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Prediction of Pharmacokinetic Parameters from Ethanolic Extract Mane Leaves (*Vitex pinnata* L.) in Geothermal Manifestation of Seulawah Agam Ie-Seu'um, Aceh

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Abstract

The Mane plant (*Vitex pinnata* L.) is traditionally used as medicine in Aceh Province, Indonesia. This study aimed to predict the pharmacokinetic parameters of compounds in the ethanolic extract of Mane leaf (EEML), including the absorption, distribution, metabolism, excretion, and toxicity (ADMET), by in-silico approach. The method used was to analyze the compounds using a web-predictor server and molecular docking. Gas chromatography-mass spectrometry (GCMS) analysis of EEML showed the presence of active compounds, including phytol (60.93%), acorenol (8.56%), n-hexadecanoic acid (4.89%), trans-Z-alpha-bisabolene epoxide (2.7%) and cedrane (2.03%). Lipinski's rule of five states that all compounds had a deviation of less than 2. Pharmacokinetic parameters suggested that phytol was moderately absorbed in the gastrointestinal tract and had a toxicity level of 5 with lethal doses (LD_{50}) >5000 mg/kg. Molecular docking results showed that phytol could be used against the targeted enzyme *Staphylococcus aureus.* In conclusion, our study suggests that the active compounds of EEML may have potential as a drug candidate.

1. Introduction

Mane plant or *Vitex pinnata* L., also known as the Laban tree in Indonesian, is a tree that may reach a height of 20 meters (Figure 1). This tree is a member of the Lamiaceae, and its leaves consist of 3-5 elliptical strands measuring 3-25 cm long and 1.5-10 cm wide. Other characteristics include terminal flowers that are white and bluish and fruits that are 5-88 mm in diameter and turn black when ripe [1, 2]. Many pharmacological studies have used the mane tree's skin, stems, leaves, and fruit. They have been used to treat stomach pain, jaundice, and high blood pressure [3, 4]. Fruit mane is used to treat boils by the people of Acehnese. Mane trees spread around Aceh Province, especially in the Area of Manifestation Geothermal Seulawah Agam (MGSA) Ie-Seu'um, Aceh Besar District, Aceh Province. This manifestation is circa 20 km from Seulawah Agam mountain and 35 km from Banda Aceh [5]. Preliminary research found that the methanol extract of mane leaf has a strong antibacterial effect on *Staphylococcus aureus* with inhibition zone 11.03

Figure 1. Mane leaves.

mm, and it contains phytochemicals such as alkaloids, flavonoids, steroids, tannins, and saponins [6]. It also reported that methanol extract of mane leaf inhibited the activity of acquired *Staphylococcus mutants* bacteria by 10.43 mm. This research aims to identify the chemicals found in ethanol extract of mane leave from MGSA Ie-Seu'um and predict pharmacokinetic parameters using the in-silico method.

2. Materials and Methods

Fresh mane leaves were collected from the MGSA Ie-Seu'um area located at 05°32'50.97" N and 095°32'55.10" E, 97 meters above sea level. Botanists and curators at the National Research and Innovation Agency (BRIN), Jakarta, recognized the specimen with the test number 47621. Fresh leaves were dried in a chamber for ten days. Simplicia was mashed until it was powdered from a sample of dry material. During the extraction process, ethanol was used as the solvent, and the maceration method was used. Then extract on evaporation until obtained dry extract is using a Rotary Evaporator (Buchi Rotavapor® R-300, Switzerland). Analysis of gas chromatography-mass spectrometry (GC-MS) performed using TRACE 1310 GC and iSQ 7000 single quadrupole MS (Thermo Fischer Scientific, USA) with TraceGOLD TG-5MS 1 (30 m x 0.25 mm x 0.25 µm). The carrier gas was helium with a maintained temperature of 75ºC for 8 minutes, 75-200ºC at 4 Cº/min, and 200ºC at 5 minutes, totaling 60 minutes. The relative quantity of components from the extract will be expressed on the resulting peak area from the GCMS chromatogram. The selected ligan will be evaluated using Classyfire [7] and the Lipinski Rule of Five (RO5) with SwissADME [8]. Absorption, distribution, metabolism, excretion, and toxicity with pKCSM [9]. Then, molecular docking was performed with PyRx, and visualization was performed with Biovia Discovery Visualizer [10–12]. Molecular docking is done on main protease of Severe Acute Respiratory Syndrome-Coronavirus 2 (SARS-CoV-2) (PDB ID: 6LU7) and the clumping factor receptors of *Staphylococcus aureus* (PDB ID: 1N76).

