hullettii* King ex Hook. f. J Atomik. 2018; 3(2):91-94.
26. Hudzicki J. Kirby-Bauer disk diffusion susceptibility test protocol. Am Soc Microbiol. 2009; 15(1):1-23.
27. Inna M, Astuti W, Saleh C. Antibacterial activity of methanolic extract of uric patch plant leaves (*Cayratia carnosa*) against *Salmonella thypi* and *Propionibacterium acnes*. J Atomik. 2022; 7(1):1-5.
28. Yulianti MF, Amat AL, Hutasoit RM, Pakan PD. Antibacterial activity of jamblang leaf ethanol extract (*Syzygium cumini*) against the growth of *Propionibacterium acnes*. Acta Biochimica Indones. 2023; 6(2):1161.
29. Hossain MR, Biplob AI, Sharif SR, Bhuiya AM, Sayem AS. Antibacterial Activity of Green Synthesized Silver Nanoparticles of *Lablab purpureus* Flowers Extract against Human Pathogenic Bacteria. Trop J Nat Prod Res. 2023;7(8):3647-3651
30. Yanda L, Tatsimo SJ, Tamokou JD, Matsute-Takongmo G, Meffo-Dongmo SC, Meli Lannang A, Sewald N. Antibacterial and antioxidant activities of isolated compounds from *Prosopis africana* leaves. Int J Anal Chem. 2022; 2022:4205823.
31. Qiu X, Janson CA, Smith WW, Green SM, McDevitt P, Johanson K, Carter P, Hibbs M, Lewis C, Chalker A, Fosberry A. Crystal structure of *Staphylococcus aureus* tyrosyl-tRNA synthetase in complex with a class of potent and specific inhibitors. Protein Sci. 2001; 10(10):2008-2016.
32. Larson MR, Rajashankar KR, Patel MH, Robinette RA, Crowley PJ, Michalek S, Brady LJ, Deivanayagam C. Elongated fibrillar structure of a *streptococcal adhesin* assembled by the high-affinity association of α - and PPII-helices. Proc Natl Acad Sci. 2010; 107(13):5983-5988.
33. Yu AC, Volkers G, Jongkees SA, Worrall LJ, Withers SG, Strynadka NC. Crystal structure of the *Propionibacterium acnes* surface sialidase, a drug target for *P. acnes*-associated diseases. Glycobiol. 2022; 32(2):162-170.
34. Jakubec D, Skoda P, Krivak R, Novotny M, Hoksza D. PrankWeb 3: accelerated ligand-binding site predictions for experimental and modelled protein structures. Nucl Acids Res. 2022; 50:(W1):W593-W597.
35. Dallakyan S and Olson AJ. Small-molecule library screening by docking with PyRx. Methods Mol Biol. 2014; 1263:243-250.
36. Trott O and Olson AJ. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. J Comput Chem. 2010; 31(2):455-461.
37. Malangu N. ed. Poisoning: From Specific Toxic Agents to Novel Rapid and Simplified Techniques for Analysis. BoD–Books on Demand; 2017.

38. Sari AA and Saleh C. Phytochemical test, toxicity and antibacterial activity of extracts of various macaranga leaf fractions (*Macaranga tanarius* (L.) MA) against *Staphylococcus aureus* and *Escherichia coli*. J Kim Mulawarman. 2015; 12(2):53-58.
39. Imelda R, Mariam M, Satari M. Effect of cassava (*Manihot esculenta* cranzt), rice (*oryza sativa* l.), and potato (*solanum tuberosum*) water extract to decrease pH phase fermentation of *streptococcus mutans* atcc 25175. Padjajaran J Dent. 2019; 31(1):14.
40. Alkhaled A, Alsabek L, Al-assaf M, Badr F. Effect of chlorhexidine, honey and propolis on *streptococcus mutans* counts: *in vitro* study. Dentistry. 2021; 9(1):a001.
41. Ren Z, Chen L, Li J, Li Y. Inhibition of *Streptococcus mutans* polysaccharide synthesis by molecules targeting glycosyltransferase activity. J Oral Microbiol. 2016; 8(1):31095.
42. Lee BS, Chen YJ, Wei TC, Ma TL, Chang CC. Comparison of antibacterial adhesion when salivary pellicle is coated on both poly (2-hydroxyethyl-methacrylate)-and polyethylene-glycol-methacrylate-grafted poly (methyl methacrylate). Int J Mol Sci. 2018; 19(9):2764.
43. Chen KC, Yang CH, Li TT, Zouboulis CC, Huang YC. Suppression of Propionibacterium acnes-stimulated proinflammatory cytokines by Chinese bayberry extracts and its active constituent myricetin in human sebocytes *in vitro*. Phytother Res. 2019; 33(4):1104-1113.
44. Xu J, Chen X, Song J, Wang C, Xu W, Tan H, Suo H. Antibacterial activity and mechanism of cell-free supernatants of *Lactocaseibacillus paracasei* against *Propionibacterium acnes*. Microb. Pathog. 2024; 189:106598
45. Warnida H, Mustika D, Supomo S, Sukawaty Y. Effectiveness of Mahang Leaf Ethanol Extract (*Macaranga Triloba*) as an anti-acne. J Penelit. Sos. Ekon. Kehuta. 2018; 4(1):9-18.
46. Schütz BA, Wright AD, Rali T, Sticher O. Prenylated flavanones from leaves of *Macaranga pleiostemona*. Phytochem. 1995; 40(4):1273-1277.
47. Lim TY, Lim YY, Yule CM. Evaluation of antioxidant, antibacterial and anti-tyrosinase activities of four Macaranga species. Food Chem. 2009; 114(2):594-599.
48. Fareza MS, Syah YM, Mujahidin D, Juliawaty LD, Kurniasih I. Antibacterial flavanones and dihydrochalcones from *Macaranga trichocarpa*. Z. Naturforsch C J Biosci. 2014; 69(9-10):375-380.
49. Hasanat A, Kabir MS, Hossain MM, Hasan M, Al Masum MA, Chowdhury TA, Bhuiyan DI, Mamur A, Kibria AS. Antibacterial activity of methanol extract of *Macaranga denticulata* leaves and in silico PASS prediction for its six secondary metabolites. World J Pharm Pharm. Sci. 2015:1258-1266.
50. O Akanbi B, Anene P, Olayanju S. Preliminary Screening Indicates Promising Antimicrobial Properties of the Stem Bark Extracts of *Macaranga rosea*. Anti-Infective Agents. 2015; 13(2):123-128.

51. Ogundajo A, Okeleye B, Ashafa AO. Chemical constituents, *in vitro* antimicrobial and cytotoxic potentials of the extracts from *Macaranga barteri* Mull-Arg. Asian Pac J Trop Biomed. 2017; 7(7):654-659.
52. Putri R, Hendra R, Teruna HY. Anti-Bacterial and Anti-Fungal Activities from *Macaranga bancana* Leaves Extract. Pharmacol Clin Pharm Res. 2019; 4(1):1-4.
53. Lee JH, Kim YG, Khadke SK, Yamano A, Woo JT, Lee J. Antimicrobial and antibiofilm activities of prenylated flavanones from *Macaranga tanarius*. Phytomed. 2019; 63:153033.
54. Pagna JI, Awazi T, Mbarga PE, Mbekou IM, Mkounga P, Fotie J, Frese M, Fabrice FB, Lenta BN, Sewald N, Nkengfack EA. Antibacterial flavonoids from the fruits of *Macaranga hurifolia*. J Asian Nat Prod Res. 2022; 24(11):1041-1051.
55. Kamso VF, Simo Fotso CC, Kanko Mbekou IM, Toussie BT, Ndjakou Lenta B, Boyom FF, Sewald N, Frese M, Ngadjui BT, Wabo Fotso G. Chemical constituents of *Macaranga occidentalis*, antimicrobial and chemophenetic studies. Molecules. 2022; 27(24):8820.
56. Rosamah E, Haqiqi MT, Putri AS, Kuspradini H, Kusuma IW, Amirta R, Yuliansyah Y, Suwinarti W, Paramita S, Ramadhan R, Tarmadi D. The potential of *Macaranga* plants as skincare cosmetic ingredients: A review. J Appl Pharm Sci. 2023; 13(7):001-12.
57. Li AP, He YH, Zhang SY, Shi YP. Antibacterial activity and action mechanism of flavonoids against phytopathogenic bacteria. Pestic Biochem Physiol. 2022; 188:105221.
58. Chen YW, Ye SR, Ting C, Yu YH. Antibacterial activity of propolins from Taiwanese green propolis. J Food Drug Anal. 2018; 26(2):761-768.
59. Inui S, Hosoya T, Shimamura Y, Masuda S, Ogawa T, Kobayashi H, Shirafuji K, Moli RT, Kozono I, Shin-ya K, Kumazawa S. Solophenols B–D and Solomonin: New Prenylated Polyphenols Isolated from Propolis Collected from The Solomon Islands and Their Antibacterial Activity. J Agric Food Chem. 2012; 60(47):11765-11770.
60. Ruga R, Kingkaew K, Tamsampaoloet K, Chavasiri W. Enhancing antibacterial activity against *Propionibacterium acnes* and *Staphylococcus aureus* by combination of tetracycline with selected compounds. Chem Lett. 2018; 47(12):1538-1541.

Table 1: Antibacterial activity of *Macaranga hullettii* King ex Hook leaf extracts and fractions against *Staphylococcus aureus*, *Streptococcus mutans*, and *Propionibacterium acnes*

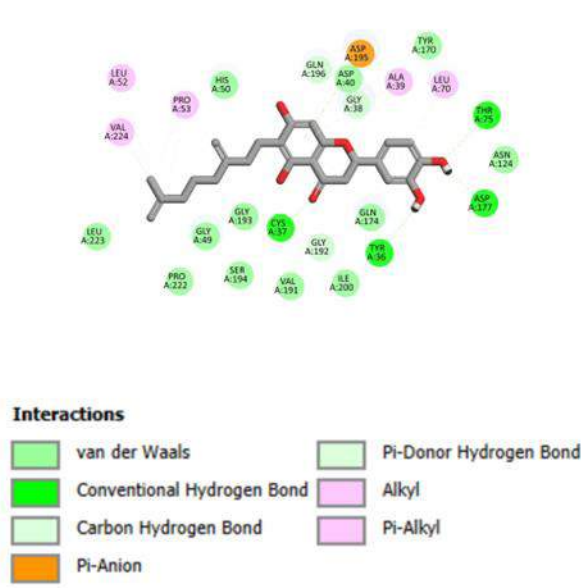
Sample	Concentration (%)	Inhibition Zone Diameter (mm)*		
		<i>Staphylococcus aureus</i>	<i>Streptococcus mutans</i>	<i>Propionibacterium acnes</i>
Methanol Extract	0	0	0	0
	0.15	6.67 ± 0.58	6.67 ± 0.58	0
	0.31	6.67 ± 0.58	6.67 ± 0.58	7.67 ± 0.58
	0.62	6.67 ± 0.58	7.0 ± 0.0	8.0 ± 1.0
	1.25	7.17 ± 0.29	7.33 ± 0.58	9.67 ± 0.58
	2.5	7.5 ± 0.5	8.0 ± 1.0	10.67 ± 0.58
	MIC (%)	<0.15	<0.15	0.15-0.31
n-Hexane Fraction	0	0	0	0
	0.15	0	0	0
	0.31	0	0	0
	0.62	0	0	0
	1.25	0	6.33 ± 0.29	6.33 ± 0.58
	2.5	6.5 ± 0.5	6.67 ± 0.58	6.67 ± 0.58
	MIC (%)	1.25-2.5	0.62-1.25	0.62-1.25
Ethyl acetate Fraction	0	0	0	0
	0.15	6.33 ± 0.58	6.33 ± 0.58	6.67 ± 0.58
	0.31	6.5 ± 0.5	6.67 ± 0.58	8.0 ± 0.0
	0.62	6.5 ± 0.5	6.83 ± 0.29	9.0 ± 0.0
	1.25	6.67 ± 0.29	7.0 ± 0.0	11.0 ± 1.0
	2.5	7.0 ± 0.0	7.67 ± 0.58	14.67 ± 0.58
	MIC (%)	<0.15	<0.15	<0.15
Ampicillin	0.01	20.33 ± 1.15	21.0 ± 1.0	21.0 ± 1.0

*Data were obtained from triplicate experiments.

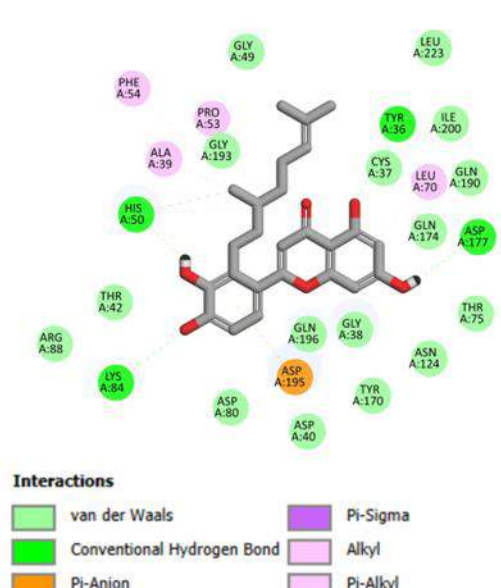
Table 2: Binding affinity of flavonoids against bacterial proteins

No.	Compound	Binding Affinity (kcal/mol)		
		1JIJ	3IPK	7LBU
1	4'-O-methyl-8-isoprenylnaringenin	-7.4	-8.1	-7.7
2	4'-O-methyl-8-isoprenyleriodictyol	-7.2	-8.1	-7.6
3	6-Isoprenyleriodictyol	-7.7	-8.7	-8.0

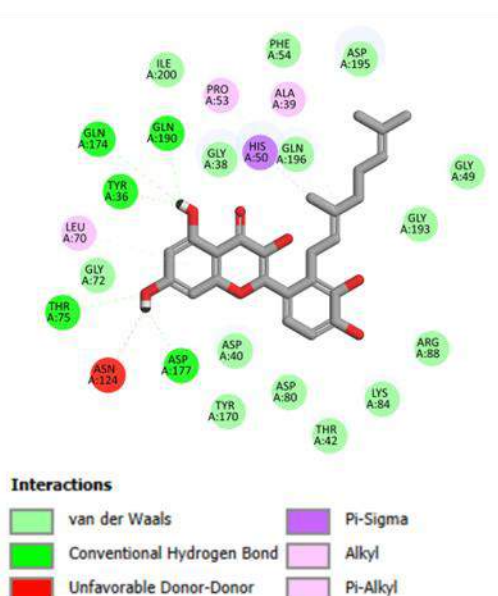
4	5-hydroxy-7,4'-dimethoxyflavone	-8.6	-7.8	-7.1
5	5-hydroxy-6,7,4'-trimethoxyflavone	-8.3	-7.5	-7.3
6	Macahuilettiin A	-7.6	-8.4	-7.1
7	Lonchocarpol A	-9.4	-7.8	-7.8
8	Nymphaeol A	-9.7	-8.9	-8.4
9	Nymphaeol B	-10.5	-8.9	-7.7
10	Nymphaeol C	-8.4	-8.3	-7.4
11	Solophenol D	-10.0	-8.7	-7.9
12	Ampicillin	-8.5	-7.7	-7.6



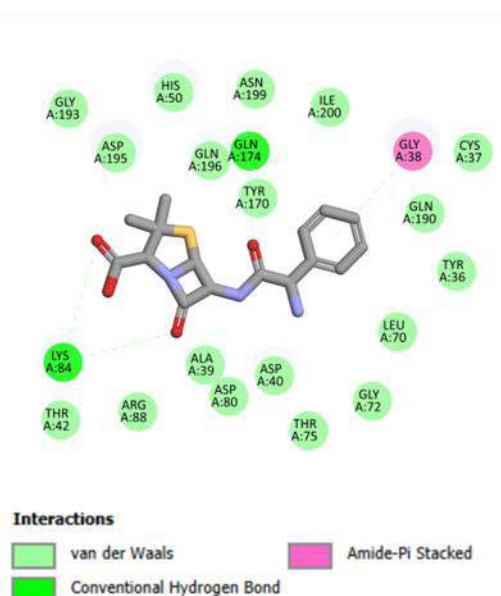
Nymphaeol A



Nymphaeol B



Solophenol D



Ampicillin

Figure 1: 2D visualization of interaction of the best docked flavonoids and ampicillin with *Staphylococcus aureus* tyrosyl-tRNA synthetase (PDB ID: 1JIJ)

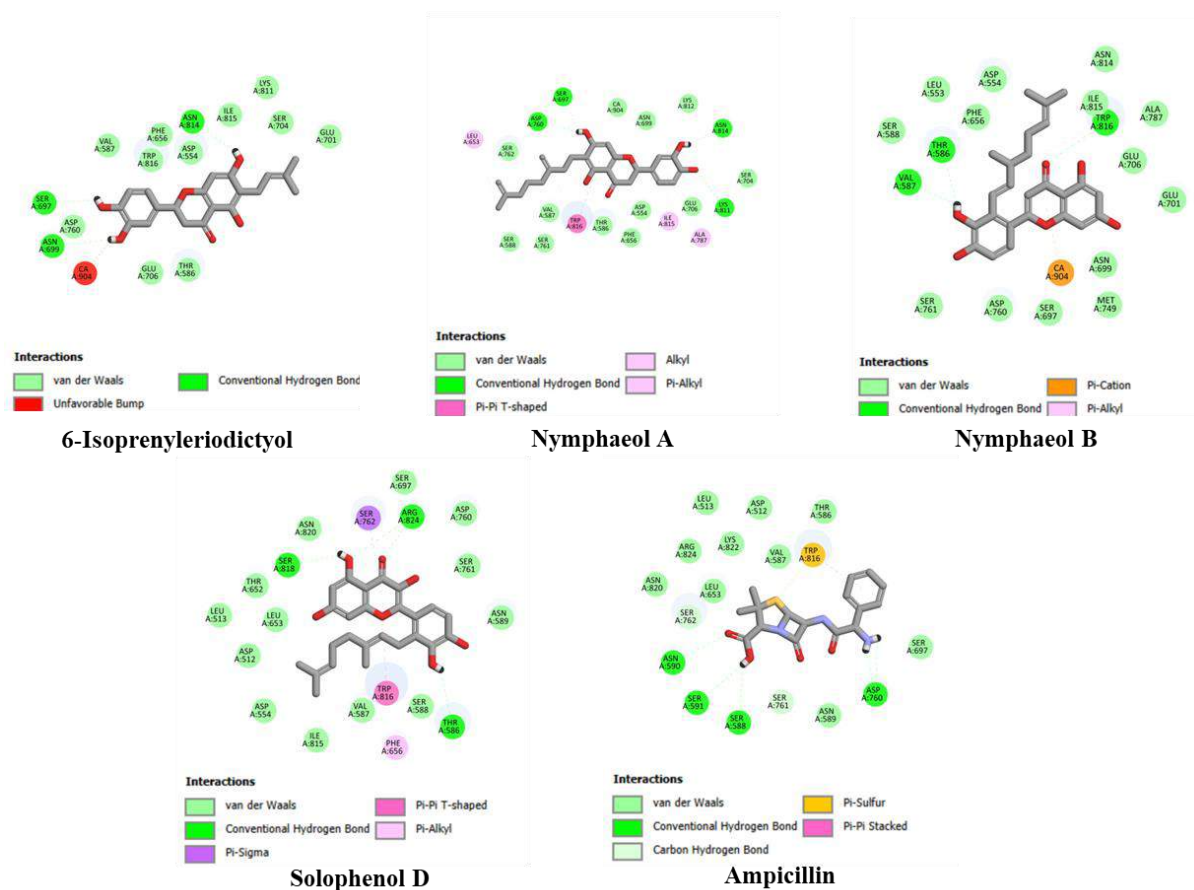


Figure 2: 2D visualization of interaction of the best-docked flavonoids and ampicillin with *Streptococcus mutans* antigen I/II (PDB ID: 3IPK).

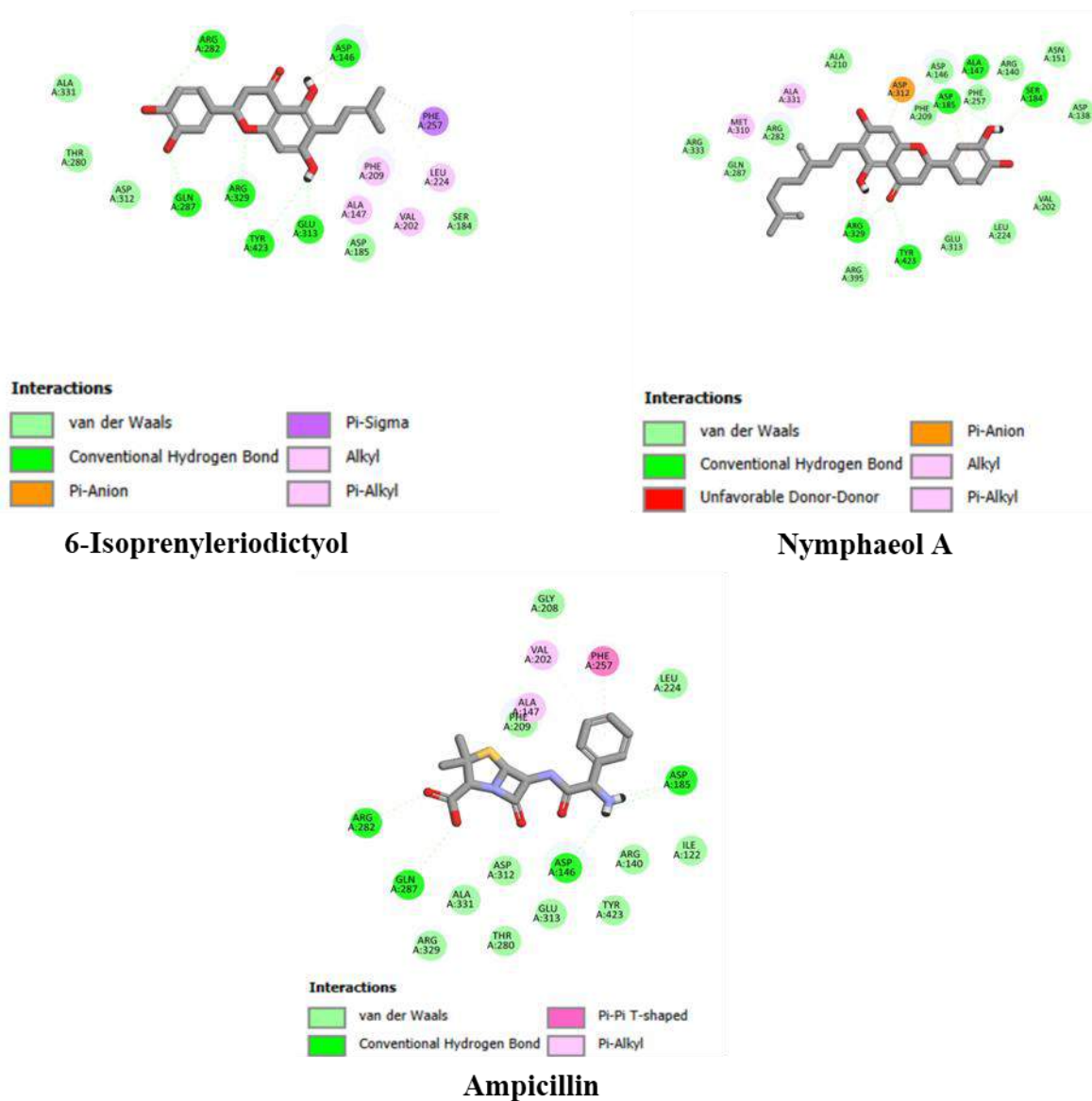


Figure 3: 2D visualization of interaction of the best-docked flavonoids and ampicillin with *Propionibacterium acnes* surface sialidase (PDB ID: 7LBU).



Outlook

English Editing from Tropical Journal of Natural Product Research

From Ade Danova <adedanova@itb.ac.id>**Date** Sun 5/11/2025 8:58 AM**To** erharuyiosaz@gmail.com <erharuyiosaz@gmail.com> 1 attachment (881 KB)

3-TJNPR-2025-420 Revised_Proofreading (1).docx;

Dear Dr Erharuyi

I am Ade Danova, would like to ask your help to revise an English for my manuscript. Here, I attach our manuscript for English Editing. Thank you very much.

Best regards,
Ade Danova

Re: Revision

From Editor-in-Chief Tjnpr <editor.tjnpr@gmail.com>

Date Wed 5/21/2025 8:24 PM

To Ade Danova <adedanova@itb.ac.id>

 1 attachment (882 KB)

5-TJNPR-2025-420 Revised_Proofreading (1)(1).docx;

You still have not done the corrections. Authors advised to carefully revise and make all corrections as directed

Best regards

Abiodun

Professor Abiodun Falodun, PhD; FAAS, FISPON

Editor-in-Chief:

Tropical Journal of Natural Product Research (TJNPR)

Head, Natural Product Research Group, University of Benin

Email: editor.tjnpr@gmail.com

www.tjnpr.org SCOPUS, SCImago SJR Q3

Professor of Pharmaceutical Chemistry, FAAS

Fellow, Fulbright (USA)

Deputy Vice-Chancellor (Academic) 2014-2016

Faculty of Pharmacy

University of Benin

[Google Scholar Citations](#)

SCOPUS <https://www.scopus.com/authid/detail.uri?>



www.tjnpr.org

***In vitro* Evaluation and Molecular Docking Study of Antibacterial Potentials of
Macaranga hulletti King ex Hook.f. Leaf Extract**

Commented [EF1]: of

Eva Marliana^{1,3}, Ade Danova^{2*}, Rita Hairani^{1,3}, Ritbey Ruga^{1,3}, Elvira Hermawati², and Ana Yulvia⁴

1. Department of Chemistry, Faculty of Mathematics and Natural Science, Mulawarman University, Samarinda 75123, East Kalimantan, Indonesia.

2. Organic Chemistry Division, Department of Chemistry, Faculty of Mathematics and Natural Sciences, Institut Teknologi Bandung Jl. Ganesha No. 10, Bandung 70132, West Java, Indonesia.

3. Research Center for Medicine and Cosmetics from Tropical Rainforest Resources, Mulawarman University, Samarinda, Indonesia

4. Department of Chemistry, Faculty of Mathematics and Natural Science, Universitas Jember, East Java, Indonesia.

*Corresponding author. E-mail: adedanova@itb.ac.id; Tel.: +6281357944698.

Abstract

Due to the resistance of bacteria to antibiotics, there has been increasing interest in the use of plant extracts to fight infections. Plants in the genus *Macaranga* contain secondary metabolites such as flavonoids that have antibacterial properties. *Macaranga hulletti* King ex Hook.f. is particularly noteworthy due to its widespread distribution in East Kalimantan. Therefore, the objective of this study was to investigate the antibacterial potential of *M. hulletti* leaf extracts through *in vitro* and molecular docking studies. The research involved testing the methanol extract, the n-hexane fraction, and the ethyl acetate fraction from *M. hulletti* leaves against

three bacterial species, *Staphylococcus aureus*, *Streptococcus mutans*, and *Propionibacterium acnes*. The results showed that both the methanol extract and ethyl acetate fraction displayed antibacterial activity against the three bacteria, with minimum inhibitory concentrations (MICs) < 0.15%, except for the methanol extract, which had an MIC of 0.15-0.31% against *Propionibacterium acnes*. In addition, molecular docking analysis showed that five flavonoids possessing prenyl or geranyl groups (6-isoprenyleriodictyol, nymphaeol A, nymphaeol B, and solophenol D) showed the highest binding affinity and dominant hydrogen bonding interactions with key amino acid residues in the binding sites of three bacterial proteins (IJJ, 3IPK, and 7LBU). The findings suggest that the methanol extract and ethyl acetate fraction from *Macaranga hulletti* have potential applications as new sources of antibacterial agents. Thus, further studies are needed to isolate and evaluate the bioactive compounds.

Keywords: Antibacterial, Extract, Flavonoid, *Macaranga hulletti*, Molecular Docking.

Introduction

Despite substantial advancements in medicine and fundamental research, infectious diseases caused by transmissible agents such as bacteria, protists, and viruses continue to present significant challenges to healthcare professionals. These challenges include the issue of antimicrobial resistance.¹ Extracts from medicinal plants present a promising approach for addressing multidrug-resistant bacteria. In addition, medicinal plants can help restore the efficacy of antibiotics to treat infectious diseases.² Therefore, it is imperative to develop more efficacious antimicrobial agents, particularly those sourced from natural sources, as these are both readily available and economically viable.

Plants in the genus *Macaranga* belong to the family Euphorbiaceae and are commonly known as "Mahang." The secondary metabolites contained in plants of this genus include flavonoids

and stilbenoids with prenylated substituents.³⁻¹³ Nine flavonoids containing either prenyl or geranyl groups and displaying antioxidant, antiparasmodial, and anticancer activities have been isolated from three species of *Macaranga* present in East Kalimantan (*Macaranga hosei*, *Macaranga pearsonii*, and *Macaranga tanarius*): 4'-*O*-methyl-8-isoprenylnaringenin, lonchocarpol A, 4'-*O*-methyl-8-isoprenyleriodytyol, 6-isoprenyleriodytyol, solophenol D, nymphaeol A, nymphaeol B, and nymphaeol C, including macahuilletin A that was isolated from *Macaranga hullettii* obtained from Central Kalimantan.¹⁴⁻¹⁸ *Macaranga tanarius* leaf extract exhibited antibacterial activity.¹⁹ The plant extracts of *Macaranga conglomerata*, *Macaranga kilimandscharica*, and *Macaranga capensis* from Kenya showed antibacterial activities against *E. coli*, *E. aerogenes*, *K. pneumoniae*, *P. stuartii*, *P. aeruginosa*, and *S. aureus*.²⁰ The flavonol rhamnetin, isolated from *Macaranga peltata*, also showed antibacterial activity against *Staphylococcus aureus*.²¹ Two flavanone groups obtained from *Macaranga hosei*, 5-hydroxy-7,4'-dimethoxyflavone and 5-hydroxy-6,7,4'-trimethoxyflavone, showed weak antibacterial activity against three pathogenic bacteria.²² In this study, the leaves of *Macaranga hullettii* King ex Hook.f. were used to identify new sources of medicinal compounds to treat infectious diseases caused by bacteria. This plant is found in all areas of East Kalimantan, where the species commonly occurs in mountain forests and logging areas in the Kalimantan region.^{23, 24} Phytochemical screening and isolation of secondary compounds from the leaf extract of *Macaranga hullettii* identified several flavonoid compounds.^{18,25} The methanol extract and its fractions derived from *Macaranga hullettii* have not yet been analyzed for their antibacterial effects. Therefore, this study aimed to assess the antibacterial activity of *Macaranga hullettii* leaf extract and its fractions using *in vitro* studies. The flavonoids isolated from *Macaranga* species in East Kalimantan, Indonesia, were evaluated for their antibacterial activity using molecular docking analysis.

Commented [EF2]: Italics

Commented [EF3]: Italics

Materials and Methods

Materials

The solvents used (methanol, n-hexane, and ethyl acetate) were purified by distillation before use. The materials used in this study were *Macaranga hullettii* leaves, *Staphylococcus aureus* ATCC 25923 and *Streptococcus mutans* ATCC 25175 obtained from Chulalongkorn University, *Propionibacterium acnes* KCCM 41747 obtained from Mulawarman University, nutrient agar (NA) from Merck, Luria Bertani liquid medium (1% tryptone, 0.5% yeast, and 0.5% NaCl), yeast extract (Oxoid 100% (w/w)), tryptone (Oxoid 100% (w/w)), ampicillin 100 mg/ml (Sigma Aldrich), NaCl (Sigma Aldrich), cotton swabs, aluminum foil, gauze, Petri dishes, Ose needles, paper discs, an autoclave (Autoclave TOMY, Model SX-700), an oven (SHARP EO-28 WH), a rotary vacuum evaporator (EYELA Rotary evaporator N-1300E V.S.Series), and a freezer (Sanyo Medicoool). For molecular docking analysis, we used a Lenovo Yoga 7 computer with AMD Ryzen AI 7-8840HS w/ Radeon 780M graphics, 3301 Mhz, 8 Core(s), and 16.0 GB of RAM.

Methods

Plant Collection and Identification:

The leaves of *Macaranga hullettii* plants were collected from the Lesan River Forest, Kelay District, Berau Regency, East Kalimantan, Indonesia (1°32'2.5919" N, 117°3'11.950" E). The plant samples were identified at the Laboratory of Anatomy and Plant Systematics, FMIPA Mulawarman University (No. 0174/UN.17.8.5.7.16/HA/XI/2017).

Extraction

Macaranga hullettii leaf powder (1.3 kg) was obtained by maceration twice for 24 h using 7.0 L methanol. A rotary vacuum evaporator was then used to obtain a methanol extract (126 gram).

Commented [EF4]: Plant Collection and Identification:

Commented [EF5]: were

After that, methanol extract (100 gram) was dissolved in the mixture of methanol and H₂O 500 mL with ratio of 8:2 and then successively fractionated using 5 x 200 mL of n-hexane and ethyl acetate to obtain n-hexane fraction (28 gram) and ethyl acetate fraction (18 gram), respectively. Each fraction was evaporated using a rotary vacuum evaporator. The methanol extract, n-hexane fraction, and ethyl acetate fraction were evaluated for antibacterial activity.

Commented [EF6]: Volume of solvents?

Pathogens

Staphylococcus aureus ATCC 25923 was obtained from the Department of Microbiology, Faculty of Science, Chulalongkorn University. *Streptococcus mutans* ATCC 25175 was obtained from the Department of Biochemistry, Faculty of Dentistry, Chulalongkorn University. Both strains were purchased from the American Type Culture Collection (ATCC). *Propionibacterium acnes* KCCM 41747 was obtained from the Faculty of Forestry, Mulawarman University, and from the Korean Culture Center of Microorganisms (KCCM). The bacteria were periodically subcultured and maintained in nutrient agar (NA) under suitable conditions.

Commented [EF7]: How? Organisms are not prepared?

In vitro antibacterial assay

The antibacterial properties of the methanol, n-hexane, and ethyl acetate extracts of *Macaranga hullettii* leaves were analyzed using the disc paper or the Kirby-Bauer method.^{26,27} *Staphylococcus aureus* ATCC 25923, *Streptococcus mutans* ATCC 25175, and *Propionibacterium acnes* KCCM 41747 were assayed. Ampicillin was used as a positive control, and the three organic solvents were used as negative controls. Nutrient Agar (NA) medium was prepared by dissolving 3.3 grams in 150 mL of distilled water. Luria Bertani (LB) liquid media was prepared by dissolving 4 grams in 500 mL of distilled water, stirring, and adding 5 mL to a test tube. The NA and LB media were sterilized using an autoclave at 121 °C

with a pressure of 1 atm for 15 minutes. Each tested bacterium was collected using an Ose needle, followed by dilution in a test tube containing sterile LB media and incubation for 24 h at 37 °C.

Nutrient Agar (25 mL) was poured into sterilized Petri dishes and maintained at room temperature for solidification. A sample of the bacterial suspension in the test tubes was then collected using a sterile cotton swab and applied to the surface of the agar in the Petri dish. Each 30 µL of the tested *M. hulleitii* leaf samples prepared in various concentrations of the organic solvents was poured onto sterile adsorbent filter paper discs (6 mm in diameter), and the solvent was removed. Afterward, the paper discs containing the tested sample were placed on the surface of the agar media and incubated at 37°C for 18-24 hours. The inhibition zone formed in each well was measured using a ruler.^{28,29} The minimum inhibitory concentration (MIC) was determined for the methanol extract as well as the n-hexane and ethyl acetate fractions. MIC was defined as the minimum concentration that completely inhibited bacterial growth.³⁰

Molecular docking

Eight flavonoid derivatives possessing either prenyl or geranyl group occurred in the three *Macaranga* species in East Kalimantan, Indonesia (*Macaranga hosei*, *Macaranga pearsonii*, and *Macaranga tanarius*): 4'-O-methyl-8-isoprenylraringenin, lonchocarpol A, 4'-O-methyl-8-isoprenylreriodytol, 6-isoprenylreriodytol, solophenol D, nymphaeol A, nymphaeol B, and nymphaeol C.¹⁴⁻¹⁷ Macahulleitiiin A is a prenylated flavonoid found in *Macaranga hulleitii* obtained from Central Kalimantan (Indonesia).¹⁸ A comparative analysis of the molecular docking results for prenylated/geranylated flavonoids with methoxylated flavonoids was

conducted, focusing on 5-hydroxy-7,4'-dimethoxyflavone and 5-hydroxy-6,7,4'-trimethoxyflavone that are found in *Macaranga hosei* from Trengganu, Malaysia²² (Table 2).

The molecular structures of the flavonoids were drawn using ChemOffice Professional 15.0 and obtained from PubChem (<https://pubchem.ncbi.nlm.nih.gov/>). Structure refinement was performed using the molecular mechanics energy minimization method with the Merck molecular force field (MMFF94) using Open Babel in PyRx V.1.1. Three proteins were downloaded from the protein data bank (PDB ID: 1JJJ, 3IPK, 7LBU),³¹⁻³³ and the locations of active sites were calculated using AlphaFold from <https://prankweb.cz/>.³⁴ Molecular docking was performed using the AutoDock Vina tool in PyRx V.1.1 software with an exhaustiveness of 32 and a mode value of 9 poses for each docked ligand.^{35,36} A protein binding site was defined as a box with dimensions of 30 × 30 × 30 Å. In the final step, the binding interactions were analyzed, and the docking results were visualized in 2D using the BIOVIA Discovery Studio Visualizer.

Statistical analysis

Data from the experiments were analyzed using Microsoft Excel.

Results and Discussion

In vitro antibacterial activity

The strength of antibacterial activity was determined based on the diameter of the clear zone, which was used to measure the inhibition zone (mm). This classification was as follows: ≤ 5 mm (weak), 5-10 mm (moderate), 10-20 mm (strong), and ≥ 20 mm (very strong). Moreover, the lower the minimum inhibitory concentration of a compound, the greater its ability to inhibit the growth of test bacteria.²⁸ In this study, ampicillin was used as a positive control against three bacteria: *Staphylococcus aureus* ATCC 25923, *Streptococcus mutans* ATCC 25175, and

Propionibacterium acnes KCCM 41747 (Table 1). The positive control served to determine whether the test bacteria could be inhibited by or were resistant to the antibiotics.

Staphylococcus aureus can cause dentoalveolar infections, jaw cysts, paroritis, oral mucosal lesions, and stomatitis. The bacteria can also produce exotoxins, lecosidine, enterotoxins, and quagulase compounds. Enterotoxins are compounds that cause food poisoning in humans.³⁷ The methanol extract, hexane fraction, and ethyl acetate fraction of *Macaranga hulettii* leaves showed moderate antibacterial activity against *S. aureus*, with an inhibition zone of 5-10 mm at concentrations of 0.15 to 2.5% (Table 1). The inhibition zone of the 0.15% methanol extract measured 6.67 mm. The 0.15% ethyl acetate fraction produced an inhibition zone of 6.33 mm, and the 2.5% n-hexane fraction produced an inhibition zone of 6.50 mm. The results indicated that the methanol extract and ethyl acetate fraction had the most potent antibacterial activity against *S. aureus*, with a minimum inhibitory concentration (MIC) of < 0.15%. Moreover, the hexane fraction of *Macaranga tanarius* was most effective against *S. aureus*, with MIC values of 0.125-0.5% and an inhibition zone of approximately 7.25 mm.³⁸

Streptococcus mutans is implicated in the pathogenesis of dental caries. This facultative anaerobe primarily ferments carbohydrates, resulting in the production of several organic acids, with lactic acid as the predominant byproduct. The acidification of the dental environment by *S. mutans* is a crucial factor contributing to enamel demineralization and the subsequent formation of carious lesions.^{39,40} The bacteria produce glucosyltransferase (GTF) to produce glucan, the compound that causes dental caries.⁴¹ *S. mutans* adheres to the salivary pellicle due to having many receptors for attachment.⁴² We tested the antibacterial activity of *Macaranga hulettii* King leaf extract against *S. mutans* (Table 1). Both extracts displayed moderate antibacterial activity, with inhibition zones of 5-10 mm at concentrations of 0.15-2.5%. The widths of the inhibition zones created by the methanol extract and ethyl acetate fraction were 6.67 and 6.33 mm at 0.15%, respectively. Meanwhile, the n-hexane fraction showed

antibacterial activity at concentrations of 1.25-2.5%, with inhibition zones of 6.33-6.67 mm. These results demonstrated that the methanol extract and ethyl acetate fraction of *Macaranga hulettii* leaves presented the highest potential antibacterial activity against *S. mutans*, with an MIC < 0.15% (Table 1).

Propionibacterium acnes is a Gram-positive anaerobic bacterium that can induce unusual keratinization within the sebaceous glands of hair follicles and increase sebum production.⁴³ The enzyme is capable of hydrolyzing triglycerides (TG) present in sebum into free fatty acids (FFA) that may subsequently induce inflammation in and around the hair follicles.⁴⁴ The bacteria also produce hyaluronidase, protease, lecithinase, and neuraminidase, that can cause inflammation of the skin. *Macaranga triloba* ethanol can prevent the growth of *P. acnes*, and the most significant inhibition value of 5.54 mm at 20%.⁴⁵ As shown in Table 1, the methanol extract and ethyl acetate fraction of *Macaranga hulettii* showed potent antibacterial activity against *P. acnes*, with respective inhibition values of 10.67 and 14.67 mm. The n-hexane fraction exhibited moderate antibacterial activity, with inhibition values of 5-10 mm at 1.25-2.5%. The methanol extract showed antibacterial activity with an inhibition value of 7.67 at 0.31%. The ethyl acetate fraction showed antibacterial activity of 0.15%, with an inhibition zone of 6.67 mm. The results demonstrated that the ethyl acetate fraction of *Macaranga hulettii* leaves presented the highest potential antibacterial activity against *P. acnes*, with a minimum inhibitory concentration (MIC) of < 0.15% (Table 1).

The methanol extract and ethyl acetate fraction exhibited good antibacterial activity against the three bacterial strains. This finding was consistent with previous studies that attributed the antibacterial effects to the presence of flavonoids and their prenylated or geranylated forms in species of *Macaranga*. These included euchrestaflavanone A, bonanione A, macarangaflavanone A, macarangaflavanone B, macatrichocarpin A, propolin D (nymphaeol B), senegalensin, schweinfurthin B, schweinfurthin O, and isomacarangain.⁴⁶⁻⁵⁶ Flavonoid

compounds can damage bacterial cell walls and denature proteases, thereby disrupting bacterial metabolism.⁵⁷ In addition, alkaloids can damage the peptidoglycan layer of bacterial cells, damaging the cell wall and inhibiting bacterial growth. Terpenoids also inhibit bacterial growth by damaging cell membranes. The phenolic compounds of secondary metabolites can damage bacterial cells by increasing the permeability of the cell membrane, resulting in cytoplasmic coagulation and cell division.²⁸

Molecular docking

Molecular docking was performed to predict the interactions of the flavonoids with the amino acid residues of the three proteins. This study used three proteins from *Staphylococcus aureus*, *Streptococcus mutans*, and *Propionibacterium acnes*.³¹⁻³³ The molecular docking results (Table 2) revealed that 11 flavonoid derivatives had binding affinities ranging from -7.2 to -10.5 kcal/mol, -7.5 to -8.9 kcal/mol, and -7.1 to -8.9 kcal/mol against *Staphylococcus aureus* tyrosyl-tRNA synthetase, *Streptococcus mutans* antigen I/II (AgI/II), and *Propionibacterium acnes* surface sialidase, respectively. The interactions of flavonoids and ampicillin with the protein targets are shown in two-dimensional graphs in Figures 1-3.

Among the compounds targeting *S. aureus* tyrosyl-tRNA synthetase, nymphaeol A-B and solophenol D had the highest binding affinities compared to ampicillin as a positive control.

Thus, the interactions of these compounds with the active site of the enzyme were modeled as a 2D structure (Figure 1). Ampicillin formed hydrogen bonds with LYS84 and GLN174 and amide- π interactions with GLY38 (Figure 1). Nymphaeol A and nymphaeol B formed hydrogen bonds with four amino acid residues, π -anions, and hydrophobic interactions, whereas nymphaeol B and ampicillin showed similar interactions with LYS84. In addition, solophenol D formed hydrogen bonds with TYR36, THR75, GLN190, ASP177, and GLN174, as well as hydrophobic interactions with HIS50, LEU70, PRO53, and ALA39 (Figure 1). These results

agreed with previous studies where nymphaeol A-B and solophenol D exhibited antibacterial activity.^{58,59}

Five compounds exhibited high antibacterial activity against *S. mutans* antigen I/II (AgI/II), including 6-isoprenyleriodictyol, nymphaeol A-B, and solophenol D, with binding affinities from -8.7 to -8.9 kcal/mol. The interactions between the ligands and amino acid residues in the binding sites of these compounds are shown in 2D views (Figure 2). Ampicillin formed hydrogen bonds with four amino acid residues, ASN590, SER591, SER588, and ASP760, as well as π -sulfur and π - π interactions with TRP 816, while 6-isoprenyleriodictyol only formed hydrogen bonds with three amino acid residues: SER697, ASN699, and ASN814. Nymphaeol A and nymphaeol B formed hydrogen bonds with four amino acid residues (Figure 2), but nymphaeol A exhibited a similar π - π interaction with TRP816, as observed with ampicillin. Solophenol D formed hydrogen bonds with SER818, ARG824, and THR586, similar to the π - π interactions (TRP816) with ampicillin, and π -sigma interactions with SER762. As 6-isoprenyleriodictyol is a prenylated flavonoid similar to nymphaeol A, and hence may have antibacterial activity.

Three compounds, including 6-isoprenyleriodictyol and nymphaeol A, displayed stronger binding affinities than ampicillin, with values of -8.0 and -8.4 kcal/mol against *P. acnes* surface sialidases. Therefore, both compounds were imaged in 2D to investigate the interactions between the ligands and the amino acid residues in the binding sites (Figure 3). Ampicillin formed hydrogen bonds with four amino acid residues, ARG282, GLN287, ASP146, ASP185, and π - π and π -alkyl interactions with PHE257, ALA147, and VAL202; 6-Isoprenyleriodictyol demonstrated π - π interactions with PHE257, π -anion interactions with ASP146, and alkyl-alkyl interactions with ALA147, PHE209, VAL202, and LEU224. This compound formed bonds with six amino acid residues, including ASP146, ARG329, TYR423, ARG282, and GLU287, that were analogous to those observed with ampicillin. Nymphaeol A formed hydrogen bonds

with five amino acid residues (ARG329, TYR423, ASP185, ALA147, and SER184), π -anion interactions with ASP312 and ASP185, alkyl-alkyl interactions with MET310, ALA331, and ALA147, and Van der Waals interactions with PHE257. Thus, these compounds may have potent antibacterial activity.

Structure-activity relationships were also studied based on the binding affinity results (Table 2). Flavonoids possessing prenyl, geranyl, and hydroxyl groups showed potential as antibacterial agents due to their strong affinity for amino acid residues in the binding sites of proteins, compared to ampicillin. However, the absence of a hydroxyl group on prenylated or geranylated flavonoids decreased the binding affinity.^{22,56,60}

Conclusion

The extracts and fractions of *Macaranga hulettii* have potential as antibacterial agents against three bacterial strains, *Staphylococcus aureus* ATCC 25923, *Streptococcus mutans* ATCC 25175, and *Propionibacterium acnes* KCCM 41747. The methanol extract demonstrated antibacterial activity against *S. aureus* and *S. mutans* with an MIC (%) of < 0.15 against *S. aureus* and *S. mutans*, and 0.15-0.31% against *P. acnes*. The n-hexane fraction had an MIC (%) of 1.25-2.5% against *S. aureus*, and 0.62-1.25% against *S. mutans* and *P. acnes*. The ethyl acetate fraction had an MIC (%) < 0.15% against the three bacterial strains. Molecular docking studies suggested that 6-isoprenyliodictyol, nymphaeol A, nymphaeol B, and solophenol D are promising antibacterial agents, as those compounds exhibited the highest binding affinity and formed hydrogen bonds with key amino acid residues in the active sites of the protein targets. Further *in vitro* and *in vivo* studies should be conducted to isolate the active secondary metabolites from this species.

Conflict of Interest

The authors declare that they have no conflicts of interest.

Author's Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims related to the content of this article will be borne by them.

Acknowledgement

This research was funded by the Islamic Development Bank (PIU-IsDB) project under contract no. 137/UN17.11/PL/2019.

References

1. Wong F, de la Fuente-Nunez C, Collins JJ. Leveraging artificial intelligence in the fight against infectious diseases. *Science*. 2023; 381(6654):164-70.
2. Seukep AJ, Kuete V, Nahar L, Sarker SD, Guo M. Plant-derived secondary metabolites as the main source of efflux pump inhibitors and methods for identification. *J. Pharm. Anal.* 2020; 10(4):277-90.
3. Magadula JJ. Phytochemistry and pharmacology of the genus *Macaranga*: a review. *J. Med. Plants Res.* 2014; 8(12):489-503.
4. Vu LT, Ngan TB, Phuong L, Huong DT, Litaudon M, Van Hung N, Thach TD, Van Cuong P. Chemical constituents from fruits of *Macaranga denticulata* (Euphorbiaceae)(Part 2). *Vietnam J. Chem.* 2018; 56(4):516-20.
5. Marlina E, Hairani R, Tjahjandarie TS, Tanjung M. Antiplasmodial activity of flavonoids from *Macaranga tanarius* leaves. *IOP Conf. Ser.: Earth Environ. Sci.* 2018; 144(1):012011.

6. Tjahjandarie TS, Tanjung M, Saputri RD, Nadar PB, Aldin MF, Marlana E, Permadi A. Flavestins K, An isoprenylated stilbene from the leaves of *Macaranga recurvata* Gage. Nat. Prod. Sci. 2019; 25(3):244-7.
7. Huong DT, Linh NT, Van TT, Litaudon M, Roussi F, Van Nam V, Van Cuong P. Stilbenes from *Macaranga tanarius* (Euphorbiaceae) growing in Vietnam. Vietnam J. Chem. 2020; 58(3):338-42.
8. Aldin MF, Tjahjandarie TS, Saputri RD, Tanjung M. Macasiamenene V, a New Stilbenoid from the Leaves of *Macaranga inermis*. Nat. Prod. Sci. 2021; 27(1):45-8.
9. Muharram A, Rachmawati DA, Mardhiyyah S, Tjahjandarie TS, Saputri RD, Ahmat N, Tanjung M. Cytotoxic and antioxidant activities of flavonoids and diterpenoids from *Macaranga involucreta* (Roxb.) Baill. J. Appl. Pharm. Sci. 2023; 13(6):087-92.
10. Tanjung M, Tjahjandarie TS, Aldin MF, Mardhiyyah S, Ahmat N, Saputri RD. Macagigantin A, a new flavonoid from *Macaranga gigantea* (Rchb. f & Zoll.) Mull. Arg. Nat. Prod. Sci. 2023; 29(4):287-94.
11. Tjahjandarie TS, Aldin MF, Saputri RD, Tanjung M. Dihydrostilbenes from *Macaranga javanica* (Blume) Müll. Arg. and their antiplasmodial activity. Nat. Prod. Sci. 2024:1-7.
12. Tanjung M, Tjahjandarie TS, Aldin MF, Mardhiyyah S, Saputri RD, Syah YM, Ahmat N. Two new flavonols from *Macaranga inermis* Pax & K. Hoffm. Nat. Prod. Sci. 2025; 39(3):498-505.
13. Tanjung M, Tjahjandarie TS, Aldin MF, Mardhiyyah S, Saputri RD, Syah YM, Ahmat N. Two new flavonols from *Macaranga inermis* Pax & K. Hoffm. Nat. Prod. Sci. 2025; 39(3):498-505.
14. Marlana E, Tjahjandarie TS, Tanjung M. Isoprenylated flavanone derivatives from *Macaranga hosei* King ex Hook f. Der Pharm. Lett. 2015; 7(3):153-6.

