Antibacterial Activity and Molecular Docking of Compounds from *Avicennia marina* Leaves Extracts: Obtained by Natural Deep Eutectic Solvents

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Abstract: *This study aims to determine the effect of using natural deep eutectic solvent in extracting compounds from young and old leaves of* Avicennia marina *on the antibacterial activity through* in vitro *and* in silico*. The research method used was experimental with different molar ratios of solvent components: citric acid and glucose (1:1, 2:1, 3:1, 4:1 v/v).* In vitro*, the paper-disk method was employed to assess percent inhibition, and* in silico*, peptide deformylase (ID 6JFQ) was the target of the investigation. The best treatment results for testing the antibacterial activity of* A. marina *leaves extracts that were extracted using natural deep eutectic solvent against* S. aureus *and* E. coli *was citric acid and glucose (molar ratio 4:1). The inhibition zone by old leaves extracts was 27.47 and 37.73 mm, and by young leaves extracts were 28.69 and 30.99 mm. Then, phytochemical compound analysis was done using liquid chromatography-mass spectrometry, and six phytochemical compounds were obtained. The docking results showed that the diosmetin 7-O-β-D-glucuronide has the best binding affinity (−9.4 kcal/mol) towards the peptide deformylase. Purification must be done to obtain pure compounds from crude extracts of* A. marina *leaves.*

Keywords: *citric acid; diosmetin 7-O-β-D-glucuronide;* Escherichia coli*; glucose;* Staphylococcus aureus

■ INTRODUCTION

Avicennia marina is an abundant mangrove type that has spread throughout Indonesia. The parts of the mangrove *A. marina* include leaves, stems, and roots, which have the potential to develop. The part of the *A. marina* type of mangrove that contains the most phytochemicals is the leaves, which consist of phenolic and flavonoid [1]. The mangrove *A. marina* leaves can be divided into old and young leaves. The old leaves contain phytochemical compounds, including alkaloids, glycosides, phenols, steroids, tannins, and terpenoids [2]. According to Al Maqtari et al. [3], young leaves contain alkaloids, flavonoids, terpenoids, phenolics, saponins, and amino acids. Rich in bioactive compounds, mangrove *A. marina* are resources that show promising biological activity such as antioxidant [4], antifungal [5], antibacterial-antifungal [6], anticancer-antiproliferative [7], antihyperglycemic [8], and antiviral [9] agents.

Traditional uses for the leaves of the mangrove *A. marina* include antiseptic and antibacterial properties. The potential for further development of this antibacterial ability exists because bacterial infections are a major cause of many ailments that plague the medical field. Bacteria such as Gram-positive *Staphylococcus aureus* and Gram-negative *Escherichia coli* could not grow when exposed to antibacterial agents [10]. However, antibiotic resistance is currently occurring in various bacteria. Bacterial resistance to antibiotics results in reduced effectiveness of therapy, which increases morbidity and mortality [11]. So, searching for new antibiotic compounds is necessary to prevent and overcome bacterial resistance. One approach that can be used to find natural ingredients as alternative antibiotic candidates is *in vitro* assay of bacteria directly and *in silico* by targeting important enzymes or proteins, such as the peptide deformylase

enzyme found in both Gram-positive and Gram-negative bacteria. The biological peptide deformylase enzyme catalyzes the deformylation phase, which is necessary for a protein's maturation and production. *N*-formylmethionine, created by formylmethionine tRNA transferase's enzymatic transformation of methionyl-tRNA, is required to produce bacterial proteins. After *N*-formylmethionine is removed, the developing protein undergoes a sequence of changes caused by protein disulfide isomerase. All bacterial species exhibit this cycle of formylation–deformylation, which is necessary for bacterial proliferation [12]. The bioactive compounds that are extracted from plant components provide the mangrove *A. marina* with its antibacterial characteristics. Ethyl acetate, methanol, and ethanol can be used as solvents in the maceration method of extraction to obtain these compounds [13-15].

The current extraction processes often use safer solvents, including natural deep eutectic solvent (NADES). NADES is a subclass of ionic liquid. NADES is a eutectic mixture of two or three constituent components, generally interacting through hydrogen bonding, with a lower melting point than each component when combined at the proper molar ratio. NADES are regarded as "natural" since the main metabolite groups that comprise the eutectic mixture include sugars, organic acids and bases, and amino acids—components the plant naturally uses for survival [16]. Ionic liquid NADES (made from an acid and a base), neutral NADES (made from sugars only or sugars and polyalcohol), neutral NADES with acids (made from sugar/polyalcohol and organic acids), neutral NADES with bases (made from sugar/polyalcohol and organic bases), and amino acid-based NADES (made from amino acids and organic acids/sugars) are the different categories into which NADES can be divided. These transparent liquid mixtures are made up of hydrogen bond donors (HBDs) and acceptors (HBAs) [17]. According to numerous research, NADES may extract phytonutrient molecules that are both hydrophilic and hydrophobic, depending on their constituent parts [18]. Recent research has demonstrated that when it comes to the extraction of flavonoids from Chinese skullcap (*Scutellaria baicalensis* Georgi), eutectic solvents based on citric acid and glucose are popular to substitute for aqueous methanol and ethanol [19]. Remarkably, unlike traditional solvents like water, hydrophilic NADES, particularly the aqueous NADES family, may dissolve certain lipophilic molecules [20]. Dai et al. [21] state that eutectic mixtures containing organic acid constituents that exhibit the highest polarity, with amino acid-based mixtures coming in second. However, compared to the first two components, a mixture of NADES containing sugar and polyalcohol has less polarity. All NADES, especially those containing organic acid, showed bacterial growth inhibition at concentrations lower than 70% ethanol, according to the microdilution technique for *in vitro* antibacterial efficacy [22].

