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Research Paper

Phytochemical Properties, In Silico and In Vitro Analysis of the Antibacterial Activity of *Cinnamomum sulavesianum* Bark from Sulawesi

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Abstract
Cinnamomum sulavesianum is an aromatic plant in Sulawesi, exhibiting significant potential as an antibacterial candidate. The ongoing search for new antibacterial agents, particularly from natural sources, is crucial due to the rising antibiotic resistance. This study aims to identify the essential oil composition of *C. sulavesianum* and evaluate its antibacterial properties in silico and in vitro. This study represents the first report on the essential oil content of *C. sulavesianum* bark and its associated antibacterial effects. The analysis of essential oil content was conducted using Gas Chromatography-Mass Spectrometry (GC-MS) with an Agilent Technologies 7890 Gas Chromatograph equipped with an Auto Sampler. The in silico analysis has been performed through molecular docking of compounds found in *C. sulavesianum*. Antibacterial testing was carried out using the disc diffusion method, targeting bacteria such as *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli* and *Pseudomonas aeruginosa*. The results from the GC-MS analysis revealed that the bark of *C. sulavesianum* consists of 10 essential oil compounds, with the primary constituents being Linalool (32.3%), Copaene (6.77%), Eugenol (5.05%), and Eucalyptol (3.17%). In silico evaluations suggest these compounds possess antibacterial potential against gram-positive and gram-negative pathogenic bacteria. Furthermore, in vitro assessments demonstrated that the bark extract of *C. sulavesianum* formed an inhibition zone that was, on average, categorized as strong based on its inhibitory efficacy. These findings indicate that *C. sulavesianum* bark holds promise for developing new antibiotics.

Keywords
Antibacterial, Bark, Biopharmaceutical, Cinnamon, Oils

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1. INTRODUCTION

Cinnamon is a tree species whose bark is rich in bioactive components, showcasing pharmacological potential and versatility for various health products (Sharifi-Rad et al., 2021; Singh et al., 2020; Wang et al., 2020). The genus Cinnamomum contains essential oils (Vasconcelos et al., 2018) that hold promise for development within the pharmaceutical and health industries. Additionally, this genus possesses secondary metabolites such as terpenoids (Wu et al., 2020), which exhibit significant bioactive potential. The bioactivity of Cinnamomum includes properties such as antibacterial effects (Shu et al., 2024; Wang et al., 2018; Yang et al., 2020), antioxidant (Ashfaq et al., 2021; Pagliari et al., 2023), anti-inflammatory (Abeysekera et al., 2022; Fazmiya et al., 2022; Xiao et al., 2021), antifungal properties (Tran et al., 2020), antidiabetic (Wariyapperuma et al., 2020), and promising anticancer activity (Banerjee and Banerjee, 2023), among various other potential bioactivities such as the use of its essential oils (Putri et al., 2025).

Cinnamon belongs to the Lauraceae family (Yang et al., 2022) and includes species such as *Cinnamomum sulavesianum* (Kostermans, 1986), which is found in Sulawesi. *C. sulavesianum* is widely distributed in ultramafic soils of the East Luwu region in South Sulawesi. This species can grow into a tree up to 20 m tall and 26 cm in diameter. The trunk's bare surface exhibits a brownish-gray coloration, while the inner bark is red. The leaves are arranged oppositely and can be either oblong with three veins measuring 4 × 10 cm or lanceolate, varying between 2 × 9 cm and 6 × 18 cm. They typically have a pointed tip and can have either a rounded or pointed base. The upper leaf surface is shiny and smooth, whereas the underside is dull or glaucous. The petiole is slender, measuring 8-12 mm long, and features a groove at the top. The bark of *C. sulavesianum* exudes a sharp and highly aromatic fragrance Figure 1.

The search for new antibacterial agents aims to discover novel antibiotics that can effectively combat various pathogenic

bacteria and address the growing issue of antibiotic resistance. Bacteria such as *Staphylococcus aureus* are responsible for a range of diseases, including skin infections, and have been identified in cases of septicemia, infective endocarditis, pneumonia, eye infections, and central nervous system infections (Ondusko and Nolt, 2018). Notably, *S. aureus* has been reported to resist several antibiotics, including methicillin (Lee et al., 2018). Another significant pathogen is *Staphylococcus epidermidis*, which is the most frequent cause of implant-related infections. Some strains of *S. epidermidis* are also implicated in skin diseases (Severn and Horswill, 2023). Both of these bacteria are gram-positive. On the other hand, *Escherichia coli* is a gram-negative bacterium that can easily colonize the human intestine and cause intestinal and extraintestinal infections, which may lead to severe invasive diseases such as bacteremia and sepsis (Bonten et al., 2021). *E. coli* also demonstrates antibiotic resistance (Poirel et al., 2018). Another gram-negative bacterium, *Pseudomonas aeruginosa*, is known to cause a wide array of infections in humans (Krell and Matilla, 2024).

The increasing prevalence of antibiotic-resistant bacteria highlights the urgent need to develop new antibiotics from diverse plant species. As an aromatic plant, *C. sulavesianum* demonstrates significant potential for its essential oils, which could be harnessed in various pharmaceutical applications, particularly as antibacterial agents. Therefore, this study aims to identify the essential oil content of *C. sulavesianum* and evaluate its antibacterial properties both in silico and in vitro. This research is the first to reveal the essential oil composition of *C. sulavesianum* and its associated antibacterial properties through computational and laboratory methods.

2. EXPERIMENTAL SECTION

2.1 Tools and Materials

The tools utilized in this study included beakers, measuring cups, stirring rods, horn spoons, scales, blenders, rulers, test tubes, test tube racks, glass jars, Petri dishes, Bunsen burners, tripods, matches, erlenmeyer flasks, clamps, tweezers, incubators, laminar airflow hoods, loop needles, refrigerators, ovens, autoclaves, vortex mixers, vials, and GC-MS. The materials used were *C. sulavesianum* bark extract, 96% ethanol, distilled water, chloramphenicol, nutrient agar, nutrient broth, spirit liquid, cotton, filter paper, syringes, parchment paper, plastic wrap, aluminum foil, and gauze.

C. sulavesianum was sampled in East Luwu Regency, South Sulawesi, based on its distribution in the area. The bark samples collected for testing were from *C. sulavesianum* and were dried before further analysis. Plants collected during field exploration were characterized and identified according to the method outlined by Kostermans (1986).

2.2 Methods

2.2.1 Essential Oil Content Analysis

The analysis of essential oil content was conducted using the Gas Chromatography-Mass Spectrometry (GC-MS) method. An Agilent Technologies 7890 Gas Chromatograph with an auto sampler, equipped with a 5975 mass selective detector and a ChemStation data system, was used for this purpose. The components of *C. sulvesianum* bark essential oils were identified by comparing the mass spectra with the NIST 2005 v.2.0 library and the Wiley 7 library from 2003 (Hurria et al., 2023).

