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Potential of clove stem essential oil (*Syzygium aromaticum* l.) as herbal medicine for antimicrobial resistance agents in livestock: GC-MS analysis and in silico study on safabl protein

Imam Taufik[1,2], Rustini Rustini[3], Friardi Ismed[4], Deddi Prima Putra[4*]

[1]*Doctoral Program of Pharmacy, Faculty of Pharmacy, Universitas Andalas, Padang 25163, West Sumatra, Indonesia.*
[2]*Indonesian Food and Drug Authority (BPOM), Regional Office in Ambon 97116, Maluku, Indonesia.*
[3]*Department of Pharmaceutical Biology, Faculty of Pharmacy, Universitas Andalas, Padang 25163, West Sumatra, Indonesia.*
[4]*Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Universitas Andalas, Padang 25163, West Sumatra, Indonesia.*

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*Correspondence:

Corresponding author: Deddi Prima Putra
E-mail address: putra_aries64@yahoo.com

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ABSTRACT

Methicillin-resistant *Staphylococcus aureus* (MRSA) cases in husbandry animals continue to increase. Antibiotic residues in livestock products are one of the causes of MRSA cases in humans. The use of natural antimicrobials is an alternative in livestock. Clove stem essential oil (*Syzygium aromaticum* L.) has been used conventionally as an antimicrobial. CSEO shows inhibitory action against MRSA. One of the mechanisms is inhibiting the SaFabl protein in the biosynthesis of lipid acids in the bacterial cell wall. This study aimed to analyze Sulawesi's CSEO (Zanzibar variety) content and predict its compounds' activity against SaFabl proteins. Samples were harvested and then hydrodistilled. GC-MS carried out compound analysis. All components of CSEO content were expected to have activity against SaFabl through docking simulations using Molegro Virtual Docker (MVD) version 5.5. The findings of this study indicate that CSEO from Sulawesi consists of three main components, namely eugenol, caryophyllene, and humulene. Meanwhile, one of the minor components is methyl 10,12-heptadecadiynoate. Docking validation has an RMSD value of 2.04±0.11. The docking results show that methyl 10,12-heptadecadiynoate is more active than chloramphenicol as a control drug, but lower than NADP as a native ligand. Methyl 10,12-heptadecadiynoate acts on the SaFabl protein by forming a hydrogen bond at the amino acid residue Val67 and two steric bonds at Val67 and Thr146. In conclusion, CSEO can potentially be a herbal medicine candidate as an antimicrobial resistance agent in husbandry animals. Further in vitro and in vivo studies are needed to validate its antibacterial efficacy against microbial resistance.

Introduction

Antimicrobial resistance (AMR) primarily contributes to global mortality (Walsh *et al*., 2023). In 2019, there were 4.95 million fatalities attributed to drug-resistant diseases, far surpassing the yearly global deaths from tuberculosis at 1.5 million, HIV/AIDS at 864 thousand, and malaria at 643 thousand (Salam *et al*., 2023). Without intervention, it is estimated that there will be 10 million deaths in 2050, with a total loss of 10 trillion USD (Tang *et al*., 2023). One of the problems in treating infections is drug resistance (World Health Organization, 2022). Resistance can occur due to many factors, such as antibiotic exposure, changes in pathogens, and changes in toxin expression (Gupta and Sharma, 2022). One of the factors is antibiotic residues in livestock products (Adegbeye *et al*., 2024) and the high prevalence of MRSA infections in husbandry animals (Barua *et al*., 2025). The use of synthetic antibiotic drugs can cause residues in livestock products (Menkem *et al*., 2019). One effort to suppress antibiotic residues and treat MRSA infections in animals with an ethnopharmacological approach is the use of antibiotics derived from herbal medicines for animals (McGaw, 2025). One of the natural ingredients that can be used as antimicrobials is essential oils (Helal *et al*., 2019).

