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In-silico evaluation of the antibiofilm potential of *Anredera cordifolia* extract, *Syzygium aromaticum,* and *Cinnamomum burmanii* essential oils against *Staphyloccocus aureus*

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Abstract: The increase of antibiotic resistance has been one of major challenges in today's healthcare. *Staphylococcus aureus* is responsible for approximately 60-80% of human infections. This research examines the anti-biofilm activity of key components of Binahong leaves (*Anredera cordifolia*), clove flower essential oil (*Syzygium aromaticum*) and cinnamon essential oil (*Cinnamomum burmanii*) against *Staphylococcus aureus* bacteria using in silico simulations. The compounds of plant secondary metabolites were analyzed using Liquid Chromatography High Resolution Mass Spectrometry (LC-HRMS) and Gas Chromatography-Mass Spectrometry (GC-MS) techniques to identify the main compounds. In silico studies employed the molecular docking method with BIOVIA Discovery Study Visualizer 2021 and iGEMDOCK V2.1 applications. The LC-HRMS analysis revealed that the binahong leaf ethanol extract contains vitexin, a flavonoid compound with a Cloud Best Match value of 97.4% and an Area of 657851019.181973. GC-MS analysis demonstrated that clove flower essential oil (*Syzygium aromaticum*) predominantly consists of eugenol, accounting for 83.89% of the oil, whereas cinnamon essential oil (*Cinnamomum burmanii*) contains cinnamaldehyde, constituting 72.44%. Through molecular docking analysis, it was observed that the vitexin compounds exhibited the highest potential for anti-biofilm activity. These findings provide valuable insights into the antibiofilm potential of *Anredera cordifolia, Syzygium aromaticum, and Cinnamomum burmanii* against *Staphylococcus aureus*.

Keywords: S*taphylococcus aureus;* vitexin; eugenol; cinnamaldehyde; *in silico*

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INTRODUCTION

Antibiotic resistance, also known as Multi-Drug Resistance (MDR), has been a major challenge in the health sector (Manjunath Sangappa, 2013). Overuse of antibiotic and non-compliance in taking antibiotics can lead to multidrug resistance. Unfortunately, the overuse of antibiotics does not only occur in the healthcare, but also extends to animal husbandry and aquaculture. The use of broad-spectrum antibiotics and the lack of good antibiotic management are major factors leading to the spread of antibiotic resistance (Vivas *et al.*, 2019). *Staphylococcus aureus* is a common gram-positive bacteria that is resistant to antibiotics (Abo-salem *et al.*, 2021; Bezlon G *et al*., 2013). Based on data from the National Institutes of Health, 80% of human bacterial infections involve microorganisms that can form biofilms (Romling and Balsalobre, 2012). Biofilm formation occurs in persistent tissues such as chronic wounds, chronic otitis media, chronic osteomyelitis, chronic rhinosinusitis, recurrent urinary tract infections, endocarditis and cystic fibrosis-related lung infections (Carbone *et al.*, 2018). The presence of this biofilm matrix provides additional resistance to bacteria, which makes it resistant to antibiotics (Divakar *et al*., 2019).

Biofilms adhere to the surface of the microbial assemblage encased in a self-synthesizing extracellular matrix (polymer material) and can grow living or dead microbes on the surface (Carbone *et al.*, 2018). Polymeric materials include exopolysaccharides, extra cellular DNA, proteins and amyloidogenic proteins (Divakar *et al*., 2019). A biofilm layer allows microbes to survive in adverse conditions and bacterial cells are 1000 times more resistant to antibiotics (Römling and Balsalobre, 2012). The increasing prevalence of pathogenic bacteria that are resistant to antibiotics has led to a reduction in antimicrobial agents to treat infections. It urges the need to look for alternatives in controlling antibiotic-resistant pathogenic bacteria. *Staphylococcus aureus* is the primary isolate that causes wound infections, including the *Methicillin-Resistant Staphylococcus aureus* (MRSA) strain resistant to methicillin and oxacillin (Thiruvengadam *et al*,, 2020). This becomes a problem in the field of medicine worldwide and may cause harm to injured patients (El-Far *et al.*, 2021). Many treatments have been developed to treat S. aureus biofilm infections, including nanosilver, a nano-sized silver particle (1-100nm) which has a good antibacterial ability and nanoemulsion gel with potential

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plant-derived antibiotic agents. Previous studies have focused on various biological effects of natural sources, including eugenol compounds, cinnamaldehyde (Ardani *et al.*, 2010) and flavonoids from binahong leaves.

One strategy that can be employed to develop potential compounds which have antimicrobial activity is developing active compounds that can inhibit bacterial enzymes, *thymidylate kinase* (TMK) (Abo-salem *et al.*, 2021). *Thymidylate kinase* is an essential enzyme in bacterial DNA biosynthesis, which is an attractive therapeutic target for developing new antibacterial agents (Kawatkar *et al.*, 2014). Natural ingredients as anti-biofilm agents need to be developed as they have lower side effects and improving therapeutic strategies can be done through a combination of more than one anti-biofilm compound from various plant sources (Mishra *et al.*, 2020).