Figure 1. *C. sulavesianum* Bark: (a) Bark Still Attached to the Stem, (b) Bark That Has Been Diced and Dried

2.2.2 In Silico Analysis of Antibacterial Compounds of *C. sulavesianum*

The tools utilized for this study include a Lenovo laptop equipped with an Intel® Core™i7-14700HX processor, featuring 28 GB of RAM and running the Windows 11 Home 64-bit operating system. An internet connection and several online resources, such as SwissADME, RCSB PDB, and PubChem, were also used. The software applications used for analysis were Chimera 1.17.3, Discovery Studios Visualizer 2019, PyMOL, and Autodock Vina. The materials involve ligands from four target bacteria: *Staphylococcus aureus* (FtsZ 12-316 complexed with TXH9179) with PDB code 8HTB; *Staphylococcus epidermidis* (the crystal structure of shikimate dehydrogenase) with PDB code 3DOO; *Escherichia coli* (the crystal structure of *E. coli* penicillin-binding protein 5 complexed with a peptide-mimetic cephalosporin) with PDB code 3BEC; and *Pseudomonas aeruginosa* (the crystal structure of penicillin-binding protein 3 complexed with the deacylated product of cefoperazone) with PDB code 5DF9. The SMILES codes for each test compound were also incorporated throughout the molecular docking process.

After identifying compounds through GC-MS, predictions were made regarding their potential as drug candidates. Physicochemical properties were be assessed based on five parameters defined by the Lipinski Rule of Five: molecular weight (MW) < 500, logarithm of the partition coefficient of octanol/water (Log P) < 5, hydrogen bond donors (HBD) < 5, hydrogen bond acceptors (HBA) < 10, and a maximum of two violations. These predictions were based on data from the Swiss-ADME online platform (Rukmana et al., 2025).

Receptor preparation was conducted using data from the RCSB PDB site for four target bacteria to be tested in silico. The ligand-receptor structures for each bacterium were saved in pdb format. The Chimera 1.17.3 application was used to

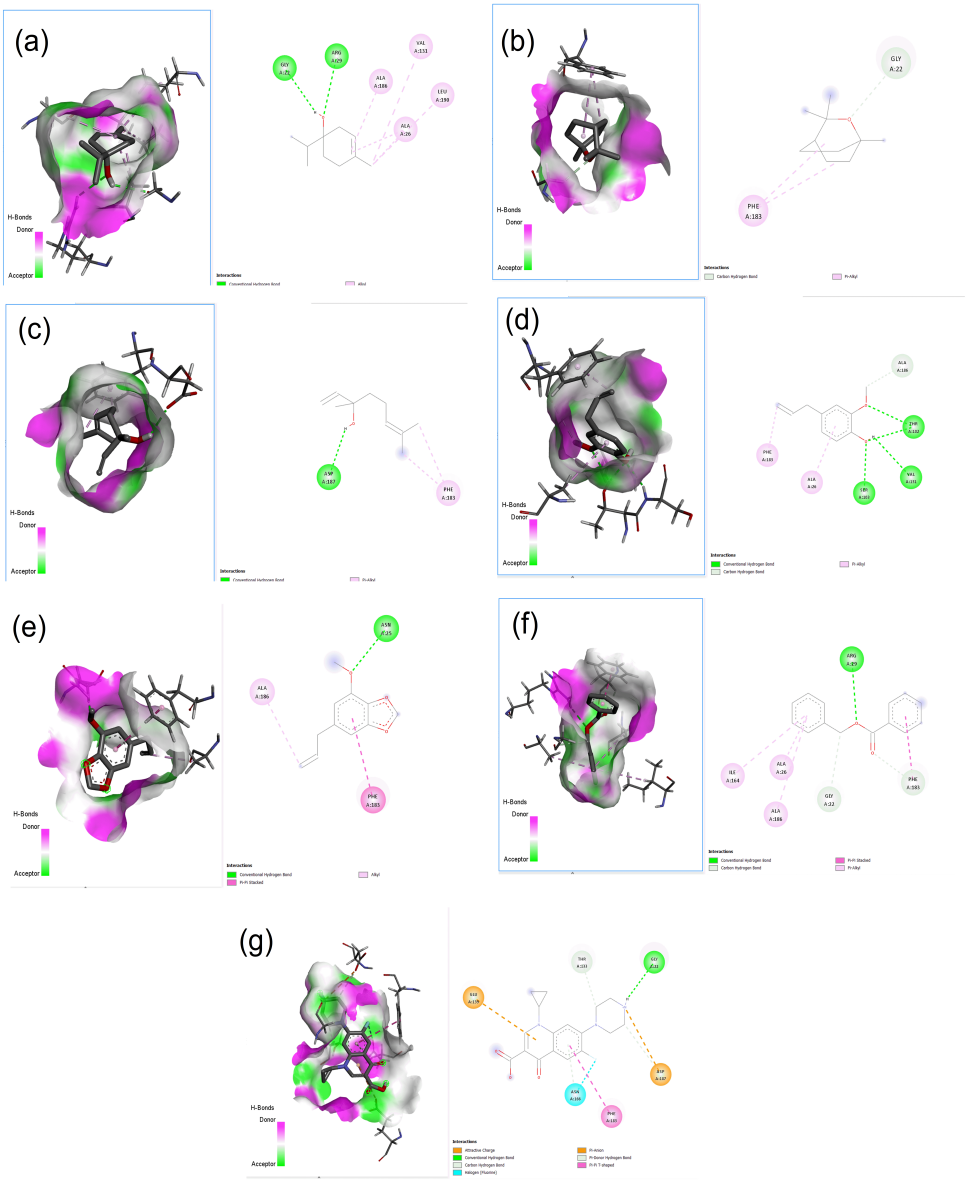


Figure 2. Visualization of Natural Ligand Binding of Compounds and Receptors (a) 8HTB-Eucalyptol, (b) 8HTB-Linalool, (c) 8HTB-Terpinen-4-ol, (d) 8HTB-Eugenol, €8HTB-Myristicine, (f) 8HTB-Benzyl Benzoate, (g) 8HTB-Ciprofloxacin (Control)

prepare the natural ligands and to analyze the interactions between the ligands and receptors by assessing the binding affinity through Autodock Vina results. This binding affinity data was saved as part of the molecular docking results. Subsequently, the molecular docking results were saved in pdb format and imported into the Discovery Studio Visualizer application. This allowed for visualization of the interactions between receptors and ligands, including amino acid bonds, and provided 3D and 2D representations of the ligand bonds. The Pymol application was also used to calculate RMSD values and perform ligand re-docking (Verma et al., 2023).

