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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**

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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 (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 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 hoesi* 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 1JIJ, 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: 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, 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.

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

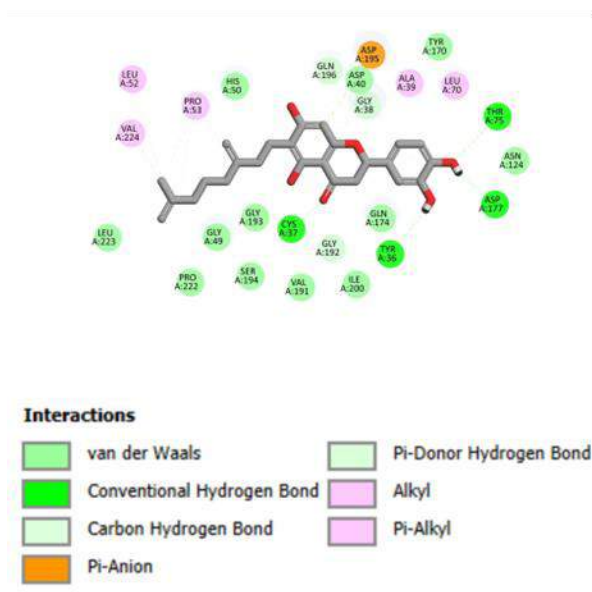
Sample	Concentration (%)	Inhibition Zone (mm)*		
		<i>Staphylococcus</i>	<i>Streptococcus</i>	<i>Propionibacterium</i>
		<i>aureus</i>	<i>mutans</i>	<i>acnes</i>
Methanol Extract	0	0	0	0
	0.15	6.67	6.67	0
	0.31	6.67	6.67	7.67
	0.62	6.67	7	8
	1.25	7.17	7.33	9.67
	2.5	7.5	8	10.67
	MIC (%)	<0.15	<0.15	0.15-0.31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

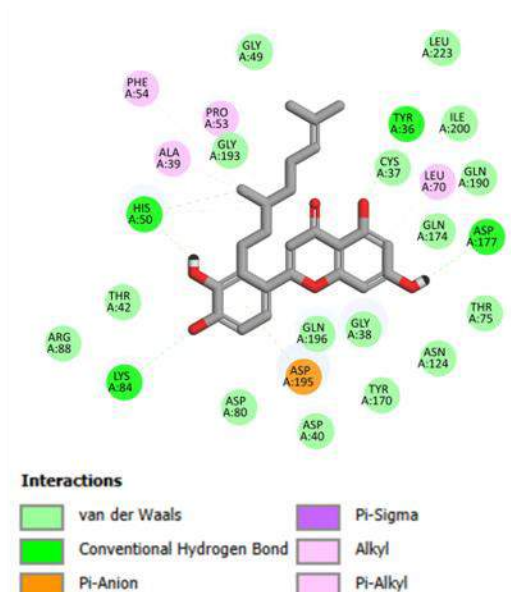
*Data were obtained from triplicate experiments.

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

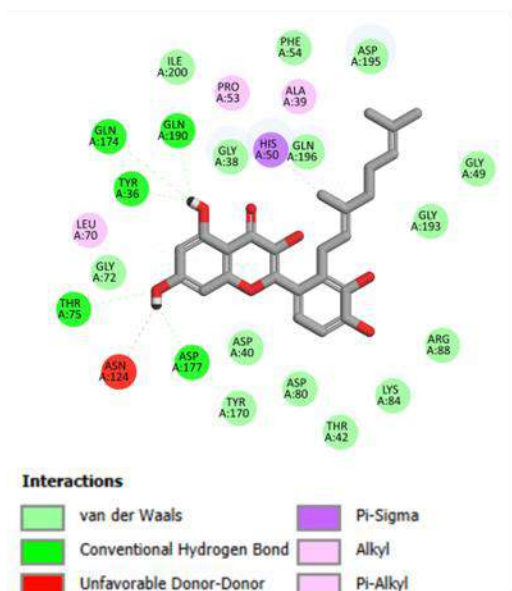
No.	Compound	Binding Affinity (kcal/mol)		
		1JIJ	3IPK	7LBU
1	4'-O-methyl-8-isoprenylnaringenin	-7.4	-8.1	-7.7
2	4'-O-methyl-8-isoprenyleriodictyol	-7.2	-8.1	-7.6
3	6-Isoprenyleriodictyol	-7.7	-8.7	-8.0
4	5-hydroxy-7,4'-dimethoxyflavone	-8.6	-7.8	-7.1
5	5-hydroxy-6,7,4'-trimethoxyflavone	-8.3	-7.5	-7.3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



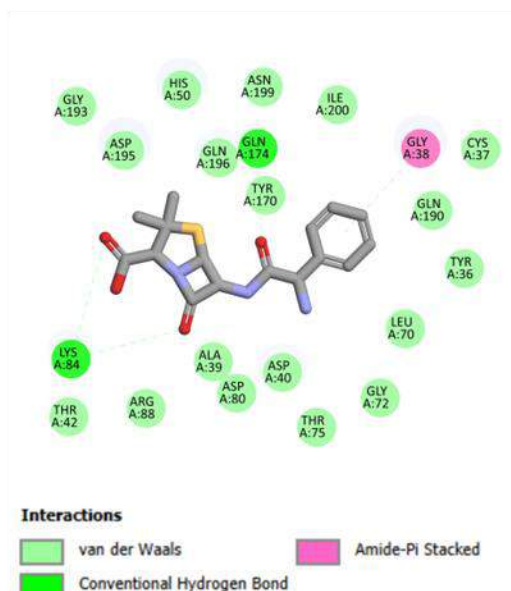
Nymphaeol A



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)

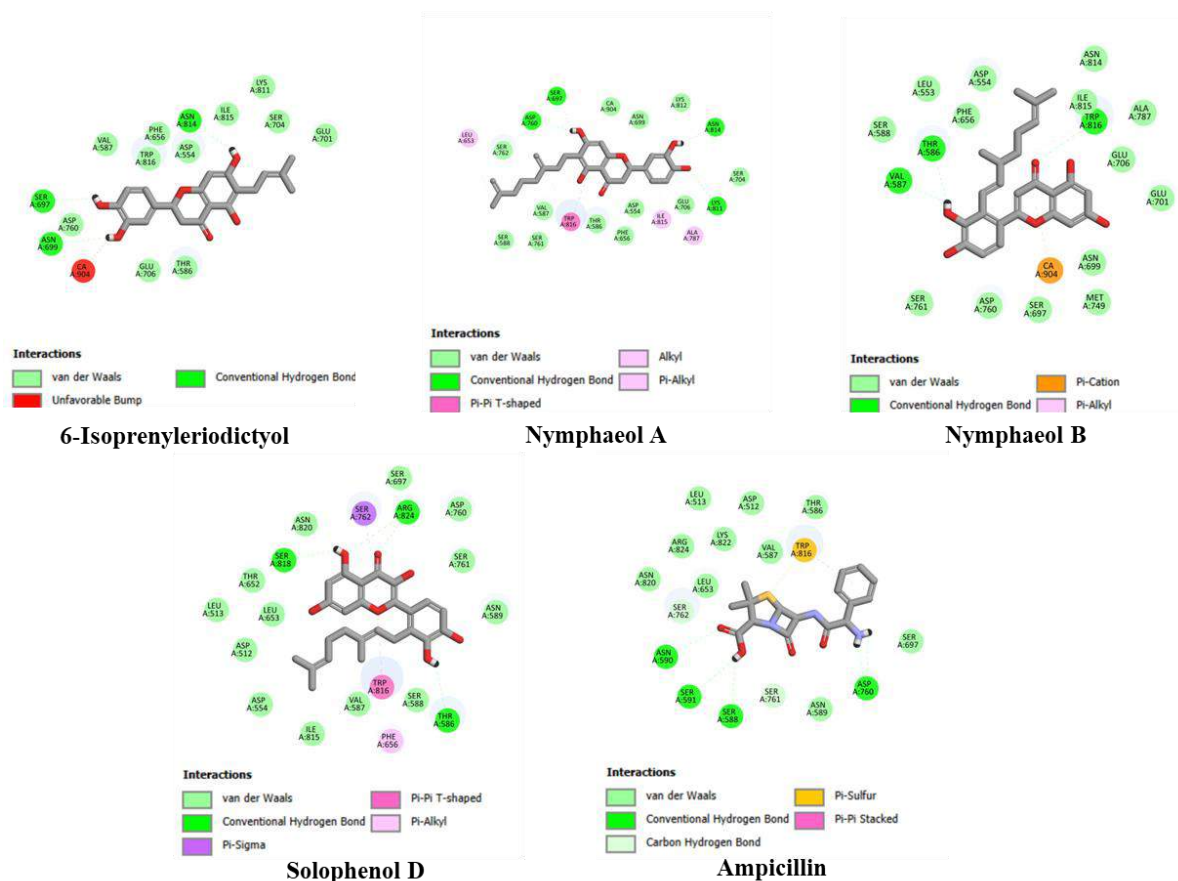


Figure 2: 2D interaction of the best-docked flavonoids and ampicillin with *Streptococcus mutans* antigen I/II (PDB ID: 3IPK).

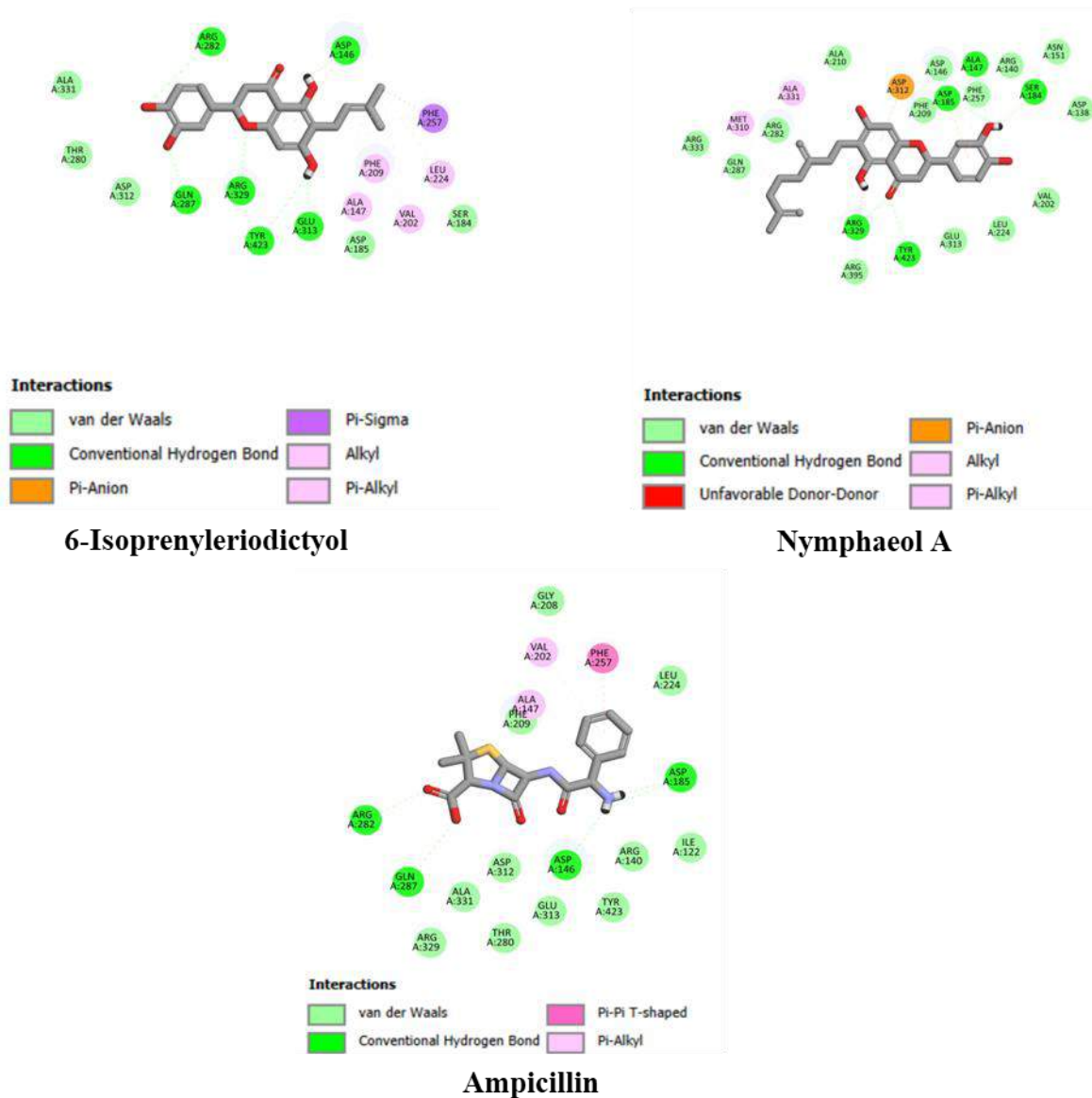


Figure 3: 2D interaction of the best-docked flavonoids and ampicillin with *Propionibacterium acnes* surface sialidase (PDB ID: 7LBU).

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>

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Best regards

Abiodun

Professor Abiodun Falodun, PhD; FAAS, FISPON

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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.
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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

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Date Sat 4/26/2025 7:47 PM

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

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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

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Best regards

Abiodun

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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

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***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.

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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).

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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.

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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.

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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

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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, 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.

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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..

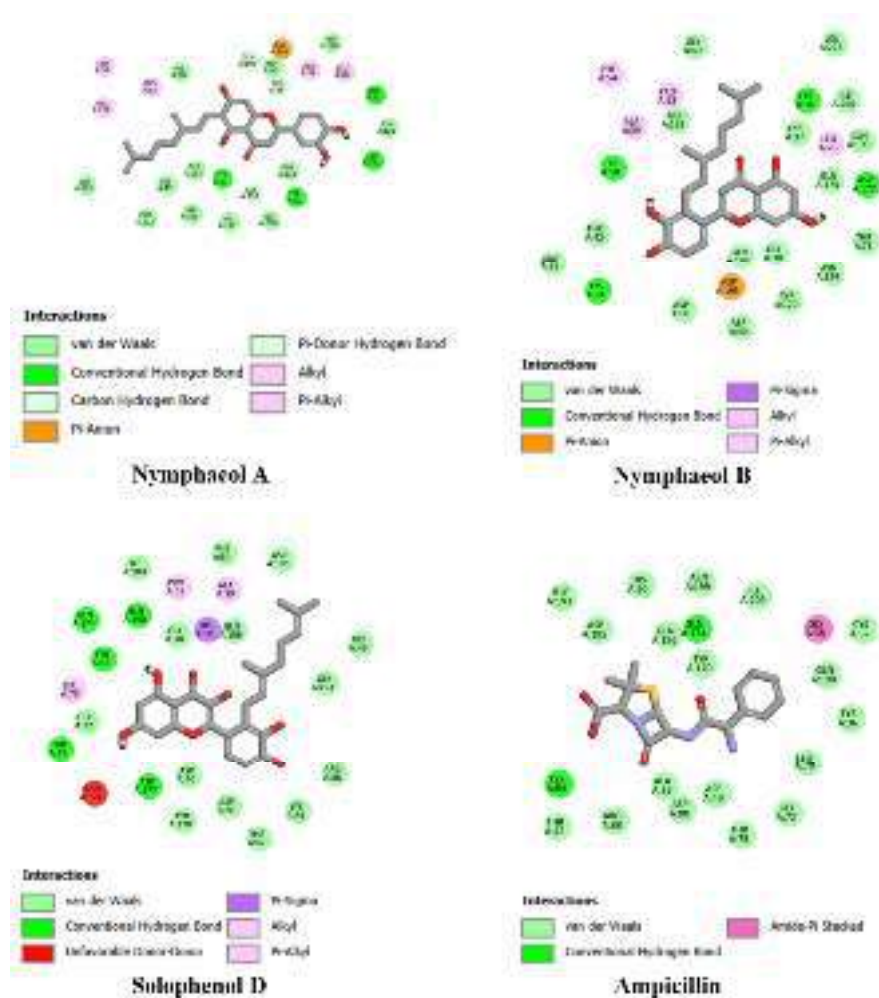


Figure 1: 2D interaction of the best docked flavonoids and ampicillin with *Staphylococcus aureus* tyrosyl-tRNA synthetase (PDB ID: 1JJJ)

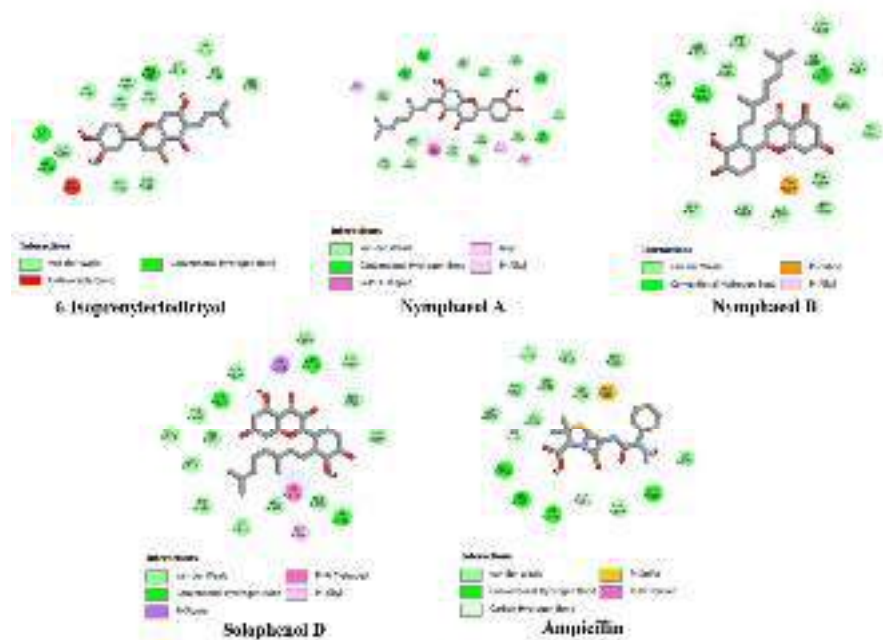


Figure 2: 2D interaction of the best-docked flavonoids and ampicillin with *Streptococcus mutans* antigen I/II (PDB ID: 3IPK).