Based on the description above, information was obtained regarding the extraction method, categorized as green-extraction, namely extraction using NADES. However, more data from research reports regarding the extraction process of mangrove *A. marina* leaves using NADES is still needed. Therefore, this study aims to use NADES as green extraction solvent to extract active compounds from young and old leaves of the mangrove *A. marina*; screening for the antibacterial activity of the crude extract as an antibacterial for *S. aureus* and *E. coli* (*in vitro*) and molecular docking on peptide deformylase enzyme as target (*in silico*).

■ EXPERIMENTAL SECTION

Materials

The materials used consisted of old and young leaves of the *A. marina* mangrove from Wonorejo Mangrove Ecotourism, D-(+)-glucose (Sigma-Aldrich), citric acid (Sigma-Aldrich), distilled water (Sigma-Aldrich), *S. aureus* ATCC®33591D-5™, *E. coli* ATCC®10798™, peptide deformylase enzyme (ID 6JFQ; source *S. aureus*), Mueller Hinton agar (MHA) media (Sigma-Aldrich), and tryptic soy broth (TSB) media (Sigma-Aldrich).

Instrumentation

The instrumentation used for LC-MS analysis consists of LC Alliance brand LC equipment 2996

(waters) with photodiode-array detector (PDA) 2996 (Waters) and MS type XEVOG2QTOF (Waters) equipment. The instrumentation used for the *in silico* process is a personal computer with Intel(R) Core™ i5-3230M CPU @2.60GHz (4 CPUs) and 4 GB of RAM installed with Open Babel GUI, PyMOL, Discovery Studio Visualizer, and PyRx software.

Procedure

NADES preparation

Making the NADES solvent refers to research by Liu et al. [23], which produces four molar ratios. The molar ratios between citric acid and glucose in making NADES solution are A (1:1), B (2:1), C (3:1), and D (4:1). The steps are to weigh the citric acid and glucose and put them in a glass beaker covered with aluminum foil and then heated using a hotplate at 80 °C for 1 h until it melts. Next, 35 mL of distilled water was added, heated again, and stirred with a magnetic stirrer at 200 rpm for 1 h at 80 °C until a homogeneous and transparent solution was formed. Next, the solution is stored at room temperature and tightly closed until it is used for extraction.

Extraction of A. marina *leaves using NADES*

The extraction process begins with sample preparation by preparing fresh *A. marina* leaves. Then, washed until clean, dried using an oven at 50 °C for 90 min, crushed using a blender, and obtained *A. marina* leaves powder. The extraction process for *A. marina* leaves refers to research by Liu et al. [23], Rebocho et al. [24], and Wang et al. [25], which was carried out by placing the sample and NADES into a glass beaker (1:20 g/mL). The extraction process uses a temperature of 50 °C with a hot plate stirrer speed of 200 rpm for 40 min. Next, the *A. marina* leaves extract was filtered using a white cloth. The extract obtained is then stored in a dark vial until the sample is used.

Preparation of MHA and TSB

MHA is prepared using the medium and distilled water (38:1000 g/mL). The medium and distilled water were put into an Erlenmeyer, covered with aluminium foil, and heated until homogeneous. The medium was then sterilized using an autoclave (121 °C; 15 min). After fixing the medium, it is poured into a petri dish and left to solidify. TSB was made by preparing the media and distilled water (30:1000 g/mL). The medium and distilled water were put into an Erlenmeyer, covered with aluminium foil, and homogenized. The homogenized TSB media was transferred into a test tube and then covered using cotton wool and plastic wrap. The TSB medium was then sterilized using an autoclave (121 °C; 15 min) [10].

Preparation of bacterial suspensions

At the bacterial suspension preparation stage, isolates containing bacterial colonies and inoculation medium are prepared. The growing bacterial colonies are taken one dose and inoculated into a test tube containing inoculation TSB medium. The suspension was then homogenized using a vortex, which was incubated for 16–18 h at 37 °C [10].

Antibacterial assay

The antibacterial assay stage using the disc method takes a bacterial suspension using a sterile cotton swab. Then, the bacterial suspension was inoculated on an MHA medium with a swab. Then, the blank disk soaked in *A. marina* (old and young) leaves extract was placed in a medium containing bacteria and incubated at 37 °C for 16–24 h. Then, the clear zone formed around the disc paper was observed and measured using a caliper. Calculation of MIC: MBC values refers to research by Okla et al. [10], which can be determined by creating a linear regression curve between the X-axis (ln Mo = ln extract concentration) and the Y-axis (Z^2 = square value of the zone of inhibition). The linear curve that intersects the X-axis is the ln Mt value. The MIC value results from multiplying 0.25 by the Mt value, while the MBC value is the MIC value times four.