Syzygium aromaticum L (*Myrtaceae*), better known in Indonesia as cloves, is an essential oil plant traditionally used as a medicine for toothache (Salsabila *et al*., 2023). In Sulawesi, clove plants are distributed in Central Sulawesi, North Sulawesi, South Sulawesi, and West Sulawesi. Cloves are used as ingredients in making kretek. Meanwhile, natural medicine uses leaf and stem waste as clove oil. In 2022, the clove production capacity in Sulawesi was ranked first in Indonesia, with a value of 68,653 tons (Ditjenbun, 2021). Cloves have also been reported to have antibacterial activity (Ningsih and Arel, 2021). Eugenol in clove essential oil has been observed to inhibit Methicillin-Resistant *S. aureus* (MRSA) bacteria with a Minimum Inhibitory Concentration (MIC) of 10 mm (Hu *et al*., 2018).

Predicting the antibacterial activity of MRSA metabolites contained in clove oil can be done in silico. One of the target proteins for MRSA antibacterial drugs is enoyl-ACP reductase (SaFabl), a protein involved in fatty acid production in the biosynthesis of the *S. aureus* bacterial cell wall (Mobolaji *et al*., 2023).

The novelty of this research is that there has been no publication on Sulawesi cloves docked with SaFabl protein for the development of herbal medicines in animals. Similar research has been conducted on cinnamon and lemongrass oil docked with eugenol (Gao *et al*., 2020; Berly and Kapelle, 2023). Meanwhile, docking of isoeugenol compounds has also been carried out on the SaFabl protein (Alnasser *et al*., 2023). This study aimed to examine the potential of clove stem essential oil as an anti-MRSA herbal medicine in animals by identifying the contents of clove oil using GC-MS and predicting molecular docking, one of its working mechanisms in inhibiting the SaFabl protein from MRSA bacteria.

Materials and methods

Samples of clove leaves (*Syzygium aromaticum* L) of the Zanzibar variety were harvested from Oro Batu Village, Tapalang, Mamuju, West Sulawesi, Indonesia (-2.52 Latitude, 118.71 Longitude, 8 m Sea Level) (Elevation Map, 2020) on July 19, 2024, in the morning (24.8°C, 87% humidity, 4149 mm rainfall, Af climate type) (Climate Data, 2024). The leaves were picked from 10-15-year-old trees, air-dried for 4-5 days, and chopped into small pieces. The dried leaves were ready for distillation. Herbarium specimens were identified at the Herbarium Materia Medika, Batu, Malang, East Java, Indonesia (voucher number: 000.9.3./3305/102.20/2024).

The analytical instruments used in the analysis of essential compounds were GC-MS (Thermo Scientific), GC (Trace-1310), Single Quadrupole MS (ISQ 7000), Autosampler (TriPlus RSH), and Library (MainLib). Other tools used are analytical balance (Mettler Toledo MS-204-TS), micropipette (Eppendorf), vortex (Digisystem VM-2000), ultrasonic (Elma-

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sonic S40H), recirculating chiller (Buchi F-314), hot plate (ITS HS-7), laboratory blender (Waring) and glassware (Pyrex).

Distilled water and anhydrous sodium sulfate (Merck) are used for clove oil distillation. The materials used for analyzing essential oil content are eugenol standard (Merck), ethanol p.a. (Merck), methanol p.a. (Merck), helium gas, nitrogen, and hydrogen. The materials used for in silico testing are Molegro Virtual Docker v.5.5 (MolegroAps), Chem Bio 3D Ultra and Chem Bio Draw v.13 (CambridgeSoft).

Distillation

The sample was chopped into small pieces, weighed 100 g, and put into a 2 l sample distillation flask; 1 l of distilled water was added to the 2 l sample flask, and hydro distillation was carried out using a hot plate at a temperature of 92.5±2.5°C for 6 hours using a magnetic stirrer. The oil and water from the distillation were collected in a closed separating funnel, separated, and purified using anhydrous sodium sulfate salt. CSEO was weighed, and the yield (% w/w) was calculated and stored in a tightly closed glass bottle in a refrigerator at a temperature of 4oC for testing (Tutuarima and Antara, 2020).

Verification of GC-MS method

Preparation of sample solution: The sample was pipetted 10.0 µl, added ethanol to the mark in a 5 ml flask (0.2% v/v), and vortexed for 20 seconds. The sample solution was pipetted 1000.0 µl, added ethanol 1000.0 µl, and vortexed for 20 seconds (0.1% v/v). Solution (A) and ethanol solvent (B) were filtered with a 0.45 µm membrane and inserted into a GC-MS vial.

