Jurnal Pijar MIPA
https://jurnalfkip.unram.ac.id/index.php/JPM

ISSN 1907-1744 (Print)
ISSN 2460-1500 (Online)

Identification of the Major Compounds from Ethanol Extract of *Scoparia dulcis* L. Leaves Based on LC-MS and Their Potential as Antibacterials Against *Escherichia coli*: A Study In Silico

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Received: May 7, 2026. Accepted: June 16, 2026. Published: October 23, 2026

Abstract: Bacterial infections remain a significant global health problem, particularly those caused by *Escherichia coli*, which is associated with various diseases such as diarrhea, urinary tract infections, and sepsis. The increasing prevalence of antibiotic resistance highlights the urgent need to explore alternative antibacterial agents derived from natural products. This study aims to identify the dominant compounds in the ethanol extract of *Scoparia dulcis* L. leaves using LC-MS and to evaluate their potential as antibacterial agents against E. coli via in silico molecular docking. The extraction was performed using the maceration method, followed by LC-MS analysis, which identified 104 secondary metabolites. Among these metabolites, flavonoids were identified as the dominant compound class, including apigenin, luteolin, acacetin, scutellarein, hispidulin, and ladanetin. Molecular docking was conducted using Autodock Vina against DNA gyrase (PDB ID: 1KZN) as the target protein. The docking protocol was validated by redocking the original ligand, yielding an RMSD value of 1.22 Å. Ligand-protein interactions were analyzed using Discovery Studio Visualizer to identify hydrogen bonds, π-interactions, and hydrophobic interactions. The docking results showed that all tested flavonoids exhibited lower binding affinity values than ciprofloxacin (-7.6 kcal/mol), with luteolin demonstrating the strongest interaction (-8.9 kcal/mol), followed by apigenin (-8.5 kcal/mol) and scutellarein (-8.4 kcal/mol). Visualization analysis revealed that these interactions played crucial roles in stabilizing ligand–protein interactions. Additionally, drug-likeness evaluation based on Lipinski's Rule of Five indicated that the selected compounds possess favorable physicochemical properties, suggesting good membrane permeability and bioavailability. The novelty of this study lies in integrating LC-MS-based compound identification with molecular docking analysis, focusing specifically on the dominant flavonoids of *Scoparia dulcis*. These findings provide a scientific basis for developing flavonoid-based antibacterial agents targeting the DNA gyrase enzyme in *E. coli*.

Keywords: Antibacterial; *Escherichia coli*; Flavonoids; Molecular Docking; *Scoparia dulcis* L.

Introduction

Bacterial infections represent a significant global health problem, as they can cause a wide range of infectious diseases in humans. These infections generally result from bacterial growth and proliferation within the human body, leading to disease [1]. One of the bacteria responsible for bacterial infections is *Escherichia coli*, which is recognised as the second-leading cause of diarrheal disease in Indonesia. Data from the Ministry of Health indicate that diarrhea remains among the highest-ranking diseases, with approximately 100.000 deaths reported [2]. In addition to causing diarrhea, this bacterium can also lead to other infections, such as urinary tract infections and sepsis [3]. E. Coli is classified as a Gram-negative bacterium, characterized by the presence of lipopolysaccharide as a major compound of its outer membrane. This structure contributes to its virulence and acts as a permeability barrier, thereby increasing its resistance to antibiotics [4].

Bacterial infections can generally be treated with antibiotics. However, improper use of antibiotics can increase antibiotic resistance. Rising infection rates, accompanied by antibiotic resistance, have become a serious issue in healthcare [5]. Antibiotic resistance reduces treatment effectiveness, increasing morbidity and mortality rates from bacterial infections. Globally, in 2019, antibacterial resistance was estimated to cause 1.27 million deaths, including 34,500 deaths in Indonesia related to resistant bacterial infections [6]. This situation highlights the need to develop new antibacterial agents as therapy alternatives. One approach that has been widely explored is the use of natural materials, since herbal plants are known to contain various bioactive compounds with antibacterial potential and relatively lower side effects [7]. Therefore, exploring bioactive compounds from medicinal plants is important for identifying new antibacterial candidates effective against resistant bacteria.

