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# Profiling Bioactive Compounds and Antimicrobial Potential of Clove (*Syzygium aromaticum* L.) Varieties of Sikotok, Siputih, and Zanzibar Extracts Using Gas Chromatography-Mass Spectrometry and Molecular Docking Analysis

**Khaerani Kiramang*[1], Gemini Alam*[2], Asmuddin Natsir*[3], Sri Purwanti*[3], A. Mujnisa*[3]**

*[1]*Islamic State University of Alauddin, Animal Husbandry Departement, Makassar, 92111, Indonesia*

*[2] *University of Hasanuddin, Pharmaceutical Science and Technology Departement, Makassar, 90245, Indonesia*

*3 University of Hasanuddin, Animal ScienceDepartement, Makassar, 90245, Indonesia*

**Abstract**

Clove (*Syzygium aromaticum* L.) is widely recognized for its rich phytochemical profile and traditional use as a natural remedy with antimicrobial properties. This study aimed to investigate the phytochemical composition of 3 clove varieties, namely Sikotok, Siputih, and Zanzibarusing gas chromatography-mass spectrometry (GC-MS). The potential antimicrobial compounds were evaluated through in molecular docking studies against *Staphylococcus aureus* (3U2D) and *Escherichia coli* (6NTZ) proteins. Clove extracts from young, mature, and fallen leaves were subjected to GC-MS analysis to identify bioactive compounds, focusing on those with a relative abundance greater than 1%. A total of 12 compounds were identified, with significant variations in relative abundances and retention times. The major compounds detected across all varieties were m-eugenol (25.65-58.49%), caryophyllene (7.63-14.39%), and hexatriacontane (0.59-43.184%). Other compounds, such as eugenol, humulene, α-farnesene, eugenol acetate, caryophyllene oxide, sandaracopimaradiene, lupeol, squalene, and nonacosane, were detected in selected samples. The result of molecular docking showed that α-farnesene (-7.2 kcal/mol and -7.4 kcal/mol), sandaracopimaradiene (-6.8 kcal/mol and -6.3 kcal/mol), and eugenol acetate (-6.2 kcal/mol) had significant binding affinities to target proteins 3U2D and 6NTZ, with other major compounds showing binding energies ranging from 5.5 to 5.8 kcal/mol. In conclusion, this study showed the importance of the clove variety in enhancing the antimicrobial potential of extracts, particularly with the Sikotok and Zanzibar varieties, which had higher concentrations of phenolic compounds and terpenoids in young leaves. These results providedvaluable insights into the future applications of natural antimicrobial agents.

Keywords: Clove; GC-MS; antimicrobial; molecular docking; phytochemicals

## 1. Introduction

The global rise of antibiotic-resistant bacteria is the most urgent public health challenge in recent times [1]. As bacteria evolve to withstand the effects of formerly effective drugs, the emergence of multidrug-resistant (MDR) and extensively drug-resistant (XDR) strains complicates treatment strategies and increases healthcare costs, morbidity, and mortality rates [2]. Common bacterial pathogens, such as *Staphylococcus aureus* and *Escherichia coli* are now resistant to multiple antibiotics, rendering standard treatments inadequate and necessitating alternative therapies [3]. The overuse and misuse of antibiotics in human medicine and agriculture have been the primary drivers of this resistance crisis [4]. In combination with poor infection control and sanitation practices, these factors have increased the evolution of bacterial defense mechanisms, including the production of β-lactamases, efflux pumps, and mutations in drug target enzymes [5]. In response to this growing threat, the World Health Organization (WHO) projected that drug-resistant infections could account for 10 million deaths per year by 2050 without immediate action [6].

The urgent need for new antimicrobial agents has led studies to explore natural products as potential sources of novel bioactive compounds [7]. Medicinal plants have long been valued for therapeutic properties, and the majority of the bioactive constituents showed promising antimicrobial activity [8]. For example, clove (*Syzygium aromaticum* L.), a spice indigenous to the Maluku Islands of Indonesia, has significant potential for use in traditional medicine due to the rich history in traditional medicine and documented efficacy in treating infections [9]. The essential oil, which is particularly rich in eugenol, has shown broad-spectrum antibacterial activity, making clove a candidate for further exploration in the fight against resistant bacterial strains [10].

Clove is the most important and widely used spices in both Indonesian and global cuisines due to strong aroma and complex flavor[11]. As a key ingredient, cloves are integral to various savory dishes, spice blends, and sweet baked goods. This plant also enhances beverages, such as tea and mulled wine. Essential oils are also often used as natural preservatives because of the antimicrobial properties. Despite widespread culinary applications, the antibacterial potential of clove varieties, especially against resistant bacteria, remains underexplored [12].