GC-MS analysis: Repeatedly injected 3x each 1 µl of solvent (B) and sample solution (A) with an autosampler. GC-MS conditions are as follows: Agilent HP-5MS column (30.0 m, 0.25 mm, 0.25 µm). Detector and injector temperature 250°C, oven temperature 50-200°C at a rate of 6°C/min, then 200-280°C at a rate of 30°C/min, post-run 280°C for 10 minutes, flow rate 1.0 ml/min He gas, use scan and split less mode. MS conditions: m/z 35-500, EI 70 eV, ion source temperature 250°C, interpreted with the MidLib database (Amelia *et al*., 2017) modified.

Specificity: Spike sample solution (A) was injected 3 times as much as 1 µl under the above GC-MS conditions. Acceptance requirements: sample solution and solvent should not produce the same peaks with m/z values (ion meter, qualification 1, and qualification 2) and ion ratios. Spike sample solution provides retention time, m/z values (ion meter, qualification 1, and qualification 2), and ion ratios in the scan method. The resolution between compound (analyte) peaks is > 1.5. Precision: Spike sample solution (A) and solvent (B) were injected repeatedly 6 times as much as 1 µl under the above GC-MS conditions (0; 24 hours). Precision was calculated using the RSD formula. Acceptance requirements: RSD value < 2.0%. Identification of all peaks (minimum area 10,000) with the main library (AOAC, 1997).

Molecular docking

Preparation of target compounds and proteins: The structure of the 4-component clove oil compound from GC-MS analysis was first drawn using the Chem Bio Draw Ultra 2D program and then converted to 3D using the Chem Bio 3D program (Cambridge Soft Corporation). The compound structure in mol2 format was then docked with the target protein that had previously been downloaded from the protein data bank (https://www.rcsb.org). One of the MRSA antibacterial target proteins used was the SaFabl protein (PDB: 4ALL), which contributes to the production of fatty acids in *S. aureus* (Bai *et al*., 2023). Before being used for molecular docking of drug compounds, SaFabl protein was first validated using the native ligand NADP (Tabassum *et al*., 2023).

SaFabl Docking: Activity prediction was carried out in silico (molecular docking) between the molecule and the target protein. The docking program can use applications such as Molegro Virtual Docker (Kesuma *et al*., 2018). The in-silico test will produce a binding energy value or re-rank score (RS). Binding energy denotes the energy required to connect the ligand and the receptor. A lower bond energy correlates with more excellent bond stability. A more stable bond correlates with an increased expected chemical activity (Mobolaji *et al*., 2023). The bond between the methyl 2-butoxy benzoic compound and the amino acid residues of the SaFabl protein is not as much as the native ligand or the control drug (Cansian *et al*., 2017). This facilitates a reduction in the energy required to establish a connection between the active chemical and the receptor (Kesuma *et al*., 2018).

Prediction of physicochemical properties and ADMET

According to the "Lipinski Rule," in predicting the physicochemical properties of a compound, when the molecular weight surpasses 500, or the Log-P value (octanol-water partition coefficient) exceeds 5, or there are more than 5 hydrogen bond donors, or there are more than 10 hydrogen bond acceptors, then there is a high probability of poor absorption or penetration of the compound (Roskoski, 2019). The SMILE code of the compound was obtained from https://pubchem.ncbi.nlm.nih.gov/. The physicochemical parameters were predicted online at (http://www.swissadme.ch/) (Illian *et al*., 2022).

Prediction of the pharmacokinetic properties of a drug compound in the body is significant. This ensures the drug compound can reach the target receptor and work according to the predicted bioactivity to produce pharmacological effects. The pharmacokinetic profile includes four perspectives of drug travel in the body: absorption, distribution, metabolism, and excretion, and the fifth perspective is toxicity (ADMET). In vivo, ADMET, and preclinical testing require a long time, expensive costs, and valid methods. Before laboratory testing, ADMET can be predicted by in-depth algorithm calculations. We carried out The ADMET prediction with online facilities from (https://biosig.lab.uq.edu.au/deeppk/) (Sulistyowaty *et al*., 2025).