Several components of active compounds from plants are known to have antimicrobial and anti-biofilm activity, one of which is vitexin, a compound from binahong leaves (*Anredera cordifolia*) (Mulia *et al*., 2017). Eugenol (El-Far *et al.*, 2021) and Cinnamaldehyde also have anti-biofilm activity (Firmino *et al.*, 2018). Previous studies showed that the eugenol compound had biofilm inhibitory activity against MRSA strains (Yadav *et al.*, 2015). Cinnamaldehyde also inhibits *Pseudomonas aeruginosa* biofilms (Topa *et al.*, 2020). Also, trans-CNMA and 4-nitro CNMA can potentially inhibit biofilms from *Escherichia coli* and *S. aureus*. (Kim *et al.*, 2022). The flavonoid compound vitexin has also been reported to have anti-biofilm activity against *Pseudomonas aeruginosa* (Das *et al.*, 2016a). However, research on the compounds responsible for clove flowers, cinnamon and binahong leaves and comparisons of the potential of these compounds as antibacterial and antibiofilm have not been conducted. Therefore, this research aims to identify the main compounds from binahong leaves (*Anredera cordifolia*), clove flower essential oil (*Syzygium aromatikum*), and cinnamon (*Cinnamomum burmanii*) essential oil in inhibiting the *thymidylate kinase enzyme*.

MATERIALS AND METHODS

Materials

The main material used in this study was clove flower essential oil obtained from the Center for Essential Oil Studies (CEOS) of the Islamic University of Indonesia, Yogyakarta. Cinnamon Essential Oil (CEO) was obtained from PT Lansida Group while binahong leaves (*Anredera cordifolia*) were obtained from Sindumartani, Ngemplak, Sleman Yogyakarta. The leaves were extracted by maceration method with 96% ethanol solvent. 223g simplisia powder was put into a glass vessel then added 96% ethanol solvent in a ratio of 1:10, close the vessel tightly and leave for 24 hours, Then after 24 hours, filtered and the results obtained in the form of liquid extract (macerate) evaporated with a rotary evaporator. Secondary metabolite profile analysis was conducted at LPPT UGM Yogyakarta.

The instruments and applications used in this study are ACER Nitro AN515-58 laptop (Windows Home Single Language 64-bit operating system; 16 GB memory; Processor: Intel® CoreTM i5-12500H 12th Generation (16 CPUs), 3.1 GHz). BIOVIA Discovery Studio Visualizer 2021 and iGEMDOCK v21 applications.

The materials used for *in-silico* studies were the main compounds from LC-HRMS and GC-MS analysis, which were processed and analyzed by accessing the server https://pubchem.ncbi.nlm.nih.gov/danenzim/protein *Thymidylate kinase* Code (4EAQ) *obtained by accessing the Protein Data Bank https://www.rcsb.org/*

GC-MS analysis of clove flower essential oil (Syzygium aromatikum) and cinnamon (Cinnamomum burmanii) essential oil

The sample was dissolved in a 1:2 ethanol solution in a microtube and vortexed for 1 minute. The resulting supernatant was used for GC-MS analysis. The injector temperature was set to 300°C, the detector temperature to 280°C and the capillary column dimensions were 30m x 0.25 mm with a film thickness of 0.25mm. The sample was dissolved in a 1:2 ethanol solution in a microtube and vortexed for 1 minute. The resulting supernatant was used for GC-MS testing. The GC-MS analysis was performed for 53 minutes, with an injector temperature of 300°C, detector temperature of 280°C and column temperature of 325°C. Helium gas was used as the carrier gas at a constant flow rate of 5 ml/min. The GC-MS tool was employed for the identification of bioactive compounds, which can be determined by analyzing the peaks in the chromatograms and examining the mass spectrum. The molecular weight of each bioactive compound can be derived from the mass spectrum data obtained.

HRMS analysis

Orbitrap high-resolution mass spectrometry (Thermo Scientific™ Q Exactive™ Hybrid Quadrupole-Orbitrap™ Hight Resolution Mass Spectrometer) and liquid chromatography (Thermo Scientific™ Vanquish™ UHPLC Binary Pump were used for the analysis. Then, an analytical column of Thermo Scientific ™ Accucore ™ Phenyl-Hexyl 100 mm x 2.1 mm ID x 2.6 µm was used in liquid chromatography. The mobile phases in this analysis used MS grade water with 0.1% formic acid (A) and MS grade methanol with 0.1% formic acid (B), employing a gradient technique at a flow rate of 0.3 ml/minute. Initially, the mobile phase B was set at 5% and gradually increased to 90% within 16 minutes. It was then held at 90% for 4 minutes before returning to the initial condition of 5% B over 25 minutes. The injection volume was 3 µL, and the column temperature was maintained at 40°C. The full MS/dd-MS2 acquisition method, utilizing either

negative or positive ionization modes, was employed for untargeted screening. Nitrogen gas was utilized for the sheath, sweep, or auxiliary gas and set at 32.8 and 4 arbitrary units (AU). The auxiliary gas heater temperature was set at 30°C, the capillary temperature at 320°C and the spray voltage at 3.30 kV. A resolution of 70,000 was used for both full MS and dd-MS2 scans in negative and positive ionization modes. The scan range was performed from 66.7 to 1000 m/z. To ensure optimal performance during ion transfer analysis, including mass accuracy (<5 ppm), instrument sensitivity, and ion isolation, the system was regulated using XCalibur 4.4 software from Thermo Scientific, Bremen, Germany (Windarsih *et al.*, 2022).

In silico procedure

This study employed an *in-silico* method which was carried out by docking the main compound molecules obtained from the results of LC-HRMS and GC-MS analysis against the enzyme/protein *Thymidylate kinase* to determine its interaction as a microbial anti-biofilm. The steps were ligand and receptor (protein) preparation, ligand and receptor validation, molecular docking using iGEMDOCK v21 (Junaidin *et al.*, 2022) and visualization using BIOVIA *Discovery Studio Visualizer*.