3. Results and Discussions

3.1. Bioactive component

The bioactive component from ethanolic extract of mane leaves (EEML) from GC-MS analysis has 19 compounds (Figure 2). Based on GCMS results, it is known that the bioactive components of EEML are terpenoids class, namely sesquiterpenoids (cedrene) and acyclic diterpenoids (phytol). Long-chain fatty acids were also found, namely n-hexadecanoic acid, the presence of tertiary alcohols, acorenol, and epoxide (cis-Z-alpha-bisabolene epoxide). Identification using GCMS to extract ethanol mane leaves from Thailand shows that the most significant components are 3,7,11,15 Tetramethylhexadecen2en1ol ($C_{20}H_{40}O$) of 16.39% and also found n hexadecanoic acid was also found with percentage 3.31% [13]. Based on abundance, there are five compounds main will be further studied, namely cedrene (2.03%), acorenol (8.56%), trans-Z-a-bisabolene epoxide (2.7%), n-hexadecanoic acid (4.89%) and phytol (60.93%) (Table 1).

3.2. Profile Druglikeness Compound

Many studies have been conducted on the potency activity of mane leaves; however, no studies on the profile pharmacokinetics of the compounds contained within. The pharmacokinetics of five compounds extracted from ethanol leaf mane include absorption, distribution, metabolism, excretion, and toxicity. Lipinski's rule was followed when testing drug similarity parameters or drug-likeness. Five parameters can be used to describe drug that could be absorbed if given orally [14]. Based on the results predicted by SwissADME, it is known that all compounds meet Lipinski's rule because the deviation is less than two (Table 2).

Phytol ($C_{20}H_{40}O$) is a member of the terpenoid family, specifically acyclic diterpenoids, also used by plants to make chlorophyll, making them widely distributed in nature. According to [15–17], phytol is a precursor to synthetic vitamins E and K, which are known to be capable of character cell toxicity in breast cancer (MCF7) and have the potential to operate as antioxidants and antinociceptives. Additionally, phytol compounds are used in molecular docking because they are the most abundant of all chemicals and meet Lipinski's rules of five.

3.3. Profile Pharmacokinetics

Pharmacokinetic parameter testing is necessary to determine the profile compound that will be analyzed.

