Predicting the potential of *Ocimum sanctum* leaf extract as an antibacterial agent for *Escherichia coli*: A crucial step towards realizing the Good Health and Well-being goal

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Abstract. Using natural compounds to treat diarrhea is a local wisdom practice in several countries. Interest in plants with antimicrobial properties, such as holy basil (*Ocimum sanctum*) leaves, has revived due to bacterial resistance associated with the inappropriate use of antibiotics. The use of natural compounds as potential affordable medicine may also support achieving the goal of good health and well-being. In this current study, selected compounds of basil leaves, i.e., eugenol, caryophyllene, and geranylgeraniol, were subjected to docking simulation to disclose their potential as an antibacterial agent to *Escherichia coli*. Molecular docking analysis was performed using *AutoDockTools*. It revealed that the geranylgeraniol compound had the lowest binding affinity energy (-6.3 kcal/mol) and RMSD value close to 2Å (1.889Å). Eugenol and geranylgeraniol have identical amino acid binding site residues as reference antibiotic ciprofloxacin and native ligand ON2. Based on these results, it can be concluded that geranylgeraniol can potentially be an antibacterial agent for *E. coli*

1 Introduction

An unhygienic environment and behavior are closely related to diarrheal diseases [1]. In 2019, infectious diarrhea was recorded in 270 out of 1000 adult people in Indonesia, while for children, it was 843 out of 1000 [2]. In 2020, pneumonia and diarrhea were the main health problems causing 73.9% and 14.5% of deaths, respectively [3]. This condition challenges the third UN SDG's Good Health and Well-being goal.

Several microorganisms, such as bacteria, fungi, and viruses, are suspected of causing the emergence of infectious diseases [4]. *Escherichia coli* is a bacterium that may cause infectious diseases in the gastrointestinal tract. Most strains of *E. coli* are microflora in the

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gut and rarely cause a health problem. However, pathogenic strains and excessive numbers of *E. coli* can cause diarrhea or extraintestinal disease [5,6].

Bacterial gastrointestinal infections can pose a significant threat, particularly in developing countries with limited access to adequate medical care. This can hinder achieving one of the targets of Sustainable Development Goal 3 (SDG 3), ensuring healthy lives and promoting well-being for all ages, emphasizing controlling infectious diseases and providing universal access to health.

In Indonesia, the consumption of antibiotics is a common prescription to cure various diseases. However, inappropriate use of antimicrobial agents often leads to multidrug resistance in microorganisms [7]. Sasongko's study [7] also indicates that *E. coli* isolates from the river and household waters alongside the Code River in the Sleman region have been more resistant to antibiotics, especially amoxicillin (80%) and streptomycin (86.7%). The bacterial resistance to antibiotics must be monitored to decide the proper prescription of antibiotics to treat or cure a particular disease [8]. Traditional medicine can be an alternative to antibiotics because the ingredients are easy to obtain and do not cause resistance. Moreover, the side effects are less severe than those caused by synthetic chemical medicine and chemical-based drugs. One of the sources of these traditional medicines is plants that contain particular compounds.

Indonesia has abundant plant resources that are used in traditional medicine. Some plants, such as holy basil (*Ocimum sanctum*), are an abundant plant resource commonly used in traditional medicine. It contains certain compounds with antibacterial properties that can be used to treat infectious diseases caused by pathogenic bacteria. Based on the phytochemical test, the ethanolic extract of holy basil leaves contains flavonoids, alkaloids, and tannins [9]. In accordance, Moeza [10] stated that holy basil leaf crude extract inhibits the growth of *E. coli*. However, that research did not reveal particular compounds which have a significant role in inhibiting bacterial growth.

The compounds from holy basil leaf extract that have a significant role as an *E. coli* antibacterial agent can be identified using molecular docking simulations. Molecular docking is the simplest structure-based in silico method commonly used to predict the interactions between two molecules, namely the active compound index and the molecular target [11]. This computationally based in silico method makes the process of finding and making drugs easier [12]. The present study investigates the potential of selected holy basil leaf extract compounds as an antibacterial agent for *E. coli*. Predicting the potential of the antibacterial properties of *O. sanctum* as an alternative medicine is essential. The development of plant-based therapies aligns with the principle of providing universal access to health. It can offer more affordable medicine and accessible solutions, especially in areas with limited access to healthcare, and support achieving the Good Health and Well-being goal, one of 17 UN SDGs Goal.