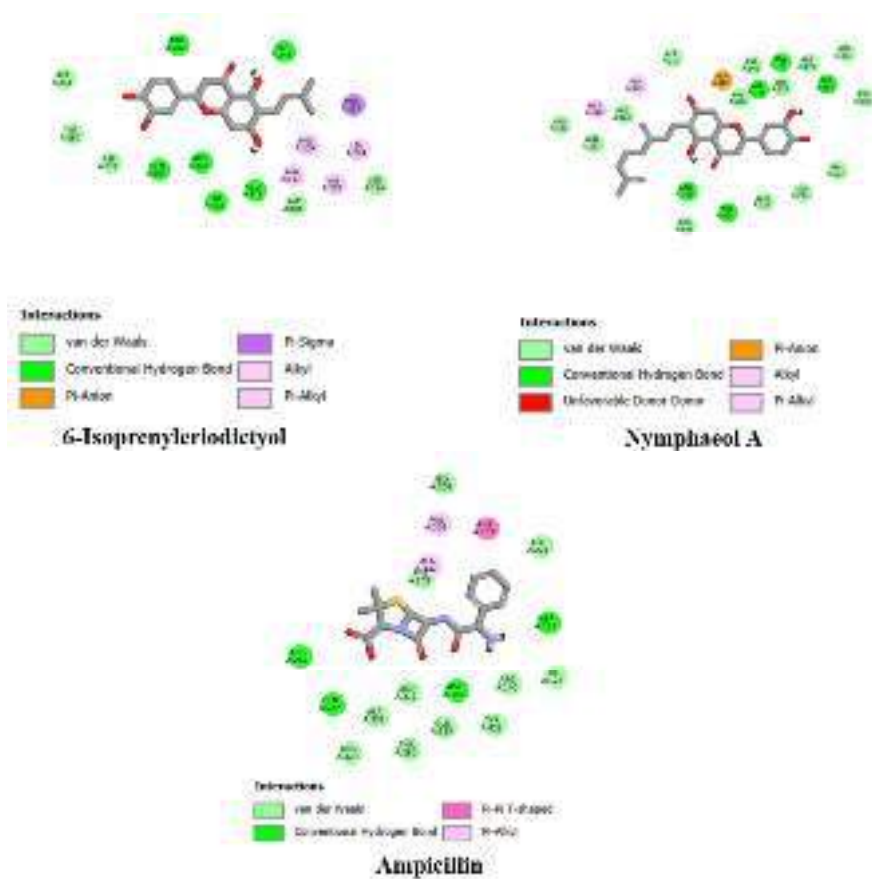


Figure 3: 2D interaction of the best-docked flavonoids and ampicillin with *Propionibacterium acnes* surface sialidase (PDB ID: 7LBU).



Revision

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

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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-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 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, 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. 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.

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

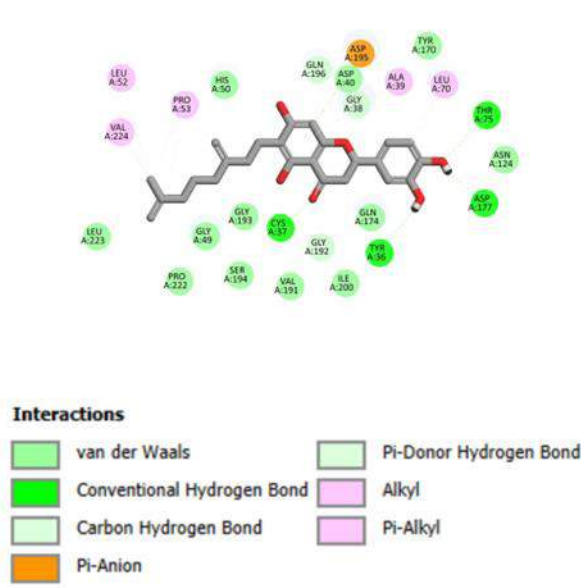
Sample	Concentration (%)	Inhibition Zone (mm)*		
		<i>Staphylococcus</i>	<i>Streptococcus</i>	<i>Propionibacterium</i>
		<i>aureus</i>	<i>mutans</i>	<i>acnes</i>
Methanol Extract	0	0	0	0
	0.15	6.67	6.67	0
	0.31	6.67	6.67	7.67
	0.62	6.67	7	8
	1.25	7.17	7.33	9.67
	2.5	7.5	8	10.67
	MIC (%)	<0.15	<0.15	0.15-0.31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

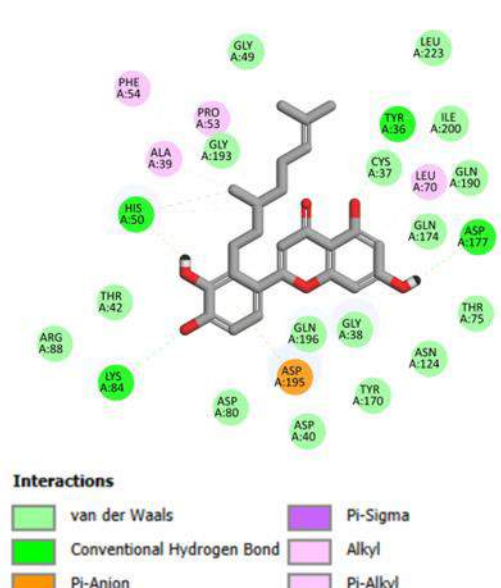
*Data were obtained from triplicate experiments.

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

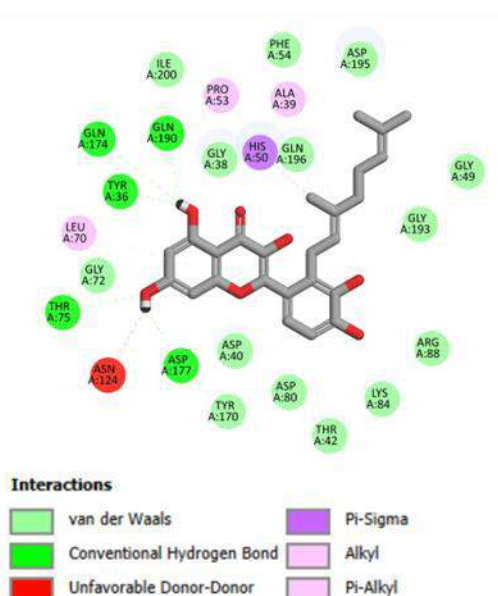
No.	Compound	Binding Affinity (kcal/mol)		
		1JJJ	3IPK	7LBU
1	4'-O-methyl-8-isoprenylnaringenin	-7.4	-8.1	-7.7
2	4'-O-methyl-8-isoprenyleriodictyol	-7.2	-8.1	-7.6
3	6-Isoprenyleriodictyol	-7.7	-8.7	-8.0
4	5-hydroxy-7,4'-dimethoxyflavone	-8.6	-7.8	-7.1
5	5-hydroxy-6,7,4'-trimethoxyflavone	-8.3	-7.5	-7.3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



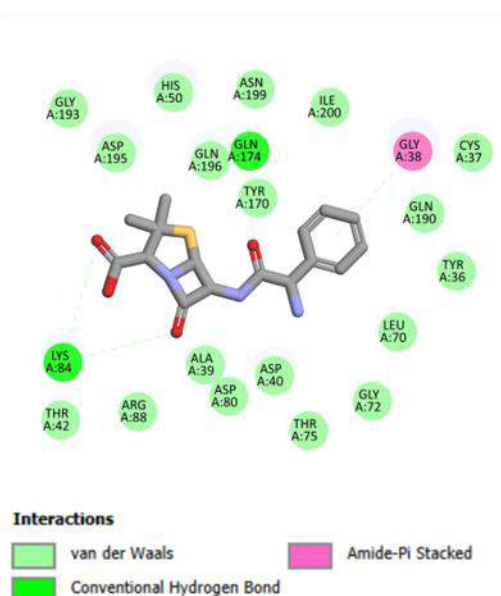
Nymphaeol A



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)

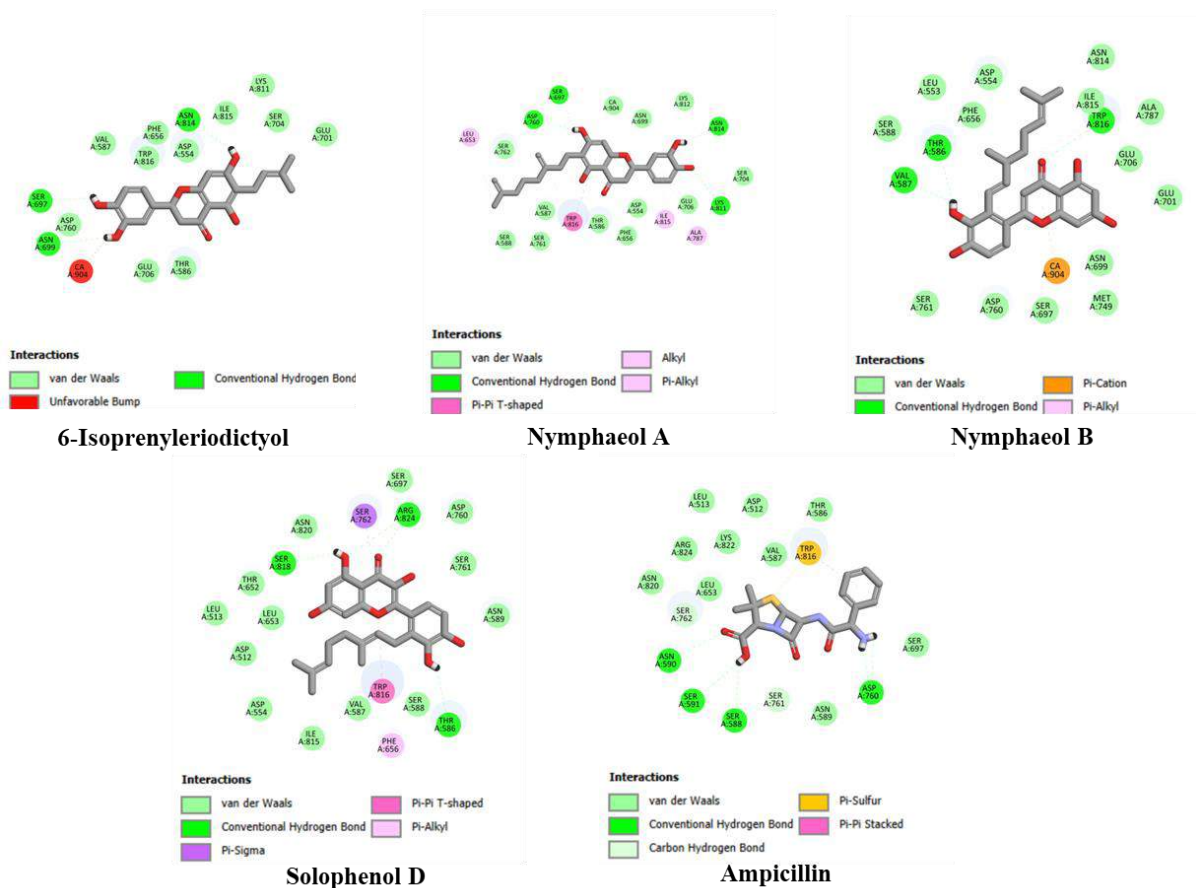


Figure 2: 2D interaction of the best-docked flavonoids and ampicillin with *Streptococcus mutans* antigen I/II (PDB ID: 3IPK).

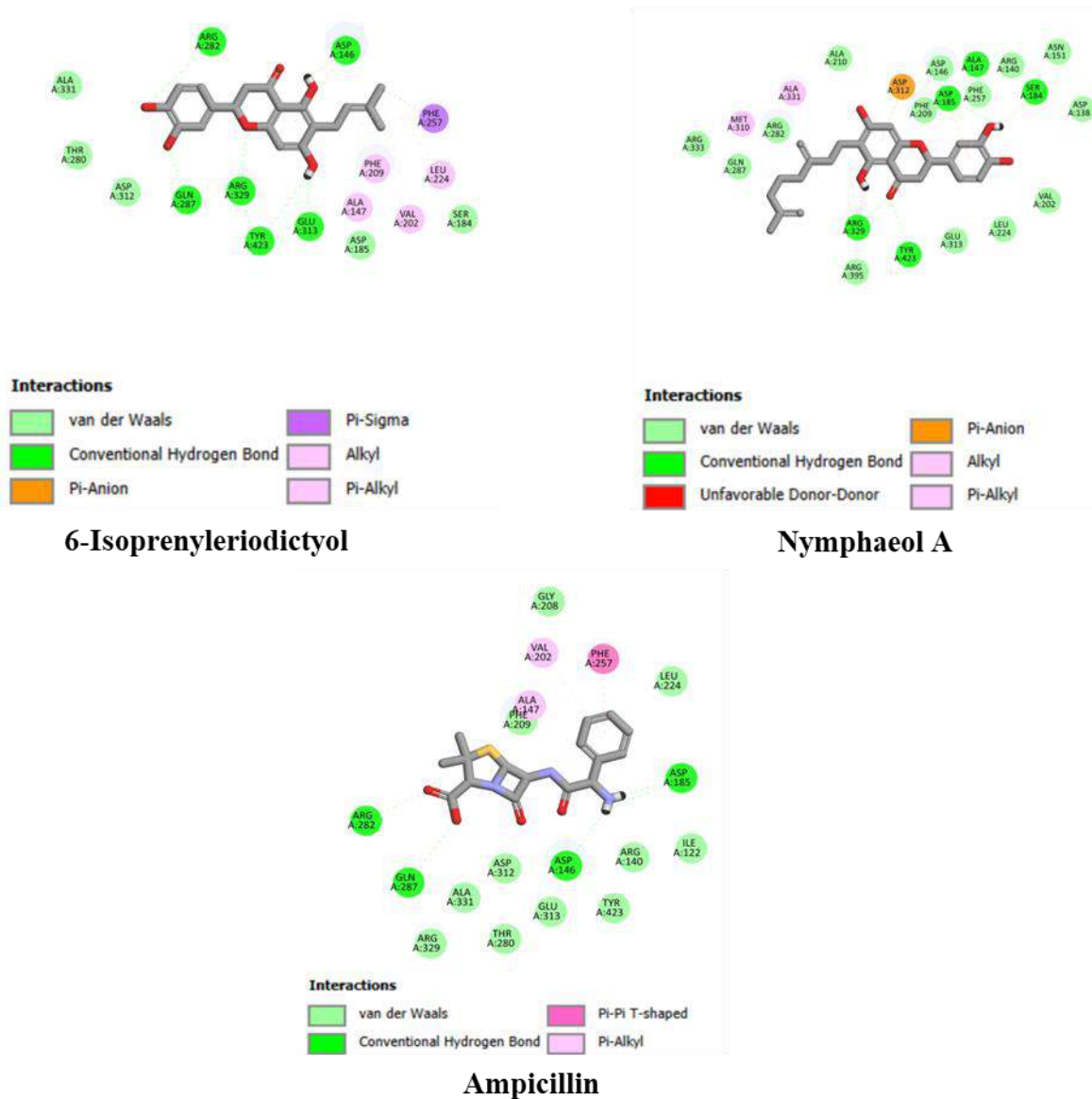


Figure 3: 2D interaction of the best-docked flavonoids and ampicillin with *Propionibacterium acnes* surface sialidase (PDB ID: 7LBU).

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***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-isoprenyleriodictyol, 6-isoprenyleriodictyol, 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*.²⁶ The flavonol rhamnetin, isolated from *Macaranga peltata*, also showed antibacterial activity against *Staphylococcus aureus*.²⁷ 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.^{19,25} 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 Medisool). 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, isolophenol D, nymphaeol A, nymphaeol B, and nymphaeol 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 1JJU, 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: 1JJU), *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 *Mucunaesga 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*.

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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-isoprenylerioidictyol	-7.2	-8.1	-7.6
3	6-Isoprenylerioidictyol	-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	Macanuleitin 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

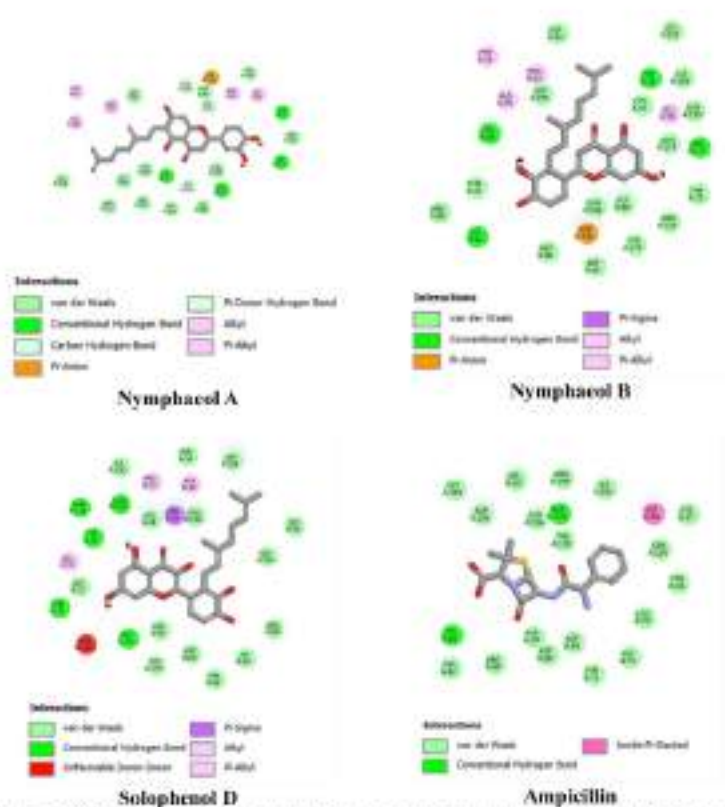


Figure 1: 2D interaction of the best docked flavonoids and ampicillin with *Staphylococcus aureus* tyrosyl-tRNA synthetase (PDB ID: 1JJJ)

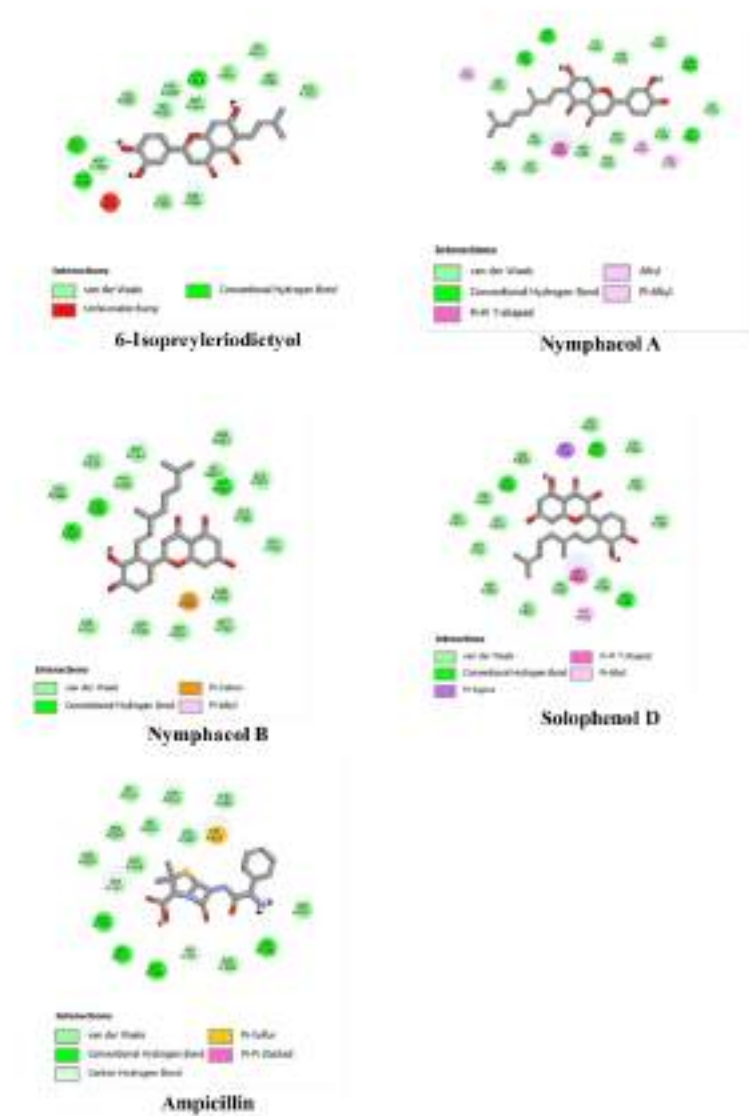


Figure 2: 2D interaction of the best-docked flavonoids and ampicillin with *Streptococcus mutans* antigen I/II (PDB ID: 3BPX).