Ligand and protein preparation

The ligand used in this study is Vitexin, obtained from LC-HRMS results. The GC-MS analysis yielded two compounds, eugenol and cinnamaldehyde, which were downloaded from the PubChem database in 3D SDF format. The formats of these compounds were then converted to mol2 in Discovery Studio. The protein utilized in the study is thymidylate kinase, identified with the code 4EAQ, obtained from the Protein Data Bank. The protein structure was saved in 3D SDF format. Water molecules were included in the analysis using Discovery Studio and saved in PDB format.

Method validation

The molecular docking method was first validated using the iGEMDOCK v2.1 software by redocking the native ligand to the receptor-*thymidylate kinase enzyme* (PDB ID 4EAQ). The binding site radius was set according to the one used. The docking accuracy setting (GA parameters) used is quick docking (rough) with population size =150, generation =70 and number of solutions =1. The observed redocking result parameter is the RMSD value, which is the deviation value of the space position of the docking ligand. The test is successful if the RMSD value is less than 2 Å.

Molecular docking screening and analysis

The docking process was carried out using the iGEMDOCK v2.1 docking protocol, which met the validity criteria based on the RMSD value. Next, an assessment was carried out based on the docking score and docking pose from the molecular docking results using *Discovery Studio* 2021.

RESULTS

Results of secondary metabolite analysis of essential oils Using GC-MS

The GC-MS analysis was performed on clove flower essential oil fig. 1 and cinnamon essential oil fig. 2. Each peak in the chromatogram was identified by comparing the corresponding MS spectrum with the Willey database to determine the compound type (Hartono *et al*., 2017). Analysis results from fig. 1. Gas chromatography can read compounds with the lowest concentrations (Al-Rubaye *et al*., 2017). The most abundant compound in clove flower essential oil (*Syzygium aromatikum)* can be seen in table 1. The results of GC-MS analysis of *cinnamomum burmanii* essential oil show 75 peaks, as indicated in fig 2. The most abundant compound components in cinnamon can be seen in table 2.

Results of analysis of 96% ethanol extract of Binahong (Anredera cordifolia) leaves using HRMS

The LC-HRMS analysis of the ethanol extract of binajong (*Anredera cordifolia*) leaves revealed 47 chromatograms fig. 3 and successfully extracted 248 possible compounds using 96% ethanol solvent.

Based on the obtained results, filtering was conducted to isolate the desired secondary metabolite group, specifically the flavonoid group. The filtering criteria included an mzCloud Best Match number ≥80 and a significant area. As a result of this filtering process, three active compounds were identified as potential active constituents in the ethanol extract of binahong (*Anredera cordifolia*) leaves table 3.

Molecular docking validation

The criterion for good validation results is RMSD value ≤ 2 Å. The results obtained are an RMSD value of 0.7081 and a binding affinity value of -125.77.

Molecular docking screening and analysis

In the next step, docking analysis was performed using iGEMDOCK software to evaluate the main compounds from binahong (*Anredera cordifolia*) leaves, namely vitexin, eugenol from clove flower essential oil (*Syzygium aromaticum*) and cinnamaldehyde from cinnamon essential oil (*Cinnamomum burmanii*). The docking method used was validated. The results of the docking analysis, showing the binding affinity (bond energy), are presented in fig. 5.

Visualization of protein-ligand interactions

The main component compounds are then visualized using Discovery Studio 2021(Shreyash D *et al*., 2023) to see which amino acid residues in the thymidylate kinase target protein to the active compound and what bonds are formed between the amino acids in the target protein and the active compound.