2.2.3 In Vitro Analysis of Antibacterial Compounds of *C.-sulavesianum*

Antibacterial testing was performed using the disc diffusion method against several bacterial strains, including *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli*, and *Pseudomonas aeruginosa*, all of which were grown on nutrient agar media. Each paper disc was soaked in different concentrations (20%, 40%, 60%, and 80%) of the test solution, along with a positive control (chloramphenicol) and a negative control. After the media had solidified, the paper discs were placed on the bacteria-inoculated agar and incubated at 37 °C for 24 hours.

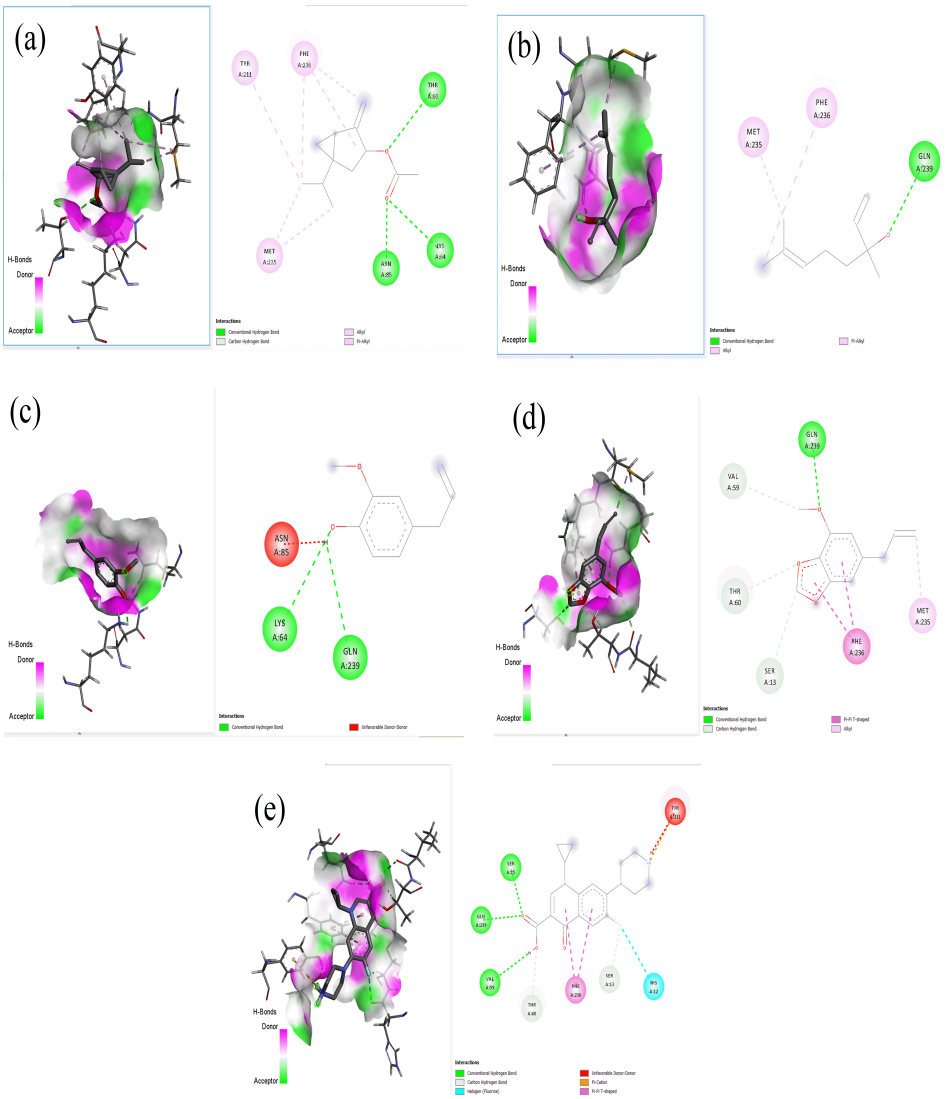


Figure 3. Visualization of Natural Ligand Binding of Compounds and Receptors (a) 3DOO- Bicyclo [3.1.0] hexane, 4-methylene-1-(1-methylethyl)-, (b) 3DOO-Linalool, (c) 3DOO-Eugenol, (d) 3DOO-Myristicine, (e) 3DOO- Ciprofloxacin (Control)

Following incubation, the inhibition zones around the discs were measured using a ruler. The measurement was taken after 24 hours, with the diameter of the inhibition zone providing information about the antibacterial sensitivity of the tested bacteria. The diameter was recorded in millimeters, measuring the zone's vertical and horizontal dimensions (Wang et al., 2018).

3. RESULTS AND DISCUSSION

3.1 *C. sulavesianum* Essential Oil Components

GC-MS Analysis Revealed that *C. sulavesianum* contains 10 distinct chemical components or essential oils, each with varying concentrations. The bark of this plant exudes a robust aroma, particularly when dried. The primary constituents of *C. sulavesianum* essential oil include Linalool (32.3%), Copaene (6.77%), Eugenol (5.05%), and Eucalyptol (3.17%) Table 1. Linalool, an aromatic monoterpene compound, is known for its diverse bioactivities, encompassing antimicrobial, anticancer, antidepressant, and anti-stress properties (An et al., 2021). Copaene, classified as a sesquiterpene, demonstrates potential bioactivities, including antimicrobial effects (Chen et al., 2024) and antioxidant properties(Türkez et al., 2014). Eugenol, a phenolic aromatic compound also prevalent in cinnamon and cloves, shows significant promise as a drug candidate, with applications as an antioxidant, antimicrobial, anesthetic, anti-inflammatory, neuroprotective, anti-diabetic, and anti-cancer agent (Pavithra, 2014). Eucalyptol, a natural prod-