The antibacterial mechanism generally involves inhibition of cell wall synthesis, disruption of bacterial cell membrane permeability, inhibition of enzymatic activity, and interference with nucleic acid and protein synthesis [8]. Various secondary metabolites, including flavonoids, have been reported to exhibit antibacterial activity through these mechanisms. Flavonoids are known to inhibit important enzymes in bacterial DNA replication, such as DNA gyrase, and also interact with cellular proteins through hydrogen bonds and hydrophobic interactions, disrupting vital bacterial functions.s [9]. Therefore, the presence of flavonoid compounds has the potential to inhibit the growth of pathogenic bacteria. Given the rising prevalence of

How to Cite:
H. Z. Firdausi and T. Tukiran, "Identification of the Major Compounds from Ethanol Extract of Scoparia dulcis L. Leaves Based on LC-MS and Their Potential as Antibacterials Against Escherichia coli: A Study In Silico", *J. Pijar.MIPA*, vol. 21, no. 5, pp. 991–998, Oct. 2026.
https://doi.org/10.29303/jpm.v21i5.12049

antibiotic resistance, exploring antibacterial compounds from natural sources, including flavonoids, has become an important approach to developing new therapeutic agents [10].

A plant suspected of having antibacterial potential is the sweet broom leaf (*Scoparia dulcis* L.), a member of the Plantaginaceae family. This plant contains various secondary metabolites, especially flavonoids, which are the main bioactive compounds [11]. Previous studies have reported that sweet broom leaves have antibacterial activity against *Haemophilus influenzae* and *Klebsiella pneumoniae* [12]. In addition, other research has also reported that sweet broom leaves (*S. dulcis* L.) show antibacterial activity against Escherichia coli and Staphylococcus aureus [13]. However, these studies generally still focus on evaluating antibacterial activity and have not specifically identified the dominant flavonoid components responsible for this activity using mass spectrometry-based analyses such as LC-MS [14]. In addition, research on the molecular interactions between flavonoid compounds from S. dulcis L. and Escherichia coli target proteins using molecular docking approaches remains very limited.

Based on the above, this study aims to analyze the flavonoid compounds present in the ethanol extract of *S. dulcis* L. Leaves. The flavonoid compounds in the ethanol extract of *S. dulcis* L. Leaves were further analyzed using an in silico approach to predict their potential antibacterial activity against *E. Coli* through interactions with target proteins [15]. Furthermore, the compounds were analysed using a drug-likeness assessment to evaluate their suitability as drug candidates based on Lipinski's Rule of Five (RO5), which includes molecular weight <500 Da, partition coefficient (LogP) <5, hydrogen-bond donors ≤5, and hydrogen-bond acceptors ≤10. Compounds that satisfy these criteria are not only expected to exhibit favorable interactions based on molecular docking results but also have a higher likelihood of possessing suitable pharmacokinetic profiles for drug development [16]. The novelty of this research lies in integrating LC-MS analysis, molecular docking, and druglikeness evaluation to examine the potential of flavonoids from S. dulcis L. as antibacterial agents against *E. coli*.

Research Methods

Materials and Tools

Materials: Scoparia dulcis L. leaves and 90% ethanol. Tools: Blender, 1000 mL glass beaker, filter paper, Buchner funnel, rotary vacuum evaporator, analytical scale, LC-MS instrument (Shimadzu). Hardware and Software: ASUS VivoBook X509MA laptop with Intel® Celeron® N4020 processor, 4 GB RAM, RCSB PDB web server, PubChem, BIOVIA Discovery Studio 2025, and PyRx Virtual Screening Tool

Procedure

Sample Preparation

The *Scoparia dulcis* plants were collected from Juwet Village, Ngronggot District, Nganjuk Regency, East Java, Indonesia. The plant was subsequently taxonomically identified at the Yayasan Generasi Biologi Indonesia. The leaves were separated from other plant parts and air-dried at room temperature. The dried leaves were then ground into a fine powder and macerated in 90% ethanol for 3 days. The resulting filtrate was concentrated using a rotary vacuum evaporator to obtain the ethanol extract of S. dulcis leaves.

Identification of Chemical Compounds

Identification of chemical compounds in the ethanol extract of Scoparia dulcis leaves was performed using Liquid Chromatography-Mass Spectrometry (LC-MS). A total of 1 µl of the sample was injected into the LC-MS instrument with a capillary voltage of 3.0 kV. Compound separation was performed using a Shimadzu Shim-Pack FC-ODS column (2mm × 150 mm with particle size 3 µm). The column temperature was maintained at 35℃. The mobile phase was used in an isocratic system with 90% ethanol at a flow rate of 0.5 mL/min, with a total analysis time of 60 minutes. Mass spectra were acquired over an m/z range of 10-1500 with a scan time of 0.6 seconds per scan. Subsequently, secondary metabolites in the extract were identified based on retention times and mass spectra from the chromatogram, using the National Institute of Standards and Technology (NIST) database [17].