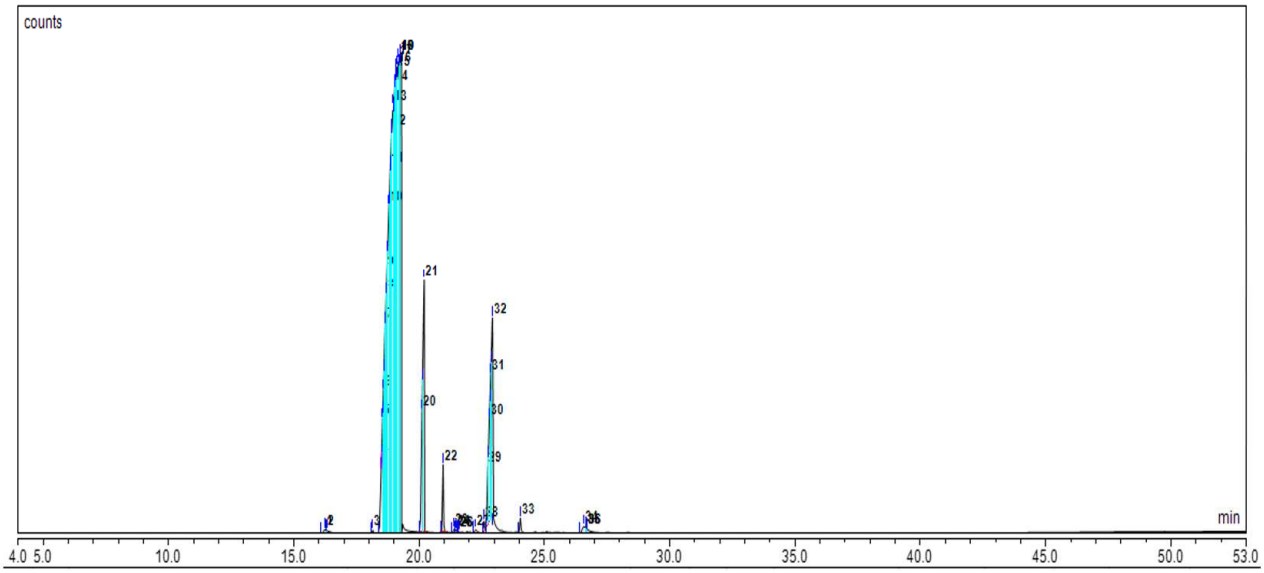

Fig. 1: Chromatogram profile of clove flower essential oil (*Syzygium aromatikum*). There are 36 chromatograms and the highest peaks are located between peaks 4 and 19.

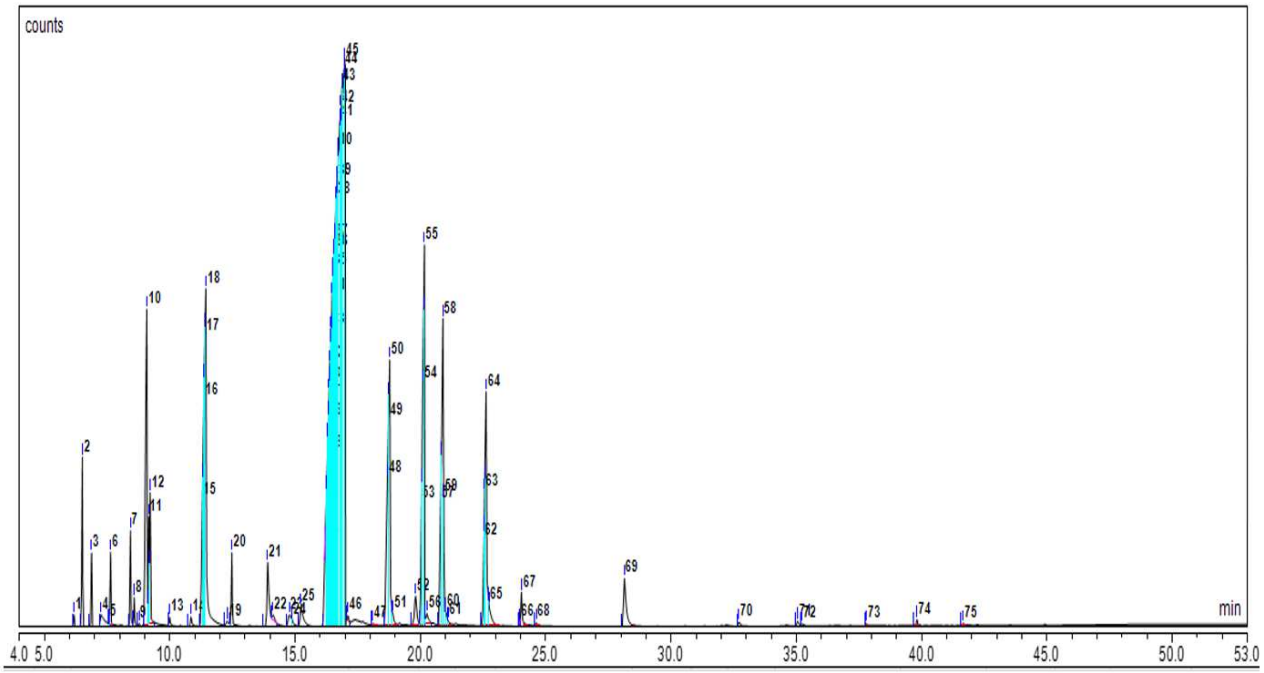

Fig. 2: Chromatogram profile of cinnamon (*Cinnamomum burmanii*) essential oil with 75 peaks, with the highest peaks located at peaks 26 to 45

Table 1: Chemical compounds and % area of Clove Flower Essential Oil (*Syzygium aromaticum*)

Rt (minutes)	Name of component	Chemical formula	Molecular weight	% area
18.84	Eugenol	$C_{10}H_{12}O_2$	164	83.89
20.20	Caryophyllene	$C_{15}H_{24}O$	220	6.98
20.95	Humulene	$C_{15}H_{24}$	204	0.90
22.93	Phenol, 2-methoxy-4-(2-propenyl)-, acetate	$C_{12}H_{14}O_3$	206	8.24

