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Potential of Several Phytochemicals of Mangrove Species (*Rhizopora stylosa*) as Inhibitor of Both Viral Gene Expression and Bacterial Nucleic Acid Synthesis

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Abstract: The mangrove family (*Rhizophora stylosa*) has been used as traditional medicine. Due to the habitat of mangroves, they develop unique phytochemicals. Thus, they have the potential to become a source of plant-based therapeutic agents. However, many of the them remain uninvestigated. The purpose of this study was to predict the potential of some phytochemicals of the mangrove family as an inhibitor of both viral gene expression and bacterial nucleic acid. Some bioactive compounds of mangrove (taraxerol, pyrethrin, 2-Furancarboxaldehyde, and avicequinone A) were used as subject of this study. The main protease (Mpro) of SARS-Cov-2 virus (PDB ID: 6y2e), the staphylococcus aureus's proteins: topoisomerase II DNA gyrase (PDB id: 2XCT), and tyrosyl-tRNA synthetase (PDB id: 1JIJ) were used as targeted protein. The drug-likeness of compounds were analyzed using Swiss ADME based on the Lipinsky rule of five. Meanwhile, the affinity value between proteins and ligands was predicted using Autodock Vina. The root means square distance (RMSD) value (<2 A) and the binding cavity of drugs (ivermectin and ciprofloxacin) were used as validation parameters. This study resulted that only taraxerol compounds have a violation of Lipinsky's rule. Even so, out of the phytochemical compound of mangrove, the taraxerol has the highest affinity in Mpro and topoisomerase II DNA gyrase protein, although still lesser than ivermectin against Mpro; while avicequinone A has the highest affinity against tyrosyl-tRNA synthetase. This concludes that some phytochemicals of the mangrove family could be developed as an antibacterial and antiviral agents of therapy.

Keywords: Phytochemical of Mangrove, Antibacterial, Antivirus, molecular docking

1. Introduction

Mangroves (Rhizophora stylosa) were one of the plants that can grow in environments with high salinity conditions like coastal areas and river estuaries. The harsh environment causes differences in morphology, physiology, and metabolic mechanisms than other higher ordo plants (Chanda et al., 2015; Mitra et al., 2021). The unique habitat of mangroves leads to the synthesis of specific bioactive compounds that only can be found on mangroves. Several metabolites have been identified as part of alkaloids, flavonoids, phenolics, tannins, terpenoid saponins (Mitra et al., 2021). This plant is highly used in several areas in Indonesia as traditional medicine, such as anti-inflammatory, antipyretic, and asthmatic (Arbiastutie et al., 2021).

Mangroves have enormous potential to be used as phytochemical agents. Many studies have been carried out to prove the pharmacological potential of the bioactive compounds of mangroves. Terpenoid, phenolic, and alkaloid compounds from extracts of the mangrove sub-family Lumnitzera racemose are known to have antibacterial effects (Das et al., 2015). The ethanolic extract of the subfamilies Rhizophora sp and Avicennia marina are known to have antifungal effects against Penicillium purpurogenum, Penicillium chrysogenum, Penicillium notatum, Aspergillus niger, Alternaria alternata, and Penicillium italicum (Rastegar and Gozari, 2016). Other studies have also shown that secondary metabolites from the mangrove subfamily Clerodendron inerme are known to have antimalarial, antioxidant, anti-inflammatory, antimicrobial, and antiviral effects (hepatitis B virus) (Chanda et al., 2015).

Mangroves have the potential to be developed as phytochemical agents. The preliminary study was needed to determine the direction of the pharmacological effects of this plant. The purpose of this study was to predict the potential of several phytochemical compounds present in mangroves as antibacterial and antiviral agents, within specific mechanism; both inhibition of viral gene expression and bacterial nucleic acid synthesis. This research is expected to provide information on the potential of mangroves as antibacterial and antiviral.

2. Materials and MethodsMaterials

The materials used in this study were the main protease (Mpro) of SARS-Cov-2 virus (PDB ID: 6y2e); The protein of Staphylococcus aureus: topoisomerase II DNA gyrase (PDB id: 2XCT), and tyrosyl-tRNA synthetase protein (PDB id: 1JIJ) which obtained from the proteins database website (RCSB.org). The ligands (Taraxerol, pyrethrin, 2-Furancarboxaldehyde, Avicequinone A) were obtained from the chemical web library database (chemspider.com). The molecular docking used hardware with specifications: Windows 10 operating system, AMD A8 7410 processor (Quad-core; 2.2 GHz), and 4 GB RAM. Meanwhile, we used the structure design tools Marvin sketch (ChemAxon), Discovery Studio Visualizer (Biovia), Preparation docking Autodock tools (ADT), and Autodock vina.