Molecular Docking Analysis

Structure 3D of the target protein (PDB ID: 1KZN) was retrieved from https://www.rcsb.org/ and prepared using BIOVIA Discovery Studio 2025 by removing the native ligand, nucleic acid molecules, and water molecules. The native ligand of the target protein was also prepared by removing the protein, nucleic acid molecules, and water molecules. DNA gyrase (PDB ID: 1KZN) was selected as the target protein because it plays a crucial role in bacterial DNA replication and is a primary target of quinolone antibiotics.

Seven flavonoid compounds, apigenin, luteolin, acacetin, scutellarein, hispidulin, jaranol, and ladanetin were selected as test ligands in molecular docking simulations because they are the dominant flavonoids identified in the ethanol extract of sweet flag leaves. The 3D structures of these compounds and the reference compound, ciprofloxacin, were downloaded from https://pubchem.ncbi.nlm.nih.gov and saved in .pdb format.

Before docking the test compounds, the docking method was validated by redocking the native ligand into the target protein's active site. Redocking was performed by re-docking the native ligand into the active site of the target protein using the grid box coordinates X: 19.150163; Y: 30.392531; and Z: 34.744776. The redocking results were evaluated using the Root Mean Square Deviation (RMSD), and the docking method was deemed valid if it yielded an RMSD ≤ 2.0 Å.

Molecular docking simulations were performed using the PyRx Virtual Screening Tool. Target proteins were loaded using the Load Molecule menu, and flavonoid compounds and drug compounds were loaded using the Open Babel feature. Energy minimization of all ligands was then performed using the Minimize All option to obtain the most stable conformation. The minimized ligands were converted to the Autodock Ligand format (.pdbqt).

The docking process was performed using AutoDock Vina through the Vina Wizard interface, with the target protein set as the macromolecule. The grid box was set at the coordinates X: 19.150163, Y: 30.392531, and Z: 34.744776. The simulation was executed using the Run Vina option, resulting in binding affinity values that indicate the strenght of interaction between the ligand and the target protein.

The docking results were analyzed comparatively against the reference compound ciprofloxacin based on the binding affinity values obtained. Compounds with lower (more negative) binding affinity values than ciprofloxacin were considered to have a stronger binding affinity for the target protein and potentially exhibit better inhibitory activity. Ligand-protein interactions were then visualized using BIOVIA Discovery Studio Visualizer to identify hydrogen bonds, hydrophobic interactions, and π interactions. Subsequently, the compounds were analyzed based on Lipinski's Rule of Five (RO5).

Results and Discussion

The powdered ethanol extract of *Scoparia dulcis* L. leaves was obtained with a total weight of 71 grams and subsequently macerated. This method was selected due to its simplicity and ease of implementation, as it does not require specialized equipment or advanced technology [18]. After maceration, the filtrate was concentrated using a rotary vacuum evaporator to obtain a viscous extract with a yield of 7.6135 grams.

The resulting extract was then anayzed using Liquid Chromatography-Mass Spectrometry (LC-MS). The LC-MS analysis of the ethanol extract of *Scoparia dulcis* L. Leaves revealed the presence of 104 secondary metabolite compounds, as illustrated in Figure 1, which presents the chromatogram of the extract.

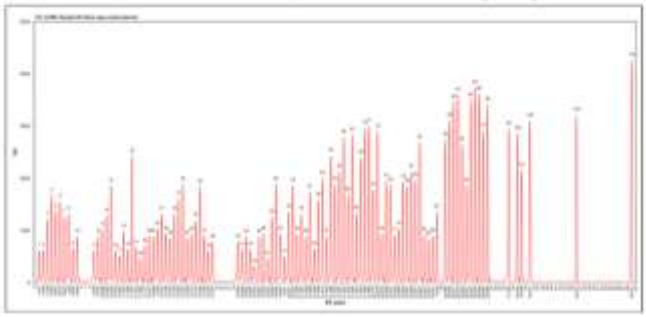


Figure 1. LC-MS Chromatogram of Ethanol Extract of *S. dulcis* L. Leaves.