Phytochemical compound analysis

LC-MS analysis using HPLC Thermo Scientific Dionex Ultimate 3000 RSLCnano with microflow meter. The mobile phase used was A: Water + 0.1% formic acid, B: acetonitrile + 0.1% formic acid. The column used is Hypersil GOLD aQ 50 × 1 × 1.9 mm particle size. Flow rate 40 L/min. The mass spectrometry used was Thermo Scientific Q Exactive with a full scan at a resolution of 70,000 and an analysis time of 30 min with positive and negative ion modes [26].

Analysis of pharmacokinetic (drug-likeness)

The drug-likeness analysis of the active compound as ligand aims to determine whether the ligand complies with Lipinski's rule. The analysis was done online through the http://www.scfbio-iitd.res.in/software/drugdesign/lipinski.jsp [27-29].

Molecular docking

Molecular docking of the active compound obtained from the LC-MS results of *A. marina* leaves extracts with peptide deformylase and its resolution of 2.25 Å as a receptor using PyRx software. The XO enzyme as a receptor was obtained from https://www.rcsb.org. The test ligands used were obtained from the active compound and gentamycin as the control ligand. The 3D structure of the ligand was obtained from PubChem (https://pubchem.ncbi.nlm.nih.go) in sdf format. Then, it is converted into pdb format using the Discovery Studio software. Receptor preparation was carried out by separating the C chain structure from the intact structure and then saving it in pdbqt format. After being stored, the water molecule was removed, and the natural ligand was separated from the enzyme chain C structure. The file was then saved in pdbqt format. The molecular docking process is carried out using the PyRx software (AutoDock Vina), and the type of docking is blinded docking. After docking is complete, the results of several docking modes along with the value of binding affinity are obtained. Next, visualize the docking results in 2D and 3D using Discovery Studio software [30].

Analysis of toxicity and bioavailability

The test ligands with the best binding affinity were tested for toxicity predictions one by one using an online toxicity test program accessed at http://tox.charite.de/protox_II/ [31]. Ligands that showed non-toxic results were tested for bioavailability (ADME) using a program that can be accessed at http://www.swissadme.ch/ [32].

Analysis of statistical

Data were analyzed using SPSS software. Analysis of variance (ANOVA) and Tukey method were used to compare any significant differences. The difference was considered significant at a p-value < 0.05.

■ RESULTS AND DISCUSSION

Antibacterial Activity

Using the paper disk method, the antibacterial activity of *A. marina* leaves extracts, both young and old, was tested against *S. aureus* and *E. coli* (Tables 1 and 2). The highest value of antibacterial activities from *A. marina* leaves extract against *S. aureus* was obtained based on the calculation of the diameter of the inhibition zone, namely treatment level D (citric acid and glucose in molar ratio of 4:1), and the lowest value based on the calculation of the diameter of the inhibition zone, namely treatment level A (citric acid and glucose in molar ratio 1:1). The results of calculating the diameter of the inhibition zone are directly proportional to the antibacterial activity, where the greater the diameter of the inhibition zone is, the higher the antibacterial activity is. Alhaddad et al. [33] reported that the inhibition zone

Table 1. Antibacterial activity by old leaves extracts of *A. marina*

Treatment	Inhibition zone (mm)	
	S. aureus	*E. coli*
Negative control (NADES only)	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
Positive control (Gentamycin)	18.60 ± 0.00^{b}	23.91 ± 0.00^{b}
Molar ratio 1:1	22.39 ± 0.96^{c}	25.63 ± 2.41^{c}
Molar ratio 2:1	24.58 ± 1.44^{c}	29.66 ± 6.02^{d}
Molar ratio 3:1	25.57 ± 0.79^{cd}	35.01 ± 4.06^{e}
Molar ratio 4:1	27.47 ± 0.98^{e}	37.73 ± 4.09^{f}

Description: different superscript letters in the same column show significant differences (P < 0.05) at the 5% test level (significance test with Tukey test)

Table 2. Antibacterial activity by young leaves extracts of *A. marina*

Treatment	Inhibition zone (mm)	
	S. aureus	*E. coli*
Negative control (NADES only)	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
Positive control (Gentamycin)	16.22 ± 0.00^{b}	23.56 ± 0.00^{b}
Molar ratio 1:1	23.49 ± 0.93^{c}	23.84 ± 0.44^{b}
Molar ratio 2:1	25.35 ± 0.09^{d}	25.44 ± 0.77^{c}
Molar ratio 3:1	26.43 ± 0.46^{d}	26.04 ± 0.47^{c}
Molar ratio 4:1	28.69 ± 0.53^{e}	30.99 ± 1.92^{d}

Description: different superscript letters in the same column show significant differences (P < 0.05) at the 5% test level (significance test with Tukey test)

for *S. aureus* and *E. coli* by ethyl acetate *A. marina* fraction were 16.97 ± 1.15 and 14.40 ± 0.46 mm, respectively. Also, the inhibition zone for *S. aureus* and *E. coli* by ethanol *A. marina* extracts was 12.20 ± 2.12 and 8.13 ± 0.42 mm, respectively. According to the followed Clinical and Laboratory Standards Institute (CSLI) methodology categorizes the strength of antibacterial power based on the inhibitory zone, namely the weak category with an inhibitory zone diameter of < 5 mm, the medium type with an inhibitory zone diameter of 5–10 mm, the strong category with an inhibitory zone diameter of 10–20 mm, and the powerful category with an inhibitory zone diameter more than 20 mm [34].