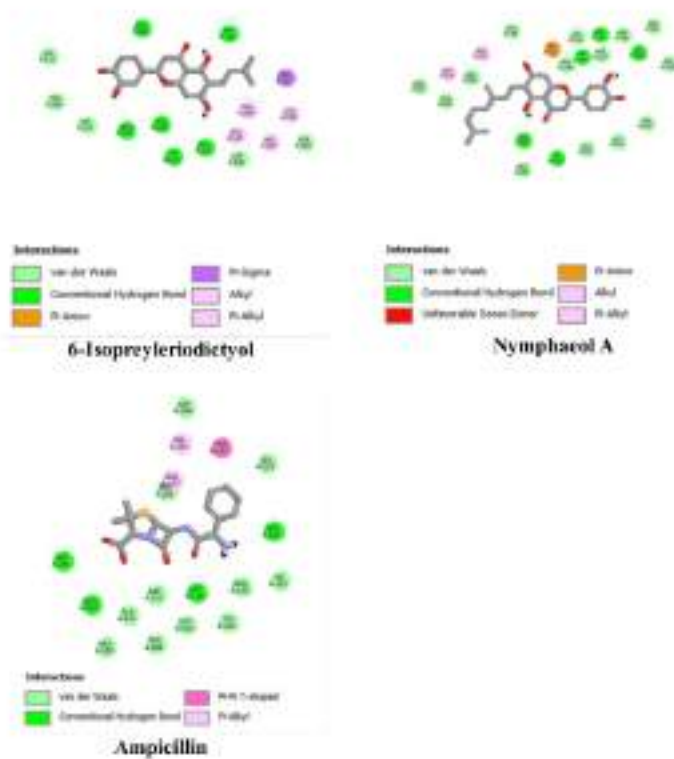


Figure 3: 2D interaction of the best-docked flavonoids and ampicillin with *Propionibacterium acnes* surface sinilidase (PDB ID: 7LBU).

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

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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**

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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*

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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.~~

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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-isoprenyleriodictyol, 6-isoprenyleriodictyol, 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

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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

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(SHARP EO-28 WH), ~~a rotary vacuum evaporator (EYELA Rotary evaporator N-1300E~~
V.S.Series), and ~~a freezer (Sanyo Medicool). 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~~.

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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).

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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.

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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~~.

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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.~~

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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

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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 Macahulleitii A was is a prenylated flavonoid found in the *Macaranga hulleitii* 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

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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).

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~~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

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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

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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

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

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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

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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

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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*,

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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

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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

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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-isoprenyleriodictyol, nymphaeol A, ~~nymphacol~~nymphameol 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.~~

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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

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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-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	Macahulleitin 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

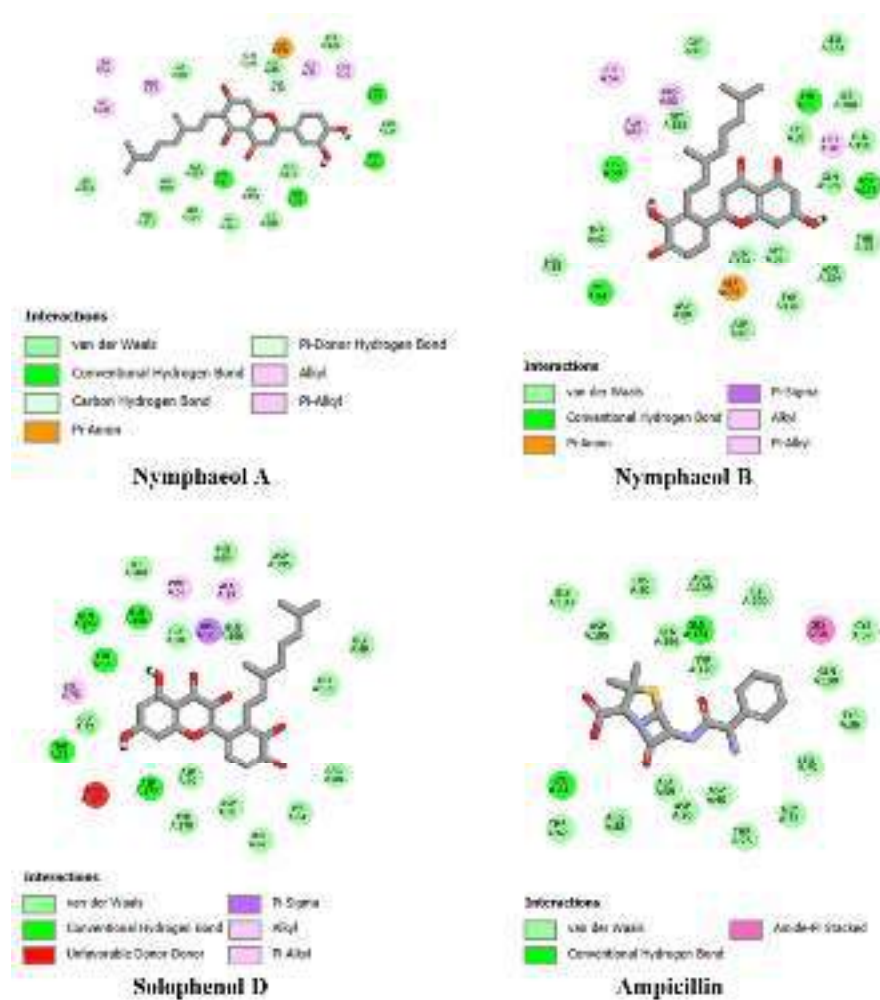


Figure 1: 2D interaction of the best docked flavonoids and ampicillin with *Staphylococcus aureus* tyrosyl-tRNA synthetase (PDB ID: 1JJJ)

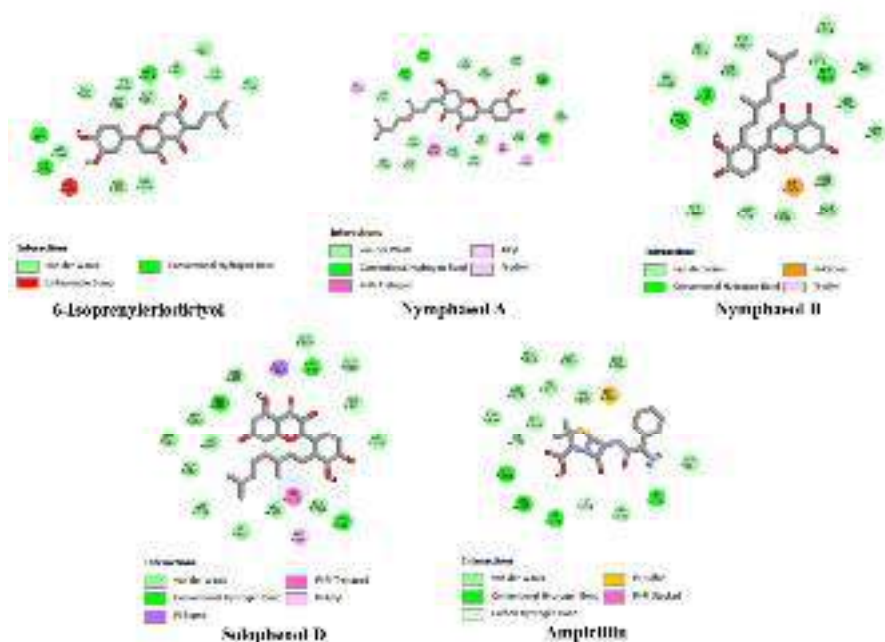


Figure 2: 2D interaction of the best-docked flavonoids and ampicillin with *Streptococcus mutans* antigen I/II (PDB ID: 3IPK).

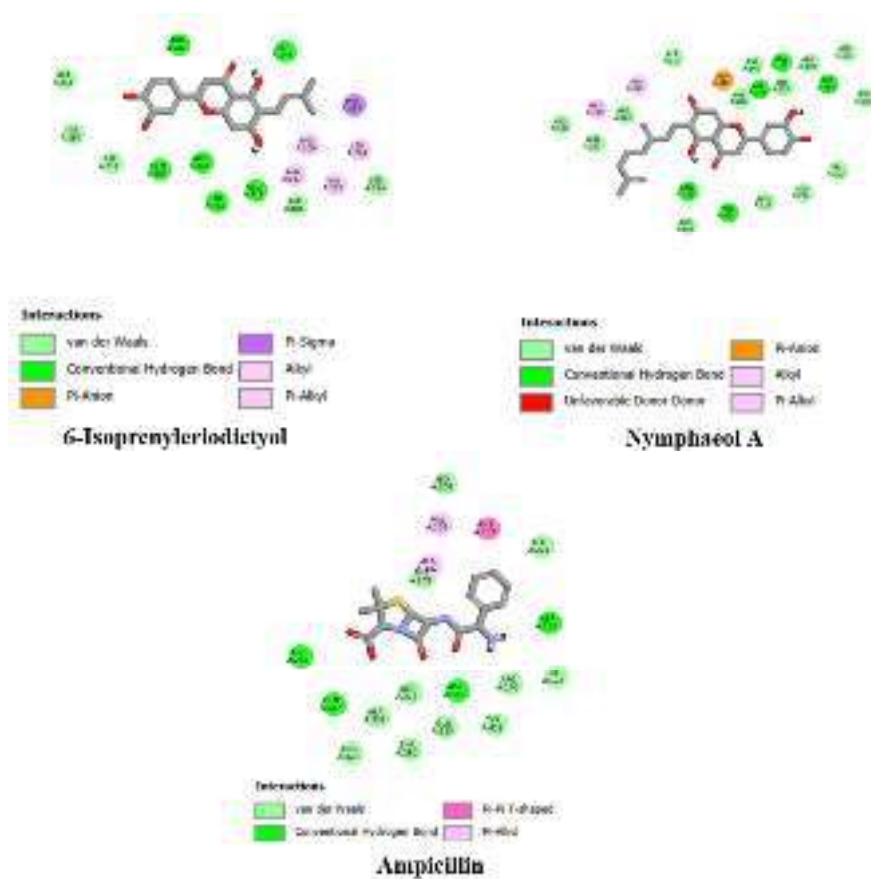


Figure 3: 2D interaction of the best-docked flavonoids and ampicillin with *Propionibacterium acnes* surface sialidase (PDB ID: 7LBU).

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 hulletti* King ex Hook.f.
Leaf Extract

Document authors:

Eva Marliana

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

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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, parodontitis, oral mucosal lesions, and stomatitis. The bacteria can also produce exotoxins, leucocidin, 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.

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

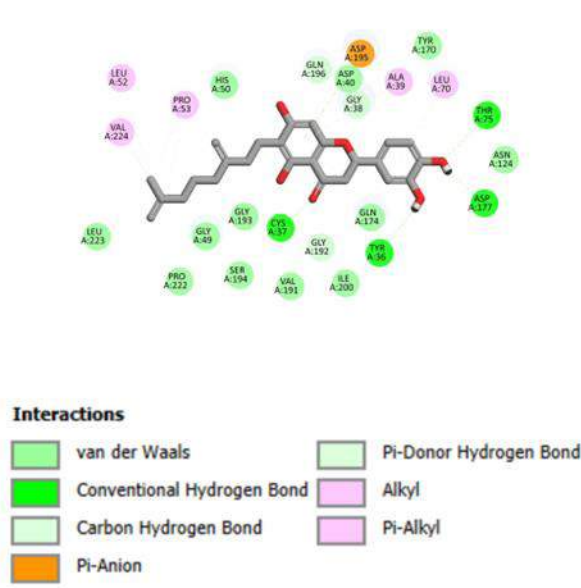
Sample	Concentration (%)	Inhibition Zone Diameter (mm)*		
		<i>Staphylococcus aureus</i>	<i>Streptococcus mutans</i>	<i>Propionibacterium acnes</i>
Methanol Extract	0	0	0	0
	0.15	6.67 ± 0.58	6.67 ± 0.58	0
	0.31	6.67 ± 0.58	6.67 ± 0.58	7.67 ± 0.58
	0.62	6.67 ± 0.58	7.0 ± 0.0	8.0 ± 1.0
	1.25	7.17 ± 0.29	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.

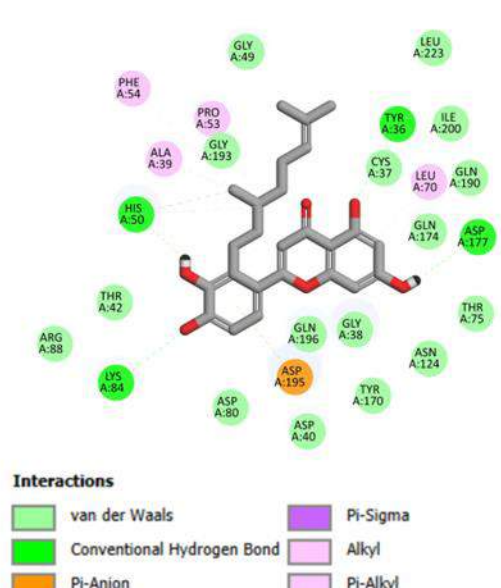
Table 2: Binding affinity of flavonoids against bacterial proteins

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

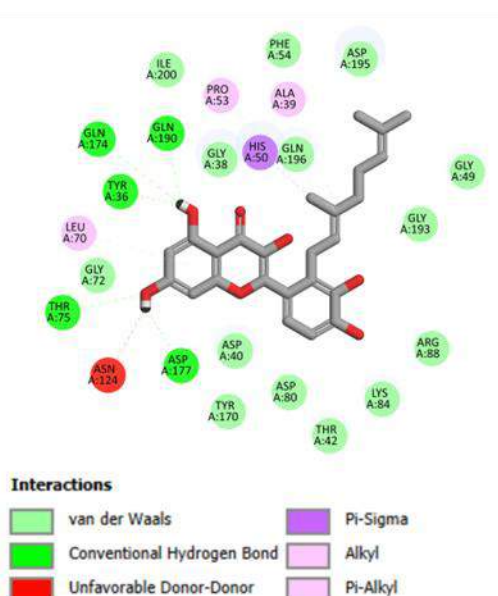
4	5-hydroxy-7,4'-dimethoxyflavone	-8.6	-7.8	-7.1
5	5-hydroxy-6,7,4'-trimethoxyflavone	-8.3	-7.5	-7.3
6	Macahuilettiin A	-7.6	-8.4	-7.1
7	Lonchocarpol A	-9.4	-7.8	-7.8
8	Nymphaeol A	-9.7	-8.9	-8.4
9	Nymphaeol B	-10.5	-8.9	-7.7
10	Nymphaeol C	-8.4	-8.3	-7.4
11	Solophenol D	-10.0	-8.7	-7.9
12	Ampicillin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



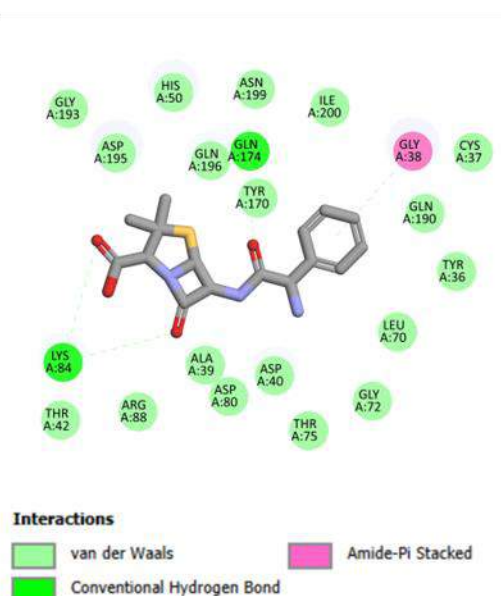
Nymphaeol A



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)

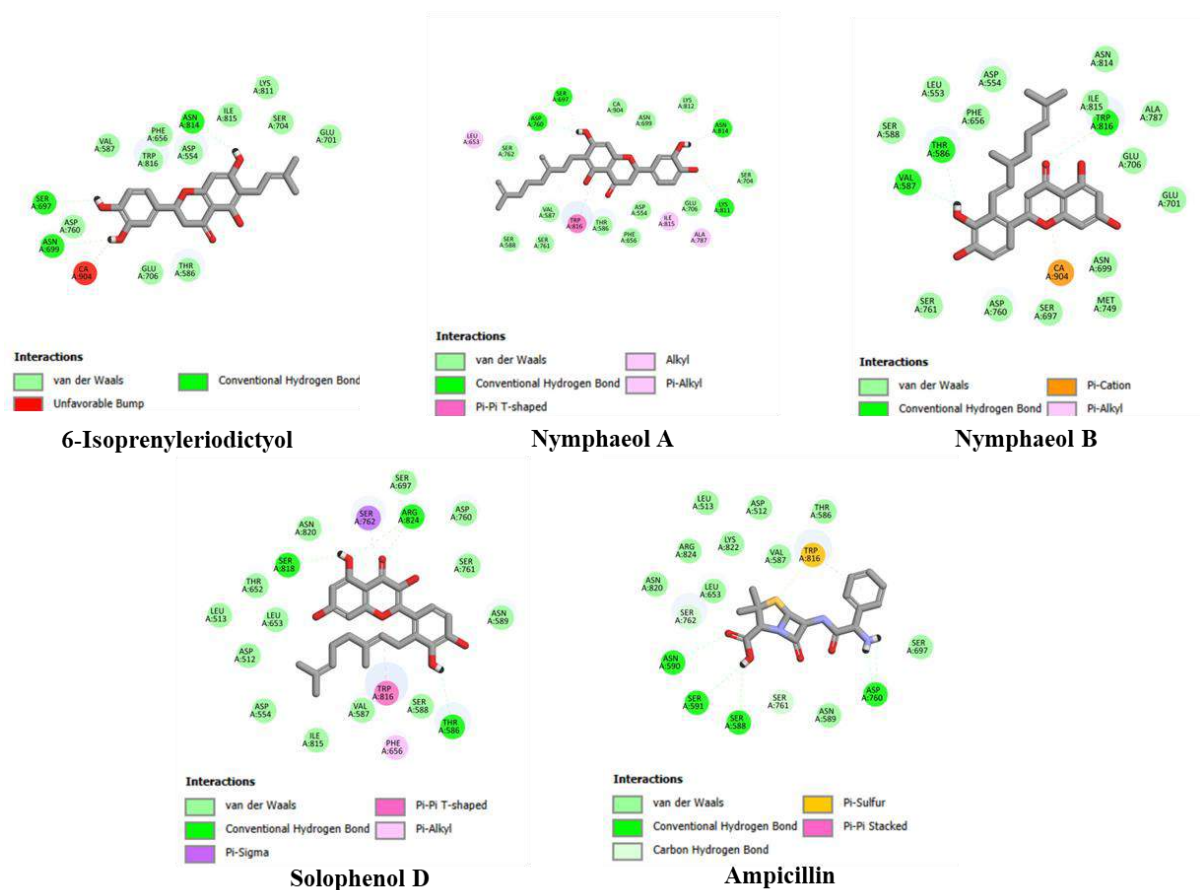


Figure 2: 2D visualization of interaction of the best-docked flavonoids and ampicillin with *Streptococcus mutans* antigen I/II (PDB ID: 3IPK).