2 Methods

The structure of the target protein *E. coli* DNA gyrase (PDB ID: 6YD9) taken from the protein data bank (https://www.rcsb.org/) is depicted in Figure 1. Meanwhile, the 3D structure of selected active compounds, eugenol, caryophyllene, and geranylgeraniol, as well as the reference antibiotics ciprofloxacin were obtained from PubChem (https://pubchem.ncbi.nlm.nih.gov/) (Figure 2).

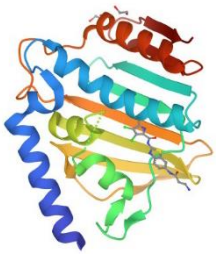

Fig. 1. The 3D structure of target protein *E. coli* DNA gyrase (PDB ID: 6YD9)

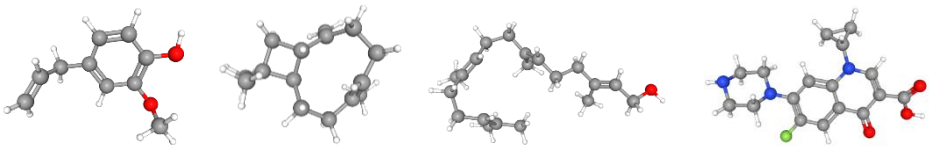

Fig. 2. The 3D chemical structure of A: eugenol, B: caryophyllene, C: geranylgeraniol, D: ciproflaxacin

2.1 Drug-likeness test of natural compound

A drug-likeness test was performed to evaluate the distributions of molecular properties of the selected active compounds, and the properties were then compared to drug-like molecules. The active compound was selected based on the Lipinski five rule, and the drug-likeness test was applied to the SwissADME web server (http://www.swissadme.ch/index.php). The canonical SMILES data of the selected active compounds of *O. sanctum* obtained from PubChem were input into the SwissADME web server. A compound is determined to have drug-like properties if it has a molecular weight (MW) of 150-500 g/mol, the value of the log P partition coefficient < 5, the number of H-bond donors < 5, the number of H-bond acceptors < 10 [13,14].

2.2 Molecular Docking analysis

Sample preparation was carried out in four stages. The first stage was to collect target protein samples from the protein data bank. The second stage was sterilization of the target protein using *AutoDockTools* to separate protein molecules with native ligands and unwanted molecules. The third stage was the collection of ligand samples of holy basil leaf extract bioactive compound and the reference antibiotic ciprofloxacin from the protein data bank. The fourth stage was minimizing the sample ligand of the compound to make it more flexible and able to produce the highest affinity with the target protein [15].

The molecular docking analysis was carried out using *PyRx*. Ligands interacted with target protein molecules to form macromolecular complexes. This analysis determined the binding energy to form macromolecular complexes and Root Mean Square Deviation (RMSD) values [15]. Docking results were visualized using *PyMOL* and *Ligplot*. At this stage, the type of formed bond and the amino acid residues can be determined.

2.3 Data analysis

Data analysis was conducted by comparing the value of binding affinity energy, RMSD, type of bond, and amino acid residues at the binding site between the holy basil leaf extract compound ligand and the reference antibiotic and the native ligand. Purnomo [16] states that the stronger the affinity, the lower the binding affinity energy and vice versa. Accordingly, the RMSD value declared valid to be used is ≤ 2Å.

3 Result and Discussion

3.1 Drug-likeness test of natural compound

The selected active compound of *O. sanctum* was evaluated using predicted ADME *(Adsorption, Distribution, Metabolism, and Excretion)* through the SwissADME web server. Table 1 shows that all selected active compounds of *O. sanctum* have a molecular weight of less than 500 g/mol and comply with Lipinski's rule of five. Hence, all selected compounds meet the first drug-likeness criteria [14]. The low molecular weight of the selected active compound *O. sanctum* indicated that the molecules could easily pass through cell membranes. Lipinski [17] stated that chemical compounds with low molecular weights are easier for oral absorption. On the other hand, the absorption of compounds with high molecular weight (MW > 500 g/mol) must take an alternative route, typically through a membrane [18].

Table 1. Characters of selected active compounds of *O. sanctum*

Molecule	PubChem ID	Formula	Canonical SMILES	MW (g/mol)
Eugenol	3314	C10H12O2	C=CCc1ccc(c(c1)OC)O	164.2
Caryophyllene	5281515	C15H24	CC1=CCCC(=C)C2C(CC1)C(C2)(C)C	204.35
Geranylgeraniol	5281365	C20H34O	OCC=C(CCC=C(CCC=C(CCC=C(C)C)C)C)C	290.48

The results of the drug-likeness test are listed in Table 2. Based on the drug-likeness test, the eugenol, caryophyllene, and geranylgeraniol had no more than 10 H-bond acceptors and no more than 5 H-bond donors. This result indicated that the body quickly absorbed the selected active compounds of *O. sanctum*. The lower number of H-bond donors and H-bond receptors indicates that the energy required to absorb a drug candidate is lower and vice versa [19].