Rt: Retention time

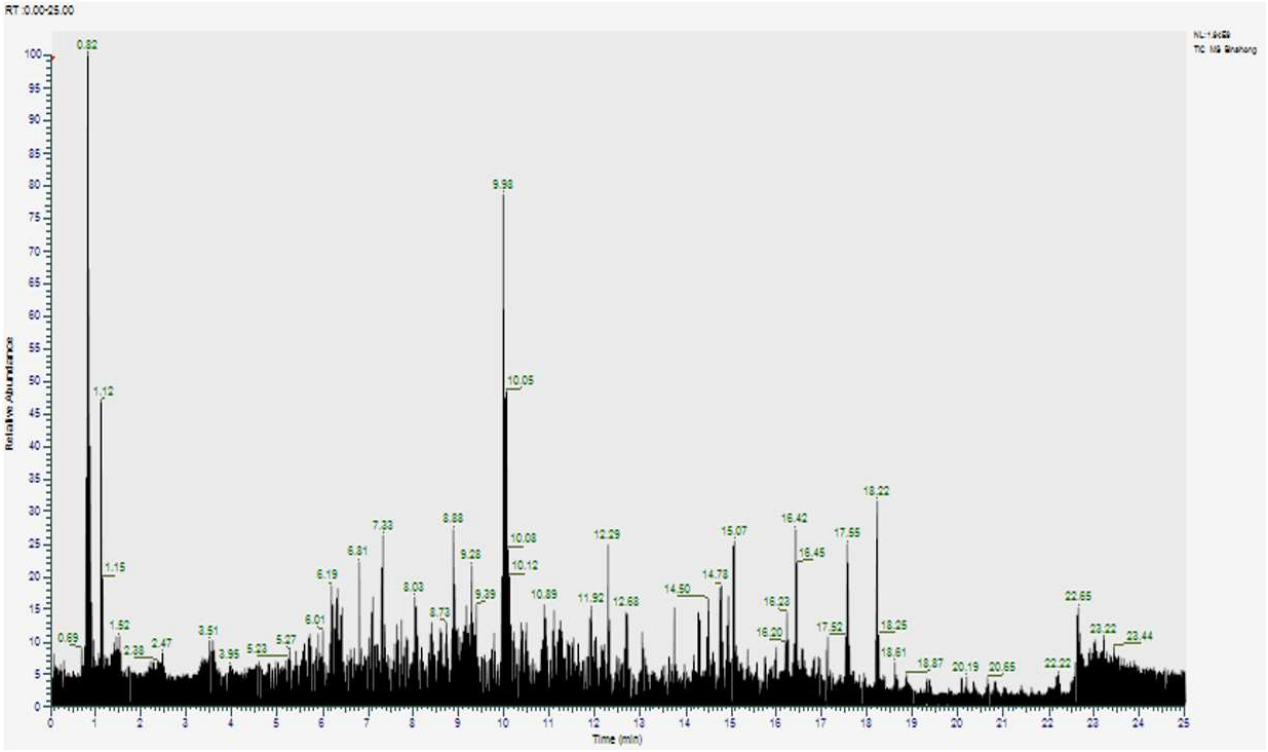

Fig. 3: Total Ion Chromatogram LC-HRMS 96% Ethanol Extract of Binahong (*Anredera cordifolia*) Leaves, there are 46 peaks.

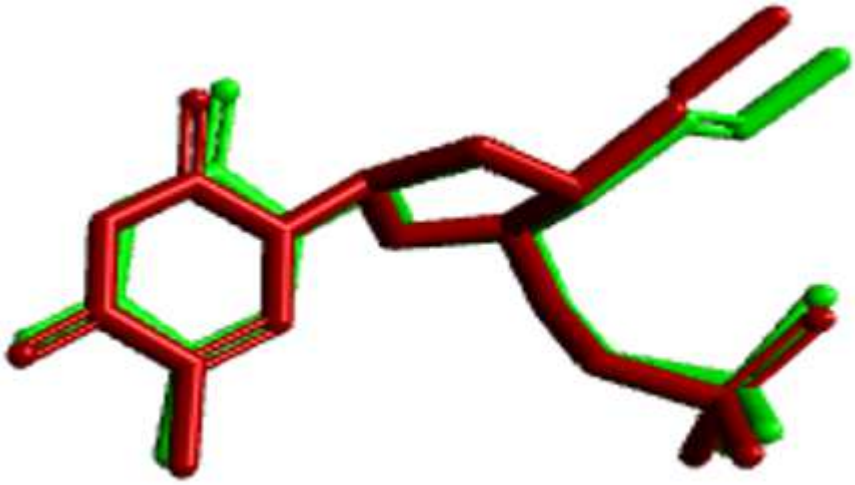

Fig. 4: Overlay redocking 3'Azido-3'-Deoxythmidine-5'-Monophosphate on *Tymydilate Kinase* with early position (green) and after redocking (red) using iGEMDOCK.

Table 2: Chemical compounds and % area of *Cinnamomum burmanii* Essential Oil

Rt (minutes)	Name of Component	Chemical Formula	Molecular Weight	% area
9.08	o-Cymene	$C_{10}H_{14}$	134	4.70
11.44	Linalool	$C_{10}H_{18}O$	154	8.26
16.59	(Z)-3-Phenylacrylaldehyde	C_9H_8O	132	77.44
18.77	Eugenol	$C_{10}H_{12}O_2$	164	6.09
20.16	Caryophyllene	$C_{15}H_{24}$	204	8.51

Rt: Retention time

Table 3: Active Compound from LC-HRMS Results of 96% Ethanol Extract of Binahong (*Anredera cordifolia*) Leaves

Name	Formulas	Molecular Weight	Retention Time (min)	Area (max)	m/zCloud Best Match
Vitexin	C_{21} H_{20} O_{10}	432,10454	5,749	657851019,181973	97,4
Glycitein	C_{16} H_{12} O_5	284,0677	10,26	16042307,8239329	90
Kaempferol-3-O-beta-D-galactoside-7-O-alpha-L-rhamnoside	C_{27} H_{30} O_{15}	594,15708	5,436	116059052,530303	85,9

Table 4: The results of molecular docking analysis between native ligand, cinnamaldehyde, eugenol and vitexin compounds against the Thymidylate kinase enzyme