*Corresponding author e-mail: khaerani.kiramang@uin-alauddin.ac.id.

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In North Indonesia, where clove cultivation originated, several varieties of clove have been developed, each distinguished by a unique chemical profile [13]. Some known varieties include Zanzibar, Sikotok, and Siputih, which differ in terms of physical characteristics and concentrations of key bioactive compounds [14; 15]. For example, Zanzibar clove is known for larger buds and higher essential oil content [16]. In contrast, the Siputih variety, unique to Maluku, has a balanced chemical composition that is versatile in traditional medicine and modern food preservation. While these varieties are prized for the culinary and medicinal applications, comprehensive profiling of the chemical composition and antibacterial, particularly against gram-positive and gram-negative bacterial, remains limited [17].

To fill this gap, this study used Gas Chromatography-Mass Spectrometry (GC-MS) to precisely identify and quantify the volatile compounds present in these clove varieties, including eugenol, β-caryophyllene, and other key phytochemicals. GC-MS profiling showed the unique chemical fingerprints of each variety and provided critical insights into the compounds responsible for the antimicrobial properties [18]. The concentration of bioactive compounds can also vary depending on the maturity of the clove leaves. This shows the importance of harvesting at the optimal time to maximize the potency of these compounds. Understanding the relationship between these chemical constituents and the antibacterial effects is essential for identifying potential pharmaceutical applications [19].

In parallel with chemical profiling, molecular docking analysis was used as a complementary method to predict the interaction of these bioactive compounds with bacterial protein targets [20]. By simulating how key compounds bind to bacterial enzymes, molecular docking provided insights into the mechanisms of action at the molecular level [21]. This computational screening process helped to identify the compounds with the highest potential for development as new antimicrobial agents, laying the groundwork for future drug discovery efforts [22].

This study investigated the antibacterial activity of clove extracts from 3 distinct Sulawesi island varieties, namely Zanzibar, Sikotok, and Siputih. Using GC-MS, the chemical composition of these extracts was profiled, and molecular docking studies assessed the interaction of major bioactive compounds with bacterial protein targets. The dual method of GC-MS profiling and molecular docking provided a comprehensive evaluation of the antibacterial potential [23] of these clove varieties, offering promising avenues for the development of new antimicrobial agents to combat antibiotic-resistant bacteria.

## 2. Methods

### *2.1 Materials*

Fresh samples of clove leaves, namely fallen, young, and mature leaves from 3 varieties (Zanzibar, Sikotok, and Siputih) were collected in Majene City, West Sulawesi, Indonesia. The botanical authentication of the plant materials was meticulously conducted at the Botanical Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Negeri Makassar, Indonesia, to ensure accurate identification and verification of the species used in this study.

### *2.2 Extraction Method*

Maceration extraction was used to extract bioactive compounds from the plant materials. For each sample, 50 g of plant material was extracted using 500 mL of hexane over a period of 3 days, maintaining a solvent-to-solid ratio of 1:10 throughout the process. After extraction, the mixtures were filtered, and the solvents were evaporated using a rotary evaporator(BUCHI) to obtain concentrated or dry extracts, respectively. The extraction yield was determined utilizing a precisely defined formula, which enabled accurate quantification of the extracted bioactive compounds [24; 25].

A total of 9 extracts were obtained for subsequent analysis, as shown in Table 1. These extracts included the hexane extracts of clove leaves, namely young (A), mature (B), fallen (C) from the Sikotok variety, young (D), mature (E), fallen (F) from the Siputih variety, young (G), mature (H), and fallen (I) from the Zanzibar variety.

$$\% \text{ Yield extract} = \frac{\text{Weight of dried extracts}}{\text{Weight of dried materials}} \times 100\%$$

### *2.3 GC MS Analysis*

GC-MS analysis was performed at the Chemical Engineering Laboratory of Politeknik Negeri Ujung Pandang (PNUP) in Makassar, Indonesia. A total of 9 extracts (1 g each) were dissolved in 5 mL of 96% ethanol (p.a). The samples were homogenized in an ultrasonic bath(BRANSON 1800)for 30 min at 55°C. Subsequently, the mixture was filtered using Whatman filter paper (No. 42), and the resulting filtrate was injected into a GC-MS Ultra QP 2010 (Shimadzu) instrument [26]. The chromatographic parameters included an injector temperature of 250 °C in splitless mode, a pressure of 76.9 kPa, and a carrier gas flow rate of 14 mL/min, along with a split ratio of 1:10. The ion source and interface temperatures were maintained at 200 °C and 280 °C, respectively, with a solvent cut-off time of 3 min and mass range of 400–700 m/z. The column was an SH-Rxi-5Sil MS, measuring 30 m in length and 0.25 mm in inner diameter. The initial temperature of the column was set to 70 °C, held for 2 min, and then ramped up at a rate of 10 °C/min until reaching 200 °C. The temperature was then increased to 280 °C at a rate of 5 °C/min and held for 9 min, resulting in a total analysis duration of 36 min. The obtained chromatographic data were analyzed using the NIST 17 and Wiley 9 libraries to identify the major compounds present in the plant extracts, specifically those with an area percentage exceeding 1% [27]. The identified major compounds were examined using in molecular docking methods to gain insights into the mechanisms of action and interactions with target proteins. The selected compounds included major constituents present in one or more samples with a relative abundance (% area) ≥ 1% and a similarity index (SI) ≥ 90% [43].