The compounds present in the ethanol extract of *Scoparia dulcis* L. Leaves consist of various classes of secondary metabolites, including phenolic acid, flavonoids, terpenoids, tannins, and glycoside derivatives. Based on the analysis results, flavonoids were identified as the most dominant group, as indicated by the presence of apigenin, luteolin, kaempferol, quercetin, naringenin, acacetin, and scutellarein. In addition, several flavonoid derivatives in the form of glycosides were also detected, including apigenin-7-O-glucoside, vitexin, isovitexin, orientin, quercetin-3-O-glucoside, hyperoside, luteolin-7-glucoside, and pectolinaroside. Phenolic acids were also identified, such as malic acid, vanillic acid, gallic acid, caffeic acid, ferulic acid, and chlorogenic acid.

In addition to flavonoids, the ethanol extract of Scoparia dulcis L. Leaves contains phenolic acids such as malic, vanillic, gallic, caffeic, ferulic, and chlorogenic acids, as well as other compounds including coumarins (scopoletin and scoparone), diterpenoids (scopadulcic acid and scoparic acid), triterpenoids (lupeol, friedelin, and betulinic acid), and tannins such as procyanidin. Among the 104 secondary metabolites identified, the 10 most abundant compounds were selected based on LC-MS results, as presented in Table 1.

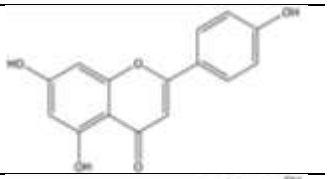
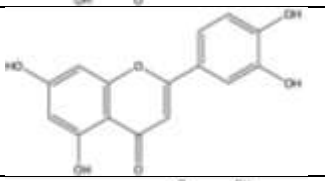
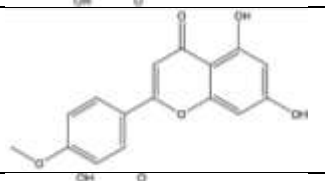
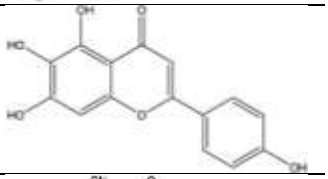
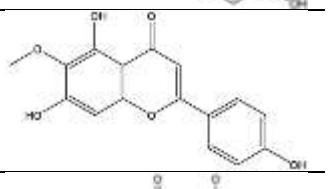
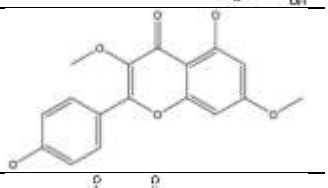
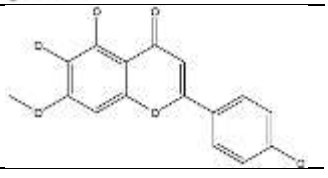
Table 1. Major compounds identified by LC-MS analysis of secondary metabolites

Compound	Composition (%)
(1R,4aS,5S,6R,7R,8S,8aR)-7-acetyloxy-8-benzoyloxy-5-[(E)-5-hydroxy-3-methylpent-3-enyl]-1,4a-dimethylspiro[3,4,5,7,8,8a-hexahydro-2H-naphthalene 6,2'-oxirane]-1-carboxylic acid	2.06
2-(4-methoxyphenyl)-7-[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-[[(2S,3R,4R,5R,6S)-3,4,5-trihydroxy-6-methyloxan-2-yl]oxymethyl]oxan-2-yl]oxychromen-4-one	2.14
[(2S,3S,4R,5R,6R)-3,5-dihydroxy-2-methyl-6-[[(2R,3S,4S,5R,6S)-3,4,5-trihydroxy-6-[5-hydroxy-2-(4 hydroxyphenyl)-4-oxochromen-7-yl]oxyoxan-2-yl]methoxy]oxan-4-yl] acetate	2.08
Pectolinaroside	2.22
[(2R,3S,4S,5R,6S)-6-[2-(3,4-dimethoxyphenyl)-5,7-dihydroxy-4-oxochromen-3-yl]oxy-3,4,5-trihydroxyoxan-2-yl]methyl (E)-3-(4-hydroxyphenyl)prop-2-enoate	2.15
[(2R,3R,4R,5S,6S)-3-acetyloxy-2-[[(2R,3S,4S,5R,6S)-6-[5,6-dihydroxy-2-(4-hydroxyphenyl)-4-oxochromen-7-yl]oxy-3,4,5-trihydroxyoxan-2-yl]methoxy]-5-hydroxy-6-methyloxan-4-yl] acetate	2.03
Procyanidin C1	2.53
Quercetin-3-glucoside-7 rhamnoside	1.85
3-O-β-D-galactopyranosyl ent-epicatechin (2 α →7,4 α→8)-epicatechin	1.91
(1S,4aR,5S,7S,8S,8aR)-7-acetyloxy-8-benzoyloxy-5-[(E)-5-hydroxy-3-methylpent-3-enyl]-1,4a-dimethyl-6 methylidene-3,4,5,7,8,8a-hexahydro-2H-naphthalene-1-carboxylic acid	1.86

