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**In vitro Evaluation and Molecular Docking Study of the Antibacterial Potential of *Macaranga hullettii* King ex Hook.f. Leaf Extract**Eva Marliana^{1,3}, Ade Danova^{2*}, Rita Hairani^{1,3}, Ritbey Ruga^{1,3}, Elvira Hermawati², Ana Yulvia⁴¹Department of Chemistry, Faculty of Mathematics and Natural Sciences, Mulawarman University, Samarinda 75123, East Kalimantan, Indonesia²Organic Chemistry Division, Department of Chemistry, Faculty of Mathematics and Natural Sciences, Institut Teknologi Bandung Jl. Ganesha No. 10, Bandung 70132, West Java, Indonesia³Research Center for Medicine and Cosmetics from Tropical Rainforest Resources, Mulawarman University, Samarinda, Indonesia⁴Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Jember, East Java, Indonesia

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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: 1JJJ, 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-isoprenyl-7,4'-dimethoxyflavone, 5-hydroxy-7,4'-dimethoxyflavone, 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.

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-isoprenyl-7,4'-dimethoxyflavone, 6-isoprenyl-7,4'-dimethoxyflavone, 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 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 Medicol). For molecular docking analysis, we used a Lenovo Yoga 7 computer with AMD Ryzen AI7-8840HS 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 the 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.^{36,37} Nutrient Agar (NA) medium was prepared by dissolving 12 g in 150 mL of distilled water. Luria Bertani (LB) liquid medium was prepared by dissolving 4 g in 500 mL of distilled water, shaking, 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, 2-O-methyl-8-isoprenyleriodictyol, 6-isoprenyleriodictyol, 5-hydroxy-7,4'-dimethoxyflavone, lonchocarpol A, 5-hydroxy-6,7,4'-trimethoxyflavone, Macahulleitin A, nymphaeol A, nymphaeol B, nymphaeol C, and solophenol D.^{14-18,22} were used for the study. Macahulleitin 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 (MMFF94s) 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 site 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 x 30 x 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 - 15 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 dental alveolar infections, jaw cysts, parodontitis, oral mucosal lesions, and stomatitis. The bacteria can also produce exotoxins, leucosidine, 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 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.³⁹ 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 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 23). 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 eucrestafavanone 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 the bacterial proteins: *Staphylococcus aureus* tyrosyl-tRNA synthetase, *Streptococcus mutans* antigen I/II (AglI/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 -8.2 kcal/mol against *Staphylococcus aureus* tyrosyl-tRNA synthetase, *Streptococcus mutans* antigen I/II (AglI/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-isoprenyliodictyol	-7.2	-8.1	-7.6
3	6-Isoprenyliodictyol	-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

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 (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-isoprenyliodictyol, nymphaeol A, nymphaeol B, and solophenol D exhibit high antibacterial activity against *S. mutans* antigen I/II (AglI/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-isoprenyliodictyol 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-isoprenyliodictyol is a prenylated flavonoid similar to nymphaeol A, and hence may have antibacterial activity. Two compounds, including 6-isoprenyliodictyol 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-Isoprenyliodictyol 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}