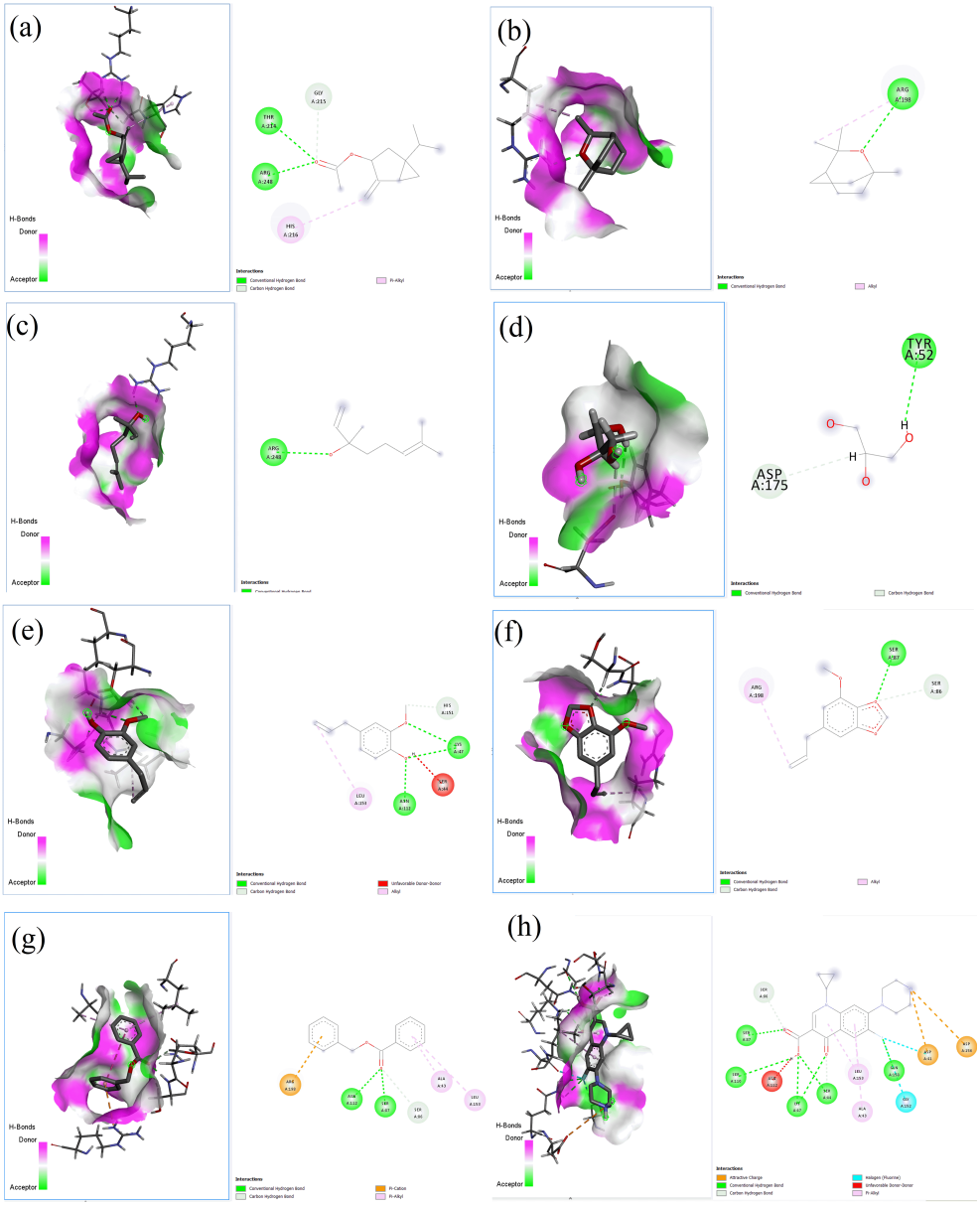


Figure 4. Visualization of Natural Ligand Binding of Compounds and Receptors (a) 3BEC-Bicyclo [3.1.0] hexane, 4-methylene-1-(1-methylethyl)-, (b) 3BEC-Eucalyptol, (c) 3BEC-Linalool, (d) 3BEC-Terpinen-4-ol, €3BEC-Eugenol, (f) 3BEC-Myristicine, (g) 3BEC-Benzyl Benzoate, (h) 3BEC- Ciprofloxacin (Control)

uct, is recognized for its numerous potential bioactivities (Campos and Berteina-Raboin, 2022). Other compounds present in *C. sulavesianum* also exhibit significant bioactive potential, particularly as antibacterial agents.

C. sulavesianum shares several essential oil components with other Cinnamomum species, including *C. zeylanicum*, which contains α-Pinene, Eugenol, Copaene, and Caryophyllene (Kaskoos, 2019). Similarly, *C. cassia* exhibits several comparable components, such as Terpinen-4-ol and Benzyl Benzoate (Jha et al., 2022). The primary compound found in *C. sulavesianum*, Linalool, is also the dominant compound in *C. burmanii* (Lewa and Gugule, 2022). As an aromatic plant, the Cinnamomum genus is widely utilized across various pharmaceutical and cosmetic industries and in food as a spice. Numerous studies have highlighted the therapeutic effects of cinnamon, which include antimicrobial, antiviral, antifungal, antioxidant, antitumor, antihypertensive, antilipemic, antidiabetic, gastroprotective, and immunomodulatory properties (Hajimonfarednejad et al., 2019).