Methods

a. Preparation of proteins and ligand

The proteins and ligands were prepared under conditions at the lowest energy possible. Another preparation on proteins was needed to avoid misread and error within the molecular docking study. The proteins need to be clean from water, ions, and other native ligands causes they could affect the affinity and binding site of the protein's amino acids

b. Analysis of potential drug-likeness properties of compounds

The drug-likeness is a term given to the ability of a chemical compound that marks its potency when taken orally. This study was performed using the web analysis Swissadme.com which is a pharmacokinetic analytical website based on several physicochemical parameters of the compounds tested (Daina et al., 2017). The calculating process will be based on the Lipinsky's rule

of five which includes lipophilicity parameters, H-bond acceptors (HBA), H-bond donors (HBD), and molecular weight (MW) (Daina et al., 2017; Prasanth et al., 2020).

c. Molecular docking of phytochemical compounds

Molecular docking is a computational method to predict the affinity between ligands and proteins. Protein components were prepared using autodock tools (ADT) to remove water components, ions, and the addition of hydrogen atoms to the polar groups of proteins to minimize errors due to tautomerization (Allam et al., 2018; Trott and Olson, 2010). Visualization of 2D and 3D bonds was carried out to clarify the possible bonds formed between ligand compounds and proteins. The Ds Visualizer software was used to show the type and form of bonds between amino acid residues and the functional groups of ligand compounds that could affect the pharmacological effects of compounds (Tallei et al., 2020).

d. Data Analysis

This study uses pre and post docking analysis using standard drug of choice as references. The validation parameters in this test used the specific binding site of the comparison drug compound (ivermectin compound on Mpro; and ciprofloxacin on protein topoisomerase II DNA gyrase and tyrosyl-tRNA synthetase). In addition, the affinity value obtained was selected with the criteria for the root mean square deviance (RMSD) <2 A (Vennila et al., 2014).

3. Results and Discussion

The development of plant-based therapeutic agents is currently increasing among researchers. One of the plants that have great potential to be developed as a phytotherapy agent is the mangrove plant. This plant has a plenty variety of secondary metabolites that have yet developed as phytochemical agents (Chanda et al., 2015; Mitra et al., 2021) This study uses computational predictions to determine the affinity of several phytochemical compounds of mangrove with certain proteins that have a role in the activation of viral gene expression pathways and DNA synthesis from bacteria. The preparation of ligand and protein used to provide optimization at the lowest energy level (Table 1). The protein component specifically was cleaned of native ligands which could cause an affinity reading error, resulting in the biased docking results.

Table 1. The Lowest Energy Estimated Within Several Phytochemical Compounds Of Mangrove

Ligands	Lowest Energy estimated
Taraxerol ($C_{30}H_{50}O$)	171.15 Kcal/Mol
Pyrethrin ($C_{18}H_{22}O_5$)	184.7Kcal/Mol
2-Furancarboxaldehyde ($C_5H_4O_2$)	16.25 Kcal/Mol
Avicequinone A ($C_{15}H_{14}O_5$)	46.52 Kcal/Mol

The compounds have different physicochemical properties; thus causing differences in pharmacokinetic profiles in the human body. Therefore, it leads to the incompatibility of drugs in the body (Chaurasia, 2016). The drug-likeness test in this study provides information regarding the potential of the drug that could be used orally following the Lipinsky rule (Lipinsky rule of five). Druglikeness analysis of the compounds showed that out of the several bioactive compounds, only taraxerol violated the lipophilicity value on Lipinsky's rule of five. It indicates that almost all the tested ligands have the potential to be used orally (Table 2). These results provide information on the need for further preformulation of the taraxerol compound for the oral route of administration.

Table 2. The Druggability Of Ligands Analyzed By Web Analysis (Swiss Admet) To Determine The Potential Of Ligands Based On Lipophilicity Of Drugs (Log $p \leq 5$); h-Bond Acceptors (Hba) < 5; h Bond Donor (Hbd) <10; And Molecular Weight (Mw) <500 Da.
*) Violation Of Lipinsky's Rule Of Five

Ligands	**MW (g/mol)**	***Oxygen count***	***H- bond acceptors (HBA)***	***H- bond donors (HBD)***	**Log-P**	**Violation of rule**
Taraxerol ($C_{30}H_{50}O$)	426.72	1	1	1	7.21*	1
pyrethrin, ($C_{18}H_{22}O_5$)	318.36	5	5	0	2.42	0
2-Furancarboxaldehyde ($C_5H_4O_2$)	96.08	2	2	0	0.69	0
Avicequinone A ($C_{15}H_{14}O_5$)	274.27	5	5	2	1.00	0

The molecular docking provides affinity values between ligands and proteins. It is influenced by gibs energy, pharmacophore groups, and the distance that occurs between ligands and the pharmacophore groups within the protein (Siti et al., 2011; Trott and Olson, 2010). The study of antiviral activity was used on the main protease (Mpro) of SARS-Cov-2. This protein cleaves polypeptide sequences in protein synthesis and functions as a "key enzyme" in the viral replication cycle (Ullrich and Nitsche, 2020). The results showed that the ligands had weaker affinity compared to the ivermectin; a drug known to be able to inhibit the Mpro in the SARS-Cov-2 virus. Interestingly, the visualization of pharmacophore and amino acid site showed a similarity site between taraxerol and ivermectin at Phenylalanine (294), valine (202), Histidine (246), proline (293), and isoleucine (249) (Fig. 1). These findings suggest that the taraxerol may have the same mechanical action as ivermectin.