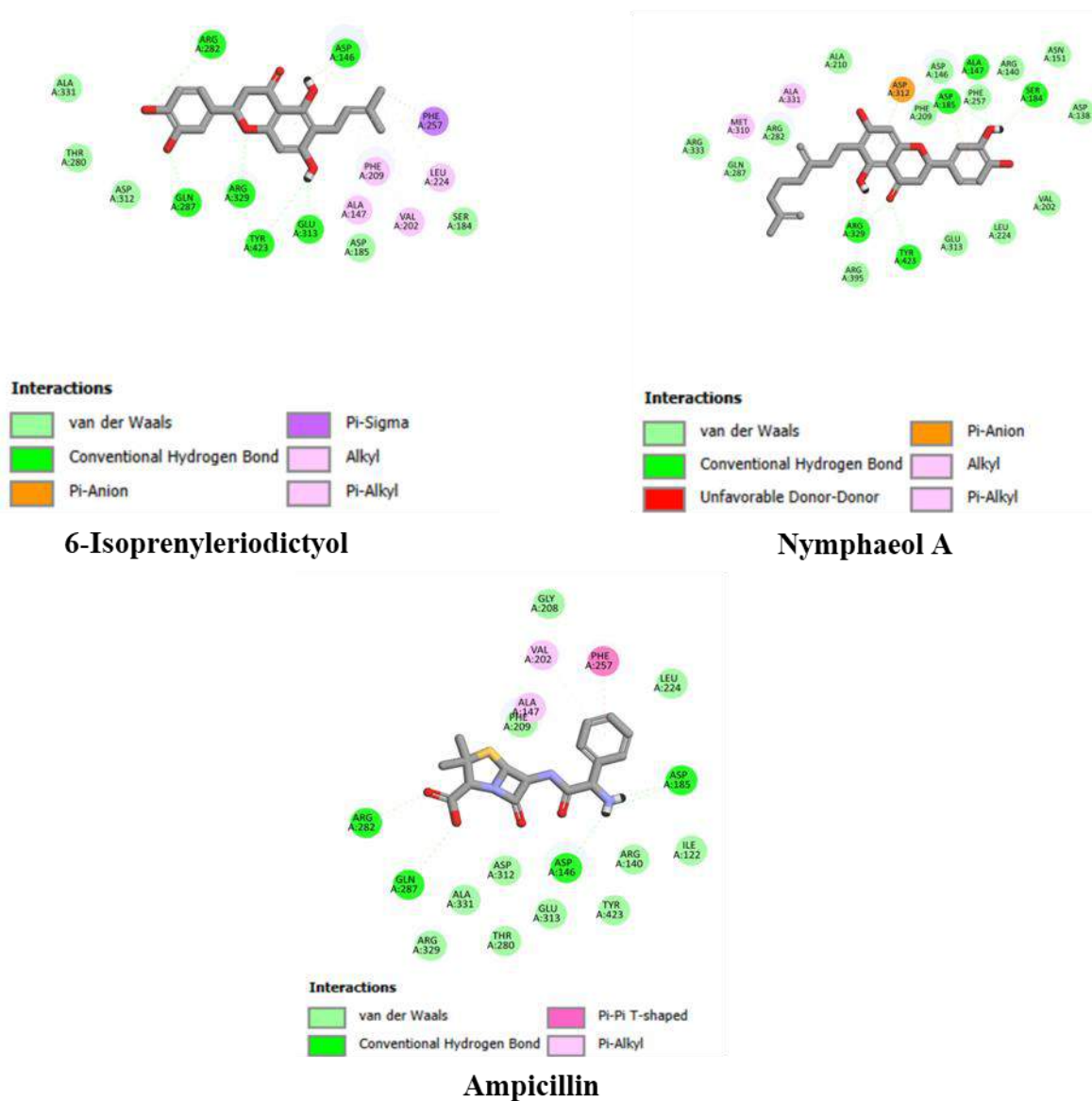


Figure 3: 2D visualization of interaction of the best-docked flavonoids and ampicillin with *Propionibacterium acnes* surface sialidase (PDB ID: 7LBU).



Re: Revision

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 1 attachment (881 KB)

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Best regards

Abiodun

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

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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.

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

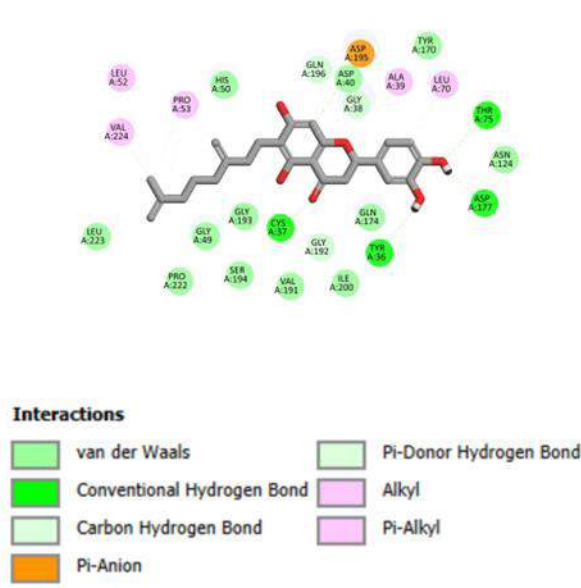
Sample	Concentration (%)	Inhibition Zone Diameter (mm)*		
		<i>Staphylococcus aureus</i>	<i>Streptococcus mutans</i>	<i>Propionibacterium acnes</i>
Methanol Extract	0	0	0	0
	0.15	6.67 ± 0.58	6.67 ± 0.58	0
	0.31	6.67 ± 0.58	6.67 ± 0.58	7.67 ± 0.58
	0.62	6.67 ± 0.58	7.0 ± 0.0	8.0 ± 1.0
	1.25	7.17 ± 0.29	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.

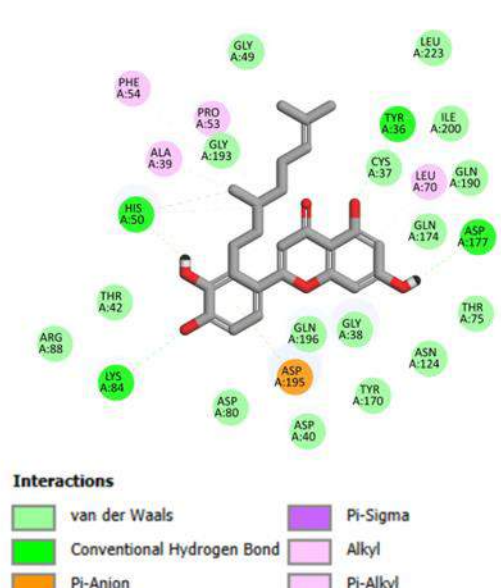
Table 2: Binding affinity of flavonoids against bacterial proteins

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

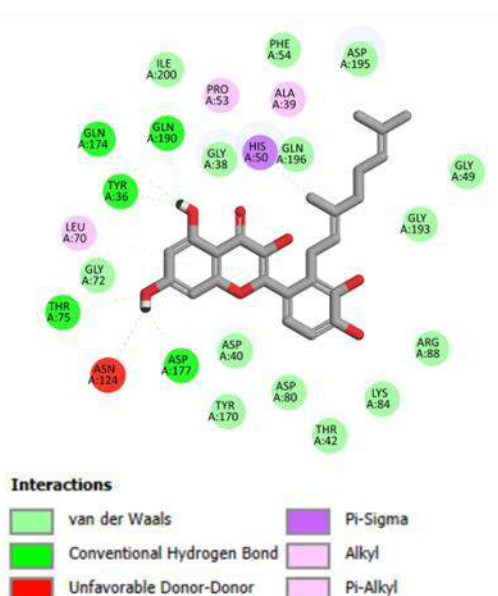
4	5-hydroxy-7,4'-dimethoxyflavone	-8.6	-7.8	-7.1
5	5-hydroxy-6,7,4'-trimethoxyflavone	-8.3	-7.5	-7.3
6	Macahuilettiin A	-7.6	-8.4	-7.1
7	Lonchocarpol A	-9.4	-7.8	-7.8
8	Nymphaeol A	-9.7	-8.9	-8.4
9	Nymphaeol B	-10.5	-8.9	-7.7
10	Nymphaeol C	-8.4	-8.3	-7.4
11	Solophenol D	-10.0	-8.7	-7.9
12	Ampicillin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



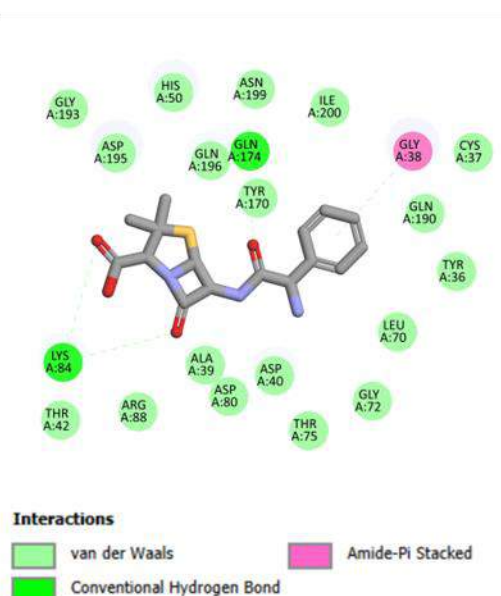
Nymphaeol A



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)

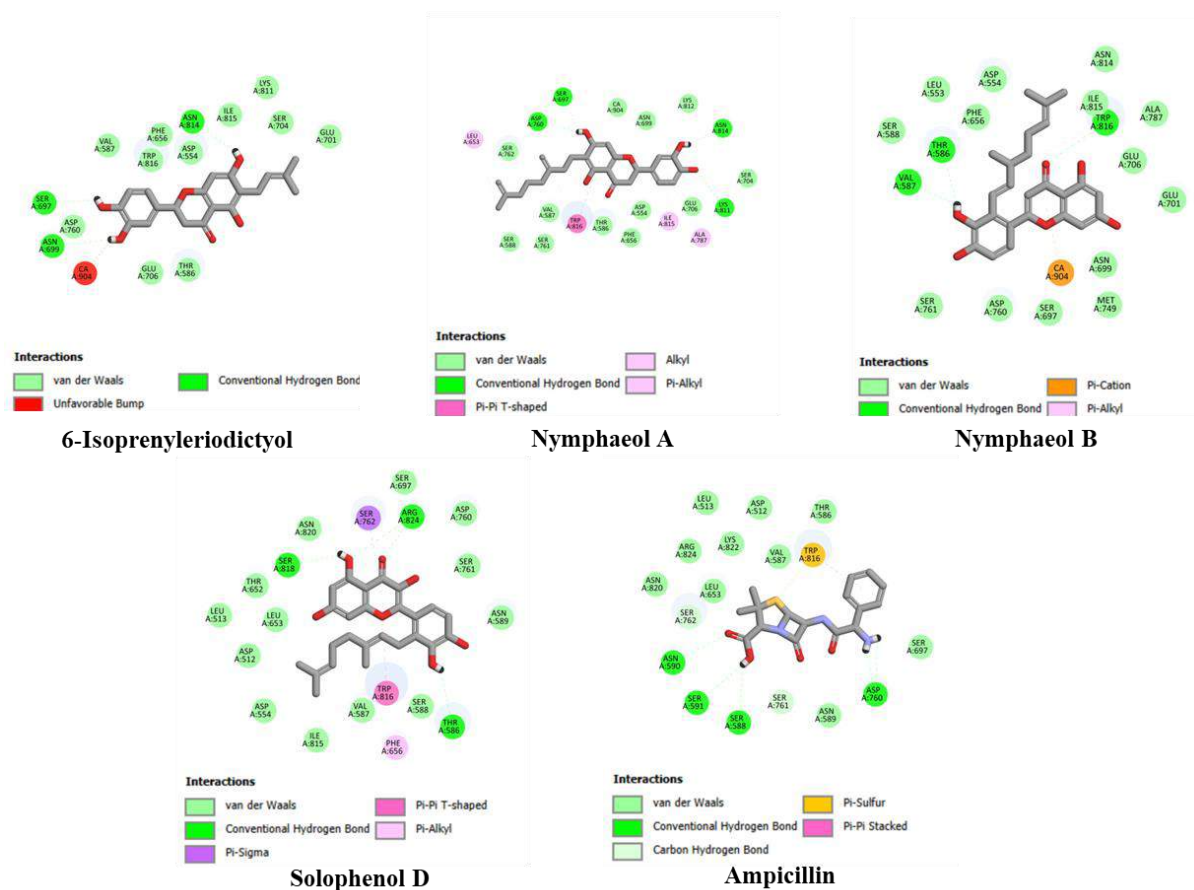


Figure 2: 2D visualization of interaction of the best-docked flavonoids and ampicillin with *Streptococcus mutans* antigen I/II (PDB ID: 3IPK).

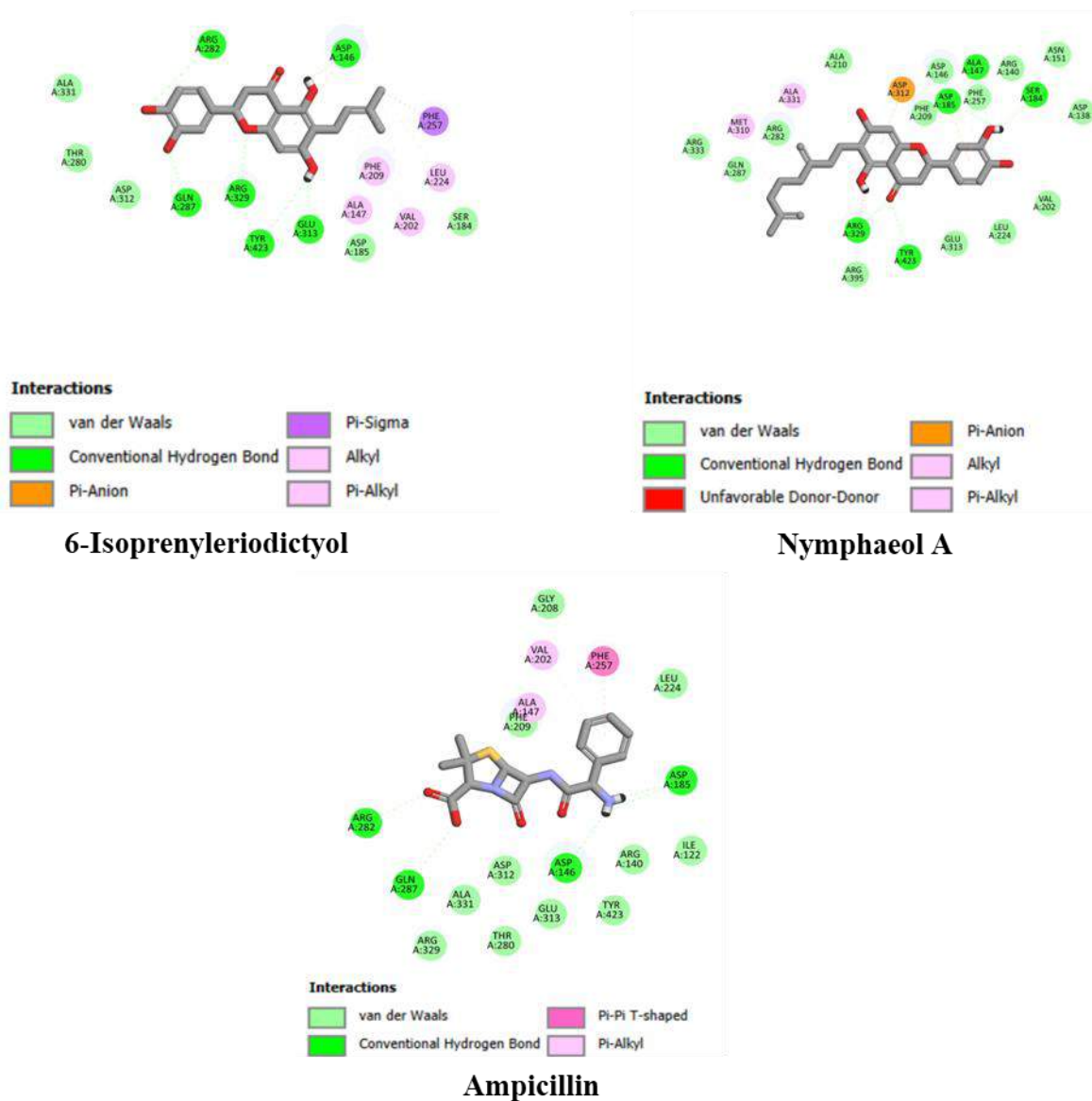


Figure 3: 2D visualization of interaction of the best-docked flavonoids and ampicillin with *Propionibacterium acnes* surface sialidase (PDB ID: 7LBU).



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

Commented [EF1]: of

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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 macahuillettin 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.

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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).

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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

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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-isoprenyleriodytol, 6-isoprenyleriodytol, solophenol D, nymphaeol A, nymphaeol B, and nymphaeol C.¹⁴⁻¹⁷ Macahuletiiin A is 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, 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.

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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	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

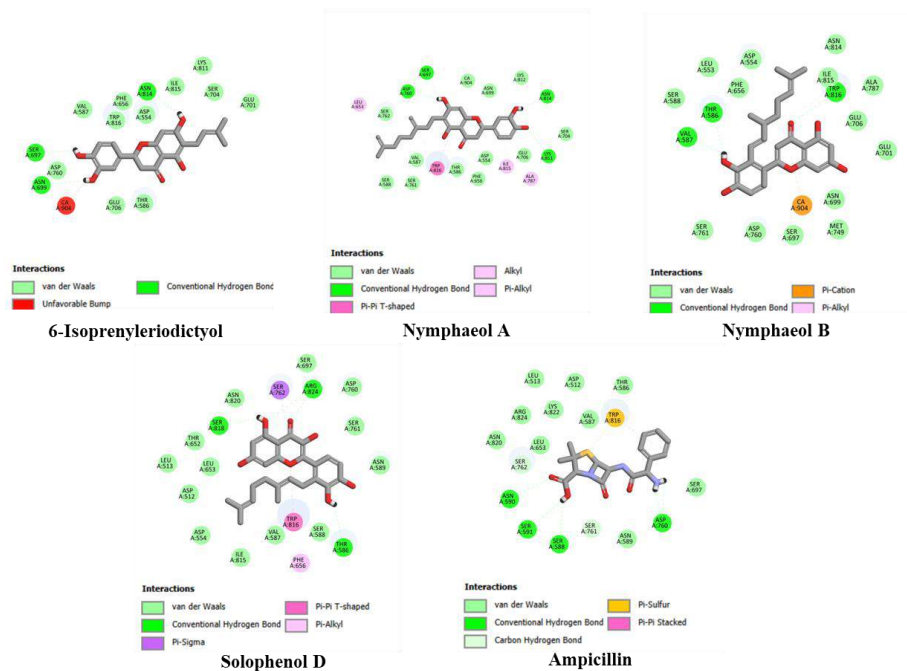


Figure 2: 2D interaction of the best-docked flavonoids and ampicillin with *Streptococcus mutans* antigen I/II (PDB ID: 3IPK).