Table 2. Drug-like properties of selected active compounds of *O. sanctum*

Molecule	H-bond acceptors	H-bond donors	XLOGP3
Eugenol	2	1	2.27
Caryophyllene	0	0	4.38
Geranylgeraniol	1	1	7.27

The log P (XLOGP3) value indicates the lipophilicity of a compound, where the greater the log P value, the greater the lipophilicity, and vice versa [20]. Eugenol and caryophyllene compounds have lower XLOGP3 values than geranylgeraniol compounds. A molecule is categorized as having good lipophilicity if the log P value ranges from -0.7 to +5.0 [14]. Eugenol and caryophyllene compounds have XLOGP3 values that meet Lipinski's rules. Hence, it can be said that eugenol and caryophyllene compounds have good lipophilicity

properties as drug candidates. Julianus et al. [21] stated that compounds with a log P value close to 1 showed good lipophilicity and hydrophilicity properties and could easily penetrate the membrane.

Geranylgeraniol has an XLOGP3 value that exceeds Lipinski's rule of five, which indicates that the compound has high lipophilicity or lipid solubility. The logarithmic value of the partition coefficient between octanol/water (log P) is closely related to the hydrophilicity or the ability to dissolve in water. Julianus et al. [21] stated that the high value of log P indicates that a molecule is difficult to dissolve in the water medium and penetrate the membrane. Fadlan et al. [22] stated that the high value of log P indicates low hydrophilicity, causing poor absorption and permeation of a molecule. The greater the log P value, the more hydrophobic a molecule or compound is. The highly hydrophobic nature indicates that the molecule tends to be toxic because it is not more widely distributed in the body, and it is retained on the lipid bilayer membrane, causing weak binding selectivity to the target enzyme [19]. On the other hand, a low log P value is also unfavored because the molecule will be challenging to penetrate the lipid bilayer membrane.

3.2 Drug-likeness test of natural compound

Molecular docking is one of the biocomputing methods (in silico) that can predict the interaction between the compound and the target protein, including its affinity and conformation. This method can assist in the discovery of new drugs, especially from natural compounds of traditional medicines that have been widely used by the community. The binding affinity and RMSD values data obtained in this study are presented in Table 3.

Table 3. Binding affinity energy and RMSD values of ligands and target protein *E. coli* DNA gyrase (PDB: 6YD9).

Ligand	Binding affinity energy (kcal/mol)	RMSD (Å)
Eugenol	-5.5	1.301
Caryophyllene	-5.5	1.385
Geranylgeraniol	-6.3	1.889
Ciprofloxacin	-7.4	2.626
Native ligand ON2	-7.4	1.871

Based on Table 3, the geranylgeraniol compound has the lowest binding affinity energy (-6.3 kcal/mol). Besides, its RMSD value was closer to 2Å than eugenol and caryophyllene. Purnomo [16] states that the lower the binding affinity energy, the higher the affinity to the macromolecular targets and vice versa. This is in line with Manna et al. [23], who state that the more negative the binding affinity energy score indicates, the more stable the bonds are. The RMSD value is declared valid if scored close to 2Å. Accordingly, the bioactive compound geranylgeraniol has the highest potential as an antibacterial for *E. coli* compared to eugenol and caryophyllene. However, the potency was still lower than that of ciprofloxacin and native ligand ON2. These results were in accordance with previous research conducted by [24], which showed that holy basil leaf extract could inhibit the growth of *E. coli*. However, its inhibitory capability was lower than the 0.5% ciprofloxacin.

The type of bond and amino acid residues at the binding site are also an important parameter in predicting a compound's potential interaction with the interacting target protein (receptor). The type of bond and amino acid residues can be determined through the visualization stage (Figure 3).