Results

Dry samples in the form of clove stem (*Syzygium aromaticum* L) of the Zanzibar variety that were chopped into small pieces were distilled using the hydro distillation method using a hot plate at a temperature of 92.5±2.5°C for 6 hours using a magnetic stirrer. The results of CSEO distillation with this method produced a yield of 1.07%±0.76. The hydro-distillation method on clove oil provides an equal yield and eugenol content to steam distillation (Nirwana and Zamrudy, 2021). In addition, hydro-distillation has the advantage of faster distillation than steam distillation. Still, the disadvantage is that it is unsuitable for unstable or quickly hydrolyzed compounds. (Tutuarima and Antara, 2020).

Compared with similar studies, the yield of clove oil derived from leaves with steam distillation method for 6-7 hours is 1.84-1.87%. Meanwhile, in hydro-distillation, pre-treatment of oven heating for 6 hours at 70°C produces a yield of 2.83% (Nirwana and Zamrudy, 2021). The results obtained in this study differ from those obtained in other studies due to differences in distillation methods, the presence of pre-treatment of oven drying, and the duration of distillation.

GC-MS analysis

The results of GC-MS analysis of CSEO samples from Sulawesi in 0.01% solution produced 12 compounds, as shown in the chromatogram in Fig 1. Three significant compounds are eugenol (57.22%±1.45), caryophyllene (38.05%±1.14), and humulene (2.79%±61). One of the minor compounds is methyl, 10,12- heptadecadynoate (0.03%±3.85). The identification of compounds and their structures is based on the identification

of m/z ion fragmentation and primary libraries, as shown in Table 1. Table 1 explains 12 compounds contained in CSEO originating from Sulawesi, consisting of eugenol, caryophyllene, and humulene. As well as minor components including methyl, 10,12- heptadecadynoate, copaene, and B-guaiene. Compound structure prediction is based on retention time, ion fragmentation m/z, and mass.

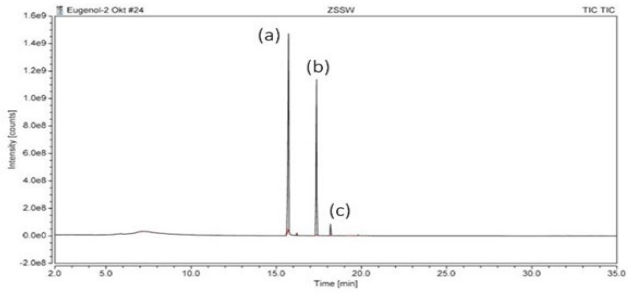


Fig 1. GC-MS Chromatogram of CSEO Sample from Sulawesi Containing Compound Peaks. Three significant compounds are: (a) eugenol (RT: 15.72 minutes, Area: 57.22%±1.45); (b) caryophyllene (RT: 17.36 minutes, Area: 38.05%±1.14); (c) humulene (RT: 18.18 minutes, Area: 2.79%±61).

GC-MS Method Verification

The results of the GC-MS method verification indicated that the specificity of eugenol, caryophyllene, and humulene (resolution: 2.48; 8.75 and 6.95), intraday precision (0 hours), and inter-day precision (24 hours) (Table 2).

Docking Validation on SaFabl Protein

Fig 2a shows a model of the SaFabl protein and its native ligand NADP. Fig 2b compares the interactions between NADP and the SaFabl protein. The cavities of the SaFabl protein and the active site of the receptor is depicted in Fig 2c.

The docking results with the native ligand are shown in Fig 3m. This Fig explains the bond between NADP as a native ligand of the SaFabl protein. NADP forms 10 hydrogen bonds at the amino acid residue Val67, Asp66, Ser44, Arg40, Ser19, Ser197, Ile20, Lys164, Ile193, Thr195, 2 electrostatic interactions at Arg40, Lys41 and 17 steric bonds at Ala95, Asp66, Val67, Ser44, Arg40, Ala15, Gly13, Ser19, Ser197, Ile20, Ser93, Ile94, Thr145, Pro192, Ile193, Thr195, and Lys164.