The results of calculating the antibacterial activities of young and old *A. marina* leaves extracts against *E. coli* at all levels of solvent treatments were included in the powerful category. The highest antibacterial activity of old *A. marina* leaves extracts against *E. coli* based on the calculation of the diameter of the inhibition zone was obtained at treatment level D (citric acid and glucose in molar ratio 4:1). For the antibacterial activity of old and young *A. marina* leaves extracts against *E. coli*, the lowest based on the calculation of the diameter of the inhibition zone was obtained at treatment level A (citric acid and glucose in molar ratio 1:1). The results of calculating the diameter of the inhibitory zone are directly proportional to the antibacterial activity, where the greater the value of the diameter of the inhibitory zone, the higher the antibacterial activity so that the old *A. marina* leaves extracts that were extracted using NADES as solvent treatment level D (citric acid and glucose in molar ratio 4:1) have the best antibacterial activity compared to other treatment levels. The results of the diameter of the inhibition zone show that both *A. marina* old and young leaves extracts are classified as having antibacterial activity in the powerful category. It has recently been evident that NADES components can be chosen to enhance the biological activity of dissolved active substances from old and young *A. marina* leaves extracts in addition to optimizing the physicochemical properties of solvents. The leaves extracts of *A. marina*, both old and young, exhibiting stronger antibacterial activities were those with NADES possessing the highest acid component ratio (in molar ratio 4:1) than those in molar ratio 1:1.

Because NADES's pH value is lower than the ideal pH for bacterial development, it exhibits extraordinary antibacterial activity when combined with organic acids, such as citric acid. The susceptibility of Gram-positive and Gram-negative bacteria to NADES extract did not correlate in this investigation, despite the literature suggesting that Gram-positive bacteria are more vulnerable to the presence of plant extracts. The information provided leads one to the conclusion that one of the main variables influencing the toxicity of NADES is the kind of HBD. In this study, increasing the ratio of citric acid as HBDs in both old and young leaves extracts of *A. marina* can increase the diameter of the inhibition zone of *S. aureus* and *E. coli*. If the NADES component only consists of sugar such as fructose, glucose or sucrose with water, it does not inhibit *E. coli.* Wikene et al. [35] reported that the presence of sugar and citric acid components as HBDs in NADES could reduce the survival of *E. coli* by 96%, *S. epidermidis* by 9%, *Pseudomonas aeruginosa* by > 99.999%, and *Klebsiella pneumoniae* by 37%. In a similar vein, Zhao et al. [36] concluded that pH shifts that interfere with cell metabolism are a possible reason Gram-negative bacteria are less resistant to acid-based NADES than Gram-positive bacteria. Tsvetov et al. [37] reported that NADES, which is based on choline chloride and several organic acids such as malonic, malic, tartaric and citric acids, is an excellent alternative to ethanol for the extraction of bioactive compounds from *Rhodiola rosea* (L.). In addition, the antibacterial activity of the solvent and extract may contribute to extending the shelf life of the extract and outline ways to use the extract further.

MIC and MBC

The lowest concentration of samples at which no bacterial growth is detected is known as MIC and MBC of extracts, as per CSLI methodology that is followed for the paper disk dilution method [34]. The results of the MIC and MBC values of old and young *A. marina* leaves extracts on the growth of *S. aureus* and *E. coli* are shown in Tables 3 and 4.

Table 3. MIC-MBC value of *A. marina* leaves extracts against *S. aureus*

Type of leaves	Treatments	MIC (g/mL)	MBC (g/mL)
Old leaves	Ratio (1:1)	28.49 ± 1.425	11.39 ± 5.702
	Ratio (2:1)	24.49 ± 1.711	97.98 ± 6.846
	Ratio (3:1)	16.41 ± 1.789	65.67 ± 7.157
	Ratio (4:1)	55.49 ± 2.487	22.22 ± 9.994
Young leaves	Ratio (1:1)	27.90 ± 8.594	11.16 ± 3.329
	Ratio (2:1)	34.62 ± 1.508	13.85 ± 6.035
	Ratio (3:1)	27.75 ± 6.430	10.40 ± 2.572
	Ratio (4:1)	34.97 ± 7.479	13.99 ± 2.991

Table 4. MIC-MBC value of *A. marina* leaves extracts against *E. coli*

Type of leaves	Treatments	MIC (g/mL)	MBC (g/mL)
Old leaves	Ratio (1:1)	24.86 ± 1.627	99.44 ± 6.510
	Ratio (2:1)	22.83 ± 2.042	91.34 ± 8.171
	Ratio (3:1)	21.30 ± 2.624	85.23 ± 1.049
	Ratio (4:1)	20.86 ± 2.396	83.47 ± 9.586
Young leaves	Ratio (1:1)	40.09 ± 6.263	16.03 ± 2.505
	Ratio (2:1)	19.88 ± 7.098	79.54 ± 2.839
	Ratio (3:1)	22.29 ± 5.239	89.16 ± 2.095
	Ratio (4:1)	44.01 ± 9.775	18.44 ± 3.863