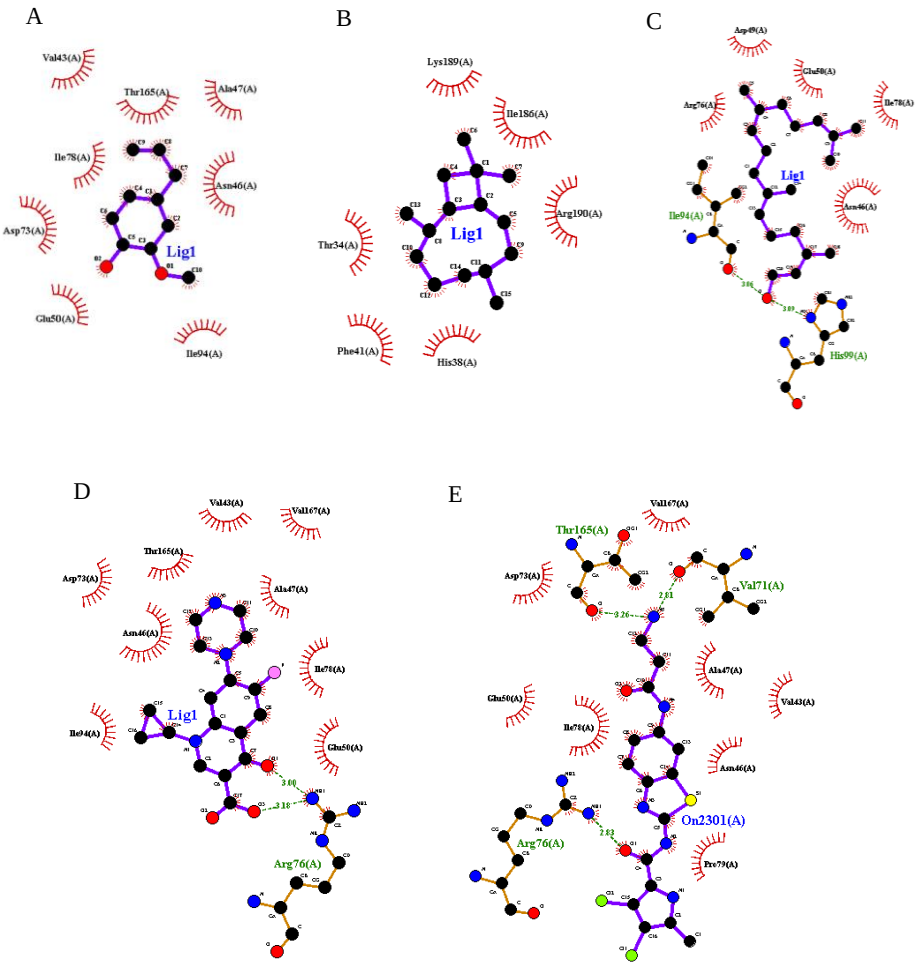

Fig. 3. Visualization of molecular docking of basil leaf extracts bioactive compounds with the target protein *E. coli* DNA gyrase. A: Eugenol, B: Caryophyllene, C: Geranylgeraniol, D: Ciprofloxacin, E: Native ligand ON2.

The type of bond and amino acid residues at the binding site are summarized in Table 4. Based on Table 4, eugenol and geranylgeraniol compounds have identical amino acid residues at the binding sites as ciprofloxacin and native ligand ON2. Eugenol compounds also have hydrophobic bonds with amino acid residues similar to ciprofloxacin and native ligands, which include Val43(A), Asn46(A), Ala47(A), Glu50(A), Asp73(A), Ile78, Ile94, and Thr 165. Therefore, the eugenol compound has the same binding site as the reference antibiotics and can potentially inhibit the growth of *E. coli*.

Table 4. Type of bond and amino acid residues at the binding site

Target protein	**Ligand**	**Type of bond**	**Amino acid residues**
E. coli DNA gyrase (PDB: 6YD9)	Eugenol	Hydrophobic	Val43(A), Asn46(A), Ala47(A), Glu50(A), Asp73(A), Ile78(A), Ile94(A), Thr165(A)

Target protein	Ligand	Type of bond	Amino acid residues
	Caryophyllene	Hydrophobic	Thr34(A), His38(A), Phe41(A), Ile186(A), Lys189(A), Arg190(A)
	Geranylgeraniol	Hydrophobic	Asn46(A), Asp49(A), Glu50(A), Arg76(A), Ile78(A)
		Hydrogen	Ile94(A), His99(A)
	Ciproflaxacin	Hydrophobic	Val43(A), Asn46(A), Ala47(A), Glu50(A), Asp73(A), Ile78(A), Ile94(A), Thr165(A), Val 167(A)
		Hydrogen	Arg76(A)
	Native Ligand ON2	Hydrophobic	Val43(A), Asn46(A), Ala47(A), Glu50(A), Asp73(A), Ile78(A), Pro79(A), Val167(A)
		Hydrogen	Arg76(A), Val71(A), Thr165(A)

Eugenol is a phenolic aromatic compound that has a calming aroma and taste. This compound belongs to the allylbenzene class of phenylpropanoids [25]. Mishra et al. [26] stated that eugenol, also known as 1-allyl-4-hydroxy-3-methoxybenzene or 4-allyl-2-methoxy phenol, is usually found in the form of a yellowish oil liquid. Eugenol also belongs to the allyl chain substituted guaiacol group [27].