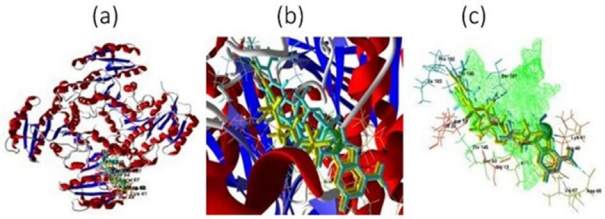


Fig 2. (a) SaFabl Protein with the Native NADP Ligand; (b) Comparison of the Interaction between the Native NADP Ligand (Yellow) and the Docking Results of NADP (Turquoise) with SaFabl Protein with RMSD 2.04288±0.11129; (c) Active Cavities of SaFabl Protein, Binding Site for Amino Acid Residues with NADP Ligand.

Table 1. GC-MS analysis and identification of CSEO component compounds.

No	RT	Product Ion m/z	Formula	Massa	Compound Prediction	% Area
1	11.36	152; 120; 92	$C_{12}H_{16}O_3$	208.11	Methyl 2-Butoxy Benzoic	0.07
2	15.72	164; 149; 77	$C_{10}H_{12}O_2$	164.08	Eugenol	57.22
3	16.2	204; 161; 105; 93	$C_{15}H_{24}$	204.19	Copaene	0,60
4	17.36	161; 105; 93; 67	$C_{15}H_{24}$	204.19	Caryophyllene	38,65
5	17.54	120; 105; 94; 59	$C_{18}H_{28}O_2$	276.21	Methyl, 10,12- Heptadecadynoate	0.03
6	18.18	204; 121; 93; 80	$C_{15}H_{24}$	204.19	Humulene	2.79
7	19.36	93; 69; 53	$C_{15}H_{24}$	204.19	1,3,6,10-Dodecatetraene 7,11-Trimethyl-ZE	0.04
8	19.82	189; 161; 131; 119	$C_{15}H_{24}$	204.19	B-Guaiene	0.31
9	21.86	164; 149; 43	$C_{12}H_{14}O_3$	206.09	Eugenyl Acetate	0.19
10	22.09	204; 161; 105; 81	$C_{15}H_{24}$	204.19	Cis-Muurola-3,5-diene	0.03
11	22.6	121; 109; 55	$C_{15}H_{24}$	204.19	Cis-a-Bisabolene	0.02
12	25.01	220; 119; 91; 77	$C_{15}H_{24}O$	220.18	Cedr-8-ene-13-ol	0.02

Area % data were obtained from the mean of 3 measurements (n=3). Peaks were confirmed with data from at least 3 m/z ions. RT: Retention Time.

Table 2. GC-MS verification results.

Compound	Specificity Resolution	Precision (RSD)			
		Intraday (0 hour)		Interday (24 hours)	
		RT	Area	RT	Area
1	n.a	0.22	1.59	0.01	4.25
2	2.48	0.19	0.84	0	2.21
3	5.61	0.18	1.81	0	2.84
4	8.75	0.18	2.38	0.01	0.59
5	1.18	0.15	2.56	0.02	0.05
6	6.95	0.15	5.25	0.01	14.46
7	4.37	0.18	3.11	0.02	0.08
8	6	0.15	0.84	0.02	2.13
9	1.72	0.15	2.2	0.01	1.12
10	2.08	0.15	1.92	0	3.6
11	2.61	0.14	0.5	0	1
12	3.04	0.14	2.33	0.01	0.06

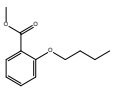
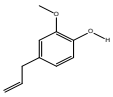
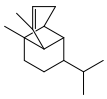
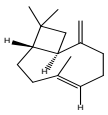
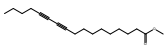
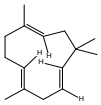
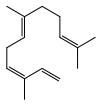
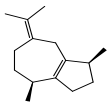
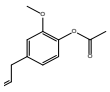
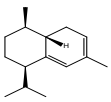
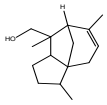
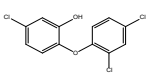
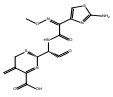
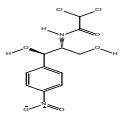
Data is displayed as mean (n=3) ± RSD. RT: Retention Time; RSD: Relative Standard Deviation. Intraday precision is performed at 0 hours; Interday precision is performed at 24 hours.