15. Marliana E, Tjahjandarie TS, Tanjung M. Antioxidant activity of flavonoid compounds from *Macaranga pearsonii* Merr. J. Kim. Mulawarman. 2016; 13(2):97-100.
16. Marliana E, Astuti W, Kosala K, Hairani R, Tjahjandarie TS, Tanjung M. Chemical composition and anticancer activity of *Macaranga hosei* leaves. Asian J. Chem. 2018; 30(4):795-8.
17. Marliana E, Ruga R, Hairani R, Tjahjandarie TS, Tanjung M. Antioxidant activity of flavonoid constituents from the leaves of *Macaranga tanarius*. IOP Conf. Ser.: J. Phys. 2019; 1277(1):012014.
18. Saputri RD, Tukiran T, Wati FA, Purnamasari AP, Wardhana MW, Tjahjandarie TS, Tanjung M. Macahuilettiin A, a new isoprenylated flavanone from the leaves of *Macaranga hullettii* King ex Hook and their antiplasmodial activity. Vietnam J. Chem. 2024; 62(3):394-8.
19. Musdalifah M, Khumaidi A, Suwastika IN. Inhibition test and phytochemical screening of leaf extracts of *Macaranga tanarius* (L.) Mull. Arg against *Salmonella typhi* as an antibacterial. Nat. Sci: J. Sci. Technol. 2017; 6(3).
20. Ibrahim H, Omosa L, Nchiozem-Ngnitedem V, Onyari J, Maru S, Guefack M. Antibacterial activities and phytochemical screening of crude extracts from Kenyan macaranga species toward MDR phenotypes expressing efflux pumps. Pharmacogn. Commn. 2021; 11(2):119-26.
21. Bijesh K, Sebastian D. Isolation and characterization of antibacterial compounds from *Macaranga peltata* against clinical isolates of *Staphylococcus aureus*. Int. J. Biol. Pharma Res. 2013; 4:1196-203.
22. Salleh WM, Razak NZ, Ahmad F. Phytochemicals and biological activities of *Macaranga hosei* and *Macaranga constricta* (Euphorbiaceae). Marmara Pharm. J. 2017;21(4):881-8.

23. Slik JW, Priyono P, Welzen PV. Key to the *Macaranga Thou.* and *Mallotus Lour.* species (Euphorbiaceae) of East Kalimantan, Indonesia. Singapore, Gardens' Bulletin (Singapore), National Parks Board, Singapore Botanic Gardens; 2000. 11-87 p.
24. Amirta R, Angi EM, Ramadhan R, Kusuma IW, Wiati CB, Haqiqi MT. Potential utilization of macaranga. Samarinda: Mulawarman University Press; 2017.
25. Rismawati R, Marlina E, Daniel D. Phytochemical test on methanol extract of leaf of *Macaranga hullettii* King ex Hook. f. J. Atomik. 2018; 3(2):91-4.
26. Hudzicki J. Kirby-Bauer disk diffusion susceptibility test protocol. American society for microbiology. 2009; 15(1):1-23.
27. Inna M, Astuti W, Saleh C. Antibacterial activity of methanolic extract of uric patch plant leaves (*Cayratia carnos*) against *Salmonella thypi* and *Propionibacterium acnes*. J. Atomik. 2022; 7(1):1-5.
28. Yulianti MF, Amat AL, Hutasoit RM, Pakan PD. Antibacterial activity of jamblang leaf ethanol extract (*Syzygium cumini*) against the growth of *Propionibacterium acnes*. Acta Biochimica Indones. 2023; 6(2):1161.
29. Hossain MR, Biplob AI, Sharif SR, Bhuiya AM, Sayem AS. Antibacterial activity of green synthesized silver nanoparticles of *Lablab purpureus* flowers extract against human pathogenic bacteria. Trop. J. Nat. Prod. Res. 2023;7(8).
30. Yanda L, Tatsimo SJ, Tamokou JD, Matsute-Takongmo G, Meffo-Dongmo SC, Meli Lannang A, Sewald N. Antibacterial and antioxidant activities of isolated compounds from *Prosopis africana* leaves. Int. J. Anal. Chem. 2022; 4205823.
31. Qiu X, Janson CA, Smith WW, Green SM, McDevitt P, Johanson K, Carter P, Hibbs M, Lewis C, Chalker A, Fosberry A. Crystal structure of *Staphylococcus aureus* tyrosyl-tRNA synthetase in complex with a class of potent and specific inhibitors. Protein Sci. 2001; 10(10):2008-16.

32. Larson MR, Rajashankar KR, Patel MH, Robinette RA, Crowley PJ, Michalek S, Brady LJ, Deivanayagam C. Elongated fibrillar structure of a *streptococcal adhesin* assembled by the high-affinity association of α -and PPII-helices. *Proc. Natl. Acad. Sci.* 2010; 107(13):5983-8.
33. Yu AC, Volkers G, Jongkees SA, Worrall LJ, Withers SG, Strynadka NC. Crystal structure of the *Propionibacterium acnes* surface sialidase, a drug target for *P. acnes*-associated diseases. *Glycobiology.* 2022; 32(2):162-70.
34. Jakubec D, Skoda P, Krivak R, Novotny M, Hoksza D. PrankWeb 3: accelerated ligand-binding site predictions for experimental and modelled protein structures. *Nucleic Acids Res.* 2022; 50:W593-7.
35. Dallakyan S, Olson AJ. Small-molecule library screening by docking with PyRx. In *Chemical biology: methods and protocols.* 2014: 243-250. New York, NY: Springer New York.
36. Trott O, Olson AJ. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *J. Comput. Chem.* 2010; 31(2):455-61.
37. Malangu N, editor. Poisoning: From specific toxic agents to novel rapid and simplified techniques for analysis. BoD–Books on Demand; 2017.
38. Sari AA, Saleh C. Phytochemical test, toxicity and antibacterial activity of extracts of various macaranga leaf fractions (*Macaranga tanarius* (L.) MA) against *Staphylococcus aureus* and *Escherichia coli*. *J. Kim. Mulawarman.* 2015; 12(2):53-8.
39. Imelda R., Mariam M., Satari M. Effect of cassava (*manihot esculenta* cranzt), rice (*oryza sativa* l.), and potato (*solanum tuberosum*) water extract to decrease pH phase fermentation of *streptococcus mutans* atcc 25175. *Padjajaran J. Dent.* 2019; 31(1):14.

40. Alkhaled A., Alsabek L., Al-assaf M. , & Badr F.. Effect of chlorhexidine, honey and propolis on *Streptococcus mutans* counts: *in vitro* study. Dentistry. 2021; 9(1).
41. Ren Z, Chen L, Li J, Li Y. Inhibition of *Streptococcus mutans* polysaccharide synthesis by molecules targeting glycosyltransferase activity. J. Oral Microbiol. 2016; 8(1):31095.
42. Lee BS, Chen YJ, Wei TC, Ma TL, Chang CC. Comparison of antibacterial adhesion when salivary pellicle is coated on both poly (2-hydroxyethyl-methacrylate)-and polyethylene-glycol-methacrylate-grafted poly (methyl methacrylate). Int. J. Mol. Sci. 2018; 19(9):2764.
43. Chen KC, Yang CH, Li TT, Zouboulis CC, Huang YC. Suppression of Propionibacterium acnes-stimulated proinflammatory cytokines by Chinese bayberry extracts and its active constituent myricetin in human sebocytes *in vitro*. Phytother. Res. 2019; 33(4):1104-13.
44. Xu J, Chen X, Song J, Wang C, Xu W, Tan H, Suo H. Antibacterial activity and mechanism of cell-free supernatants of *Lactocaseibacillus paracasei* against *Propionibacterium acnes*. Microb. Pathog. 2024; 189:106598
45. Warnida H, Mustika D, Supomo S, Sukawaty Y. Effectiveness of Mahang Leaf Ethanol Extract (*Macaranga Triloba*) as an anti-acne. J. Penelit. Sos. Ekon. Kehuta. 2018; 4(1):9-18.
46. Schütz BA, Wright AD, Rali T, Sticher O. Prenylated flavanones from leaves of *Macaranga pleiostemona*. Phytochemistry. 1995; 40(4):1273-7.
47. Lim TY, Lim YY, Yule CM. Evaluation of antioxidant, antibacterial and anti-tyrosinase activities of four Macaranga species. Food Chem. 2009; 114(2):594-9.
48. Fareza MS, Syah YM, Mujahidin D, Juliawaty LD, Kurniasih I. Antibacterial flavanones and dihydrochalcones from *Macaranga trichocarpa*. Z. Naturforsch C J. Biosci. 2014; 69(9-10):375-80.

49. Hasanat A, Kabir MS, Hossain MM, Hasan M, Al Masum MA, Chowdhury TA, Bhuiyan DI, Mamur A, Kibria AS. Antibacterial activity of methanol extract of *Macaranga denticulata* leaves and in silico PASS prediction for its six secondary metabolites. World J. Pharm. Pharm. Sci. 2015;1258-66.
50. O Akanbi B, Anene P, Olayanju S. Preliminary Screening Indicates Promising antimicrobial properties of the stem bark extracts of *Macaranga rosea*. Anti-Infective Agents. 2015; 13(2):123-8.
51. Ogundajo A, Okeleye B, Ashafa AO. Chemical constituents, *in vitro* antimicrobial and cytotoxic potentials of the extracts from *Macaranga barteri* Mull-Arg. Asian Pac. J. Trop. Biomed. 2017; 7(7):654-9.
52. Putri R, Hendra R, Teruna HY. Anti-bacterial and anti-fungal activities from *Macaranga bancana* leaves extract. Pharmacol. Clin. Pharm. Res. 2019; 4(1):1-4.
53. Lee JH, Kim YG, Khadke SK, Yamano A, Woo JT, Lee J. Antimicrobial and antibiofilm activities of prenylated flavanones from *Macaranga tanarius*. Phytomedicine. 2019; 63:153033.
54. Pagna JI, Awazi T, Mbarga PE, Mbekou IM, Mkounga P, Fotie J, Frese M, Fabrice FB, Lenta BN, Sewald N, Nkengfack EA. Antibacterial flavonoids from the fruits of *Macaranga hurifolia*. J. Asian Nat. Prod. Res. 2022; 24(11):1041-51.
55. Kamso VF, Simo Fotso CC, Kanko Mbekou IM, Toussie BT, Ndjakou Lenta B, Boyom FF, Sewald N, Frese M, Ngadjui BT, Wabo Fotso G. Chemical constituents of *Macaranga occidentalis*, antimicrobial and chemophenetic studies. Molecules. 2022; 27(24):8820.
56. Rosamah E, Haqiqi MT, Putri AS, Kuspradini H, Kusuma IW, Amirta R, Yuliansyah Y, Suwinarti W, Paramita S, Ramadhan R, Tarmadi D. The potential of macaranga plants as skincare cosmetic ingredients: A review. J. Appl. Pharm. Sci. 2023; 13(7):001-12.

57. Li AP, He YH, Zhang SY, Shi YP. Antibacterial activity and action mechanism of flavonoids against phytopathogenic bacteria. *Pestic. Biochem. Phys.* 2022; 188:105221.
58. Chen YW, Ye SR, Ting C, Yu YH. Antibacterial activity of propolins from Taiwanese green propolis. *J. Food Drug Anal.* 2018; 26(2):761-8.
59. Inui S, Hosoya T, Shimamura Y, Masuda S, Ogawa T, Kobayashi H, Shirafuji K, Moli RT, Kozono I, Shin-ya K, Kumazawa S. Solophenols B–D and Solomonin: New Prenylated Polyphenols Isolated from Propolis Collected from The Solomon Islands and Their Antibacterial Activity. *J. Agri. Food Chem.* 2012; 60(47):11765-70.
60. Ruga R, Kingkaew K, Tamsampaoloet K, Chavasiri W. Enhancing antibacterial activity against *Propionibacterium acnes* and *Staphylococcus aureus* by combination of tetracycline with selected compounds. *Chem. Lett.* 2018; 47(12):1538-41.

Table 1: Antibacterial Activity of *Macaranga hullettii* King ex Hook Leaf Extracts and Fractions against *Staphylococcus aureus*, *Streptococcus mutans*, and *Propionibacterium acnes*

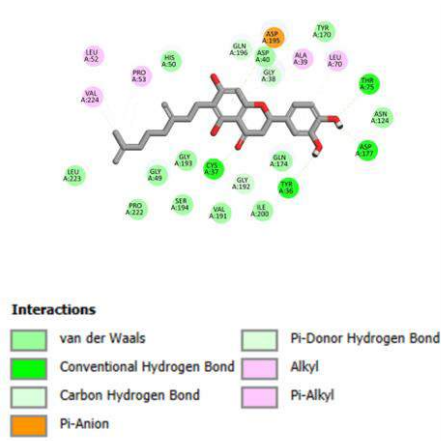
Sample	Concentration (%)	Inhibition Zone (mm)*		
		<i>Staphylococcus</i>	<i>Streptococcus</i>	<i>Propionibacterium</i>
		<i>aureus</i>	<i>mutans</i>	<i>acnes</i>
Methanol Extract	0	0	0	0
	0.15	6.67±0.58	6.67±0.58	0
	0.31	6.67±0.58	6.67±0.58	7.67±0.58
	0.62	6.67±0.58	7.0±0.0	8.0±1.0
	1.25	7.17±0.29	7.33±0.58	9.67±0.58
	2.5	7.5±0.5	8.0±1.0	10.67±0.58
	MIC (%)	<0.15	<0.15	0.15-0.31
n-Hexane Fraction	0	0	0	0
	0.15	0	0	0
	0.31	0	0	0
	0.62	0	0	0
	1.25	0	6.33±0.29	6.33±0.58
	2.5	6.5±0.5	6.67±0.58	6.67±0.58
	MIC (%)	1.25-2.5	0.62-1.25	0.62-1.25
Ethyl acetate Fraction	0	0	0	0
	0.15	6.33±0.58	6.33±0.58	6.67±0.58
	0.31	6.5±0.5	6.67±0.58	8.0±0.0
	0.62	6.5±0.5	6.83±0.29	9.0±0.0
	1.25	6.67±0.29	7.0±0.0	11.0±1.0
	2.5	7.0±0.0	7.67±0.58	14.67±0.58

	MIC (%)	<0.15	<0.15	<0.15
Ampicillin	0.01	20.33±1.15	21.0±1.0	21.0±1.0

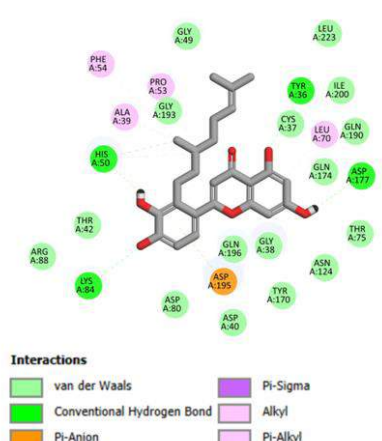
*Data were obtained from triplicate experiments.

Table 2: Binding affinity of flavonoids against three proteins (1JIJ, 3IPK, and 7LBU)

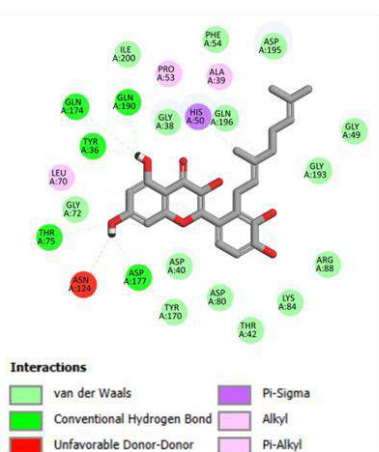
No.	Compound	Binding Affinity (kcal/mol)		
		1JIJ	3IPK	7LBU
1	4'- <i>O</i> -methyl-8-isoprenylnaringenin	-7.4	-8.1	-7.7
2	4'- <i>O</i> -methyl-8-isoprenylriodictyol	-7.2	-8.1	-7.6
3	6-Isoprenylriodictyol	-7.7	-8.7	-8.0
4	5-hydroxy-7,4'-dimethoxyflavone	-8.6	-7.8	-7.1
5	5-hydroxy-6,7,4'-trimethoxyflavone	-8.3	-7.5	-7.3
6	Macahuiettiin A	-7.6	-8.4	-7.1
7	Lonchocarpol A	-9.4	-7.8	-7.8
8	Nymphaeol A	-9.7	-8.9	-8.4
9	Nymphaeol B	-10.5	-8.9	-7.7
10	Nymphaeol C	-8.4	-8.3	-7.4
11	Solophenol D	-10.0	-8.7	-7.9
12	Ampicillin	-8.5	-7.7	-7.6



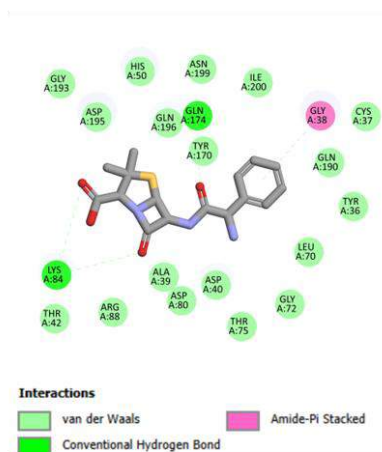
Nymphaeol A



Nymphaeol B



Solphenol D



Ampicillin

Figure 1: 2D interaction of the best docked flavonoids and ampicillin with *Staphylococcus aureus* tyrosyl-tRNA synthetase (PDB ID: 1JJJ)

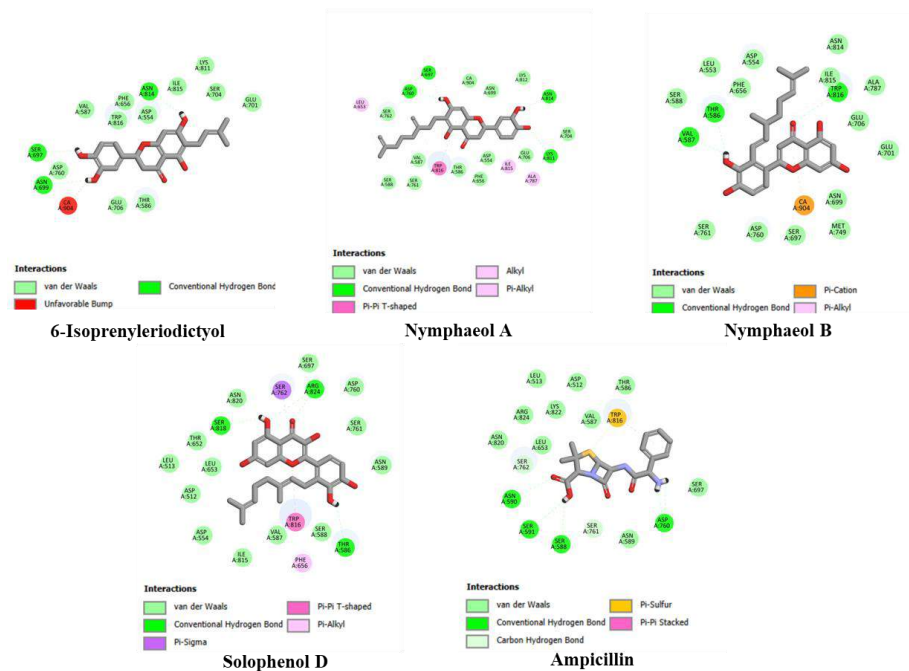


Figure 2: 2D interaction of the best-docked flavonoids and ampicillin with *Streptococcus mutans* antigen I/II (PDB ID: 3IPK).

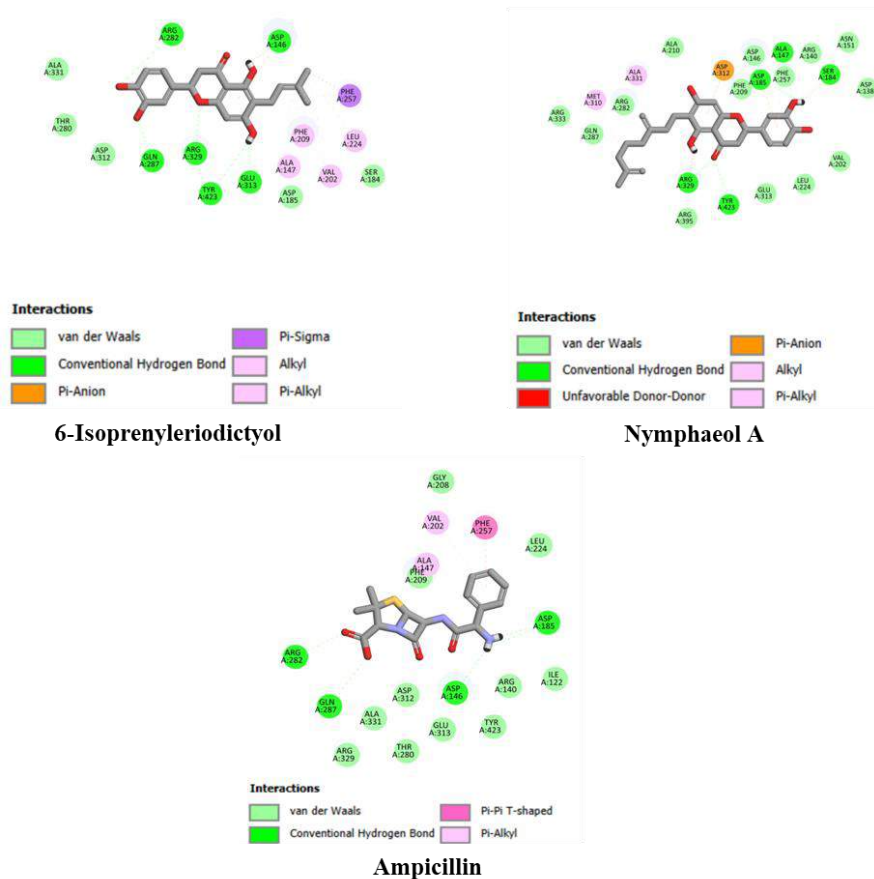


Figure 3: 2D interaction of the best-docked flavonoids and ampicillin with *Propionibacterium acnes* surface sialidase (PDB ID: 7LBU).



Outlook

Re: Revision

From Ade Danova <adedanova@itb.ac.id>

Date Sun 5/25/2025 11:25 AM

To Editor-in-Chief Tjnpr <editor.tjnpr@gmail.com>

 2 attachments (909 KB)

5-TJNPR-2025-420 Revised_25052025.docx; Respond to the reviewer 2.docx;

Dear Editor-in-Chief

Hope you are well

Thank you very much for your review of our manuscript. We have already revised it based on the reviewer comments and suggestions. Here, I attach the revised manuscript and respond to the reviewer. Moreover, I would like to inform you that I am also still waiting for English editing result from Dr. Erharuyi based on your suggestion.

Best regards,
Ade Danova

***In vitro* Evaluation and Molecular Docking Study of Antibacterial Potentials of
Macaranga hulletti King ex Hook.f. Leaf Extract**

Eva Marlina^{1,3}, Ade Danova^{2*}, Rita Hairani^{1,3}, Ritbey Ruga^{1,3}, Elvira Hermawati², and Ana
Yulvia⁴

1. Department of Chemistry, Faculty of Mathematics and Natural Science, Mulawarman University, Samarinda 75123, East Kalimantan, Indonesia.

2. Organic Chemistry Division, Department of Chemistry, Faculty of Mathematics and Natural Sciences, Institut Teknologi Bandung Jl. Ganesha No. 10, Bandung 70132, West Java, Indonesia.

3. Research Center for Medicine and Cosmetics from Tropical Rainforest Resources, Mulawarman University, Samarinda, Indonesia

4. Department of Chemistry, Faculty of Mathematics and Natural Science, Universitas Jember, East Java, Indonesia.

*Corresponding author. E-mail: adedanova@itb.ac.id; Tel.: +6281357944698.

Abstract

Due to the resistance of bacteria to antibiotics, there has been increasing interest in the use of plant extracts to fight infections. Plants in the genus *Macaranga* contain secondary metabolites such as flavonoids that have antibacterial properties. *Macaranga hullettii* King ex Hook.f. is particularly noteworthy due to its widespread distribution in East Kalimantan. Therefore, the objective of this study was to investigate the antibacterial potential of *M. hullettii* leaf extracts through *in vitro* and molecular docking studies. The research involved testing the methanol extract, the n-hexane fraction, and the ethyl acetate fraction from *M. hullettii* leaves against

three bacterial species, *Staphylococcus aureus*, *Streptococcus mutans*, and *Propionibacterium acnes*. The results showed that both the methanol extract and ethyl acetate fraction displayed antibacterial activity against the three bacteria, with minimum inhibitory concentrations (MICs) < 0.15%, except for the methanol extract, which had an MIC of 0.15-0.31% against *Propionibacterium acnes*. In addition, molecular docking analysis showed that five flavonoids possessing prenyl or geranyl groups (6-isoprenyleriodictyol, nymphaeol A, nymphaeol B, and solophenol D) showed the highest binding affinity and dominant hydrogen bonding interactions with key amino acid residues in the binding sites of three bacterial proteins (IJJJ, 3IPK, and 7LBU). The findings suggest that the methanol extract and ethyl acetate fraction from *Macaranga hulletti* have potential applications as new sources of antibacterial agents. Thus, further studies are needed to isolate and evaluate the bioactive compounds.

Keywords: Antibacterial, Extract, Flavonoid, *Macaranga hulletti*, Molecular Docking.

Introduction

Despite substantial advancements in medicine and fundamental research, infectious diseases caused by transmissible agents such as bacteria, protists, and viruses continue to present significant challenges to healthcare professionals. These challenges include the issue of antimicrobial resistance.¹ Extracts from medicinal plants present a promising approach for addressing multidrug-resistant bacteria. In addition, medicinal plants can help restore the efficacy of antibiotics to treat infectious diseases.² Therefore, it is imperative to develop more efficacious antimicrobial agents, particularly those sourced from natural sources, as these are both readily available and economically viable.

Plants in the genus *Macaranga* belong to the family Euphorbiaceae and are commonly known as "Mahang." The secondary metabolites contained in plants of this genus include flavonoids

and stilbenoids with prenylated substituents.³⁻¹³ Nine flavonoids containing either prenyl or geranyl groups and displaying antioxidant, antiplasmodial, and anticancer activities have been isolated from three species of *Macaranga* present in East Kalimantan (*Macaranga hosei*, *Macaranga pearsonii*, and *Macaranga tanarius*): 4'-*O*-methyl-8-isoprenylnaringenin, lonchocarpol A, 4'-*O*-methyl-8-isoprenyleriodictyol, 6-isoprenyleriodictyol, solophenol D, nymphaeol A, nymphaeol B, and nymphaeol C, including macahuilletin A that was isolated from *Macaranga hullettii* obtained from Central Kalimantan.¹⁴⁻¹⁸ *Macaranga tanarius* leaf extract exhibited antibacterial activity.¹⁹ The plant extracts of *Macaranga conglomerata*, *Macaranga kilimandscharica*, and *Macaranga capensis* from Kenya showed antibacterial activities against *E. coli*, *E. aerogenes*, *K. pneumoniae*, *P. stuartii*, *P. aeruginosa*, and *S. aureus*.²⁰ The flavonol rhamnetin, isolated from *Macaranga peltata*, also showed antibacterial activity against *Staphylococcus aureus*.²¹ Two flavanone groups obtained from *Macaranga hosei*, 5-hydroxy-7,4'-dimethoxyflavone and 5-hydroxy-6,7,4'-trimethoxyflavone, showed weak antibacterial activity against three pathogenic bacteria.²² In this study, the leaves of *Macaranga hullettii* King ex Hook.f. were used to identify new sources of medicinal compounds to treat infectious diseases caused by bacteria. This plant is found in all areas of East Kalimantan, where the species commonly occurs in mountain forests and logging areas in the Kalimantan region.^{23, 24} Phytochemical screening and isolation of secondary compounds from the leaf extract of *Macaranga hulletti* identified several flavonoid compounds.^{18,25} The methanol extract and its fractions derived from *Macaranga hulletti* have not yet been analyzed for their antibacterial effects. Therefore, this study aimed to assess the antibacterial activity of *Macaranga hullettii* leaf extract and its fractions using *in vitro* studies. The flavonoids isolated from *Macaranga* species in East Kalimantan, Indonesia, were evaluated for their antibacterial activity using molecular docking analysis.

Materials and Methods

Materials

The solvents used (methanol, n-hexane, and ethyl acetate) were purified by distillation before use. The materials used in this study were *Macaranga hullettii* leaves, *Staphylococcus aureus* ATCC 25923 and *Streptococcus mutans* ATCC 25175 obtained from Chulalongkorn University, *Propionibacterium acnes* KCCM 41747 obtained from Mulawarman University, nutrient agar (NA) from Merck, Luria Bertani liquid medium (1% tryptone, 0.5% yeast, and 0.5% NaCl), yeast extract (Oxoid 100% (w/w)), tryptone (Oxoid 100% (w/w)), ampicillin 100 mg/ml (Sigma Aldrich), NaCl (Sigma Aldrich), cotton swabs, aluminum foil, gauze, Petri dishes, Ose needles, paper discs, an autoclave (Autoclave TOMY, Model SX-700), an oven (SHARP EO-28 WH), a rotary vacuum evaporator (EYELA Rotary evaporator N-1300E V.S.Series), and a freezer (Sanyo Medicoool). For molecular docking analysis, we used a Lenovo Yoga 7 computer with AMD Ryzen AI 7-8840HS w/ Radeon 780M graphics, 3301 Mhz, 8 Core(s), and 16.0 GB of RAM.

Methods

Plant Collection and Identification:

The leaves of *Macaranga hullettii* plants were collected from the Lesan River Forest, Kelay District, Berau Regency, East Kalimantan, Indonesia (1°32'2.5919" N, 117°3'11.950" E). The plant samples were identified at the Laboratory of Anatomy and Plant Systematics, FMIPA Mulawarman University (No. 0174/UN.17.8.5.7.16/HA/XI/2017).

Extraction

Macaranga hullettii leaf powder (1.3 kg) was obtained by maceration twice for 24 h using 7.0 L methanol. A rotary vacuum evaporator was then used to obtain a methanol extract (126 gram).

After that, methanol extract (100 gram) was dissolved in the mixture of methanol and H₂O 500 mL with ratio of 8:2 and then successively fractionated using 5 x 200 mL of n-hexane and ethyl acetate to obtain n-hexane fraction (28 gram) and ethyl acetate fraction (18 gram), respectively. Each fraction was evaporated using a rotary vacuum evaporator. The methanol extract, n-hexane fraction, and ethyl acetate fraction were evaluated for antibacterial activity.

Pathogens

Staphylococcus aureus ATCC 25923 was obtained from the Department of Microbiology, Faculty of Science, Chulalongkorn University. *Streptococcus mutans* ATCC 25175 was obtained from the Department of Biochemistry, Faculty of Dentistry, Chulalongkorn University. Both strains were purchased from the American Type Culture Collection (ATCC). *Propionibacterium acnes* KCCM 41747 was obtained from the Faculty of Forestry, Mulawarman University, and from the Korean Culture Center of Microorganisms (KCCM). The bacteria were periodically subcultured and maintained in nutrient agar (NA) under suitable conditions.

In vitro antibacterial assay

The antibacterial properties of the methanol, n-hexane, and ethyl acetate extracts of *Macaranga hullettii* leaves were analyzed using the disc paper or the Kirby-Bauer method.^{26,27} *Staphylococcus aureus* ATCC 25923, *Streptococcus mutans* ATCC 25175, and *Propionibacterium acnes* KCCM 41747 were assayed. Ampicillin was used as a positive control, and the three organic solvents were used as negative controls. Nutrient Agar (NA) medium was prepared by dissolving 3.3 grams in 150 mL of distilled water. Luria Bertani (LB) liquid media was prepared by dissolving 4 grams in 500 mL of distilled water, stirring, and adding 5 mL to a test tube. The NA and LB media were sterilized using an autoclave at 121 °C

with a pressure of 1 atm for 15 minutes. Each tested bacterium was collected using an Ose needle, followed by dilution in a test tube containing sterile LB media and incubation for 24 h at 37 °C.

Nutrient Agar (25 mL) was poured into sterilized Petri dishes and maintained at room temperature for solidification. A sample of the bacterial suspension in the test tubes was then collected using a sterile cotton swab and applied to the surface of the agar in the Petri dish. Each 30 µL of the tested *M. hulleitii* leaf samples prepared in various concentrations of the organic solvents was poured onto sterile adsorbent filter paper discs (6 mm in diameter), and the solvent was removed. Afterward, the paper discs containing the tested sample were placed on the surface of the agar media and incubated at 37°C for 18-24 hours. The inhibition zone formed in each well was measured using a ruler.^{28,29} The minimum inhibitory concentration (MIC) was determined for the methanol extract as well as the n-hexane and ethyl acetate fractions. MIC was defined as the minimum concentration that completely inhibited bacterial growth.³⁰

Molecular docking

Eight flavonoid derivatives possessing either prenyl or geranyl group occurred in the three *Macaranga* species in East Kalimantan, Indonesia (*Macaranga hosei*, *Macaranga pearsonii*, and *Macaranga tanarius*): 4'-O-methyl-8-isoprenylnaringenin, lonchocarpol A, 4'-O-methyl-8-isoprenyleriodictyol, 6-isoprenyleriodictyol, solophenol D, nymphaeol A, nymphaeol B, and nymphaeol C.¹⁴⁻¹⁷ Macahuilettiin A is a prenylated flavonoid found in *Macaranga hulleitii* obtained from Central Kalimantan (Indonesia).¹⁸ A comparative analysis of the molecular docking results for prenylated/geranylated flavonoids with methoxylated flavonoids was

conducted, focusing on 5-hydroxy-7,4'-dimethoxyflavone and 5-hydroxy-6,7,4'-trimethoxyflavone that are found in *Macaranga hosei* from Trengganu, Malaysia²² (Table 2). The molecular structures of the flavonoids were drawn using ChemOffice Professional 15.0 and obtained from PubChem (<https://pubchem.ncbi.nlm.nih.gov/>). Structure refinement was performed using the molecular mechanics energy minimization method with the Merck molecular force field (MMFF94) using Open Babel in PyRx V.1.1. Three proteins were downloaded from the protein data bank (PDB ID: 1JIJ, 3IPK, 7LBU),³¹⁻³³ and the locations of active sites were calculated using AlphaFold from <https://prankweb.cz/>.³⁴ Molecular docking was performed using the AutoDock Vina tool in PyRx V.1.1 software with an exhaustiveness of 32 and a mode value of 9 poses for each docked ligand.^{35,36} A protein binding site was defined as a box with dimensions of 30 × 30 × 30 Å. In the final step, the binding interactions were analyzed, and the docking results were visualized in 2D using the BIOVIA Discovery Studio Visualizer.

Statistical analysis

Data from the experiments were analyzed using Microsoft Excel.

Results and Discussion

***In vitro* antibacterial activity**

The strength of antibacterial activity was determined based on the diameter of the clear zone, which was used to measure the inhibition zone (mm). This classification was as follows: ≤ 5 mm (weak), 5-10 mm (moderate), 10-20 mm (strong), and ≥ 20 mm (very strong). Moreover, the lower the minimum inhibitory concentration of a compound, the greater its ability to inhibit the growth of test bacteria.²⁸ In this study, ampicillin was used as a positive control against three bacteria: *Staphylococcus aureus* ATCC 25923, *Streptococcus mutans* ATCC 25175, and

Propionibacterium acnes KCCM 41747 (Table 1). The positive control served to determine whether the test bacteria could be inhibited by or were resistant to the antibiotics.

Staphylococcus aureus can cause dentoalveolar infections, jaw cysts, paroritis, oral mucosal lesions, and stomatitis. The bacteria can also produce exotoxins, lecosidine, enterotoxins, and quagulase compounds. Enterotoxins are compounds that cause food poisoning in humans.³⁷

The methanol extract, hexane fraction, and ethyl acetate fraction of *Macaranga hulettii* leaves showed moderate antibacterial activity against *S. aureus*, with an inhibition zone of 5-10 mm at concentrations of 0.15 to 2.5% (Table 1). The inhibition zone of the 0.15% methanol extract measured 6.67 mm. The 0.15% ethyl acetate fraction produced an inhibition zone of 6.33 mm, and the 2.5% n-hexane fraction produced an inhibition zone of 6.50 mm. The results indicated that the methanol extract and ethyl acetate fraction had the most potent antibacterial activity against *S. aureus*, with a minimum inhibitory concentration (MIC) of < 0.15%. Moreover, the hexane fraction of *Macaranga tanarius* was most effective against *S. aureus*, with MIC values of 0.125-0.5% and an inhibition zone of approximately 7.25 mm.³⁸

Streptococcus mutans is implicated in the pathogenesis of dental caries. This facultative anaerobe primarily ferments carbohydrates, resulting in the production of several organic acids, with lactic acid as the predominant byproduct. The acidification of the dental environment by *S. mutans* is a crucial factor contributing to enamel demineralization and the subsequent formation of carious lesions.^{39,40} The bacteria produce glucosyltransferase (GTF) to produce glucan, the compound that causes dental caries.⁴¹ *S. mutans* adheres to the salivary pellicle due to having many receptors for attachment.⁴² We tested the antibacterial activity of *Macaranga hulettii* King leaf extract against *S. mutans* (Table 1). Both extracts displayed moderate antibacterial activity, with inhibition zones of 5-10 mm at concentrations of 0.15-2.5%. The widths of the inhibition zones created by the methanol extract and ethyl acetate fraction were 6.67 and 6.33 mm at 0.15%, respectively. Meanwhile, the n-hexane fraction showed

antibacterial activity at concentrations of 1.25-2.5%, with inhibition zones of 6.33-6.67 mm. These results demonstrated that the methanol extract and ethyl acetate fraction of *Macaranga hulettii* leaves presented the highest potential antibacterial activity against *S. mutans*, with an MIC < 0.15% (Table 1).

Propionibacterium acnes is a Gram-positive anaerobic bacterium that can induce unusual keratinization within the sebaceous glands of hair follicles and increase sebum production.⁴³ The enzyme is capable of hydrolyzing triglycerides (TG) present in sebum into free fatty acids (FFA) that may subsequently induce inflammation in and around the hair follicles.⁴⁴ The bacteria also produce hyaluronidase, protease, lecithinase, and neuraminidase, that can cause inflammation of the skin. *Macaranga triloba* ethanol can prevent the growth of *P. acnes*, and the most significant inhibition value of 5.54 mm at 20%.⁴⁵ As shown in Table 1, the methanol extract and ethyl acetate fraction of *Macaranga hulettii* showed potent antibacterial activity against *P. acnes*, with respective inhibition values of 10.67 and 14.67 mm. The n-hexane fraction exhibited moderate antibacterial activity, with inhibition values of 5-10 mm at 1.25-2.5%. The methanol extract showed antibacterial activity with an inhibition value of 7.67 at 0.31%. The ethyl acetate fraction showed antibacterial activity of 0.15%, with an inhibition zone of 6.67 mm. The results demonstrated that the ethyl acetate fraction of *Macaranga hulettii* leaves presented the highest potential antibacterial activity against *P. acnes*, with a minimum inhibitory concentration (MIC) of < 0.15% (Table 1).

The methanol extract and ethyl acetate fraction exhibited good antibacterial activity against the three bacterial strains. This finding was consistent with previous studies that attributed the antibacterial effects to the presence of flavonoids and their prenylated or geranylated forms in species of *Macaranga*. These included euchrestaflavanone A, bonanione A, macarangaflavanone A, macarangaflavanone B, macatrichocarpin A, propolin D (nymphaeol B), senegalensin, schweinfurthin B, schweinfurthin O, and isomacarangain.⁴⁶⁻⁵⁶ Flavonoid

compounds can damage bacterial cell walls and denature proteases, thereby disrupting bacterial metabolism.⁵⁷ In addition, alkaloids can damage the peptidoglycan layer of bacterial cells, damaging the cell wall and inhibiting bacterial growth. Terpenoids also inhibit bacterial growth by damaging cell membranes. The phenolic compounds of secondary metabolites can damage bacterial cells by increasing the permeability of the cell membrane, resulting in cytoplasmic coagulation and cell division.²⁸

Molecular docking

Molecular docking was performed to predict the interactions of the flavonoids with the amino acid residues of the three proteins. This study used three proteins from *Staphylococcus aureus*, *Streptococcus mutans*, and *Propionibacterium acnes*.³¹⁻³³ The molecular docking results (Table 2) revealed that 11 flavonoid derivatives had binding affinities ranging from -7.2 to -10.5 kcal/mol, -7.5 to -8.9 kcal/mol, and -7.1 to -8.9 kcal/mol against *Staphylococcus aureus* tyrosyl-tRNA synthetase, *Streptococcus mutans* antigen I/II (AgI/II), and *Propionibacterium acnes* surface sialidase, respectively. The interactions of flavonoids and ampicillin with the protein targets are shown in two-dimensional graphs in Figures 1-3.

Among the compounds targeting *S. aureus* tyrosyl-tRNA synthetase, nymphaeol A-B and solophenol D had the highest binding affinities compared to ampicillin as a positive control.

Thus, the interactions of these compounds with the active site of the enzyme were modeled as a 2D structure (Figure 1). Ampicillin formed hydrogen bonds with LYS84 and GLN174 and amide- π interactions with GLY38 (Figure 1). Nymphaeol A and nymphaeol B formed hydrogen bonds with four amino acid residues, π -anions, and hydrophobic interactions, whereas nymphaeol B and ampicillin showed similar interactions with LYS84. In addition, solophenol D formed hydrogen bonds with TYR36, THR75, GLN190, ASP177, and GLN174, as well as hydrophobic interactions with HIS50, LEU70, PRO53, and ALA39 (Figure 1). These results

agreed with previous studies where nymphaeol A-B and solophenol D exhibited antibacterial activity.^{58,59}

Five compounds exhibited high antibacterial activity against *S. mutans* antigen I/II (AgI/II), including 6-isoprenyleriodictyol, nymphaeol A-B, and solophenol D, with binding affinities from -8.7 to -8.9 kcal/mol. The interactions between the ligands and amino acid residues in the binding sites of these compounds are shown in 2D views (Figure 2). Ampicillin formed hydrogen bonds with four amino acid residues, ASN590, SER591, SER588, and ASP760, as well as π -sulfur and π - π interactions with TRP 816, while 6-isoprenyleriodictyol only formed hydrogen bonds with three amino acid residues: SER697, ASN699, and ASN814. Nymphaeol A and nymphaeol B formed hydrogen bonds with four amino acid residues (Figure 2), but nymphaeol A exhibited a similar π - π interaction with TRP816, as observed with ampicillin. Solophenol D formed hydrogen bonds with SER818, ARG824, and THR586, similar to the π - π interactions (TRP816) with ampicillin, and π -sigma interactions with SER762. As 6-isoprenyleriodictyol is a prenylated flavonoid similar to nymphaeol A, and hence may have antibacterial activity.

Three compounds, including 6-isoprenyleriodictyol and nymphaeol A, displayed stronger binding affinities than ampicillin, with values of -8.0 and -8.4 kcal/mol against *P. acnes* surface sialidases. Therefore, both compounds were imaged in 2D to investigate the interactions between the ligands and the amino acid residues in the binding sites (Figure 3). Ampicillin formed hydrogen bonds with four amino acid residues, ARG282, GLN287, ASP146, ASP185, and π - π and π -alkyl interactions with PHE257, ALA147, and VAL202; 6-Isoprenyleriodictyol demonstrated π - π interactions with PHE257, π -anion interactions with ASP146, and alkyl-alkyl interactions with ALA147, PHE209, VAL202, and LEU224. This compound formed bonds with six amino acid residues, including ASP146, ARG329, TYR423, ARG282, and GLU287, that were analogous to those observed with ampicillin. Nymphaeol A formed hydrogen bonds

with five amino acid residues (ARG329, TYR423, ASP185, ALA147, and SER184), π -anion interactions with ASP312 and ASP185, alkyl-alkyl interactions with MET310, ALA331, and ALA147, and Van der Waals interactions with PHE257. Thus, these compounds may have potent antibacterial activity.

Structure-activity relationships were also studied based on the binding affinity results (Table 2). Flavonoids possessing prenyl, geranyl, and hydroxyl groups showed potential as antibacterial agents due to their strong affinity for amino acid residues in the binding sites of proteins, compared to ampicillin. However, the absence of a hydroxyl group on prenylated or geranylated flavonoids decreased the binding affinity.^{22,56,60}

Conclusion

The extracts and fractions of *Macaranga hulettii* have potential as antibacterial agents against three bacterial strains, *Staphylococcus aureus* ATCC 25923, *Streptococcus mutans* ATCC 25175, and *Propionibacterium acnes* KCCM 41747. The methanol extract demonstrated antibacterial activity against *S. aureus* and *S. mutans* with an MIC (%) of < 0.15 against *S. aureus* and *S. mutans*, and 0.15-0.31% against *P. acnes*. The n-hexane fraction had an MIC (%) of 1.25-2.5% against *S. aureus*, and 0.62-1.25% against *S. mutans* and *P. acnes*. The ethyl acetate fraction had an MIC (%) < 0.15% against the three bacterial strains. Molecular docking studies suggested that 6-isoprenyleriodictyol, nymphaeol A, nymphaeol B, and solophenol D are promising antibacterial agents, as those compounds exhibited the highest binding affinity and formed hydrogen bonds with key amino acid residues in the active sites of the protein targets. Further *in vitro* and *in vivo* studies should be conducted to isolate the active secondary metabolites from this species.

Conflict of Interest

The authors declare that they have no conflicts of interest.

Author's Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims related to the content of this article will be borne by them.

Acknowledgement

This research was funded by the Islamic Development Bank (PIU-IsDB) project under contract no. 137/UN17.11/PL/2019.

References

1. Wong F, de la Fuente-Nunez C, Collins JJ. Leveraging artificial intelligence in the fight against infectious diseases. *Science*. 2023; 381(6654):164-70.
2. Seukep AJ, Kuete V, Nahar L, Sarker SD, Guo M. Plant-derived secondary metabolites as the main source of efflux pump inhibitors and methods for identification. *J. Pharm. Anal.* 2020; 10(4):277-90.
3. Magadula JJ. Phytochemistry and pharmacology of the genus *Macaranga*: a review. *J. Med. Plants Res.* 2014; 8(12):489-503.
4. Vu LT, Ngan TB, Phuong L, Huong DT, Litaudon M, Van Hung N, Thach TD, Van Cuong P. Chemical constituents from fruits of *Macaranga denticulata* (Euphorbiaceae)(Part 2). *Vietnam J. Chem.* 2018; 56(4):516-20.
5. Marliana E, Hairani R, Tjahjandarie TS, Tanjung M. Antiplasmodial activity of flavonoids from *Macaranga tanarius* leaves. *IOP Conf. Ser.: Earth Environ. Sci.* 2018; 144(1):012011.

6. Tjahjendarie TS, Tanjung M, Saputri RD, Nadar PB, Aldin MF, Marlina E, Permadi A. Flavestins K, An isoprenylated stilbene from the leaves of *Macaranga recurvata* Gage. Nat. Prod. Sci. 2019; 25(3):244-7.
7. Huong DT, Linh NT, Van TT, Litaudon M, Roussi F, Van Nam V, Van Cuong P. Stilbenes from *Macaranga tanarius* (Euphorbiaceae) growing in Vietnam. Vietnam J. Chem. 2020; 58(3):338-42.
8. Aldin MF, Tjahjendarie TS, Saputri RD, Tanjung M. Macasiamenene V, a New Stilbenoid from the Leaves of *Macaranga inermis*. Nat. Prod. Sci. 2021; 27(1):45-8.
9. Muharram A, Rachmawati DA, Mardhiyyah S, Tjahjendarie TS, Saputri RD, Ahmat N, Tanjung M. Cytotoxic and antioxidant activities of flavonoids and diterpenoids from *Macaranga involucrata* (Roxb.) Baill. J. Appl. Pharm. Sci. 2023; 13(6):087-92.
10. Tanjung M, Tjahjendarie TS, Aldin MF, Mardhiyyah S, Ahmat N, Saputri RD. Macagigantin A, a new flavonoid from *Macaranga gigantea* (Rchb. f & Zoll.) Mull. Arg. Nat. Prod. Sci. 2023; 29(4):287-94.
11. Tjahjendarie TS, Aldin MF, Saputri RD, Tanjung M. Dihydrostilbenes from *Macaranga javanica* (Blume) Müll. Arg. and their antiplasmodial activity. Nat. Prod. Sci. 2024:1-7.
12. Tanjung M, Tjahjendarie TS, Aldin MF, Mardhiyyah S, Saputri RD, Syah YM, Ahmat N. Two new flavonols from *Macaranga inermis* pax & K. Hoffm. Nat. Prod. Sci. 2025; 39(3):498-505.
13. Tanjung M, Tjahjendarie TS, Aldin MF, Mardhiyyah S, Saputri RD, Syah YM, Ahmat N. Two new flavonols from *Macaranga inermis* pax & K. Hoffm. Nat. Prod. Sci. 2025; 39(3):498-505.
14. Marlina E, Tjahjendarie TS, Tanjung M. Isoprenylated flavanone derivatives from *Macaranga hosei* King ex Hook F. Der Pharm. Lett. 2015; 7(3):153-6.

15. Marliana E, Tjahjandarie TS, Tanjung M. Antioxidant activity of flavonoid compounds from *Macaranga pearsonii* Merr. J. Kim. Mulawarman. 2016; 13(2):97-100.
16. Marliana E, Astuti W, Kosala K, Hairani R, Tjahjandarie TS, Tanjung M. Chemical composition and anticancer activity of *Macaranga hosei* leaves. Asian J. Chem. 2018; 30(4):795-8.
17. Marliana E, Ruga R, Hairani R, Tjahjandarie TS, Tanjung M. Antioxidant activity of flavonoid constituents from the leaves of *Macaranga tanarius*. IOP Conf. Ser.: J. Phys. 2019; 1277(1):012014.
18. Saputri RD, Tukiran T, Wati FA, Purnamasari AP, Wardhana MW, Tjahjandarie TS, Tanjung M. Macahuilettiin A, a new isoprenylated flavanone from the leaves of *Macaranga hullettii* King ex Hook and their antiplasmodial activity. Vietnam J. Chem. 2024; 62(3):394-8.
19. Musdalifah M, Khumaidi A, Suwastika IN. Inhibition test and phytochemical screening of leaf extracts of *Macaranga tanarius* (L.) Mull. Arg against *Salmonella typhi* as an antibacterial. Nat. Sci. J. Sci. Technol. 2017; 6(3).
20. Ibrahim H, Omosa L, Nchiozem-Ngnitedem V, Onyari J, Maru S, Guefack M. Antibacterial activities and phytochemical screening of crude extracts from Kenyan macaranga species toward MDR phenotypes expressing efflux pumps. Pharmacogn. Commn. 2021; 11(2):119-26.
21. Bijesh K, Sebastian D. Isolation and characterization of antibacterial compounds from *Macaranga peltata* against clinical isolates of *Staphylococcus aureus*. Int. J. Biol. Pharma Res. 2013; 4:1196-203.
22. Salleh WM, Razak NZ, Ahmad F. Phytochemicals and biological activities of *Macaranga hosei* and *Macaranga constricta* (Euphorbiaceae). Marmara Pharm. J. 2017;21(4):881-8.