### *2.4 Molecular Docking Studies*

#### *2.4.1**Sample Preparation (Virtual Screening)***

The compounds identified from the GC-MS analysis of the extracts were analyzed using molecular docking methods. The initial step was to search and download the structures of these compounds from PubChem (acces on 10 september 2024

linkhttps://pubchem.ncbi.nlm.nih.gov). This platform provides comprehensive information on compound structures, including SMILES (Simplified Molecular Input Line Entry System) data, which can be used to download 3D structures in PDF for docking purposes. The target proteins for molecular docking were sourced from the Protein Data Bank (acces on 10 september 2024 linkhttps://www.rcsb.org) in PDF [28]. In this study, the bacterial target proteins 3U2D (*Staphylococcus aureus*) and 6NTZ (*Escherichia coli*) were prepared for docking analysis.

*2.4.2**Molecular Docking***

In total, 12 major compounds from the various extracts were selected for docking analysis. Ligand optimization was carried out using Chimera 1.17.3 (UCSF Chimera; acces on 14 september 2024 linkhttps://www.cgl.ucsf.edu/chimera). PyMOL version 2.5 (acces on 14 september 2024 linkhttps://pymol.org/2) was used to refine protein structures, remove ligands, and eliminate water molecules. The docking simulations were executed using PyRxAutoDock Vina (acces on 14 september 2024 linkhttps://pyrx.sourceforge.io), which calculated the binding affinity, RMSD, amino acid residues, and bond types between the optimized ligands and the corresponding receptors. Additionally, the interactions between the ligands and receptors were visualized using the Biovia Discovery Studio Visualizer (acces on 14 september 2024 linkhttps://www.3dsbiovia.com). The ligand exhibiting the lowest binding energy or docking score, with significant hydrogen bonding interactions, was chosen as the most promising candidate [29; 30].

## 3. Result and Discussion

*3.1 Clove Extracts Analysis*

Antibacterial profiling of the clove (*Syzygium aromaticum* L.) varieties Sikotok, Siputih, and Zanzibar was conducted using extracts obtained by maceration with hexane. The yields of these extracts, as summarized in **Table 1**, showed significant variation among the different varieties and leaves types, suggesting thepotential bioactivity and suitability for further analysis. Extraction yields varied across the 3 clove varieties, with the young leaves of the Zanzibar variety yielding the highest extract of 3.25% (Code H). This result suggests that young leaves may possess higher concentrations of bioactive compounds than mature and fallen leaves [31].

**Table 1:** Percentage yields of extracts from different types of clove leaves

| Code | Cloves | *Leaves* | Simplicia (g) | Extract (g) | Yield (%) |
|---|---|---|---|---|---|
| A | Sikotok | Fallen | 50 | 0.8993 | 1.80 |
| B | Sikotok | Young | 50 | 0.6916 | 1.38 |
| C | Sikotok | Mature | 50 | 0.6198 | 1.24 |
| D | Siputih | Fallen | 50 | 0.6554 | 1.31 |
| E | Siputih | Young | 50 | 1.1361 | 2.27 |
| F | Siputih | Mature | 50 | 0.7050 | 1.41 |
| G | Zanzibar | Fallen | 50 | 0.9628 | 1.93 |
| H | Zanzibar | Young | 50 | 1.6248 | 3.25 |
| I | Zanzibar | Mature | 50 | 0.7867 | 1.57 |

In contrast, mature leaves of the Sikotok variety produced the lowest extract of 1.24% (Code C). The yields suggest that both genetic factors inherent in each leaves type and the developmental stage play significant roles in the extraction efficiency of bioactive compounds. This result is consistent with the existing studies, which showed the influence of plant maturity and variety on phytochemical content and extraction efficiency. High-yield extracts are critical for subsequent analyses, such as GC-MS, due to the provision of a more concentrated source of potential antimicrobial agents [32]. Compounds such as eugenol, acetyl eugenol, and caryophyllene can be isolated from *Syzygium aromaticum* extracts [44] and have been identified as major constituents [45].