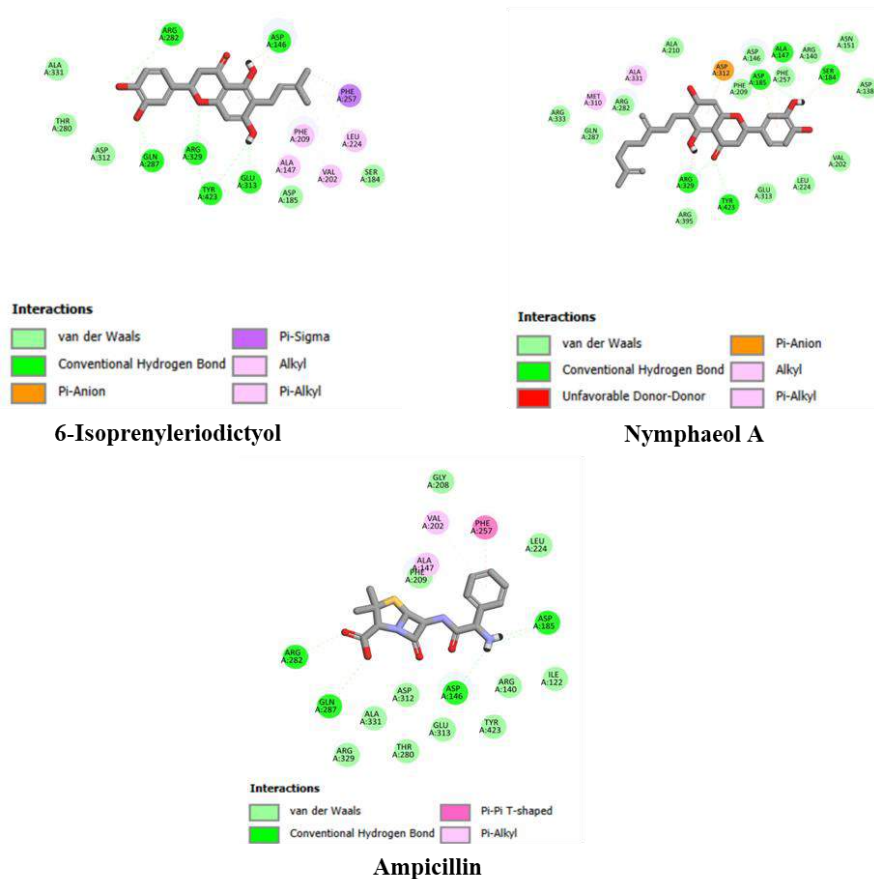


Figure 3: 2D interaction of the best-docked flavonoids and ampicillin with *Propionibacterium acnes* surface sialidase (PDB ID: 7LBU).



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**

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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 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, 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

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

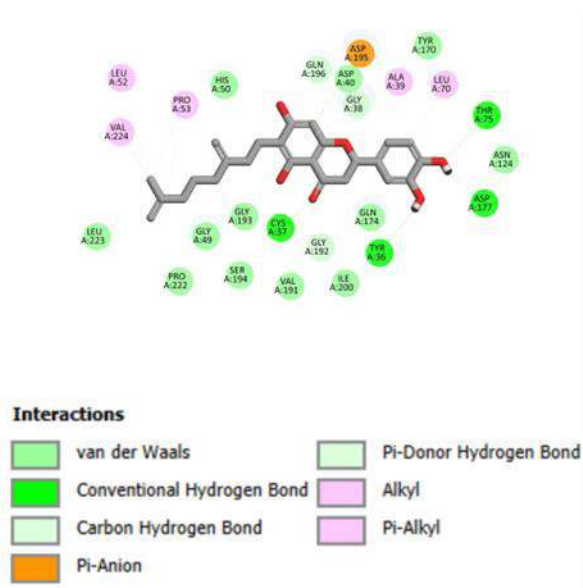
Sample	Concentration (%)	Inhibition Zone (mm)*		
		<i>Staphylococcus</i>	<i>Streptococcus</i>	<i>Propionibacterium</i>
		<i>aureus</i>	<i>mutans</i>	<i>acnes</i>
Methanol Extract	0	0	0	0
	0.15	6.67±0.58	6.67±0.58	0
	0.31	6.67±0.58	6.67±0.58	7.67±0.58
	0.62	6.67±0.58	7.0±0.0	8.0±1.0
	1.25	7.17±0.29	7.33±0.58	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

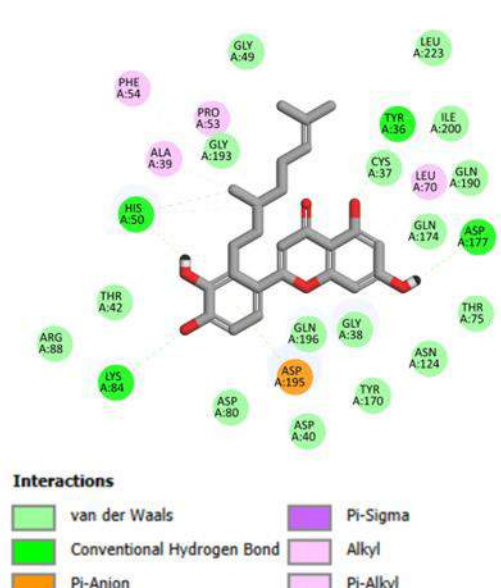
*Data were obtained from triplicate experiments.

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

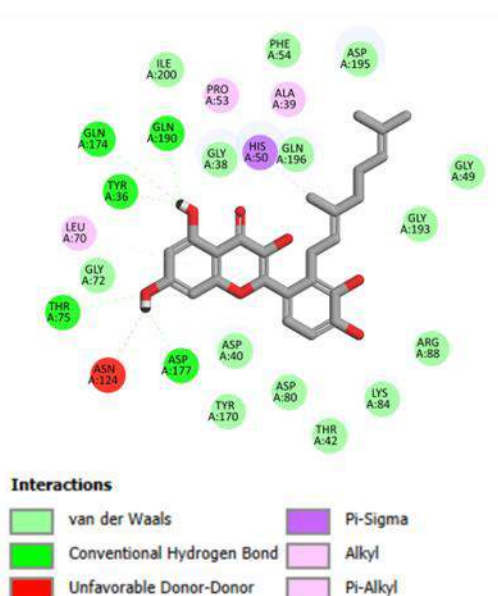
No.	Compound	Binding Affinity (kcal/mol)		
		1JJJ	3IPK	7LBU
1	4'- <i>O</i> -methyl-8-isoprenylnaringenin	-7.4	-8.1	-7.7
2	4'- <i>O</i> -methyl-8-isoprenyleriodictyol	-7.2	-8.1	-7.6
3	6-Isoprenyleriodictyol	-7.7	-8.7	-8.0
4	5-hydroxy-7,4'-dimethoxyflavone	-8.6	-7.8	-7.1
5	5-hydroxy-6,7,4'-trimethoxyflavone	-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



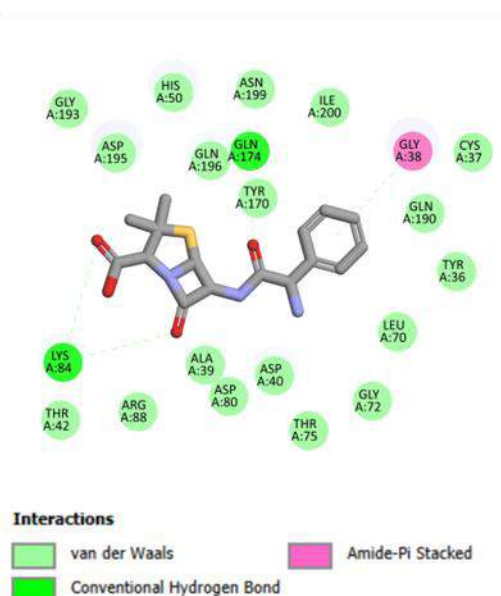
Nymphaeol A



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)

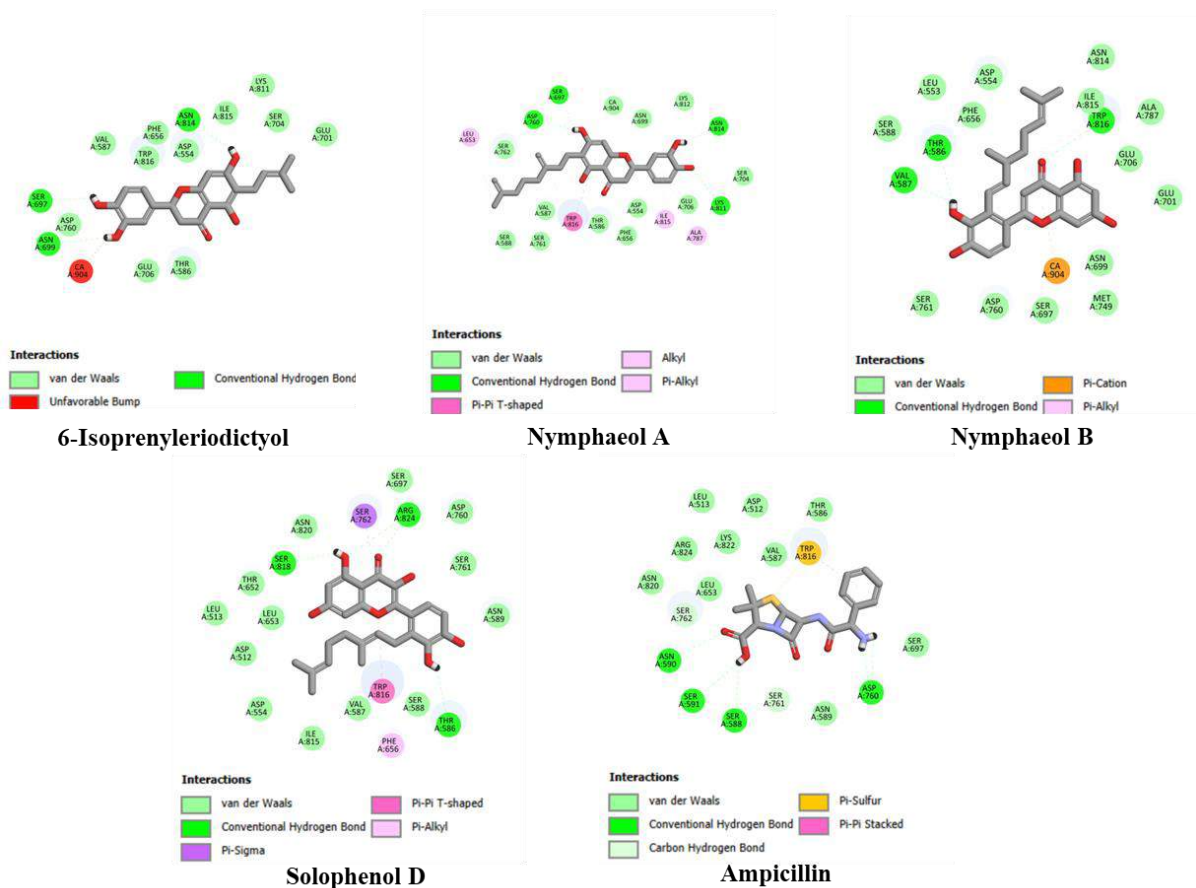


Figure 2: 2D interaction of the best-docked flavonoids and ampicillin with *Streptococcus mutans* antigen I/II (PDB ID: 3IPK).

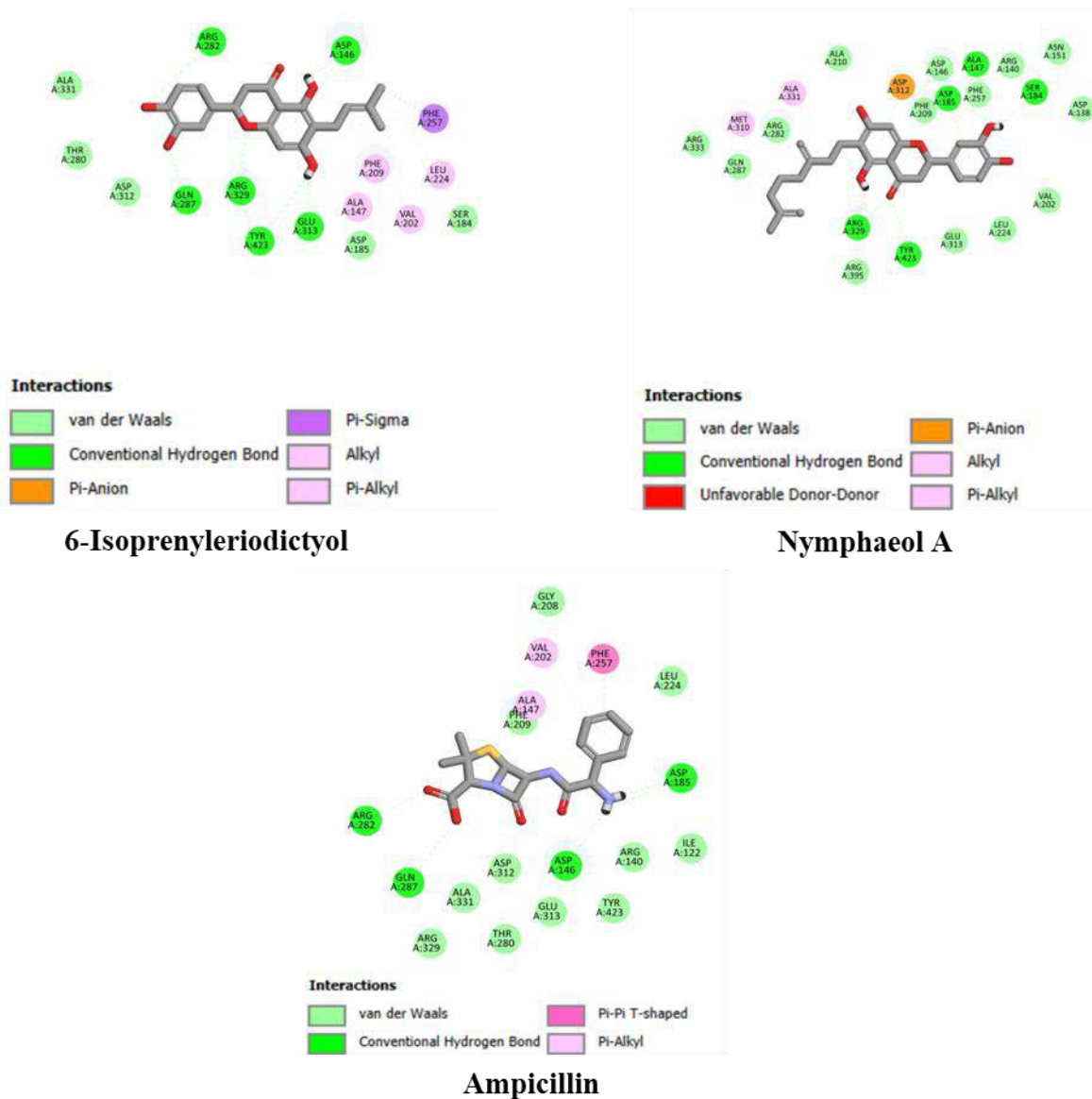


Figure 3: 2D interaction of the best-docked flavonoids and ampicillin with *Propionibacterium acnes* surface sialidase (PDB ID: 7LBU).

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

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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.



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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**

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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 (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, antiparasitic, 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 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, 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.

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

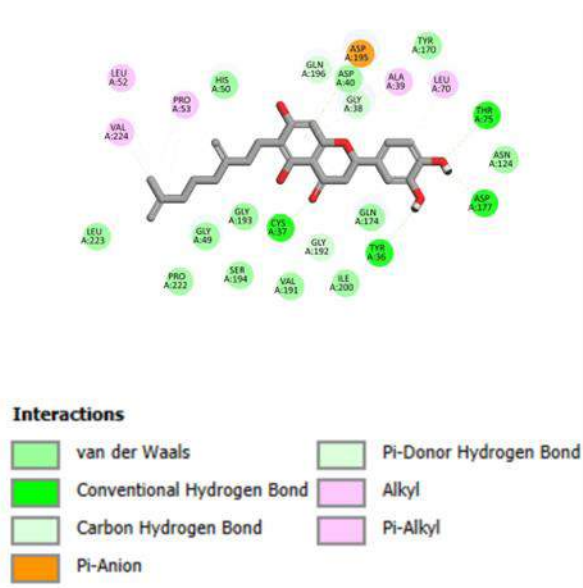
Sample	Concentration (%)	Inhibition Zone (mm)*		
		<i>Staphylococcus</i>	<i>Streptococcus</i>	<i>Propionibacterium</i>
		<i>aureus</i>	<i>mutans</i>	<i>acnes</i>
Methanol Extract	0	0	0	0
	0.15	6.67±0.58	6.67±0.58	0
	0.31	6.67±0.58	6.67±0.58	7.67±0.58
	0.62	6.67±0.58	7.0±0.0	8.0±1.0
	1.25	7.17±0.29	7.33±0.58	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

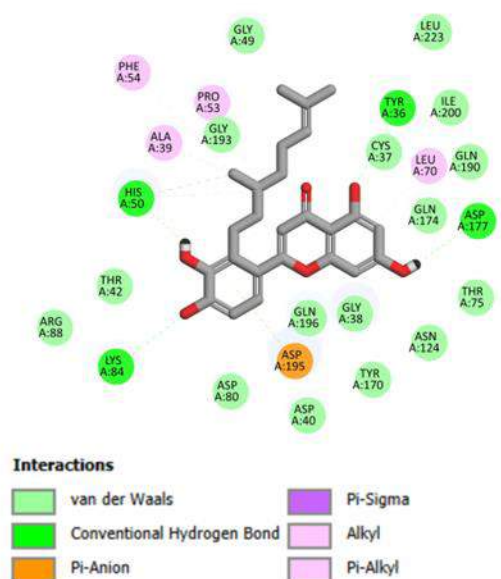
*Data were obtained from triplicate experiments.