23. Slik JW, Priyono P, Welzen PV. Key to the *Macaranga Thou.* and *Mallotus Lour.* species (Euphorbiaceae) of East Kalimantan, Indonesia. Singapore, Gardens' Bulletin (Singapore), National Parks Board, Singapore Botanic Gardens; 2000. 11-87 p.
24. Amirta R, Angi EM, Ramadhan R, Kusuma IW, Wiati CB, Haqiqi MT. Potential utilization of macaranga. Samarinda: Mulawarman University Press; 2017.
25. Rismawati R, Marliana E, Daniel D. Phytochemical test on methanol extract of leaf of *Macaranga hullettii* King ex Hook. f. J. Atomik. 2018; 3(2):91-4.
26. Hudzicki J. Kirby-Bauer disk diffusion susceptibility test protocol. American society for microbiology. 2009; 15(1):1-23.
27. Inna M, Astuti W, Saleh C. Antibacterial activity of methanolic extract of uric patch plant leaves (*Cayratia carnosa*) against *Salmonella thypi* and *Propionibacterium acnes*. J. Atomik. 2022; 7(1):1-5.
28. Yulianti MF, Amat AL, Hutasoit RM, Pakan PD. Antibacterial activity of jamblang leaf ethanol extract (*Syzygium cumini*) against the growth of *Propionibacterium acnes*. Acta Biochimica Indones. 2023; 6(2):1161.
29. Hossain MR, Biplob AI, Sharif SR, Bhuiya AM, Sayem AS. Antibacterial activity of green synthesized silver nanoparticles of *Lablab purpureus* flowers extract against human pathogenic bacteria. Trop. J. Nat. Prod. Res. 2023;7(8).
30. Yanda L, Tatsimo SJ, Tamokou JD, Matsute-Takongmo G, Meffo-Dongmo SC, Meli Lannang A, Sewald N. Antibacterial and antioxidant activities of isolated compounds from *Prosopis africana* leaves. Int. J. Anal. Chem. 2022; 4205823.
31. Qiu X, Janson CA, Smith WW, Green SM, McDevitt P, Johanson K, Carter P, Hibbs M, Lewis C, Chalker A, Fosberry A. Crystal structure of *Staphylococcus aureus* tyrosyl-tRNA synthetase in complex with a class of potent and specific inhibitors. Protein Sci. 2001; 10(10):2008-16.

32. Larson MR, Rajashankar KR, Patel MH, Robinette RA, Crowley PJ, Michalek S, Brady LJ, Deivanayagam C. Elongated fibrillar structure of a *streptococcal adhesin* assembled by the high-affinity association of α -and PPII-helices. *Proc. Natl. Acad. Sci.* 2010; 107(13):5983-8.
33. Yu AC, Volkers G, Jongkees SA, Worrall LJ, Withers SG, Strynadka NC. Crystal structure of the *Propionibacterium acnes* surface sialidase, a drug target for *P. acnes*-associated diseases. *Glycobiology*. 2022; 32(2):162-70.
34. Jakubec D, Skoda P, Krivak R, Novotny M, Hoksza D. PrankWeb 3: accelerated ligand-binding site predictions for experimental and modelled protein structures. *Nucleic Acids Res.* 2022; 50:W593-7.
35. Dallakyan S, Olson AJ. Small-molecule library screening by docking with PyRx. In *Chemical biology: methods and protocols*. 2014: 243-250. New York, NY: Springer New York.
36. Trott O, Olson AJ. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *J. Comput. Chem.* 2010; 31(2):455-61.
37. Malangu N, editor. *Poisoning: From specific toxic agents to novel rapid and simplified techniques for analysis*. BoD–Books on Demand; 2017.
38. Sari AA, Saleh C. Phytochemical test, toxicity and antibacterial activity of extracts of various macaranga leaf fractions (*Macaranga tanarius* (L.) MA) against *Staphylococcus aureus* and *Escherichia coli*. *J. Kim. Mulawarman*. 2015; 12(2):53-8.
39. Imelda R., Mariam M., Satari M. Effect of cassava (*manihot esculenta* cranzt), rice (*oryza sativa* l.), and potato (*solanum tuberosum*) water extract to decrease pH phase fermentation of *streptococcus mutans* atcc 25175. *Padjajaran J. Dent.* 2019; 31(1):14.

40. Alkhaled A., Alsabek L., Al-assaf M. , & Badr F.. Effect of chlorhexidine, honey and propolis on *streptococcus mutans* counts: *in vitro* study. Dentistry. 2021; 9(1).
41. Ren Z, Chen L, Li J, Li Y. Inhibition of *Streptococcus mutans* polysaccharide synthesis by molecules targeting glycosyltransferase activity. J. Oral Microbiol. 2016; 8(1):31095.
42. Lee BS, Chen YJ, Wei TC, Ma TL, Chang CC. Comparison of antibacterial adhesion when salivary pellicle is coated on both poly (2-hydroxyethyl-methacrylate)-and polyethylene-glycol-methacrylate-grafted poly (methyl methacrylate). Int. J. Mol. Sci. 2018; 19(9):2764.
43. Chen KC, Yang CH, Li TT, Zouboulis CC, Huang YC. Suppression of Propionibacterium acnes-stimulated proinflammatory cytokines by Chinese bayberry extracts and its active constituent myricetin in human sebocytes *in vitro*. Phytother. Res. 2019; 33(4):1104-13.
44. Xu J, Chen X, Song J, Wang C, Xu W, Tan H, Suo H. Antibacterial activity and mechanism of cell-free supernatants of *Lactocaseibacillus paracasei* against *Propionibacterium acnes*. Microb. Pathog. 2024; 189:106598
45. Warnida H, Mustika D, Supomo S, Sukawaty Y. Effectiveness of Mahang Leaf Ethanol Extract (*Macaranga Triloba*) as an anti-acne. J. Penelit. Sos. Ekon. Kehuta. 2018; 4(1):9-18.
46. Schütz BA, Wright AD, Rali T, Sticher O. Prenylated flavanones from leaves of *Macaranga pleiostemona*. Phytochemistry. 1995; 40(4):1273-7.
47. Lim TY, Lim YY, Yule CM. Evaluation of antioxidant, antibacterial and anti-tyrosinase activities of four Macaranga species. Food Chem. 2009; 114(2):594-9.
48. Fareza MS, Syah YM, Mujahidin D, Juliawaty LD, Kurniasih I. Antibacterial flavanones and dihydrochalcones from *Macaranga trichocarpa*. Z. Naturforsch C J. Biosci. 2014; 69(9-10):375-80.

49. Hasanat A, Kabir MS, Hossain MM, Hasan M, Al Masum MA, Chowdhury TA, Bhuiyan DI, Mamur A, Kibria AS. Antibacterial activity of methanol extract of *Macaranga denticulata* leaves and in silico PASS prediction for its six secondary metabolites. World J. Pharm. Pharm. Sci. 2015;1258-66.
50. O Akanbi B, Anene P, Olayanju S. Preliminary Screening Indicates Promising antimicrobial properties of the stem bark extracts of *Macaranga rosea*. Anti-Infective Agents. 2015; 13(2):123-8.
51. Ogundajo A, Okeleye B, Ashafa AO. Chemical constituents, *in vitro* antimicrobial and cytotoxic potentials of the extracts from *Macaranga barteri* Mull-Arg. Asian Pac. J. Trop. Biomed. 2017; 7(7):654-9.
52. Putri R, Hendra R, Teruna HY. Anti-bacterial and anti-fungal activities from *Macaranga bancana* leaves extract. Pharmacol. Clin. Pharm. Res. 2019; 4(1):1-4.
53. Lee JH, Kim YG, Khadke SK, Yamano A, Woo JT, Lee J. Antimicrobial and antibiofilm activities of prenylated flavanones from *Macaranga tanarius*. Phytomedicine. 2019; 63:153033.
54. Pagna JI, Awazi T, Mbarga PE, Mbekou IM, Mkounga P, Fotie J, Frese M, Fabrice FB, Lenta BN, Sewald N, Nkengfack EA. Antibacterial flavonoids from the fruits of *Macaranga hurifolia*. J. Asian Nat. Prod. Res. 2022; 24(11):1041-51.
55. Kamso VF, Simo Fotso CC, Kanko Mbekou IM, Toussie BT, Ndjakou Lenta B, Boyom FF, Sewald N, Frese M, Ngadjui BT, Wabo Fotso G. Chemical constituents of *Macaranga occidentalis*, antimicrobial and chemophenetic studies. Molecules. 2022; 27(24):8820.
56. Rosamah E, Haqiqi MT, Putri AS, Kuspradini H, Kusuma IW, Amirta R, Yuliansyah Y, Suwinarti W, Paramita S, Ramadhan R, Tarmadi D. The potential of macaranga plants as skincare cosmetic ingredients: A review. J. Appl. Pharm. Sci. 2023; 13(7):001-12.

57. Li AP, He YH, Zhang SY, Shi YP. Antibacterial activity and action mechanism of flavonoids against phytopathogenic bacteria. *Pestic. Biochem. Phys.* 2022; 188:105221.
58. Chen YW, Ye SR, Ting C, Yu YH. Antibacterial activity of propolins from Taiwanese green propolis. *J. Food Drug Anal.* 2018; 26(2):761-8.
59. Inui S, Hosoya T, Shimamura Y, Masuda S, Ogawa T, Kobayashi H, Shirafuji K, Moli RT, Kozono I, Shin-ya K, Kumazawa S. Solophenols B–D and Solomonin: New Prenylated Polyphenols Isolated from Propolis Collected from The Solomon Islands and Their Antibacterial Activity. *J. Agri. Food Chem.* 2012; 60(47):11765-70.
60. Ruga R, Kingkaew K, Tamsampaoloet K, Chavasiri W. Enhancing antibacterial activity against *Propionibacterium acnes* and *Staphylococcus aureus* by combination of tetracycline with selected compounds. *Chem. Lett.* 2018; 47(12):1538-41.

Table 1: Antibacterial Activity of *Macaranga hullettii* King ex Hook Leaf Extracts and Fractions against *Staphylococcus aureus*, *Streptococcus mutans*, and *Propionibacterium acnes*

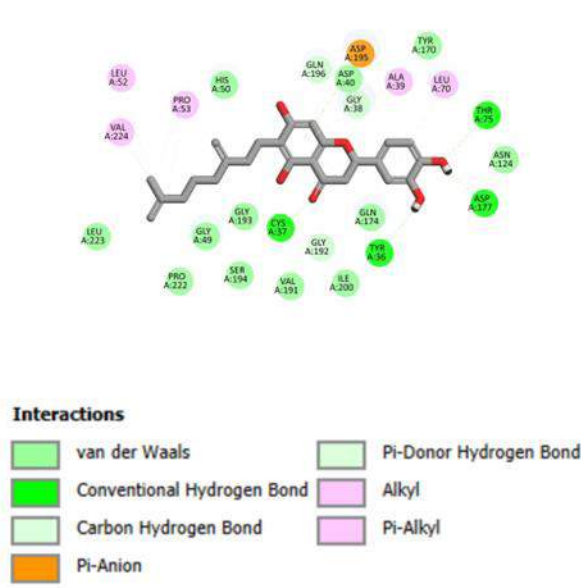
Sample	Concentration (%)	Inhibition Zone (mm)*		
		<i>Staphylococcus</i>	<i>Streptococcus</i>	<i>Propionibacterium</i>
		<i>aureus</i>	<i>mutans</i>	<i>acnes</i>
Methanol Extract	0	0	0	0
	0.15	6.67±0.58	6.67±0.58	0
	0.31	6.67±0.58	6.67±0.58	7.67±0.58
	0.62	6.67±0.58	7.0±0.0	8.0±1.0
	1.25	7.17±0.29	7.33±0.58	9.67±0.58
	2.5	7.5±0.5	8.0±1.0	10.67±0.58
	MIC (%)	<0.15	<0.15	0.15-0.31
n-Hexane Fraction	0	0	0	0
	0.15	0	0	0
	0.31	0	0	0
	0.62	0	0	0
	1.25	0	6.33±0.29	6.33±0.58
	2.5	6.5±0.5	6.67±0.58	6.67±0.58
	MIC (%)	1.25-2.5	0.62-1.25	0.62-1.25
Ethyl acetate Fraction	0	0	0	0
	0.15	6.33±0.58	6.33±0.58	6.67±0.58
	0.31	6.5±0.5	6.67±0.58	8.0±0.0
	0.62	6.5±0.5	6.83±0.29	9.0±0.0
	1.25	6.67±0.29	7.0±0.0	11.0±1.0
	2.5	7.0±0.0	7.67±0.58	14.67±0.58

	MIC (%)	<0.15	<0.15	<0.15
Ampicillin	0.01	20.33±1.15	21.0±1.0	21.0±1.0

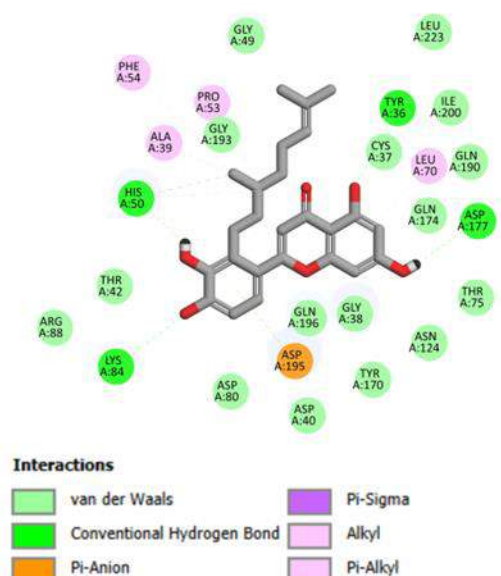
*Data were obtained from triplicate experiments.

Table 2: Binding affinity of flavonoids against three proteins (1JJJ, 3IPK, and 7LBU)

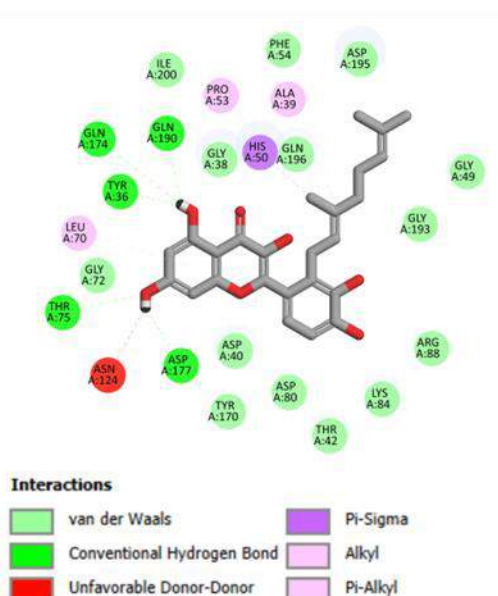
No.	Compound	Binding Affinity (kcal/mol)		
		1JJJ	3IPK	7LBU
1	4'- <i>O</i> -methyl-8-isoprenylnaringenin	-7.4	-8.1	-7.7
2	4'- <i>O</i> -methyl-8-isoprenyleriodictyol	-7.2	-8.1	-7.6
3	6-Isoprenyleriodictyol	-7.7	-8.7	-8.0
4	5-hydroxy-7,4'-dimethoxyflavone	-8.6	-7.8	-7.1
5	5-hydroxy-6,7,4'-trimethoxyflavone	-8.3	-7.5	-7.3
6	Macahuilettiin A	-7.6	-8.4	-7.1
7	Lonchocarpol A	-9.4	-7.8	-7.8
8	Nymphaeol A	-9.7	-8.9	-8.4
9	Nymphaeol B	-10.5	-8.9	-7.7
10	Nymphaeol C	-8.4	-8.3	-7.4
11	Solophenol D	-10.0	-8.7	-7.9
12	Ampicillin	-8.5	-7.7	-7.6



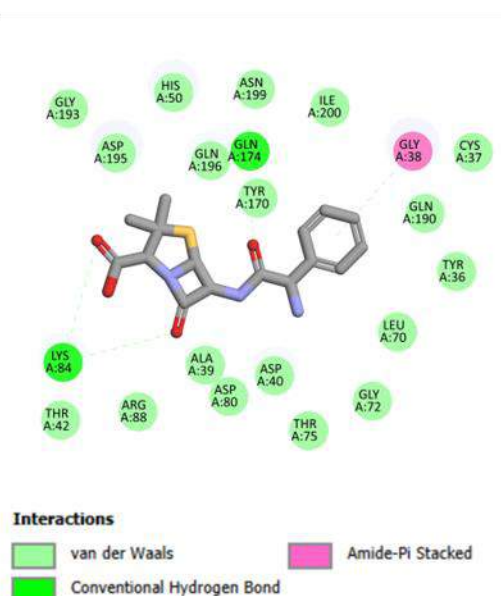
Nymphaeol A



Nymphaeol B

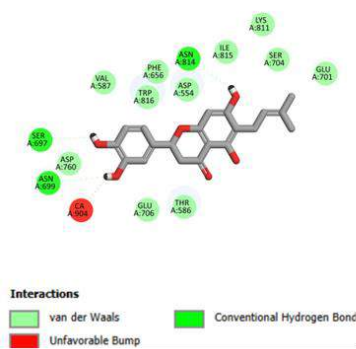


Solophenol D

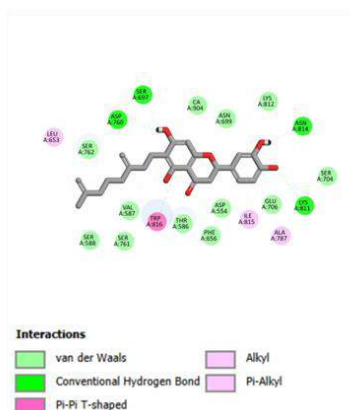


Ampicillin

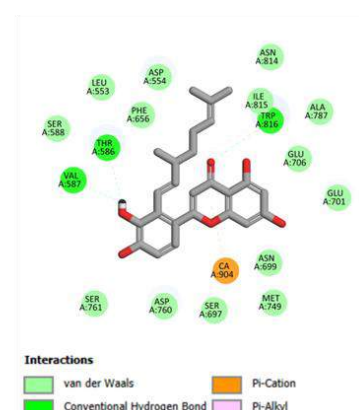
Figure 1: 2D interaction of the best docked flavonoids and ampicillin with *Staphylococcus aureus* tyrosyl-tRNA synthetase (PDB ID: 1JIJ)



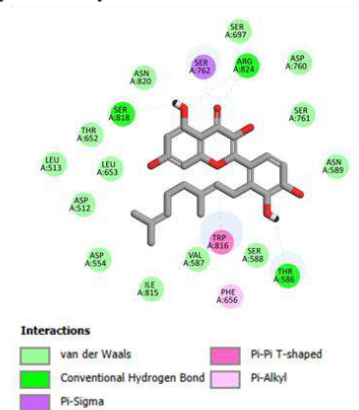
6-Isoprenylioridictyol



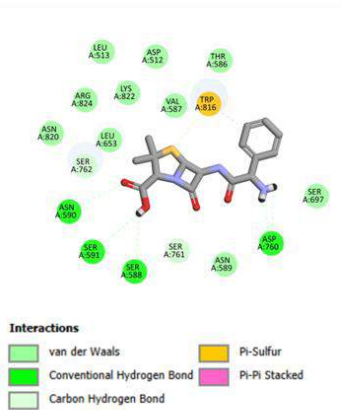
Nymphaeol A



Nymphaeol B



Solophenol D



Ampicillin

Figure 2: 2D interaction of the best-docked flavonoids and ampicillin with *Streptococcus mutans* antigen I/II (PDB ID: 3IPK).

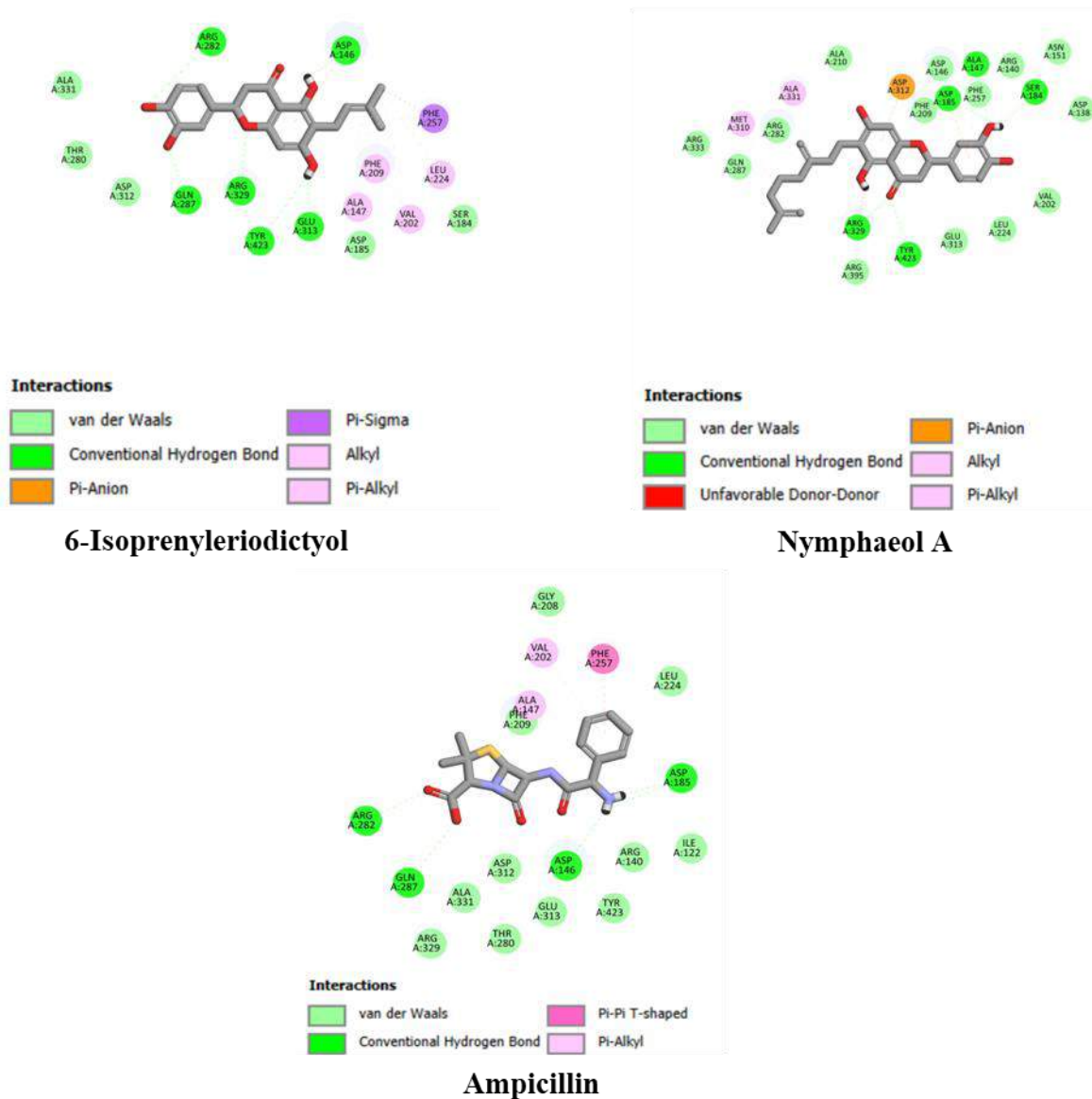


Figure 3: 2D interaction of the best-docked flavonoids and ampicillin with *Propionibacterium acnes* surface sialidase (PDB ID: 7LBU).

Respond

Dear Reviewer,

Thank you very much for reviewing our manuscript. We have already revised it based on your suggestion as follows:

1. On --> of
2. Sample preparation --> *Plant Collection and Identification:*
3. The research samples used in this study were leaves of *Macaranga hullettii* plants --> The leaves of *Macaranga hullettii* plants were
4. 4'-O-methyl-8-isoprenylnaringenin --> 4'-O-methyl-8-isoprenylnaringenin
5. 4'-O-methyl-8-isoprenyleryodictyol --> 4'-O-methyl-8-isoprenyleryodictyol
6. The methanol extract was successively fractionated using n-hexane and ethyl acetate --> After that, methanol extract (100 gram) was dissolved in the mixture of methanol and H₂O 500 mL with ratio of 8:2 and then successively fractionated using 5 x 200 mL of n-hexane and ethyl acetate to obtain n-hexane fraction (28 gram) and ethyl acetate fraction (18 gram), respectively.
7. was prepared by --> was obtained from
8. All references in Indonesia, I have already translated in English.

From: Editor-in-Chief Tjnpr <editor.tjnpr@gmail.com>

Sent: Sunday, May 25, 2025 10:33 PM

To: Ade Danova <adedanova@itb.ac.id>

Subject: Re: Revision

It will be better if all documents are submitted once

Best regards

Abiodun

Professor Abiodun Falodun, PhD; FAAS, FISPON

Editor-in-Chief:

Tropical Journal of Natural Product Research (TJNPR)

Head, Natural Product Research Group, University of Benin

Email: editor.tjnpr@gmail.com

www.tjnpr.org SCOPUS, SCImago SJR Q3

Professor of Pharmaceutical Chemistry, FAAS

Fellow, Fulbright (USA)

Deputy Vice-Chancellor (Academic) 2014-2016



Outlook

Re: English Editing from Tropical Journal of Natural Product Research

From Osayemwenre Erharuyi <erharuyiosaz@gmail.com>

Date Sun 5/25/2025 10:50 PM

To Ade Danova <adedanova@itb.ac.id>

Dear Author,

The editing, formatting, and proofreading of your manuscript is at the final stage, please provide the following information to enable us complete the editing process:

1. volume of methanol use for extraction
2. weight of methanol extract fractionated
3. volume of n-hexane and ethyl acetate used for fractionation.



Outlook

Re: English Editing from Tropical Journal of Natural Product Research

From Ade Danova <adedanova@itb.ac.id>

Date Mon 5/26/2025 5:56 AM

To Osayemwenre Erharuyi <erharuyiosaz@gmail.com>

 1 attachment (888 KB)

5-TJNPR-2025-420 Revised_25052025.docx;

Dear Dr. Erharuyi

Here I send new revised manuscripts. I already added volume and weight as well as references in Indonesia is already changed to English. Thank you very much for your kind help.

Best regards,
Ade Danova

***In vitro* Evaluation and Molecular Docking Study of Antibacterial Potentials of
Macaranga hulletti King ex Hook.f. Leaf Extract**

Eva Marlina^{1,3}, Ade Danova^{2*}, Rita Hairani^{1,3}, Ritbey Ruga^{1,3}, Elvira Hermawati², and Ana
Yulvia⁴

1. Department of Chemistry, Faculty of Mathematics and Natural Science, Mulawarman University, Samarinda 75123, East Kalimantan, Indonesia.

2. Organic Chemistry Division, Department of Chemistry, Faculty of Mathematics and Natural Sciences, Institut Teknologi Bandung Jl. Ganesha No. 10, Bandung 70132, West Java, Indonesia.

3. Research Center for Medicine and Cosmetics from Tropical Rainforest Resources, Mulawarman University, Samarinda, Indonesia

4. Department of Chemistry, Faculty of Mathematics and Natural Science, Universitas Jember, East Java, Indonesia.

*Corresponding author. E-mail: adedanova@itb.ac.id; Tel.: +6281357944698.

Abstract

Due to the resistance of bacteria to antibiotics, there has been increasing interest in the use of plant extracts to fight infections. Plants in the genus *Macaranga* contain secondary metabolites such as flavonoids that have antibacterial properties. *Macaranga hullettii* King ex Hook.f. is particularly noteworthy due to its widespread distribution in East Kalimantan. Therefore, the objective of this study was to investigate the antibacterial potential of *M. hullettii* leaf extracts through *in vitro* and molecular docking studies. The research involved testing the methanol extract, the n-hexane fraction, and the ethyl acetate fraction from *M. hullettii* leaves against

three bacterial species, *Staphylococcus aureus*, *Streptococcus mutans*, and *Propionibacterium acnes*. The results showed that both the methanol extract and ethyl acetate fraction displayed antibacterial activity against the three bacteria, with minimum inhibitory concentrations (MICs) < 0.15%, except for the methanol extract, which had an MIC of 0.15-0.31% against *Propionibacterium acnes*. In addition, molecular docking analysis showed that five flavonoids possessing prenyl or geranyl groups (6-isoprenylerydiol, nymphaeol A, nymphaeol B, and solophenol D) showed the highest binding affinity and dominant hydrogen bonding interactions with key amino acid residues in the binding sites of three bacterial proteins (IJJ, 3IPK, and 7LBU). The findings suggest that the methanol extract and ethyl acetate fraction from *Macaranga hulletti* have potential applications as new sources of antibacterial agents. Thus, further studies are needed to isolate and evaluate the bioactive compounds.

Keywords: Antibacterial, Extract, Flavonoid, *Macaranga hulletti*, Molecular Docking.

Introduction

Despite substantial advancements in medicine and fundamental research, infectious diseases caused by transmissible agents such as bacteria, protists, and viruses continue to present significant challenges to healthcare professionals. These challenges include the issue of antimicrobial resistance.¹ Extracts from medicinal plants present a promising approach for addressing multidrug-resistant bacteria. In addition, medicinal plants can help restore the efficacy of antibiotics to treat infectious diseases.² Therefore, it is imperative to develop more efficacious antimicrobial agents, particularly those sourced from natural sources, as these are both readily available and economically viable.

Plants in the genus *Macaranga* belong to the family Euphorbiaceae and are commonly known as "Mahang." The secondary metabolites contained in plants of this genus include flavonoids

and stilbenoids with prenylated substituents.³⁻¹³ Nine flavonoids containing either prenyl or geranyl groups and displaying antioxidant, antiplasmodial, and anticancer activities have been isolated from three species of *Macaranga* present in East Kalimantan (*Macaranga hosei*, *Macaranga pearsonii*, and *Macaranga tanarius*): 4'-*O*-methyl-8-isoprenylnaringenin, lonchocarpol A, 4'-*O*-methyl-8-isoprenyleriodictyol, 6-isoprenyleriodictyol, solophenol D, nymphaeol A, nymphaeol B, and nymphaeol C, including macahuilletin A that was isolated from *Macaranga hullettii* obtained from Central Kalimantan.¹⁴⁻¹⁸ *Macaranga tanarius* leaf extract exhibited antibacterial activity.¹⁹ The plant extracts of *Macaranga conglomerata*, *Macaranga kilimandscharica*, and *Macaranga capensis* from Kenya showed antibacterial activities against *E. coli*, *E. aerogenes*, *K. pneumoniae*, *P. stuartii*, *P. aeruginosa*, and *S. aureus*.²⁰ The flavonol rhamnetin, isolated from *Macaranga peltata*, also showed antibacterial activity against *Staphylococcus aureus*.²¹ Two flavanone groups obtained from *Macaranga hosei*, 5-hydroxy-7,4'-dimethoxyflavone and 5-hydroxy-6,7,4'-trimethoxyflavone, showed weak antibacterial activity against three pathogenic bacteria.²² In this study, the leaves of *Macaranga hullettii* King ex Hook.f. were used to identify new sources of medicinal compounds to treat infectious diseases caused by bacteria. This plant is found in all areas of East Kalimantan, where the species commonly occurs in mountain forests and logging areas in the Kalimantan region.^{23, 24} Phytochemical screening and isolation of secondary compounds from the leaf extract of *Macaranga hulletti* identified several flavonoid compounds.^{18,25} The methanol extract and its fractions derived from *Macaranga hulletti* have not yet been analyzed for their antibacterial effects. Therefore, this study aimed to assess the antibacterial activity of *Macaranga hullettii* leaf extract and its fractions using *in vitro* studies. The flavonoids isolated from *Macaranga* species in East Kalimantan, Indonesia, were evaluated for their antibacterial activity using molecular docking analysis.

Materials and Methods

Materials

The solvents used (methanol, n-hexane, and ethyl acetate) were purified by distillation before use. The materials used in this study were *Macaranga hullettii* leaves, *Staphylococcus aureus* ATCC 25923 and *Streptococcus mutans* ATCC 25175 obtained from Chulalongkorn University, *Propionibacterium acnes* KCCM 41747 obtained from Mulawarman University, nutrient agar (NA) from Merck, Luria Bertani liquid medium (1% tryptone, 0.5% yeast, and 0.5% NaCl), yeast extract (Oxoid 100% (w/w)), tryptone (Oxoid 100% (w/w)), ampicillin 100 mg/ml (Sigma Aldrich), NaCl (Sigma Aldrich), cotton swabs, aluminum foil, gauze, Petri dishes, Ose needles, paper discs, an autoclave (Autoclave TOMY, Model SX-700), an oven (SHARP EO-28 WH), a rotary vacuum evaporator (EYELA Rotary evaporator N-1300E V.S.Series), and a freezer (Sanyo Medicoool). For molecular docking analysis, we used a Lenovo Yoga 7 computer with AMD Ryzen AI 7-8840HS w/ Radeon 780M graphics, 3301 Mhz, 8 Core(s), and 16.0 GB of RAM.

Methods

Plant Collection and Identification:

The leaves of *Macaranga hullettii* plants were collected from the Lesan River Forest, Kelay District, Berau Regency, East Kalimantan, Indonesia (1°32'2.5919" N, 117°3'11.950" E). The plant samples were identified at the Laboratory of Anatomy and Plant Systematics, FMIPA Mulawarman University (No. 0174/UN.17.8.5.7.16/HA/XI/2017).

Extraction

Macaranga hullettii leaf powder (1.3 kg) was obtained by maceration twice for 24 h using 7.0 L methanol. A rotary vacuum evaporator was then used to obtain a methanol extract (126 gram).

After that, methanol extract (100 gram) was dissolved in the mixture of methanol and H₂O 500 mL with ratio of 8:2 and then successively fractionated using 5 x 200 mL of n-hexane and ethyl acetate to obtain n-hexane fraction (28 gram) and ethyl acetate fraction (18 gram), respectively. Each fraction was evaporated using a rotary vacuum evaporator. The methanol extract, n-hexane fraction, and ethyl acetate fraction were evaluated for antibacterial activity.

Pathogens

Staphylococcus aureus ATCC 25923 was obtained from the Department of Microbiology, Faculty of Science, Chulalongkorn University. *Streptococcus mutans* ATCC 25175 was obtained from the Department of Biochemistry, Faculty of Dentistry, Chulalongkorn University. Both strains were purchased from the American Type Culture Collection (ATCC). *Propionibacterium acnes* KCCM 41747 was obtained from the Faculty of Forestry, Mulawarman University, and from the Korean Culture Center of Microorganisms (KCCM). The bacteria were periodically subcultured and maintained in nutrient agar (NA) under suitable conditions.

In vitro antibacterial assay

The antibacterial properties of the methanol, n-hexane, and ethyl acetate extracts of *Macaranga hullettii* leaves were analyzed using the disc paper or the Kirby-Bauer method.^{26,27} *Staphylococcus aureus* ATCC 25923, *Streptococcus mutans* ATCC 25175, and *Propionibacterium acnes* KCCM 41747 were assayed. Ampicillin was used as a positive control, and the three organic solvents were used as negative controls. Nutrient Agar (NA) medium was prepared by dissolving 3.3 grams in 150 mL of distilled water. Luria Bertani (LB) liquid media was prepared by dissolving 4 grams in 500 mL of distilled water, stirring, and adding 5 mL to a test tube. The NA and LB media were sterilized using an autoclave at 121 °C

with a pressure of 1 atm for 15 minutes. Each tested bacterium was collected using an Ose needle, followed by dilution in a test tube containing sterile LB media and incubation for 24 h at 37 °C.

Nutrient Agar (25 mL) was poured into sterilized Petri dishes and maintained at room temperature for solidification. A sample of the bacterial suspension in the test tubes was then collected using a sterile cotton swab and applied to the surface of the agar in the Petri dish. Each 30 µL of the tested *M. hullettii* leaf samples prepared in various concentrations of the organic solvents was poured onto sterile adsorbent filter paper discs (6 mm in diameter), and the solvent was removed. Afterward, the paper discs containing the tested sample were placed on the surface of the agar media and incubated at 37°C for 18-24 hours. The inhibition zone formed in each well was measured using a ruler.^{28,29} The minimum inhibitory concentration (MIC) was determined for the methanol extract as well as the n-hexane and ethyl acetate fractions. MIC was defined as the minimum concentration that completely inhibited bacterial growth.³⁰

Molecular docking

Eight flavonoid derivatives possessing either prenyl or geranyl group occurred in the three *Macaranga* species in East Kalimantan, Indonesia (*Macaranga hosei*, *Macaranga pearsonii*, and *Macaranga tanarius*): 4'-O-methyl-8-isoprenylnaringenin, lonchocarpol A, 4'-O-methyl-8-isoprenyleriodictyol, 6-isoprenyleriodictyol, solophenol D, nymphaeol A, nymphaeol B, and nymphaeol C.¹⁴⁻¹⁷ Macahuilettiin A is a prenylated flavonoid found in *Macaranga huletti* obtained from Central Kalimantan (Indonesia).¹⁸ A comparative analysis of the molecular docking results for prenylated/geranylated flavonoids with methoxylated flavonoids was

conducted, focusing on 5-hydroxy-7,4'-dimethoxyflavone and 5-hydroxy-6,7,4'-trimethoxyflavone that are found in *Macaranga hosei* from Trengganu, Malaysia²² (Table 2). The molecular structures of the flavonoids were drawn using ChemOffice Professional 15.0 and obtained from PubChem (<https://pubchem.ncbi.nlm.nih.gov/>). Structure refinement was performed using the molecular mechanics energy minimization method with the Merck molecular force field (MMFF94) using Open Babel in PyRx V.1.1. Three proteins were downloaded from the protein data bank (PDB ID: 1JIJ, 3IPK, 7LBU),³¹⁻³³ and the locations of active sites were calculated using AlphaFold from <https://prankweb.cz/>.³⁴ Molecular docking was performed using the AutoDock Vina tool in PyRx V.1.1 software with an exhaustiveness of 32 and a mode value of 9 poses for each docked ligand.^{35,36} A protein binding site was defined as a box with dimensions of 30 × 30 × 30 Å. In the final step, the binding interactions were analyzed, and the docking results were visualized in 2D using the BIOVIA Discovery Studio Visualizer.

Statistical analysis

Data from the experiments were analyzed using Microsoft Excel.

Results and Discussion

***In vitro* antibacterial activity**

The strength of antibacterial activity was determined based on the diameter of the clear zone, which was used to measure the inhibition zone (mm). This classification was as follows: ≤ 5 mm (weak), 5-10 mm (moderate), 10-20 mm (strong), and ≥ 20 mm (very strong). Moreover, the lower the minimum inhibitory concentration of a compound, the greater its ability to inhibit the growth of test bacteria.²⁸ In this study, ampicillin was used as a positive control against three bacteria: *Staphylococcus aureus* ATCC 25923, *Streptococcus mutans* ATCC 25175, and

Propionibacterium acnes KCCM 41747 (Table 1). The positive control served to determine whether the test bacteria could be inhibited by or were resistant to the antibiotics.

Staphylococcus aureus can cause dentoalveolar infections, jaw cysts, paroritis, oral mucosal lesions, and stomatitis. The bacteria can also produce exotoxins, lecosidine, enterotoxins, and quagulase compounds. Enterotoxins are compounds that cause food poisoning in humans.³⁷

The methanol extract, hexane fraction, and ethyl acetate fraction of *Macaranga hulettii* leaves showed moderate antibacterial activity against *S. aureus*, with an inhibition zone of 5-10 mm at concentrations of 0.15 to 2.5% (Table 1). The inhibition zone of the 0.15% methanol extract measured 6.67 mm. The 0.15% ethyl acetate fraction produced an inhibition zone of 6.33 mm, and the 2.5% n-hexane fraction produced an inhibition zone of 6.50 mm. The results indicated that the methanol extract and ethyl acetate fraction had the most potent antibacterial activity against *S. aureus*, with a minimum inhibitory concentration (MIC) of < 0.15%. Moreover, the hexane fraction of *Macaranga tanarius* was most effective against *S. aureus*, with MIC values of 0.125-0.5% and an inhibition zone of approximately 7.25 mm.³⁸

Streptococcus mutans is implicated in the pathogenesis of dental caries. This facultative anaerobe primarily ferments carbohydrates, resulting in the production of several organic acids, with lactic acid as the predominant byproduct. The acidification of the dental environment by *S. mutans* is a crucial factor contributing to enamel demineralization and the subsequent formation of carious lesions.^{39,40} The bacteria produce glucosyltransferase (GTF) to produce glucan, the compound that causes dental caries.⁴¹ *S. mutans* adheres to the salivary pellicle due to having many receptors for attachment.⁴² We tested the antibacterial activity of *Macaranga hulettii* King leaf extract against *S. mutans* (Table 1). Both extracts displayed moderate antibacterial activity, with inhibition zones of 5-10 mm at concentrations of 0.15-2.5%. The widths of the inhibition zones created by the methanol extract and ethyl acetate fraction were 6.67 and 6.33 mm at 0.15%, respectively. Meanwhile, the n-hexane fraction showed

antibacterial activity at concentrations of 1.25-2.5%, with inhibition zones of 6.33-6.67 mm. These results demonstrated that the methanol extract and ethyl acetate fraction of *Macaranga hulettii* leaves presented the highest potential antibacterial activity against *S. mutans*, with an MIC < 0.15% (Table 1).

Propionibacterium acnes is a Gram-positive anaerobic bacterium that can induce unusual keratinization within the sebaceous glands of hair follicles and increase sebum production.⁴³ The enzyme is capable of hydrolyzing triglycerides (TG) present in sebum into free fatty acids (FFA) that may subsequently induce inflammation in and around the hair follicles.⁴⁴ The bacteria also produce hyaluronidase, protease, lecithinase, and neuraminidase, that can cause inflammation of the skin. *Macaranga triloba* ethanol can prevent the growth of *P. acnes*, and the most significant inhibition value of 5.54 mm at 20%.⁴⁵ As shown in Table 1, the methanol extract and ethyl acetate fraction of *Macaranga hulettii* showed potent antibacterial activity against *P. acnes*, with respective inhibition values of 10.67 and 14.67 mm. The n-hexane fraction exhibited moderate antibacterial activity, with inhibition values of 5-10 mm at 1.25-2.5%. The methanol extract showed antibacterial activity with an inhibition value of 7.67 at 0.31%. The ethyl acetate fraction showed antibacterial activity of 0.15%, with an inhibition zone of 6.67 mm. The results demonstrated that the ethyl acetate fraction of *Macaranga hulettii* leaves presented the highest potential antibacterial activity against *P. acnes*, with a minimum inhibitory concentration (MIC) of < 0.15% (Table 1).

The methanol extract and ethyl acetate fraction exhibited good antibacterial activity against the three bacterial strains. This finding was consistent with previous studies that attributed the antibacterial effects to the presence of flavonoids and their prenylated or geranylated forms in species of *Macaranga*. These included euchrestaflavanone A, bonanione A, macarangaflavanone A, macarangaflavanone B, macatrichocarpin A, propolin D (nymphaeol B), senegalensin, schweinfurthin B, schweinfurthin O, and isomacarangain.⁴⁶⁻⁵⁶ Flavonoid

compounds can damage bacterial cell walls and denature proteases, thereby disrupting bacterial metabolism.⁵⁷ In addition, alkaloids can damage the peptidoglycan layer of bacterial cells, damaging the cell wall and inhibiting bacterial growth. Terpenoids also inhibit bacterial growth by damaging cell membranes. The phenolic compounds of secondary metabolites can damage bacterial cells by increasing the permeability of the cell membrane, resulting in cytoplasmic coagulation and cell division.²⁸

Molecular docking

Molecular docking was performed to predict the interactions of the flavonoids with the amino acid residues of the three proteins. This study used three proteins from *Staphylococcus aureus*, *Streptococcus mutans*, and *Propionibacterium acnes*.³¹⁻³³ The molecular docking results (Table 2) revealed that 11 flavonoid derivatives had binding affinities ranging from -7.2 to -10.5 kcal/mol, -7.5 to -8.9 kcal/mol, and -7.1 to -8.9 kcal/mol against *Staphylococcus aureus* tyrosyl-tRNA synthetase, *Streptococcus mutans* antigen I/II (AgI/II), and *Propionibacterium acnes* surface sialidase, respectively. The interactions of flavonoids and ampicillin with the protein targets are shown in two-dimensional graphs in Figures 1-3.

Among the compounds targeting *S. aureus* tyrosyl-tRNA synthetase, nymphaeol A-B and solophenol D had the highest binding affinities compared to ampicillin as a positive control.

Thus, the interactions of these compounds with the active site of the enzyme were modeled as a 2D structure (Figure 1). Ampicillin formed hydrogen bonds with LYS84 and GLN174 and amide- π interactions with GLY38 (Figure 1). Nymphaeol A and nymphaeol B formed hydrogen bonds with four amino acid residues, π -anions, and hydrophobic interactions, whereas nymphaeol B and ampicillin showed similar interactions with LYS84. In addition, solophenol D formed hydrogen bonds with TYR36, THR75, GLN190, ASP177, and GLN174, as well as hydrophobic interactions with HIS50, LEU70, PRO53, and ALA39 (Figure 1). These results

agreed with previous studies where nymphaeol A-B and solophenol D exhibited antibacterial activity.^{58,59}

Five compounds exhibited high antibacterial activity against *S. mutans* antigen I/II (AgI/II), including 6-isoprenyleriodictyol, nymphaeol A-B, and solophenol D, with binding affinities from -8.7 to -8.9 kcal/mol. The interactions between the ligands and amino acid residues in the binding sites of these compounds are shown in 2D views (Figure 2). Ampicillin formed hydrogen bonds with four amino acid residues, ASN590, SER591, SER588, and ASP760, as well as π -sulfur and π - π interactions with TRP 816, while 6-isoprenyleriodictyol only formed hydrogen bonds with three amino acid residues: SER697, ASN699, and ASN814. Nymphaeol A and nymphaeol B formed hydrogen bonds with four amino acid residues (Figure 2), but nymphaeol A exhibited a similar π - π interaction with TRP816, as observed with ampicillin. Solophenol D formed hydrogen bonds with SER818, ARG824, and THR586, similar to the π - π interactions (TRP816) with ampicillin, and π -sigma interactions with SER762. As 6-isoprenyleriodictyol is a prenylated flavonoid similar to nymphaeol A, and hence may have antibacterial activity.

Three compounds, including 6-isoprenyleriodictyol and nymphaeol A, displayed stronger binding affinities than ampicillin, with values of -8.0 and -8.4 kcal/mol against *P. acnes* surface sialidases. Therefore, both compounds were imaged in 2D to investigate the interactions between the ligands and the amino acid residues in the binding sites (Figure 3). Ampicillin formed hydrogen bonds with four amino acid residues, ARG282, GLN287, ASP146, ASP185, and π - π and π -alkyl interactions with PHE257, ALA147, and VAL202; 6-Isoprenyleriodictyol demonstrated π - π interactions with PHE257, π -anion interactions with ASP146, and alkyl-alkyl interactions with ALA147, PHE209, VAL202, and LEU224. This compound formed bonds with six amino acid residues, including ASP146, ARG329, TYR423, ARG282, and GLU287, that were analogous to those observed with ampicillin. Nymphaeol A formed hydrogen bonds

with five amino acid residues (ARG329, TYR423, ASP185, ALA147, and SER184), π -anion interactions with ASP312 and ASP185, alkyl-alkyl interactions with MET310, ALA331, and ALA147, and Van der Waals interactions with PHE257. Thus, these compounds may have potent antibacterial activity.

Structure-activity relationships were also studied based on the binding affinity results (Table 2). Flavonoids possessing prenyl, geranyl, and hydroxyl groups showed potential as antibacterial agents due to their strong affinity for amino acid residues in the binding sites of proteins, compared to ampicillin. However, the absence of a hydroxyl group on prenylated or geranylated flavonoids decreased the binding affinity.^{22,56,60}

Conclusion

The extracts and fractions of *Macaranga hulettii* have potential as antibacterial agents against three bacterial strains, *Staphylococcus aureus* ATCC 25923, *Streptococcus mutans* ATCC 25175, and *Propionibacterium acnes* KCCM 41747. The methanol extract demonstrated antibacterial activity against *S. aureus* and *S. mutans* with an MIC (%) of < 0.15 against *S. aureus* and *S. mutans*, and 0.15-0.31% against *P. acnes*. The n-hexane fraction had an MIC (%) of 1.25-2.5% against *S. aureus*, and 0.62-1.25% against *S. mutans* and *P. acnes*. The ethyl acetate fraction had an MIC (%) < 0.15% against the three bacterial strains. Molecular docking studies suggested that 6-isoprenyleriodictyol, nymphaeol A, nymphaeol B, and solophenol D are promising antibacterial agents, as those compounds exhibited the highest binding affinity and formed hydrogen bonds with key amino acid residues in the active sites of the protein targets. Further *in vitro* and *in vivo* studies should be conducted to isolate the active secondary metabolites from this species.

Conflict of Interest

The authors declare that they have no conflicts of interest.

Author's Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims related to the content of this article will be borne by them.

Acknowledgement

This research was funded by the Islamic Development Bank (PIU-IsDB) project under contract no. 137/UN17.11/PL/2019.

References

1. Wong F, de la Fuente-Nunez C, Collins JJ. Leveraging artificial intelligence in the fight against infectious diseases. *Science*. 2023; 381(6654):164-70.
2. Seukep AJ, Kuete V, Nahar L, Sarker SD, Guo M. Plant-derived secondary metabolites as the main source of efflux pump inhibitors and methods for identification. *J. Pharm. Anal.* 2020; 10(4):277-90.
3. Magadula JJ. Phytochemistry and pharmacology of the genus *Macaranga*: a review. *J. Med. Plants Res.* 2014; 8(12):489-503.
4. Vu LT, Ngan TB, Phuong L, Huong DT, Litaudon M, Van Hung N, Thach TD, Van Cuong P. Chemical constituents from fruits of *Macaranga denticulata* (Euphorbiaceae)(Part 2). *Vietnam J. Chem.* 2018; 56(4):516-20.
5. Marliana E, Hairani R, Tjahjandarie TS, Tanjung M. Antiplasmodial activity of flavonoids from *Macaranga tanarius* leaves. *IOP Conf. Ser.: Earth Environ. Sci.* 2018; 144(1):012011.

6. Tjahjandarie TS, Tanjung M, Saputri RD, Nadar PB, Aldin MF, Marlina E, Permadi A. Flavestins K, An isoprenylated stilbene from the leaves of *Macaranga recurvata* Gage. Nat. Prod. Sci. 2019; 25(3):244-7.
7. Huong DT, Linh NT, Van TT, Litaudon M, Roussi F, Van Nam V, Van Cuong P. Stilbenes from *Macaranga tanarius* (Euphorbiaceae) growing in Vietnam. Vietnam J. Chem. 2020; 58(3):338-42.
8. Aldin MF, Tjahjandarie TS, Saputri RD, Tanjung M. Macasiamenene V, a New Stilbenoid from the Leaves of *Macaranga inermis*. Nat. Prod. Sci. 2021; 27(1):45-8.
9. Muharram A, Rachmawati DA, Mardhiyyah S, Tjahjandarie TS, Saputri RD, Ahmat N, Tanjung M. Cytotoxic and antioxidant activities of flavonoids and diterpenoids from *Macaranga involucreata* (Roxb.) Baill. J. Appl. Pharm. Sci. 2023; 13(6):087-92.
10. Tanjung M, Tjahjandarie TS, Aldin MF, Mardhiyyah S, Ahmat N, Saputri RD. Macagigantin A, a new flavonoid from *Macaranga gigantea* (Rchb. f & Zoll.) Mull. Arg. Nat. Prod. Sci. 2023; 29(4):287-94.
11. Tjahjandarie TS, Aldin MF, Saputri RD, Tanjung M. Dihydrostilbenes from *Macaranga javanica* (Blume) Müll. Arg. and their antiplasmodial activity. Nat. Prod. Sci. 2024:1-7.
12. Tanjung M, Tjahjandarie TS, Aldin MF, Mardhiyyah S, Saputri RD, Syah YM, Ahmat N. Two new flavonols from *Macaranga inermis* pax & K. Hoffm. Nat. Prod. Sci. 2025; 39(3):498-505.
13. Tanjung M, Tjahjandarie TS, Aldin MF, Mardhiyyah S, Saputri RD, Syah YM, Ahmat N. Two new flavonols from *Macaranga inermis* pax & K. Hoffm. Nat. Prod. Sci. 2025; 39(3):498-505.
14. Marlina E, Tjahjandarie TS, Tanjung M. Isoprenylated flavanone derivatives from *Macaranga hosei* King ex Hook F. Der Pharm. Lett. 2015; 7(3):153-6.