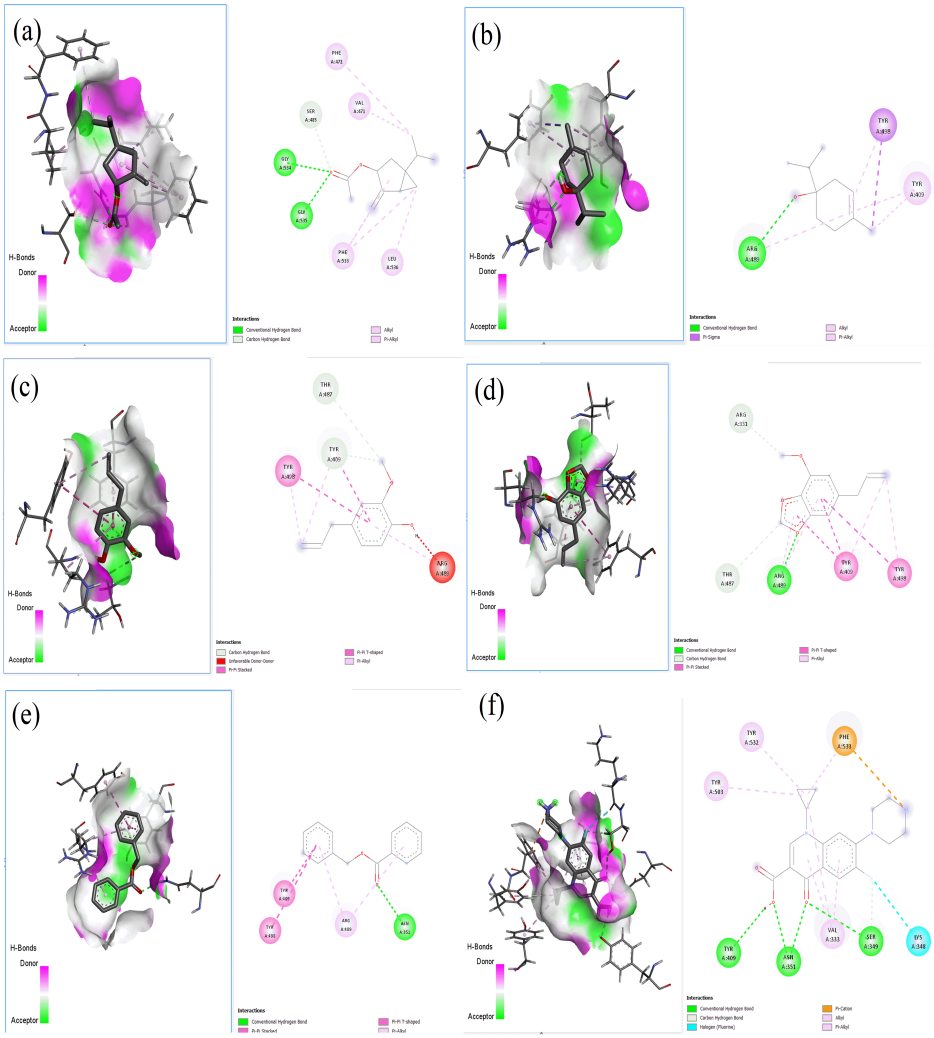


Figure 5. Visualization of Natural Ligand Binding of Compounds and Receptors (a) 5DF9-Bicyclo [3.1.0] hexane, 4-methylene-1-(1-methylethyl)-, (b) 5DF9- Terpinen-4-ol, (c) 5DF9-Eugenol, (d) 5DF9- Myristicine, (e) 5DF9-Benzyl Benzoate, (f) 5DF9- Ciprofloxacin (Control)

3.2 In Silico Analysis of Antibacterial Activity of *C. sulavesianum* Bark

In silico analysis was conducted on active compounds derived from the GC-MS analysis of *C. sulavesianum* bark. Additionally, in silico tests were performed on four pathogenic bacteria: two gram-positive strains, *S. aureus* and *S. epidermidis*, and two gram-negative strains, *E. coli* and *P. aeruginosa*. Before conducting further in silico analysis, the physicochemical properties of the compounds identified through GC-MS analysis were assessed based on Lipinski's Five Rules, utilizing SwissADME for prediction. The results indicated that all tested compounds adhered to the criteria outlined by Lipinski's rules Table 2. Specifically, the maximum limits of Lipinski's Five Rules are a molecular weight < 500, several hydrogen bond acceptors (HBA) < 10, several hydrogen bond donors (HBD) < 5, and a partition coefficient (log *P*) < 5 (Ivanović et al., 2020). These findings suggest that none of the compounds violated Lipinski's criteria. A compound or drug meets Lipinski's rules if it does not violate more than two of these criteria (Kalontong et al., 2022).

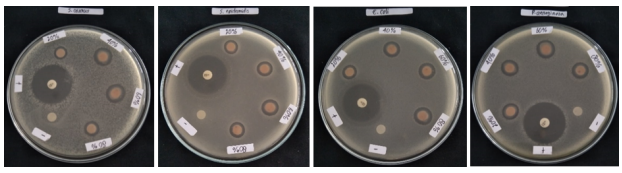
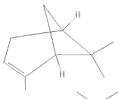
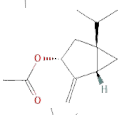
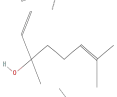
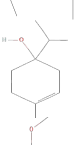
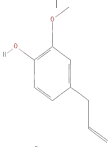
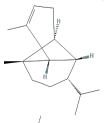
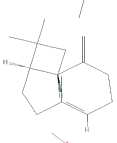
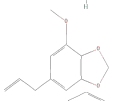
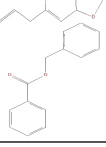


Figure 6. Examples of In Vitro Test Results

3.3 Ligand Preparation and Molecular Docking Validation

The ligands used in this study were selected based on the bacteria tested, specifically *S. aureus*, *S. epidermidis*, *E. coli*, and *P.*

Table 1. Essential Oils Component of *C. sulawesianum* Bark

Chemical Compound	Retention Times	Percentage (%)	Chemical Structure
α-Pinene	3.459	2.45	
Bicyclo[3.1.0] hexane, 4-methylene-1-(1-methylethyl)-	3.956	1.93	
Eucalyptol	4.818	3.17	
Linalool	6.521	32.30	
Terpinen-4-ol	8.362	1.79	
Eugenol	14.733	5.05	
Copaene	14.871	6.77	
Caryophyllene	16.450	2.65	
Myristicine	20.897	2.53	
Benzyl Benzoate	29.392	3.60	

aeruginosa. To validate the accuracy of the molecular docking method, a re-docking process was conducted using PyMol software. The results of the ligand validation indicated that all the Root Mean Square Deviation (RMSD) values were below 2 Å, demonstrating successful ligand docking Table 3. A smaller RMSD value signifies that the ligand's position is closer to the original natural ligand (Nursamsiar et al., 2020). This confirms that the grid box size and coordinates used for docking were appropriate.

The RMSD values for the interactions between ligands and each bacterium range from 0.001 to 0.043, indicating a very small variation and less than 2 Å. In the ligand images, blue represents the natural ligand, while violet indicates the ligand generated from docking Table 3. The binding affinity reveals that *P. aeruginosa* has the lowest binding affinity value. A smaller binding affinity value signifies a more potent ligand and receptor interaction. This interaction is crucial for drug design as it provides insights into the nature of the interactions formed (Puspitasari et al., 2023).

3.4 In Silico Results of *C. sulavesianum* Compounds Against *S. aureus* (8HTB)

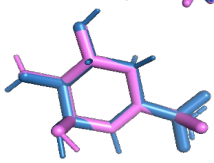
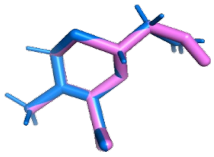
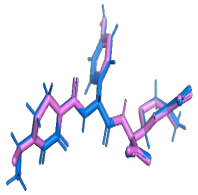
The docking results of compounds from *C. sulavesianum* with the 8HTB receptor indicated the formation of bonds through amino acid interactions. The compounds displaying hydrogen bond interactions included Eucalyptol, Linalool, Terpinen-4-ol, Eugenol, Myristicin, Benzyl Benzoate, and the control substance, Ciprofloxacin. These compounds have the potential to position *C. sulavesianum* bark as a candidate for antibacterial agents against S. aureus. Among the compounds that formed hydrogen bonds with the same amino acid residue (GLY 22) as the control (Ciprofloxacin) were Eucalyptol, Terpinen-4-ol, and Benzyl Benzoate. The interaction of these compounds with the identical amino acid residues as the reference com-

Table 2. Results of the Prediction of Physicochemical Properties of Compounds Using Lipinski's Rules