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

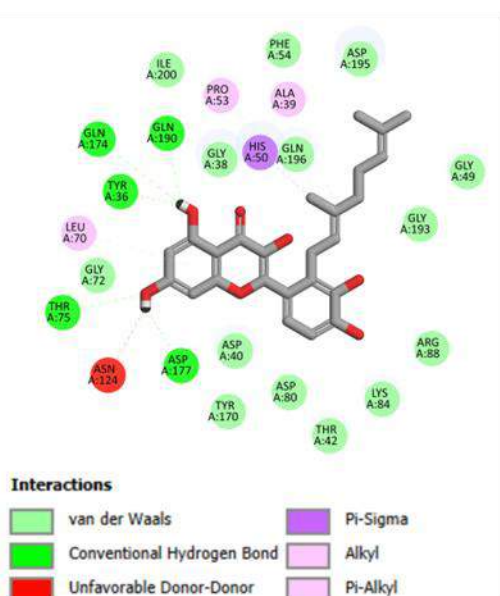
No.	Compound	Binding Affinity (kcal/mol)		
		1JJJ	3IPK	7LBU
1	4'- <i>O</i> -methyl-8-isoprenylnaringenin	-7.4	-8.1	-7.7
2	4'- <i>O</i> -methyl-8-isoprenyleriodictyol	-7.2	-8.1	-7.6
3	6-Isoprenyleriodictyol	-7.7	-8.7	-8.0
4	5-hydroxy-7,4'-dimethoxyflavone	-8.6	-7.8	-7.1
5	5-hydroxy-6,7,4'-trimethoxyflavone	-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



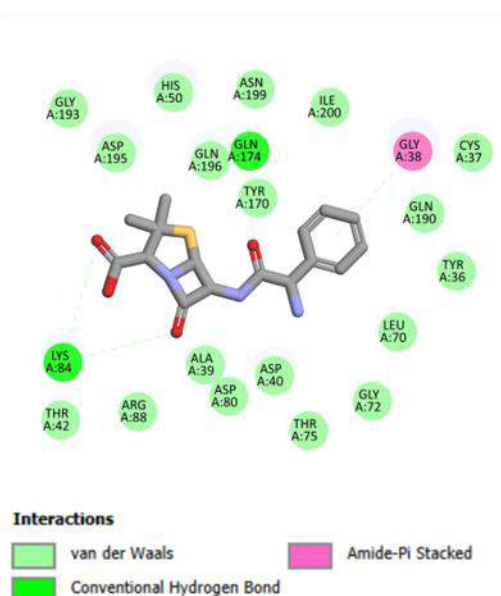
Nymphaeol A



Nymphaeol B

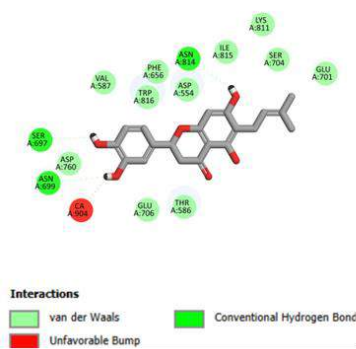


Solophenol D

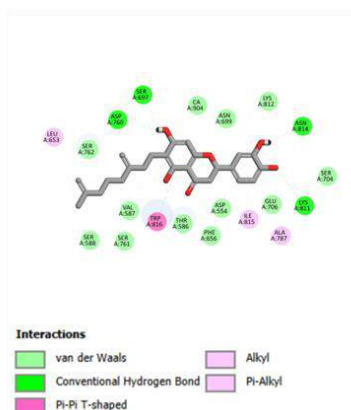


Ampicillin

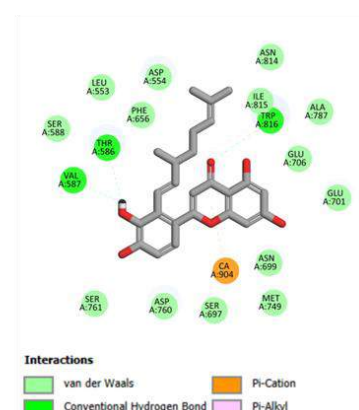
Figure 1: 2D interaction of the best docked flavonoids and ampicillin with *Staphylococcus aureus* tyrosyl-tRNA synthetase (PDB ID: 1JIJ)



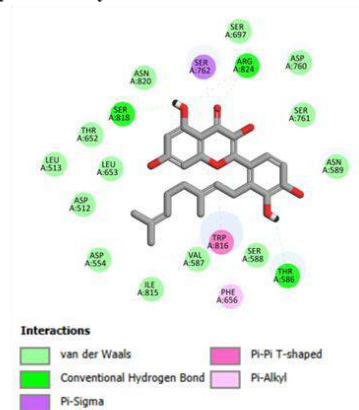
6-Isoprenylioridictyol



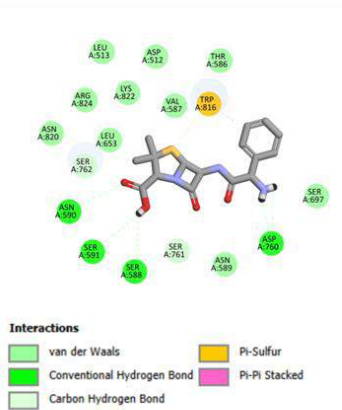
Nymphaeol A



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).

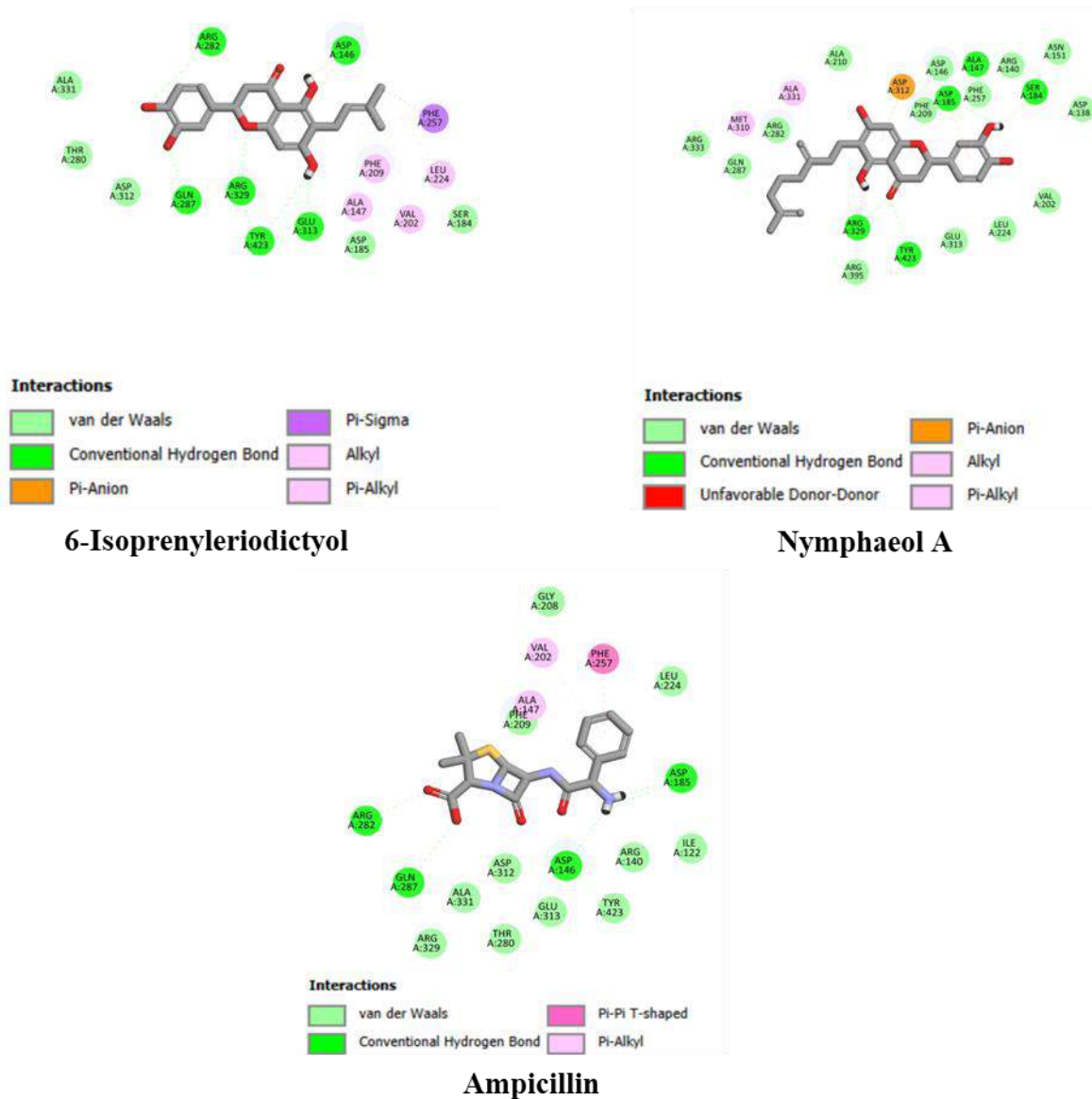


Figure 3: 2D interaction of the best-docked flavonoids and ampicillin with *Propionibacterium acnes* surface sialidase (PDB ID: 7LBU).



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Leaf Extract**

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

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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**

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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 hulleitii* 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. hulleitii* 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.

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

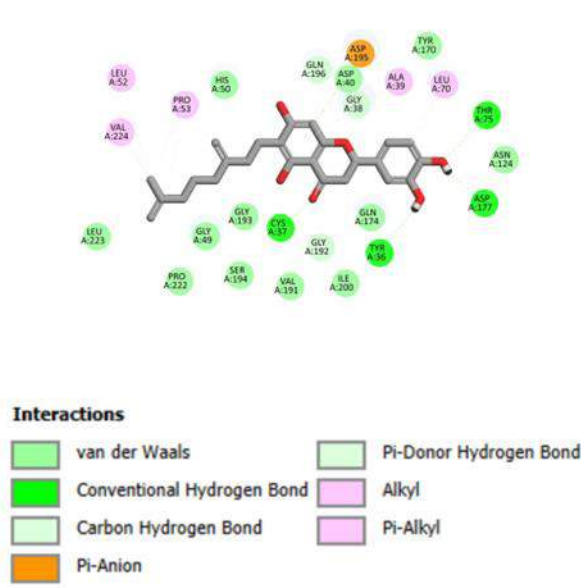
Sample	Concentration (%)	Inhibition Zone Diameter (mm)*		
		<i>Staphylococcus aureus</i>	<i>Streptococcus mutans</i>	<i>Propionibacterium acnes</i>
Methanol Extract	0	0	0	0
	0.15	6.67 ± 0.58	6.67 ± 0.58	0
	0.31	6.67 ± 0.58	6.67 ± 0.58	7.67 ± 0.58
	0.62	6.67 ± 0.58	7.0 ± 0.0	8.0 ± 1.0
	1.25	7.17 ± 0.29	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.

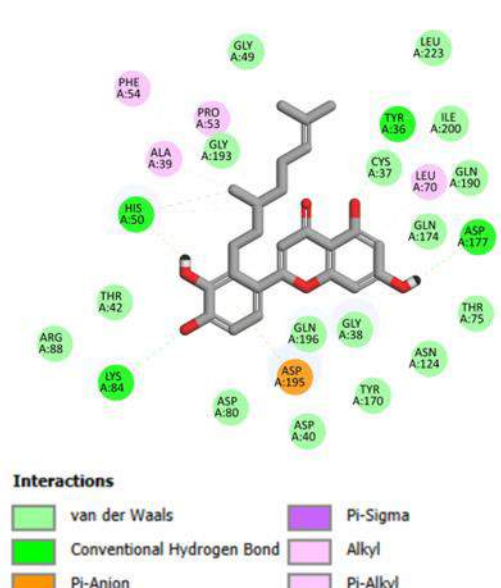
Table 2: Binding affinity of flavonoids against bacterial proteins

No.	Compound	Binding Affinity (kcal/mol)		
		1JIJ	3IPK	7LBU
1	4'- <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

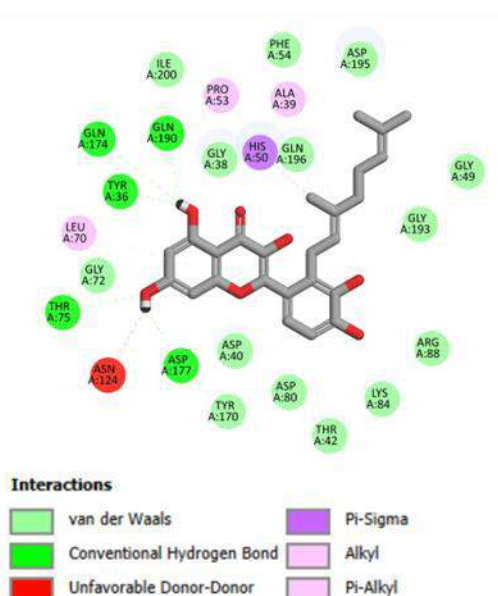
4	5-hydroxy-7,4'-dimethoxyflavone	-8.6	-7.8	-7.1
5	5-hydroxy-6,7,4'-trimethoxyflavone	-8.3	-7.5	-7.3
6	Macahuilettiin A	-7.6	-8.4	-7.1
7	Lonchocarpol A	-9.4	-7.8	-7.8
8	Nymphaeol A	-9.7	-8.9	-8.4
9	Nymphaeol B	-10.5	-8.9	-7.7
10	Nymphaeol C	-8.4	-8.3	-7.4
11	Solophenol D	-10.0	-8.7	-7.9
12	Ampicillin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



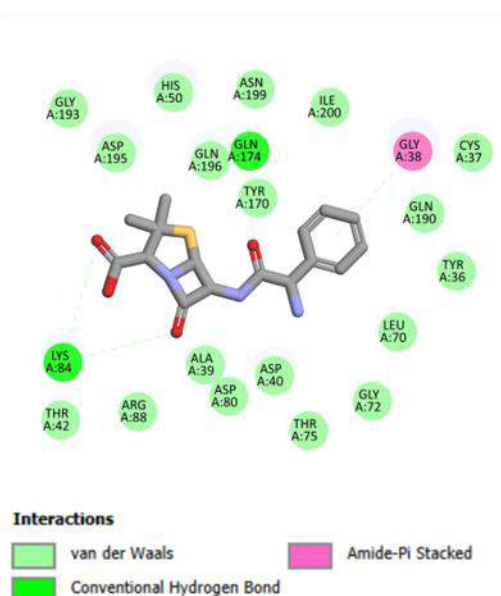
Nymphaeol A



Nymphaeol B



Solophenol D



Ampicillin

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

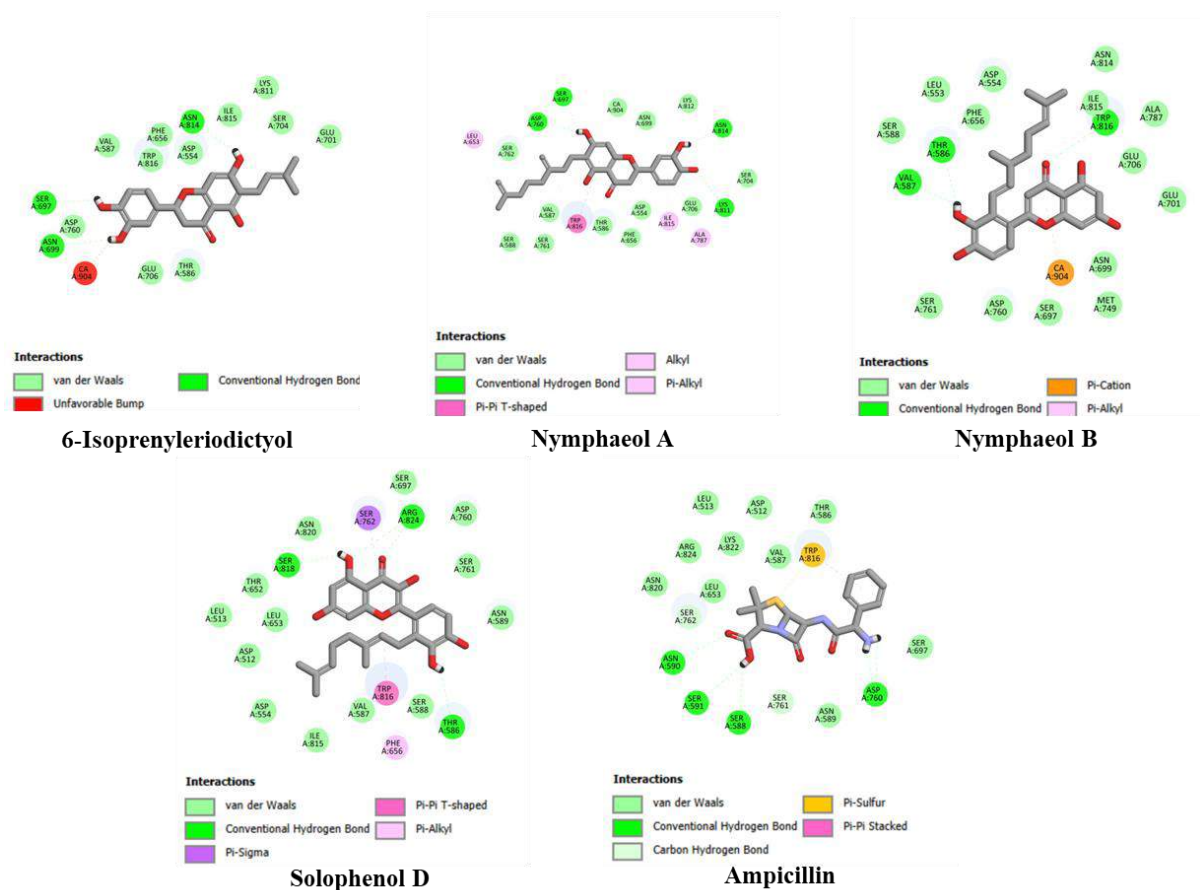


Figure 2: 2D visualization of interaction of the best-docked flavonoids and ampicillin with *Streptococcus mutans* antigen I/II (PDB ID: 3IPK).

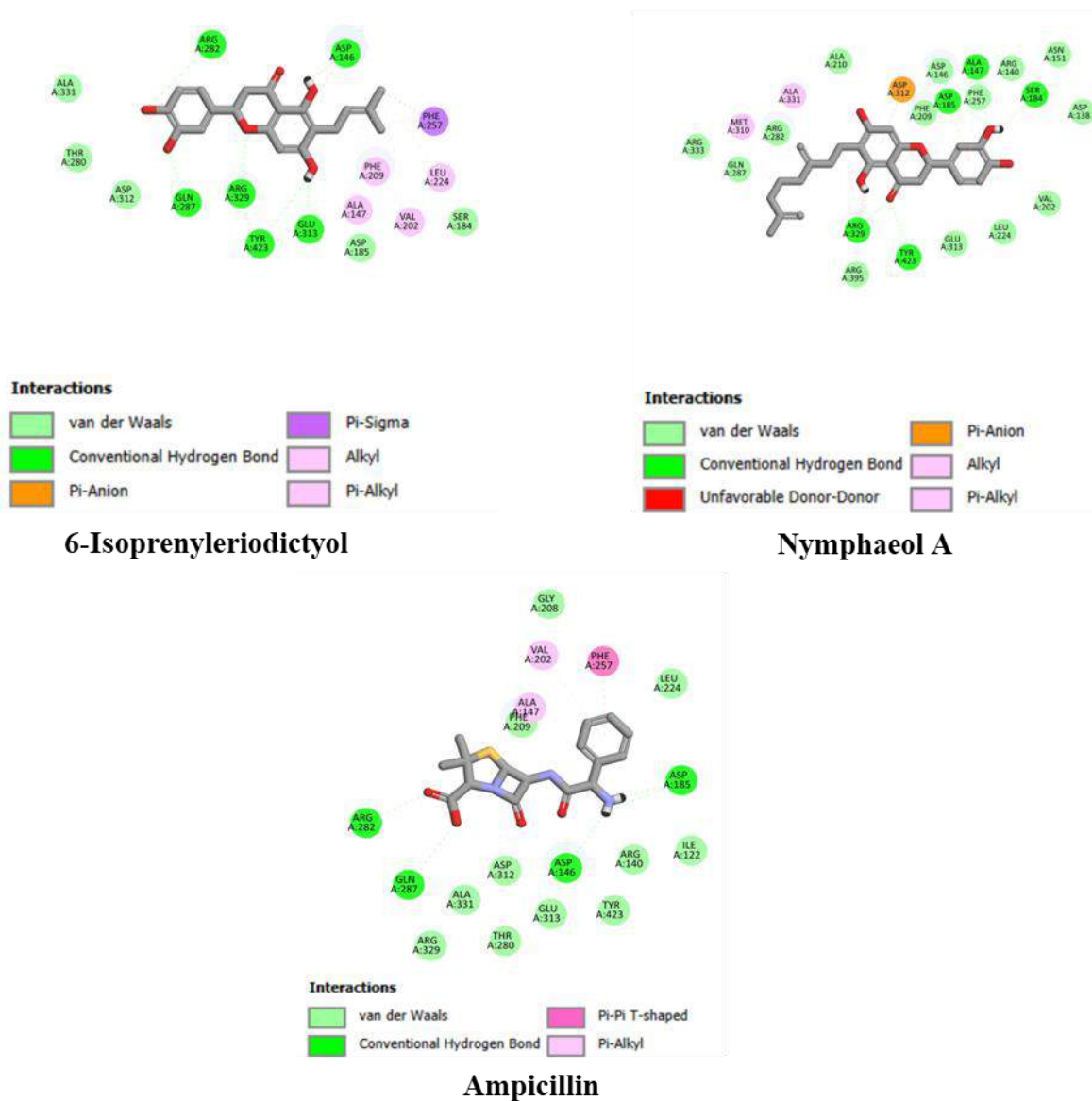


Figure 3: 2D visualization of interaction of the best-docked flavonoids and ampicillin with *Propionibacterium acnes* surface sialidase (PDB ID: 7LBU).