15. Marliana E, Tjahjandarie TS, Tanjung M. Antioxidant activity of flavonoid compounds from *Macaranga pearsonii* Merr. J. Kim. Mulawarman. 2016; 13(2):97-100.
16. Marliana E, Astuti W, Kosala K, Hairani R, Tjahjandarie TS, Tanjung M. Chemical composition and anticancer activity of *Macaranga hosei* leaves. Asian J. Chem. 2018; 30(4):795-8.
17. Marliana E, Ruga R, Hairani R, Tjahjandarie TS, Tanjung M. Antioxidant activity of flavonoid constituents from the leaves of *Macaranga tanarius*. IOP Conf. Ser.: J. Phys. 2019; 1277(1):012014.
18. Saputri RD, Tukiran T, Wati FA, Purnamasari AP, Wardhana MW, Tjahjandarie TS, Tanjung M. Macahuilettiin A, a new isoprenylated flavanone from the leaves of *Macaranga hullettii* King ex Hook and their antiplasmodial activity. Vietnam J. Chem. 2024; 62(3):394-8.
19. Musdalifah M, Khumaidi A, Suwastika IN. Inhibition test and phytochemical screening of leaf extracts of *Macaranga tanarius* (L.) Mull. Arg against *Salmonella typhi* as an antibacterial. Nat. Sci. J. Sci. Technol. 2017; 6(3).
20. Ibrahim H, Omosa L, Nchiozem-Ngnitedem V, Onyari J, Maru S, Guefack M. Antibacterial activities and phytochemical screening of crude extracts from Kenyan macaranga species toward MDR phenotypes expressing efflux pumps. Pharmacogn. Commn. 2021; 11(2):119-26.
21. Bijesh K, Sebastian D. Isolation and characterization of antibacterial compounds from *Macaranga peltata* against clinical isolates of *Staphylococcus aureus*. Int. J. Biol. Pharma Res. 2013; 4:1196-203.
22. Salleh WM, Razak NZ, Ahmad F. Phytochemicals and biological activities of *Macaranga hosei* and *Macaranga constricta* (Euphorbiaceae). Marmara Pharm. J. 2017;21(4):881-8.

23. Slik JW, Priyono P, Welzen PV. Key to the *Macaranga Thou.* and *Mallotus Lour.* species (Euphorbiaceae) of East Kalimantan, Indonesia. Singapore, Gardens' Bulletin (Singapore), National Parks Board, Singapore Botanic Gardens; 2000. 11-87 p.
24. Amirta R, Angi EM, Ramadhan R, Kusuma IW, Wiati CB, Haqiqi MT. Potential utilization of macaranga. Samarinda: Mulawarman University Press; 2017.
25. Rismawati R, Marliana E, Daniel D. Phytochemical test on methanol extract of leaf of *Macaranga hullettii* King ex Hook. f. J. Atomik. 2018; 3(2):91-4.
26. Hudzicki J. Kirby-Bauer disk diffusion susceptibility test protocol. American society for microbiology. 2009; 15(1):1-23.
27. Inna M, Astuti W, Saleh C. Antibacterial activity of methanolic extract of uric patch plant leaves (*Cayratia carnosa*) against *Salmonella thypi* and *Propionibacterium acnes*. J. Atomik. 2022; 7(1):1-5.
28. Yulianti MF, Amat AL, Hutasoit RM, Pakan PD. Antibacterial activity of jamblang leaf ethanol extract (*Syzygium cumini*) against the growth of *Propionibacterium acnes*. Acta Biochimica Indones. 2023; 6(2):1161.
29. Hossain MR, Biplob AI, Sharif SR, Bhuiya AM, Sayem AS. Antibacterial activity of green synthesized silver nanoparticles of *Lablab purpureus* flowers extract against human pathogenic bacteria. Trop. J. Nat. Prod. Res. 2023;7(8).
30. Yanda L, Tatsimo SJ, Tamokou JD, Matsute-Takongmo G, Meffo-Dongmo SC, Meli Lannang A, Sewald N. Antibacterial and antioxidant activities of isolated compounds from *Prosopis africana* leaves. Int. J. Anal. Chem. 2022; 4205823.
31. Qiu X, Janson CA, Smith WW, Green SM, McDevitt P, Johanson K, Carter P, Hibbs M, Lewis C, Chalker A, Fosberry A. Crystal structure of *Staphylococcus aureus* tyrosyl-tRNA synthetase in complex with a class of potent and specific inhibitors. Protein Sci. 2001; 10(10):2008-16.

32. Larson MR, Rajashankar KR, Patel MH, Robinette RA, Crowley PJ, Michalek S, Brady LJ, Deivanayagam C. Elongated fibrillar structure of a *streptococcal adhesin* assembled by the high-affinity association of α -and PPII-helices. *Proc. Natl. Acad. Sci.* 2010; 107(13):5983-8.
33. Yu AC, Volkers G, Jongkees SA, Worrall LJ, Withers SG, Strynadka NC. Crystal structure of the *Propionibacterium acnes* surface sialidase, a drug target for *P. acnes*-associated diseases. *Glycobiology*. 2022; 32(2):162-70.
34. Jakubec D, Skoda P, Krivak R, Novotny M, Hoksza D. PrankWeb 3: accelerated ligand-binding site predictions for experimental and modelled protein structures. *Nucleic Acids Res.* 2022; 50:W593-7.
35. Dallakyan S, Olson AJ. Small-molecule library screening by docking with PyRx. In *Chemical biology: methods and protocols*. 2014: 243-250. New York, NY: Springer New York.
36. Trott O, Olson AJ. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *J. Comput. Chem.* 2010; 31(2):455-61.
37. Malangu N, editor. *Poisoning: From specific toxic agents to novel rapid and simplified techniques for analysis*. BoD–Books on Demand; 2017.
38. Sari AA, Saleh C. Phytochemical test, toxicity and antibacterial activity of extracts of various macaranga leaf fractions (*Macaranga tanarius* (L.) MA) against *Staphylococcus aureus* and *Escherichia coli*. *J. Kim. Mulawarman*. 2015; 12(2):53-8.
39. Imelda R., Mariam M., Satari M. Effect of cassava (*manihot esculenta* cranzt), rice (*oryza sativa* l.), and potato (*solanum tuberosum*) water extract to decrease pH phase fermentation of *streptococcus mutans* atcc 25175. *Padjajaran J. Dent.* 2019; 31(1):14.

40. Alkhaled A., Alsabek L., Al-assaf M. , & Badr F.. Effect of chlorhexidine, honey and propolis on *streptococcus mutans* counts: *in vitro* study. Dentistry. 2021; 9(1).
41. Ren Z, Chen L, Li J, Li Y. Inhibition of *Streptococcus mutans* polysaccharide synthesis by molecules targeting glycosyltransferase activity. J. Oral Microbiol. 2016; 8(1):31095.
42. Lee BS, Chen YJ, Wei TC, Ma TL, Chang CC. Comparison of antibacterial adhesion when salivary pellicle is coated on both poly (2-hydroxyethyl-methacrylate)-and polyethylene-glycol-methacrylate-grafted poly (methyl methacrylate). Int. J. Mol. Sci. 2018; 19(9):2764.
43. Chen KC, Yang CH, Li TT, Zouboulis CC, Huang YC. Suppression of Propionibacterium acnes-stimulated proinflammatory cytokines by Chinese bayberry extracts and its active constituent myricetin in human sebocytes *in vitro*. Phytother. Res. 2019; 33(4):1104-13.
44. Xu J, Chen X, Song J, Wang C, Xu W, Tan H, Suo H. Antibacterial activity and mechanism of cell-free supernatants of *Lactocaseibacillus paracasei* against *Propionibacterium acnes*. Microb. Pathog. 2024; 189:106598
45. Warnida H, Mustika D, Supomo S, Sukawaty Y. Effectiveness of Mahang Leaf Ethanol Extract (*Macaranga Triloba*) as an anti-acne. J. Penelit. Sos. Ekon. Kehuta. 2018; 4(1):9-18.
46. Schütz BA, Wright AD, Rali T, Sticher O. Prenylated flavanones from leaves of *Macaranga pleiostemona*. Phytochemistry. 1995; 40(4):1273-7.
47. Lim TY, Lim YY, Yule CM. Evaluation of antioxidant, antibacterial and anti-tyrosinase activities of four Macaranga species. Food Chem. 2009; 114(2):594-9.
48. Fareza MS, Syah YM, Mujahidin D, Juliawaty LD, Kurniasih I. Antibacterial flavanones and dihydrochalcones from *Macaranga trichocarpa*. Z. Naturforsch C J. Biosci. 2014; 69(9-10):375-80.

49. Hasanat A, Kabir MS, Hossain MM, Hasan M, Al Masum MA, Chowdhury TA, Bhuiyan DI, Mamur A, Kibria AS. Antibacterial activity of methanol extract of *Macaranga denticulata* leaves and in silico PASS prediction for its six secondary metabolites. World J. Pharm. Pharm. Sci. 2015;1258-66.
50. O Akanbi B, Anene P, Olayanju S. Preliminary Screening Indicates Promising antimicrobial properties of the stem bark extracts of *Macaranga rosea*. Anti-Infective Agents. 2015; 13(2):123-8.
51. Ogundajo A, Okeleye B, Ashafa AO. Chemical constituents, *in vitro* antimicrobial and cytotoxic potentials of the extracts from *Macaranga barteri* Mull-Arg. Asian Pac. J. Trop. Biomed. 2017; 7(7):654-9.
52. Putri R, Hendra R, Teruna HY. Anti-bacterial and anti-fungal activities from *Macaranga bancana* leaves extract. Pharmacol. Clin. Pharm. Res. 2019; 4(1):1-4.
53. Lee JH, Kim YG, Khadke SK, Yamano A, Woo JT, Lee J. Antimicrobial and antibiofilm activities of prenylated flavanones from *Macaranga tanarius*. Phytomedicine. 2019; 63:153033.
54. Pagna JI, Awazi T, Mbarga PE, Mbekou IM, Mkounga P, Fotie J, Frese M, Fabrice FB, Lenta BN, Sewald N, Nkengfack EA. Antibacterial flavonoids from the fruits of *Macaranga hurifolia*. J. Asian Nat. Prod. Res. 2022; 24(11):1041-51.
55. Kamso VF, Simo Fotso CC, Kanko Mbekou IM, Toussie BT, Ndjakou Lenta B, Boyom FF, Sewald N, Frese M, Ngadjui BT, Wabo Fotso G. Chemical constituents of *Macaranga occidentalis*, antimicrobial and chemophenetic studies. Molecules. 2022; 27(24):8820.
56. Rosamah E, Haqiqi MT, Putri AS, Kuspradini H, Kusuma IW, Amirta R, Yuliansyah Y, Suwinarti W, Paramita S, Ramadhan R, Tarmadi D. The potential of macaranga plants as skincare cosmetic ingredients: A review. J. Appl. Pharm. Sci. 2023; 13(7):001-12.

57. Li AP, He YH, Zhang SY, Shi YP. Antibacterial activity and action mechanism of flavonoids against phytopathogenic bacteria. *Pestic. Biochem. Phys.* 2022; 188:105221.
58. Chen YW, Ye SR, Ting C, Yu YH. Antibacterial activity of propolins from Taiwanese green propolis. *J. Food Drug Anal.* 2018; 26(2):761-8.
59. Inui S, Hosoya T, Shimamura Y, Masuda S, Ogawa T, Kobayashi H, Shirafuji K, Moli RT, Kozono I, Shin-ya K, Kumazawa S. Solophenols B–D and Solomonin: New Prenylated Polyphenols Isolated from Propolis Collected from The Solomon Islands and Their Antibacterial Activity. *J. Agri. Food Chem.* 2012; 60(47):11765-70.
60. Ruga R, Kingkaew K, Tamsampaoloet K, Chavasiri W. Enhancing antibacterial activity against *Propionibacterium acnes* and *Staphylococcus aureus* by combination of tetracycline with selected compounds. *Chem. Lett.* 2018; 47(12):1538-41.

Table 1: Antibacterial Activity of *Macaranga hullettii* King ex Hook Leaf Extracts and Fractions against *Staphylococcus aureus*, *Streptococcus mutans*, and *Propionibacterium acnes*

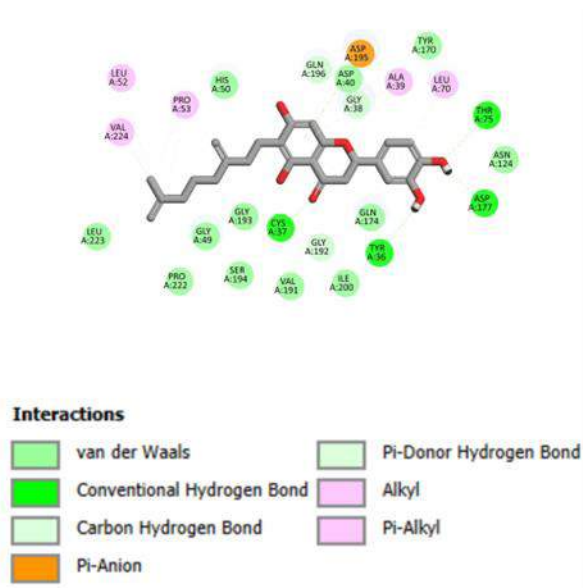
Sample	Concentration (%)	Inhibition Zone (mm)*		
		<i>Staphylococcus</i>	<i>Streptococcus</i>	<i>Propionibacterium</i>
		<i>aureus</i>	<i>mutans</i>	<i>acnes</i>
Methanol Extract	0	0	0	0
	0.15	6.67±0.58	6.67±0.58	0
	0.31	6.67±0.58	6.67±0.58	7.67±0.58
	0.62	6.67±0.58	7.0±0.0	8.0±1.0
	1.25	7.17±0.29	7.33±0.58	9.67±0.58
	2.5	7.5±0.5	8.0±1.0	10.67±0.58
	MIC (%)	<0.15	<0.15	0.15-0.31
n-Hexane Fraction	0	0	0	0
	0.15	0	0	0
	0.31	0	0	0
	0.62	0	0	0
	1.25	0	6.33±0.29	6.33±0.58
	2.5	6.5±0.5	6.67±0.58	6.67±0.58
	MIC (%)	1.25-2.5	0.62-1.25	0.62-1.25
Ethyl acetate Fraction	0	0	0	0
	0.15	6.33±0.58	6.33±0.58	6.67±0.58
	0.31	6.5±0.5	6.67±0.58	8.0±0.0
	0.62	6.5±0.5	6.83±0.29	9.0±0.0
	1.25	6.67±0.29	7.0±0.0	11.0±1.0
	2.5	7.0±0.0	7.67±0.58	14.67±0.58

	MIC (%)	<0.15	<0.15	<0.15
Ampicillin	0.01	20.33±1.15	21.0±1.0	21.0±1.0

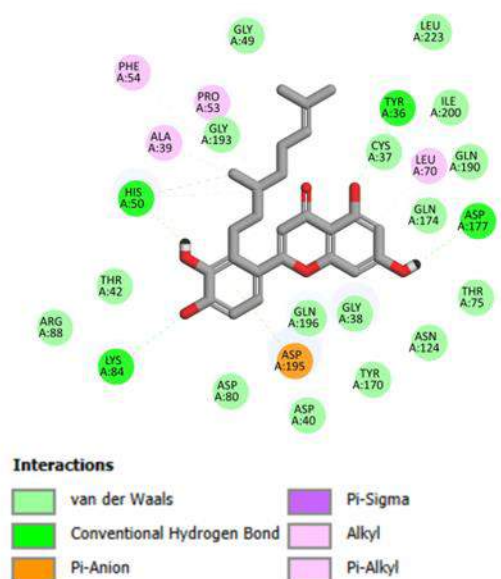
*Data were obtained from triplicate experiments.

Table 2: Binding affinity of flavonoids against three proteins (1JJJ, 3IPK, and 7LBU)

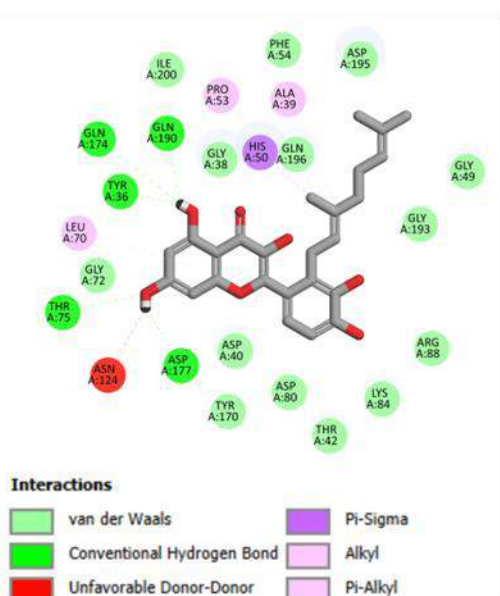
No.	Compound	Binding Affinity (kcal/mol)		
		1JJJ	3IPK	7LBU
1	4'- <i>O</i> -methyl-8-isoprenylnaringenin	-7.4	-8.1	-7.7
2	4'- <i>O</i> -methyl-8-isoprenyleriodictyol	-7.2	-8.1	-7.6
3	6-Isoprenyleriodictyol	-7.7	-8.7	-8.0
4	5-hydroxy-7,4'-dimethoxyflavone	-8.6	-7.8	-7.1
5	5-hydroxy-6,7,4'-trimethoxyflavone	-8.3	-7.5	-7.3
6	Macahuilettiin A	-7.6	-8.4	-7.1
7	Lonchocarpol A	-9.4	-7.8	-7.8
8	Nymphaeol A	-9.7	-8.9	-8.4
9	Nymphaeol B	-10.5	-8.9	-7.7
10	Nymphaeol C	-8.4	-8.3	-7.4
11	Solophenol D	-10.0	-8.7	-7.9
12	Ampicillin	-8.5	-7.7	-7.6



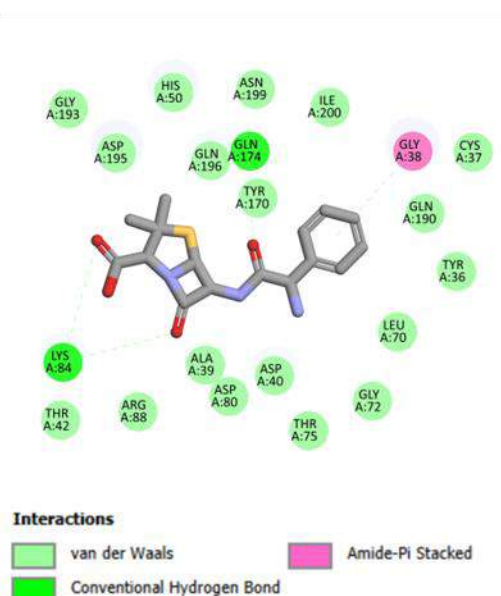
Nymphaeol A



Nymphaeol B

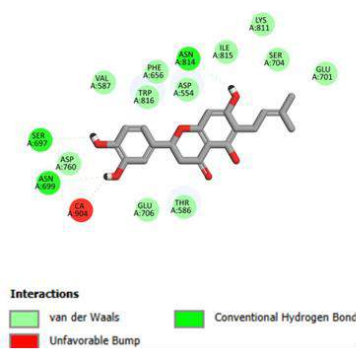


Solophenol D

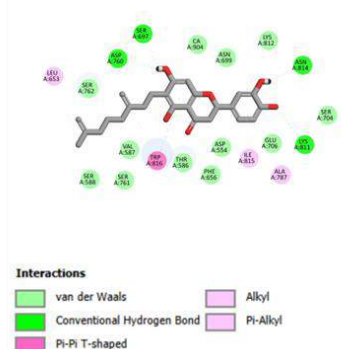


Ampicillin

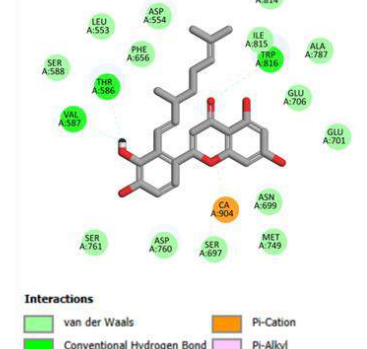
Figure 1: 2D interaction of the best docked flavonoids and ampicillin with *Staphylococcus aureus* tyrosyl-tRNA synthetase (PDB ID: 1JIJ)



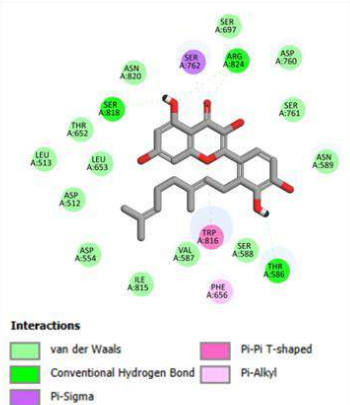
6-Isoprenylioridictyol



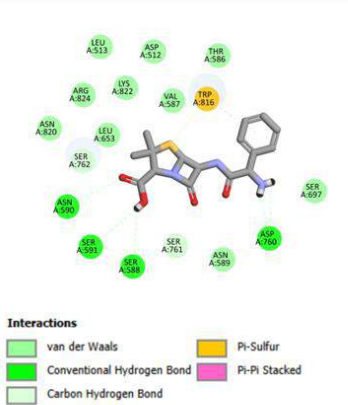
Nymphaeol A



Nymphaeol B



Solophenol D



Ampicillin

Figure 2: 2D interaction of the best-docked flavonoids and ampicillin with *Streptococcus mutans* antigen I/II (PDB ID: 3IPK).

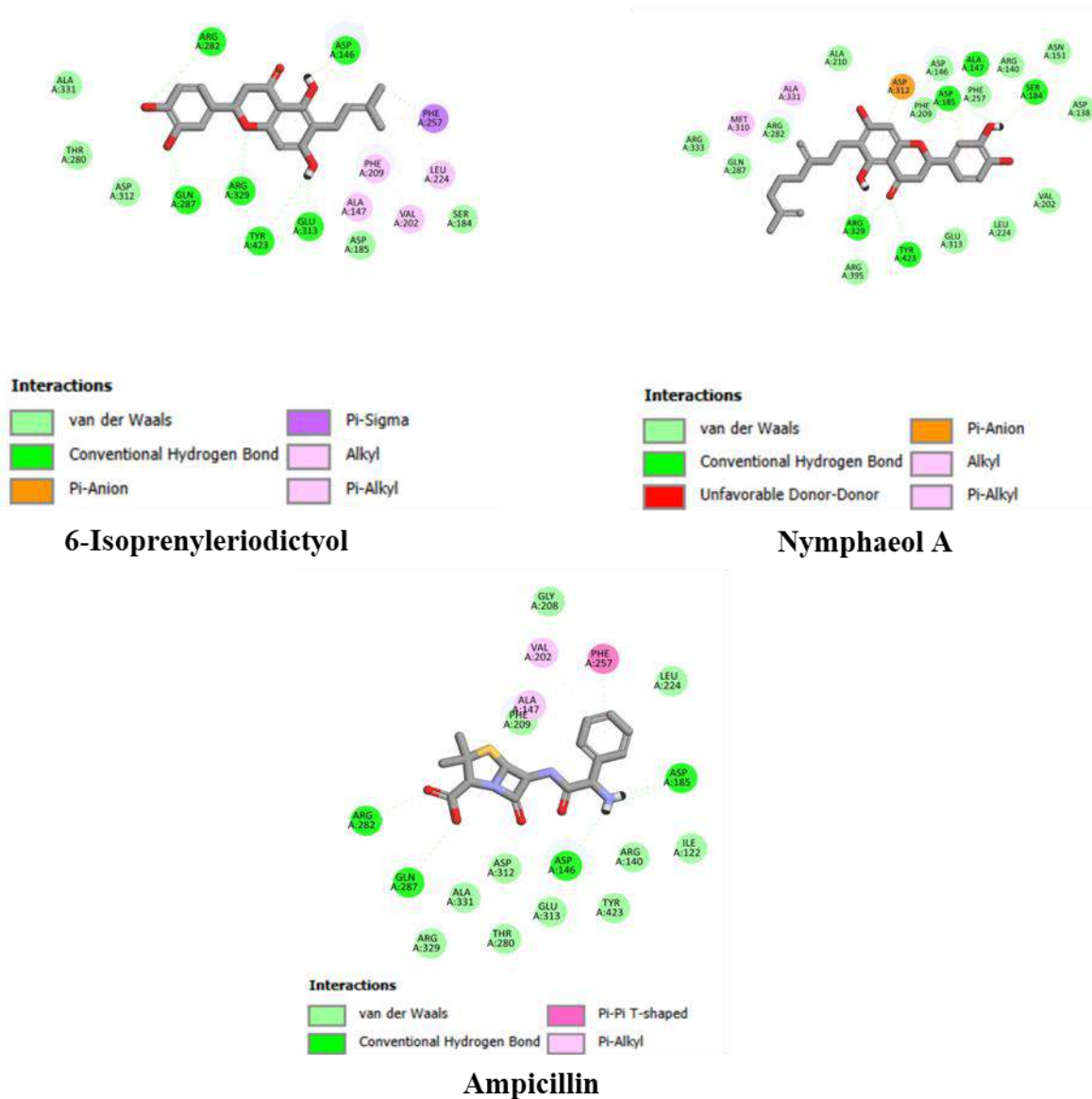


Figure 3: 2D interaction of the best-docked flavonoids and ampicillin with *Propionibacterium acnes* surface sialidase (PDB ID: 7LBU).



Outlook

English Editing of Your Manuscript

From Osayemwenre Erharuyi <erharuyiosaz@gmail.com>

Date Tue 5/27/2025 7:08 AM

To Ade Danova <adedanova@itb.ac.id>

 2 attachments (1,020 KB)

3-TJNPR-2025-420 Revised_Proofreading - Edited.docx; Certificate of English Editing - Marliana et al..pdf;

Dear Author,

Please find attached the edited version of your manuscript, and the editing certificate.

Best wishes.

Caution: This Message was sent from outside of Institute Technology Bandung (ITB). Please do not click Link or open attachment unless you recognize the source of this email and know the content is safe. Please report all suspicious email to "it-helpdesk@itb.ac.id" as an attachment.

Certificate of English Editing

Ref No.: EO/EES/Cert/M003/05/25

This is to certify that the manuscript titled:

***In vitro* Evaluation and Molecular Docking Study on
Antibacterial Potential of *Macaranga hullei* King ex Hook.f.
Leaf Extract**

Authored by Eva Marlina, Ade Danova, Rita Hairani, Ritbey Ruga, Elvira Hermawati and Ana Yulvia

has been edited for English Language; Grammar, Spelling, Punctuation, Sentence Structure and Formatting Style. The editing process improved on the Scientific Quality of the Manuscript without affecting the Authors' intended message.



Osayemwenre Erharuyi, PhD

Associate Professor and Research Fellow,
Department of Pharmaceutical Chemistry,
University of Benin, Nigeria

Issued on 27th Day of May, 2025

Re: Revision

From Ade Danova <adedanova@itb.ac.id>

Date Tue 5/27/2025 10:57 AM

To Editor-in-Chief Tjnpr <editor.tjnpr@gmail.com>

Cc evakia@fmipa.unmul.ac.id <evakia@fmipa.unmul.ac.id>; ritahairani@fmipa.unmul.ac.id <ritahairani@fmipa.unmul.ac.id>; ritbey.r@fmipa.unmul.ac.id <ritbey.r@fmipa.unmul.ac.id>; Dr. Elvira Hermawati, S.Si., M.Si. <elvira.hermawati@itb.ac.id>; anayulvia@unej.ac.id <anayulvia@unej.ac.id>

 2 attachments (1,021 KB)

3-TJNPR-2025-420 Revised_Proofreading - Edited.docx; Certificate of English Editing - Marlana et al..pdf;

Dear Editor-in-Chief
Tropical Journal of Natural Product Research

Thank you very much for your patience to review this manuscript. I would like to submit our final manuscript after reviewing and editing English. Here, I attach two files: manuscript and certificate of editing English.

Best regards,
Ade Danova

Certificate of English Editing

Ref No.: EO/EES/Cert/M003/05/25

This is to certify that the manuscript titled:

***In vitro* Evaluation and Molecular Docking Study on
Antibacterial Potential of *Macaranga hulletti* King ex Hook.f.
Leaf Extract**

Authored by Eva Marlina, Ade Danova, Rita Hairani, Ritbey Ruga, Elvira Hermawati and Ana Yulvia

has been edited for English Language; Grammar, Spelling, Punctuation, Sentence Structure and Formatting Style. The editing process improved on the Scientific Quality of the Manuscript without affecting the Authors' intended message.



Osayemwenre Erharuyi, PhD

Associate Professor and Research Fellow,
Department of Pharmaceutical Chemistry,
University of Benin, Nigeria

Issued on 27th Day of May, 2025

***In vitro* Evaluation and Molecular Docking Study of the Antibacterial Potential of
Macaranga hulletti King ex Hook.f. Leaf Extract**

Eva Marlina^{1,3}, Ade Danova^{2*}, Rita Hairani^{1,3}, Ritbey Ruga^{1,3}, Elvira Hermawati², Ana Yulvia⁴

¹*Department of Chemistry, Faculty of Mathematics and Natural Sciences, Mulawarman University, Samarinda 75123, East Kalimantan, Indonesia*

²*Organic Chemistry Division, Department of Chemistry, Faculty of Mathematics and Natural Sciences, Institut Teknologi Bandung Jl. Ganesha No. 10, Bandung 70132, West Java, Indonesia*

³*Research Center for Medicine and Cosmetics from Tropical Rainforest Resources, Mulawarman University, Samarinda, Indonesia*

⁴*Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Jember, East Java, Indonesia*

***Corresponding author. E-mail: adedanova@itb.ac.id**

Tel.: +6281357944698.

Abstract

Due to the resistance of bacteria to antibiotics, there has been increasing interest in the use of plant extracts to combat infections. Plants in the genus *Macaranga* contain secondary metabolites such as flavonoids with antibacterial properties. *Macaranga hullettii* King ex Hook.f. are particularly noteworthy due to their widespread distribution in East Kalimantan. This study aimed to investigate the antibacterial potential of *M. hullettii* leaf extract through *in vitro* and molecular docking studies. The antibacterial activity of the methanol extract, n-hexane, and ethyl acetate fractions from *M. hullettii* leaves was evaluated against three bacterial species, *Staphylococcus aureus*, *Streptococcus mutans*, and *Propionibacterium acnes* using the disc diffusion assay. Molecular docking of eleven flavonoid derivatives presents in three *Macaranga* species was performed against selected bacterial proteins (PDB ID: IJIJ, 3IPK, and 7LBU). The results showed that both the methanol extract and ethyl acetate fraction displayed antibacterial activity against the three bacteria strains, with minimum inhibitory concentrations (MICs) < 0.15%, except for the methanol extract, which had MIC of 0.15-0.31% against *Propionibacterium acnes*. In addition, molecular docking study showed that four flavonoids possessing prenyl or geranyl groups (6-isoprenyleriodictyol, nymphaeol A, nymphaeol B, and solophenol D) showed the highest binding affinity and dominant hydrogen bonding interactions with key amino acid residues in the binding sites of three bacterial proteins. The findings suggest that the methanol extract and its ethyl acetate fraction from *Macaranga hulletti* leaves could be a potential source of new antibacterial agents. Further studies are needed to isolate and evaluate the bioactive compounds.

Keywords: Antibacterial, Extract, Flavonoid, *Macaranga hulletti*, Molecular Docking.

Introduction

Despite substantial advancements in medicine and fundamental research, infectious diseases caused by transmissible agents such as bacteria, protists, and viruses continue to present significant challenges to healthcare professionals. These challenges include the issue of antimicrobial resistance.¹ Extracts from medicinal plants present a promising approach to addressing multidrug-resistant bacteria. In addition, medicinal plants can augment the efficacy of antibiotics for the treatment of infectious diseases.² Therefore, it is imperative to develop more efficacious antimicrobial agents, particularly those sourced from natural sources, as these are both readily available and economically viable.

Plants in the genus *Macaranga* belong to the family Euphorbiaceae and are commonly known as "Mahang." The secondary metabolites contained in plants of this genus include flavonoids and stilbenoids with prenylated substituents.³⁻¹³ Nine flavonoids containing either prenyl or geranyl groups and displaying antioxidant, antiparasitic, and anticancer activities have been isolated from three species (*Macaranga hosei*, *Macaranga pearsonii*, and *Macaranga tanarius*) of *Macaranga* present in East Kalimantan: 4'-*O*-methyl-8-isoprenylnaringenin, 4'-*O*-methyl-8-isoprenyleriodictyol, 6-isoprenyleriodictyol, solophenol D, nymphaeol A, and nymphaeol B, including lonchocarpol A, nymphaeol C, and macahuillettin A that was isolated from *Macaranga hullettii* obtained from Central Kalimantan.¹⁴⁻¹⁸ *Macaranga tanarius* leaf extract has been shown to exhibit antibacterial activity.¹⁹ The extracts of *Macaranga conglomerata*, *Macaranga kilimandscharica*, and *Macaranga capensis* from Kenya has shown antibacterial activity against *E. coli*, *E. aerogenes*, *K. pneumoniae*, *P. stuartii*, *P. aeruginosa*, and *S. aureus*.²⁰ The flavonol rhamnetin, isolated from *Macaranga peltata*, also showed antibacterial activity against *Staphylococcus aureus*.²¹ Two flavanone groups; 5-hydroxy-7,4'-dimethoxyflavone and 5-hydroxy-6,7,4'-trimethoxyflavone obtained from *Macaranga hosei* showed weak antibacterial activity against three pathogenic bacteria.²² In this study, the leaves of *Macaranga hullettii* King ex Hook.f. were identified as potential new source of medicinal compounds against bacterial infection. This plant is found in all areas of East Kalimantan, where the species commonly occurs in mountain forests and logging areas in the Kalimantan region.^{23,24} Phytochemical screening and isolation of secondary metabolites from the leaf extract of *Macaranga hullettii* identified several flavonoid compounds.^{18,25} The methanol extract and its fractions derived from *Macaranga hullettii* have not yet been analyzed for their antibacterial activity. Therefore, this study aimed to assess the antibacterial activity of *Macaranga hullettii* leaf extract and its fractions using *in vitro* studies. The flavonoids isolated

from *Macaranga* species in East Kalimantan, Indonesia, were evaluated for their antibacterial activity using molecular docking analysis.

Materials and Methods

Chemicals and equipment

The solvents used (methanol, n-hexane, and ethyl acetate) were purified by distillation before use, nutrient agar (NA) from Merck, Luria Bertani liquid medium (1% tryptone, 0.5% yeast, and 0.5% NaCl), yeast extract (Oxoid 100% (w/w)), tryptone (Oxoid 100% (w/w)), ampicillin 100 mg/ml (Sigma Aldrich), NaCl (Sigma Aldrich), cotton swabs, aluminum foil, gauze, Petri dishes, Ose needles, paper discs, an autoclave (Autoclave TOMY, Model SX-700), an oven (SHARP EO-28 WH), a rotary vacuum evaporator (EYELA Rotary evaporator N-1300E V.S.Series), and a freezer (Sanyo Medicoool). For molecular docking analysis, we used a Lenovo Yoga 7 computer with AMD Ryzen AI 7-8840HS w/ Radeon 780M graphics, 3301 Mhz, 8 Core(s), and 16.0 GB RAM.

Plant collection and identification

Fresh leaves of *Macaranga hullettii* were collected from the Lesan River Forest, Kelay District, Berau Regency, East Kalimantan, Indonesia (1°32'2.5919" N, 117°3'11.950" E). The plant samples were identified at the Laboratory of Anatomy and Plant Systematics, FMIPA Mulawarman University where voucher No. 0174/UN.17.8.5.7.16/HA/XI/2017 was assigned.

Extraction and fractionation

Macaranga hullettii leaf powder (1.3 kg) was extracted by maceration in methanol (2 x 7.0 L) at room temperature for 24 h. The extract was filtered, and filtrate was evaporated *in vacuo* using a rotary vacuum evaporator to obtain the crude methanol extract (126 g). The crude methanol extract (100 g) was dissolved in 500 mL of methanol-water mixture (4:1), and then successively fractionated with 5 x 200 mL each of n-hexane and ethyl acetate to obtain n-hexane fraction (28 g) and ethyl acetate fraction (18 g), respectively. Each fraction was evaporated using a rotary vacuum evaporator. The methanol extract, n-hexane fraction, and ethyl acetate fraction were evaluated for antibacterial activity.

Test organisms

Staphylococcus aureus ATCC 25923 was obtained from the Department of Microbiology, Faculty of Science, Chulalongkorn University. *Streptococcus mutans* ATCC 25175 was obtained from the Department of Biochemistry, Faculty of Dentistry, Chulalongkorn University. Both strains were purchased from the American Type Culture Collection (ATCC). *Propionibacterium acnes* KCCM 41747 was obtained from the Faculty of Forestry, Mulawarman University, and from the Korean Culture Center of Microorganisms (KCCM). The bacteria were periodically sub-cultured and maintained in nutrient agar (NA) under suitable conditions.

In vitro antibacterial assay

The antibacterial activity of the methanol extract, n-hexane, and ethyl acetate fractions of *Macaranga hullettii* leaves was evaluated using the disc diffusion or the Kirby-Bauer method.^{26,27} Nutrient Agar (NA) medium was prepared by dissolving 3.3 g in 150 mL of distilled water. Luria Bertani (LB) liquid medium was prepared by dissolving 4 g in 500 mL of distilled water, stirring, and adding 5 mL to a test tube. The NA and LB media were sterilized in an autoclave at 121°C with a pressure of 1 atm for 15 minutes. Each test bacterium was collected using an Ose needle, followed by dilution in a test tube containing sterile LB media and incubated at 37 °C for 24 h.

Nutrient Agar (25 mL) was poured into sterilized Petri dishes and maintained at room temperature for solidification. A sample of the bacterial suspension in the test tubes was then collected using a sterile cotton swab and applied to the surface of the agar in the Petri dish. Each 30 µL of the test *M. hullettii* leaf samples prepared in various concentrations in the organic solvents (methanol, n-hexane, and ethyl acetate) was poured onto sterile adsorbent filter paper discs (6 mm in diameter), and the solvent was removed. Afterward, the paper discs containing the test sample were placed on the surface of the agar media and incubated at 37 °C for 18-24 hours. The inhibition zone formed in each well was measured using a ruler.^{28,29} The minimum inhibitory concentration (MIC) was determined for the methanol extract as well as the n-hexane and ethyl acetate fractions. MIC was defined as the minimum concentration that completely inhibited bacterial growth.³⁰ Ampicillin was used as a positive control, and the three organic solvents were used as negative controls.

Molecular docking

Molecular docking was conducted to eleven (11) flavonoid derivatives possessing either prenyl or geranyl group presented in the three *Macaranga* species in East Kalimantan, Indonesia (*Macaranga hosei*, *Macaranga pearsonii*, and *Macaranga tanarius*), *Macaranga hulettii* in Central Kalimantan (Indonesia), and *Macaranga hosei* in Trengganu (Malaysia): 4'-*O*-methyl-8-isoprenylnaringenin, 4'-*O*-methyl-8-isoprenyleriodictyol, 6-isoprenyleriodictyol, 5-hydroxy-7,4'-dimethoxyflavone, lonchocarpol A, 5-hydroxy-6,7,4'-trimethoxyflavone, Macahuilettiin A, nymphaeol A, nymphaeol B, nymphaeol C, and solophenol D,^{14-18, 22} were used for the study. Macahuilettiin A, together with lonchocarpol A and nymphaeol C, were a prenylated flavonoid found in *Macaranga hulettii* obtained from Central Kalimantan (Indonesia).¹⁸ A comparative analysis of the molecular docking results for prenylated/geranylated flavonoids with methoxylated flavonoids was conducted, focusing on 5-hydroxy-7,4'-dimethoxyflavone and 5-hydroxy-6,7,4'-trimethoxyflavone that are found in *Macaranga hosei* from Trengganu, Malaysia.²²

The molecular structures of the flavonoids were drawn using ChemOffice Professional 15.0 and obtained from PubChem (<https://pubchem.ncbi.nlm.nih.gov/>). Structure refinement was performed using the molecular mechanics energy minimization method with the Merck molecular force field (MMFF94) using Open Babel in PyRx V.1.1. Three bacterial proteins, including *Staphylococcus aureus* tyrosyl-tRNA synthetase (PDB ID: 1JIJ), *Streptococcus mutans* antigen I/II (AgI/II) (PDB ID: 3IPK), and *Propionibacterium acnes* surface sialidase (PDB ID: 7LBU) were downloaded from the protein data bank,³¹⁻³³ and the locations of active sites were calculated using AlphaFold from <https://prankweb.cz/>.³⁴ Molecular docking was performed using the AutoDock Vina tool in PyRx V.1.1 software with an exhaustiveness of 32 and a mode value of 9 poses for each docked ligand.^{35,36} A protein binding site was defined as a box with dimensions of 30 × 30 × 30 Å. In the final step, the binding interactions were analyzed, and the docking results were visualized in 2D using the BIOVIA Discovery Studio Visualizer.

Statistical analysis

Data from the experiments were analyzed using Microsoft Excel.

Results and Discussion

In vitro antibacterial activity

The strength of antibacterial activity of the test samples was determined based on the diameter of the clear zone of inhibition (mm). The antibacterial activity was classified as follows: ≤ 5 mm (weak), 5 - 10 mm (moderate), 10 - 20 mm (strong), and ≥ 20 mm (very strong). Moreover, the lower the minimum inhibitory concentration of a compound, the greater its ability to inhibit the growth of test bacteria.²⁸ In this study, ampicillin was used as a positive control against three bacteria: *Staphylococcus aureus* ATCC 25923, *Streptococcus mutans* ATCC 25175, and *Propionibacterium acnes* KCCM 41747 (Table 1). The positive control served to determine whether the test bacteria could be inhibited by or were resistant to the antibiotics.

Staphylococcus aureus can cause dentoalveolar infections, jaw cysts, paroritis, oral mucosal lesions, and stomatitis. The bacteria can also produce exotoxins, lecosidine, enterotoxins, and coagulase enzyme. Enterotoxins are compounds that cause food poisoning in humans.³⁷ The methanol extract, hexane fraction, and ethyl acetate fraction of *Macaranga hulettii* leaves showed moderate antibacterial activity against *S. aureus*, with an inhibition zone diameter of 5 - 10 mm at concentrations of 0.15 to 2.5% (Table 1). The inhibition zone diameter of the 0.15% methanol extract was 6.67 mm. The 0.15% ethyl acetate fraction produced an inhibition zone diameter of 6.33 mm, and the 2.5% n-hexane fraction produced an inhibition zone diameter of 6.50 mm. The results indicated that the methanol extract and ethyl acetate fraction had the most potent antibacterial activity against *S. aureus*, with a minimum inhibitory concentration (MIC) of $< 0.15\%$. Moreover, the study of Sari and Saleh (2015) showed that ethyl acetate fraction of *Macaranga tanarius* was most effective against *S. aureus*, with MIC values of 0.125-0.5% and an inhibition zone of approximately 7.25 mm.³⁸

Streptococcus mutans is implicated in the pathogenesis of dental caries. This facultative anaerobe primarily ferments carbohydrates, resulting in the production of several organic acids, with lactic acid as the predominant byproduct. The acidification of the dental environment by *S. mutans* is a crucial factor contributing to enamel demineralization and the subsequent formation of carious lesions.^{39,40} The bacteria produce glucosyltransferase (GTF) which produces glucan, the compound that causes dental caries.⁴¹ *S. mutans* adheres to the salivary pellicle via its numerous receptors.⁴² The antibacterial activity of *Macaranga hulettii* King leaf extract and fractions was tested against *S. mutans*. Both extract and fractions displayed moderate antibacterial activity, with inhibition zone diameters of 5 - 10 mm at concentrations of 0.15 - 2.5%. The diameters of the inhibition zones produced by the methanol extract and ethyl acetate fraction were 6.67 and 6.33 mm, respectively at 0.15%. Meanwhile, the n-hexane fraction showed antibacterial activity at concentrations of 1.25 - 2.5%, with inhibition zone diameter ranging from 6.33 - 6.67 mm. These results demonstrated that the methanol extract

and ethyl acetate fraction of *Macaranga hulettii* leaves produced the highest antibacterial activity against *S. mutans*, with an MIC < 0.15% (Table 1).

Propionibacterium acnes is a Gram-positive anaerobic bacterium that can induce unusual keratinization within the sebaceous glands of hair follicles and increase sebum production.⁴³ The enzyme is capable of hydrolyzing triglycerides (TG) present in sebum into free fatty acids (FFA) that may subsequently induce inflammation in and around the hair follicles.⁴⁴ The bacteria also produce hyaluronidase, protease, lecithinase, and neuraminidase, that can cause inflammation of the skin. *Macaranga triloba* ethanol extract has been shown to prevent the growth of *P. acnes*, resulting in an inhibition zone diameter of 5.54 mm at 20%.⁴⁵ As shown in Table 1, the methanol extract and ethyl acetate fraction of *Macaranga hulettii* showed potent antibacterial activity against *P. acnes*, with inhibition zone diameters of 10.67 and 14.67 mm, respectively. The n-hexane fraction exhibited moderate antibacterial activity, with inhibition zone diameter of 5 - 10 mm at 1.25 - 2.5%. At concentration of 0.31%, the methanol extract showed antibacterial activity with an inhibition zone diameter of 7.67 mm, whereas at 0.15% concentration, the ethyl acetate fraction showed antibacterial activity with inhibition zone diameter of 6.67 mm. The results demonstrated that the ethyl acetate fraction of *Macaranga hulettii* leaves presented the highest antibacterial activity against *P. acnes*, with a minimum inhibitory concentration (MIC) of < 0.15% (Table 1).

The methanol extract and ethyl acetate fraction of *Macaranga hulettii* leaves exhibited good antibacterial activity against the three bacterial strains. This finding was consistent with previous studies that attributed the antibacterial effects to the presence of flavonoids and their prenylated or geranylated forms in species of *Macaranga*. These included euchrestaflavanone A, bonanione A, macarangaflavanone A, macarangaflavanone B, macatrichocarpin A, propolin D (nymphaeol B), senegalensin, schweinfurthin B, schweinfurthin O, and isomacarangain.⁴⁶⁻⁵⁶ Flavonoids can damage bacterial cell walls and denature proteases, thereby disrupting bacterial metabolism.⁵⁷

Molecular docking result

Molecular docking was performed to predict the interactions of the flavonoids with the amino acid residues of three bacterial proteins: *Staphylococcus aureus* tyrosyl-tRNA synthetase, *Streptococcus mutans* antigen I/II (AgI/II), and *Propionibacterium acnes* surface sialidase.³¹⁻³³ The molecular docking results (Table 2) revealed that 11 flavonoid derivatives had binding affinities ranging from -7.2 to -10.5 kcal/mol, -7.5 to -8.9 kcal/mol, and -7.1 to -8.9 kcal/mol against *Staphylococcus aureus* tyrosyl-tRNA synthetase, *Streptococcus mutans* antigen I/II

(AgI/II), and *Propionibacterium acnes* surface sialidase, respectively. The interactions of flavonoids and ampicillin with the protein targets are shown in two-dimensional graphs in Figures 1-3.

Among the compounds targeting *S. aureus* tyrosyl-tRNA synthetase, nymphaeol A, nymphaeol B, and solophenol D had the highest binding affinities, higher than that of the positive control ligand (ampicillin). Thus, the interactions of these compounds with the active site of the enzyme were modeled as a 2D structure (Figure 1). Ampicillin formed hydrogen bonds with LYS84 and GLN174 and amide- π interactions with GLY38 (Figure 1). Nymphaeol A and nymphaeol B formed hydrogen bonds as well as π -anions, and hydrophobic interactions with four amino acid residues. Nymphaeol B and ampicillin showed similar interactions with LYS84. In addition, solophenol D formed hydrogen bonds with TYR36, THR75, GLN190, ASP177, and GLN174, as well as hydrophobic interactions with HIS50, LEU70, PRO53, and ALA39 (Figure 1). These results agreed with previous studies where nymphaeol A-B and solophenol D exhibited antibacterial activity.^{58,59}

Four compounds including 6-isoprenyleriodictyol, nymphaeol A, nymphaeol B, and solophenol D exhibited high antibacterial activity against *S. mutans* antigen I/II (AgI/II) with binding affinities ranging from -8.7 to -8.9 kcal/mol. The interactions between the ligands and amino acid residues in the binding sites of these compounds are shown in 2D format (Figure 2). Ampicillin formed hydrogen bonds with four amino acid residues, ASN590, SER591, SER588, and ASP760, as well as π -sulfur and π - π interactions with TRP 816, while 6-isoprenyleriodictyol only formed hydrogen bonds with three amino acid residues: SER697, ASN699, and ASN814. Nymphaeol A and nymphaeol B formed hydrogen bonds with four amino acid residues (Figure 2). Nymphaeol A exhibited a similar π - π interaction with TRP816, as observed with ampicillin. Solophenol D formed hydrogen bonds with SER818, ARG824, and THR586, similar π - π interaction as ampicillin with TRP816), and π -sigma interaction with SER762. The compound 6-isoprenyleriodictyol is a prenylated flavonoid similar to nymphaeol A, and hence may have antibacterial activity.

Two compounds, including 6-isoprenyleriodictyol and nymphaeol A, displayed stronger binding affinities than ampicillin, with values of -8.0 and -8.4 kcal/mol against *P. acnes* surface sialidases. Therefore, both compounds were visualized in 2D format to investigate the interactions between the ligands and the amino acid residues in the protein binding sites (Figure 3). Ampicillin formed hydrogen bonds with four amino acid residues, ARG282, GLN287, ASP146, ASP185, and π - π and π -alkyl interactions with PHE257, ALA147, and VAL202; 6-Isoprenyleriodictyol demonstrated π - π interactions with PHE257, π -anion interactions with

ASP146, and alkyl-alkyl interactions with ALA147, PHE209, VAL202, and LEU224. This compound formed bonds with five amino acid residues, including ASP146, ARG329, TYR423, ARG282, and GLU287, that were analogous to those observed with ampicillin. Nymphaeol A formed hydrogen bonds with five amino acid residues (ARG329, TYR423, ASP185, ALA147, and SER184), π -anion interactions with ASP312 and ASP185, alkyl-alkyl interactions with MET310, ALA331, and ALA147, and Van der Waals interactions with PHE257. Thus, these compounds may have potent antibacterial activity.