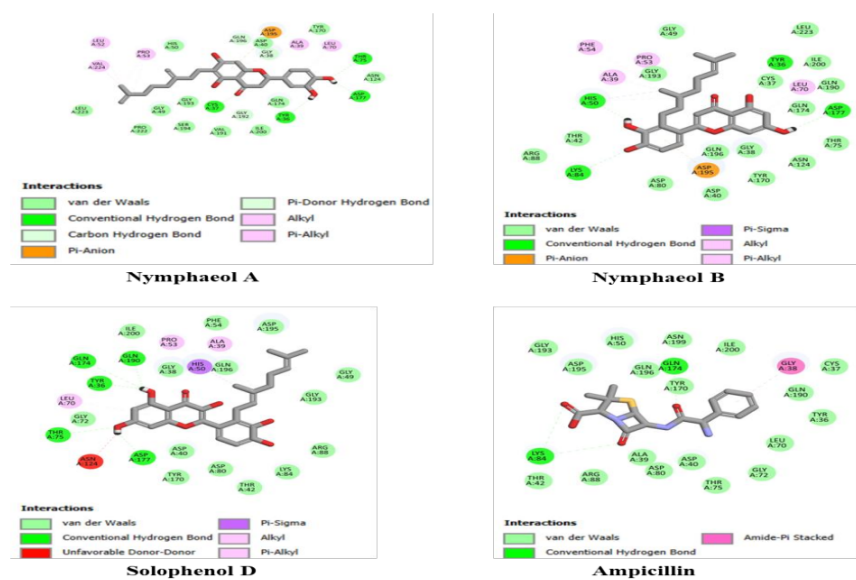


Figure 1: 2D visualization of interaction of the best docked flavonoids and ampicillin with *Staphylococcus aureus* tyrosyl-tRNA synthetase (PDB ID: 1JJJ)

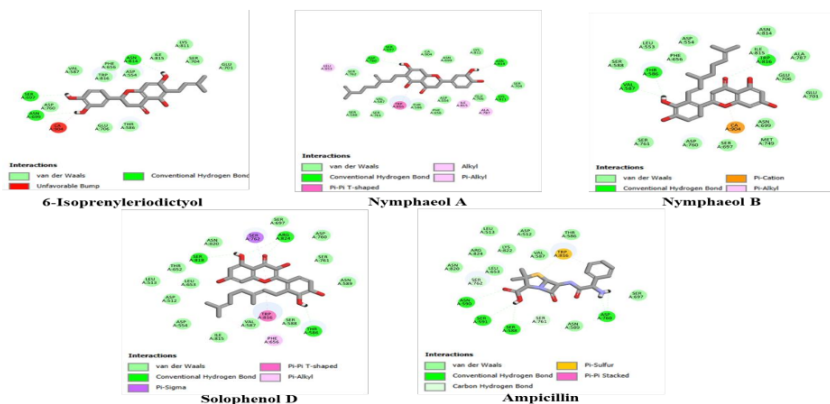


Figure 2: 2D visualization of interaction of the best-docked flavonoids and ampicillin with *Streptococcus mutans* antigen I/II (PDB ID: 3IPK).

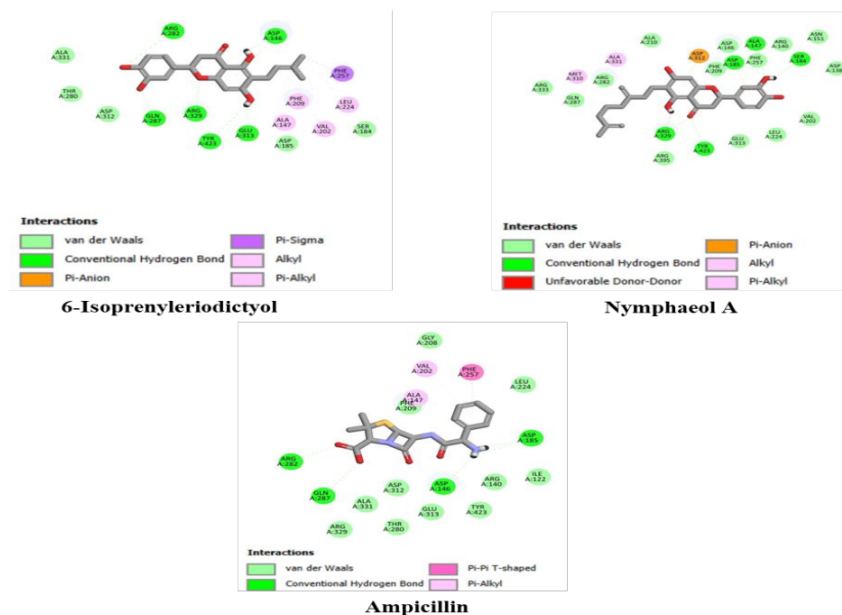


Figure 3: 2D visualization of interaction of the best-docked flavonoids and ampicillin with *Propionibacterium acnes* surface sialidase (PDB ID: 7LBU)

Conclusion

The extract and fractions of *Macaranga hulletii* 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 a 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 exhibit 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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