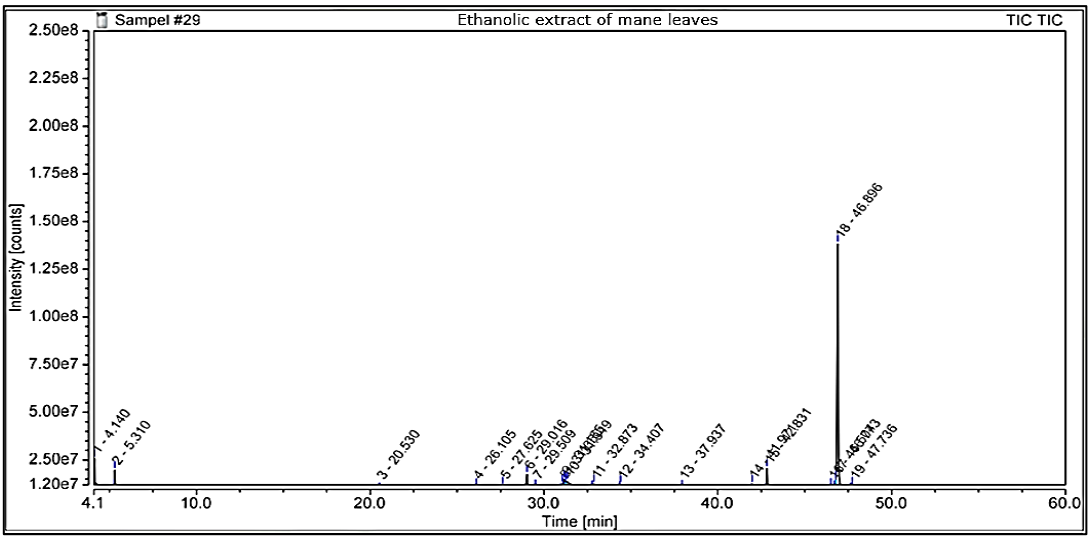

Figure 2. Chromatogram of ethanolic extract mane leaves.

Table 1. Components of ethanolic extract mane leaves.

Compounds Name	Molecular Formula	Retention Time (Min)	Relative Area (%)	(Match Fair) SI
Cedrene	($C_{15}H_{24}$)	26.105	2.03	813
Acorenol	($C_{15}H_{26}O$)	29.016	8.56	759
trans-Za-bisabolene epoxide	($C_{15}H_{24}O$)	32.87	2.7	753
n-hexadecanoic acid	($C_{16}H_{32}O_2$)	42.83	4.89	669
Phytol	($C_{20}H_{40}O$)	46.89	60.93	914

This can be tested using in-silico method with a predictor server, such as pKCSM. pKCSM can predict absorption, distribution, metabolism, excretion, and toxicity with a simplified molecular-input line-entry system (SMILES) structure. SMILES is a standard for a line notation for defining the structure of chemical species using short ASCII strings. The molecules' three-dimensional models or two-dimensional drawings can be recreated using SMILES strings. Phytol has a SMILES form CC(C)CCCC(C)CCCC(C)CCCC(=CCO)C. Table 3 shows the pharmacokinetic parameter test results for Phytol compounds.

Absorption of a drug candidate has five parameters (Table 3). Solubility in water (log S) is essential because it can inform if a compound can dissolve in water at 25°C. Based on testing with pKCSM, it is known that phytol compounds are moderately soluble in water because their log S value is greater than -4.5. Table 2 Lipinski test also shows a high score for lipophilicity (log P>4), which means that the substance is lipid-soluble. The molecule's polarity is affected by the structure of the carbon atom in phytol. Despite difficulty dissolving in water, phytol compounds can penetrate lipid bilayers because they contain molecules less than 500 mg/mol and hydrogen acceptors and donors less than 5 (Table 2). Additionally, human intestinal absorption (HIA+) becomes crucial when a drug is administered orally. Phytol had moderate absorption with a percentage is >30% or 91.904%. Phytol potential for absorption in the route of human intestinal is accomplished by absorption or can be orally administrated.

After absorption, the distribution compound plays a crucial role in assisting the delivery of the drug to the receptors. The volume of distribution (VD), the permeability of the blood-brain barrier (BBB), and the permeability of the central nervous system (CNS) are all general aspects of aspect distribution. The total amount of liquid a person can transport in a compartment to carry chemical medications is known as VD. Phytol has a high VD of 2.97 L/kg or log 0.473. BBB permeability of phytol is 0.815, and CNS permeability is -1.593 (Table 3). This value indicates that phytol has no permeability to BBB and CNS.

Total clearance and binding to an organic cation transporter 2 (OCT2) substrate are the two parameters of excretion. A clearance drug is a substance that the body eliminates without raising a fuss about the mechanism involved. Total clearance is based on the assumption that the liver's metabolism and kidney clearance completely clean the body. Phytol is not bound to the renal substrate OCT2 and has a Log 1,686 mL/min/kg or 48.5 mL/min/kg

Table 2. Lipinski rule of five.

No	Compounds name	PubChem CID	Molecular weight (g/mol)	H-bond acceptor	H-bond donor	Log P	Molar refractivity	Meet Lipinski rule
1	Cedrene	521207	204.35	0	0	4.36	66.88	Yes
2	Acorenol	11972555	222.37	1	1	3.52	70.46	Yes
3	cis-Z-alpha-bisabolene epoxide	91753574	220.35	1	0	3.91	70.17	Yes
4	n-hexadecanoic acid	985	256.42	2	1	5.20	80.80	Yes
5	Phytol	5280435	296.53	1	1	6.25	96.94	Yes

Table 3. Pharmacokinetics of phytol.