Structure-activity relationships were also studied based on the binding affinity results (Table 2). Flavonoids possessing prenyl, geranyl, and hydroxyl groups showed potential as antibacterial agents due to their strong affinity for amino acid residues in the binding sites of the proteins, compared to ampicillin. However, the absence of a hydroxyl group on prenylated or geranylated flavonoids decreased the binding affinity.^{56,60}

Conclusion

The extract and fractions of *Macaranga hulettii* leaves have potential as antibacterial agents against three bacterial strains, *Staphylococcus aureus* ATCC 25923, *Streptococcus mutans* ATCC 25175, and *Propionibacterium acnes* KCCM 41747. The methanol extract demonstrated antibacterial activity against *S. aureus* and *S. mutans* with MIC of < 0.15% against *S. aureus* and *S. mutans*, and 0.15-0.31% against *P. acnes*. The n-hexane fraction had an MIC of 1.25-2.5% against *S. aureus*, and 0.62-1.25% against *S. mutans* and *P. acnes*. The ethyl acetate fraction had an MIC < 0.15% against the three bacterial strains. Molecular docking studies suggested that 6-isoprenyleriodictyol, nymphaeol A, nymphaeol B, and solophenol D are promising antibacterial agents, as these compounds exhibited the highest binding affinity and formed hydrogen bonds with key amino acid residues in the active sites of the protein targets. Further *in vitro* and *in vivo* studies should be conducted by isolating the active secondary metabolites from this species.

Conflict of Interest

The authors declare no conflict of interest.

Author's Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims related to the content of this article will be borne by them.

Acknowledgements

This research was funded by the Islamic Development Bank (PIU-IsDB) project under contract no. 137/UN17.11/PL/2019.

References

1. Wong F, de la Fuente-Nunez C, Collins JJ. Leveraging artificial intelligence in the fight against infectious diseases. *Science*. 2023; 381(6654):164-170.
2. Seukep AJ, Kuete V, Nahar L, Sarker SD, Guo M. Plant-derived secondary metabolites as the main source of efflux pump inhibitors and methods for identification. *J Pharm Anal*. 2020; 10(4):277-290.
3. Magadula JJ. Phytochemistry and pharmacology of the genus *Macaranga*: a review. *J Med Plants Res*. 2014; 8(12):489-503.
4. Vu LT, Ngan TB, Phuong L, Huong DT, Litaudon M, Van Hung N, Thach TD, Van Cuong P. Chemical constituents from fruits of *Macaranga denticulata* (Euphorbiaceae) (Part 2). *Vietnam J Chem*. 2018; 56(4):516-520.
5. Marliana E, Hairani R, Tjahjandarie TS, Tanjung M. Antiplasmodial activity of flavonoids from *Macaranga tanarius* leaves. *IOP Conf Ser Earth Environ Sci*. 2018; 144(1):012011
6. Tjahjandarie TS, Tanjung M, Saputri RD, Nadar PB, Aldin MF, Marliana E, Permadi A. Flavestins K, An isoprenylated stilbene from the leaves of *Macaranga recurvata* Gage. *Nat Prod Sci*. 2019; 25(3):244-247.
7. Huong DT, Linh NT, Van TT, Litaudon M, Roussi F, Van Nam V, Van Cuong P. Stilbenes from *Macaranga tanarius* (Euphorbiaceae) growing in Vietnam. *Vietnam J Chem*. 2020; 58(3):338-342.
8. Aldin MF, Tjahjandarie TS, Saputri RD, Tanjung M. Macasiamenene V, a New Stilbenoid from the Leaves of *Macaranga inermis*. *Nat Prod Sci*. 2021; 27(1):45-48.
9. Muharram A, Rachmawati DA, Mardhiyyah S, Tjahjandarie TS, Saputri RD, Ahmat N, Tanjung M. Cytotoxic and antioxidant activities of flavonoids and diterpenoids from *Macaranga involucreata* (Roxb.) Baill. *J Appl Pharm Sci*. 2023; 13(6):087-92.
10. Tanjung M, Tjahjandarie TS, Aldin MF, Mardhiyyah S, Ahmat N, Saputri RD. Macagigantin A, A New Flavonoid from *Macaranga gigantea* (Rchb. f & Zoll.) Mull Arg *Nat Prod Sci*. 2023; 29(4):287-294.
11. Tjahjandarie TS, Aldin MF, Saputri RD, Tanjung M. Dihydrostilbenes from *Macaranga javanica* (Blume) Müll. Arg. and their antiplasmodial activity. *Nat Prod Sci*. 2024:1-7.
12. Toko EG, Tchapo CED, Tsamo AT, Kemzeu R, Wang Y, Fekam FB, Ndinteh DT, Choudhary MI, Nkengfack EA, Mmutlane EM, Mkounga P. Three New Polyphenol Derivatives from the Fruits of *Macaranga Monandra* and their Antioxidant Potential. *Chem. Biodivers*. 2024;21(7):e202301816.

13. Tanjung M, Tjahjandarie TS, Aldin MF, Mardhiyyah S, Saputri RD, Syah YM, Ahmat N. Two new flavonols from *Macaranga inermis* pax & K. Hoffm. Nat Prod Sci. 2025; 39(3):498-505.
14. Marliana E, Tjahjandarie TS, Tanjung M. Isoprenylated flavanone derivatives from *Macaranga hosei* King ex Hook F. Der Pharm Lett. 2015; 7(3):153-156.
15. Marliana E, Tjahjandarie TS, Tanjung M. Antioxidant activity of flavonoids from *Macaranga pearsonii* Merr. J Kim Mulawarman. 2016; 13(2):97-100.
16. Marliana E, Astuti W, Kosala K, Hairani R, Tjahjandarie TS, Tanjung M. Chemical composition and anticancer activity of *Macaranga hosei* leaves. Asian J Chem. 2018; 30(4):795-798.
17. Marliana E, Ruga R, Hairani R, Tjahjandarie TS, Tanjung M. Antioxidant activity of flavonoid constituents from the leaves of *Macaranga tanarius*. IOP Conf Ser J Phys. 2019; 1277(1):012014.
18. Saputri RD, Tukiran T, Wati FA, Purnamasari AP, Wardhana MW, Tjahjandarie TS, Tanjung M. Macahuilettiin A, a new isoprenylated flavanone from the leaves of *Macaranga hullettii* King ex Hook and their antiplasmodial activity. Vietnam J Chem 2024; 62(3):394-398.
19. Musdalifah M, Khumaidi A, Suwastika IN. Inhibition test and phytochemical screening of leaf extracts of *Macaranga tanarius* (L.) Mull. Arg against *Salmonella typhi* as an antibacterial. Nat Sci J Sci Technol. 2017; 6(3):9194.
20. Ibrahim H, Omosa L, Nchiozem-Ngnitedem V, Onyari J, Maru S, Guefack M. Antibacterial activities and phytochemical screening of crude extracts from Kenyan macaranga species toward MDR phenotypes expressing efflux pumps. Pharmacogn Commun. 2021; 11(2):119-126.
21. Bijesh K and Sebastian D. Isolation and characterization of antibacterial compounds from *Macaranga peltata* against clinical isolates of *Staphylococcus aureus*. Int J Biol Pharm Res. 2013; 4(12):1196-1203.
22. Salleh WM, Razak NZ, Ahmad F. Phytochemicals and biological activities of *Macaranga hosei* and *Macaranga constricta* (Euphorbiaceae). Marmara Pharm J. 2017; 21(4):881-888.
23. Slik JW, Priyono P, Welzen PV. Key to the *Macaranga Thou.* and *Mallotus Lour.* species (Euphorbiaceae) of East Kalimantan, Indonesia. Singapore, Gardens' Bulletin (Singapore), National Parks Board, Singapore Botanic Gardens; 2000. 11-87 p.
24. Amirta R, Angi EM, Ramadhan R, Kusuma IW, Wiati CB, Haqiqi MT. Potential utilization of macaranga. Samarinda: Mulawarman University Press; 2017.

25. Rismawati R, Marlina E, Daniel D. Phytochemical Test on Methanol Extract of Leaf of *Macaranga hullettii* King ex Hook. f. J Atomik. 2018; 3(2):91-94.
26. Hudzicki J. Kirby-Bauer disk diffusion susceptibility test protocol. Am Soc Microbiol. 2009; 15(1):1-23.
27. Inna M, Astuti W, Saleh C. Antibacterial activity of methanolic extract of uric patch plant leaves (*Cayratia carnosa*) against *Salmonella thypi* and *Propionibacterium acnes*. J Atomik. 2022; 7(1):1-5.
28. Yulianti MF, Amat AL, Hutasoit RM, Pakan PD. Antibacterial activity of jamblang leaf ethanol extract (*Syzygium cumini*) against the growth of *Propionibacterium acnes*. Acta Biochimica Indones. 2023; 6(2):1161.
29. Hossain MR, Biplob AI, Sharif SR, Bhuiya AM, Sayem AS. Antibacterial Activity of Green Synthesized Silver Nanoparticles of *Lablab purpureus* Flowers Extract against Human Pathogenic Bacteria. Trop J Nat Prod Res. 2023;7(8):3647-3651
30. Yanda L, Tatsimo SJ, Tamokou JD, Matsute-Takongmo G, Meffo-Dongmo SC, Meli Lannang A, Sewald N. Antibacterial and antioxidant activities of isolated compounds from *Prosopis africana* leaves. Int J Anal Chem. 2022; 2022:4205823.
31. Qiu X, Janson CA, Smith WW, Green SM, McDevitt P, Johanson K, Carter P, Hibbs M, Lewis C, Chalker A, Fosberry A. Crystal structure of *Staphylococcus aureus* tyrosyl-tRNA synthetase in complex with a class of potent and specific inhibitors. Protein Sci. 2001; 10(10):2008-2016.
32. Larson MR, Rajashankar KR, Patel MH, Robinette RA, Crowley PJ, Michalek S, Brady LJ, Deivanayagam C. Elongated fibrillar structure of a *streptococcal adhesin* assembled by the high-affinity association of α - and PPII-helices. Proc Natl Acad Sci. 2010; 107(13):5983-5988.
33. Yu AC, Volkers G, Jongkees SA, Worrall LJ, Withers SG, Strynadka NC. Crystal structure of the *Propionibacterium acnes* surface sialidase, a drug target for *P. acnes*-associated diseases. Glycobiol. 2022; 32(2):162-170.
34. Jakubec D, Skoda P, Krivak R, Novotny M, Hoksza D. PrankWeb 3: accelerated ligand-binding site predictions for experimental and modelled protein structures. Nucl Acids Res. 2022; 50:(W1):W593-W597.
35. Dallakyan S and Olson AJ. Small-molecule library screening by docking with PyRx. Methods Mol Biol. 2014; 1263:243-250.
36. Trott O and Olson AJ. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. J Comput Chem. 2010; 31(2):455-461.
37. Malangu N. ed. Poisoning: From Specific Toxic Agents to Novel Rapid and Simplified Techniques for Analysis. BoD–Books on Demand; 2017.

38. Sari AA and Saleh C. Phytochemical test, toxicity and antibacterial activity of extracts of various macaranga leaf fractions (*Macaranga tanarius* (L.) MA) against *Staphylococcus aureus* and *Escherichia coli*. J Kim Mulawarman. 2015; 12(2):53-58.
39. Imelda R, Mariam M, Satari M. Effect of cassava (*Manihot esculenta* cranzt), rice (*oryza sativa* L.), and potato (*solanum tuberosum*) water extract to decrease pH phase fermentation of *streptococcus mutans* atcc 25175. Padjajaran J Dent. 2019; 31(1):14.
40. Alkhaled A, Alsabek L, Al-assaf M, Badr F. Effect of chlorhexidine, honey and propolis on *streptococcus mutans* counts: *in vitro* study. Dentistry. 2021; 9(1):a001.
41. Ren Z, Chen L, Li J, Li Y. Inhibition of *Streptococcus mutans* polysaccharide synthesis by molecules targeting glycosyltransferase activity. J Oral Microbiol. 2016; 8(1):31095.
42. Lee BS, Chen YJ, Wei TC, Ma TL, Chang CC. Comparison of antibacterial adhesion when salivary pellicle is coated on both poly (2-hydroxyethyl-methacrylate)-and polyethylene-glycol-methacrylate-grafted poly (methyl methacrylate). Int J Mol Sci. 2018; 19(9):2764.
43. Chen KC, Yang CH, Li TT, Zouboulis CC, Huang YC. Suppression of Propionibacterium acnes-stimulated proinflammatory cytokines by Chinese bayberry extracts and its active constituent myricetin in human sebocytes *in vitro*. Phytother Res. 2019; 33(4):1104-1113.
44. Xu J, Chen X, Song J, Wang C, Xu W, Tan H, Suo H. Antibacterial activity and mechanism of cell-free supernatants of *Lactocaseibacillus paracasei* against *Propionibacterium acnes*. Microb. Pathog. 2024; 189:106598
45. Warnida H, Mustika D, Supomo S, Sukawaty Y. Effectiveness of Mahang Leaf Ethanol Extract (*Macaranga Triloba*) as an anti-acne. J Penelit. Sos. Ekon. Kehuta. 2018; 4(1):9-18.
46. Schütz BA, Wright AD, Rali T, Sticher O. Prenylated flavanones from leaves of *Macaranga pleiostemona*. Phytochem. 1995; 40(4):1273-1277.
47. Lim TY, Lim YY, Yule CM. Evaluation of antioxidant, antibacterial and anti-tyrosinase activities of four Macaranga species. Food Chem. 2009; 114(2):594-599.
48. Fareza MS, Syah YM, Mujahidin D, Juliawaty LD, Kurniasih I. Antibacterial flavanones and dihydrochalcones from *Macaranga trichocarpa*. Z. Naturforsch C J Biosci. 2014; 69(9-10):375-380.
49. Hasanat A, Kabir MS, Hossain MM, Hasan M, Al Masum MA, Chowdhury TA, Bhuiyan DI, Mamur A, Kibria AS. Antibacterial activity of methanol extract of *Macaranga denticulata* leaves and in silico PASS prediction for its six secondary metabolites. World J Pharm Pharm. Sci. 2015:1258-1266.
50. O Akanbi B, Anene P, Olayanju S. Preliminary Screening Indicates Promising Antimicrobial Properties of the Stem Bark Extracts of *Macaranga rosea*. Anti-Infective Agents. 2015; 13(2):123-128.

51. Ogundajo A, Okeleye B, Ashafa AO. Chemical constituents, *in vitro* antimicrobial and cytotoxic potentials of the extracts from *Macaranga barteri* Mull-Arg. Asian Pac J Trop Biomed. 2017; 7(7):654-659.
52. Putri R, Hendra R, Teruna HY. Anti-Bacterial and Anti-Fungal Activities from *Macaranga bancana* Leaves Extract. Pharmacol Clin Pharm Res. 2019; 4(1):1-4.
53. Lee JH, Kim YG, Khadke SK, Yamano A, Woo JT, Lee J. Antimicrobial and antibiofilm activities of prenylated flavanones from *Macaranga tanarius*. Phytomed. 2019; 63:153033.
54. Pagna JI, Awazi T, Mbarga PE, Mbekou IM, Mkounga P, Fotie J, Frese M, Fabrice FB, Lenta BN, Sewald N, Nkengfack EA. Antibacterial flavonoids from the fruits of *Macaranga hurifolia*. J Asian Nat Prod Res. 2022; 24(11):1041-1051.
55. Kamso VF, Simo Fotso CC, Kanko Mbekou IM, Toussie BT, Ndjakou Lenta B, Boyom FF, Sewald N, Frese M, Ngadjui BT, Wabo Fotso G. Chemical constituents of *Macaranga occidentalis*, antimicrobial and chemophenetic studies. Molecules. 2022; 27(24):8820.
56. Rosamah E, Haqiqi MT, Putri AS, Kuspradini H, Kusuma IW, Amirta R, Yuliansyah Y, Suwinarti W, Paramita S, Ramadhan R, Tarmadi D. The potential of Macaranga plants as skincare cosmetic ingredients: A review. J Appl Pharm Sci. 2023; 13(7):001-12.
57. Li AP, He YH, Zhang SY, Shi YP. Antibacterial activity and action mechanism of flavonoids against phytopathogenic bacteria. Pestic Biochem Physiol. 2022; 188:105221.
58. Chen YW, Ye SR, Ting C, Yu YH. Antibacterial activity of propolins from Taiwanese green propolis. J Food Drug Anal. 2018; 26(2):761-768.
59. Inui S, Hosoya T, Shimamura Y, Masuda S, Ogawa T, Kobayashi H, Shirafuji K, Moli RT, Kozono I, Shin-ya K, Kumazawa S. Solophenols B–D and Solomonin: New Prenylated Polyphenols Isolated from Propolis Collected from The Solomon Islands and Their Antibacterial Activity. J Agric Food Chem. 2012; 60(47):11765-11770.
60. Ruga R, Kingkaew K, Tamsampaoloet K, Chavasiri W. Enhancing antibacterial activity against *Propionibacterium acnes* and *Staphylococcus aureus* by combination of tetracycline with selected compounds. Chem Lett. 2018; 47(12):1538-1541.

Table 1: Antibacterial activity of *Macaranga hullettii* King ex Hook leaf extracts and fractions against *Staphylococcus aureus*, *Streptococcus mutans*, and *Propionibacterium acnes*

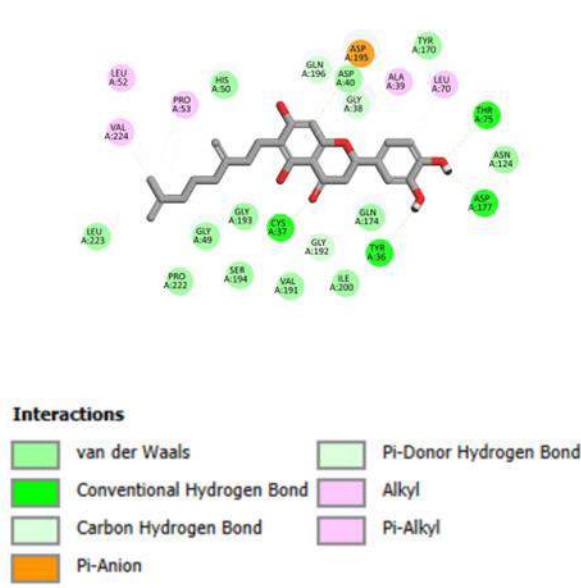
Sample	Concentration (%)	Inhibition Zone Diameter (mm)*		
		<i>Staphylococcus aureus</i>	<i>Streptococcus mutans</i>	<i>Propionibacterium acnes</i>
Methanol Extract	0	0	0	0
	0.15	6.67 ± 0.58	6.67 ± 0.58	0
	0.31	6.67 ± 0.58	6.67 ± 0.58	7.67 ± 0.58
	0.62	6.67 ± 0.58	7.0 ± 0.0	8.0 ± 1.0
	1.25	7.17 ± 0.29	7.33 ± 0.58	9.67 ± 0.58
	2.5	7.5 ± 0.5	8.0 ± 1.0	10.67 ± 0.58
	MIC (%)	<0.15	<0.15	0.15-0.31
n-Hexane Fraction	0	0	0	0
	0.15	0	0	0
	0.31	0	0	0
	0.62	0	0	0
	1.25	0	6.33 ± 0.29	6.33 ± 0.58
	2.5	6.5 ± 0.5	6.67 ± 0.58	6.67 ± 0.58
	MIC (%)	1.25-2.5	0.62-1.25	0.62-1.25
Ethyl acetate Fraction	0	0	0	0
	0.15	6.33 ± 0.58	6.33 ± 0.58	6.67 ± 0.58
	0.31	6.5 ± 0.5	6.67 ± 0.58	8.0 ± 0.0
	0.62	6.5 ± 0.5	6.83 ± 0.29	9.0 ± 0.0
	1.25	6.67 ± 0.29	7.0 ± 0.0	11.0 ± 1.0
	2.5	7.0 ± 0.0	7.67 ± 0.58	14.67 ± 0.58
	MIC (%)	<0.15	<0.15	<0.15
Ampicillin	0.01	20.33 ± 1.15	21.0 ± 1.0	21.0 ± 1.0

*Data were obtained from triplicate experiments.

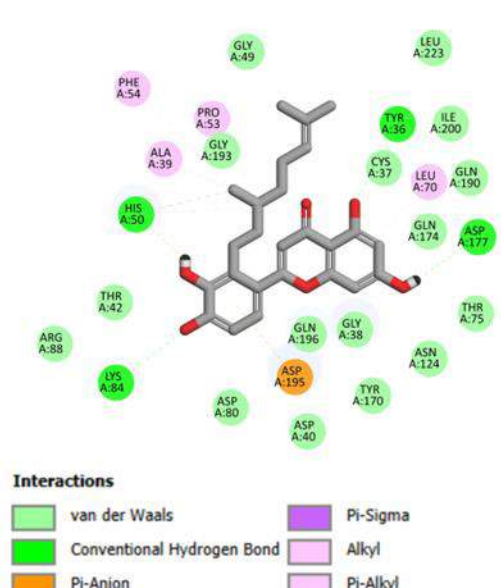
Table 2: Binding affinity of flavonoids against bacterial proteins

No.	Compound	Binding Affinity (kcal/mol)		
		1JIJ	3IPK	7LBU
1	4'-O-methyl-8-isoprenylnaringenin	-7.4	-8.1	-7.7
2	4'-O-methyl-8-isoprenyleriodictyol	-7.2	-8.1	-7.6
3	6-Isoprenyleriodictyol	-7.7	-8.7	-8.0

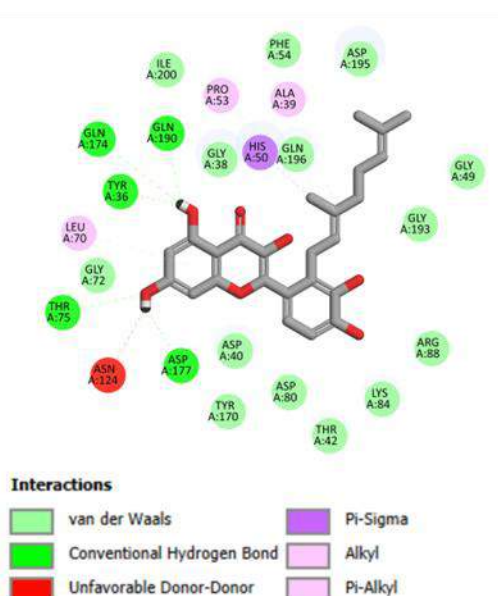
4	5-hydroxy-7,4'-dimethoxyflavone	-8.6	-7.8	-7.1
5	5-hydroxy-6,7,4'-trimethoxyflavone	-8.3	-7.5	-7.3
6	Macahuilettiin A	-7.6	-8.4	-7.1
7	Lonchocarpol A	-9.4	-7.8	-7.8
8	Nymphaeol A	-9.7	-8.9	-8.4
9	Nymphaeol B	-10.5	-8.9	-7.7
10	Nymphaeol C	-8.4	-8.3	-7.4
11	Solophenol D	-10.0	-8.7	-7.9
12	Ampicillin	-8.5	-7.7	-7.6



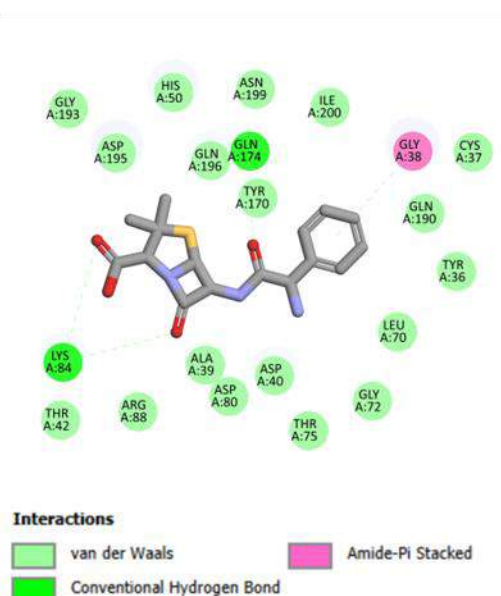
Nymphaeol A



Nymphaeol B



Solophenol D



Ampicillin

Figure 1: 2D visualization of interaction of the best docked flavonoids and ampicillin with *Staphylococcus aureus* tyrosyl-tRNA synthetase (PDB ID: 1JIJ)

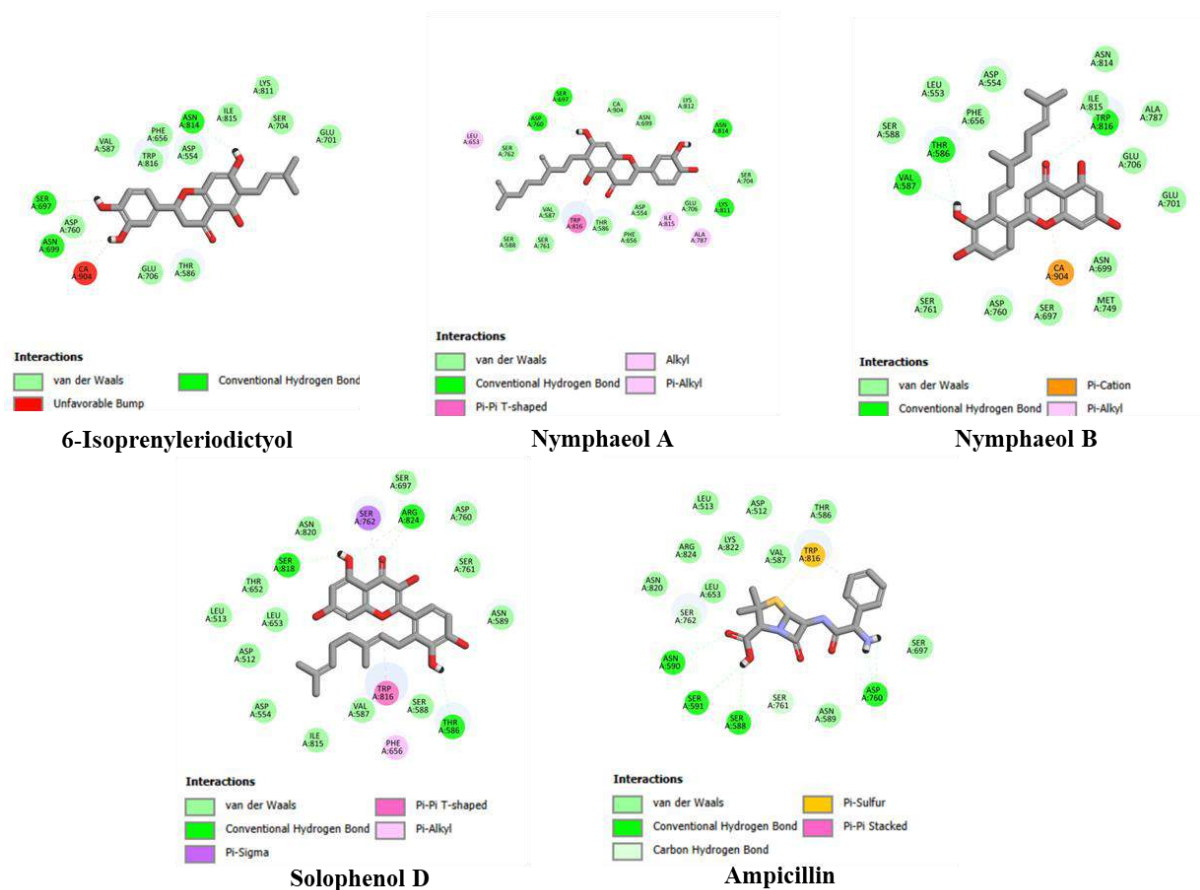


Figure 2: 2D visualization of interaction of the best-docked flavonoids and ampicillin with *Streptococcus mutans* antigen I/II (PDB ID: 3IPK).

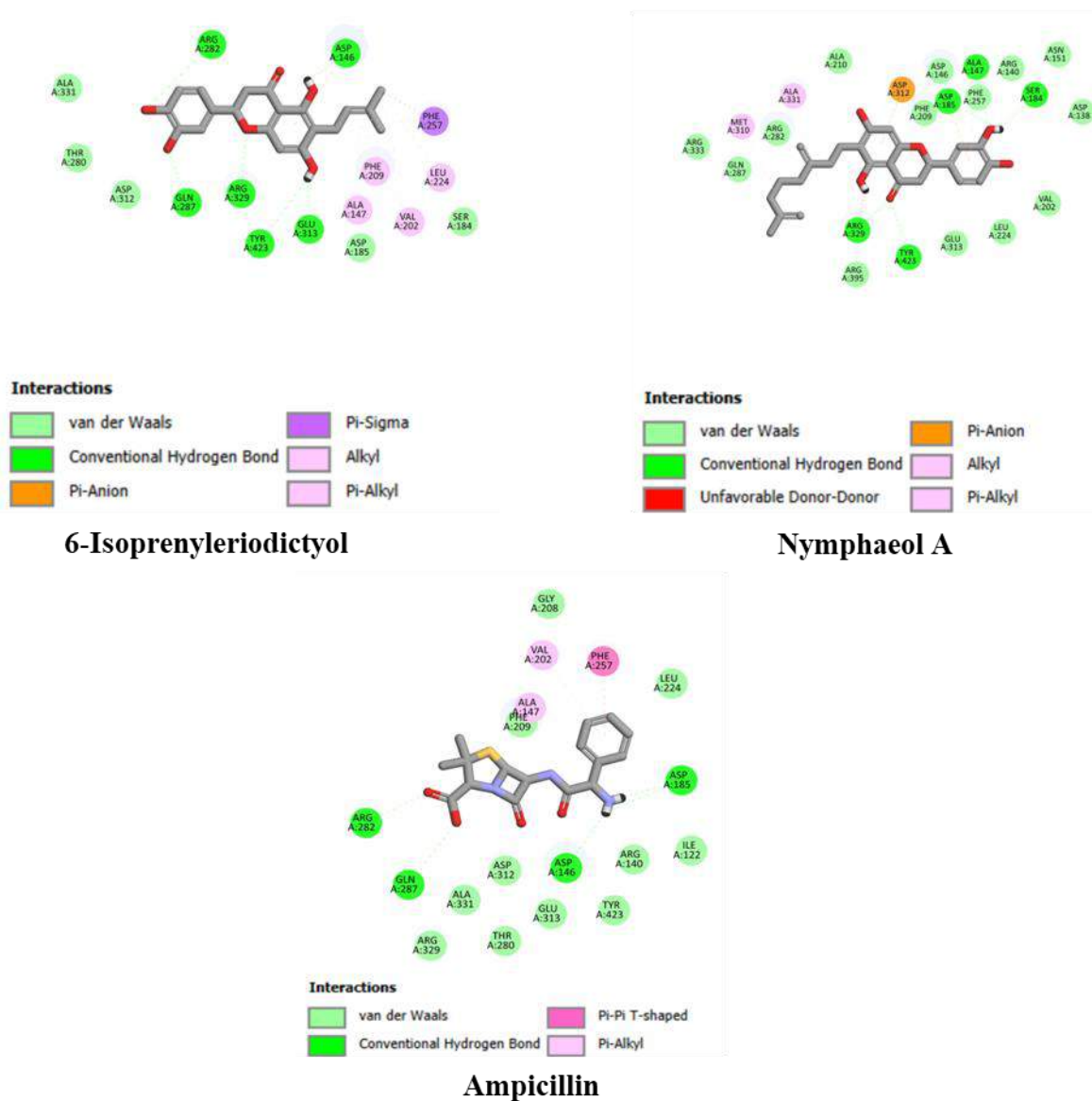


Figure 3: 2D visualization of interaction of the best-docked flavonoids and ampicillin with *Propionibacterium acnes* surface sialidase (PDB ID: 7LBU).



Check Galley Proof

From Editorial Team <et.tjnpr@gmail.com>
Date Sun 6/1/2025 10:26 PM
To Ade Danova <adedanova@itb.ac.id>
Cc editor.tjnpr@gmail.com <editor.tjnpr@gmail.com>

 1 attachment (896 KB)
TJNPR-2025-420 STA.docx;

Dear Author,

Please, find attached the galley proof of your manuscript titled “ ***In vitro* Evaluation and Molecular Docking Study of the Antibacterial Potential of *Macaranga hulletti* King ex Hook.f. Leaf Extract** ”

You are kindly requested to **carefully read** the galley proof, make your comments and observations/corrections. Also, respond to the comment(s) where indicated.

Carefully review the formatting of names, affiliations, figures, tables, citations and the entire text.

Note:

1. If all comments are not addressed, the manuscript will be withdrawn from consideration for publication in this issue.
2. Return the revised galley proof within **48 hours** to prevent the manuscript from being withdrawn from publication in this issue.
3. **All corrections/changes made in the manuscript should be highlighted ONLY in yellow ink when submitting the revised manuscript as soon as possible (not later than 48 hours).**
4. **The revised galley proof is scheduled for publication in Volume 9 Issue 7**

Thank you.

Caution: This Message was sent from outside of Institute Technology Bandung (ITB). Please do not click Link or open attachment unless you recognize the source of this email and know the content is safe. Please report all suspicious email to "it-helpdesk@itb.ac.id" as an attachment.



In vitro Evaluation and Molecular Docking Study of the Antibacterial Potential of *Macaranga hulletti* King ex Hook.f. Leaf Extract

Eva Marliana^{1,3}, Ade Danova^{2*}, Rita Hairani^{1,3}, Ritbey Ruga^{1,3}, Elvira Hermawati², Ana Yulvia⁴

¹Department of Chemistry, Faculty of Mathematics and Natural Sciences, Mulawarman University, Samarinda 75123, East Kalimantan, Indonesia

²Organic Chemistry Division, Department of Chemistry, Faculty of Mathematics and Natural Sciences, Institut Teknologi Bandung Jl. Ganesha No. 10, Bandung 70132, West Java, Indonesia

³Research Center for Medicine and Cosmetics from Tropical Rainforest Resources, Mulawarman University, Samarinda, Indonesia

⁴Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Jember, East Java, Indonesia

ARTICLE INFO

Article history:

Received

Revised

Accepted

Published online 01 May 2025

ABSTRACT

Due to the resistance of bacteria to antibiotics, there has been increasing interest in the use of plant extracts to combat infections. Plants in the genus *Macaranga* contain secondary metabolites such as flavonoids with antibacterial properties. *Macaranga hulletti* King ex Hook.f. are particularly noteworthy due to their widespread distribution in East Kalimantan. This study aimed to investigate the antibacterial potential of *M. hulletti* leaf extract through *in vitro* and molecular docking studies. The antibacterial activity of the methanol extract, n-hexane, and ethyl acetate fractions from *M. hulletti* leaves was evaluated against three bacterial species, *Staphylococcus aureus*, *Streptococcus mutans*, and *Propionibacterium acnes* using the disc diffusion assay. Molecular docking of eleven flavonoid derivatives presents in three *Macaranga* species was performed against selected bacterial proteins (PDB ID: IJJ, 3IPK, and 7LBU). The results showed that both the methanol extract and ethyl acetate fraction displayed antibacterial activity against the three bacteria strains, with minimum inhibitory concentrations (MICs) < 0.15%, except for the methanol extract, which had MIC of 0.15-0.31% against *Propionibacterium acnes*. In addition, molecular docking study showed that four flavonoids possessing prenyl or geranyl groups (6-isoprenyleriodictyol, nymphaeol A, nymphaeol B, and solophenol D) showed the highest binding affinity and dominant hydrogen bonding interactions with key amino acid residues in the binding sites of three bacterial proteins. The findings suggest that the methanol extract and its ethyl acetate fraction from *Macaranga hulletti* leaves could be a potential source of new antibacterial agents. Further studies are needed to isolate and evaluate the bioactive compounds.

Copyright: © 2025 Zulaikhah *et al.* This is an open-access article distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Keywords: Antibacterial, Extract, Flavonoid, *Macaranga hulletti*, Molecular Docking

Introduction

Despite substantial advancements in medicine and fundamental research, infectious diseases caused by transmissible agents such as bacteria, protists, and viruses continue to present significant challenges to healthcare professionals. These challenges include the issue of antimicrobial resistance.¹ Extracts from medicinal plants present a promising approach to addressing multidrug-resistant bacteria. In addition, medicinal plants can augment the efficacy of antibiotics for the treatment of infectious diseases.² Therefore, it is imperative to develop more efficacious antimicrobial agents, particularly those sourced from natural sources, as these are both readily available and economically viable. Plants in the genus *Macaranga* belong to the family Euphorbiaceae and are commonly known as "Mahang." The secondary metabolites contained in plants of this genus include flavonoids and stilbenoids with prenylated substituents.³⁻¹³

*Corresponding author. Email: adedanova@itb.ac.id,

Tel: +6281357944698

Citation: Zulaikhah ST, Ratnawati R, Husaana A. Nephroprotective Effect of a Combination of Probiotics, Tender Coconut Water, and Vitamin E in Gentamicin-Induced Acute Kidney Injury in Rats. Trop J Nat Prod Res. 2025; 9(7).

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

Nine flavonoids containing either prenyl or geranyl groups and displaying antioxidant, antiparasitic, and anticancer activities have been isolated from three species (*Macaranga hoesi*, *Macaranga pearsonii*, and *Macaranga tanarius*) of *Macaranga* present in East Kalimantan: 4'-O-methyl-8-isoprenylnaringenin, 4'-O-methyl-8-isoprenyleriodictyol, 6-isoprenyleriodictyol, solophenol D, nymphaeol A, and nymphaeol B, including lonchocarpol A, nymphaeol C, and macahuillettin A that was isolated from *Macaranga hulletti* obtained from Central Kalimantan.¹⁴⁻¹⁸ *Macaranga tanarius* leaf extract has been shown to exhibit antibacterial activity.¹⁹ The extracts of *Macaranga conglomerata*, *Macaranga kilimandscharica*, and *Macaranga capensis* from Kenya has shown antibacterial activity against *E. coli*, *E. aerogenes*, *K. pneumoniae*, *P. stuartii*, *P. aeruginosa*, and *S. aureus*.²⁰ The flavonol rhamnetin, isolated from *Macaranga peltata*, also showed antibacterial activity against *Staphylococcus aureus*.²¹ Two flavanone groups; 5-hydroxy-7,4'-dimethoxyflavone and 5-hydroxy-6,7,4'-trimethoxyflavone obtained from *Macaranga hoesi* showed weak antibacterial activity against three pathogenic bacteria.²² In this study, the leaves of *Macaranga hulletti* King ex Hook.f. were identified as potential new source of medicinal compounds against bacterial infection. This plant is found in all areas of East Kalimantan, where the species commonly occurs in mountain forests and logging areas in the Kalimantan region.^{23,24} Phytochemical screening and isolation of secondary metabolites from the leaf extract of *Macaranga hulletti* identified several flavonoid compounds.^{18,25} The methanol extract and its fractions derived from *Macaranga hulletti* have not yet been analyzed for their antibacterial activity. Therefore, this study aimed to

assess the antibacterial activity of *Macaranga hullettii* leaf extract and its fractions using *in vitro* studies. The flavonoids isolated from *Macaranga* species in East Kalimantan, Indonesia, were evaluated for their antibacterial activity using molecular docking analysis.

Materials and Methods

Chemicals and equipment

The solvents used (methanol, n-hexane, and ethyl acetate) were purified by distillation before use, nutrient agar (NA) from Merck, Luria Bertani liquid medium (1% tryptone, 0.5% yeast, and 0.5% NaCl), yeast extract (Oxoid 100% (w/w)), tryptone (Oxoid 100% (w/w)), ampicillin 100 mg/ml (Sigma Aldrich), NaCl (Sigma Aldrich), cotton swabs, aluminum foil, gauze, Petri dishes, Ose needles, paper discs, an autoclave (Autoclave TOMY, Model SX-700), an oven (SHARP EO-28 WH), a rotary vacuum evaporator (EYELA Rotary evaporator N-1300E V.S.Series), and a freezer (Sanyo Medicoool). For molecular docking analysis, we used a Lenovo Yoga 7 computer with AMD Ryzen AI 7-8840HS w/ Radeon 780M graphics, 3301 Mhz, 8 Core(s), and 16.0 GB RAM.

Plant collection and identification

Fresh leaves of *Macaranga hullettii* were collected from the Lesan River Forest, Kelay District, Berau Regency, East Kalimantan, Indonesia (1°32'2.5919" N, 117°3'11.950" E). The plant samples were identified at the Laboratory of Anatomy and Plant Systematics, FMIPA Mulawarman University where voucher No. 0174/UN.17.8.5.7.16/HA/XI/2017 was assigned.

Extraction and fractionation

Macaranga hullettii leaf powder (1.3 kg) was extracted by maceration in methanol (2 x 7.0 L) at room temperature for 24 h. The extract was filtered, and filtrate was evaporated *in vacuo* using a rotary vacuum evaporator to obtain the crude methanol extract (126 g). The crude methanol extract (100 g) was dissolved in 500 mL of methanol-water mixture (4:1), and then successively fractionated with 5 x 200 mL each of n-hexane and ethyl acetate to obtain n-hexane fraction (28 g) and ethyl acetate fraction (18 g), respectively. Each fraction was evaporated using a rotary vacuum evaporator. The methanol extract, n-hexane fraction, and ethyl acetate fraction were evaluated for antibacterial activity.

Test organisms

Staphylococcus aureus ATCC 25923 was obtained from the Department of Microbiology, Faculty of Science, Chulalongkorn University. *Streptococcus mutans* ATCC 25175 was obtained from the Department of Biochemistry, Faculty of Dentistry, Chulalongkorn University. Both strains were purchased from the American Type Culture Collection (ATCC). *Propionibacterium acnes* KCCM 41747 was obtained from the Faculty of Forestry, Mulawarman University, and from the Korean Culture Center of Microorganisms (KCCM). The bacteria were periodically sub-cultured and maintained in nutrient agar (NA) under suitable conditions.

In vitro antibacterial assay

The antibacterial activity of the methanol extract, n-hexane, and ethyl acetate fractions of *Macaranga hullettii* leaves was evaluated using the disc diffusion or the Kirby-Bauer method.^{26,27} Nutrient Agar (NA) medium was prepared by dissolving 3.3 g in 150 mL of distilled water. Luria Bertani (LB) liquid medium was prepared by dissolving 4 g in 500 mL of distilled water, stirring, and adding 5 mL to a test tube. The NA and LB media were sterilized in an autoclave at 121°C with a pressure of 1 atm for 15 minutes. Each test bacterium was collected using an Ose needle, followed by dilution in a test tube containing sterile LB media and incubated at 37 °C for 24 h.

Nutrient Agar (25 mL) was poured into sterilized Petri dishes and maintained at room temperature for solidification. A sample of the bacterial suspension in the test tubes was then collected using a sterile cotton swab and applied to the surface of the agar in the Petri dish. Each 30 µL of the test *M. hullettii* leaf samples prepared in various

concentrations in the organic solvents (methanol, n-hexane, and ethyl acetate) was poured onto sterile adsorbent filter paper discs (6 mm in diameter), and the solvent was removed. Afterward, the paper discs containing the test sample were placed on the surface of the agar media and incubated at 37 °C for 18-24 hours. The inhibition zone formed in each well was measured using a ruler.^{28,29} The minimum inhibitory concentration (MIC) was determined for the methanol extract as well as the n-hexane and ethyl acetate fractions. MIC was defined as the minimum concentration that completely inhibited bacterial growth.³⁰ Ampicillin was used as a positive control, and the three organic solvents were used as negative controls.

Molecular docking

Eleven (11) flavonoid derivatives possessing either prenyl or geranyl group present in the three *Macaranga* species in East Kalimantan, Indonesia (*Macaranga hosei*, *Macaranga pearsonii*, and *Macaranga tanarius*): 4'-O-methyl-8-isoprenylnaringenin, 4'-O-methyl-8-isoprenyleriodictyol, 6-isoprenyleriodictyol, 5-hydroxy-7,4'-dimethoxyflavone, lonchocarpol A, 5-hydroxy-6,7,4'-trimethoxyflavone, Macahulleitiin A, nymphaeol A, nymphaeol B, nymphaeol C, and solophenol D,¹⁴⁻¹⁷ were used for the study. Macahulleitiin A, together with lonchocarpol A and nymphaeol C, are a prenylated flavonoid found in *Macaranga hullettii* obtained from Central Kalimantan (Indonesia).¹⁸ A comparative analysis of the molecular docking results for prenylated/geranylated flavonoids with methoxylated flavonoids was conducted, focusing on 5-hydroxy-7,4'-dimethoxyflavone and 5-hydroxy-6,7,4'-trimethoxyflavone that are found in *Macaranga hosei* from Trengganu, Malaysia.²²

The molecular structures of the flavonoids were drawn using ChemOffice Professional 15.0 and obtained from PubChem (<https://pubchem.ncbi.nlm.nih.gov/>). Structure refinement was performed using the molecular mechanics energy minimization method with the Merck molecular force field (MMFF94) using Open Babel in PyRx V.1.1. Three bacterial proteins, including *Staphylococcus aureus* tyrosyl-tRNA synthetase (PDB ID: 1JIJ), *Streptococcus mutans* antigen I/II (AgI/II) (PDB ID: 3IPK), and *Propionibacterium acnes* surface sialidase (PDB ID: 7LBU) were downloaded from the protein data bank,³¹⁻³³ and the locations of active sites were calculated using AlphaFold from <https://prankweb.cz/>.³⁴ Molecular docking was performed using the AutoDock Vina tool in PyRx V.1.1 software with an exhaustiveness of 32 and a mode value of 9 poses for each docked ligand.^{35,36} A protein binding site was defined as a box with dimensions of 30 × 30 × 30 Å. In the final step, the binding interactions were analyzed, and the docking results were visualized in 2D using the BIOVIA Discovery Studio Visualizer.

Statistical analysis

Data from the experiments were analyzed using Microsoft Excel.

Results and Discussion

In vitro antibacterial activity

The strength of antibacterial activity of the test samples was determined based on the diameter of the clear zone of inhibition (mm). The antibacterial activity was classified as follows: ≤ 5 mm (weak), 5 - 10 mm (moderate), 10 - 20 mm (strong), and ≥ 20 mm (very strong). Moreover, the lower the minimum inhibitory concentration of a compound, the greater its ability to inhibit the growth of test bacteria.²⁸ In this study, ampicillin was used as a positive control against three bacteria: *Staphylococcus aureus* ATCC 25923, *Streptococcus mutans* ATCC 25175, and *Propionibacterium acnes* KCCM 41747 (Table 1). The positive control served to determine whether the test bacteria could be inhibited by or were resistant to the antibiotics.

Staphylococcus aureus can cause dentoalveolar infections, jaw cysts, parodontitis, oral mucosal lesions, and stomatitis. The bacteria can also produce exotoxins, lecosidine, enterotoxins, and coagulase enzyme. Enterotoxins are compounds that cause food poisoning in humans.³⁷ The methanol extract, hexane fraction, and ethyl acetate fraction of *Macaranga hullettii* leaves showed moderate antibacterial activity against *S. aureus*, with an inhibition zone diameter of 5 - 10 mm at concentrations of 0.15 to 2.5% (Table 1).

Table 1: Antibacterial activity of *Macaranga hulettii* King ex Hook leaf extracts and fractions against *Staphylococcus aureus*, *Streptococcus mutans*, and *Propionibacterium acnes*

Sample	Concentration (%)	Inhibition Zone Diameter (mm)*		
		<i>Staphylococcus aureus</i>	<i>Streptococcus mutans</i>	<i>Propionibacterium acnes</i>
Methanol Extract	0	0	0	0
	0.15	6.67 ± 0.58	6.67 ± 0.58	0
	0.31	6.67 ± 0.58	6.67 ± 0.58	7.67 ± 0.58
	0.62	6.67 ± 0.58	7.0 ± 0.0	8.0 ± 1.0
	1.25	7.17 ± 0.29	7.33 ± 0.58	9.67 ± 0.58
	2.5	7.5 ± 0.5	8.0 ± 1.0	10.67 ± 0.58
	MIC (%)	<0.15	<0.15	0.15-0.31
n-Hexane Fraction	0	0	0	0
	0.15	0	0	0
	0.31	0	0	0
	0.62	0	0	0
	1.25	0	6.33 ± 0.29	6.33 ± 0.58
	2.5	6.5 ± 0.5	6.67 ± 0.58	6.67 ± 0.58
	MIC (%)	1.25-2.5	0.62-1.25	0.62-1.25
Ethyl acetate Fraction	0	0	0	0
	0.15	6.33 ± 0.58	6.33 ± 0.58	6.67 ± 0.58
	0.31	6.5 ± 0.5	6.67 ± 0.58	8.0 ± 0.0
	0.62	6.5 ± 0.5	6.83 ± 0.29	9.0 ± 0.0
	1.25	6.67 ± 0.29	7.0 ± 0.0	11.0 ± 1.0
	2.5	7.0 ± 0.0	7.67 ± 0.58	14.67 ± 0.58
	MIC (%)	<0.15	<0.15	<0.15
Ampicillin	0.01	20.33 ± 1.15	21.0 ± 1.0	21.0 ± 1.0

*Data were obtained from triplicate experiments.

The inhibition zone diameter of the 0.15% methanol extract was 6.67 mm. The 0.15% ethyl acetate fraction produced an inhibition zone diameter of 6.33 mm, and the 2.5% n-hexane fraction produced an inhibition zone diameter of 6.50 mm. The results indicated that the methanol extract and ethyl acetate fraction had the most potent antibacterial activity against *S. aureus*, with a minimum inhibitory concentration (MIC) of < 0.15%. Moreover, the study of Sari and Saleh (2015) showed that ethyl acetate fraction of *Macaranga tanarius* was most effective against *S. aureus*, with MIC values of 0.125-0.5% and an inhibition zone of approximately 7.25 mm.³⁸

Streptococcus mutans is implicated in the pathogenesis of dental caries. This facultative anaerobe primarily ferments carbohydrates, resulting in the production of several organic acids, with lactic acid as the predominant byproduct. The acidification of the dental environment by *S. mutans* is a crucial factor contributing to enamel demineralization and the subsequent formation of carious lesions.^{39,40} The bacteria produce glucosyltransferase (GTF) which produces glucan, the compound that causes dental caries.⁴¹ *S. mutans* adheres to the salivary pellicle via its numerous receptors.⁴² The antibacterial activity of *Macaranga hulettii* King leaf extract and fractions was tested against *S. mutans*. Both extract and fractions displayed moderate antibacterial activity, with inhibition zone diameters of 5 - 10 mm at concentrations of 0.15 - 2.5%. The diameters of the inhibition zones produced by the methanol extract and ethyl acetate fraction were 6.67 and 6.33 mm, respectively at 0.15%. Meanwhile, the n-hexane fraction showed antibacterial activity at concentrations of 1.25 - 2.5%, with inhibition zone diameter ranging from 6.33 - 6.67 mm. These results demonstrated that the methanol extract and ethyl acetate fraction of *Macaranga hulettii* leaves produced the highest antibacterial activity against *S.*

mutans, with an MIC < 0.15% (Table 1). *Propionibacterium acnes* is a Gram-positive anaerobic bacterium that can induce unusual keratinization within the sebaceous glands of hair follicles and increase sebum production.⁴³ The enzyme is capable of hydrolyzing triglycerides (TG) present in sebum into free fatty acids (FFA) that may subsequently induce inflammation in and around the hair follicles.⁴⁴ The bacteria also produce hyaluronidase, protease, lecithinase, and neuraminidase, that can cause inflammation of the skin. *Macaranga triloba* ethanol extract has been shown to prevent the growth of *P. acnes*, resulting in an inhibition zone diameter of 5.54 mm at 20%.⁴⁵ As shown in Table 1, the methanol extract and ethyl acetate fraction of *Macaranga hulettii* showed potent antibacterial activity against *P. acnes*, with inhibition zone diameters of 10.67 and 14.67 mm, respectively. The n-hexane fraction exhibited moderate antibacterial activity, with inhibition zone diameter of 5 - 10 mm at 1.25 - 2.5%. At concentration of 0.31%, the methanol extract showed antibacterial activity with an inhibition zone diameter of 7.67 mm, whereas at 0.15% concentration, the ethyl acetate fraction showed antibacterial activity with inhibition zone diameter of 6.67 mm. The results demonstrated that the ethyl acetate fraction of *Macaranga hulettii* leaves presented the highest antibacterial activity against *P. acnes*, with a minimum inhibitory concentration (MIC) of < 0.15% (Table 1).