The action of novel antimicrobial medicines was assessed, and bacteria' susceptibility to medications was ascertained using MIC values. Any drug's antibacterial activity could be confirmed using MBC, which is a confirmatory measure [10]. In this study, the determination of MIC and MBC was based on the results of calculating the inhibitory zone and the concentration of the old and young *A. marina* leaves extracts was used. Based on Table 3, it can be seen that old *A. marina* leaves extracts have the highest MIC value against *S. aureus* at treatment level A (1:1 ratio of citric acid and glucose) and the lowest MIC value at treatment level D (4:1 of citric acid and glucose). It means that the respective concentrations of old *A. marina* leaves extracts can inhibit the growth of these bacteria. The highest MBC value for *S. aureus* was at treatment level A (1:1 ratio of citric acid and glucose), and the lowest MBC value was at treatment level D (4:1 of citric acid and glucose). It means that each concentration of old *A. marina* leaves extracts can kill the growth of these bacteria. In the research of Nwobodo et al. [38], the ability of an antibacterial to inhibit or kill microorganisms depends on the concentration of the antibacterial used. The resulting inhibitory power will be greater if the concentration is higher.

Based on Table 4, it can be seen that *A. marina* leaves extracts have the highest MIC value against *E. coli* at the treatment level (1:1 ratio of citric acid and glucose) and the lowest MIC value at the treatment level (4:1 ratio of citric acid and glucose). This means that each concentration of *A. marina* leaves extracts can inhibit the growth of these bacteria. The highest MBC value for *E. coli* was at treatment level A (1:1 ratio of citric acid and glucose), and the lowest MBC value was at treatment level D (4:1 ratio of citric acid and glucose). This means that each concentration of old *A. marina* leaves extracts can kill the growth of these bacteria. Jurić et al. [22] stated that the low pH values of NADES, which are below the ideal pH for bacterial growth, are primarily responsible for the outstanding MIC and MBC of NADES containing organic acids, such as citric acid. The growth of *S. aureus* and *E. coli* was inhibited by NADES extracts containing sugar and organic acid as HBDs. The results of the ability to inhibit will be more significant if the concentration used is higher [37].

Active Compounds of Extracts from *A. marina* Leaves

The results of the LC-MS analysis of active compounds from extracts of old and young leaves of *A. marina* are presented in Tables 5 and 6. Also, the chromatograms of LC-MS for old and young leaves extracts *of A. marina* are presented in Fig. 1 and 2. Based on the results of analysis using LC-MS, the old leaves extracts of *A. marina* contain five compounds from the flavonoid group, namely diosmetin 7-O-β-D-glucuronide, kaempferol, rhamnocitrin, rhamnazin, and pectolinarigenin. The young leaves extracts of *A. marina* contain one compound from the flavonoid group, namely pectolinarigenin and one compound from the alkaloid group, namely betaine. It can be seen that the active compounds of the flavonoid group dominate in the leaves of *A. marina*, which are extracted using NADES with the component citric acid: glucose (ratio 4:1) as solvent. Thatoi et al. [39] and Hidayati et al. [40] also reported that the active compounds in *A. marina*

Table 5. Active compounds from extracts of old leaves of *A. marina*

Compound group	RT (min)	Molecular formula and molecular mass (*m/z*)	Compound	Similarity index (%)	Abundance (%)
Flavonoid	5.70	$C_{22}H_{20}O_{12}$ (477.1033)	Diosmetin 7-O-β-D-glucuronide	100	3.59
	6.95	$C_{15}H_{10}O_6$ (287.0556)	Kaempferol	100	1.08
	8.00	$C_{16}H_{12}O_6$ (301.0712)	Rhamnocitrin	100	7.17
	9.04	$C_{17}H_{14}O_7$ (331.0818)	Rhamnazin	100	1.98
	10.09	$C_{17}H_{14}O_6$ (315.0869)	Pectolinarigenin	100	6.12

Table 6. Active compounds from extracts of young leaves of *A. marina*

Compound group	RT (min)	Molecular formula and molecular mass (*m/z*)	Compound	Similarity index (%)	Abundance (%)
Alkaloid	1.28	$C_5H_{11}NO_2$ (117.15)	Betaine	100	17.50
Flavonoid	10.09	$C_{17}H_{14}O_6$ (315.0869)	Pectolinarigenin	96.69	10.16