*3.1 GC-MS Analysis**Compounds in Clove Extracts***

The GC-MS analysis of clove extracts from the Sikotok, Siputih, and Zanzibar varieties showed significant variations in active compound composition. Based on data from **Table 2 and Figure1**, compounds such as m-eugenol, caryophyllene, and humulene were detected in varying proportions in each sample. From the analysis, 25.65% - 58.49%, 7.63% - 14.39 %, and 0.59% - 43.184% of m-eugenol, caryophyllene, andhexatriacontane, respectively, were found in all samples with varying concentrations.

These compounds often exhibit a higher percentage area compared to other compounds, with concentrations ranging from 13.614% to 42.59% in varieties such as Sikotok, Zanzibar, and Siputih. Humulene was also consistently detected, with concentrations ranging from 1.34% to 2.47%. Other compounds, such as farnesene0.25% - 1.06%, eugenol acetate 0.45% - 11.22%, caryophyllene oxide 1.18% - 16.554%, and sandaracopimaradiene0.21% - 1.11% were only detected in some sample and were not found

in all varieties. Additionally, several samples contained concentrations of specific compounds, including lupeol, squalene, and nonacosane at 0.82%-12.21%, 0.84%-3.95%, and 0.12%-1.09%, respectively. Eugenol was detected only in sample code E from the Siputih variety at a very low concentration, showing that the compound is inconsistent across all samples. The

collection of clove leaves at different times, namely young, mature, and fallen leaves, had a significant impact on the composition of compounds detected in the GC-MS analysis.

Young leaves generally contain higher concentrations of active compounds, such as m-eugenol and eugenol, ranging from 21.84% to 58.49%. These compounds tended to accumulate more during the active growth phase, as shown by the high percentage of m-eugenols in the Sikotok and Zanzibar arieties. The high concentration of bioactive compounds may render these young leaves a more effective source for medicinal applications, particularly as antibacterial and anti-inflammatory agents [33].

In mature leaves, the composition of compounds is more variable. Although active compounds, such as caryophyllene and humulene are still detected, with lower concentrations compared to young leaves, ranging from 7.96% - 14.393% and 1.34% - 2.25%, respectively. This reduction in active compounds in mature leaves may be attributed to inefficient metabolic processes and an increase in complex secondary compounds, which could affect the bioactivity of the extracts [34].

Fallen leaves typically exhibit the lowest concentrations of active compounds. Biochemical degradation of fallen leaves leads to the loss of active compounds that accumulate during the growth phase. In the analysis, many of the most significant compounds were identified as non-flavonoids. This showed a marked decrease in the concentration of bioactive compounds. This decline shows the impact of environmental factors and degradation processes on the phytochemical profile of fallen leaves [35]. The results of the GC-MS analysis showed that the timing of clove leaves collection significantly affected the composition of the compounds. Young leaves had higher concentrations of active compounds than mature and fallen leaves [36]. Therefore, in further studies and practical applications, it is important to consider the timing and conditions of leaves collection to maximize the bioactive potential of clove extract. This knowledge can contribute to the development of more effective and high-quality herbal products.