Chemical Compounds	Lipinski's Rule of Five				Lipinski Rules
	MW (g/mol)	HBA	HBD	Log *P*	
α-Pinene	136.23	0	0	2.63	Yes
Bicyclo[3.1.0] hexane, 4-methylene-1-(1-methylethyl)-	194.27	2	0	2.54	Yes
Eucalyptol	154.25	1	0	2.58	Yes
Linalool	154.25	1	1	2.70	Yes
Terpinen-4-ol	154.25	1	1	2.51	Yes
Eugenol	164.20	2	1	2.37	Yes
Copaene	204.35	0	0	3.40	Yes
Caryophyllene	204.35	0	0	3.25	Yes
Myristicine	192.21	3	0	2.67	Yes
Benzyl Benzoate	212.24	2	0	2.68	Yes
Ciprofloxacin (Control)	331.34	5	2	2.24	Yes

Note: MW: Molecular weight, HBA: Number of H-Bond Acceptors, HBD: Number of H-Bond donors, log *P*: Partition coefficient

Table 3. Molecular Docking Results

Bacteria	Ligand Docking Validation	PDB Code	Ligan Interaction	RMSD Value (Å)	Binding Affinity (kcal/mol)
Staphylococcus aureus (EtsZ 12-316 complexed with TXH9179)		8HTB	GDP	0.043	−10.4
Staphylococcus epidermidis (Crystal structure of shikimate dehydrogenase)		3DOO	SKM	0.001	−6.7
Escherichia coli (Crystal structure of *E. coli* penicillin-binding protein 5 in complex with a peptide-mimetic cephalosporin)		3BEC	HJ2	0.001	−5.5
Pseudomonas aeruginosa (Crystal structure of penicillin-binding protein 3 in complex with deacylated product of cefoperazone)		5DF9	59J	0.001	−11.7

Note: Root Mean Standard Deviation (RMSD) is considered valid if it is less than 2 Å

Table 4. Molecular Docking Results of *C. sulavesianum* Compounds with 8HTB Receptors

Chemical Compounds	Binding Affinity (kcal/mol)	Amino Acid Interactions		
		Hydrophobic Amino Acid Residues	Hydrogen Bond	Hydrogen Bond Distance (Å)
α-Pinene	-4.9	Alkyl Bond: ALA186, PHE183	-	-
Bicyclo[3.1.0]hexane, 4-methylene-1-(1-methylethyl)-	-6.3	Pi-Sigma Bond: PHE183; Alkyl Bond: ALA186	-	-
Eucalyptol	-4.8	Pi-Alkyl Bond: PHE183	GLY22	2.53
Linalool	-5.8	Pi-Alkyl Bond: PHE183	ASP187	2.07
Terpinen-4-ol	-6.6	Alkyl Bond: ALA186, ALA26, VAL131, LEU190	GLY22 ARG29 THR102	2.49 2.66 2.22
Eugenol	-6.6	Pi-Alkyl Bond: PHE183, ALA26	VAL131 SER103 ALA186	2.72 2.37 3.33
Copaene	-6.7	Pi-Sigma Bond: PHE183; Alkyl Bond: ALA26, ALA186, PHE136	-	-
Caryophyllene	-5.8	Pi-Sigma Bond: PHE183	ASN25	2.35
Myristicine	-5.8	Pi-Pi Stacked Bond: PHE183; Alkyl Bond: ALA186	-	-
Benzyl Benzoate	-7.5	Pi-Alkyl Bond: ILE164, ALA26, ALA186	ARG29 GLY22 PHE183	2.73 2.90 2.90
Ciprofloxacin (Control)	-7.8	Pi-Anion Bond: ASP187, GLU139; Halogen: ASN166	GLY22 THR133	2.07 3.18

pound indicates that they may exhibit similar biological activity to that of the reference compound Table 4.

Visualizing molecular docking results is essential for understanding the interactions between reference ligands and test ligands with the 8HTB receptor. This analysis allows us to evaluate the types of bonds formed between the ligand and the receptor. Key parameters that indicate the strength of these interactions include hydrogen bonds, hydrophobic interactions, and the distances of hydrogen bonds. Hydrogen bonds play a crucial role in stabilizing the interaction between the ligand and the receptor; they are considered stable if the bond length is less than 2.7 Å. Additionally, hydrophobic interactions contribute to maintaining the binding conformation (Kalontong et al., 2022).

The interactions between the compounds of *C. sulavesianum* and ligands and receptors were visualized in both 2D and 3D using Discovery Studio Visualizer Figure 2. The results of this visualization revealed that six compounds formed hydrogen bonds, indicating a strong interaction between the ligands and receptors. These six compounds, which established hydrogen bonds, demonstrate significant potential as antibacterial agents derived from the bark of *C. sulavesianum*. Eugenol formed the most hydrogen bonds among them, surpassing even the control compound, ciprofloxacin.

3.5 In Silico Results of *C. sulavesianum* Compounds Against *S. epidermidis* (3DOO)

The docking results of *C. sulavesianum* compounds with the 3DOO receptor revealed the formation of bonds through their amino acid interactions. The compounds displaying hydrogen bond interactions included Bicyclo [3.1.0] hexane, 4-methylene-1-(1-methylethyl)-, Linalool, Eugenol, Myristicine, and the control drug Ciprofloxacin. These compounds demonstrate potential as antibacterial agents, positioning *C. sulavesianum* bark as a candidate against *S. epidermidis*. Notably, Myristicine forms hydrogen bonds with the same amino acid

Table 5. Molecular Docking Results of *C. sulavesianum* Compounds with the 3DOO Receptor

Chemical Compound	Binding Affinity (kcal/mol)	Amino Acid Interactions		
		Hydrophobic Amino Acid Residues	Hydrogen Bond	Hydrogen Bond Distance (Å)
α-Pinene	-4.7	Alkyl Bond: PHE236, MET235, ILE209	-	-
Bicyclo[3.1.0]hexane, 4-methylene-1-(1-methylethyl)-	-5.6	Alkyl Bond: TYR211, PHE236, MET235	PHE60 LYS64 ASN85	2.91 2.77 2.13
Eucalyptol	-4.8	Alkyl Bond: MET235, TYR211, PHE236	-	-
Linalool	-5.1	Alkyl Bond: MET235, PHE236	GLN239	2.07
Terpinen-4-ol	-5.1	Alkyl Bond: VAL81, ALA128	-	-
Eugenol	-5.7	Unfavorable Donor–Donor: ASN85	LYS64 GLN239	2.87 2.95
Copaene	-5.9	Alkyl Bond: PHE236, TYR211, MET235	-	-
Caryophyllene	-6.2	Alkyl Bond: VAL210, TYR211, PHE236, MET235	-	-
Myristicine	-5.9	Pi–Pi T-Shaped: PHE236; Alkyl Bond: MET235	GLN239 VAL59 THR60 SER13	2.64 3.58 2.90 3.50
Benzyl Benzoate	-6.6	Alkyl Bond: MET235, PHE236	-	-
Ciprofloxacin (Control)	-7.6	Halogen: HIS12; Unfavorable Donor–Donor: TYR211; Pi–Pi T-Shaped: PHE236	SER15 GLN239 VAL59 THR60 SER13	2.88 2.55 3.53 2.31 2.42

interactions (SER 13, THR 60, VAL 59) as the control compound, Ciprofloxacin. This similarity in amino acid residue interactions suggests that Myristicine may exhibit comparable biological activity to the reference compound. Additionally, the interaction of amino acid GLN 239 in Ciprofloxacin is the same as that of Linalool, Eugenol, and Myristicine Table 5.