Absorption						
Water solubility (log mol/L)	$CaC0_2$ permeability (log Papp in 10^-6 cm/s)	Intestinal absorption (%absorbed)	Skin permeability (log Kp)	P-gp substrate[a] (Yes/No)	P-gp I inhibitor (Yes/No)	P-gp II inhibitor (Yes/No)
-7.559	1.52	91,904	-2.585	No	No	Yes
Distribution						
VDss[b] (Log L/kg)		Fraction unbound (Fu)	BBB permeability[c] (Log BB)		CNS permeability[d] (PS logs)	
0.473		0	0.815		-1.593	
Metabolism						
CYP2D6 substrate (Yes/No)	CYP3A4 substrate (Yes/No)	CYP1A2 inhibitors (Yes/No)	CYP2C19 inhibitors (Yes/No)	CYP2C9 inhibitors (Yes/No)	CYP2D6 inhibitors (Yes/No)	CYP3A4 inhibitors (Yes/No)
No	Yes	Yes	No	No	No	No
Excretion						
Total clearance (Log ml/min/kg)				Renal OCT2 substrate (Yes/No)		
1,686				No		

[a]P-gp = P-glycoprotein; [b]VDss = volume distribution on stady state; [c]BBB = blood brain barrier; [d]CNS = central nervous system

Table 4. Toxicity of phytol.

Toxicity									
AMES toxicity (Yes/No)	Max. tolerated dose (Log mg/kg/day)	hERG I inhibitor (Yes/No)	hERG II inhibitor (Yes/No)	Oral rat acute toxicity (LD50) (mol/kg)	Oral rat chronic toxicity (LAOLEL) (Log mg/kg /day)	Hepatotoxicity (Yes/No)	Skin sensitization (Yes/No)	*T. pyriformis* toxicity (Log ug/L)	Minnow toxicity (Log mM)
No	0.088	No	Yes	1.621	1.05	No	Yes	1.844	-1.614

total clearance rate (Table 3). Clearance and distribution volume are connected. If a patient's distribution volume for phytol is 2.97 L/kg and phytol clearance is 48.5 mL/min/kg, then each minute, 48.5 ml of 2.97 liters of phytol are cleansed from the patient. Toxicity testing was conducted showing that AMES using the *Salmonella typhus* reverse mutation assay (Table 4). Predictions that have been conducted show that phytol did not character toxic in this test, indicating that the compounds present in EEML are not potential for mutagenic. Hepatotoxicity tests also show that phytol is non-toxic in the liver and liver metabolizing enzymes. The maximum tolerated dose of phytol is log 0.088 mg/kg/day. Calculating the lethal dose 50% (LD_{50}) using protox online also shows that phytol is not toxic to the liver or immune system and is not mutagenic. Phytol's toxicity level is 5, with an LD_{50} value of 5000 mg/kg. The results of probit analysis showed that the LC_{50} value of Mane fruit extract was reported to be 600.608 mg/L with an interval ranging from 529.713 mg/L to 689.886 mg/L indicating toxicity activity [18]. These results also show that Phytol is not hepatotoxic, carcinogenic, or immunotoxic, making it a potential medicinal plant.