Some of the compounds identified in the ethanol extract of broomstick leaves are known to have various biological activities. Therefore, a molecular docking study was conducted on flavonoid compounds in the flavone,

flavonol, and flavanone groups, which were identified as the main components in the ethanol extract of broomstick leaves suspected to have antibacterial activity. The flavonoid compounds selected as test ligands for molecular docking analysis are presented in Table 2.

Table 2. Flavonoid compounds in ethanol extract of S. dulcis L. Leaves.

Compound	RT (minute)	Structure
Apigenin	9.365	
Luteolin	10.265	
Acacetin	10.008	
Scutellarein	10.329	
Hispidulin	10.532	
Jaranol	11.434	
Ladanetin	11.033	

Molecular docking analysis was performed to assess interactions between flavonoid compounds and an antibacterial drug. Ciprofloxacin, against a target protein with PDB ID 1KZN, as shown in Figure 3. Flavonoid compounds were chosen for molecular docking analysis because they are one of the dominant groups of secondary metabolites identified in ethanol extracts of broomstick leaves. Various studies have reported that flavonoids exhibit antibacterial activity through several mechanisms, including disrupting bacterial cell membrane integrity, inhibiting nucleic acid synthesis, interfering with energy metabolism, and inhibiting the function of key bacterial enzymes such as DNA gyrase. The presence of hydroxyl and methoxy groups in the flavonoid structure also enhances interactions with amino acid residues in the active sites of target proteins, potentially boosting their inhibitory activity [19].

In this study, the protein with PDB ID 1KZN was selected as the target because it represents the crystal structure of the DNA gyrase enzyme complexed with a fluoroquinolone inhibitor. DNA gyrase enzyme was chosen as the target due to its essential role in bacterial DNA replication. This structure is considered biologically relevant because it represents the active complex among the enzyme, DNA, and inhibitor, thereby providing a more realistic depiction of the enzyme-binding mechanism.

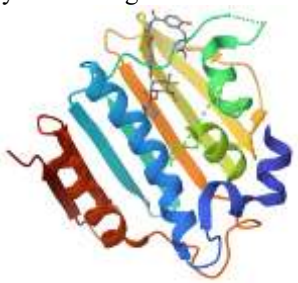


Figure 2. Structure Protein Target PDB ID 1KZN

Before molecular docking analysis was performed, a redocking procedure was conducted to assess the docking method's accuracy. Redocking refers to the process of repositioning the native ligand into the protein's active site using the coordinates from the crystal structure. The reliability of the docking method is assessed using the Root Mean Square Deviation (RMSD), with an RMSD of less than 2 Å indicating good accuracy [20]. The redocking results showed an RMSD of 1.22 Å, indicating that the docking method used in this study is accurate and suitable for further analysis.

The redocking process was performed using the same grid box coordinates as defined in the docking setup. The docking results were evaluated using binding affinity values, which represent the strength of interaction between the test ligands and the target protein. Lower binding affinity values indicate a more stable ligand-receptor complex and stronger binding interactions [21].

Furthermore, the interactions between the ligands and the receptor involve various noncovalent interactions, including hydrogen bonds, ionic interactions, van der Waals forces, and hydrophobic interactions [22]. The binding affinity data are presented in Table 3.