Compounds/Ligands	*Binding Affinity* (Kcal/mol)	Bond Interaction				
		Van der Waals	Hydrogen	Electrostatic	Hydrophobic	Alkyl
3'Azido-3'-Deoxythmidine-5'-Monophosphate (Natif Ligand)	-125.77	-	GLU 11 GLN 101 ARG 70 SER 97	ARG 92 LYS 15 ARG 36	PHE 66 TYR 100	-
Cinnamaldehyde	-63.42	-	-	-	-	ARG 92
Eugenol	-71.67	-	SER 97 ARG 70	-	PHE 66	TYR 100 ARG 92
Vitexin	-131.61	-	GLN 101 ARG 70 GLU 37 ARG 48 ASP 91 LYS 15	ARG 92	PHE 66 TYR 100	-

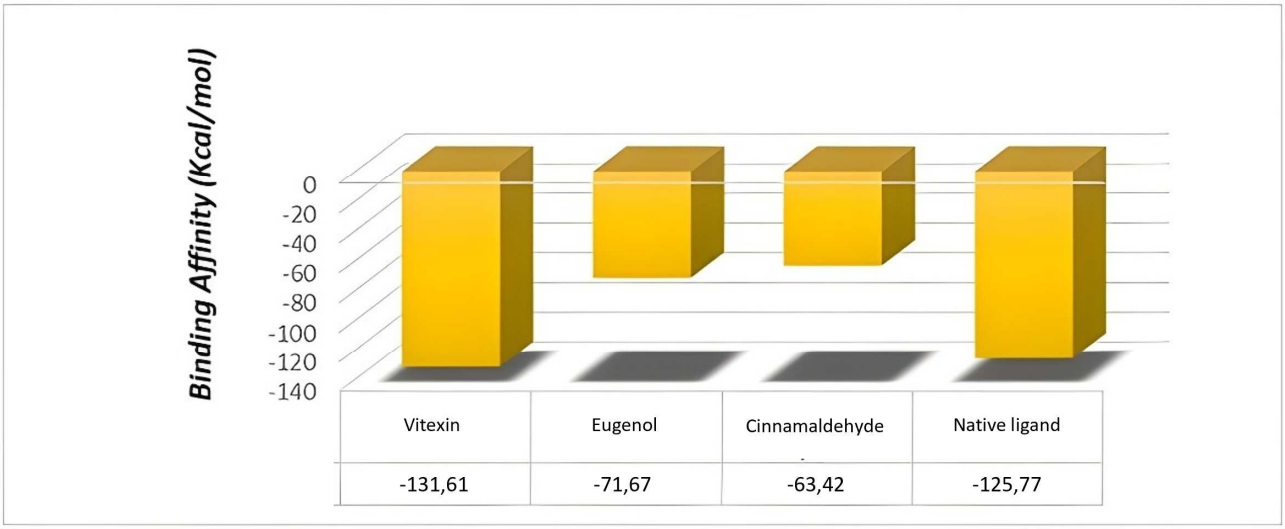

Fig. 5: *Binding Affinity* shows that vitexin has the closest score to the ligand score of -131.61

DISCUSSION

Analysis of essential oil compounds was conducted by using GC-MS method to determine the compouds and the percentage of essential oil compound content (Tuslinah *et al.*, 2023). The chromatogram results in fig. 1 show that the most abundant main compounds are located between peaks 4 to 19. The importance of determining eugenol content is related to the required conditions and its dominant content in clove flower essential oil below 78% indicates a quality not meeting the standard by SNI 06-2387-2006 (Loppies *et al.*, 2021). The clove flower eugenol compound obtained in this study was 83.89% (table 1).

The second largest component is *phenol, 2-methoxy-4-(2-propenyl)-, acetate*, which appears at peaks between 29 and 32 and clove flower essential oil contains caryophyllene compounds. This is also in line with the study which reported that clove essential oil has eight main compounds what has been reported that clove essential oil has eight main compounds: *eugenol, phenol,*

2-methoxy-4-(2-propenyl)-, acetate, caryophyllene, humulen, Caryophyllene oxide, Phenol-4-(2 -prpenyl)-acetate, methyl salicylate, and 6-methyl-2-heptanol. These compounds also have *in silico* activity to bind to proteins which can inhibit protein synthesis essential in microbial growth (Mobolaji *et al.*, 2023). Eugenol compounds and their derivatives have also been shown *in vitro* and *in silico* to have the potential to be developed as antibacterials in bacterial strains that have NorA efflux pumps (Muniz *et al.*, 2021).

Cinnamaldehyde is the primary compound in cinnamon (*Cinnamomum burmanii*) essential oil, identified at peaks 26 to 45 (fig. 2), accounting for 72.44% of the total area (table 2). These levels meet the SNI 8891: 2020 standard, requiring a minimum of 50%. The second major component is Caryophyllene, detected at peaks 52 to 55 with an area of 8.51%, followed by the presence of Linalool at 8.26%. Prior research has also highlighted cinnamaldehyde as the predominant active compound in cinnamon essential oil (Marissa Rijoice and Saragih, 2022).