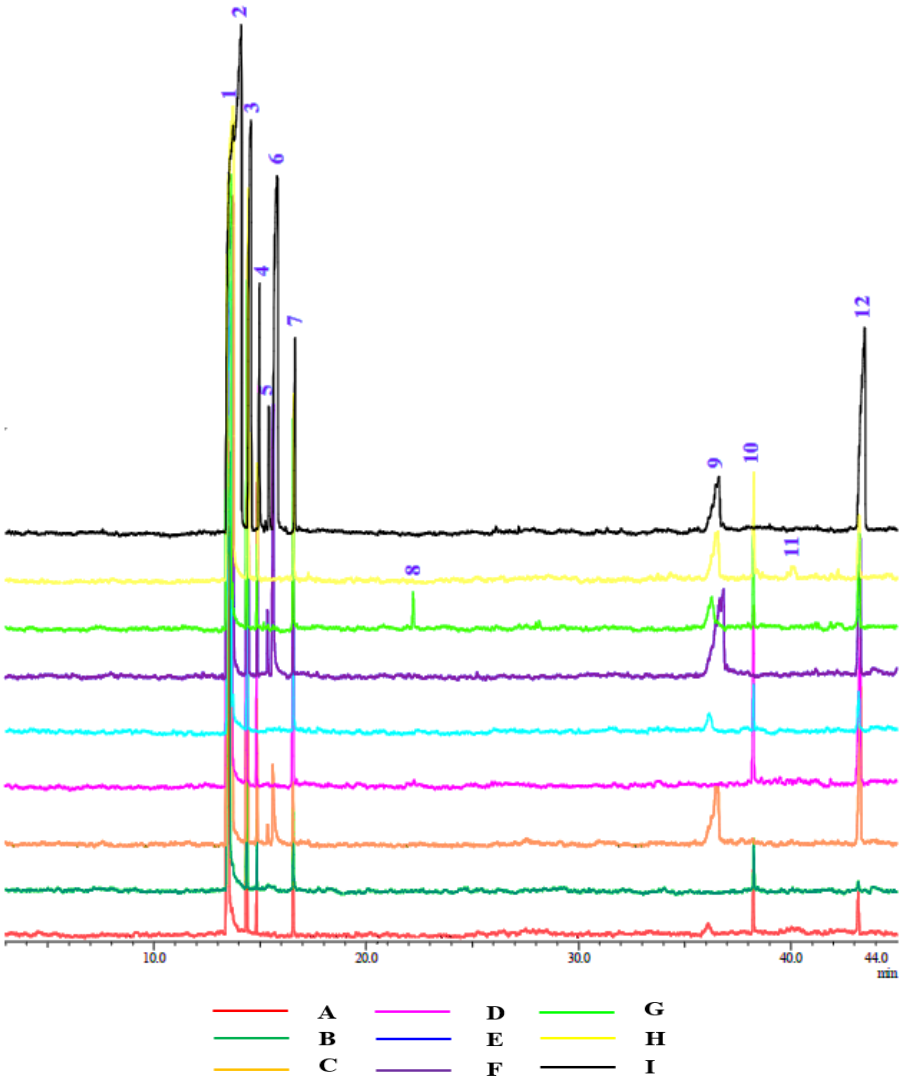


**Figure 1: GC-MS spectra of hexane extracts from Clove containing varieties of Sikotok, Siputih, and Zanzibar.**Peaks 1-12 represent major compounds found in the extracts at concentrations above 1%, as detailed in Tables 2. Samples A–I correspond to the experimental procedure described in Table 1.

**Table 2:** GC-MS analysis of clove extracts from the Sikotok, Siputih, and Zanzibar varieties

| Peaks | Compounds | MS (g/mol) | Area (%) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | A | B | C | D | E | F | G | H | I |
| 1 | Eugenol | 164 | NF | NF | NF | NF | 21.84 | NF | NF | NF | NF |
| 2 | m-eugenol | 164 | 51.60 | 47.21 | 58.49 | 42.59 | 25.65 | 13.614 | 41.04 | 47.11 | 43.92 |
| | Caryophyllene | 204 | 12.87 | 12.68 | 13.96 | 8.74 | 12.65 | 14.393 | 7.96 | 9.38 | 7.63 |
| 4 | Humulene | 204 | 1.81 | 2.47 | 2.15 | 1.55 | 2.25 | NF | 1.45 | 2.34 | 1.34 |
| 5 | α-farnesene | 204 | NF | 0.53 | NF | NF | 0.87 | 0.25 | NF | 1.06 | 0.34 |
| 6 | Eugenol acetate | 206 | NF | 3.41 | NF | NF | 6.35 | NF | NF | 11.22 | 0.45 |
| 7 | Caryophyllene oxide | 220 | NF | 1.18 | NF | 5.02 | 1.33 | 16.554 | NF | 1.64 | 2.52 |
| 8 | Sandaracopimaradiene | 272 | NF | NF | NF | 0.21 | NF | NF | 1.11 | NF | NF |
| 9 | Lupeol | 426 | 0.82 | 6.10 | NF | NF | 12.21 | 2.94 | 4.01 | 3.66 | 4.91 |
| 10 | Squalene | 410 | 3.27 | 0.84 | 2.84 | 3.95 | NF | 1.32 | 2.58 | NF | 2.31 |
| 11 | Nonacosane | 410 | NF | NF | NF | NF | NF | 0.81 | NF | 0.12 | 1.09 |
| 12 | Hexatriacontane | 506 | 3.31 | 7.42 | 0.59 | 5.98 | 5.26 | 43.184 | 3.88 | 7.80 | 2.04 |