The interactions between these compounds, ligands, and receptors were visualized in 2D and 3D using Discovery Studio Visualizer Figure 3. The results indicated that five compounds formed hydrogen bonds, which reflects the strength of the interactions between the ligands and receptors. These five compounds formed hydrogen bonds and showed great potential as antibacterial agents against *S. epidermidis* derived from *C. sulavesianum* bark. Among them, myristicine exhibited the highest number of hydrogen bonds compared to the others, and all of its hydrogen bonds matched those of the control compound, ciprofloxacin.

3.6 In Silico Results of *C. sulavesianum* Compounds Against *Escherichia coli* (3BEC)

The docking results of compounds from *C. sulavesianum* with the 3BEC receptors indicated the formation of bonds through amino acid interactions. The compounds exhibiting hydrogen bond interactions included Bicyclo [3.1.0] hexane, 4-methylene-1-(1-methylethyl), Eucalyptol, Linalool, Terpinen-4-ol, Euge-nol, Myristicine, Benzyl Benzoate, and the control sub-

Table 6. Molecular Docking Results of *C. sulavesianum* Compounds with 3BEC Receptors

Chemical Compound	Binding Affinity (kcal/mol)	Amino Acid Interactions		
		Hydrophobic Amino Acid Residues	Hydrogen Bond	Hydrogen Bond Distance (Å)
α-Pinene	-4.2	Alkyl Bond: ARG 198	-	-
Bicyclo[3.1.0]hexane, 4-methylene-1-(1-methylethyl)-	-5.3	Pi-Alkyl Bond: HIS 216	THR 214	2.51
			ARG 248	2.17
			GLY 215	2.88
Eucalyptol	-4.3	-	ARG 198	2.35
Linalool	-4.2	-	ARG 248	2.13
Terpinen-4-ol	-4.8	-	TYR 52	2.30
			ASP 175	2.81
Eugenol	-5.7	Alkyl Bond: LEU 153 Unfavorable Donor-Donor: SER 44	LYS 47	2.47
			ASN 112	1.95
			HIS 151	3.41
Copaene	-5.2	Alkyl Bond: ARG 198, TRP 203, LEU 196	-	-
Caryophyllene	-5.1	Alkyl Bond: ARG 198	-	-
Myristicine	-5.2	Alkyl Bond: ARG 198	SER 87	1.99
			SER 86	2.72
Benzyl Benzoate	-5.6	Pi-Alkyl Bond: ALA 43, LEU 153 Pi-Cation: ARG 198	ASN 112	1.89
			SER 87	1.80
			SER 86	2.64
Ciprofloxacin (Control)	-7.3	Pi-Alkyl Bond: LEU 153, ALA 43 Unfavorable Donor-Donor: ASN 112 Attractive Charge: ASP 41, ASP 156	GLN 158	2.84
			SER 44	2.42
			LYS 47	2.41
			SER 110	2.85
			SER 87	1.98
			SER 86	3.00

stance Cipro-floxacin. These compounds are potential agents for developing *C. sulavesianum* bark into an antibacterial treatment against *E. coli*. Myristicine and Benzyl Benzoate exhibited hydrogen bonds with the identical amino acid residues (SER 86 and SER 87) as those found in the control substance, Ciprofloxacin. This similarity in amino acid interactions suggests that these compounds may possess comparable biological activity to the reference compound. Additionally, the interaction of the amino acid LYS 47 in Ciprofloxacin is identical to that of Eugenol Table 6.

The interactions of these compounds with ligands and receptors were visualized in both 2D and 3D using Discovery Studio Visualizer Figure 4. The results indicated that eight compounds (including one control) formed hydrogen bonds, highlighting the strength of the interaction between ligands and receptors. Among these, seven compounds demonstrated significant potential as antibacterial agents against *E. coli* found in *C. sulavesianum* bark. Notably, Bicyclo[3.1.0]hexane, 4-methylene-1-(1-methylethyl)-, Eugenol, and Benzyl Benzoate had the highest number of hydrogen bonds compared to the others.

3.7 In Silico Results of *C. sulavesianum* Compounds Against *Pseudomonas aeruginosa* (5DF9)

The docking results of *C. sulavesianum* compounds with the 5DF9 receptor demonstrated the formation of bonds through amino acid interactions. Compounds exhibiting hydrogen bond interactions include Bicyclo[3.1.0] hexane, 4-methylene-1-(1-methylethyl)-, Terpinen-4-ol, Eugenol, Myristicine, Benzyl Benzoate, and the control compound Ciprofloxacin. These

Table 7. Molecular Docking Results of Compound with 5DF9 Receptor

Chemical Compound	Binding Affinity (kcal/mol)	Amino Acid Interactions		
		Hydrophobic Amino Acid Residues	Hydrogen Bond	Hydrogen Bond Distance (Å)
α-Pinene	-5.7	Pi Sigma: TYR 498 Alkyl Bond: TYR 409, ARG 489	-	-
Bicyclo[3.1.0]hexane, 4-methylene-1-(1-methylethyl)-	-5.6	Alkyl Bond: PHE 472, VAL 471, PHE 533, LEU 536	GLY 535 GLY 534 SER 485	2.25 2.47 2.64
Eucalyptol	-5.6	Alkyl Bond: TYR 407, ARG 489, TYR 409 Pi-Sigma: TYR 498	-	-
Linalool	-4.9	Alkyl Bond: ARG 489 Pi-Sigma: TYR 498, TYR 409	-	-
Terpinen-4-ol	-5.8	Alkyl Bond: TYR 409 Pi-Sigma: TYR 498	ARG 489	2.53
Eugenol	-5.6	Pi-Alkyl: TYR 498 Unfavorable Donor-Donor: ARG 489	TYR 409 THR 487	3.40 3.78
Copaene	-7.0	Alkyl Bond: TYR 498, TYR 409, TYR 407, ARG 489	-	-
Caryophyllene	-7.2	Pi-Alkyl Bond: Tyr 407, TYR 328 Pi-Sigma: Tyr 409, Tyr 498	-	-
Myristicine	-6.3	Pi-Alkyl Bond: TYR 409, TYR 498	ARG 489 THR 487 ARG 331	2.08 3.51 3.54
Benzyl Benzoate	-7.5	Pi-Alkyl Bond: ARG 489 Pi-Pi T-Shaped: TYR 409, TYR 498	ASN 351	2.18
Ciprofloxacin (Control)	-8.1	Alkyl Bond: VAL 333, TYR 532, TYR 503 Pi-Cation: PHE 533 Halogen: LYS 348	SER349 ASN 351 TYR 409	2.27 2.07 2.46

compounds can position *C. sulavesianum* bark as a candidate for antibacterial agents against *P. aeruginosa*. Among these, the compound Eugenol forms a hydrogen bond with the same amino acid interaction as Ciprofloxacin, the control substance. This similarity in interaction suggests that Eugenol may possess comparable biological activity to Ciprofloxacin. Additionally, the amino acid interaction of Ciprofloxacin aligns with that of Benzyl Benzoate Table 7.