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

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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-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. 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.

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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

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Nine flavonoids containing either prenyl or geranyl groups and displaying antioxidant, antiparasitic, and anticancer activities have been isolated from three species (*Macaranga husei*, *Macaranga pearsonii*, and *Macaranga tanarius*) of *Macaranga* present in East Kalimantan: 4'-O-methyl-8-isoprenylnaringenin, 4'-O-methyl-8-isoprenylerydiol, 6-isoprenylerydiol, 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 husei* 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: 1JJJ), *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}

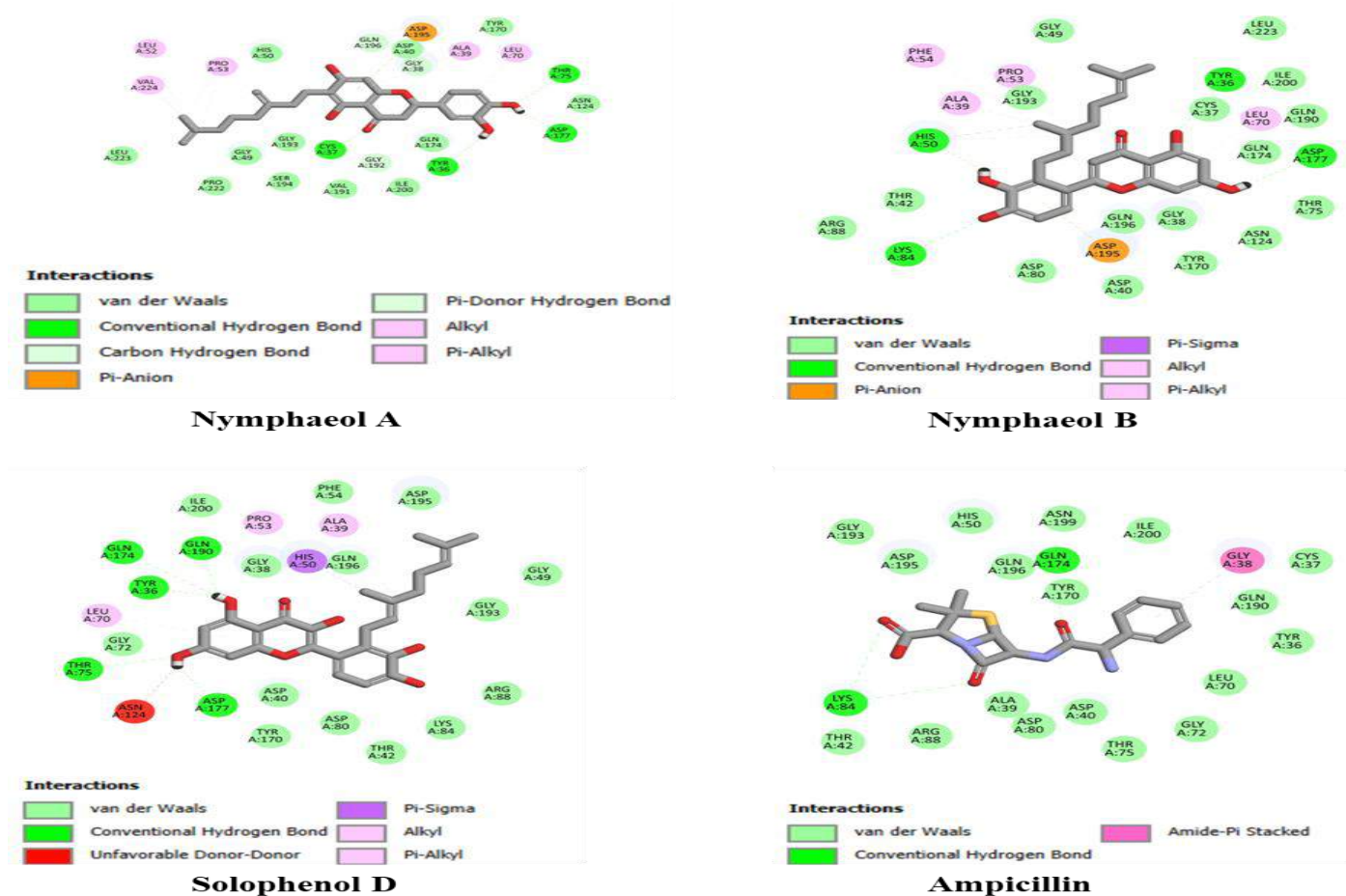


Figure 1: 2D visualization of interaction of the best docked flavonoids and ampicillin with *Staphylococcus aureus* tyrosyl-tRNA synthetase (PDB ID: 1JIJ)

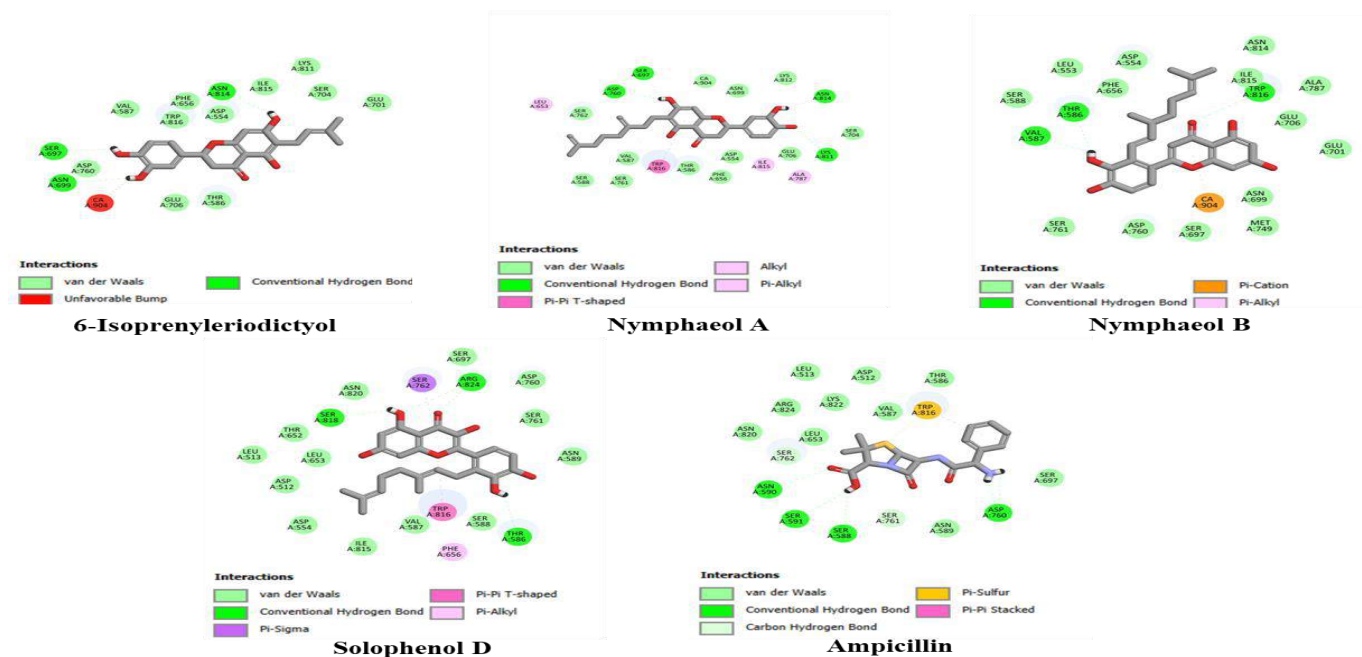


Figure 2: 2D visualization of interaction of the best-docked flavonoids and ampicillin with *Streptococcus mutans* antigen I/II (PDB ID: 3IPK).

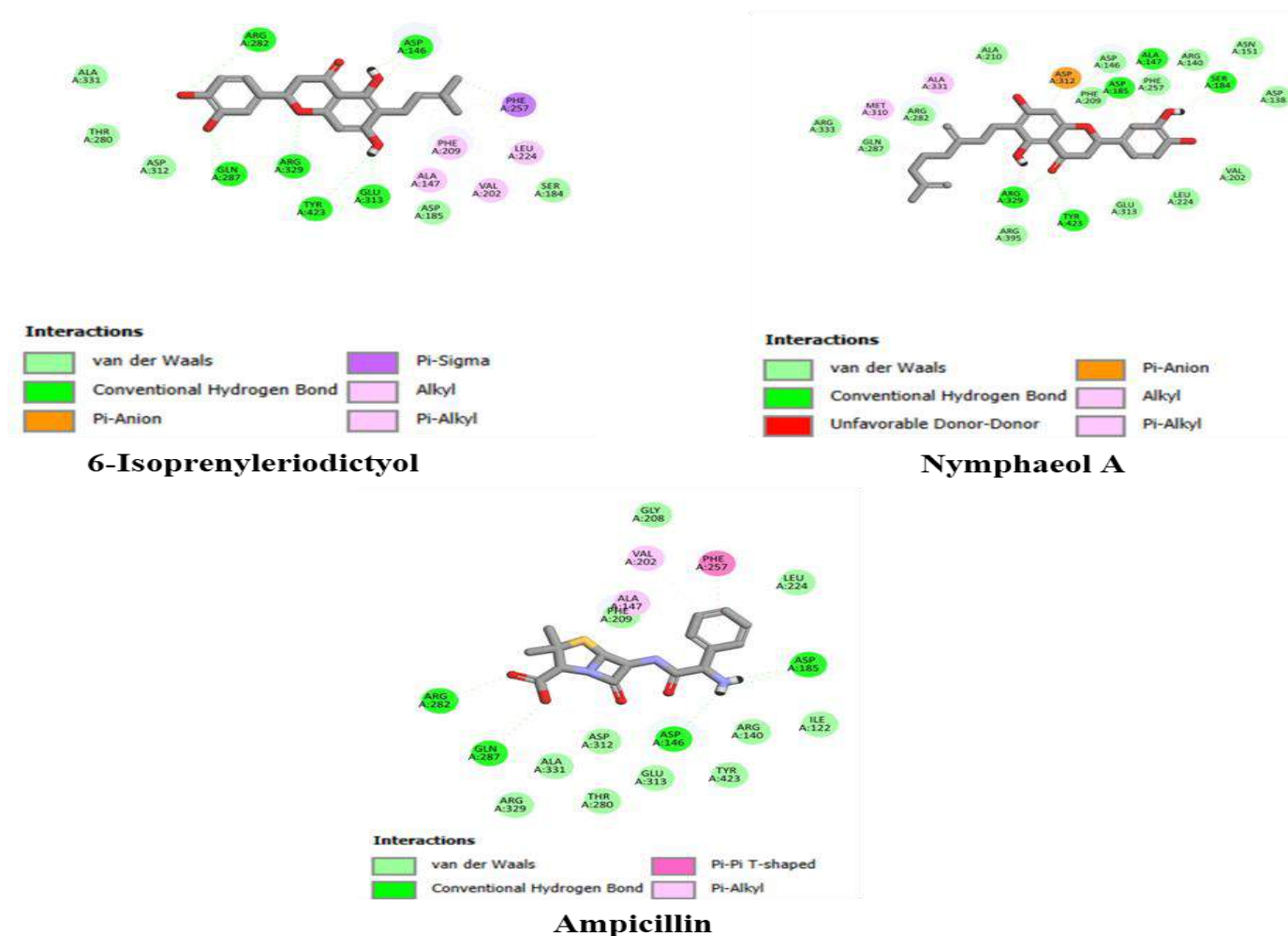


Figure 3: 2D visualization of interaction of the best-docked flavonoids and ampicillin with *Propionibacterium acnes* surface sialidase (PDB ID: 7LBU)

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.

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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.

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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 and 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**

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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.



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Original Research Article

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

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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.

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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

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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 (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)		
		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 (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}

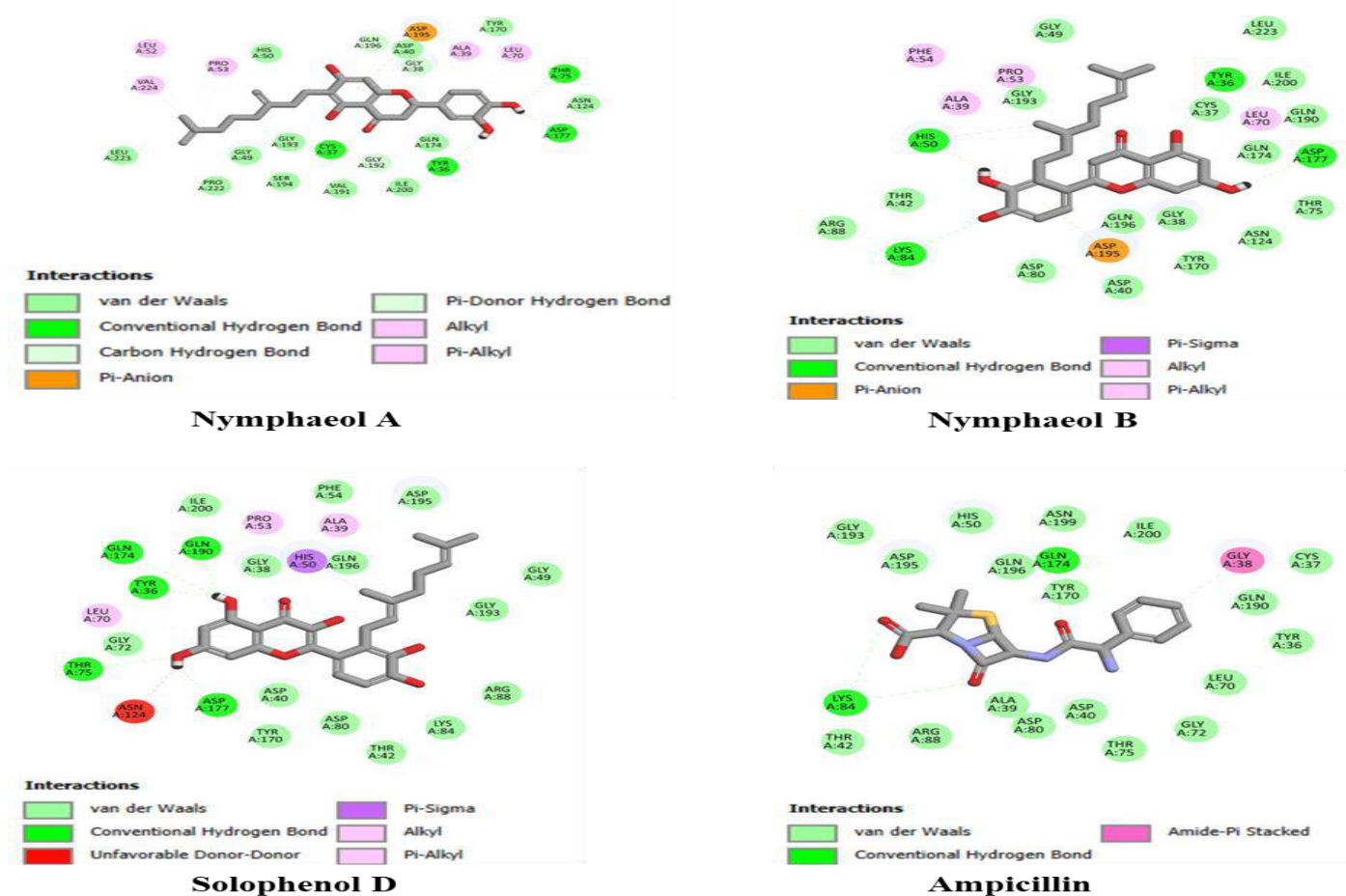


Figure 1: 2D visualization of interaction of the best docked flavonoids and ampicillin with *Staphylococcus aureus* tyrosyl-tRNA synthetase (PDB ID: 1JIJ)

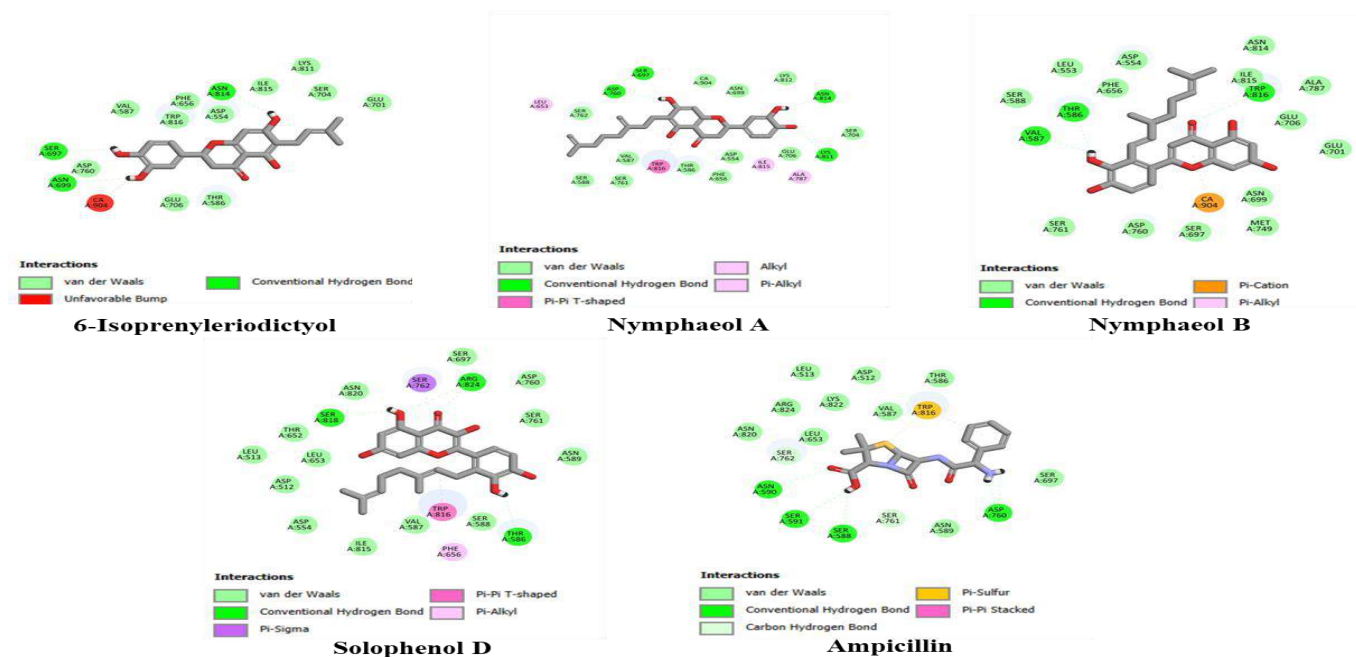


Figure 2: 2D visualization of interaction of the best-docked flavonoids and ampicillin with *Streptococcus mutans* antigen I/II (PDB ID: 3IPK).