The LC-HRMS analysis revealed that the flavonoid compound Vitexin, with significant antimicrobial properties, had the highest concentration in the 96% ethanol extract table 3. Vitexin is a compound of the flavonoid group (G. Shanthini Nachiar *et al*, 2023) with the main framework of the flavone ring (Ramasamy Arivukkarasu *et al*., 2021), which has antimicrobial activity. Vitexin showed significant antimicrobial and antibiofilm activity against Pseudomonas aeroginosa (Das *et al.*, 2016). The results showed that the flavonoid compound vitexin provides antibacterial activity against *Staphylococcus mutans* by inhibiting the production of Extracellular polysaccharides (EPS), which can cause membrane damage in bacteria, preventing biofilm formation and downregulating the expression of the spaP, strA, gtfB, brpA and luxS genes related to biofilm formation (Yan *et al.*, 2023). Vitexin is also reported to reduce the hydrophobicity of the surface of S. aureus, which can cause disruption of aggregation during biofilm formation and disrupt the biomass of biofilm-associated proteins: Aureus, EPS production and suppression of protease production. The compound Kaempferol -3,7-O-α-l- dirhamnoside is very effective against S. aureus. Glycitein is a compound of the isoflavone aglycone group, known to have antioxidant, anti-inflammatory, and antibacterial activity. Glycitein is an isoflavone aglycone compound with antioxidant, anti-inflammatory and antibacterial activity (Zhen Yang *et al*., 2012). The content of genistein in the ethanol extract of edamame seeds (Glycine max L. Merril) accelerates the wound healing process (Zhen Yang*, Kaustubh Kulkarni, 2012).

Fig. 4 presents the results of Validation, aiming to assess the software's performance in conducting calculations and its comparison with test compounds during the docking process. A key validation metric is the RMSD values ≤ 2Å. Lower RMSD values indicate better accuracy, reflecting closer alignment with the natural ligand conformation (Dwi Agistia *et al*., 2013). In the fig. the green portion represents the native ligand pre-docking, while the red portion signifies the ligand after docking. Observing minimal displacement between the pre-docking and post-docking ligands indicates the software's accuracy in calculations, demonstrating the efficacy of the iGEMDOCK docking method.

The docking process yields the binding energy reflecting the stability of interactions between the ligand, receptor, and the bonding interactions (Subramnian *et al*., 2020). Binding affinity, crucial for forming protein-ligand complexes, is influenced by the total binding energy within this complex. A lower binding affinity indicates easier and more stable ligand binding to the protein, with greater potential for influencing the protein, negative binding affinities suggest more favorable bonds and spontaneous reactions (Ekawasti *et al.*, 2021). Fig. 5 vitexin, eugenol, cinnamaldehyde, and native ligand exhibit values of -131.61, -71.67, -63.42 and -125.77, respectively. These results suggest that vitexin interact most effectively with the active site of thymidylate kinase, potentially inhibiting the enzym's activity. Flavonoids have been reported in insilico studies to show potential in treating psoriasis and effectively combating various S. aureus strains (Sagar Pol *et al*., 2023) (Adak *et al.*, 2018).

Visualization of these result using discovery Studio 2021 reveals the involved amino acid residues and bonds, predominantly at the active site. This visualization aids in predicting compound activity and identifying the most effective compounds based on their interaction with the protein molecule (Ekawasti *et al.*, 2021).

Based on fig. 6, The interaction post docking between the native ligand and Thymidylate kinase enzyme showcase nine binding amino acid residues. Notably, four hydrogen bonds (GLU 11, GLN 101, ARG 70, SER 97), two hydrophobic interaction bonds (PHE 66, TYR 100) and three electrostatic bonds (ARG 92, LYS 15, ARG 36) were observed.

Post-docking interaction with the thymidylate kinase receptor revealed eugenol binding to five amino acid residues, forming two hydrogen bonds (SER 97, ARG 70), one hydrophobic bond (PHE 66) and two alkyl bonds (TYR 100, ARG 92). Cinnamaldehyde displayed an alkyl bond with one amino acid residue (ARG 92), While vitexin bonded with ten residues, including six hydrogen bonds (GLN 101, ARG 70, GLU 37, ARG 48, ASP 91, LYS 15), two hydrophobic bonds (PHE 66, TYR 100), one electrostatic bond (ARG 92).