Eugenol exerts beneficial effects on human health. One of them is the antimicrobial and antifungal properties that target a broad spectrum of microorganisms [25]. Devendran and Balasubramanian [28] found that basil leaf extracts contain eugenol with a percentage of 43.88%.

Eugenol inhibits *E. coli* growth by interrupting the lipid fraction of the bacterial membrane. This disturbance causes changes in membrane permeability, leading to intracellular leakage and bacterial cell death [29]. In addition, eugenol also inhibits the growth of *Staphylococcus aureus* [30]. Eugenol is classified as a robust antibacterial because of its ability to inhibit the growth of *S. aureus* with an optimum diameter of inhibition zone (22.27 mm) obtained at a low concentration (8%).

The visualization of caryophyllene indicated the interaction model in the form of hydrophobic bonds with amino acid residues different from reference antibiotics ciprofloxacin and native ligands. These results indicated that caryophyllene has lower antibacterial properties than other compounds. In a study by Dahham et al. [31], caryophyllene showed a robust antibacterial effect against all tested bacterial strains. However, these compounds showed more significant antibacterial activity against gram-positive than gram-negative bacteria. Accordingly, the antibacterial effect of caryophyllene against *E. coli* was less susceptible than that of *S. aureus*.

The visualization results of geranylgeraniol showed the presence of hydrophobic and hydrogen bonds with amino acid residues at the same binding site as the reference antibiotics, which included Asn46, Glu50(A), Arg76(A), Ile78(A), and Ile94(A). These results indicated that the compound geranylgeraniol interacts with the target protein, and the binding site was the same as the reference antibiotics ciprofloxacin and native ligand. Consistently, the geranylgeraniol has the lowest binding affinity energy value (-6.3 kcal/mol), not much different from the binding affinity energy of ciprofloxacin (-7.4 kcal/mol) and native ligand (-7.4 kcal/mol). In addition, the RMSD value of the geranylgeraniol compound was closer to the value of 2Å.

Geranylgeraniol is a compound that belongs to the diterpenoid group, namely hexadeca-2,6,10,14-tetraene, which is substituted by a methyl group at positions 3, 7, 11, and 15 and a hydroxy group at position 1. The mechanism of geranylgeraniol as an antibacterial agent is by disrupting the cell membrane leading to the leakage of K ions. Consistently, Inoue et al.

[32] found that geranylgeraniol showed its inhibition of the growth of *S. aureus* by disrupting its cell membrane.

The results of the log P value of the geranylgeraniol compound were inverse to the docking results, where this compound had a higher drug potential in terms of docking results. However, this can be tolerated because each compound only violates 1 rule, but modifying the structure to improve the physicochemical properties is necessary. According to Lipinski's rules of five, a drug can be given orally if it does not violate more than one criterion [33]. In addition, reducing the value of lipophilicity in a compound can be done by modifying the structure of the compound by adding carbon to its structure. In a study conducted by Degorce et al. [34], one bridge carbon was added to reduce the lipophilicity of Morpholin and Piperazine compounds and found to increase the polarity of these compounds.

4 Conclusion

Selected compounds of ethanolic extract from holy basil leaves used in this present study, namely eugenol, geranylgeraniol, and caryophyllene, have the potential to be an antibacterial agent for *E. coli*. The RMSD value of geranylgeraniol is 1.889Å, which is close to 2Å. Moreover, this compound has the lowest binding affinity energy. Correspondingly, it can be concluded that geranylgeraniol may be the most potent compound to be used as an antibacterial agent. Drug-likeness tests show that eugenol and caryophyllene complied with Lipinski's rule of five. The log P value of geranylgeraniol is not in accordance with Lipinski's rule, but it can be used as a drug candidate by modifying the structure to meet Lipinski's rules. However, further research studies are needed to confirm the potency of this compound as an antibacterial agent to other bacteria to a better impact the goal of good health and well-being.

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