The interactions of these compounds with ligands and receptors were visualized in both 2D and 3D using Discovery Studio Visualizer Figure 5. The results indicated that six compounds, including one control, formed hydrogen bonds, highlighting the strength of the interactions between the ligands and receptors. Five compounds exhibited significant potential as antibacterial agents against *P. aeruginosa* derived from *C. sulavesianum* bark. Bicyclo[3.1.0]hexane and Myristicine were the two compounds that formed the highest number of hydrogen bonds compared to the others.

In silico testing results suggest that the compounds present in the bark of *C. sulavesianum* demonstrate hydrogen bonding. This interaction takes place between receptors and ligands (the compounds). It can predict which compounds exhibit antibacterial properties by analyzing binding affinity, amino acid interactions, and hydrogen bonding. These compounds

Table 8. Inhibition Zone of *C. sulavesignum* Ethanol Extract

Bacteria	Diameter of Inhibition Zone (mm)				
	20%	40%	60%	80%	K+ (Chloramphenicol)
Staphylococcus aureus	11.7 ± 1.4^{a}	13.0 ± 0.1^{a}	13.3 ± 0.6^{a}	12.0 ± 1.0^{a}	26.8 ± 0.8^{b}
Staphylococcus epidermidis	9.7 ± 0.6^{a}	11.7 ± 0.6^{b}	12.7 ± 0.6^{bc}	13.2 ± 0.3^{c}	28 ± 0.1^{d}
Escherichia coli	11.0 ± 1.7^{a}	12.3 ± 0.6^{a}	13.8 ± 1.9^{a}	12.8 ± 0.8^{a}	28 ± 0.6^{b}
Pseudomonas aeruginosa	12.5 ± 1.3^{a}	12.3 ± 0.3^{a}	12.8 ± 0.8^{a}	12.5 ± 0.5^{a}	29 ± 0.1^{b}

are likely key contributors to the antibacterial efficacy of *C. sulavesianum* bark, positioning it as a promising candidate for antibacterial applications.

3.8 In vitro Analysis of Antibacterial *Cinnamomum sulavesianum*

In vitro testing of the bark from *C. sulavesianum* demonstrated antibacterial activity against gram-positive bacteria (*S. aureus* and *S. epidermidis*) and gram-negative bacteria (*E. coli* and *P. aeruginosa*) Figure 6. This testing utilized an ethanol extract of the bark. The results align with in silico findings, indicating that compounds from *C. sulavesianum* possess potential antibacterial properties against these four bacterial strains. When tested against *S. aureus*, the inhibitory effect of the extract was categorized as strong, with inhibition zones ranging from 11 to 20 mm in diameter. For *S. epidermidis*, strong inhibition was observed only at extract concentrations of 40% to 80%; at a concentration of 20%, the inhibition was categorized as moderate. In contrast, all tested concentrations against *E. coli* exhibited strong inhibition, as did all concentrations against *P. aeruginosa*. The classification of inhibition zones is defined as follows: >20 mm is considered very strong, 11-20 mm is strong, 5-10 mm is moderate, and <5 mm is low Table 8.

The antibacterial properties of ethanol extract are attributed to the presence of specific compounds. In silico analyses of compounds identified through GC-MS from the bark of *C. sulavesianum* indicate potential antibacterial activity. Notable compounds such as Eucalyptol have been shown to exhibit antibacterial effects against bacteria such as *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* (Yoro et al., 2020). Additionally, Linalool, which is the primary compound with the highest concentration, has demonstrated antibacterial activity against *Pseudomonas aeruginosa* (Liu et al., 2020), *Shewanella putrefaciens* (Guo et al., 2021), *Listeria monocytogenes* (He et al., 2022), and *Shigella sonnei* (Su et al., 2022). Given its substantial Linalool content, *C. sulavesianum* shows promise as an antibacterial agent and is currently being developed for use in various pharmaceutical industries.

Another *C. sulavesianum* compound that can inhibit bacterial growth is Terpinen-4-ol, which can fight *Streptococcus agalactiae* (Zhang et al., 2018)) and *S. aureus* (Cordeiro et al., 2020). One of the main compounds of *C. sulavesianum* bark is Eugenol, which can fight *E. coli* (Jeyakumar and Lawrence, 2021), *Shigella flexneri* (Bai et al., 2022), and *Vibrio parahaemolyticus* (Ashrafudoulla et al., 2020). The Myristicine compound has antibacterial properties, such as *Bacillus subtilis*, *S. aureus*, and *E. coli* (Wibowo et al., 2018). Benzyl Benzoate is the last compound that results in silicon forming hydrogen bonds, and as a supporting compound in the antibacterial activity of *C. sulavesianum* bark. This compound is reported to have antibacterial properties against *E. coli*, *Enterococcus aerogenes*, *Bacillus cereus*, and *S. aereus* (Diastuti et al., 2020).

In vitro testing using the disc diffusion method has demonstrated that *C. sulavesianum* possesses significant potential as an antibacterial agent and may even serve as a new antibiotic candidate in the future. The Cinnamomum genus also shows considerable promise as an antibacterial agent. Several species within this genus have been reported to exhibit antibacterial properties, including *C. zeylanicum* against Porphyromonas gingivalis (Wang et al., 2018), *C. validinerve* against *Propionibacterium acnes* (Yang et al., 2020), *C. camphora* against *S. aureus*, *Enterococcus faecalis*, *Bacillus subtilis*, *Salmonella gallinarum*, and *E. coli* (Chen et al., 2020), and *C. burmannii* against *S. aureus* (Hakim et al., 2020).

4. CONCLUSIONS

Cinnamomum sulavesianum bark contains 10 essential oil compounds, with the primary ones being linalool (32.3%), copaene (6.77%), eugenol (5.05%), and eucalyptol (3.17%). In silico studies indicate that the compounds in *C. sulavesianum* have antibacterial potential against gram-positive and gram-negative pathogenic bacteria. Additionally, in vitro testing has demonstrated the formation of inhibition zones in extracts of *C. sulavesianum* bark, which are categorized as having strong inhibitory power.

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