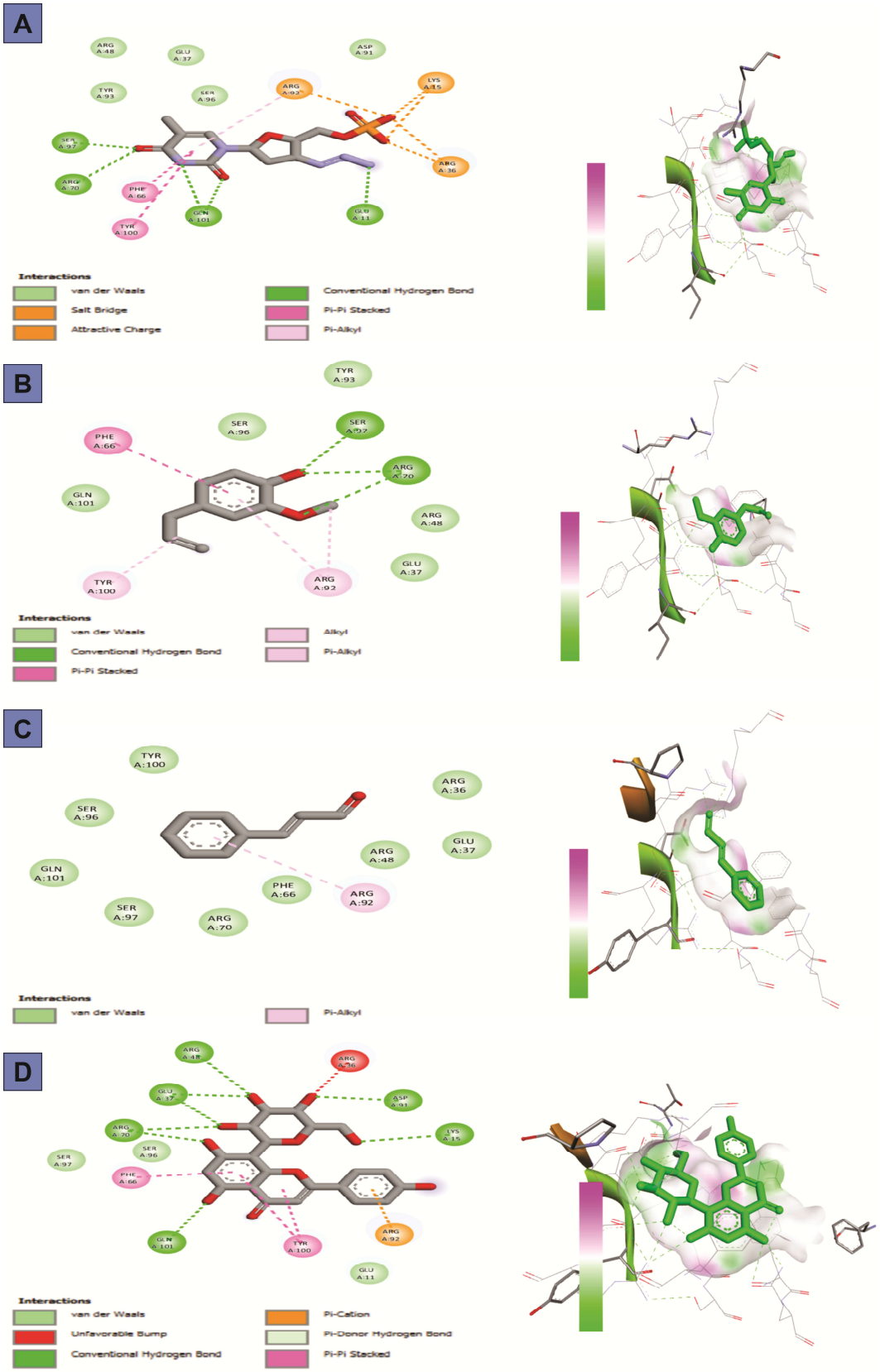

Fig. 6: Ligand Native Docking Pose to Thymidylate Kinase Enzyme (A), Eugenol Docking Pose to Thymidylate kinase Enzyme (B), Cinnamaldehyde Docking Pose to Thymidylate kinase Enzyme (C), Vitexin Docking Pose to Thymidylate kinase Enzyme (D).

Active site amino acid residues in the thymidylate kinase enzyme notably from hydrogen bonds with ARG 70, GLN 101, hydrophobic bonds with VAL 51, LEU 52 and PHE 66 and a salt-bridge binding with ARG 48 (Kawatkar *et al.*, 2014). The molecular docking in table 4 exhibit a binding energy affinity value of - 125.77, indicating robust binding with the enzyme. Formation of hydrogen bonds plays a critical role in stabilizing the protein structure. Bonds at the enzyme's active site indicates its cellular penetration capability affectively (Kawatkar *et al.*, 2014) (Ryan Syahputra *et al*., 2022).

Cinnamaldehyde exhibits a binding energy of -63.42 with the thymidylate kinase enzyme, indicating lower affinity compared to the native ligand. No hydrogen, electrostatic or hydrophobic bonds are seen in the docking results, with only μ-alkyl bonds forming at residue ARG 92, suggesting decreased cell penetration ability. Eugenol demonstrates a binding energy of -71.67 with the thymidylate kinase enzyme, showcasing lower affinity compared to the native ligand. While fewer hydrogen bonds are formed compared to the native ligand, eugenol display better efficacy than cinnamaldehyde. Vitexin exhibits a binding energy of -131.61 with the thymidylate kinase enzyme, displaying superior affinity to the enzyme compared to eugenol and cinnamaldehyde. The docking results reveal the formation of hydrogen, electrostatic and hydrophobic bonds, particulary an abundance of hydrogen bonds with amino acid residues, indicating potential for inhibiting the enzyme.

In drug candidates, ligand-protein interaction typically occur via non-covalent forces, such as

Electromagnetic or quantum mechanical forces. These interaction involve electrostatic forces like hydrogen bonds and Van der Waals forces. Hydrogen bonds play a vital role in physical properties like melting point and solubility in water, influencing the compound's biological activity (Junaidin *et al.*, 2022). Additionally, hydrogen bonds are robust and stable. Hydrophobic interactions involve nonpolar molecules that do not form hydrogen bonds with water, while Van der Waals interactions are weak, non specificattractive forces between close atoms (Ekawasti *et al.*, 2021).

CONCLUSION

The ethanol extract from binahong (*Anredera cordifolia*) leaves, clove flower essential oil (*Syzygium aromaticum)*, and (*Cinnamomum burmanii*) essential oil exhibit promising antibiofilm properties against *Staphylococcus aureus*. These compounds, especially vitexin from binahong (Anredera cordifolia) leaf extract, show potential as antibiotic agents for biofilm-related infections. Further studies are required to substantiate these results and explore the precise mechanisms behind inhibiting the Thymidylate kinase enzyme in *Staphylococcus aureus*, potentially necessitating animal model testing.

Conflict of interest

The authors have no conflicts of interest to declare.

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