The methanol extract and ethyl acetate fraction of *Macaranga hulettii* leaves exhibited good antibacterial activity against the three bacterial strains. This finding was consistent with previous studies that attributed the antibacterial effects to the presence of flavonoids and their prenylated or geranylated forms in species of *Macaranga*. These included euchrestaflavanone A, bonanione A, macarangaflavanone A, macarangaflavanone B, macatrichocarpin A, propolin D (nymphaeol

B), senegalensin, schweinfurthin B, schweinfurthin O, and isomacarangain.⁴⁶⁻⁵⁶ Flavonoids can damage bacterial cell walls and denature proteases, thereby disrupting bacterial metabolism.⁵⁷

Molecular docking result

Molecular docking was performed to predict the interactions of the flavonoids with the amino acid residues of three bacterial proteins: *Staphylococcus aureus* tyrosyl-tRNA synthetase, *Streptococcus mutans* antigen I/II (AgI/II), and *Propionibacterium acnes* surface sialidase.³¹⁻³³ The molecular docking results (Table 2) revealed that 11 flavonoid derivatives had binding affinities ranging from -7.2 to -10.5 kcal/mol, -7.5 to -8.9 kcal/mol, and -7.1 to -8.9 kcal/mol against *Staphylococcus aureus* tyrosyl-tRNA synthetase, *Streptococcus mutans* antigen I/II (AgI/II), and *Propionibacterium acnes* surface sialidase, respectively. The interactions of flavonoids and ampicillin with the protein targets are shown in two-dimensional graphs in Figures 1-3.

Table 2: Binding affinity of flavonoids against bacterial proteins

No.	Compound	Binding (kcal/mol)		Affinity
		1JIJ	3IPK	7LBU
1	4'-O-methyl-8-isoprenylnaringenin	-7.4	-8.1	-7.7
2	4'-O-methyl-8-isoprenyleriodictyol	-7.2	-8.1	-7.6
3	6-Isoprenyleriodictyol	-7.7	-8.7	-8.0
4	5-hydroxy-7,4'-dimethoxyflavone	-8.6	-7.8	-7.1
5	5-hydroxy-6,7,4'-trimethoxyflavone	-8.3	-7.5	-7.3
6	Macahulettiin A	-7.6	-8.4	-7.1
7	Lonchocarpol A	-9.4	-7.8	-7.8
8	Nymphaeol A	-9.7	-8.9	-8.4
9	Nymphaeol B	-10.5	-8.9	-7.7
10	Nymphaeol C	-8.4	-8.3	-7.4
11	Solophenol D	-10.0	-8.7	-7.9
12	Ampicillin	-8.5	-7.7	-7.6

Among the compounds targeting *S. aureus* tyrosyl-tRNA synthetase, nymphaeol A, nymphaeol B, and solophenol D had the highest binding affinities, higher than that of the positive control ligand (ampicillin). Thus, the interactions of these compounds with the active site of the

enzyme were modeled as a 2D structure (Figure 1). Ampicillin formed hydrogen bonds with LYS84 and GLN174 and amide- π interactions with GLY38 (Figure 1). Nymphaeol A and nymphaeol B formed hydrogen bonds as well as π -anions, and hydrophobic interactions with four amino acid residues. Nymphaeol B and ampicillin showed similar interactions with LYS84. In addition, solophenol D formed hydrogen bonds with TYR36, THR75, GLN190, ASP177, and GLN174, as well as hydrophobic interactions with HIS50, LEU70, PRO53, and ALA39 (Figure 1). These results agreed with previous studies where nymphaeol A-B and solophenol D exhibited antibacterial activity.^{58,59}

Four compounds including 6-isoprenyleriodictyol, nymphaeol A, nymphaeol B, and solophenol D exhibited high antibacterial activity against *S. mutans* antigen I/II (AgI/II) with binding affinities ranging from -8.7 to -8.9 kcal/mol. The interactions between the ligands and amino acid residues in the binding sites of these compounds are shown in 2D format (Figure 2). Ampicillin formed hydrogen bonds with four amino acid residues, ASN590, SER591, SER588, and ASP760, as well as π -sulfur and π - π interactions with TRP 816, while 6-isoprenyleriodictyol only formed hydrogen bonds with three amino acid residues: SER697, ASN699, and ASN814. Nymphaeol A and nymphaeol B formed hydrogen bonds with four amino acid residues (Figure 2). Nymphaeol A exhibited a similar π - π interaction with TRP816, as observed with ampicillin. Solophenol D formed hydrogen bonds with SER818, ARG824, and THR586, similar π - π interaction as ampicillin with TRP816), and π -sigma interaction with SER762. The compound 6-isoprenyleriodictyol is a prenylated flavonoid similar to nymphaeol A, and hence may have antibacterial activity.

Two compounds, including 6-isoprenyleriodictyol and nymphaeol A, displayed stronger binding affinities than ampicillin, with values of -8.0 and -8.4 kcal/mol against *P. acnes* surface sialidases. Therefore, both compounds were visualized in 2D format to investigate the interactions between the ligands and the amino acid residues in the protein binding sites (Figure 3). Ampicillin formed hydrogen bonds with four amino acid residues, ARG282, GLN287, ASP146, ASP185, and π - π and π -alkyl interactions with PHE257, ALA147, and VAL202; 6-Isoprenyleriodictyol demonstrated π - π interactions with PHE257, π -anion interactions with ASP146, and alkyl-alkyl interactions with ALA147, PHE209, VAL202, and LEU224. This compound formed bonds with five amino acid residues, including ASP146, ARG329, TYR423, ARG282, and GLU287, that were analogous to those observed with ampicillin. Nymphaeol A formed hydrogen bonds with five amino acid residues (ARG329, TYR423, ASP185, ALA147, and SER184), π -anion interactions with ASP312 and ASP185, alkyl-alkyl interactions with MET310, ALA331, and ALA147, and Van der Waals interactions with PHE257. Thus, these compounds may have potent antibacterial activity.

Structure-activity relationships were also studied based on the binding affinity results (Table 2). Flavonoids possessing prenyl, geranyl, and hydroxyl groups showed potential as antibacterial agents due to their strong affinity for amino acid residues in the binding sites of the proteins, compared to ampicillin. However, the absence of a hydroxyl group on prenylated or geranylated flavonoids decreased the binding affinity.^{56,60}

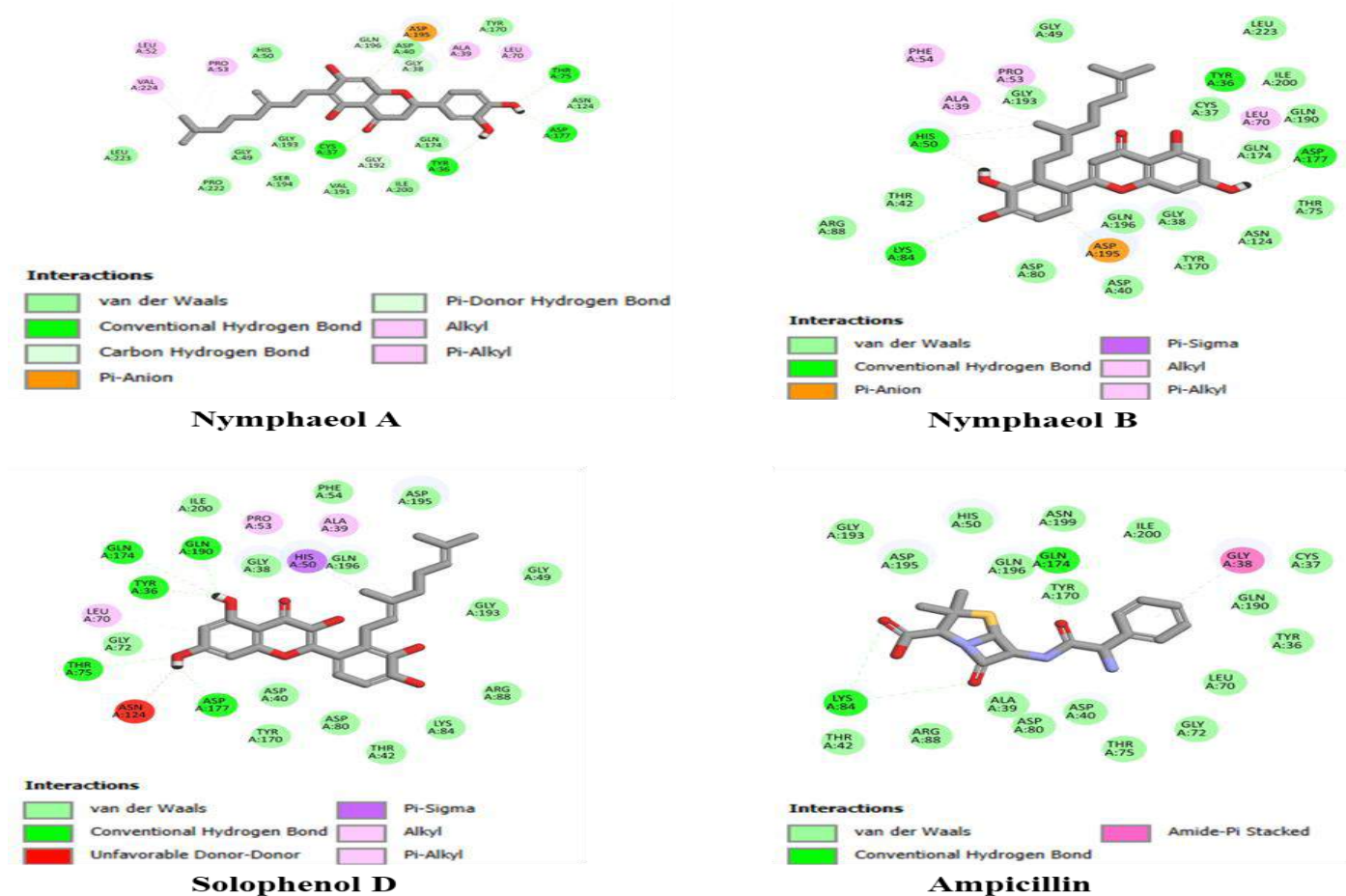


Figure 1: 2D visualization of interaction of the best docked flavonoids and ampicillin with *Staphylococcus aureus* tyrosyl-tRNA synthetase (PDB ID: 1JIJ)

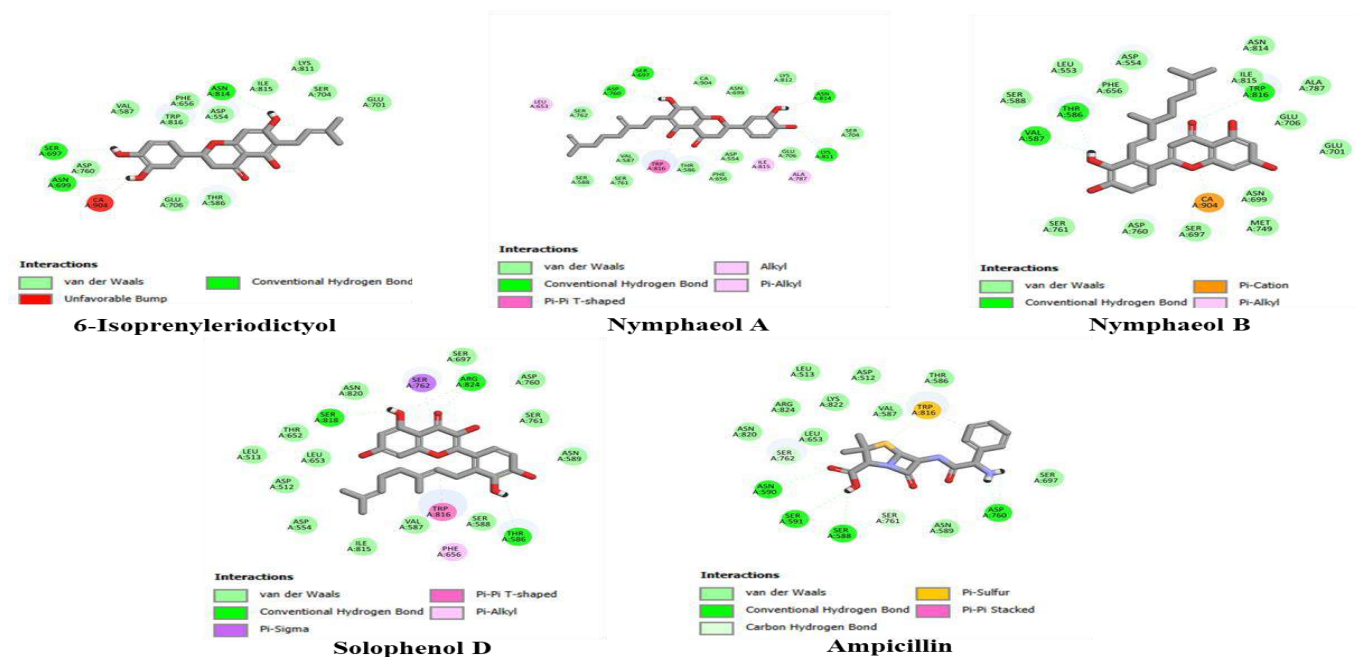


Figure 2: 2D visualization of interaction of the best-docked flavonoids and ampicillin with *Streptococcus mutans* antigen I/II (PDB ID: 3IPK).

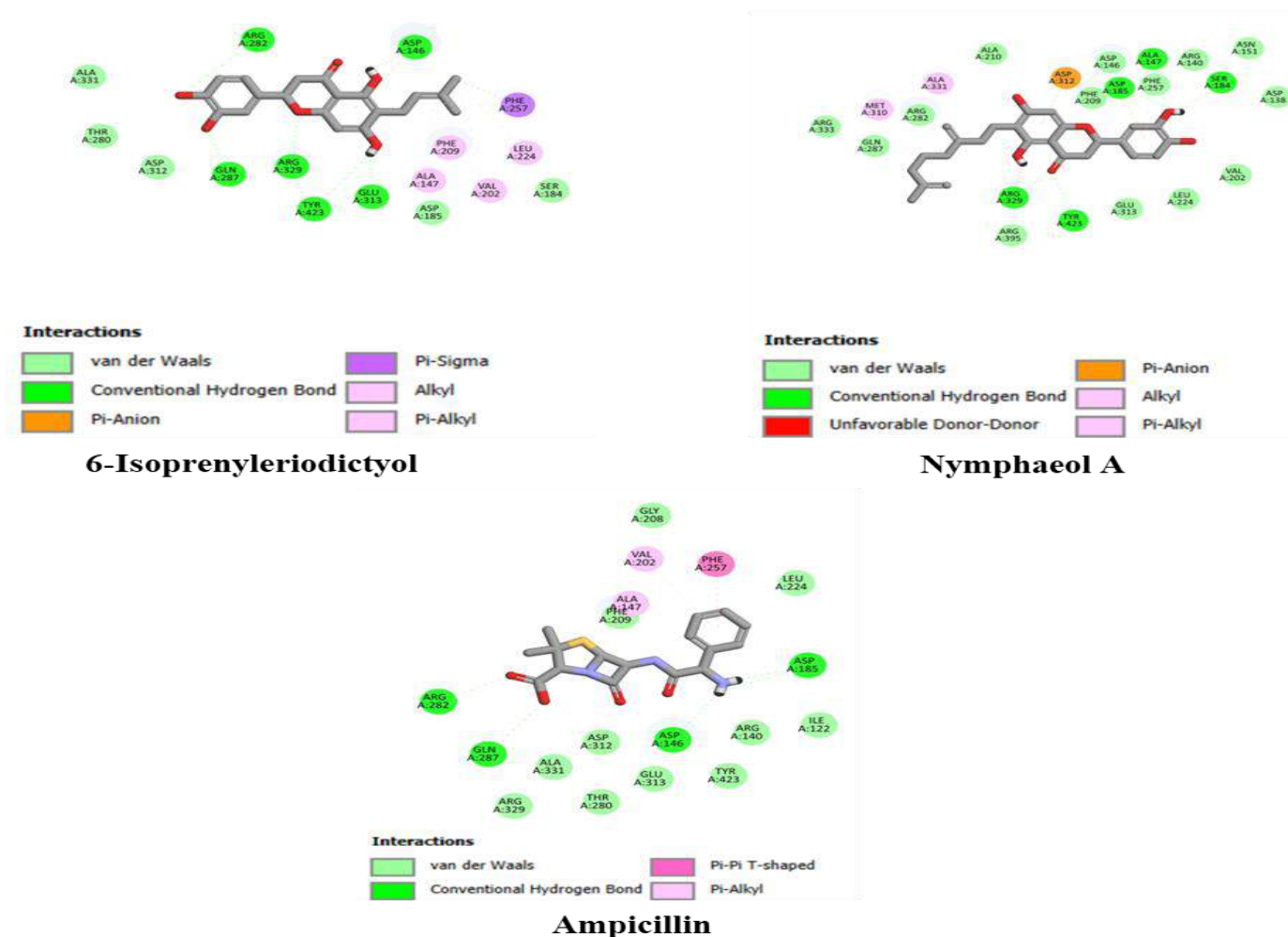


Figure 3: 2D visualization of interaction of the best-docked flavonoids and ampicillin with *Propionibacterium acnes* surface sialidase (PDB ID: 7LBU)

Conclusion

The extract and fractions of *Macaranga hulettii* leaves have potential as antibacterial agents against three bacterial strains, *Staphylococcus aureus* ATCC 25923, *Streptococcus mutans* ATCC 25175, and *Propionibacterium acnes* KCCM 41747. The methanol extract demonstrated antibacterial activity against *S. aureus* and *S. mutans* with MIC of < 0.15% against *S. aureus* and *S. mutans*, and 0.15-0.31% against *P. acnes*. The n-hexane fraction had an MIC of 1.25-2.5% against *S. aureus*, and 0.62-1.25% against *S. mutans* and *P. acnes*. The ethyl acetate fraction had an MIC < 0.15% against the three bacterial strains. Molecular docking studies suggested that 6-isoprenyleriodictyol, nymphaeol A, nymphaeol B, and solophenol D are promising antibacterial agents, as these compounds exhibited the highest binding affinity and formed hydrogen bonds with key amino acid residues in the active sites of the protein targets. Further *in vitro* and *in vivo* studies should be conducted by isolating the active secondary metabolites from this species.

Conflict of Interest

Authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgement

This research was funded by the Islamic Development Bank (PIU-IsDB) project under contract no. 137/UN17.11/PL/2019.

References

- Wong F, de la Fuente-Nunez C, Collins JJ. Leveraging artificial intelligence in the fight against infectious diseases. *Science*. 2023; 381(6654):164-170.
- Seukep AJ, Kuete V, Nahar L, Sarker SD, Guo M. Plant-derived secondary metabolites as the main source of efflux pump inhibitors and methods for identification. *J Pharm Anal*. 2020; 10(4):277-290.
- Magadula JJ. Phytochemistry and pharmacology of the genus *Macaranga*: a review. *J Med Plants Res*. 2014; 8(12):489-503.
- Vu LT, Ngan TB, Phuong L, Huong DT, Litaudon M, Van Hung N, Thach TD, Van Cuong P. Chemical constituents from fruits of *Macaranga denticulata* (Euphorbiaceae) (Part 2). *Vietnam J Chem*. 2018; 56(4):516-520.
- Marliana E, Hairani R, Tjahjandarie TS, Tanjung M. Antiplasmodial activity of flavonoids from *Macaranga tanarius* leaves. *IOP Conf Ser Earth Environ Sci*. 2018; 144(1):012011.
- Tjahjandarie TS, Tanjung M, Saputri RD, Nadar PB,

- Aldin MF, Marlina E, Permadi A, Flavestin K, An isoprenylated stilbene from the leaves of *Macaranga recurvata* Gage. Nat Prod Sci. 2019; 25(3):244-247.
7. Huang DT, Linh NT, Van TT, Litaudon M, Roussi F, Van Nam V, Van Cuong P. Stilbenes from *Macaranga tanarius* (Euphorbiaceae) growing in Vietnam. Vietnam J Chem. 2020; 58(3):338-342.
 8. Aldin MF, Tjahjandarie TS, Saputri RD, Tanjung M. Macasiamenene V, a New Stilbenoid from the Leaves of *Macaranga inermis*. Nat Prod Sci. 2021; 27(1):45-48.
 9. Muharram A, Rachmawati DA, Mardhiyyah S, Tjahjandarie TS, Saputri RD, Ahmat N, Tanjung M. Cytotoxic and antioxidant activities of flavonoids and diterpenoids from *Macaranga involucrata* (Roxb.) Baill. J Appl Pharm Sci. 2023; 13(6):087-92.
 10. Tanjung M, Tjahjandarie TS, Aldin MF, Mardhiyyah S, Ahmat N, Saputri RD. Macagigantin A, A New Flavonoid from *Macaranga gigantea* (Rchb. f & Zoll.) Mull Arg Nat Prod Sci. 2023; 29(4):287-294.
 11. Tjahjandarie TS, Aldin MF, Saputri RD, Tanjung M. Dihydrostilbenes from *Macaranga javanica* (Blume) Müll. Arg. and their antiplasmodial activity. Nat Prod Sci. 2024;1-7.
 12. Tanjung M, Tjahjandarie TS, Aldin MF, Mardhiyyah S, Saputri RD, Syah YM, Ahmat N. Two new flavonols from *Macaranga inermis* pax & K. Hoffm. Nat Prod Sci. 2025; 39(3):498-505.
 13. Tanjung M, Tjahjandarie TS, Aldin MF, Mardhiyyah S, Saputri RD, Syah YM, Ahmat N. Two new flavonols from *Macaranga inermis* pax & K. Hoffm. Nat Prod Sci. 2025; 39(3):498-505.
 14. Marlina E, Tjahjandarie TS, Tanjung M. Isoprenylated flavanone derivatives from *Macaranga hosei* King ex Hook F. Der Pharm Lett. 2015; 7(3):153-156.
 15. Marlina E, Tjahjandarie TS, Tanjung M. Antioxidant activity of flavonoids from *Macaranga pearsonii* Merr. J Kim Mulawarman. 2016; 13(2):97-100.
 16. Marlina E, Astuti W, Kosala K, Hairani R, Tjahjandarie TS, Tanjung M. Chemical composition and anticancer activity of *Macaranga hosei* leaves. Asian J Chem. 2018; 30(4):795-798.
 17. Marlina E, Ruga R, Hairani R, Tjahjandarie TS, Tanjung M. Antioxidant activity of flavonoid constituents from the leaves of *Macaranga tanarius*. IOP Conf Ser J Phys. 2019; 1277(1):012014.
 18. Saputri RD, Tukiran T, Wati FA, Purnamasari AP, Wardhana MW, Tjahjandarie TS, Tanjung M. Macahuilettiin A, a new isoprenylated flavanone from the leaves of *Macaranga hullettii* King ex Hook and their antiplasmodial activity. Vietnam J Chem 2024; 62(3):394-398.
 19. Musdalifah M, Khumaidi A, Suwastika IN. Inhibition test and phytochemical screening of leaf extracts of *Macaranga tanarius* (L.) Mull. Arg against *Salmonella typhi* as an antibacterial. Nat Sci J Sci Technol. 2017; 6(3):9194.
 20. Ibrahim H, Omosa L, Nchiozem-Ngnitedem V, Onyari J, Maru S, Guefack M. Antibacterial activities and phytochemical screening of crude extracts from Kenyan macaranga species toward MDR phenotypes expressing efflux pumps. Pharmacogn Commun. 2021; 11(2):119-126.
 21. Bijesh K and Sebastian D. Isolation and characterization of antibacterial compounds from *Macaranga peltata* against clinical isolates of *Staphylococcus aureus*. Int J Biol Pharm Res. 2013; 4(12):1196-1203.
 22. Salleh WM, Razak NZ, Ahmad F. Phytochemicals and biological activities of *Macaranga hosei* and *Macaranga constricta* (Euphorbiaceae). Marmara Pharm J. 2017; 21(4):881-888.
 23. Slik JW, Priyono P, Welzen PV. Key to the *Macaranga Thou. and Mallotus Lour.* species (Euphorbiaceae) of East Kalimantan, Indonesia. Singapore, Gardens' Bulletin (Singapore), National Parks Board, Singapore Botanic Gardens; 2000. 11-87 p.
 24. Amirta R, Angi EM, Ramadhan R, Kusuma IW, Wiati CB, Haqiqi MT. Potential utilization of macaranga. Samarinda: Mulawarman University Press; 2017.
 25. Rismawati R, Marlina E, Daniel D. Phytochemical Test on Methanol Extract of Leaf of *Macaranga hullettii* King ex Hook. f. J Atomik. 2018; 3(2):91-94.
 26. Hudzicki J. Kirby-Bauer disk diffusion susceptibility test protocol. Am Soc Microbiol. 2009; 15(1):1-23.
 27. Inna M, Astuti W, Saleh C. Antibacterial activity of methanolic extract of uric patch plant leaves (*Cayratia carnosa*) against *Salmonella thypi* and *Propionibacterium acnes*. J Atomik. 2022; 7(1):1-5.
 28. Yulianti MF, Amat AL, Hutasoit RM, Pakan PD. Antibacterial activity of jambang leaf ethanol extract (*Syzygium cumini*) against the growth of *Propionibacterium acnes*. Acta Biochimica Indones. 2023; 6(2):1161.
 29. Hossain MR, Biplob AI, Sharif SR, Bhuiya AM, Sayem AS. Antibacterial Activity of Green Synthesized Silver Nanoparticles of *Lablab purpureus* Flowers Extract against Human Pathogenic Bacteria. Trop J Nat Prod Res. 2023;7(8):3647-3651
 30. Yanda L, Tatsimo SJ, Tamokou JD, Matsuet-Takongmo G, Meffo-Dongmo SC, Meli Lannang A, Sewald N. Antibacterial and antioxidant activities of isolated compounds from *Prosopis africana* leaves. Int J Anal Chem. 2022; 2022:4205823.
 31. Qiu X, Janson CA, Smith WW, Green SM, McDevitt P, Johanson K, Carter P, Hibbs M, Lewis C, Chalker A, Fosberry A. Crystal structure of *Staphylococcus aureus* tyrosyl-tRNA synthetase in complex with a class of potent and specific inhibitors. Protein Sci. 2001; 10(10):2008-2016.
 32. Larson MR, Rajashankar KR, Patel MH, Robinette RA, Crowley PJ, Michalek S, Brady LJ, Deivanayagam C. Elongated fibrillar structure of a *streptococcal adhesin* assembled by the high-affinity association of α - and PPII-helices. Proc Natl Acad Sci. 2010; 107(13):5983-5988.
 33. Yu AC, Volkers G, Jongkees SA, Worrall LJ, Withers SG, Strynadka NC. Crystal structure of the *Propionibacterium acnes* surface sialidase, a drug target for *P. acnes*-associated diseases. Glycobiol. 2022; 32(2):162-170.
 34. Jakubec D, Skoda P, Krivak R, Novotny M, Hoksza D. PrankWeb 3: accelerated ligand-binding site predictions for experimental and modelled protein structures. Nucl Acids Res. 2022; 50(W1):W593-W597.
 35. Dallakyan S and Olson AJ. Small-molecule library screening by docking with PyRx. Methods Mol Biol. 2014; 1263:243-250.
 36. Trott O and Olson AJ. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. J Comput Chem. 2010; 31(2):455-461.
 37. Malangu N. ed. Poisoning: From Specific Toxic Agents to Novel Rapid and Simplified Techniques for Analysis. BoD-Books on Demand; 2017.
 38. Sari AA and Saleh C. Phytochemical test, toxicity and antibacterial activity of extracts of various macaranga leaf fractions (*Macaranga tanarius* (L.) MA) against *Staphylococcus aureus* and *Escherichia coli*. J Kim Mulawarman. 2015; 12(2):53-58.
 39. Imelda R, Mariam M, Satari M. Effect of cassava (*Manihot esculenta* crantz), rice (*oryza sativa* L.), and potato (*solanum tuberosum*) water extract to decrease pH phase fermentation of *streptococcus mutans* atcc 25175. Padjajaran J Dent. 2019; 31(1):14.
 40. Alkhaled A, Alsabek L, Al-assaf M, Badr F. Effect of

- chlorhexidine, honey and propolis on *streptococcus mutans* counts: *in vitro* study. *Dentistry*. 2021; 9(1):a001.
41. Ren Z, Chen L, Li J, Li Y. Inhibition of *Streptococcus mutans* polysaccharide synthesis by molecules targeting glycosyltransferase activity. *J Oral Microbiol*. 2016; 8(1):31095.
 42. Lee BS, Chen YJ, Wei TC, Ma TL, Chang CC. Comparison of antibacterial adhesion when salivary pellicle is coated on both poly (2-hydroxyethyl-methacrylate)-and polyethylene-glycol-methacrylate-grafted poly (methyl methacrylate). *Int J Mol Sci*. 2018; 19(9):2764.
 43. Chen KC, Yang CH, Li TT, Zouboulis CC, Huang YC. Suppression of *Propionibacterium acnes*-stimulated proinflammatory cytokines by Chinese bayberry extracts and its active constituent myricetin in human sebocytes *in vitro*. *Phytother Res*. 2019; 33(4):1104-1113.
 44. Xu J, Chen X, Song J, Wang C, Xu W, Tan H, Suo H. Antibacterial activity and mechanism of cell-free supernatants of *Lactocaseibacillus paracasei* against *Propionibacterium acnes*. *Microb. Pathog.* 2024; 189:106598
 45. Warnida H, Mustika D, Supomo S, Sukawaty Y. Effectiveness of Mahang Leaf Ethanol Extract (*Macaranga Triloba*) as an anti-acne. *J Penelit. Sos. Ekon. Kehuta*. 2018; 4(1):9-18.
 46. Schütz BA, Wright AD, Rali T, Sticher O. Prenylated flavanones from leaves of *Macaranga pleiostemona*. *Phytochem*. 1995; 40(4):1273-1277.
 47. Lim TY, Lim YY, Yule CM. Evaluation of antioxidant, antibacterial and anti-tyrosinase activities of four *Macaranga* species. *Food Chem*. 2009; 114(2):594-599.
 48. Fareza MS, Syah YM, Mujahidin D, Juliawaty LD, Kurniasih I. Antibacterial flavanones and dihydrochalcones from *Macaranga trichocarpa*. *Z. Naturforsch C J Biosci*. 2014; 69(9-10):375-380.
 49. Hasanat A, Kabir MS, Hossain MM, Hasan M, Al Masum MA, Chowdhury TA, Bhuiyan DI, Mamur A, Kibria AS. Antibacterial activity of methanol extract of *Macaranga denticulata* leaves and *in silico* PASS prediction for its six secondary metabolites. *World J Pharm Pharm. Sci*. 2015:1258-1266.
 50. O Akanbi B, Anene P, Olayanju S. Preliminary Screening Indicates Promising Antimicrobial Properties of the Stem Bark Extracts of *Macaranga rosea*. *Anti-Infective Agents*. 2015; 13(2):123-128.
 51. Ogundajo A, Okeleye B, Ashafa AO. Chemical constituents, *in vitro* antimicrobial and cytotoxic potentials of the extracts from *Macaranga barteri* Mull-Arg. *Asian Pac J Trop Biomed*. 2017; 7(7):654-659.
 52. Putri R, Hendra R, Teruna HY. Anti-Bacterial and Anti-Fungal Activities from *Macaranga bancana* Leaves Extract. *Pharmacol Clin Pharm Res*. 2019; 4(1):1-4.
 53. Lee JH, Kim YG, Khadke SK, Yamano A, Woo JT, Lee J. Antimicrobial and antibiofilm activities of prenylated flavanones from *Macaranga tanarius*. *Phytomed*. 2019; 63:153033.
 54. Pagna JI, Awazi T, Mbarga PE, Mbekou IM, Mkounga P, Fotie J, Frese M, Fabrice FB, Lenta BN, Sewald N, Nkengfack EA. Antibacterial flavonoids from the fruits of *Macaranga hurifolia*. *J Asian Nat Prod Res*. 2022; 24(11):1041-1051.
 55. Kamsi VF, Simo Fotso CC, Kanko Mbekou IM, Toussie BT, Ndjakou Lenta B, Boyom FF, Sewald N, Frese M, Ngadjui BT, Wabo Fotso G. Chemical constituents of *Macaranga occidentalis*, antimicrobial and chemophenetic studies. *Molecules*. 2022; 27(24):8820.
 56. Rosamah E, Haqiqi MT, Putri AS, Kuspradini H, Kusuma IW, Amirta R, Yuliansyah Y, Suwinarti W, Paramita S, Ramadhan R, Tarmadi D. The potential of *Macaranga* plants as skincare cosmetic ingredients: A review. *J Appl Pharm Sci*. 2023; 13(7):001-12.
 57. Li AP, He YH, Zhang SY, Shi YP. Antibacterial activity and action mechanism of flavonoids against phytopathogenic bacteria. *Pestic Biochem Physiol*. 2022; 188:105221.
 58. Chen YW, Ye SR, Ting C, Yu YH. Antibacterial activity of propolis from Taiwanese green propolis. *J Food Drug Anal*. 2018; 26(2):761-768.
 59. Inui S, Hosoya T, Shimamura Y, Masuda S, Ogawa T, Kobayashi H, Shirafuji K, Moli RT, Kozono I, Shin-ya K, Kumazawa S. Solophenols B–D and Solomonin: New Prenylated Polyphenols Isolated from Propolis Collected from The Solomon Islands and Their Antibacterial Activity. *J Agric Food Chem*. 2012; 60(47):11765-11770.
 60. Ruga R, Kingkaew K, Tamsampaoloet K, Chavasiri W. Enhancing antibacterial activity against *Propionibacterium acnes* and *Staphylococcus aureus* by combination of tetracycline with selected compounds. *Chem Lett*. 2018; 47(12):1538-1541.

Re: Check Galley Proof

From Ade Danova <adedanova@itb.ac.id>

Date Mon 6/2/2025 12:05 PM

To Editorial Team <et.tjnpr@gmail.com>

Cc Editor-in-Chief Tjnpr <editor.tjnpr@gmail.com>

 2 attachments (970 KB)

Respond to the editor.docx; TJNPR-2025-420 STA-Revised.docx;

Dear Editorial Team

Thank you very much for giving us to do some correction in this manuscript before published. Here, we attach the revised manuscript that has been revised it.

Best regards,
Ade Danova

Respond to the editor

Thank you very much for your help that has edited our manuscript to the journal format. Here, I have some corrections dan have already revised them.

1. Abstract: Molecular docking of eleven flavonoid derivatives presents in **three** Macaranga species

Correction: Molecular docking of eleven flavonoid derivatives presents in **four** Macaranga species

2. Introduction: The flavonoids isolated from Macaranga species in East Kalimantan, Indonesia, were evaluated for their antibacterial activity using molecular docking analysis.

Correction: The flavonoids isolated from Macaranga species in East Kalimantan and Central Kalimantan (Indonesia) as well as Trengganu (Malaysia)

3. A typo for species name: *Macaranga* **huletti**

Correction: *Macaranga* **hullettii**

4. Copyright: © 2025 Zulaikhah et al.

Correction: Copyright: © 2025 Danova et al.

5. Citation: Zulaikhah ST, Ratnawati R, Husaana A. Nephroprotective Effect of a Combination of Probiotics, Tender Coconut Water, and Vitamin E in Gentamicin-Induced Acute Kidney Injury in Rats. Trop J Nat Prod Res. 2025; 9(7).

Correction:

Marliana E, Danova A, Hairani R, Ruga R, Hermawati E, Yulvia A. *In Vitro* Evaluation and Molecular Docking Study of the Antibacterial Potential of *Macaranga hullettii* King ex Hook.f. Leaf Extract. Trop J Nat Prod Res. 2025; 9(7).

6. Nutrient Agar (25 mL)

Correction: Nutrient Agar (**15 mL**)

7. Eleven (11) flavonoid derivatives possessing either prenyl or geranyl group present in the three *Macaranga* species in East Kalimantan, Indonesia (*Macaranga hosei*, *Macaranga pearsonii*, and *Macaranga tanarius*):

Correction:

Molecular docking was conducted to eleven (11) flavonoid derivatives possessing either prenyl or geranyl group presented in the three *Macaranga* species in East Kalimantan, Indonesia (*Macaranga hosei*, *Macaranga pearsonii*, and *Macaranga tanarius*), *Macaranga hullettii* in Central Kalimantan (Indonesia), and *Macaranga hosei* in Trengganu (Malaysia):

8. Citation: nymphaeol A, nymphaeol B, nymphaeol C, and solophenol D,¹⁴⁻¹⁷ were used for the study

Correction: nymphaeol A, nymphaeol B, nymphaeol C, and solophenol D,^{14-18,22} were used for the study

9. References no 12:

Tanjung M, Tjahjandarie TS, Aldin MF, Mardhiyyah S, Saputri RD, Syah YM, Ahmat N. Two new flavonols from *Macaranga inermis* pax & K. Hoffm. Nat Prod Sci. 2025; 39(3):498-505.

Correction:

Toko EG, Tchapo CED, Tsamo AT, Kemzeu R, Wang Y, Fekam FB, Ndinteh DT, Choudhary MI, Nkengfack EA, Mmutlane EM, Mkounga P. Three New Polyphenol Derivatives from the Fruits of *Macaranga Monandra* and their Antioxidant Potential. Chem. Biodivers. 2024;21(7):e202301816.



Tropical Journal of Natural Product Research

Available online at <https://www.tjnpr.org>

Original Research Article

In vitro Evaluation and Molecular Docking Study of the Antibacterial Potential of *Macaranga hullettii* King ex Hook.f. Leaf Extract

Eva Marlina^{1,3}, Ade Danova^{2*}, Rita Hairani^{1,3}, Ritbey Ruga^{1,3}, Elvira Hermawati², Ana Yulvia⁴¹Department of Chemistry, Faculty of Mathematics and Natural Sciences, Mulawarman University, Samarinda 75123, East Kalimantan, Indonesia²Organic Chemistry Division, Department of Chemistry, Faculty of Mathematics and Natural Sciences, Institut Teknologi Bandung Jl. Ganesha No. 10, Bandung 70132, West Java, Indonesia³Research Center for Medicine and Cosmetics from Tropical Rainforest Resources, Mulawarman University, Samarinda, Indonesia⁴Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Jember, East Java, Indonesia

ARTICLE INFO

Article history:

Received

Revised

Accepted

Published online 01 May 2025

ABSTRACT

Due to the resistance of bacteria to antibiotics, there has been increasing interest in the use of plant extracts to combat infections. Plants in the genus *Macaranga* contain secondary metabolites such as flavonoids with antibacterial properties. *Macaranga hullettii* King ex Hook.f. are particularly noteworthy due to their widespread distribution in East Kalimantan. This study aimed to investigate the antibacterial potential of *M. hullettii* leaf extract through *in vitro* and molecular docking studies. The antibacterial activity of the methanol extract, n-hexane, and ethyl acetate fractions from *M. hullettii* leaves was evaluated against three bacterial species, *Staphylococcus aureus*, *Streptococcus mutans*, and *Propionibacterium acnes* using the disc diffusion assay. Molecular docking of eleven flavonoid derivatives presents in *four* *Macaranga* species was performed against selected bacterial proteins (PDB ID: IJII, 3IPK, and 7LBU). The results showed that both the methanol extract and ethyl acetate fraction displayed antibacterial activity against the three bacteria strains, with minimum inhibitory concentrations (MICs) < 0.15%, except for the methanol extract, which had MIC of 0.15-0.31% against *Propionibacterium acnes*. In addition, molecular docking study showed that four flavonoids possessing prenyl or geranyl groups (6-isoprenyleriodictyol, nymphaeol A, nymphaeol B, and solophenol D) showed the highest binding affinity and dominant hydrogen bonding interactions with key amino acid residues in the binding sites of three bacterial proteins. The findings suggest that the methanol extract and its ethyl acetate fraction from *Macaranga hullettii* leaves could be a potential source of new antibacterial agents. Further studies are needed to isolate and evaluate the bioactive compounds.

Copyright: © 2025 Danova et al. This is an open-access article distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Keywords: Antibacterial, Extract, Flavonoid, *Macaranga hullettii*, Molecular Docking

Introduction

Despite substantial advancements in medicine and fundamental research, infectious diseases caused by transmissible agents such as bacteria, protists, and viruses continue to present significant challenges to healthcare professionals. These challenges include the issue of antimicrobial resistance.¹ Extracts from medicinal plants present a promising approach to addressing multidrug-resistant bacteria. In addition, medicinal plants can augment the efficacy of antibiotics for the treatment of infectious diseases.² Therefore, it is imperative to develop more efficacious antimicrobial agents, particularly those sourced from natural sources, as these are both readily available and economically viable. Plants in the genus *Macaranga* belong to the family Euphorbiaceae and are commonly known as "Mahang." The secondary metabolites contained in plants of this genus include flavonoids and stilbenoids with prenylated substituents.³⁻¹³

*Corresponding author. Email: adedanova@itb.ac.id,
Tel: +6281357944698

Citation: Marlina E, Danova A, Hairani R, Ruga R, Hermawati E, Yulvia A. *In Vitro* Evaluation and Molecular Docking Study of the Antibacterial Potential of *Macaranga hullettii* King ex Hook.f. Leaf Extract. Trop J Nat Prod Res. 2025; 9(7).

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

Nine flavonoids containing either prenyl or geranyl groups and displaying antioxidant, antiparasitic, and anticancer activities have been isolated from three species (*Macaranga hosei*, *Macaranga pearsonii*, and *Macaranga tanarius*) of *Macaranga* present in East Kalimantan: 4'-O-methyl-8-isoprenylnaringenin, 4'-O-methyl-8-isoprenyleriodictyol, 6-isoprenyleriodictyol, solophenol D, nymphaeol A, and nymphaeol B, including lonchocarpol A, nymphaeol C, and macahulleitin A that was isolated from *Macaranga hullettii* obtained from Central Kalimantan.¹⁴⁻¹⁸ *Macaranga tanarius* leaf extract has been shown to exhibit antibacterial activity.¹⁹ The extracts of *Macaranga conglomerata*, *Macaranga kilimandscharica*, and *Macaranga capensis* from Kenya has shown antibacterial activity against *E. coli*, *E. aerogenes*, *K. pneumoniae*, *P. stuartii*, *P. aeruginosa*, and *S. aureus*.²⁰ The flavonol rhamnetin, isolated from *Macaranga peltata*, also showed antibacterial activity against *Staphylococcus aureus*.²¹ Two flavanone groups; 5-hydroxy-7,4'-dimethoxyflavone and 5-hydroxy-6,7,4'-trimethoxyflavone obtained from *Macaranga hosei* showed weak antibacterial activity against three pathogenic bacteria.²² In this study, the leaves of *Macaranga hullettii* King ex Hook.f. were identified as potential new source of medicinal compounds against bacterial infection. This plant is found in all areas of East Kalimantan, where the species commonly occurs in mountain forests and logging areas in the Kalimantan region.^{23,24} Phytochemical screening and isolation of secondary metabolites from the leaf extract of *Macaranga hullettii* identified several flavonoid compounds.^{18,25} The methanol extract and its fractions derived from *Macaranga hullettii* have not yet been analyzed for their antibacterial activity. Therefore, this study aimed to assess the antibacterial activity of *Macaranga hullettii* leaf extract and

its fractions using *in vitro* studies. The flavonoids isolated from *Macaranga* species in East Kalimantan and Central Kalimantan (Indonesia) as well as Trengganu (Malaysia) were evaluated for their antibacterial activity using molecular docking analysis.

Materials and Methods

Chemicals and equipment

The solvents used (methanol, n-hexane, and ethyl acetate) were purified by distillation before use, nutrient agar (NA) from Merck, Luria Bertani liquid medium (1% tryptone, 0.5% yeast, and 0.5% NaCl), yeast extract (Oxoid 100% (w/w)), tryptone (Oxoid 100% (w/w)), ampicillin 100 mg/ml (Sigma Aldrich), NaCl (Sigma Aldrich), cotton swabs, aluminum foil, gauze, Petri dishes, Ose needles, paper discs, an autoclave (Autoclave TOMY, Model SX-700), an oven (SHARP EO-28 WH), a rotary vacuum evaporator (EYELA Rotary evaporator N-1300E V.S.Series), and a freezer (Sanyo Medicoool). For molecular docking analysis, we used a Lenovo Yoga 7 computer with AMD Ryzen AI 7-8840HS w/ Radeon 780M graphics, 3301 Mhz, 8 Core(s), and 16.0 GB RAM.

Plant collection and identification

Fresh leaves of *Macaranga hullettii* were collected from the Lesan River Forest, Kelay District, Berau Regency, East Kalimantan, Indonesia (1°32'2.5919" N, 117°3'11.950" E). The plant samples were identified at the Laboratory of Anatomy and Plant Systematics, FMIPA Mulawarman University where voucher No. 0174/UN.17.8.5.7.16/HA/XI/2017 was assigned.

Extraction and fractionation

Macaranga hullettii leaf powder (1.3 kg) was extracted by maceration in methanol (2 x 7.0 L) at room temperature for 24 h. The extract was filtered, and filtrate was evaporated *in vacuo* using a rotary vacuum evaporator to obtain the crude methanol extract (126 g). The crude methanol extract (100 g) was dissolved in 500 mL of methanol-water mixture (4:1), and then successively fractionated with 5 x 200 mL each of n-hexane and ethyl acetate to obtain n-hexane fraction (28 g) and ethyl acetate fraction (18 g), respectively. Each fraction was evaporated using a rotary vacuum evaporator. The methanol extract, n-hexane fraction, and ethyl acetate fraction were evaluated for antibacterial activity.

Test organisms

Staphylococcus aureus ATCC 25923 was obtained from the Department of Microbiology, Faculty of Science, Chulalongkorn University. *Streptococcus mutans* ATCC 25175 was obtained from the Department of Biochemistry, Faculty of Dentistry, Chulalongkorn University. Both strains were purchased from the American Type Culture Collection (ATCC). *Propionibacterium acnes* KCCM 41747 was obtained from the Faculty of Forestry, Mulawarman University, and from the Korean Culture Center of Microorganisms (KCCM). The bacteria were periodically sub-cultured and maintained in nutrient agar (NA) under suitable conditions.

In vitro antibacterial assay

The antibacterial activity of the methanol extract, n-hexane, and ethyl acetate fractions of *Macaranga hullettii* leaves was evaluated using the disc diffusion or the Kirby-Bauer method.^{26,27} Nutrient Agar (NA) medium was prepared by dissolving 3.3 g in 150 mL of distilled water. Luria Bertani (LB) liquid medium was prepared by dissolving 4 g in 500 mL of distilled water, stirring, and adding 5 mL to a test tube. The NA and LB media were sterilized in an autoclave at 121°C with a pressure of 1 atm for 15 minutes. Each test bacterium was collected using an Ose needle, followed by dilution in a test tube containing sterile LB media and incubated at 37 °C for 24 h.

Nutrient Agar (15 mL) was poured into sterilized Petri dishes and maintained at room temperature for solidification. A sample of the bacterial suspension in the test tubes was then collected using a sterile cotton swab and applied to the surface of the agar in the Petri dish. Each 30 µL of the test *M. hullettii* leaf samples prepared in various

concentrations in the organic solvents (methanol, n-hexane, and ethyl acetate) was poured onto sterile adsorbent filter paper discs (6 mm in diameter), and the solvent was removed. Afterward, the paper discs containing the test sample were placed on the surface of the agar media and incubated at 37 °C for 18-24 hours. The inhibition zone formed in each well was measured using a ruler.^{28,29} The minimum inhibitory concentration (MIC) was determined for the methanol extract as well as the n-hexane and ethyl acetate fractions. MIC was defined as the minimum concentration that completely inhibited bacterial growth.³⁰ Ampicillin was used as a positive control, and the three organic solvents were used as negative controls.

Molecular docking

Molecular docking was conducted to eleven (11) flavonoid derivatives possessing either prenyl or geranyl group presented in the three *Macaranga* species in East Kalimantan, Indonesia (*Macaranga hosei*, *Macaranga pearsonii*, and *Macaranga tanarius*), *Macaranga hullettii* in Central Kalimantan (Indonesia), and *Macaranga hosei* in Trengganu (Malaysia): 4'-O-methyl-8-isoprenylnaringenin, 4'-O-methyl-8-isoprenyleriodictyol, 6-isoprenyleriodictyol, 5-hydroxy-7,4'-dimethoxyflavone, lonchocarpol A, 5-hydroxy-6,7,4'-trimethoxyflavone, Macahulleitiin A, nymphaeol A, nymphaeol B, nymphaeol C, and solophenol D,^{14-18,22} were used for the study. Macahulleitiin A, together with lonchocarpol A and nymphaeol C, are a prenylated flavonoid found in *Macaranga hullettii* obtained from Central Kalimantan (Indonesia).¹⁸ A comparative analysis of the molecular docking results for prenylated/geranylated flavonoids with methoxylated flavonoids was conducted, focusing on 5-hydroxy-7,4'-dimethoxyflavone and 5-hydroxy-6,7,4'-trimethoxyflavone that are found in *Macaranga hosei* from Trengganu, Malaysia.²²

The molecular structures of the flavonoids were drawn using ChemOffice Professional 15.0 and obtained from PubChem (<https://pubchem.ncbi.nlm.nih.gov/>). Structure refinement was performed using the molecular mechanics energy minimization method with the Merck molecular force field (MMFF94) using Open Babel in PyRx V.1.1. Three bacterial proteins, including *Staphylococcus aureus* tyrosyl-tRNA synthetase (PDB ID: 1JIJ), *Streptococcus mutans* antigen I/II (AgI/II) (PDB ID: 3IPK), and *Propionibacterium acnes* surface sialidase (PDB ID: 7LBU) were downloaded from the protein data bank,³¹⁻³³ and the locations of active sites were calculated using AlphaFold from <https://prankweb.cz/>.³⁴ Molecular docking was performed using the AutoDock Vina tool in PyRx V.1.1 software with an exhaustiveness of 32 and a mode value of 9 poses for each docked ligand.^{35,36} A protein binding site was defined as a box with dimensions of 30 × 30 × 30 Å. In the final step, the binding interactions were analyzed, and the docking results were visualized in 2D using the BIOVIA Discovery Studio Visualizer.

Statistical analysis

Data from the experiments were analyzed using Microsoft Excel.

Results and Discussion

In vitro antibacterial activity

The strength of antibacterial activity of the test samples was determined based on the diameter of the clear zone of inhibition (mm). The antibacterial activity was classified as follows: ≤ 5 mm (weak), 5 - 10 mm (moderate), 10 - 20 mm (strong), and ≥ 20 mm (very strong). Moreover, the lower the minimum inhibitory concentration of a compound, the greater its ability to inhibit the growth of test bacteria.²⁸ In this study, ampicillin was used as a positive control against three bacteria: *Staphylococcus aureus* ATCC 25923, *Streptococcus mutans* ATCC 25175, and *Propionibacterium acnes* KCCM 41747 (Table 1). The positive control served to determine whether the test bacteria could be inhibited by or were resistant to the antibiotics.

Staphylococcus aureus can cause dentoalveolar infections, jaw cysts, parotitis, oral mucosal lesions, and stomatitis. The bacteria can also produce exotoxins, lecosidine, enterotoxins, and coagulase enzyme. Enterotoxins are compounds that cause food poisoning in humans.³⁷ The methanol extract, hexane fraction, and ethyl acetate fraction of *Macaranga hullettii* leaves showed moderate antibacterial activity

against *S. aureus*, with an inhibition zone diameter of 5 - 10 mm at

concentrations of 0.15 to 2.5% (Table 1).