**Note**: NF (Not Found)

*3.2 Molecular Docking Analysis*

The data in **Table 3, and Figures 2, and 3** show the binding affinities and interactions of various compounds with the target proteins of *Staphylococcus aureus* (3U2D) [37] and *Escherichia coli* (6NTZ) [38]. These results provide insights into the potential antimicrobial properties of the analyzed compounds, with a particular focus on the correlation between structural features and biological activities. Binding free energy values (in kcal/mol) are crucial for determining the affinity of each compound for the target protein. Generally, lower (more negative) values show stronger binding affinity [39]. The native ligand for *Staphylococcus aureus*has a binding free energy of -8.1 kcal/mol, showing strong interaction with residues, such as Glu58, Asp81, and Pro87. For *Escherichia coli*, the native ligand binding free energy is -7.4 kcal/mol, with interactions including residues, such as Ser 73, Lys 76, Ser 115, Ser 116, Ser 139, Asn 141, Thr 243, and His245.Compounds, such as α-farnesene and sandalacopimaradiene showed promising binding energies (-7.2 and -6.8 kcal/mol, respectively), although not through hydrogen bond interactions. This result shows the potential efficacy against targeted Staphylococcus aureus. Research indicates that α-farnesene (20.27%) in the hexane extract of the aerial parts of *Conyza sumatrensis* exhibits inhibition against *Staphylococcus aureus* at moderate to high concentrations [46].Meanwhile, compounds, such as hexatriacontane and nonacosane showed weaker binding affinities (-5.0 and -4.8 kcal/mol, respectively), suggesting less effectiveness against inhibition.

The identified compounds can be connected to the chemical composition of various clove varieties. The variations in bioactive compound contents significantly influence the binding affinity andpotential antimicrobial activity.The Sikotok and Zanzibar varieties had higher concentrations of m-eugenol (25.65% - 58.49%)and α-farnesene - 7.2 kcal/mol and – 7.4 kcal/mol, respectively), known for the antimicrobial properties. These results suggested that the strains were more effective against *Staphylococcus aureus* and *Escherichia coli*[40].

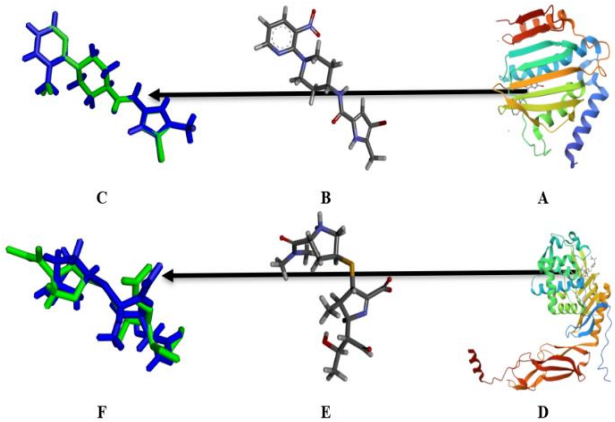


**Figure 2:** Structure of the *Staphylococcus aureus* target protein (3U2D) (**C**) along with its native ligand, 4-bromo-5-methyl-N-[1-(3-nitropyridin-2-yl)piperidin-4-yl]-1H-pyrrole-2-carboxamide (**B**). The re-docking of the native ligand (co-crystal) into the 3U2D protein target pocket validates the docking method, resulting in a root mean square deviation (RMSD) value of 0.072 Å (**A**). Similarly, the structure of the *E. coli* target protein (6NTZ) (**F**) is shown along with its native ligand (2S,3R,4S)-4-[(3S,5R)-5-(dimethylcarbamoyl)pyrrolidin-3-yl]sulfanyl-2-[(2S,3R)-3-hydroxy-1-oxobutan-2-yl]-3-methyl-3,4-dihydro-2H-pyrrole-5-carboxylic acid (**E**). The re-docking of the native ligand (co-crystal) into the 6NTZ target pocket validates the docking method, with an RMSD value of 1.997 Å (**D**).

**Table 3:** Bond-free energy values and amino acid residues binding to protein 3U2D (*Staphylococcus aureus*) and 6NTZ (*Esherichia coli*)