Table 3. Result of Molecular Docking

Compound	Binding Affinity (kcal/mol)
Ciprofloxacin (positive control)	-7,6
Apigenin	-8,5
Luteolin	-8,9
Acacetin	-8,1
Scutellarein	-8,4
Hispidulin	-8,4
Jaranol	-7,3
Ladanetin	-7,9

The molecular docking results showed that all tested flavonoid, including flavones, flavonols, and flavanones,

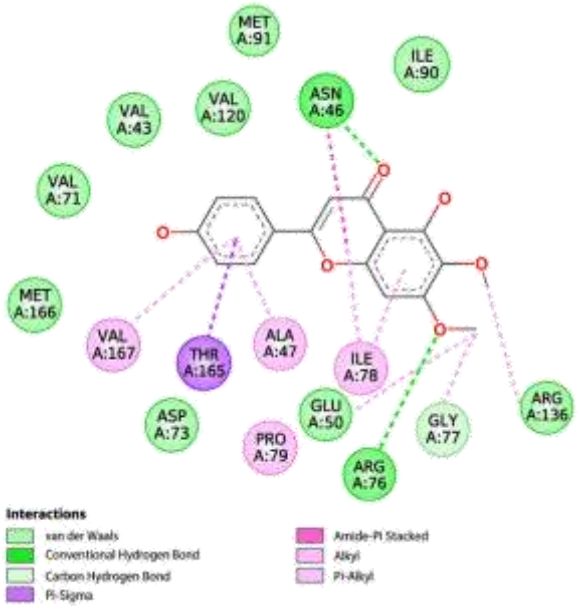
exhibited lower binding affinity values compared to the positive control, ciprofloxacin (-7.6 kcal/mol). Among the tested compounds, luteolin demonstrated the lowest binding affinity (-8.9 kcal/mol), followed by apigenin (-8.5 kcal/mol), scutellarein (-8.4 kcal/mol), hispidulin (-8.4 kcal/mol), and acacetin (-8.1 kcal/mol). These results indicate that flavonoid compounds potentially have antibacterial activity. Various studies report that flavonoids can inhibit bacterial growth through several mechanisms, including disrupting cell membrane integrity, inhibiting nucleic acid synthesis, and blocking the activity of essential enzymes such as DNA gyrase [23].

The results of this study are in line with previous research reporting that luteolin has strong inhibitory activity against the bacterial DNA gyrase protein. Luteolin's low binding affinity indicates that it can interact strongly with amino acid residues at the target protein's active site. These findings strengthen the potential of luteolin as a candidate DNA gyrase inhibitor for development as an antibacterial agent [24].

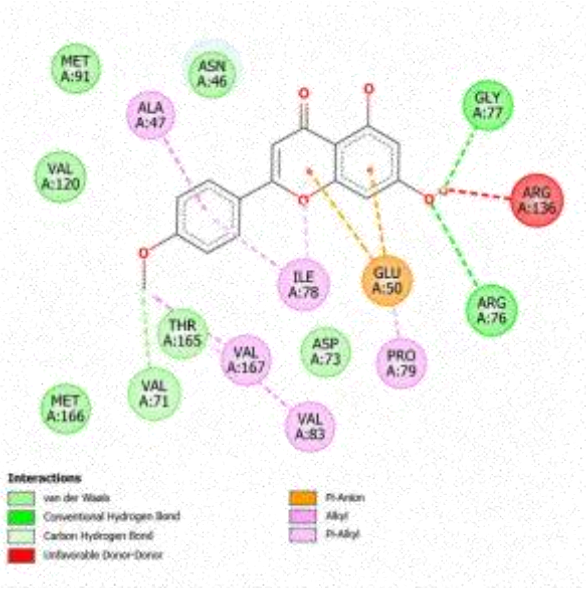
Luteolin shows the strongest binding affinity compared to other flavonoids. This is suspected to be related to the presence of four hydroxyl groups in luteolin's structure, which allows for the formation of more hydrogen bonds with amino acid residues on the active site of DNA gyrase. Previous studies have shown that the hydroxyl groups on flavonoids play an important role in forming hydrogen bonds and affect inhibitory activity against DNA gyrase. In addition, variations in the number and position of hydroxyl or methoxy groups in the flavonoid structure can influence the strength of interaction with target proteins and the resulting biological activity [25].

However, these results remain predictive in silico and require further analysis to better understand the interactions between ligands an the target protein. Therefore, a ligand-protein interaction visualization analysis was performed to identify the types of interactions formed between the compounds and amino acid residues. The interactions between flavonoid compounds and amino acid residues were analysed using BIOVIA Discovery Studio Visualizer 2025, as illustrated in Figure 4.