Molecular Docking

The docking results on the SaFabl protein showed that methyl, 10,12- heptadecadynoate was more active than chloramphenicol as a control drug but lower than NADP as a native ligand with a re-rank score of -98.56±6.83, -80.21±3.65 and -177.74±0.27 respectively. Methyl 10,12-heptadecadiynoate works on the SaFabl protein by forming a hydrogen bond at the amino acid residue Val67 and two steric bonds at Val67 and Thr146, as shown in Figure 3e. Chloramphenicol, as a control drug, binds to SaFabl, as in Figure 3o

Table 3 shows the molecular docking results. The methyl 2-butoxy benzoic compound has a lower re-rank score value than the three major components, including the native ligand and chloramphenicol as a control drug. The re-rank score for methyl 2-butoxy benzoic was -98.56±6.83,

Table 3. Molecular docking results of clove oil compounds on safabl protein.

Compound	Structure	RS (kcal/mol)	Types of Interaction with Amino Acid Residues		
			Hydrogen	Electrostatic	Steric
1		-75.44	Tyr157; 1le193	-	Tyr157; 1le193
2		-65.55	Ile193	-	Ile193; Gly191; Ile207
3		-65.07	-	-	Arg40; Gly13
4		-68.47	-	-	Gly191; Pro192; Phe204; Tyr157
5		-91.73	Val67	-	Val67; Thr146
6		-71.20	-	-	Pro192
7		-78.13	-	-	Gly13
8		-72.31	-	-	Val201; Tyr147; Pro192
9		-69.54	Ser44; Arg40	-	Ser44; Arg40; Ile94; Ile65
10		-75.26	-	-	Val67; Ile65; Gly13
11		-76.71	-	-	Ile193; Pro192; Val201; Tyr157
12		-62.08	Ile193	-	Tyr147; Thr146; Ile193; Thr195
13		-177.47 RMSD: 2.0428±0.1113 A	Val67; Asp66; Ser44; Arg40; Ser19; Ser197; Ile20; Lys164; Ile193; Thr195	Arg40, Lys41	Ala95; Asp66; Val67; Ser44; Arg40; Ala15; Gly13; Ser19; Ser197; Ile20; Ser93; Ile94; Thr145; Pro192; Ile193; Thr195; and Lys164
14		-107.19	Ser197; Ser198; Ser44; Thr38; Ile65	-	Ser197; Gly13; Ser44; Thr38; Ile94; Arg40; Tyr39; Ile65; Ala15; Ser19; Asn16
15		-76.55	Ser93; Ala95; Lys164; Ser19; Leu196	-	Ser93; Ala95; Lys164; Ser19; Leu196; Thr195

RSD and RMSD Data are displayed as mean (n=5) ± SD. SD: Standard Deviation; RS: Re-rank Score; RMSD: Root Mean Square Deviation.

chloramphenicol was -80.21±3.65, and NADP was -177.74±0.27. A lower re-rank score indicates more stability of the chemical or the higher its activity.

Prediction of Physicochemical Properties and ADMET

Based on the results of CSEO compound docking, ADMET predicted five compounds with the highest activity. These compounds are [5] Methyl, 10,12- Heptadecadynoate; [7] 1,3,6,10-Dodecatetraene 3,7,11-Trimethyl-ZE; [11] Cis-a-Bisabolene; [10] Cis-Muurola-3,5-diene [1] Methyl 2-Butoxy Benzoic. From the online prediction results (http://www.swissadme.ch/), the five compounds fulfil Lipinski's rule (Table 4), namely hydrogen bond donor (HBD) ≤ 5, molecular weight (MW) ≤ 500, Log-P <5, and hydrogen bond acceptor (HBA) ≤ 10 (Table 4).