Table 1: Antibacterial activity of *Macaranga hullettii* King ex Hook leaf extracts and fractions against *Staphylococcus aureus*, *Streptococcus mutans*, and *Propionibacterium acnes*

Sample	Concentration (%)	Inhibition Zone Diameter (mm)*		
		<i>Staphylococcus aureus</i>	<i>Streptococcus mutans</i>	<i>Propionibacterium acnes</i>
Methanol Extract	0	0	0	0
	0.15	6.67 ± 0.58	6.67 ± 0.58	0
	0.31	6.67 ± 0.58	6.67 ± 0.58	7.67 ± 0.58
	0.62	6.67 ± 0.58	7.0 ± 0.0	8.0 ± 1.0
	1.25	7.17 ± 0.29	7.33 ± 0.58	9.67 ± 0.58
	2.5	7.5 ± 0.5	8.0 ± 1.0	10.67 ± 0.58
	MIC (%)	<0.15	<0.15	0.15-0.31
n-Hexane Fraction	0	0	0	0
	0.15	0	0	0
	0.31	0	0	0
	0.62	0	0	0
	1.25	0	6.33 ± 0.29	6.33 ± 0.58
	2.5	6.5 ± 0.5	6.67 ± 0.58	6.67 ± 0.58
	MIC (%)	1.25-2.5	0.62-1.25	0.62-1.25
Ethyl acetate Fraction	0	0	0	0
	0.15	6.33 ± 0.58	6.33 ± 0.58	6.67 ± 0.58
	0.31	6.5 ± 0.5	6.67 ± 0.58	8.0 ± 0.0
	0.62	6.5 ± 0.5	6.83 ± 0.29	9.0 ± 0.0
	1.25	6.67 ± 0.29	7.0 ± 0.0	11.0 ± 1.0
	2.5	7.0 ± 0.0	7.67 ± 0.58	14.67 ± 0.58
	MIC (%)	<0.15	<0.15	<0.15
Ampicillin	0.01	20.33 ± 1.15	21.0 ± 1.0	21.0 ± 1.0

*Data were obtained from triplicate experiments.

The inhibition zone diameter of the 0.15% methanol extract was 6.67 mm. The 0.15% ethyl acetate fraction produced an inhibition zone diameter of 6.33 mm, and the 2.5% n-hexane fraction produced an inhibition zone diameter of 6.50 mm. The results indicated that the methanol extract and ethyl acetate fraction had the most potent antibacterial activity against *S. aureus*, with a minimum inhibitory concentration (MIC) of < 0.15%. Moreover, the study of Sari and Saleh (2015) showed that ethyl acetate fraction of *Macaranga tanarius* was most effective against *S. aureus*, with MIC values of 0.125-0.5% and an inhibition zone of approximately 7.25 mm.³⁸

Streptococcus mutans is implicated in the pathogenesis of dental caries. This facultative anaerobe primarily ferments carbohydrates, resulting in the production of several organic acids, with lactic acid as the predominant byproduct. The acidification of the dental environment by *S. mutans* is a crucial factor contributing to enamel demineralization and the subsequent formation of carious lesions.^{39,40} The bacteria produce glucosyltransferase (GTF) which produces glucan, the compound that causes dental caries.⁴¹ *S. mutans* adheres to the salivary pellicle via its numerous receptors.⁴² The antibacterial activity of *Macaranga hullettii* King leaf extract and fractions was tested against *S. mutans*. Both extract and fractions displayed moderate antibacterial activity, with inhibition zone diameters of 5 - 10 mm at concentrations of 0.15 - 2.5%. The diameters of the inhibition zones produced by the methanol extract and ethyl acetate fraction were 6.67 and 6.33 mm, respectively at 0.15%. Meanwhile, the n-hexane fraction showed antibacterial activity at concentrations of 1.25 - 2.5%, with inhibition zone diameter ranging from 6.33 - 6.67 mm. These results demonstrated that the methanol extract and ethyl acetate fraction of *Macaranga*

hullettii leaves produced the highest antibacterial activity against *S. mutans*, with an MIC < 0.15% (Table 1). *Propionibacterium acnes* is a Gram-positive anaerobic bacterium that can induce unusual keratinization within the sebaceous glands of hair follicles and increase sebum production.⁴³ The enzyme is capable of hydrolyzing triglycerides (TG) present in sebum into free fatty acids (FFA) that may subsequently induce inflammation in and around the hair follicles.⁴⁴ The bacteria also produce hyaluronidase, protease, lecithinase, and neuraminidase, that can cause inflammation of the skin. *Macaranga triloba* ethanol extract has been shown to prevent the growth of *P. acnes*, resulting in an inhibition zone diameter of 5.54 mm at 20%.⁴⁵ As shown in Table 1, the methanol extract and ethyl acetate fraction of *Macaranga hullettii* showed potent antibacterial activity against *P. acnes*, with inhibition zone diameters of 10.67 and 14.67 mm, respectively. The n-hexane fraction exhibited moderate antibacterial activity, with inhibition zone diameter of 5 - 10 mm at 1.25 - 2.5%. At concentration of 0.31%, the methanol extract showed antibacterial activity with an inhibition zone diameter of 7.67 mm, whereas at 0.15% concentration, the ethyl acetate fraction showed antibacterial activity with inhibition zone diameter of 6.67 mm. The results demonstrated that the ethyl acetate fraction of *Macaranga hullettii* leaves presented the highest antibacterial activity against *P. acnes*, with a minimum inhibitory concentration (MIC) of < 0.15% (Table 1).

The methanol extract and ethyl acetate fraction of *Macaranga hullettii* leaves exhibited good antibacterial activity against the three bacterial strains. This finding was consistent with previous studies that attributed the antibacterial effects to the presence of flavonoids and their prenylated or geranylated forms in species of *Macaranga*. These

included euchrestaflavanone A, bonanione A, macarangaflavanone A, macarangaflavanone B, macatrichocarpin A, propolin D (nymphaeol B), senegalensin, schweinfurthin B, schweinfurthin O, and isomacarangain.⁴⁶⁻⁵⁶ Flavonoids can damage bacterial cell walls and denature proteases, thereby disrupting bacterial metabolism.⁵⁷

Molecular docking result

Molecular docking was performed to predict the interactions of the flavonoids with the amino acid residues of three bacterial proteins: *Staphylococcus aureus* tyrosyl-tRNA synthetase, *Streptococcus mutans* antigen I/II (Agl/II), and *Propionibacterium acnes* surface sialidase.³¹⁻³³ The molecular docking results (Table 2) revealed that 11 flavonoid derivatives had binding affinities ranging from -7.2 to -10.5 kcal/mol, -7.5 to -8.9 kcal/mol, and -7.1 to -8.9 kcal/mol against *Staphylococcus aureus* tyrosyl-tRNA synthetase, *Streptococcus mutans* antigen I/II (Agl/II), and *Propionibacterium acnes* surface sialidase, respectively. The interactions of flavonoids and ampicillin with the protein targets are shown in two-dimensional graphs in Figures 1-3.

Table 2: Binding affinity of flavonoids against bacterial proteins

No.	Compound	Binding Affinity (kcal/mol)		
		1JIJ	3IPK	7LBU
1	4'-O-methyl-8-isoprenylnaringenin	-7.4	-8.1	-7.7
2	4'-O-methyl-8-isoprenyleriodictyol	-7.2	-8.1	-7.6
3	6-Isoprenyleriodictyol	-7.7	-8.7	-8.0
4	5-hydroxy-7,4'-dimethoxyflavone	-8.6	-7.8	-7.1
5	5-hydroxy-6,7,4'-trimethoxyflavone	-8.3	-7.5	-7.3
6	Macahuilettiin A	-7.6	-8.4	-7.1
7	Lonchocarpol A	-9.4	-7.8	-7.8
8	Nymphaeol A	-9.7	-8.9	-8.4
9	Nymphaeol B	-10.5	-8.9	-7.7
10	Nymphaeol C	-8.4	-8.3	-7.4
11	Solophenol D	-10.0	-8.7	-7.9
12	Ampicillin	-8.5	-7.7	-7.6

Among the compounds targeting *S. aureus* tyrosyl-tRNA synthetase, nymphaeol A, nymphaeol B, and solophenol D had the highest binding affinities, higher than that of the positive control ligand (ampicillin).

Thus, the interactions of these compounds with the active site of the enzyme were modeled as a 2D structure (Figure 1). Ampicillin formed hydrogen bonds with LYS84 and GLN174 and amide- π interactions with GLY38 (Figure 1). Nymphaeol A and nymphaeol B formed hydrogen bonds as well as π -anions, and hydrophobic interactions with four amino acid residues. Nymphaeol B and ampicillin showed similar interactions with LYS84. In addition, solophenol D formed hydrogen bonds with TYR36, THR75, GLN190, ASP177, and GLN174, as well as hydrophobic interactions with HIS50, LEU70, PRO53, and ALA39 (Figure 1). These results agreed with previous studies where nymphaeol A-B and solophenol D exhibited antibacterial activity.^{58,59}

Four compounds including 6-isoprenyleriodictyol, nymphaeol A, nymphaeol B, and solophenol D exhibited high antibacterial activity against *S. mutans* antigen I/II (Agl/II) with binding affinities ranging from -8.7 to -8.9 kcal/mol. The interactions between the ligands and amino acid residues in the binding sites of these compounds are shown in 2D format (Figure 2). Ampicillin formed hydrogen bonds with four amino acid residues, ASN590, SER591, SER588, and ASP760, as well as π -sulfur and π - π interactions with TRP 816, while 6-isoprenyleriodictyol only formed hydrogen bonds with three amino acid residues: SER697, ASN699, and ASN814. Nymphaeol A and nymphaeol B formed hydrogen bonds with four amino acid residues (Figure 2). Nymphaeol A exhibited a similar π - π interaction with TRP816, as observed with ampicillin. Solophenol D formed hydrogen bonds with SER818, ARG824, and THR586, similar π - π interaction as ampicillin with TRP816), and π -sigma interaction with SER762. The compound 6-isoprenyleriodictyol is a prenylated flavonoid similar to nymphaeol A, and hence may have antibacterial activity.

Two compounds, including 6-isoprenyleriodictyol and nymphaeol A, displayed stronger binding affinities than ampicillin, with values of -8.0 and -8.4 kcal/mol against *P. acnes* surface sialidases. Therefore, both compounds were visualized in 2D format to investigate the interactions between the ligands and the amino acid residues in the protein binding sites (Figure 3). Ampicillin formed hydrogen bonds with four amino acid residues, ARG282, GLN287, ASP146, ASP185, and π - π and π -alkyl interactions with PHE257, ALA147, and VAL202; 6-Isoprenyleriodictyol demonstrated π - π interactions with PHE257, π -anion interactions with ASP146, and alkyl-alkyl interactions with ALA147, PHE209, VAL202, and LEU224. This compound formed bonds with five amino acid residues, including ASP146, ARG329, TYR423, ARG282, and GLU287, that were analogous to those observed with ampicillin. Nymphaeol A formed hydrogen bonds with five amino acid residues (ARG329, TYR423, ASP185, ALA147, and SER184), π -anion interactions with ASP312 and ASP185, alkyl-alkyl interactions with MET310, ALA331, and ALA147, and Van der Waals interactions with PHE257. Thus, these compounds may have potent antibacterial activity.

Structure-activity relationships were also studied based on the binding affinity results (Table 2). Flavonoids possessing prenyl, geranyl, and hydroxyl groups showed potential as antibacterial agents due to their strong affinity for amino acid residues in the binding sites of the proteins, compared to ampicillin. However, the absence of a hydroxyl group on prenylated or geranylated flavonoids decreased the binding affinity.^{56,60}

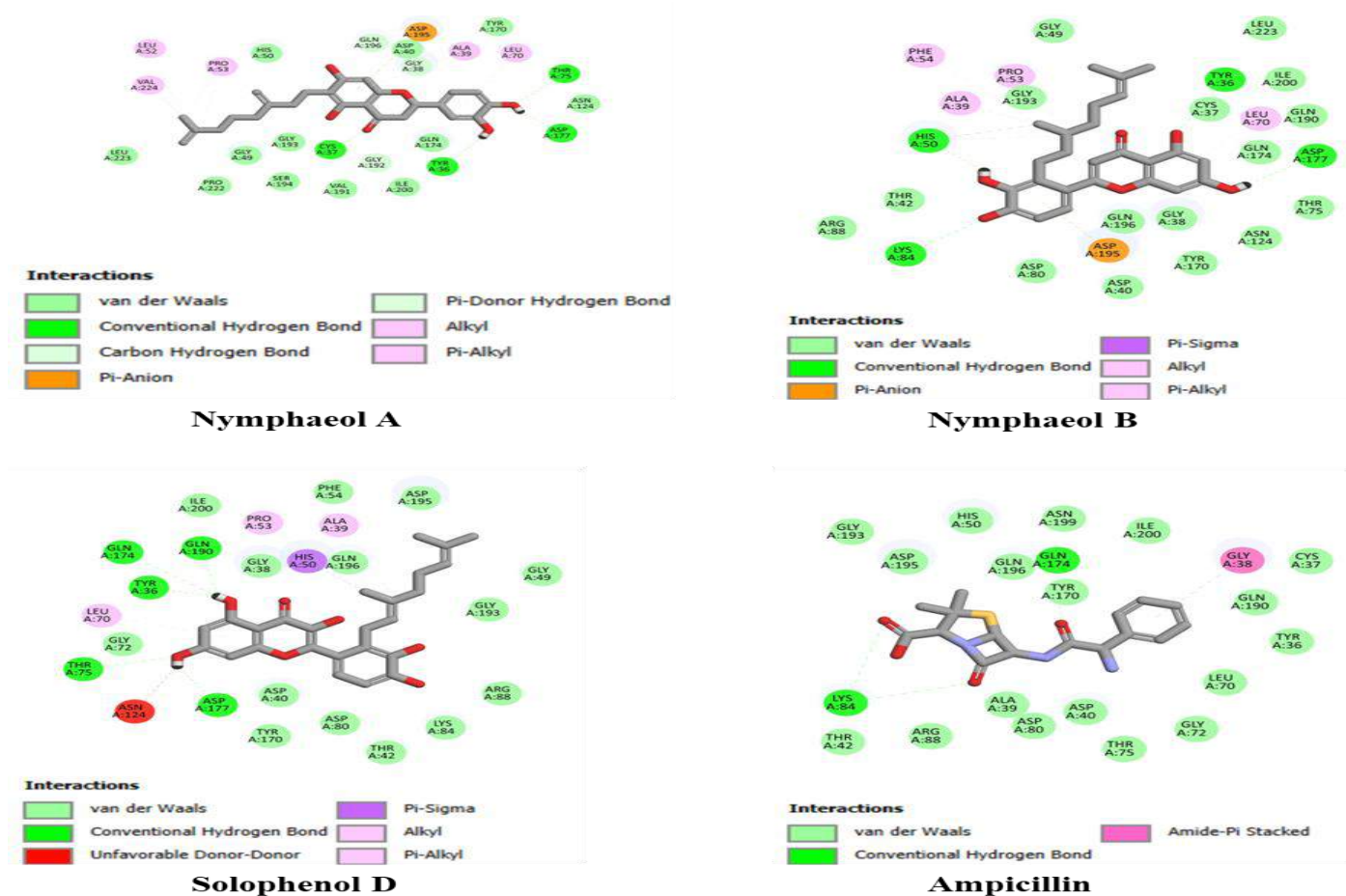


Figure 1: 2D visualization of interaction of the best docked flavonoids and ampicillin with *Staphylococcus aureus* tyrosyl-tRNA synthetase (PDB ID: 1JIJ)

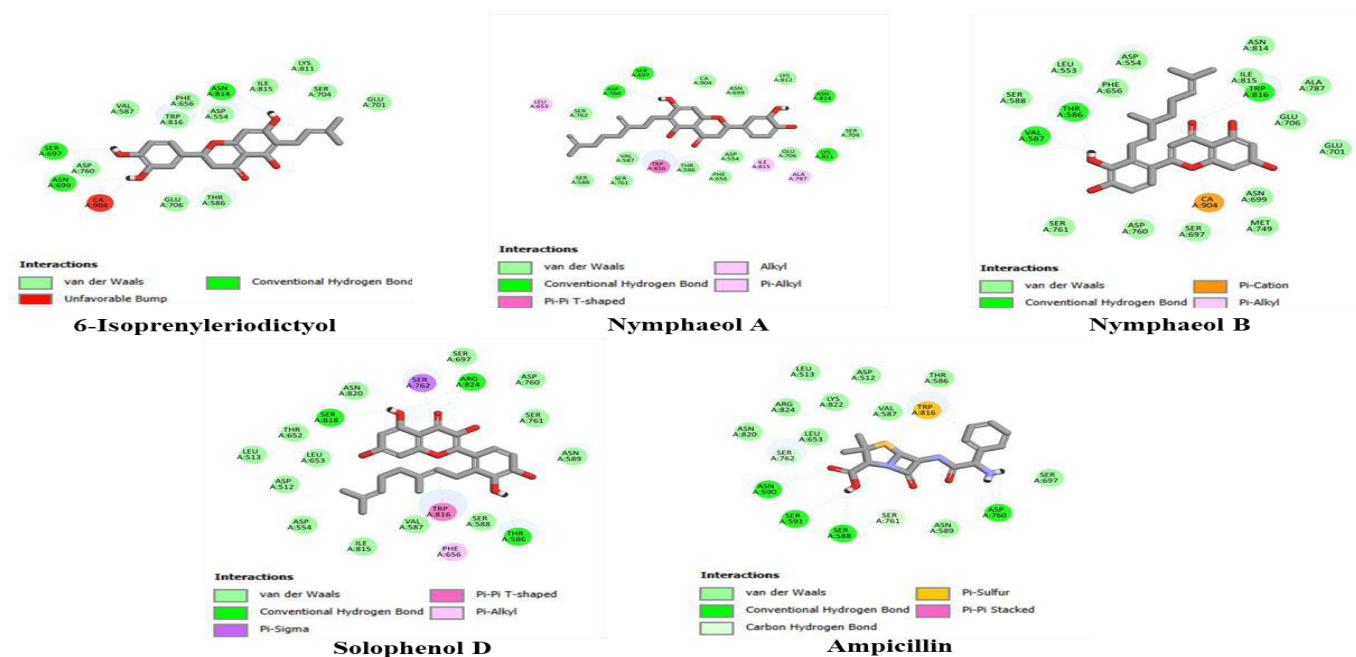


Figure 2: 2D visualization of interaction of the best-docked flavonoids and ampicillin with *Streptococcus mutans* antigen I/II (PDB ID: 3IPK).

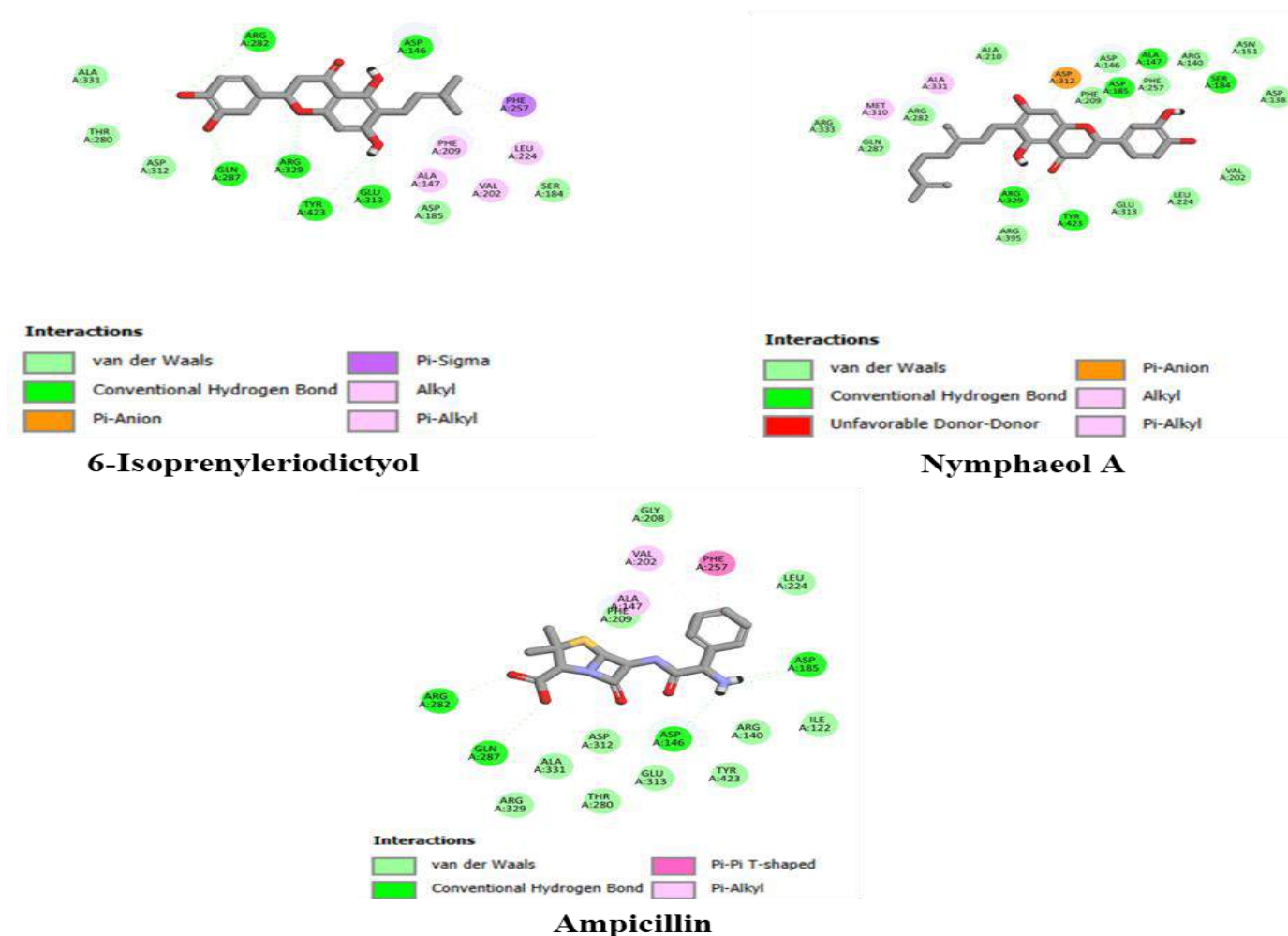


Figure 3: 2D visualization of interaction of the best-docked flavonoids and ampicillin with *Propionibacterium acnes* surface sialidase (PDB ID: 7LBU)

Conclusion

The extract and fractions of *Macaranga hullettii* leaves have potential as antibacterial agents against three bacterial strains, *Staphylococcus aureus* ATCC 25923, *Streptococcus mutans* ATCC 25175, and *Propionibacterium acnes* KCCM 41747. The methanol extract demonstrated antibacterial activity against *S. aureus* and *S. mutans* with MIC of < 0.15% against *S. aureus* and *S. mutans*, and 0.15-0.31% against *P. acnes*. The n-hexane fraction had an MIC of 1.25-2.5% against *S. aureus*, and 0.62-1.25% against *S. mutans* and *P. acnes*. The ethyl acetate fraction had an MIC < 0.15% against the three bacterial strains. Molecular docking studies suggested that 6-isoprenyleriodictyol, nymphaeol A, nymphaeol B, and solophenol D are promising antibacterial agents, as these compounds exhibited the highest binding affinity and formed hydrogen bonds with key amino acid residues in the active sites of the protein targets. Further *in vitro* and *in vivo* studies should be conducted by isolating the active secondary metabolites from this species.

Conflict of Interest

Authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgement

This research was funded by the Islamic Development Bank (PIU-IsDB) project under contract no. 137/UN17.11/PL/2019.

References

- Wong F, de la Fuente-Nunez C, Collins JJ. Leveraging artificial intelligence in the fight against infectious diseases. *Science*. 2023; 381(6654):164-170.
- Seukep AJ, Kuete V, Nahar L, Sarker SD, Guo M. Plant-derived secondary metabolites as the main source of efflux pump inhibitors and methods for identification. *J Pharm Anal*. 2020; 10(4):277-290.
- Magadula JJ. Phytochemistry and pharmacology of the genus *Macaranga*: a review. *J Med Plants Res*. 2014; 8(12):489-503.
- Vu LT, Ngan TB, Phuong L, Huong DT, Litaudon M, Van Hung N, Thach TD, Van Cuong P. Chemical constituents from fruits of *Macaranga denticulata* (Euphorbiaceae) (Part 2). *Vietnam J Chem*. 2018; 56(4):516-520.
- Marliana E, Hairani R, Tjahjandarie TS, Tanjung M. Antiplasmodial activity of flavonoids from *Macaranga tanarius* leaves. *IOP Conf Ser Earth Environ Sci*. 2018; 144(1):012011.
- Tjahjandarie TS, Tanjung M, Saputri RD, Nadar PB,

- Aldin MF, Marlina E, Permadi A, Flavestin K, An isoprenylated stilbene from the leaves of *Macaranga recurvata* Gage. Nat Prod Sci. 2019; 25(3):244-247.
7. Huang DT, Linh NT, Van TT, Litaudon M, Roussi F, Van Nam V, Van Cuong P. Stilbenes from *Macaranga tanarius* (Euphorbiaceae) growing in Vietnam. Vietnam J Chem. 2020; 58(3):338-342.
 8. Aldin MF, Tjahjandarie TS, Saputri RD, Tanjung M. Macasiamenene V, a New Stilbenoid from the Leaves of *Macaranga inermis*. Nat Prod Sci. 2021; 27(1):45-48.
 9. Muharram A, Rachmawati DA, Mardhiyyah S, Tjahjandarie TS, Saputri RD, Ahmat N, Tanjung M. Cytotoxic and antioxidant activities of flavonoids and diterpenoids from *Macaranga involucrata* (Roxb.) Baill. J Appl Pharm Sci. 2023; 13(6):087-92.
 10. Tanjung M, Tjahjandarie TS, Aldin MF, Mardhiyyah S, Ahmat N, Saputri RD. Macagigantin A, A New Flavonoid from *Macaranga gigantea* (Rchb. f & Zoll.) Mull Arg Nat Prod Sci. 2023; 29(4):287-294.
 11. Tjahjandarie TS, Aldin MF, Saputri RD, Tanjung M. Dihydrostilbenes from *Macaranga javanica* (Blume) Müll. Arg. and their antiparasitic activity. Nat Prod Sci. 2024;1:7.
 12. Toko EG, Tchapo CED, Tsamo AT, Kemzeu R, Wang Y, Fekam FB, Ndinteh DT, Choudhary MI, Nkengfack EA, Mmutlane EM, Mkounga P. Three New Polyphenol Derivatives from the Fruits of *Macaranga Monandra* and their Antioxidant Potential. Chem. Biodivers. 2024;21(7):e202301816.
 13. Tanjung M, Tjahjandarie TS, Aldin MF, Mardhiyyah S, Saputri RD, Syah YM, Ahmat N. Two new flavonols from *Macaranga inermis* pax & K. Hoffm. Nat Prod Sci. 2025; 39(3):498-505.
 14. Marlina E, Tjahjandarie TS, Tanjung M. Isoprenylated flavanone derivatives from *Macaranga hosei* King ex Hook F. Der Pharm Lett. 2015; 7(3):153-156.
 15. Marlina E, Tjahjandarie TS, Tanjung M. Antioxidant activity of flavonoids from *Macaranga pearsonii* Merr. J Kim Mulawarman. 2016; 13(2):97-100.
 16. Marlina E, Astuti W, Kosala K, Hairani R, Tjahjandarie TS, Tanjung M. Chemical composition and anticancer activity of *Macaranga hosei* leaves. Asian J Chem. 2018; 30(4):795-798.
 17. Marlina E, Ruga R, Hairani R, Tjahjandarie TS, Tanjung M. Antioxidant activity of flavonoid constituents from the leaves of *Macaranga tanarius*. IOP Conf Ser J Phys. 2019; 1277(1):012014.
 18. Saputri RD, Tukiran T, Wati FA, Purnamasari AP, Wardhana MW, Tjahjandarie TS, Tanjung M. Macahuilettiin A, a new isoprenylated flavanone from the leaves of *Macaranga hullettii* King ex Hook and their antiparasitic activity. Vietnam J Chem 2024; 62(3):394-398.
 19. Musdalifah M, Khumaidi A, Suwastika IN. Inhibition test and phytochemical screening of leaf extracts of *Macaranga tanarius* (L.) Mull. Arg against *Salmonella typhi* as an antibacterial. Nat Sci J Sci Technol. 2017; 6(3):9194.
 20. Ibrahim H, Omosa L, Nchiozem-Ngnitedem V, Onyari J, Maru S, Guefack M. Antibacterial activities and phytochemical screening of crude extracts from Kenyan macaranga species toward MDR phenotypes expressing efflux pumps. Pharmacogn Commun. 2021; 11(2):119-126.
 21. Bijesh K and Sebastian D. Isolation and characterization of antibacterial compounds from *Macaranga peltata* against clinical isolates of *Staphylococcus aureus*. Int J Biol Pharm Res. 2013; 4(12):1196-1203.
 22. Salleh WM, Razak NZ, Ahmad F. Phytochemicals and biological activities of *Macaranga hosei* and *Macaranga constricta* (Euphorbiaceae). Marmara Pharm J. 2017; 21(4):881-888.
 23. Slik JW, Priyono P, Welzen PV. Key to the *Macaranga Thou.* and *Mallotus Lour.* species (Euphorbiaceae) of East Kalimantan, Indonesia. Singapore, Gardens' Bulletin (Singapore), National Parks Board, Singapore Botanic Gardens; 2000. 11-87 p.
 24. Amirta R, Angi EM, Ramadhan R, Kusuma IW, Wiati CB, Haqiqi MT. Potential utilization of macaranga. Samarinda: Mulawarman University Press; 2017.
 25. Rismawati R, Marlina E, Daniel D. Phytochemical Test on Methanol Extract of Leaf of *Macaranga hullettii* King ex Hook. f. J Atomik. 2018; 3(2):91-94.
 26. Hudzicki J. Kirby-Bauer disk diffusion susceptibility test protocol. Am Soc Microbiol. 2009; 15(1):1-23.
 27. Inna M, Astuti W, Saleh C. Antibacterial activity of methanolic extract of uric patch plant leaves (*Cayratia carnososa*) against *Salmonella typhi* and *Propionibacterium acnes*. J Atomik. 2022; 7(1):1-5.
 28. Yulianti MF, Amat AL, Hutasoit RM, Pakan PD. Antibacterial activity of jambang leaf ethanol extract (*Syzygium cumini*) against the growth of *Propionibacterium acnes*. Acta Biochimica Indones. 2023; 6(2):1161.
 29. Hossain MR, Biplob AI, Sharif SR, Bhuiya AM, Sayem AS. Antibacterial Activity of Green Synthesized Silver Nanoparticles of *Lablab purpureus* Flowers Extract against Human Pathogenic Bacteria. Trop J Nat Prod Res. 2023;7(8):3647-3651
 30. Yanda L, Tatsimo SJ, Tamokou JD, Matsuet-Takongmo G, Meffo-Dongmo SC, Meli Lannang A, Sewald N. Antibacterial and antioxidant activities of isolated compounds from *Prosopis africana* leaves. Int J Anal Chem. 2022; 2022:4205823.
 31. Qiu X, Janson CA, Smith WW, Green SM, McDevitt P, Johanson K, Carter P, Hibbs M, Lewis C, Chalker A, Fosberry A. Crystal structure of *Staphylococcus aureus* tyrosyl-tRNA synthetase in complex with a class of potent and specific inhibitors. Protein Sci. 2001; 10(10):2008-2016.
 32. Larson MR, Rajashankar KR, Patel MH, Robinette RA, Crowley PJ, Michalek S, Brady LJ, Deivanayagam C. Elongated fibrillar structure of a *streptococcal adhesin* assembled by the high-affinity association of α - and PPII-helices. Proc Natl Acad Sci. 2010; 107(13):5983-5988.
 33. Yu AC, Volkens G, Jongkees SA, Worrall LJ, Withers SG, Strynadka NC. Crystal structure of the *Propionibacterium acnes* surface sialidase, a drug target for *P. acnes*-associated diseases. Glycobiol. 2022; 32(2):162-170.
 34. Jakubec D, Skoda P, Krivak R, Novotny M, Hoksza D. PrankWeb 3: accelerated ligand-binding site predictions for experimental and modelled protein structures. Nucl Acids Res. 2022; 50(W1):W593-W597.
 35. Dallakyan S and Olson AJ. Small-molecule library screening by docking with PyRx. Methods Mol Biol. 2014; 1263:243-250.
 36. Trott O and Olson AJ. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. J Comput Chem. 2010; 31(2):455-461.
 37. Malangu N. ed. Poisoning: From Specific Toxic Agents to Novel Rapid and Simplified Techniques for Analysis. BoD—Books on Demand; 2017.
 38. Sari AA and Saleh C. Phytochemical test, toxicity and antibacterial activity of extracts of various macaranga leaf fractions (*Macaranga tanarius* (L.) MA) against *Staphylococcus aureus* and *Escherichia coli*. J Kim Mulawarman. 2015; 12(2):53-58.
 39. Imelda R, Mariani M, Satari M. Effect of cassava (*Manihot esculenta* crantz), rice (*Oryza sativa* L.), and potato (*Solanum tuberosum*) water extract to decrease pH phase fermentation of *Streptococcus mutans* atcc 25175.

- Padjajaran J Dent. 2019; 31(1):14.
40. Alkhaled A, Alsabek L, Al-assaf M, Badr F. Effect of chlorhexidine, honey and propolis on *streptococcus mutans* counts: *in vitro* study. Dentistry. 2021; 9(1):a001.
 41. Ren Z, Chen L, Li J, Li Y. Inhibition of *Streptococcus mutans* polysaccharide synthesis by molecules targeting glycosyltransferase activity. J Oral Microbiol. 2016; 8(1):31095.
 42. Lee BS, Chen YJ, Wei TC, Ma TL, Chang CC. Comparison of antibacterial adhesion when salivary pellicle is coated on both poly (2-hydroxyethyl-methacrylate)-and polyethylene-glycol-methacrylate-grafted poly (methyl methacrylate). Int J Mol Sci. 2018; 19(9):2764.
 43. Chen KC, Yang CH, Li TT, Zouboulis CC, Huang YC. Suppression of *Propionibacterium acnes*-stimulated proinflammatory cytokines by Chinese bayberry extracts and its active constituent myricetin in human sebocytes *in vitro*. Phytother Res. 2019; 33(4):1104-1113.
 44. Xu J, Chen X, Song J, Wang C, Xu W, Tan H, Suo H. Antibacterial activity and mechanism of cell-free supernatants of *Lactocaseibacillus paracasei* against *Propionibacterium acnes*. Microb. Pathog. 2024; 189:106598.
 45. Warnida H, Mustika D, Supomo S, Sukawaty Y. Effectiveness of Mahang Leaf Ethanol Extract (*Macaranga Triloba*) as an anti-acne. J Penelit. Sos. Ekon. Kehuta. 2018; 4(1):9-18.
 46. Schütz BA, Wright AD, Rali T, Sticher O. Prenylated flavanones from leaves of *Macaranga pleiostemona*. Phytochem. 1995; 40(4):1273-1277.
 47. Lim TY, Lim YY, Yule CM. Evaluation of antioxidant, antibacterial and anti-tyrosinase activities of four *Macaranga* species. Food Chem. 2009; 114(2):594-599.
 48. Fareza MS, Syah YM, Mujahidin D, Juliawaty LD, Kurniasih I. Antibacterial flavanones and dihydrochalcones from *Macaranga trichocarpa*. Z. Naturforsch C J Biosci. 2014; 69(9-10):375-380.
 49. Hasanat A, Kabir MS, Hossain MM, Hasan M, Al Masum MA, Chowdhury TA, Bhuiyan DI, Mamur A, Kibria AS. Antibacterial activity of methanol extract of *Macaranga denticulata* leaves and *in silico* PASS prediction for its six secondary metabolites. World J Pharm Pharm. Sci. 2015:1258-1266.
 50. O Akanbi B, Anene P, Olayanju S. Preliminary Screening Indicates Promising Antimicrobial Properties of the Stem Bark Extracts of *Macaranga rosea*. Anti-Infective Agents. 2015; 13(2):123-128.
 51. Ogundajo A, Okeleye B, Ashafa AO. Chemical constituents, *in vitro* antimicrobial and cytotoxic potentials of the extracts from *Macaranga barteri* Mull-Arg. Asian Pac J Trop Biomed. 2017; 7(7):654-659.
 52. Putri R, Hendra R, Teruna HY. Anti-Bacterial and Anti-Fungal Activities from *Macaranga bancana* Leaves Extract. Pharmacol Clin Pharm Res. 2019; 4(1):1-4.
 53. Lee JH, Kim YG, Khadke SK, Yamano A, Woo JT, Lee J. Antimicrobial and antibiofilm activities of prenylated flavanones from *Macaranga tanarius*. Phytomed. 2019; 63:153033.
 54. Pagna JI, Awazi T, Mbarga PE, Mbekou IM, Mkounga P, Fotie J, Frese M, Fabrice FB, Lenta BN, Sewald N, Nkengfack EA. Antibacterial flavonoids from the fruits of *Macaranga hurifolia*. J Asian Nat Prod Res. 2022; 24(11):1041-1051.
 55. Kamsi VF, Simo Fotso CC, Kanko Mbekou IM, Toussie BT, Ndjakou Lenta B, Boyom FF, Sewald N, Frese M, Ngadjui BT, Wabo Fotso G. Chemical constituents of *Macaranga occidentalis*, antimicrobial and chemophenetic studies. Molecules. 2022; 27(24):8820.
 56. Rosamah E, Haqiqi MT, Putri AS, Kuspradini H, Kusuma IW, Amirta R, Yuliansyah Y, Suwinarti W, Paramita S, Ramadhan R, Tarmadi D. The potential of *Macaranga* plants as skincare cosmetic ingredients: A review. J Appl Pharm Sci. 2023; 13(7):001-12.
 57. Li AP, He YH, Zhang SY, Shi YP. Antibacterial activity and action mechanism of flavonoids against phytopathogenic bacteria. Pestic Biochem Physiol. 2022; 188:105221.
 58. Chen YW, Ye SR, Ting C, Yu YH. Antibacterial activity of propolis from Taiwanese green propolis. J Food Drug Anal. 2018; 26(2):761-768.
 59. Inui S, Hosoya T, Shimamura Y, Masuda S, Ogawa T, Kobayashi H, Shirafuji K, Moli RT, Kozono I, Shin-ya K, Kumazawa S. Solophenols B-D and Solomonin: New Prenylated Polyphenols Isolated from Propolis Collected from The Solomon Islands and Their Antibacterial Activity. J Agric Food Chem. 2012; 60(47):11765-11770.
 60. Ruga R, Kingkaew K, Tamsampaoloet K, Chavasiri W. Enhancing antibacterial activity against *Propionibacterium acnes* and *Staphylococcus aureus* by combination of tetracycline with selected compounds. Chem Lett. 2018; 47(12):1538-1541.



Tropical Journal of Natural Product Research

Available online at <https://www.tjnpr.org>

Original Research Article

In vitro Evaluation and Molecular Docking Study of the Antibacterial Potential of *Macaranga hullettii* King ex Hook.f. Leaf Extract

Eva Marlina^{1,3}, Ade Danova^{2*}, Rita Hairani^{1,3}, Ritbey Ruga^{1,3}, Elvira Hermawati², Ana Yulvia⁴¹Department of Chemistry, Faculty of Mathematics and Natural Sciences, Mulawarman University, Samarinda 75123, East Kalimantan, Indonesia²Organic Chemistry Division, Department of Chemistry, Faculty of Mathematics and Natural Sciences, Institut Teknologi Bandung Jl. Ganesha No. 10, Bandung 70132, West Java, Indonesia³Research Center for Medicine and Cosmetics from Tropical Rainforest Resources, Mulawarman University, Samarinda, Indonesia⁴Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Jember, East Java, Indonesia

ARTICLE INFO

Article history:

Received 26 April 2025

Revised 27 May 2025

Accepted 01 June 2025

Published online 01 August 2025

ABSTRACT

Due to the resistance of bacteria to antibiotics, there has been increasing interest in the use of plant extracts to combat infections. Plants in the genus *Macaranga* contain secondary metabolites such as flavonoids with antibacterial properties. *Macaranga hullettii* King ex Hook.f. are particularly noteworthy due to their widespread distribution in East Kalimantan. This study aimed to investigate the antibacterial potential of *M. hullettii* leaf extract through *in vitro* and molecular docking studies. The antibacterial activity of the methanol extract, n-hexane, and ethyl acetate fractions from *M. hullettii* leaves was evaluated against three bacterial species, *Staphylococcus aureus*, *Streptococcus mutans*, and *Propionibacterium acnes* using the disc diffusion assay. Molecular docking of eleven flavonoid derivatives presents in four *Macaranga* species was performed against selected bacterial proteins (PDB ID: IJII, 3IPK, and 7LBU). The results showed that both the methanol extract and ethyl acetate fraction displayed antibacterial activity against the three bacteria strains, with minimum inhibitory concentrations (MICs) < 0.15%, except for the methanol extract, which had MIC of 0.15-0.31% against *Propionibacterium acnes*. In addition, molecular docking study showed that four flavonoids possessing prenyl or geranyl groups (6-isoprenyleriodictyol, nymphaeol A, nymphaeol B, and solophenol D) showed the highest binding affinity and dominant hydrogen bonding interactions with key amino acid residues in the binding sites of three bacterial proteins. The findings suggest that the methanol extract and its ethyl acetate fraction from *Macaranga hullettii* leaves could be a potential source of new antibacterial agents. Further studies are needed to isolate and evaluate the bioactive compounds.

Copyright: © 2025 Danova *et al.* This is an open-access article distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Keywords: Antibacterial, Extract, Flavonoid, *Macaranga hullettii*, Molecular Docking

Introduction

Despite substantial advancements in medicine and fundamental research, infectious diseases caused by transmissible agents such as bacteria, protists, and viruses continue to present significant challenges to healthcare professionals. These challenges include the issue of antimicrobial resistance.¹ Extracts from medicinal plants present a promising approach to addressing multidrug-resistant bacteria. In addition, medicinal plants can augment the efficacy of antibiotics for the treatment of infectious diseases.² Therefore, it is imperative to develop more efficacious antimicrobial agents, particularly those sourced from natural sources, as these are both readily available and economically viable. Plants in the genus *Macaranga* belong to the family Euphorbiaceae and are commonly known as "Mahang." The secondary metabolites contained in plants of this genus include flavonoids and stilbenoids with prenylated substituents.³⁻¹³

*Corresponding author. Email: adedanova@itb.ac.id,
Tel: +6281357944698

Citation: Marlina E, Danova A, Hairani R, Ruga R, Hermawati E, Yulvia A. *In Vitro* Evaluation and Molecular Docking Study of the Antibacterial Potential of *Macaranga hullettii* King ex Hook.f. Leaf Extract. Trop J Nat Prod Res. 2025; 9(7): 3050 – 3057 <https://doi.org/10.26538/tjnpr/v9i7.15>

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

Nine flavonoids containing either prenyl or geranyl groups and displaying antioxidant, antiparasitic, and anticancer activities have been isolated from three species (*Macaranga hoesi*, *Macaranga pearsonii*, and *Macaranga tanarius*) of *Macaranga* present in East Kalimantan: 4'-O-methyl-8-isoprenylnaringenin, 4'-O-methyl-8-isoprenyleriodictyol, 6-isoprenyleriodictyol, solophenol D, nymphaeol A, and nymphaeol B, including lonchocarpol A, nymphaeol C, and macahulletin A that was isolated from *Macaranga hullettii* obtained from Central Kalimantan.¹⁴⁻¹⁸ *Macaranga tanarius* leaf extract has been shown to exhibit antibacterial activity.¹⁹ The extracts of *Macaranga conglomerata*, *Macaranga kilimandscharica*, and *Macaranga capensis* from Kenya has shown antibacterial activity against *E. coli*, *E. aerogenes*, *K. pneumoniae*, *P. stuartii*, *P. aeruginosa*, and *S. aureus*.²⁰ The flavonol rhamnetin, isolated from *Macaranga peltata*, also showed antibacterial activity against *Staphylococcus aureus*.²¹ Two flavanone groups; 5-hydroxy-7,4'-dimethoxyflavone and 5-hydroxy-6,7,4'-trimethoxyflavone obtained from *Macaranga hoesi* showed weak antibacterial activity against three pathogenic bacteria.²² In this study, the leaves of *Macaranga hullettii* King ex Hook.f. were identified as potential new source of medicinal compounds against bacterial infection. This plant is found in all areas of East Kalimantan, where the species commonly occurs in mountain forests and logging areas in the Kalimantan region.^{23,24} Phytochemical screening and isolation of secondary metabolites from the leaf extract of *Macaranga hullettii* identified several flavonoid compounds.^{18,25} The methanol extract and its fractions derived from *Macaranga hullettii* have not yet been analyzed for their antibacterial activity. Therefore, this study aimed to assess the antibacterial activity of *Macaranga hullettii* leaf extract and its fractions using *in vitro* studies. The flavonoids isolated from

Macaranga species in East Kalimantan and Central Kalimantan (Indonesia) as well as Trengganu (Malaysia) were evaluated for their antibacterial activity using molecular docking analysis.

Materials and Methods

Chemicals and equipment

The solvents used (methanol, n-hexane, and ethyl acetate) were purified by distillation before use, nutrient agar (NA) from Merck, Luria Bertani liquid medium (1% tryptone, 0.5% yeast, and 0.5% NaCl), yeast extract (Oxoid 100% (w/w)), tryptone (Oxoid 100% (w/w)), ampicillin 100 mg/ml (Sigma Aldrich), NaCl (Sigma Aldrich), cotton swabs, aluminum foil, gauze, Petri dishes, Ose needles, paper discs, an autoclave (Autoclave TOMY, Model SX-700), an oven (SHARP EO-28 WH), a rotary vacuum evaporator (EYELA Rotary evaporator N-1300E V.S.Series), and a freezer (Sanyo Medicoool). For molecular docking analysis, we used a Lenovo Yoga 7 computer with AMD Ryzen AI 7-8840HS w/ Radeon 780M graphics, 3301 Mhz, 8 Core(s), and 16.0 GB RAM.

Plant collection and identification

Fresh leaves of *Macaranga hullettii* were collected from the Lesan River Forest, Kelay District, Berau Regency, East Kalimantan, Indonesia (1°32'2.5919" N, 117°3'11.950" E). The plant samples were identified at the Laboratory of Anatomy and Plant Systematics, FMIPA Mulawarman University where voucher No. 0174/UN.17.8.5.7.16/HA/XI/2017 was assigned.

Extraction and fractionation

Macaranga hullettii leaf powder (1.3 kg) was extracted by maceration in methanol (2 x 7.0 L) at room temperature for 24 h. The extract was filtered, and filtrate was evaporated *in vacuo* using a rotary vacuum evaporator to obtain the crude methanol extract (126 g). The crude methanol extract (100 g) was dissolved in 500 mL of methanol-water mixture (4:1), and then successively fractionated with 5 x 200 mL each of n-hexane and ethyl acetate to obtain n-hexane fraction (28 g) and ethyl acetate fraction (18 g), respectively. Each fraction was evaporated using a rotary vacuum evaporator. The methanol extract, n-hexane fraction, and ethyl acetate fraction were evaluated for antibacterial activity.

Test organisms

Staphylococcus aureus ATCC 25923 was obtained from the Department of Microbiology, Faculty of Science, Chulalongkorn University. *Streptococcus mutans* ATCC 25175 was obtained from the Department of Biochemistry, Faculty of Dentistry, Chulalongkorn University. Both strains were purchased from the American Type Culture Collection (ATCC). *Propionibacterium acnes* KCCM 41747 was obtained from the Faculty of Forestry, Mulawarman University, and from the Korean Culture Center of Microorganisms (KCCM). The bacteria were periodically sub-cultured and maintained in nutrient agar (NA) under suitable conditions.

In vitro antibacterial assay

The antibacterial activity of the methanol extract, n-hexane, and ethyl acetate fractions of *Macaranga hullettii* leaves was evaluated using the disc diffusion or the Kirby-Bauer method.^{26,27} Nutrient Agar (NA) medium was prepared by dissolving 3.3 g in 150 mL of distilled water. Luria Bertani (LB) liquid medium was prepared by dissolving 4 g in 500 mL of distilled water, stirring, and adding 5 mL to a test tube. The NA and LB media were sterilized in an autoclave at 121°C with a pressure of 1 atm for 15 minutes. Each test bacterium was collected using an Ose needle, followed by dilution in a test tube containing sterile LB media and incubated at 37 °C for 24 h.

Nutrient Agar (15 mL) was poured into sterilized Petri dishes and maintained at room temperature for solidification. A sample of the bacterial suspension in the test tubes was then collected using a sterile cotton swab and applied to the surface of the agar in the Petri dish. Each 30 µL of the test *M. hullettii* leaf samples prepared in various concentrations in the organic solvents (methanol, n-hexane, and ethyl acetate) was poured onto sterile adsorbent filter paper discs (6 mm in

diameter), and the solvent was removed. Afterward, the paper discs containing the test sample were placed on the surface of the agar media and incubated at 37 °C for 18-24 hours. The inhibition zone formed in each well was measured using a ruler.^{28,29} The minimum inhibitory concentration (MIC) was determined for the methanol extract as well as the n-hexane and ethyl acetate fractions. MIC was defined as the minimum concentration that completely inhibited bacterial growth.³⁰ Ampicillin was used as a positive control, and the three organic solvents were used as negative controls.

Molecular docking

Molecular docking was conducted to eleven (11) flavonoid derivatives possessing either prenyl or geranyl group presented in the three *Macaranga* species in East Kalimantan, Indonesia (*Macaranga hosei*, *Macaranga pearsonii*, and *Macaranga tanarius*), *Macaranga hullettii* in Central Kalimantan (Indonesia), and *Macaranga hosei* in Trengganu (Malaysia): 4'-O-methyl-8-isoprenylnaringenin, 4'-O-methyl-8-isoprenyleriodictyol, 6-isoprenyleriodictyol, 5-hydroxy-7,4'-dimethoxyflavone, lonchocarpol A, 5-hydroxy-6,7,4'-trimethoxyflavone, Macahulleitii A, nymphaeol A, nymphaeol B, nymphaeol C, and solophenol D,^{14-18,22} were used for the study. Macahulleitii A, together with lonchocarpol A and nymphaeol C, are a prenylated flavonoid found in *Macaranga hullettii* obtained from Central Kalimantan (Indonesia).¹⁸ A comparative analysis of the molecular docking results for prenylated/geranylated flavonoids with methoxylated flavonoids was conducted, focusing on 5-hydroxy-7,4'-dimethoxyflavone and 5-hydroxy-6,7,4'-trimethoxyflavone that are found in *Macaranga hosei* from Trengganu, Malaysia.²²

The molecular structures of the flavonoids were drawn using ChemOffice Professional 15.0 and obtained from PubChem (<https://pubchem.ncbi.nlm.nih.gov/>). Structure refinement was performed using the molecular mechanics energy minimization method with the Merck molecular force field (MMFF94) using Open Babel in PyRx V.1.1. Three bacterial proteins, including *Staphylococcus aureus* tyrosyl-tRNA synthetase (PDB ID: 1JIJ), *Streptococcus mutans* antigen I/II (Agl/II) (PDB ID: 3IPK), and *Propionibacterium acnes* surface sialidase (PDB ID: 7LBU) were downloaded from the protein data bank,³¹⁻³³ and the locations of active sites were calculated using AlphaFold from <https://prankweb.cz/>.³⁴ Molecular docking was performed using the AutoDock Vina tool in PyRx V.1.1 software with an exhaustiveness of 32 and a mode value of 9 poses for each docked ligand.^{35,36} A protein binding site was defined as a box with dimensions of 30 × 30 × 30 Å. In the final step, the binding interactions were analyzed, and the docking results were visualized in 2D using the BIOVIA Discovery Studio Visualizer.