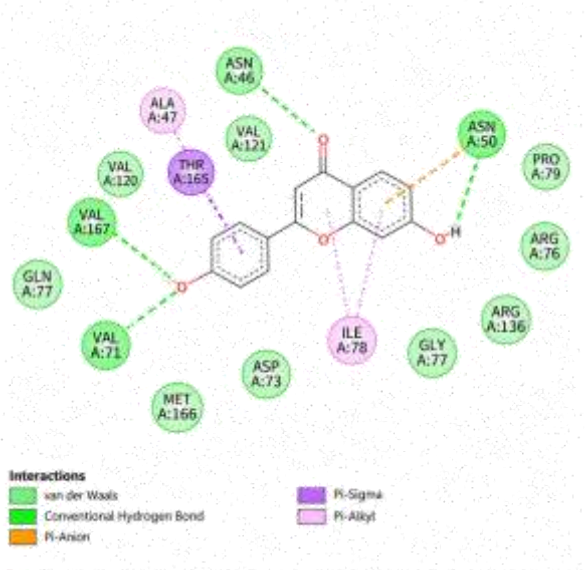
a) Ladanetin



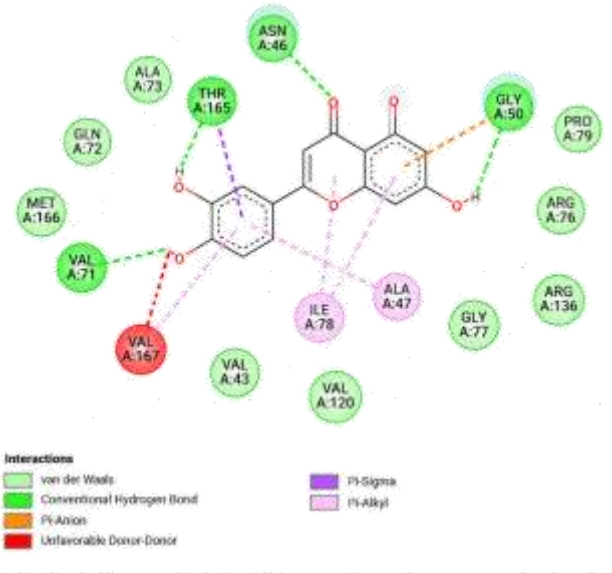
b) Acacetin



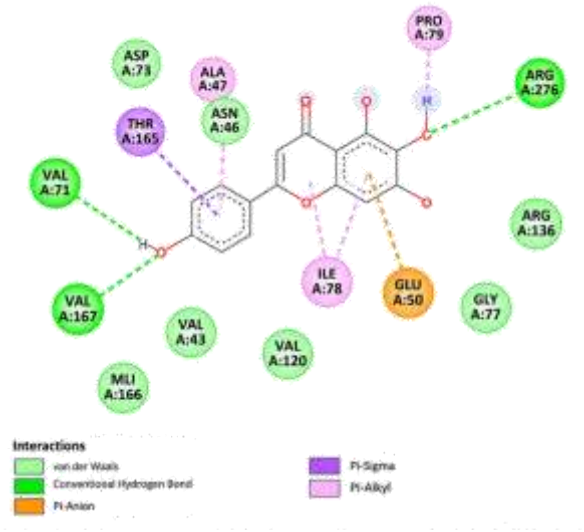
c) Apigenin



d) Luteolin



e) Hispidulin



f) Scutellarein

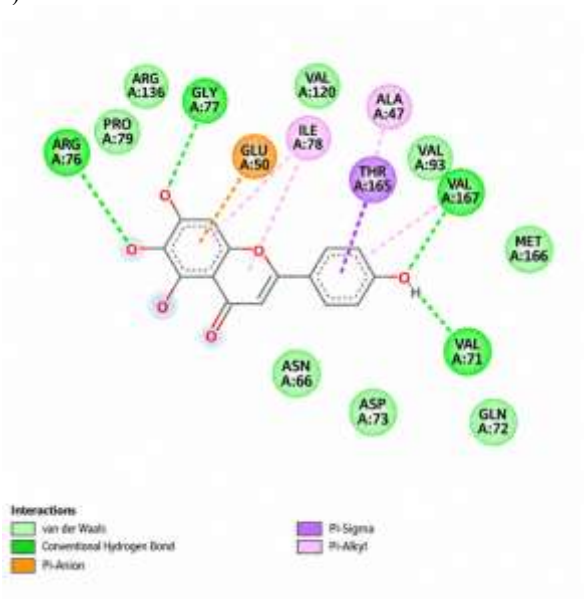


Figure 4. Visualization of the interactions between the flavonoid compounds and the target protein (1KZN)

The interactions between ligands and the DNA gyrase enzyme involve various types of interactions, including hydrogen bonds, π-alkyl, π-sigma, π-anion, unfavorable interactions and van der Waals forces. The analysis indicates that hydrogen bonding plays a crucial role in stabilizing the ligand-protein complex. Compounds such as luteolin and apigenin form hydrogen bonds with amino acid residues at the enzyme's active site, including GLY, ASN, and ARG. These interactions suggest that the ligands can bind to functionally relevant regions, potentially interfering with DNA gyrase activity.