Table 1. The Molecular Docking Study Resulted In Affinity Values Between Ligands And Proteins (a) Affinity Between Ligands And The Main Viral Protease Sars-Cov-2; (b) Affinity Between Ligands With Topoisomerase Ii Dna Gyrase And Tyrosyl-Trna Synthethase
*)Rmsd <2a; **) Best Score

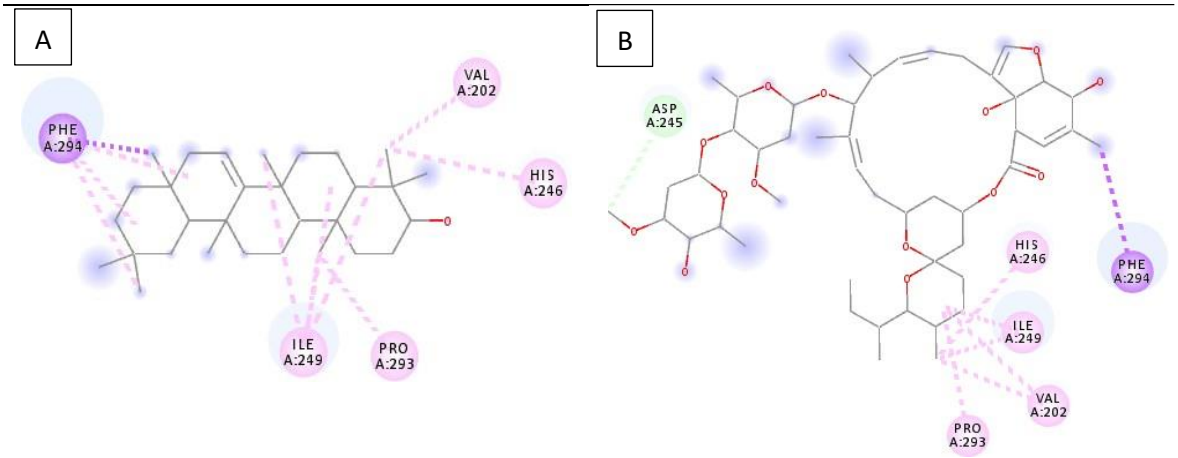
A. Main Protease of SARS-Cov-2 (PDB ID: 6y2e)

Proteins	Affinity of ligands*				
	Taraxerol	**pyrethrin**	**2-Furancarboxaldehyde**	**Avicequinone A**	**Ivermectin**
Mpro	-7.8	-6.8	-3.4	-6.6	-8.1**

B. Topoisomerase II DNA gyrase (PDB id: 2XCT) and tyrosyl-tRNA synthetase (PDB id: 1JIJ)

Proteins	Affinity of ligands*				
	Taraxerol	**pyrethrin**	**2-Furancarboxaldehyde**	**Avicequinone A**	**Ciprofloxacin**
topoisomerase II DNA gyrase	-9.4**	-7.2	-3.8	-7.6	-7.5
tyrosyl-tRNA synthetase	-7.1	-5.3	-4.6	-8.7**	-8.7**

The antibacterial investigation was performed on topoisomerase II DNA gyrase and tyrosyl-tRNA synthetase proteins. Both proteins play a role in the nucleic acid synthesis of bacteria. The topoisomerase II enzyme DNA gyrase affects the cell cycle of bacteria, while tyrosyl-tRNA synthetase catalyzes the covalent binding of tRNA (Islam and Pillay, 2020; Pisano et al., 2019). The taraxerol and avicequinone A had a better affinity than the antibiotic ciprofloxacin (Table 3). There are similarities in the pharmacophore and amino acid-binding site of ciprofloxacin and the ligand. In topoisomerase II DNA gyrase the similarity is in the amino acids histidine (1081), phenylalanine (1123), proline (1080), tyrosine (1150); while the tyrosyl-tRNA synthetase is in Aspartic acid (195), cysteine (37). Based on these results, the taraxerol and avicequinone A possibility has activity as an inhibitor of bacterial nucleic acid synthetase. Furthermore, avicequinone A coupled with drug-likeness properties has the highest potential to be developed as an antibacterial agent.



Amino acid	bond	Amino acid	bond
Phenylalanine (294)	Pi-sigma	Aspartic acid (245)	Carbon-hydrogen
Valine (202)	alkyl	Phenylalanine (294)	Pi-sigma
Histidine (246)	alkyl	Histidine (246)	alkyl
Isoleucine (249)	alkyl	Isoleucine (249)	alkyl
Proline (293)	alkyl	Valine (202)	alkyl
		Prloline (293)	alkyl

Fig 1. Visualization Of Binding Site Formed Between Pharmacophore And Amino Acid Site On Main Protease Of Sars-Cov-2 (a). Taraxerol (b). Ivermectin

4. Conclusion

The potential of mangroves has not been fully explored. This study concluded that several bioactive compounds of mangroves potentially developed as antimicrobial and antiviral. Still, the practical use of these plants remains yet uncovered. Thus, extensive research was required for unmasking the potentiality of mangroves

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Conflict of Interest

The authors has no conflict of interest.

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