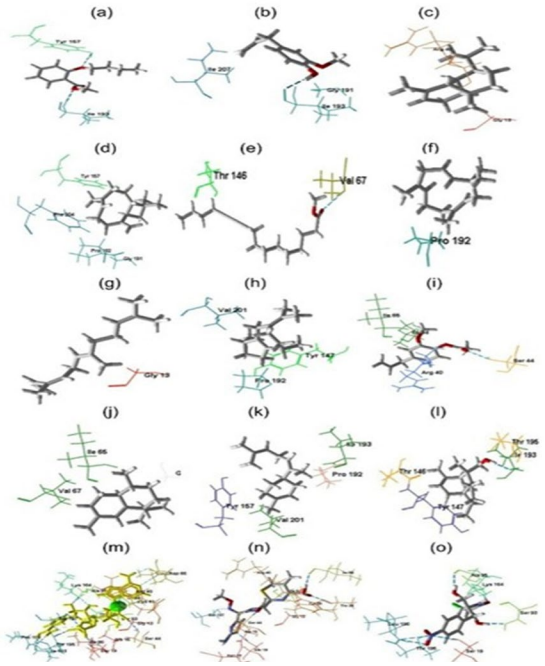


Fig. 3. Interaction Between Ligand and SaFabl Protein: (a) Methyl 2-Butoxy Benzoic; (b) Eugenol; (c) Copaene; (d) Caryophyllene; (e) Methyl, 10,12- Heptadecadynoate; (f) Humulene; (g) 1,3,6,10-Dodecatetraene 3,7,11-Trimethyl-ZE; (h) B-Guaiene; (i) Eugenyl Acetate; (j) Cis-Muurola-3,5-diene; (k) Cis-a-Bisabolene; (l) Cedr-8-ene-13-ol; (m) NADP; (n) Cefotaxime; (o) Chloramphenicol.

Discussion

In GC-MS analysis, the results obtained in the analysis of clove oil content from Sulawesi have similarities in significant content with the research results in other publications. Clove bud oil from Manado and Java has the same major components, namely eugenol (55.60%), caryophyllene (14.84%), humulene (2.75%), and eugenyl acetate (8.70%) (Amelia *et al*., 2017). In a similar study conducted on the analysis of clove oil from Saparua Maluku, it also contained trans-caryophyllene (74.59-91.34%), eugenyl acetate (21.66-34.67%) and eugenol (46.69-64.91%) (Berly and Kapelle, 2023). The difference between clove oil from Java, Manado, and Maluku lies in the content of its main compounds. Furthermore, the substance of minor compounds is also different. These differences may be due to a variety (Lee *et al*., 2015), growing location (Hashim *et al*., 2020), Climate (Mishra, 2016) and soil mineral content (Taufik *et al*., 2021).

In verification of GC-MS method, because it is an analysis with non-target compounds, verification was carried out on the parameters: specificity, intra-day and inter-day precision (AOAC, 1997). The verification results of this method's verification meet the requirements, namely specificity with a resolution value of >1.5 and intraday and inter-day precision < 2.0. Similar studies on stability testing have been conducted. Stability testing at a particular time is still acceptable if 80% of the samples are within the acceptance limit (Brown *et al*., 2015). In our study, the acceptance limit was statistically tested at 5% against the percentage of significant content of clove oil compounds.

Docking validation on SaFabl (PDB: 4ALL) has an RMSD value of 2.04288±0.11129 A. The results of validating the SaFabl protein before being used for docking showed that the validation value for the native ligand NADP met the requirements <2.0 A. This follows the resolution of the SaFabl protein (PDB: 4ALL) in PDB RCSB of 2.80 A (Masumi *et al*., 2022).

A similar study has been conducted docking between isoeugenol compound and SaFabl protein, resulting in a re-rank score of -4.55 kcal/mol and hydrogen bonding with amino acid residues, namely Asn146 and Gly296. While allosteric bonds with amino acid residues are Lys316, Ile309, Asn146, Tyr297, Glu294, Lys273, Asp295, Gly296, Tyr105, and Asn104 (Alnasser *et al*., 2023). These results are not the same as the current study because the isomer of the eugenol compound was docked. Meanwhile, eugenol and eugenyl acetate compounds have never been published with SaFabl protein.