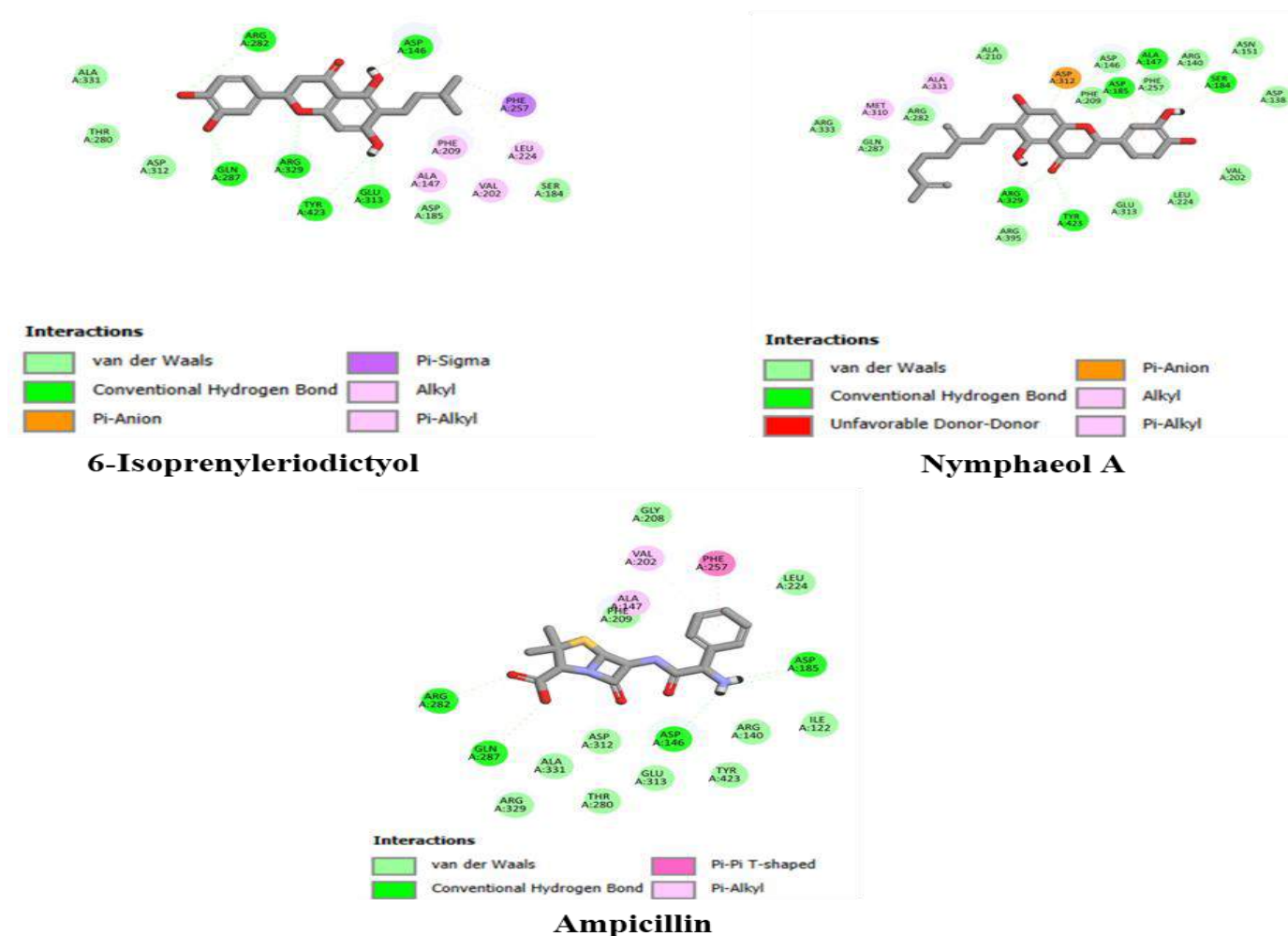


Figure 3: 2D visualization of interaction of the best-docked flavonoids and ampicillin with *Propionibacterium acnes* surface sialidase (PDB ID: 7LBU)

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.

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Original Research Article

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

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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.

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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

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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, 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 (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, 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 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}

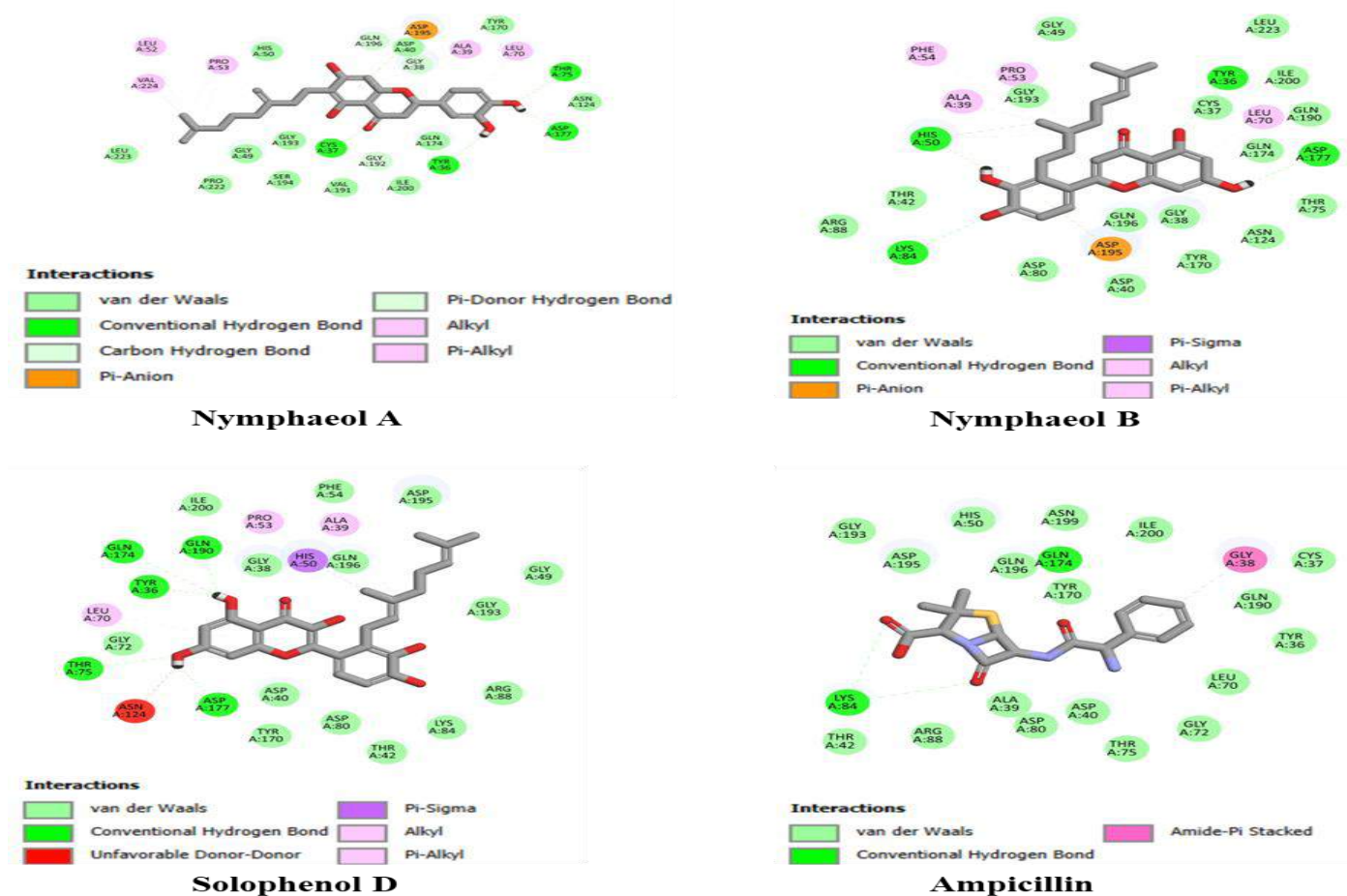


Figure 1: 2D visualization of interaction of the best docked flavonoids and ampicillin with *Staphylococcus aureus* tyrosyl-tRNA synthetase (PDB ID: 1JIJ)

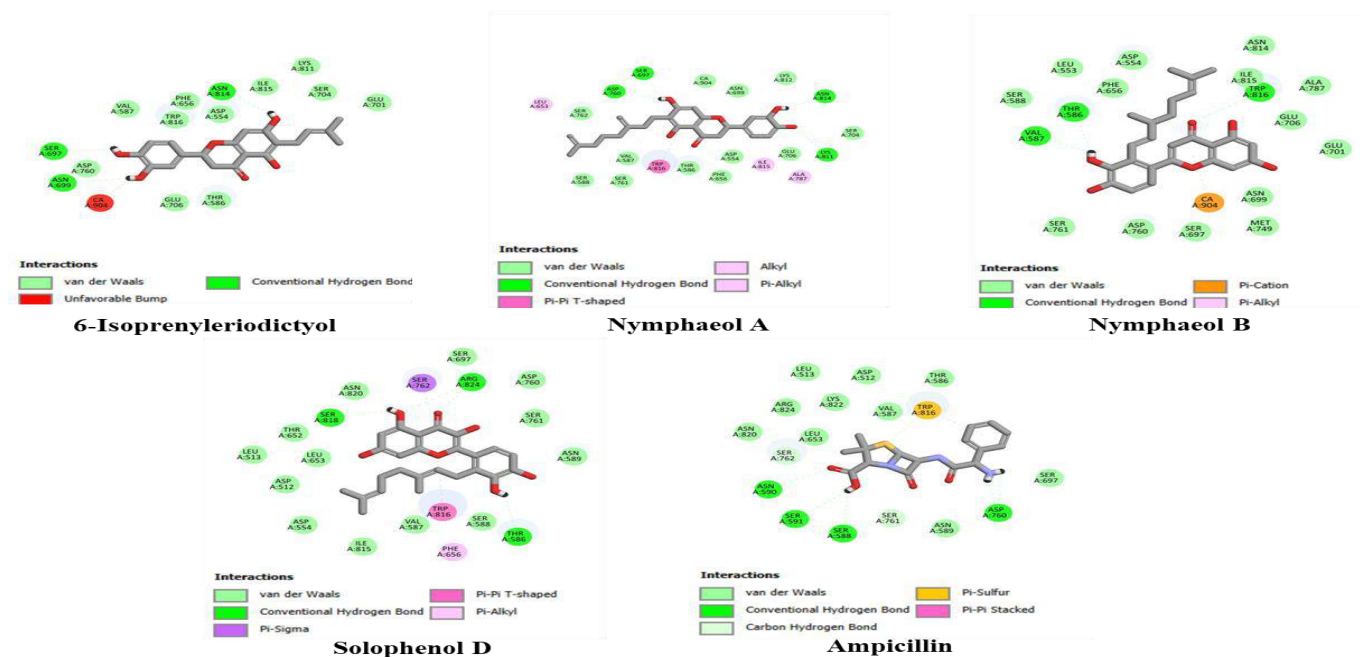


Figure 2: 2D visualization of interaction of the best-docked flavonoids and ampicillin with *Streptococcus mutans* antigen I/II (PDB ID: 3IPK).

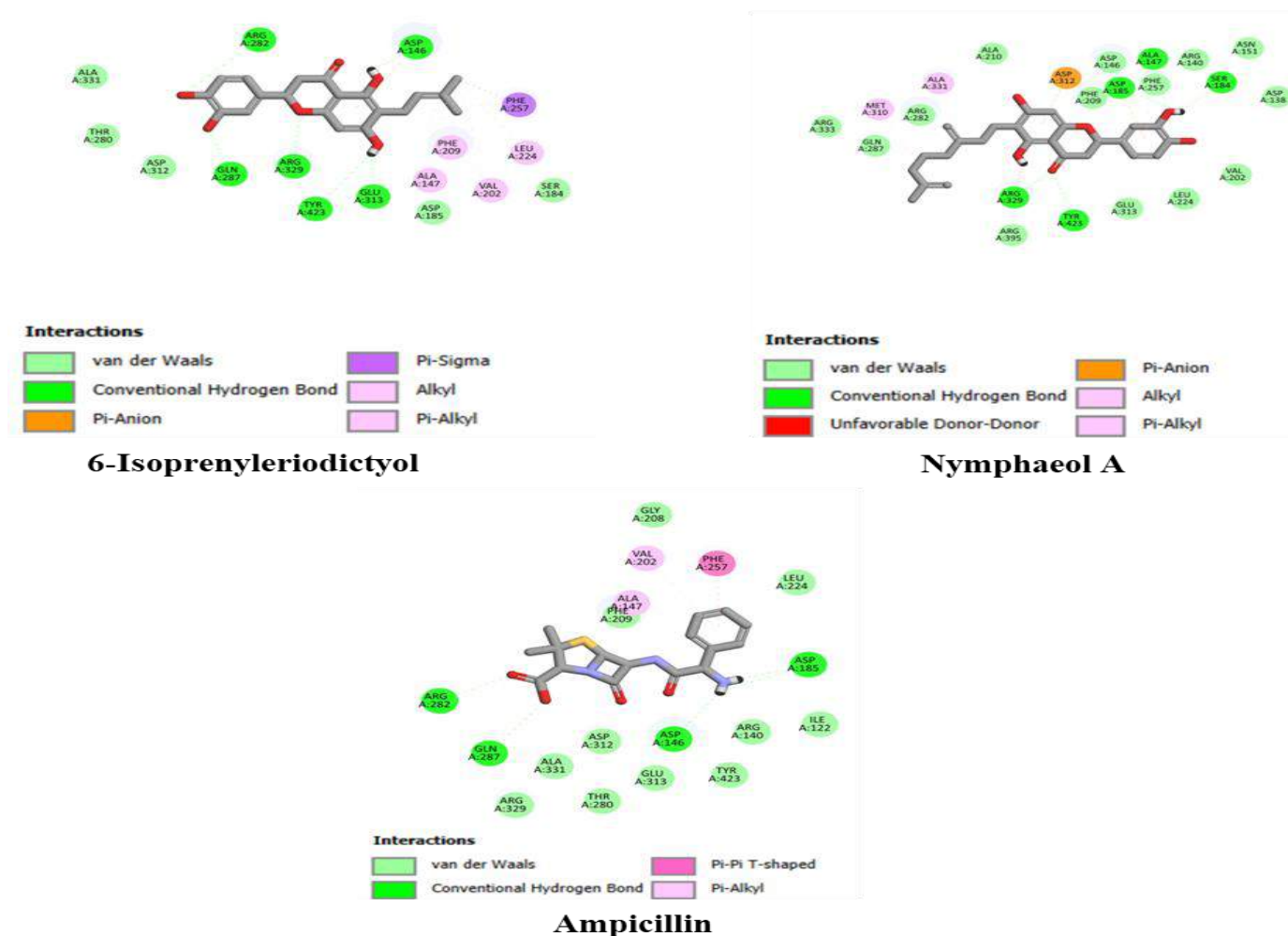


Figure 3: 2D visualization of interaction of the best-docked flavonoids and ampicillin with *Propionibacterium acnes* surface sialidase (PDB ID: 7LBU)

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.

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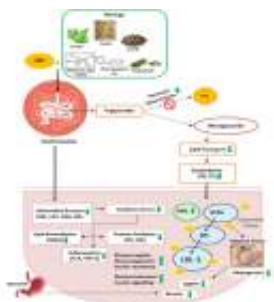
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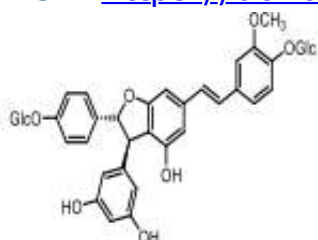
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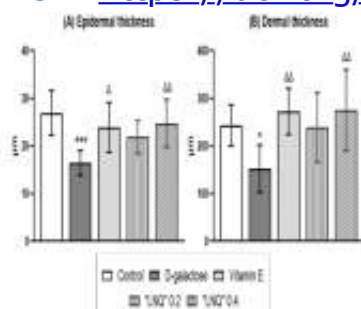
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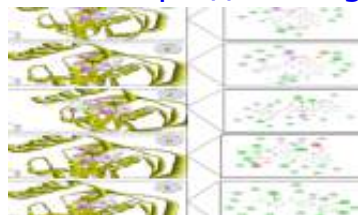
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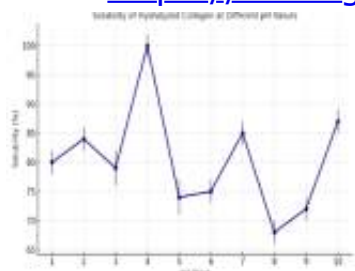
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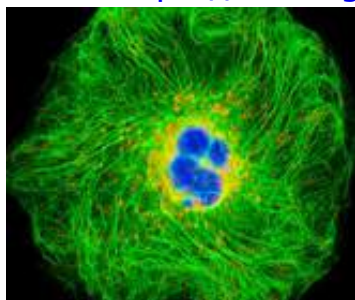
Characterization and Formulation of Anti-Aging Cream Using Collagen Derived from Sardine (*Sardinella spp.*) Fish Scales

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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

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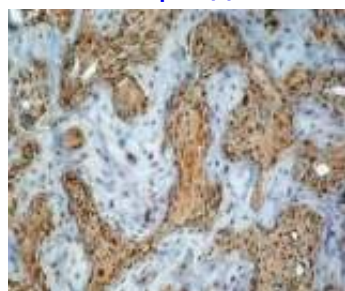
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Noer Ulfah, Chiquita Prahasanti, Theresia I. Budhy, Apriani W. Nelly, Esi Y. Fitrina, Zalfaa F. Yuda

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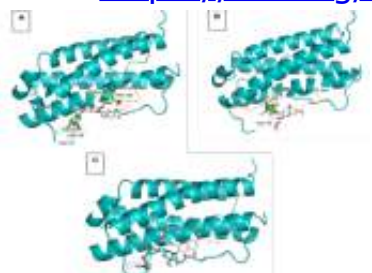
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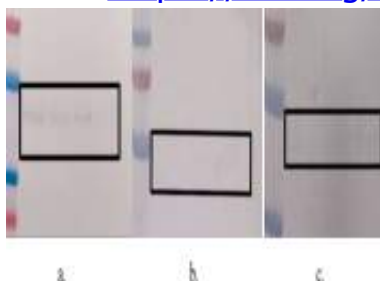
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Dira Hefni, Dachriyanus, Rahmad Abdillah, Nonda Riaknes, Fatma S Wahyuni

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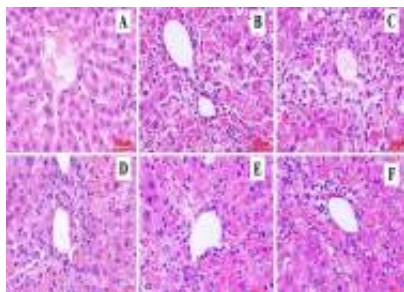
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