| Peaks/ Compounds | Protein Target | Compounds | *Bond-Free Energy (kcal/mol)* | H-Bond Interaction |
|---|---|---|---|---|
| Native ligan | *S. aureus* (3U2D) | **(4-bromo-5-methyl-N-[1-(3-nitropyridin-2-yl)piperidin-4-yl]-1H-pyrrole-2-carboxamide)** | -8.1 | Glu 58, Asp 81, Pro 87 |
| 1. | | Eugenol | -5.8 | Ser 55, Val 79, Thr 81 |
| 2. | | m- eugenol | -5.7 | Glu 58, Gly 85, Thr 173 |
| 3. | | Caryophyllene | -5.7 | NI |
| 4. | | Humulene | -5.3 | NI |
| 5. | | α-farnesene | -7.2 | NI |
| 6. | | Eugenol acetate | -6.2 | Asn 54, Gly 58, Thr 173 |
| 7. | | Caryophyllene oxide | -5.9 | NI |
| 8. | | Sandaracopimaradiene | -6.8 | NI |
| 9. | | Lupeol | -5.9 | Glu 58 |
| 10. | | Squalene | -7.4 | NI |
| 11. | | Nonacosane | -5.4 | Gly 85, Thr 173 |
| 12. | | Hexatriacontane | -5.0 | NI |
| Native ligan | *E. coli* (6NTZ) | (2S,3R,4S)-4-{[(3S,5R)-5-(dimethylcarbamoyl)pyrrolidin-3-yl]sulfanyl}-2-[(2S,3R)-3-hydroxy-1-oxobutan-2-yl]-3-methyl-3,4-dihydro-2H-pyrrole-5-carboxylic acid) | -7.4 | Ser 73, Lys 76, Ser 115, Ser 116, Ser 139, Asn 141, Thr 243, His 245 |
| 1. | | Eugenol | -5.7 | Lys 76, Ser 139, Ser 116 |
| 2. | | m- eugenol | -5.5 | Ser 116, Asn 141, Ser 73 |
| 3. | | Caryophyllene | -5.8 | NI |
| 4. | | Humulene | -5.5 | NI |
| 5. | | α-farnesene | -7.4 | Ser 73, His 245 |
| 6. | | Eugenol acetate | -6.2 | Ser 73, Leu 137, Asn 226, Lys 242, Gly 244, His 245 |
| 7. | | Caryophyllene oxide | -5.9 | Ser 73 |
| 8. | | Sandaracopimaradiene | -6.3 | NI |
| 9. | | Lupeol | -6.2 | NI |
| 10. | | Squalene | -5.9 | NI |
| 11. | | Nonacosane | -4.8 | Arg 227 |
| 12. | | Hexatriacontane | -4.1 | NI |

Conversely, the Siputih variety contained a lower concentration of bioactive compounds, such as eugenol (detected only at very low concentrations), potentially limiting the antimicrobial efficacy. Eugenol, m-eugenol, and eugenol acetate are phenolic compounds with broad-spectrum antimicrobial activity [41]. Eugenol at 57 µg/disk was able to inhibit *Staphylococcus aureus* with a zone of 3.8 ± 0.7 mm through hydrogen bond interactions with Ser 55, Val 79, Thr 81, Glu 58, Gly 85, and Thr 173, compared to Cefotaxime at 30 µg/disk, which showed no inhibition[47]. For *Escherichia coli*, eugenol formed hydrogen bonds at Lys 76, Ser 139, Ser 116, and at Ser 116, Asn 141, and Ser 73, similar to the native ligand. Eugenol acetate was effective against both bacteria with a MIC of 25-50 µg/Mland exhibited hydrogen bonding with Asn 54, Gly 58, Thr 173, Ser 73, Leu 137, Asn 226, Lys 242, Gly 244, and His 245, resembling the native ligand. The presence of these compounds at higher concentrations in certain clove varieties was correlated with better binding to target proteins, thereby enhancing the antibacterial activity.

Caryophyllene and humulene, which were detected consistently across the samples, also contributed to the antimicrobial potential of the clove extracts. This result suggests that the compounds may exhibit effective antimicrobial activity. The binding interactions in the table and figure show potential mechanisms by which these compounds can inhibit bacterial growth. The presence of hydrogen bonds and van der waals interactions with key amino acid residues in proteins was critical for the stability of the compound-protein complex. For instance, the interaction of m-eugenol with Asp 81 and Thr 173enhances binding, potentially disrupting bacterial metabolic processes [42]. These interactions mayinhibit critical enzymes or metabolic pathways in bacteria, providing a viable strategy for developing new antibacterial agents based on clove-derived compounds.