Statistical analysis

Data from the experiments were analyzed using Microsoft Excel.

Results and Discussion

In vitro antibacterial activity

The strength of antibacterial activity of the test samples was determined based on the diameter of the clear zone of inhibition (mm). The antibacterial activity was classified as follows: ≤ 5 mm (weak), 5 - 10 mm (moderate), 10 - 20 mm (strong), and ≥ 20 mm (very strong). Moreover, the lower the minimum inhibitory concentration of a compound, the greater its ability to inhibit the growth of test bacteria.²⁸ In this study, ampicillin was used as a positive control against three bacteria: *Staphylococcus aureus* ATCC 25923, *Streptococcus mutans* ATCC 25175, and *Propionibacterium acnes* KCCM 41747 (Table 1). The positive control served to determine whether the test bacteria could be inhibited by or were resistant to the antibiotics.

Staphylococcus aureus can cause dentoalveolar infections, jaw cysts, paroritis, oral mucosal lesions, and stomatitis. The bacteria can also produce exotoxins, lecosidine, enterotoxins, and coagulase enzyme. Enterotoxins are compounds that cause food poisoning in humans.³⁷ The methanol extract, hexane fraction, and ethyl acetate fraction of *Macaranga hullettii* leaves showed moderate antibacterial activity against *S. aureus*, with an inhibition zone diameter of 5 - 10 mm at concentrations of 0.15 to 2.5% (Table 1).

Table 1: Antibacterial activity of *Macaranga hullettii* King ex Hook leaf extracts and fractions against *Staphylococcus aureus*, *Streptococcus mutans*, and *Propionibacterium acnes*

Sample	Concentration (%)	Inhibition Zone Diameter (mm)*		
		<i>Staphylococcus aureus</i>	<i>Streptococcus mutans</i>	<i>Propionibacterium acnes</i>
Methanol Extract	0	0	0	0
	0.15	6.67 ± 0.58	6.67 ± 0.58	0
	0.31	6.67 ± 0.58	6.67 ± 0.58	7.67 ± 0.58
	0.62	6.67 ± 0.58	7.0 ± 0.0	8.0 ± 1.0
	1.25	7.17 ± 0.29	7.33 ± 0.58	9.67 ± 0.58
	2.5	7.5 ± 0.5	8.0 ± 1.0	10.67 ± 0.58
	MIC (%)	<0.15	<0.15	0.15-0.31
n-Hexane Fraction	0	0	0	0
	0.15	0	0	0
	0.31	0	0	0
	0.62	0	0	0
	1.25	0	6.33 ± 0.29	6.33 ± 0.58
	2.5	6.5 ± 0.5	6.67 ± 0.58	6.67 ± 0.58
	MIC (%)	1.25-2.5	0.62-1.25	0.62-1.25
Ethyl acetate Fraction	0	0	0	0
	0.15	6.33 ± 0.58	6.33 ± 0.58	6.67 ± 0.58
	0.31	6.5 ± 0.5	6.67 ± 0.58	8.0 ± 0.0
	0.62	6.5 ± 0.5	6.83 ± 0.29	9.0 ± 0.0
	1.25	6.67 ± 0.29	7.0 ± 0.0	11.0 ± 1.0
	2.5	7.0 ± 0.0	7.67 ± 0.58	14.67 ± 0.58
	MIC (%)	<0.15	<0.15	<0.15
Ampicillin	0.01	20.33 ± 1.15	21.0 ± 1.0	21.0 ± 1.0

*Data were obtained from triplicate experiments.

The inhibition zone diameter of the 0.15% methanol extract was 6.67 mm. The 0.15% ethyl acetate fraction produced an inhibition zone diameter of 6.33 mm, and the 2.5% n-hexane fraction produced an inhibition zone diameter of 6.50 mm. The results indicated that the methanol extract and ethyl acetate fraction had the most potent antibacterial activity against *S. aureus*, with a minimum inhibitory concentration (MIC) of < 0.15%. Moreover, the study of Sari and Saleh (2015) showed that ethyl acetate fraction of *Macaranga tanarius* was most effective against *S. aureus*, with MIC values of 0.125-0.5% and an inhibition zone of approximately 7.25 mm.³⁸

Streptococcus mutans is implicated in the pathogenesis of dental caries. This facultative anaerobe primarily ferments carbohydrates, resulting in the production of several organic acids, with lactic acid as the predominant byproduct. The acidification of the dental environment by *S. mutans* is a crucial factor contributing to enamel demineralization and the subsequent formation of carious lesions.^{39,40} The bacteria produce glucosyltransferase (GTF) which produces glucan, the compound that causes dental caries.⁴¹ *S. mutans* adheres to the salivary pellicle via its numerous receptors.⁴² The antibacterial activity of *Macaranga hullettii* King leaf extract and fractions was tested against *S. mutans*. Both extract and fractions displayed moderate antibacterial activity, with inhibition zone diameters of 5 - 10 mm at concentrations of 0.15 - 2.5%. The diameters of the inhibition zones produced by the methanol extract and ethyl acetate fraction were 6.67 and 6.33 mm, respectively at 0.15%. Meanwhile, the n-hexane fraction showed antibacterial activity at concentrations of 1.25 - 2.5%, with inhibition zone diameter ranging from 6.33 - 6.67 mm. These results demonstrated that the methanol extract and ethyl acetate fraction of *Macaranga hullettii* leaves produced the highest antibacterial activity against *S.*

mutans, with an MIC < 0.15% (Table 1). *Propionibacterium acnes* is a Gram-positive anaerobic bacterium that can induce unusual keratinization within the sebaceous glands of hair follicles and increase sebum production.⁴³ The enzyme is capable of hydrolyzing triglycerides (TG) present in sebum into free fatty acids (FFA) that may subsequently induce inflammation in and around the hair follicles.⁴⁴ The bacteria also produce hyaluronidase, protease, lecithinase, and neuraminidase, that can cause inflammation of the skin. *Macaranga triloba* ethanol extract has been shown to prevent the growth of *P. acnes*, resulting in an inhibition zone diameter of 5.54 mm at 20%.⁴⁵ As shown in Table 1, the methanol extract and ethyl acetate fraction of *Macaranga hullettii* showed potent antibacterial activity against *P. acnes*, with inhibition zone diameters of 10.67 and 14.67 mm, respectively. The n-hexane fraction exhibited moderate antibacterial activity, with inhibition zone diameter of 5 - 10 mm at 1.25 - 2.5%. At concentration of 0.31%, the methanol extract showed antibacterial activity with an inhibition zone diameter of 7.67 mm, whereas at 0.15% concentration, the ethyl acetate fraction showed antibacterial activity with inhibition zone diameter of 6.67 mm. The results demonstrated that the ethyl acetate fraction of *Macaranga hullettii* leaves presented the highest antibacterial activity against *P. acnes*, with a minimum inhibitory concentration (MIC) of < 0.15% (Table 1).

The methanol extract and ethyl acetate fraction of *Macaranga hullettii* leaves exhibited good antibacterial activity against the three bacterial strains. This finding was consistent with previous studies that attributed the antibacterial effects to the presence of flavonoids and their prenylated or geranylated forms in species of *Macaranga*. These included eucarestaflavanone A, bonanione A, macarangaflavanone A, macarangaflavanone B, macatrichocarpin A, propolin D (nymphaeol

B), senegalensin, schweinfurthin B, schweinfurthin O, and isomacaranganin.⁴⁶⁻⁵⁶ Flavonoids can damage bacterial cell walls and denature proteases, thereby disrupting bacterial metabolism.⁵⁷

Molecular docking result

Molecular docking was performed to predict the interactions of the flavonoids with the amino acid residues of three bacterial proteins: *Staphylococcus aureus* tyrosyl-tRNA synthetase, *Streptococcus mutans* antigen I/II (AgI/II), and *Propionibacterium acnes* surface sialidase.³¹⁻

³³ The molecular docking results (Table 2) revealed that 11 flavonoid derivatives had binding affinities ranging from -7.2 to -10.5 kcal/mol, -7.5 to -8.9 kcal/mol, and -7.1 to -8.9 kcal/mol against *Staphylococcus aureus* tyrosyl-tRNA synthetase, *Streptococcus mutans* antigen I/II (AgI/II), and *Propionibacterium acnes* surface sialidase, respectively. The interactions of flavonoids and ampicillin with the protein targets are shown in two-dimensional graphs in Figures 1-3.

Table 2: Binding affinity of flavonoids against bacterial proteins

No.	Compound	Binding Affinity (kcal/mol)		
		1JJJ	3IPK	7LBU
1	4'-O-methyl-8-isoprenylnaringenin	-7.4	-8.1	-7.7
2	4'-O-methyl-8-isoprenyleriodictyol	-7.2	-8.1	-7.6
3	6-Isoprenyleriodictyol	-7.7	-8.7	-8.0
4	5-hydroxy-7,4'-dimethoxyflavone	-8.6	-7.8	-7.1
5	5-hydroxy-6,7,4'-trimethoxyflavone	-8.3	-7.5	-7.3
6	Macahulettiin A	-7.6	-8.4	-7.1
7	Lonchocarpol A	-9.4	-7.8	-7.8
8	Nymphaeol A	-9.7	-8.9	-8.4
9	Nymphaeol B	-10.5	-8.9	-7.7
10	Nymphaeol C	-8.4	-8.3	-7.4
11	Solophenol D	-10.0	-8.7	-7.9
12	Ampicillin	-8.5	-7.7	-7.6

Among the compounds targeting *S. aureus* tyrosyl-tRNA synthetase, nymphaeol A, nymphaeol B, and solophenol D had the highest binding affinities, higher than that of the positive control ligand (ampicillin). Thus, the interactions of these compounds with the active site of the enzyme were modeled as a 2D structure (Figure 1). Ampicillin formed hydrogen bonds with LYS84 and GLN174 and amide- π interactions with GLY38 (Figure 1). Nymphaeol A and nymphaeol B formed hydrogen bonds as well as π -anions, and hydrophobic interactions with four amino acid residues. Nymphaeol B and ampicillin showed similar interactions with LYS84. In addition, solophenol D formed hydrogen bonds with TYR36, THR75, GLN190, ASP177, and GLN174, as well as hydrophobic interactions with HIS50, LEU70, PRO53, and ALA39 (Figure 1). These results agreed with previous studies where nymphaeol A-B and solophenol D exhibited antibacterial activity.^{58,59}

Four compounds including 6-isoprenyleriodictyol, nymphaeol A, nymphaeol B, and solophenol D exhibited high antibacterial activity against *S. mutans* antigen I/II (AgI/II) with binding affinities ranging from -8.7 to -8.9 kcal/mol. The interactions between the ligands and amino acid residues in the binding sites of these compounds are shown in 2D format (Figure 2). Ampicillin formed hydrogen bonds with four amino acid residues, ASN590, SER591, SER588, and ASP760, as well as π -sulfur and π - π interactions with TRP 816, while 6-isoprenyleriodictyol only formed hydrogen bonds with three amino acid residues: SER697, ASN699, and ASN814. Nymphaeol A and nymphaeol B formed hydrogen bonds with four amino acid residues (Figure 2). Nymphaeol A exhibited a similar π - π interaction with TRP816, as observed with ampicillin. Solophenol D formed hydrogen bonds with SER818, ARG824, and THR586, similar π - π interaction as ampicillin with TRP816), and π -sigma interaction with SER762. The

compound 6-isoprenyleriodictyol is a prenylated flavonoid similar to nymphaeol A, and hence may have antibacterial activity.

Two compounds, including 6-isoprenyleriodictyol and nymphaeol A, displayed stronger binding affinities than ampicillin, with values of -8.0 and -8.4 kcal/mol against *P. acnes* surface sialidases. Therefore, both compounds were visualized in 2D format to investigate the interactions between the ligands and the amino acid residues in the protein binding sites (Figure 3). Ampicillin formed hydrogen bonds with four amino acid residues, ARG282, GLN287, ASP146, ASP185, and π - π and π -alkyl interactions with PHE257, ALA147, and VAL202; 6-Isoprenyleriodictyol demonstrated π - π interactions with PHE257, π -anion interactions with ASP146, and alkyl-alkyl interactions with ALA147, PHE209, VAL202, and LEU224. This compound formed bonds with five amino acid residues, including ASP146, ARG329, TYR423, ARG282, and GLU287, that were analogous to those observed with ampicillin. Nymphaeol A formed hydrogen bonds with five amino acid residues (ARG329, TYR423, ASP185, ALA147, and SER184), π -anion interactions with ASP312 and ASP185, alkyl-alkyl interactions with MET310, ALA331, and ALA147, and Van der Waals interactions with PHE257. Thus, these compounds may have potent antibacterial activity.

Structure-activity relationships were also studied based on the binding affinity results (Table 2). Flavonoids possessing prenyl, geranyl, and hydroxyl groups showed potential as antibacterial agents due to their strong affinity for amino acid residues in the binding sites of the proteins, compared to ampicillin. However, the absence of a hydroxyl group on prenylated or geranylated flavonoids decreased the binding affinity.^{56,60}

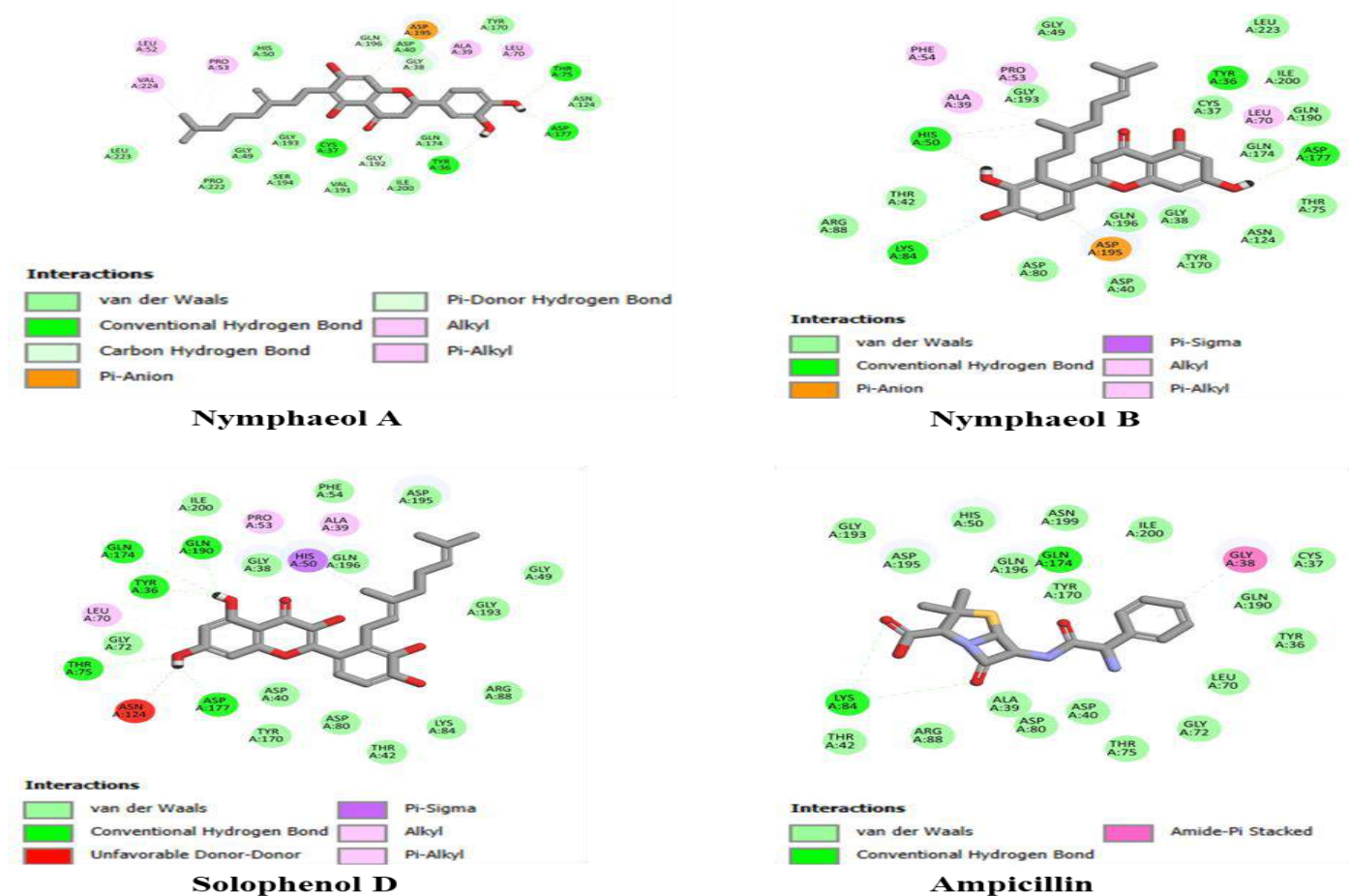


Figure 1: 2D visualization of interaction of the best docked flavonoids and ampicillin with *Staphylococcus aureus* tyrosyl-tRNA synthetase (PDB ID: 1JIJ)

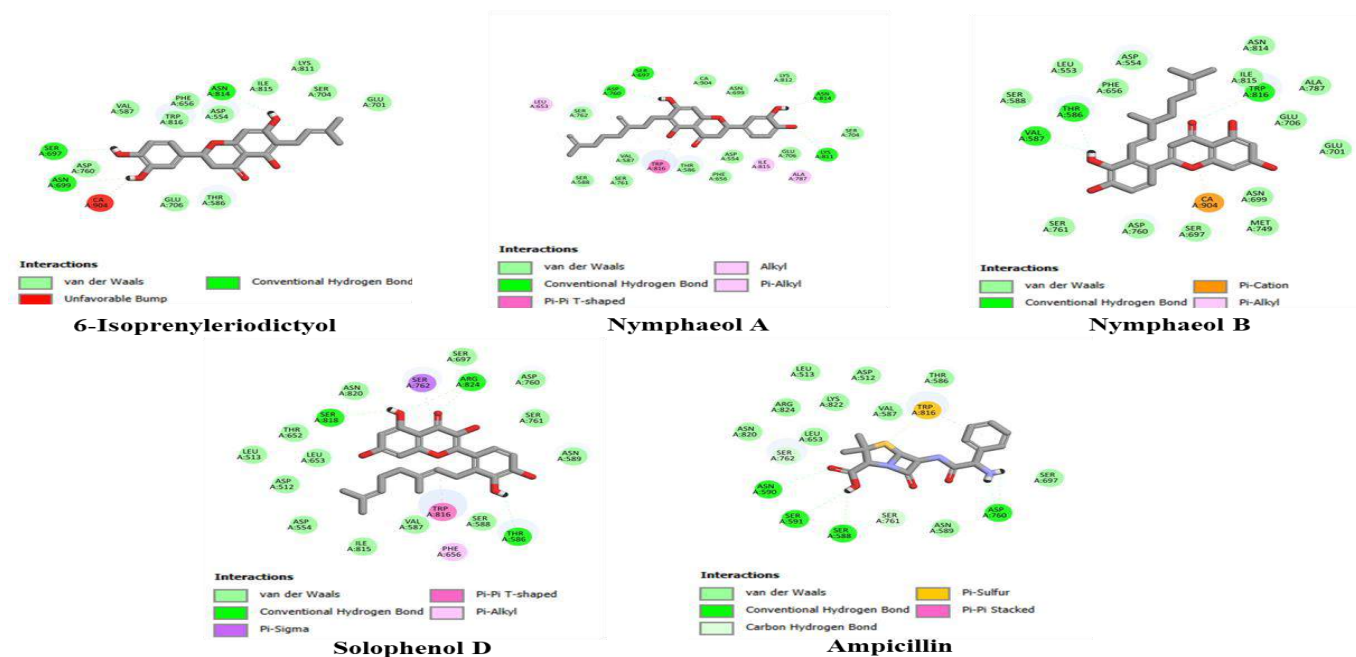


Figure 2: 2D visualization of interaction of the best-docked flavonoids and ampicillin with *Streptococcus mutans* antigen I/II (PDB ID: 3IPK).

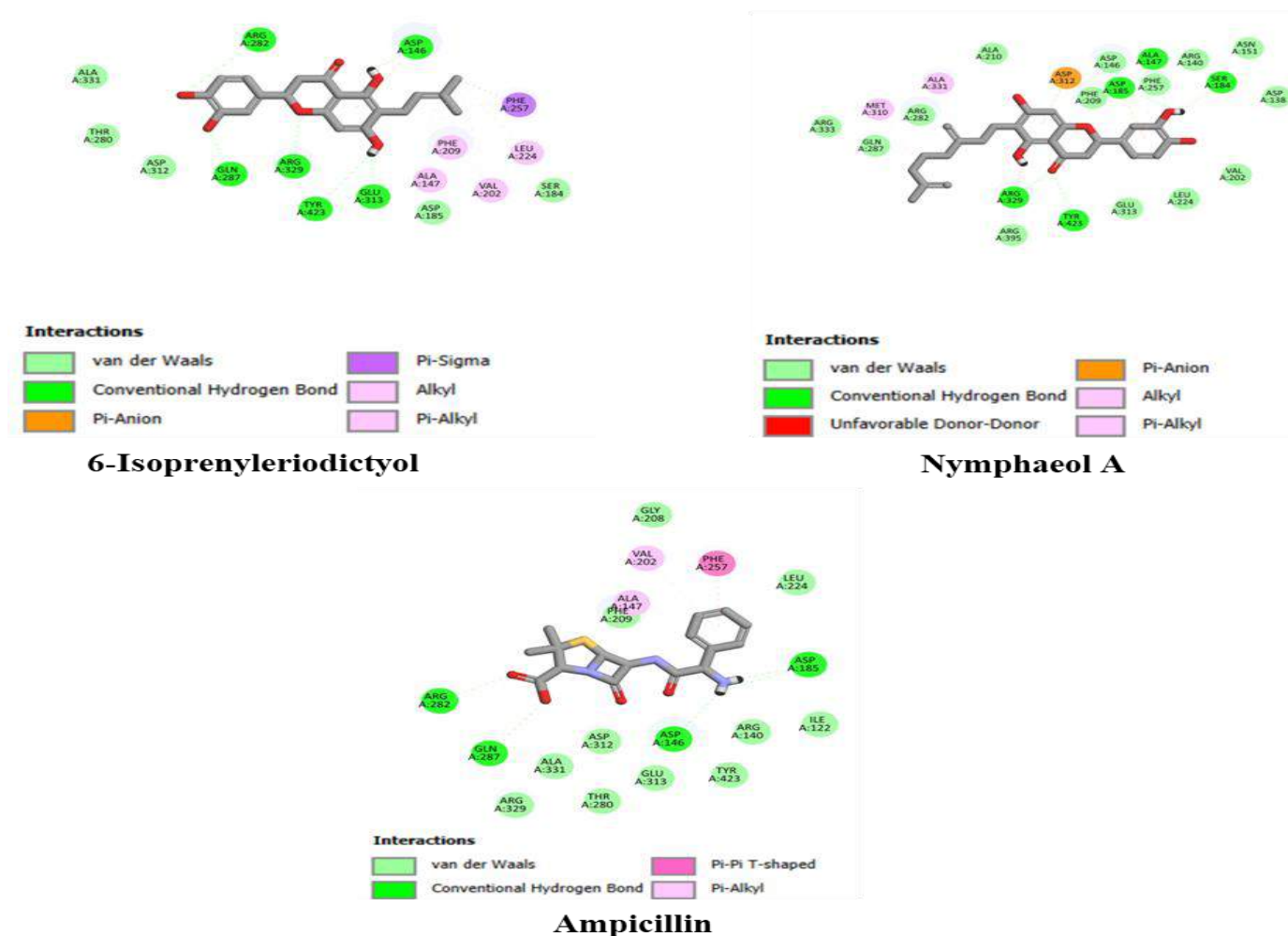


Figure 3: 2D visualization of interaction of the best-docked flavonoids and ampicillin with *Propionibacterium acnes* surface sialidase (PDB ID: 7LBU)

Conclusion

The extract and fractions of *Macaranga hullettii* leaves have potential as antibacterial agents against three bacterial strains, *Staphylococcus aureus* ATCC 25923, *Streptococcus mutans* ATCC 25175, and *Propionibacterium acnes* KCCM 41747. The methanol extract demonstrated antibacterial activity against *S. aureus* and *S. mutans* with MIC of < 0.15% against *S. aureus* and *S. mutans*, and 0.15-0.31% against *P. acnes*. The n-hexane fraction had an MIC of 1.25-2.5% against *S. aureus*, and 0.62-1.25% against *S. mutans* and *P. acnes*. The ethyl acetate fraction had an MIC < 0.15% against the three bacterial strains. Molecular docking studies suggested that 6-isoprenyleriodictyol, nymphaeol A, nymphaeol B, and solophenol D are promising antibacterial agents, as these compounds exhibited the highest binding affinity and formed hydrogen bonds with key amino acid residues in the active sites of the protein targets. Further *in vitro* and *in vivo* studies should be conducted by isolating the active secondary metabolites from this species.

Conflict of Interest

Authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgement

This research was funded by the Islamic Development Bank (PIU-IsDB) project under contract no. 137/UN17.11/PL/2019.

References

- Wong F, de la Fuente-Nunez C, Collins JJ. Leveraging artificial intelligence in the fight against infectious diseases. *Science*. 2023; 381(6654):164-170.
- Seukep AJ, Kuete V, Nahar L, Sarker SD, Guo M. Plant-derived secondary metabolites as the main source of efflux pump inhibitors and methods for identification. *J Pharm Anal*. 2020; 10(4):277-290.
- Magadula JJ. Phytochemistry and pharmacology of the genus *Macaranga*: a review. *J Med Plants Res*. 2014; 8(12):489-503.
- Vu LT, Ngan TB, Phuong L, Huong DT, Litaudon M, Van Hung N, Thach TD, Van Cuong P. Chemical constituents from fruits of *Macaranga denticulata* (Euphorbiaceae) (Part 2). *Vietnam J Chem*. 2018; 56(4):516-520.
- Marliana E, Hairani R, Tjahjandarie TS, Tanjung M. Antiplasmodial activity of flavonoids from *Macaranga tanarius* leaves. *IOP Conf Ser Earth Environ Sci*. 2018; 144(1):012011.
- Tjahjandarie TS, Tanjung M, Saputri RD, Nadar PB, Aldin MF, Marliana E, Permadi A. Flavestins K, An

- isoprenylated stilbene from the leaves of *Macaranga recurvata* Gage. Nat Prod Sci. 2019; 25(3):244-247.
7. Huong DT, Linh NT, Van TT, Litaudon M, Roussi F, Van Nam V, Van Cuong P. Stilbenes from *Macaranga tanarius* (Euphorbiaceae) growing in Vietnam. Vietnam J Chem. 2020; 58(3):338-342.
 8. Aldin MF, Tjahjandarie TS, Saputri RD, Tanjung M. Macasiamenene V, a New Stilbenoid from the Leaves of *Macaranga inermis*. Nat Prod Sci. 2021; 27(1):45-48.
 9. Muharram A, Rachmawati DA, Mardhiyyah S, Tjahjandarie TS, Saputri RD, Ahmat N, Tanjung M. Cytotoxic and antioxidant activities of flavonoids and diterpenoids from *Macaranga involucrata* (Roxb.) Baill. J Appl Pharm Sci. 2023; 13(6):087-92.
 10. Tanjung M, Tjahjandarie TS, Aldin MF, Mardhiyyah S, Ahmat N, Saputri RD. Macagigantin A, A New Flavonoid from *Macaranga gigantea* (Rchb. f & Zoll.) Mull Arg Nat Prod Sci. 2023; 29(4):287-294.
 11. Tjahjandarie TS, Aldin MF, Saputri RD, Tanjung M. Dihydrostilbenes from *Macaranga javanica* (Blume) Müll. Arg. and their antiparasmodial activity. Nat Prod Sci. 2024;1-7.
 12. Toko EG, Tchapo CED, Tsamo AT, Kemzeu R, Wang Y, Fekam FB, Ndinteh DT, Choudhary MI, Nkengfack EA, Mmutlane EM, Mkounga P. Three New Polyphenol Derivatives from the Fruits of *Macaranga Monandra* and their Antioxidant Potential. Chem. Biodivers. 2024;21(7):e202301816.
 13. Tanjung M, Tjahjandarie TS, Aldin MF, Mardhiyyah S, Saputri RD, Syah YM, Ahmat N. Two new flavonols from *Macaranga inermis* pax & K. Hoffm. Nat Prod Sci. 2025; 39(3):498-505.
 14. Marlina E, Tjahjandarie TS, Tanjung M. Isoprenylated flavanone derivatives from *Macaranga hoesi* King ex Hook F. Der Pharm Lett. 2015; 7(3):153-156.
 15. Marlina E, Tjahjandarie TS, Tanjung M. Antioxidant activity of flavonoids from *Macaranga pearsonii* Merr. J Kim Mulawarman. 2016; 13(2):97-100.
 16. Marlina E, Astuti W, Kosala K, Hairani R, Tjahjandarie TS, Tanjung M. Chemical composition and anticancer activity of *Macaranga hoesi* leaves. Asian J Chem. 2018; 30(4):795-798.
 17. Marlina E, Ruga R, Hairani R, Tjahjandarie TS, Tanjung M. Antioxidant activity of flavonoid constituents from the leaves of *Macaranga tanarius*. IOP Conf Ser J Phys. 2019; 1277(1):012014.
 18. Saputri RD, Tukiran T, Wati FA, Purnamasari AP, Wardhana MW, Tjahjandarie TS, Tanjung M. Macahuilettiin A, a new isoprenylated flavanone from the leaves of *Macaranga hullettii* King ex Hook and their antiparasmodial activity. Vietnam J Chem 2024; 62(3):394-398.
 19. Musdalifah M, Khumaidi A, Suwastika IN. Inhibition test and phytochemical screening of leaf extracts of *Macaranga tanarius* (L.) Mull. Arg against *Salmonella typhi* as an antibacterial. Nat Sci J Sci Technol. 2017; 6(3):9194.
 20. Ibrahim H, Omosa L, Nchiozem-Ngnitedem V, Onyari J, Maru S, Guefack M. Antibacterial activities and phytochemical screening of crude extracts from Kenyan macaranga species toward MDR phenotypes expressing efflux pumps. Pharmacogn Commun. 2021; 11(2):119-126.
 21. Bijesh K and Sebastian D. Isolation and characterization of antibacterial compounds from *Macaranga peltata* against clinical isolates of *Staphylococcus aureus*. Int J Biol Pharm Res. 2013; 4(12):1196-1203.
 22. Salleh WM, Razak NZ, Ahmad F. Phytochemicals and biological activities of *Macaranga hoesi* and *Macaranga constricta* (Euphorbiaceae). Marmara Pharm J. 2017; 21(4):881-888.
 23. Slik JW, Priyono P, Welzen PV. Key to the *Macaranga Thou.* and *Mallotus Lour.* species (Euphorbiaceae) of East Kalimantan, Indonesia. Singapore, Gardens' Bulletin (Singapore), National Parks Board, Singapore Botanic Gardens; 2000. 11-87 p.
 24. Amirta R, Angi EM, Ramadhan R, Kusuma IW, Wiati CB, Haqiqi MT. Potential utilization of macaranga. Samarinda: Mulawarman University Press; 2017.
 25. Rismawati R, Marlina E, Daniel D. Phytochemical Test on Methanol Extract of Leaf of *Macaranga hullettii* King ex Hook. f. J Atomik. 2018; 3(2):91-94.
 26. Hudzicki J. Kirby-Bauer disk diffusion susceptibility test protocol. Am Soc Microbiol. 2009; 15(1):1-23.
 27. Inna M, Astuti W, Saleh C. Antibacterial activity of methanolic extract of uric patch plant leaves (*Cayratia carnososa*) against *Salmonella typhi* and *Propionibacterium acnes*. J Atomik. 2022; 7(1):1-5.
 28. Yulianti MF, Amat AL, Hutasoit RM, Pakan PD. Antibacterial activity of jambang leaf ethanol extract (*Syzygium cumini*) against the growth of *Propionibacterium acnes*. Acta Biochimica Indones. 2023; 6(2):1161.
 29. Hossain MR, Biplob AI, Sharif SR, Bhuiya AM, Sayem AS. Antibacterial Activity of Green Synthesized Silver Nanoparticles of *Lablab purpureus* Flowers Extract against Human Pathogenic Bacteria. Trop J Nat Prod Res. 2023;7(8):3647-3651
 30. Yanda L, Tatsimo SJ, Tamokou JD, Matsuet-Takongmo G, Meffo-Dongmo SC, Meli Lannang A, Sewald N. Antibacterial and antioxidant activities of isolated compounds from *Prosopis africana* leaves. Int J Anal Chem. 2022; 2022:4205823.
 31. Qiu X, Janson CA, Smith WW, Green SM, McDevitt P, Johanson K, Carter P, Hibbs M, Lewis C, Chalker A, Fosberry A. Crystal structure of *Staphylococcus aureus* tyrosyl-tRNA synthetase in complex with a class of potent and specific inhibitors. Protein Sci. 2001; 10(10):2008-2016.
 32. Larson MR, Rajashankar KR, Patel MH, Robinette RA, Crowley PJ, Michalek S, Brady LJ, Deivanayagam C. Elongated fibrillar structure of a streptococcal adhesin assembled by the high-affinity association of α - and PPII-helices. Proc Natl Acad Sci. 2010; 107(13):5983-5988.
 33. Yu AC, Volkens G, Jongkees SA, Worrall LJ, Withers SG, Strynadka NC. Crystal structure of the *Propionibacterium acnes* surface sialidase, a drug target for *P. acnes*-associated diseases. Glycobiol. 2022; 32(2):162-170.
 34. Jakubec D, Skoda P, Krivak R, Novotny M, Hoksza D. PrankWeb 3: accelerated ligand-binding site predictions for experimental and modelled protein structures. Nucl Acids Res. 2022; 50(W1):W593-W597.
 35. Dallakyan S and Olson AJ. Small-molecule library screening by docking with PyRx. Methods Mol Biol. 2014; 1263:243-250.
 36. Trott O and Olson AJ. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. J Comput Chem. 2010; 31(2):455-461.
 37. Malangu N. ed. Poisoning: From Specific Toxic Agents to Novel Rapid and Simplified Techniques for Analysis. BoD-Books on Demand; 2017.
 38. Sari AA and Saleh C. Phytochemical test, toxicity and antibacterial activity of extracts of various macaranga leaf fractions (*Macaranga tanarius* (L.) MA) against *Staphylococcus aureus* and *Escherichia coli*. J Kim Mulawarman. 2015; 12(2):53-58.
 39. Imelda R, Mariam M, Satari M. Effect of cassava (*Manihot esculenta* crantz), rice (*oryza sativa* L.), and potato (*solanum tuberosum*) water extract to decrease pH phase fermentation of *streptococcus mutans* atcc 25175. Padjajaran J Dent. 2019; 31(1):14.

40. Alkhaled A, Alsabek L, Al-assaf M, Badr F. Effect of chlorhexidine, honey and propolis on *streptococcus mutans* counts: *in vitro* study. Dentistry. 2021; 9(1):a001.
41. Ren Z, Chen L, Li J, Li Y. Inhibition of *Streptococcus mutans* polysaccharide synthesis by molecules targeting glycosyltransferase activity. J Oral Microbiol. 2016; 8(1):31095.
42. Lee BS, Chen YJ, Wei TC, Ma TL, Chang CC. Comparison of antibacterial adhesion when salivary pellicle is coated on both poly (2-hydroxyethyl-methacrylate)-and polyethylene-glycol-methacrylate-grafted poly (methyl methacrylate). Int J Mol Sci. 2018; 19(9):2764.
43. Chen KC, Yang CH, Li TT, Zouboulis CC, Huang YC. Suppression of *Propionibacterium acnes*-stimulated proinflammatory cytokines by Chinese bayberry extracts and its active constituent myricetin in human sebocytes *in vitro*. Phytother Res. 2019; 33(4):1104-1113.
44. Xu J, Chen X, Song J, Wang C, Xu W, Tan H, Suo H. Antibacterial activity and mechanism of cell-free supernatants of *Lactocaseibacillus paracasei* against *Propionibacterium acnes*. Microb. Pathog. 2024; 189:106598.
45. Warnida H, Mustika D, Supomo S, Sukawaty Y. Effectiveness of Mahang Leaf Ethanol Extract (*Macaranga Triloba*) as an anti-acne. J Penelit. Sos. Ekon. Kehuta. 2018; 4(1):9-18.
46. Schütz BA, Wright AD, Rali T, Sticher O. Prenylated flavanones from leaves of *Macaranga pleiostemona*. Phytochem. 1995; 40(4):1273-1277.
47. Lim TY, Lim YY, Yule CM. Evaluation of antioxidant, antibacterial and anti-tyrosinase activities of four *Macaranga* species. Food Chem. 2009; 114(2):594-599.
48. Fareza MS, Syah YM, Mujahidin D, Juliawaty LD, Kurniasih I. Antibacterial flavanones and dihydrochalcones from *Macaranga trichocarpa*. Z. Naturforsch C J Biosci. 2014; 69(9-10):375-380.
49. Hasanat A, Kabir MS, Hossain MM, Hasan M, Al Masum MA, Chowdhury TA, Bhuiyan DI, Mamur A, Kibria AS. Antibacterial activity of methanol extract of *Macaranga denticulata* leaves and *in silico* PASS prediction for its six secondary metabolites. World J Pharm Pharm. Sci. 2015:1258-1266.
50. O Akanbi B, Anene P, Olayanju S. Preliminary Screening Indicates Promising Antimicrobial Properties of the Stem Bark Extracts of *Macaranga rosea*. Anti-Infective Agents. 2015; 13(2):123-128.
51. Ogundajo A, Okeleye B, Ashafa AO. Chemical constituents, *in vitro* antimicrobial and cytotoxic potentials of the extracts from *Macaranga barteri* Mull-Arg. Asian Pac J Trop Biomed. 2017; 7(7):654-659.
52. Putri R, Hendra R, Teruna HY. Anti-Bacterial and Anti-Fungal Activities from *Macaranga bancana* Leaves Extract. Pharmacol Clin Pharm Res. 2019; 4(1):1-4.
53. Lee JH, Kim YG, Khadke SK, Yamano A, Woo JT, Lee J. Antimicrobial and antibiofilm activities of prenylated flavanones from *Macaranga tanarius*. Phytomed. 2019; 63:153033.
54. Pagna JI, Awazi T, Mbarga PE, Mbekou IM, Mkounga P, Fotie J, Frese M, Fabrice FB, Lenta BN, Sewald N, Nkengfack EA. Antibacterial flavonoids from the fruits of *Macaranga hurifolia*. J Asian Nat Prod Res. 2022; 24(11):1041-1051.
55. Kamso VF, Simo Fotso CC, Kanko Mbekou IM, Toussie BT, Ndjakou Lenta B, Boyom FF, Sewald N, Frese M, Ngadjui BT, Wabo Fotso G. Chemical constituents of *Macaranga occidentalis*, antimicrobial and chemophenetic studies. Molecules. 2022; 27(24):8820.
56. Rosamah E, Haqiqi MT, Putri AS, Kuspradini H, Kusuma IW, Amirta R, Yuliansyah Y, Suwinarti W, Paramita S, Ramadhan R, Tarmadi D. The potential of *Macaranga* plants as skincare cosmetic ingredients: A review. J Appl Pharm Sci. 2023; 13(7):001-12.
57. Li AP, He YH, Zhang SY, Shi YP. Antibacterial activity and action mechanism of flavonoids against phytopathogenic bacteria. Pestic Biochem Physiol. 2022; 188:105221.
58. Chen YW, Ye SR, Ting C, Yu YH. Antibacterial activity of propolins from Taiwanese green propolis. J Food Drug Anal. 2018; 26(2):761-768.
59. Inui S, Hosoya T, Shimamura Y, Masuda S, Ogawa T, Kobayashi H, Shirafuji K, Moli RT, Kozono I, Shin-ya K, Kumazawa S. Solophenols B-D and Solomonin: New Prenylated Polyphenols Isolated from Propolis Collected from The Solomon Islands and Their Antibacterial Activity. J Agric Food Chem. 2012; 60(47):11765-11770.
60. Ruga R, Kingkaew K, Tamsampaolot K, Chavasiri W. Enhancing antibacterial activity against *Propionibacterium acnes* and *Staphylococcus aureus* by combination of tetracycline with selected compounds. Chem Lett. 2018; 47(12):1538-1541.

Publication of your article Vol 9 issue 7

From: Editor-in-Chief Tjnpr editor.tjnpr@gmail.com

To: Daddy Falodun faloabi@uniben.edu

Date: Sab, 2 Agu 2025, 16.03

Dear esteemed author,

Your article is available online www.tjnpr.org Vol 9 issue 8 (1st August 2025)

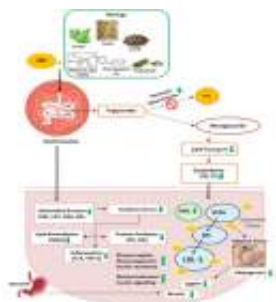
Please, check if you have not made your APC payment, do so urgently.

Current Issue

Vol. 9 No. 7 (2025): Tropical Journal of Natural Product Research (TJNPR)

Published: 2025-07-31

Articles



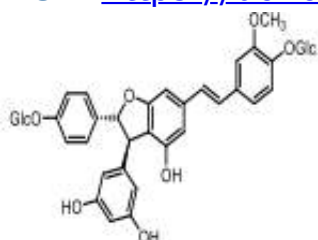
Potential of Isothiocyanates from Moringa as Antiobesity: A Review

Surahmat Hamid, Nurfaika hardjo, Sukaeni Ibrahim, Ilhamuddin Azis, Mirna Muis, Ika Yustisia, Syahrijuita Kadir, Marhaen Hardjo

2933 – 2940

Abstract Views | PDF/EPUB Download: 17 / 14 / 1

DOI: <https://doi.org/10.26538/tjnpr/v9i7.1>



Gnetum Genus: Review on its Traditional Uses, Phytochemistry and Pharmacological Activities

Kamsirah Jim Shamsudin, Nurulfazlina Edayah Rasol, Nurunajah Ab. Ghani

2941 – 2963

Abstract Views | PDF/EPUB Download: 6 / 2 / 2

DOI: <https://doi.org/10.26538/tjnpr/v9i7.2>



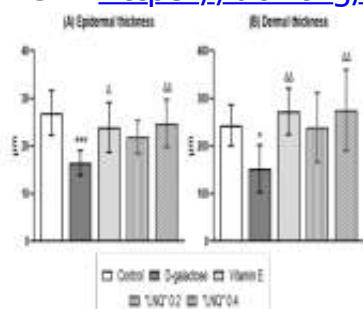
Goniothalamus macrophyllus: A Comprehensive Review of Its Phytochemistry, Pharmacological Activities, and Therapeutic Potential

Rollando Rollando, Eva Monica, Dodi Iskandar, Viol Dhea Kharisma, Arif Nur Muhammad Ansori

2964 – 2972

Abstract Views | PDF/EPUB Download: 7 / 4 / 1

DOI: <https://doi.org/10.26538/tjnpr/v9i7.3>



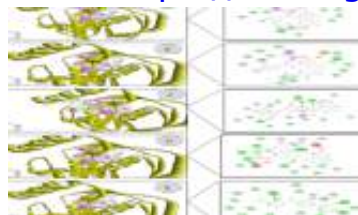
Topical Virgin Coconut Oil Ameliorates Oxidative Stress and Improves Skin Structure in a Mouse Model of D-Galactose-Induced Aging

Ha TT. Nguyen, Trang Tran, Phong X. Pham, Hanh M. Pham, Phuong HT. Tran, Chau Anh T. Phan, Quang V. Trinh, Van Anh T. Pham

2973 – 2980

Abstract Views | PDF/EPUB Download: 7 / 2 / 1

DOI: <https://doi.org/10.26538/tjnpr/v9i7.4>



Phytochemical Composition, Antioxidant Activity, and Metabolite Profiling of Sequential Extracts from *Vitex trifolia* Leaves with Antibacterial Potential: An Integrated In vitro and In silico Study

Masruri Masruri, Anna Safitri, Siti M. Ulfa, Revian F. Ferdian, Ahmad R. A. Fabri, I Wayan Karta, Muhammad Harrist, Aprilita A. Shalsadila

2981 – 2992

Abstract Views | PDF/EPUB Download: 8 / 5 / 1

DOI: <https://doi.org/10.26538/tjnpr/v9i7.5>



The Anticancer Potential of Plant Essential Oils: Mechanisms, Applications, and Challenges

Mohd NAN Yuseri, Abd NZ Wahab, HS Asmara, Wan WR Taib, Abdul AS Manap

2992 – 3006

Abstract Views | PDF/EPUB Download: 9 / 5 / 0

DOI: <https://doi.org/10.26538/tjnpr/v9i7.6>



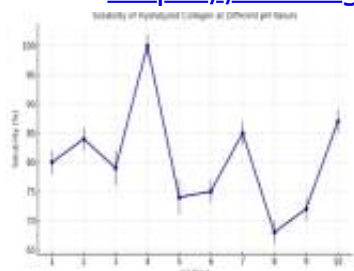
Alpha-Glucosidase Inhibitory Activity and Analysis of Eleuthoside B from Eleutherine bulbosa Urb. Bulbs Purified Extract from Lampo Donggala, Central Sulawesi, Indonesia

Syariful Anam, Yonelian Yuyun, Siti Nurhasana Tanaijo, Friska R. Langkoda, Yusriadi, Asriana Sultan, David Pakaya

3007 – 3012

Abstract Views | PDF/EPUB Download: 4 / 4 / 2

DOI: <https://doi.org/10.26538/tjnpr/v9i7.7>



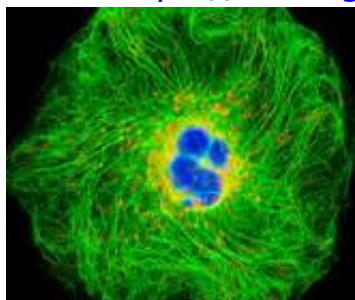
Characterization and Formulation of Anti-Aging Cream Using Collagen Derived from Sardine (*Sardinella spp.*) Fish Scales

Herlina Jusuf, Hartono Hadjarati, Mohamad Adam Mustapa, Widy Susanti Abdulkadir, Ahmad M. Andy Suryadi, Muhammad Taupik, Mohamad Aprianto Paneo

3013 – 3020

Abstract Views | PDF/EPUB Download: 4 / 2 / 1

DOI: <https://doi.org/10.26538/tjnpr/v9i7.8>



Cytotoxic Activity of N-Hexane, Ethyl Acetate, and Ethanol Extract of Hibiscus tiliaceus against A549 Lung Cancer Cells

Nilsya F. Zebua, Nerdy Nerdy, Abdi W. Septama, Des Suryani, Jesika R. Sipayung, Nazwa N.E. Purba, Mhd A.T. Mardia, Elisabeth P. Nahulae, Iksen Iksen, Suthasinee Seephan

3021 – 3025

Abstract Views | PDF/EPUB Download: 5 / 4 / 1

DOI: <https://doi.org/10.26538/tjnpr/v9i7.9>



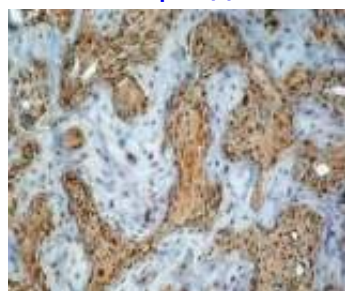
In Silico Study of Java Cardamom (*Amomum compactum*) Fruit Derivative Compounds as Antihyperuricemia

Ali Nofriyaldi, Nita Astuti Rahmawati, Mochamad H. Nurzaman, Nitya N. Fadilah, Selvy Isnaeni

3026 – 3030

Abstract Views | PDF/EPUB Download: 2 / 2 / 1

DOI: <https://doi.org/10.26538/tjnpr/v9i7.10>



Type 1 Collagen from Scales of *Osphronemus gouramy* Enhances Osteocalcin Expression: An In Vivo Study

Noer Ulfah, Chiquita Prahasanti, Theresia I. Budhy, Apriani W. Nelly, Esi Y. Fitrina, Zalfaa F. Yuda

3031 – 3034

Abstract Views | PDF/EPUB Download: 7 / 6 / 0

DOI: <https://doi.org/10.26538/tjnpr/v9i7.11>



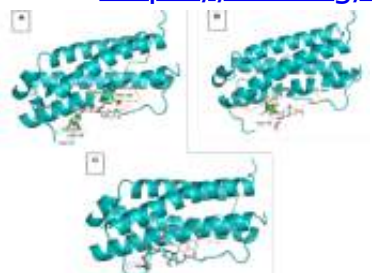
Triterpenoid Extraction from *Ficus racemosa* Leaves: Impact of Key Processing Factors

Thuan N. Nguyen, Mai S. Dam, Luong T. Nguyen, Phuong V. Do

3035 – 3039

Abstract Views | PDF/EPUB Download: 4 / 3 / 1

DOI: <https://doi.org/10.26538/tjnpr/v9i7.12>



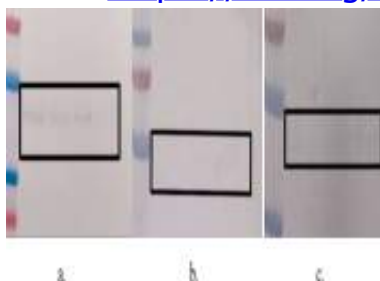
In silico Evaluation of the Active Compounds of *Hibiscus sabdariffa* Linn as IL-11 Inhibitor

Yeniar Fitrianingrum, Nurhadi Ibrahim, Trinovita Andraini, Linda Erlina

3040 – 3044

Abstract Views | PDF/EPUB Download: 6 / 3 / 1

DOI: <https://doi.org/10.26538/tjnpr/v9i7.13>



The Effect of Cowanin on ERK1/2 and p-ERK1/2 Protein Expression in T47D Breast Cancer Cells by The Western Blot Method

Dira Hefni, Dachriyanus, Rahmad Abdillah, Nonda Riaknes, Fatma S Wahyuni

3045 – 3049

Abstract Views | PDF/EPUB Download: 2 / 3 / 1

DOI: <https://doi.org/10.26538/tjnpr/v9i7.14>



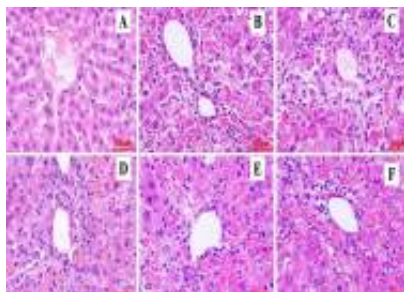
In vitro Evaluation and Molecular Docking Study of the Antibacterial Potential of Macaranga hullettii King ex Hook.f. Leaf Extract

Eva Marliana, Ade Danova, Rita Hairani, Ritbey Ruga, Elvira Hermawati, Ana Yulvia

3050 – 3057

Abstract Views | PDF/EPUB Download: 27 / 8 / 1

DOI: <https://doi.org/10.26538/tjnpr/v9i7.15>



Hepatoprotective and Antioxidant Activities of Selected Helicteres Species in a CCl₄-Induced Acute Liver Injury Mouse Model

Tran N. Quy, Phan T. Dat, Nguyen H. Anh, Nguyen T. Hieu, Phung T. Hang, Nguyen N. Hien, Tran T. T. Thao, Nguyen T. H. Phuc

3058 – 3066

Abstract Views | PDF/EPUB Download: 2 / 3 / 1

DOI: <https://doi.org/10.26538/tjnpr/v9i7.16>