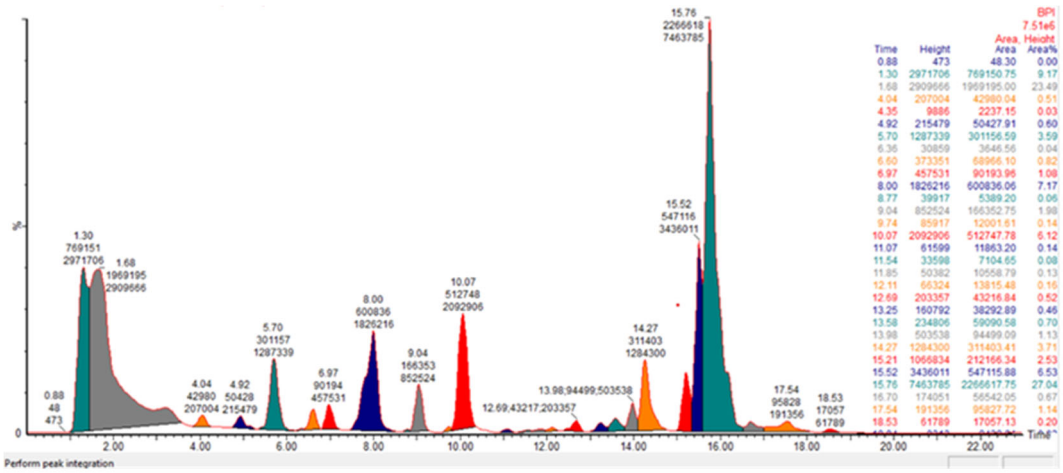

Fig 1. Chromatogram of NADES extract of old leaves *A. marina* (ratio 4:1)

leaves extracts include kaempferol and pectolinarigenin from flavonoid group.

Pharmacokinetic Drug-Likeness Lipinski's Rule

Each ligand of the active compounds from the extracts of *A. marina* leaves was examined using Lipinski's rule before docking the ligand to the enzyme target. If a ligand meets the following criteria, it can proceed with the docking process: (1) molecular weight (MW) less than 500 g/mol; (2) log P value less than 5; (3) number of HBDs less than 5; and (4) HBAs fewer than 10 [27]. Before molecular docking, it is necessary to ensure that the test ligand characteristics are suitable within the bounds of Lipinski's rule. The physicochemical characteristics of a ligand that can pass across bodily cell membranes can be analyzed using Lipinski's rule. Table 7 depicts the findings of examining the compatibility of the test ligand properties for the parameters of Lipinski's rule. Based on

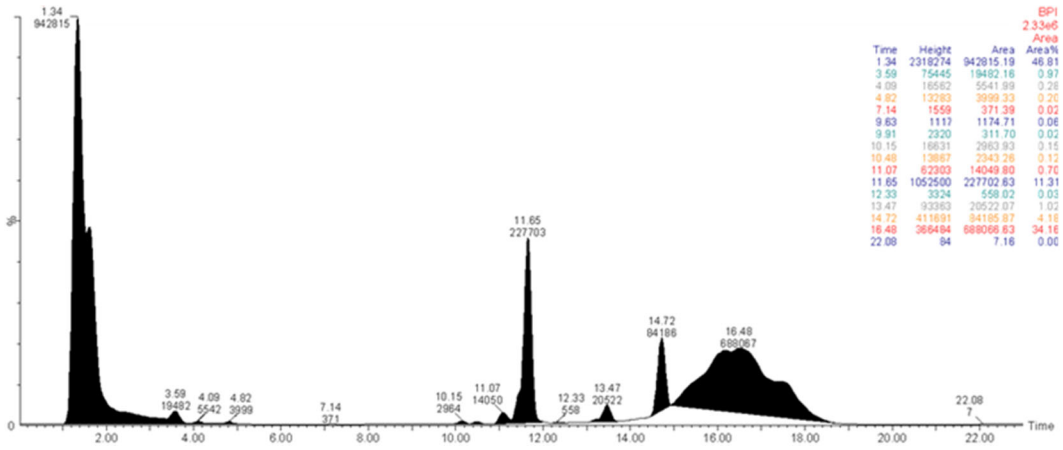

Fig 2. Chromatogram of NADES extract of young leaves *A. marina* (ratio 4:1)

Table 7. Ligand parameters to comply with Lipinski's rules

Item	Diosmetin 7-O-β-D-glucuronide	Kaempferol	Rhamnocitrin	Rhamnazin	Pectolinarigenin	Betaine	Lipinski's rules	Violation
MW (g/mol)	476	286	300	330	314	117	< 500	0
Log P	−0.006400	2.305299	2.608299	2.616899	2.731199	−1.557501	< 5	0
HBDs	4	4	3	3	2	0	< 5	0
HBAs	9	6	6	7	6	2	< 10	0

the analysis results according to Lipinski's rules, the six active compounds from the extracts of both old and young leaves of *A. marina* met Lipinski's Rules or zero violation and can be continued for molecular docking. Thus, the active compounds of *A. marina* leaves, both young and old, that NADES extracted has the potential to be pharmacologically useful. Owoloye et al. [41] reported that sylibin, hyperoside and harpagoside compounds did not violate any of Lipinski's rules and potentially druggable.