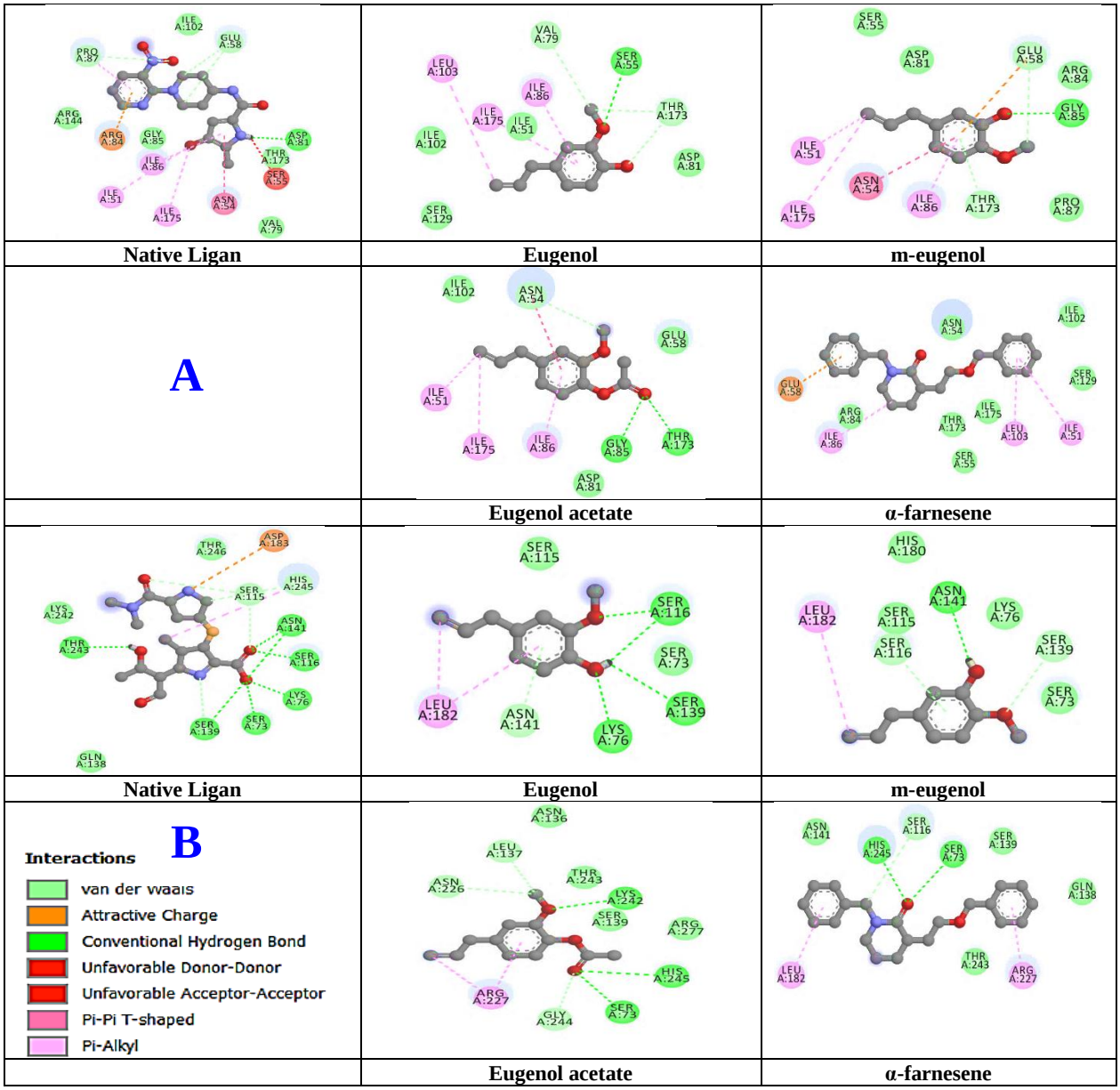


**Figure 3:** H-bond interactions of compounds with the (A) *Staphylococcus aureus* target protein (3U2D) and (B) *Escherichia coli* target protein (6NTZ)

## 4. Conclusion

In conclusion, the GC-MS profiling analysis of clove extracts from the Sikotok, Siputih, and Zanzibar varieties showed significant variations in bioactive compound composition. Key compounds such as m-eugenol, α-farnesene, and caryophyllene showed different concentrations across the varieties. This result suggested that the clove variety significantly affected the therapeutic efficacy of the extract. Binding affinity studies with the target proteins *S. aureus* (3U2D) and *E. coli* (6NTZ) showed strong energies for both native ligands and selected bioactive compounds, suggesting the antimicrobial potential. Compounds, such as eugenol, m-eugenol, eugenol acetate, and α-farnesene exhibited significant interactions with essential amino acid residues, showing the effectiveness as bacterial growth inhibitors. Furthermore, younger leaves were suitable for pharmaceutical application due to the higher concentrations of bioactive constituents. This study showed the significance of varietal selection and optimal harvesting conditions for maximizing the antimicrobial potential of clove extracts, providing insights for future investigations and the development of effective natural antimicrobial agents.

## 5. Conflicts of interest

The authors have no conflict of interest.

## 6. Author Contributions

KhaeraniKiramang conceptualized and designed the study; KhaeraniKiramang and AsmuddinNatsir performed the experiments and collected the data; KhaeraniKiramang, and Gemini Alam analyzed and interpreted the results;

KhaeraniKiramang, and A. Mujnisa prepared the initial manuscript; and KhaeraniKiramang, AsmuddinNatsir and Sri Purwanti reviewed and approved the final version of the manuscript.

**7. Acknowledgments**

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