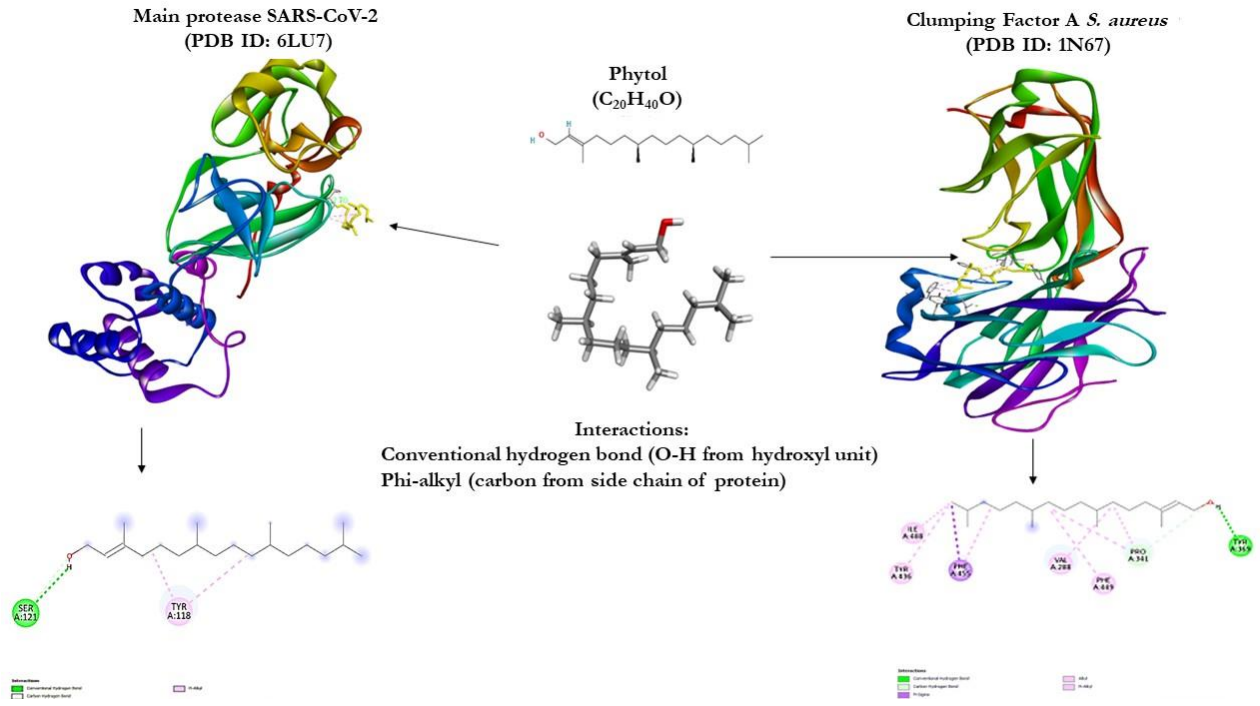

Figure 3. Interactions of phytol in receptors.

Table 5. Binding affinity and visualization.

Ligand	Main protease (6LU7)	Clumping factor A (IN67)
Phytol	-3.3 Kcal/mol Hydrogen bond (Serine:121) and interactions π-Alkyl (Tyrosine:118)	-6.3 -Kcal/mol Hydrogen bond (Tyrosine:369), carbon-hydrogen bond, interaction π-σ, π-Alkyl and alkyl

3.4. Molecular Docking

Molecular docking was carried out on two receptors, main protease of SARS-CoV-2 (PDB ID: 6LU7) and clumping factor A *S. aureus* (PDB ID: 1N67). The results of molecular docking are presented in Table 5 and Figure 3. The interaction that occurs in phytol as a ligand is the interaction of hydrogen bonds caused by the O-H group of the hydroxyl and the π-alkyl of the side chain (Table 5). The binding affinity value at the 6LU7 receptor -3.3 kcal/mol and IN67 -6.3 kcal/mol. Phytol has a carbon chain with a single bond type which causes an increase in the number of rotatable bonds. The number of rotatable bonds of phytol is 13. This makes it easy for the conformational change of the phytol to move according to the accompanying conditions.

4. Conclusions

Our data suggest phytol is the compound with the most abundance in the ethanolic extract of mane leaves which has a good absorption profile in the human intestine, a high volume of distribution, is not bound to renal OCT2, and has a level 5 toxicity. Molecular docking of phytol showed inhibitory activity on the receptor of *S. aureus* clumping factor and the main protease SARS-CoV-2. Phytol is potentially not hepatotoxic, carcinogenic, or immunotoxic so that it can be one of the potential medicinal plants from Ie-Seu'um geothermal area of Aceh Province.

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