Molecular Docking

Molecular docking is one of the most important methods for determining the ideal ligand-protein/enzyme interaction [42-43]. Docking method validation must be completed before molecular docking. The molecular docking method validation aims to redocking the ligand on the peptide deformylase enzyme's active site by choosing a conformation close to the known natural ligand conformation by re-docking. Grid box measurements are now being taken to identify the region where the test and control ligands will attach. According to the grid box that was obtained, the two types of ligands will connect to the dimensions (Å) of X 40.2566, Y 46.3354, and Z 45.9629, as well as the center positions (X 32.168, Y 18.1052, and Z 6.5603). The docking results are chosen by comparing the conformation that most closely resembles the native ligand after ten iterations of the docking process. The root mean square deviation (RMSD) value of the chosen overall ligand conformation is computed using the Discovery Studio. In this study, RMSD values varied between 1 and 1.5753 Å, and the binding pattern was retained. The average RMSD value obtained is less than 2 Å, which indicates that this molecular docking validation is adequate [44].

The binding affinity score ranged from −6.6 to −9.4 kcal/mol for active compounds from *A. marina* leaves extracts that were molecularly docked against the active site of *S. aureus* and *E. coli*'s peptide deformylase enzyme (Table 8). Two-dimensional representations of compounds' binding interactions can be viewed in Fig. 3. Diosmetin-7-O-β-D-glucuronide had the lowest binding affinity (−9.4 kcal/mol), followed by pectolinarigenin (−8.2 kcal/mol) and kaempferol (−7.8 kcal/mol). For the positive control, gentamicin has a binding affinity toward peptide deformylase enzyme was −5.9 kcal/mol. According to Toppo et al. [45], a ligand's capacity to bind

Table 8. Binding affinity of active compounds toward peptide deformylase

Compound	Binding affinity (kcal/mol)
Diosmetin-7-O-β-D-glucuronide	−9.4
Pectolinarigenin	−8.2
Kaempferol	−7.8
Rhamnocitrin	−7.9
Rhamnazin	−7.5
Betaine	−6.6
Gentamycin (positive control)	−5.9

and its ability to inhibit the target enzyme is stronger when the ligand-receptor complexes binding affinity score is lower. The molecular docking results suit the results of *in vitro* antibacterial study, which show that both old and young leaves extracts from *A. marina* have higher *S. aureus* and *E. coli* inhibition values than gentamicin as a positive control.

Diosmetin 7-O-β-D-glucuronide was reported to have a broad-spectrum antibacterial effect against various pathogenic bacteria like *Streptococcus pneumoniae*, *Bacillus subtilis*, *K. pneumonia*, *S. aureus*, and *E. coli* [46]. Pectolinarigenin was active against three bacterial (*E. coli*, *S. aureus*, *K. pneumonia*) [47]. Kaempferol was found to have some antibacterial activity against *Micrococcus luteus*, *B. subtillis*, *Bacillus cereus*, *S. aureus*, *Enterobacter aerogenes*, *K. pneumoniae*, *E. coli* and *P. aeruginosa*, albeit at relatively low bacterial loads [48]. This evidence