In a prediction of physicochemical properties and ADMET, The forecasts of chemical toxicity classes were categorized into the following segments: Class 1 = extremely lethal (lethal dose 50 (LD50) ≤ 5); Class 2 = fatal (5 < LD50≤ 50); Class 3 = toxic (50 < LD50 ≤ 300); Class 4 = harmful (300 < LD50 ≤ 2,000); Class 5 = possibly hazardous (2,000 < LD50 ≤ 5,000); and Class 6 = nontoxic (LD50 > 5,000). Based on the toxicity prediction results at (https://biosig.lab.uq.edu.au/deeppk/), the four compounds, except Cis-Muurola-3,5-diene, are safe from mutagenic

Table 4. Prediction of physicochemical properties, toxicity and adme of 5 active compounds in cseo.

CP	MW	Log P	HBD	HBA	RB	LR	Toxicity			
							AMES	CR	ORAT	DILI
5	276.41	4.47	0	2	11	Yes	Safe	Toxic	1.82	Safe
7	204.35	4.96	0	0	6	Yes	Safe	Toxic	1.57	Safe
11	204.35	4.77	0	0	3	Yes	Safe	Toxic	1.5	Safe
10	204.35	4.15	0	0	1	Yes	Toxic	Toxic	1.47	Safe
1	208.25	2.81	0	3	6	Yes	Safe	Safe	1.82	Safe

CP	Absorption		Distribution		Metabolism (CYP Inhibitor)					Excretion	
	$CaCO_2$	SP	VD	PB	2D6	3A4	1A2	2C9	2C19	Total Cl	$T_{1/2}$
5	-4.37	-2.87	1.39	34.33	Non	Non	Inhibit	Inhibit	Inhibit	8.19	<3
7	-4.25	-3.45	4.54	30.08	Non	Non	Non	Non	Non	7.32	>3
11	-4.34	-3.2	3.4	19.07	Non	Non	Non	Non	Non	8.93	≥3
10	-4.46	-3.22	2.69	50.3	Non	Non	Non	Inhibit	Inhibit	10.23	<3
1	-4.47	-2.89	1.15	17.54	Non	Non	Inhibit	Inhibit	Inhibit	6.67	<3

CP: Compounds; MW: Molecular Weight (g/mol); HBD: H-Bond Donor; HBA: H-Bond Acceptor; RB: Rotatable Bond; LR: Lipinski's Rule; AMES: Mutagen; CR: Carcinogen; ORAT: LD50 (mol/kg); DILI: Hepatotoxicity; SP: Skin Permeable; VD: Volume Distribution (L/kg); PB: Protein Binding; T1/2: Half Time (hours).

properties. Meanwhile, due to its carcinogenic properties, only methyl 2-butoxybenzoic is safe (Table 4) (Illian *et al*., 2022).

Before laboratory testing, ADMET can be predicted by in-depth algorithm calculations. We carried out the ADMET prediction with online facilities from (https://biosig.lab.uq.edu.au/deeppk/) (Sulistyowaty *et al*., 2025). In ADMET prediction, from the absorption properties, the five compounds have low permeability (P value of $CaCO_2$ <4) (Yamashita *et al*., 2000). In terms of distribution properties, the compound with the highest form bound to plasma protein is Cis-Muurola-3,5-diene, and the lowest is Methyl 2-Butoxy Benzoic. For metabolic properties, 2 compounds do not inhibit the CYP 2C9 enzyme, an enzyme that plays a role in metabolic clearance (molecules), namely compounds 1,3,6,10-Dodecatetraene 3,7,11-Trimethyl-ZE and Cis-a-Bisabolene. Meanwhile, from the excretion properties, the compound with the highest clearance is Cis-Muurola-3,5-diene, while the compound with the lowest clearance is Methyl 2-Butoxy Benzoic (Niu *et al*., 2024) (Table 4).

Conclusion

The finding of this study is that clove stem essential oil (CSEO) from Sulawesi, analyzed by GC-MS, contains 12 compounds. The three main compounds are eugenol, caryophyllene, and humulene. One of the minor components is methyl 10,12-heptadecadinoate, which is a compound that is more active than chloramphenicol as a control drug and has the potential as a candidate herbal medicine for livestock as an antimicrobial resistance agent by acting on the SaFabl protein. Furthermore, in vitro and in vivo studies are recommended to prove the activity of CSEO in inhibiting the growth of MRSA in husbandry animals.

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Conflict of interest

The authors have no conflict of interest to declare.

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