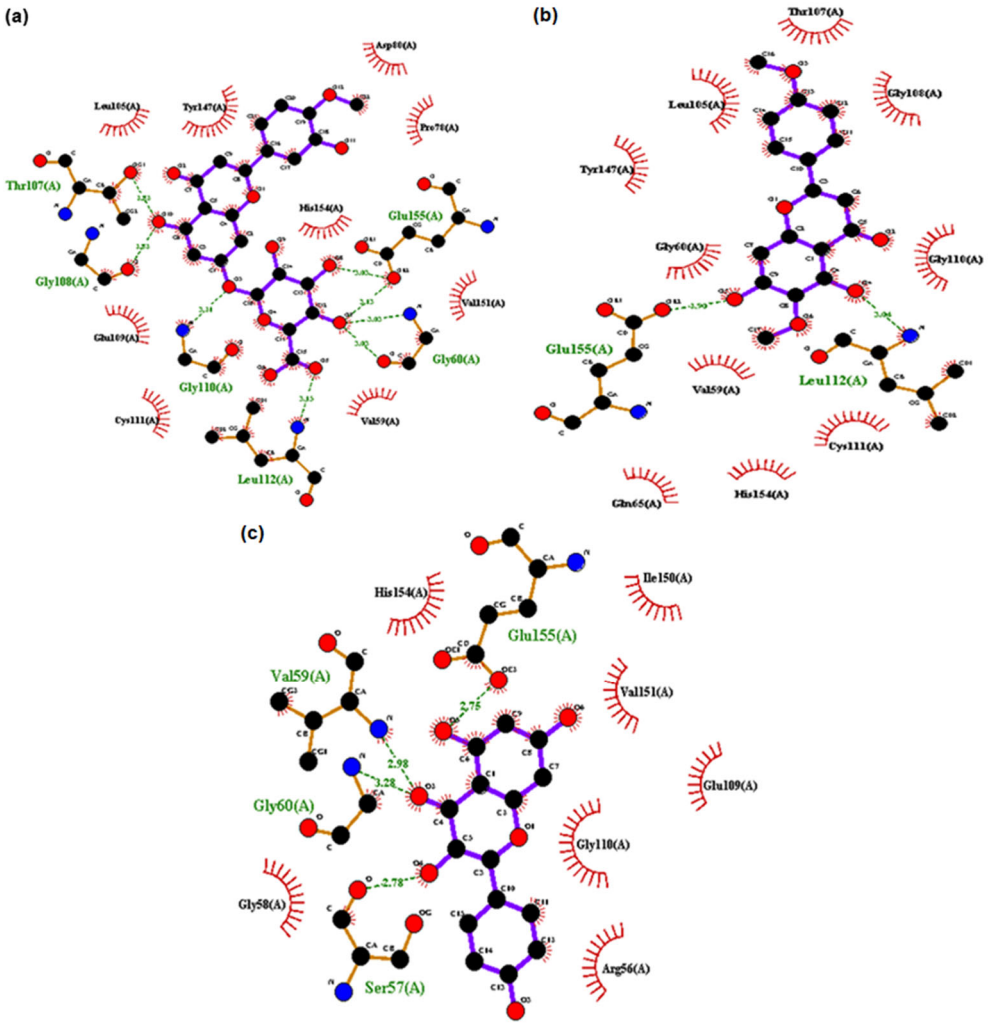

Fig 3. The 2D visualization of the interaction of the ligand (a) diosmetin 7-O-β-D-glucuronide, (b) pectolinarigenin, and (c) kaempferol toward peptide deformylase enzyme as receptor

strengthens that the antibacterial activity (*in vitro*) and molecular docking study (*in silico*) of active compounds from old and young *A. marina* leaves extracts against *S. aureus* and *E. coli* in this study is due to the content of these flavonoid compounds.

Based on the 2D visualization results, it can be seen that the ligand diosmetin 7-O-β-D-glucuronide has more hydrogen bonds with the amino acid residues of the peptide deformylation enzyme than other ligands. In addition, the form of hydrophobic interactions (pi-sigma, pi-pi stacked, alkyl, pi-alkyl, and van der Waals) between the ligand diosmetin 7-O-β-D-glucuronide and the amino acid residues of the peptide deformylase is more numerous than other ligands. In theory, hydrogen bonds on the active site of the peptide deformylase enzyme will be formed through the amino acid residues Arg56, Leu112, and Asn117, while hydrophobic interactions will occur on the amino acid residues Ser57, Gly58, Val59, Gly60, Leu105, Gly108, Glu109, Tyr147, Val151, Glu155, His159, and Glu185 [49]. There are similarities between the theory and the results of this study, which the type of hydrogen bond between diosmetin 7-O-β-D-glucuronide and the peptide deformylase is through the amino acid residues Gly60, Thr107, Gly108, Gly110, Leu112, and Glu155. At the same time, hydrophobic interaction occurs at amino acid residues Val59, Pro78, Asp80, Leu105, Glu109, Cys111, Tyr147, Val151, and His154.

Toxicity and Bioavailability

The results of the toxicity and bioavailability analysis of the ligand diosmetin 7-O-β-D-glucuronide are presented in Table 9. According to the analysis's findings, ligand diosmetin 7-O-β-D-glucuronide has a high category of gastrointestinal absorption in humans. Chen et al. [50] reported that diosmin (diosmetin-7-O-rutinoside) and its aglycone diosmetin (diosmetin 7-O-β-D-glucuronide) were easily digested, absorbed, and metabolized by the rat digestive system. The material taken orally should be easily absorbed by the organs of the digestive system, able to be distributed precisely to target sites, metabolized by the body, and eliminated without causing harm to the organs [51].

Table 9. Toxicity and bioavailability of diosmetin 7-O-β-D-glucuronide

Parameter	Prediction
GI absorption	High
Hepatotoxicity	No
Immunotoxicity	No
Carcinogenicity	No
Mutagenicity	No
Cytotoxicity	No

■ CONCLUSION

The best treatment results for testing the antibacterial activity of *A. marina* leaves extracts that were extracted using NADES against *S. aureus* and *E. coli* was citric acid and glucose in ratio 4:1. The inhibition zone by old leaves extracts on *S. aureus* and *E. coli* were 27.47 ± 0.98 and 37.73 ± 4.09 mm, respectively. The inhibition zone by young leaves extracts on *S. aureus* and *E. coli* were 28.69 ± 0.53 and 30.99 ± 1.92 mm, respectively. Then, phytochemical compound analysis was using LC-MS, active compounds diosmetin-7-O-β-D-glucuronide, pectolinarigenin, kaempferol, rhamnocitrin, rhamnazin, and betaine were obtained. The docking results showed that the diosmetin 7-O-β-D-glucuronide has the best binding affinity (−9.4 kcal/mol) towards the peptide deformylase enzyme through hydrogen bond on Gly60, Thr107, Gly108, Gly110, Leu112, Glu155 and hydrophobic interaction on Val59, Pro78, Asp80, Leu105, Glu109, Cys111, Tyr147, Val151, and His154 amino acid residues. As a potential compound, diosmetin-7-O-β-D-glucuronide meets Lipinski's rules, is a high category of human gastrointestinal absorption, and is not potentially toxic to the body.

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■ CONFLICT OF INTEREST

The authors declare that they hold no competing interests.

■ AUTHOR CONTRIBUTIONS

Hartati Kartikaningsih assisted in designing and conducting the experiments and wrote the manuscript. Heder Djamaludin and Nanda Audina conducted all of the experiments and wrote the manuscript. Jihan Nur Fauziyah conducted the data visualization and wrote the manuscript. All authors have read and approved of the final manuscript.

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