π-interactions, including π-alkyl, π-sigma, π-anion interactions, also contribute to enhancing the stability of the ligand-protein complex. These interactions generally occur between the aromatic rings of flavonoids and amino acid residues such as GLU and ARG. In addition, hydrophobic interactions involving residues such as VAL, ILE, and PRO help maintain ligand stability within the active site through non polar interactions.

However, some compounds, such as acacetin, exhibit unfavorable interactions, specifically donor-donor interactions with ARG residues. These interactions may cause electrostatic repulsion, which can potentially reduce the stability of the ligand-protein complex. Compounds that form more hydrogen bonds, such as luteolin, tend to exhibit greater complex stability, consistent with their lower binding affinity values. Overall, the visualization results indicate that flavonoid compounds have the potential to act as inhibitors of the DNA gyrase enzyme through the formation of stable interactions at the protein's active site. This mechanism is suggested to inhibit bacterial DNA synthesis, thereby contributing to the antibacterial potential of these compounds against E. coli.

After visualization analysis of ligand-DNA gyrase interactions, further evaluation is required to assess the suitability of these compounds as drug candidates. This evaluation was conducted using Lipinski's Rule of Five, which includes parameters such as molecular weight (≤500 Da), partition coefficient (log P ≤5), number of hydrogen bond donors (≤5), and number of hydrogen bond acceptors (≤10). Compounds that meet these criteria are considered not only to have good interaction potential based on docking results but also to possess physicochemical properties that support their drug-likeness and potential bioavailability. The results of the drug-likeness analysis are presented in **Table 4**.

Table 4. Lipinski's Rule of Five druglikeness analysis

Compound	MW[a]	Log P[b]	HBD[c]	HBA[d]
Apigenin	270,24	2,11	3	5
Luteolin	286,24	1,73	4	6
Acacetin	284,26	2,56	2	5
Scutellarein	286,24	1,81	4	6
Hispidulin	300,26	2,12	3	6
Ladanetin	300,26	2,11	3	6

[a] Molecular Weight, [b] Log P, [c] Hydrogen Bond Donor, [d] Hydrogen Bond Acceptor.

Based on the drug-likeness analysis, the compounds with potential antibacterial activity fall within the acceptable range defined by Lipinski's Rule of Five. The molecular weight of the compounds is below 500 Da, the log P is less than 5, the number of hydrogen bond donors is fewer than 5, and the number of hydrogen bond acceptors is fewer than 10. These findings indicate that the compounds have the potential to penetrate biological membranes and are likely to be readily absorbed in the body [26].

This study has several limitations that need to be considered. Molecular docking analysis is a predictive computational approach, so the results obtained cannot fully represent actual biological conditions. The binding affinity values and ligand-protein interaction patterns generated reflect only potential interactions under simulated conditions and do not account for other biological factors, such as absorption, distribution, metabolism, excretion, and compound toxicity in a biological system. In addition, this study has not been complemented with experimental validation through in vitro or in vivo testing to confirm the antibacterial activity of flavonoid compounds against *Escherichia coli*. Therefore, further research is needed to

validate the molecular docking predictions and evaluate the biological efficacy of the identified compounds.

Conclusion

Based on the in silico analysis, flavonoid compounds in the ethanol extract of *S. dulcis* L. Leaves exhibit potential as antibacterial agents against *E. coli,* as indicated by lower binding affinity values than the positive control, ciprofloxacin, with luteolin identified as the most active compound. Visualization analysis revealed that the stability of the ligand-protein complex is influenced by hydrogen bonds, π-interactions, and hydrophobic interactions at the active site of the DNA gyrase enzyme. Furthermore, all compounds satisfied Lipinski's Rule of Five, suggesting good potential for bioavailability as drug candidates. However, further studies are required to confirm their biological activity. These findings provide a scientific basis for developing flavonoid-based antibacterial agents and support the use of lemongrass leaves as a source of bioactive compounds for pharmaceutical preparations. Further research is needed through in vitro and in vivo testing, toxicity evaluation, pharmacokinetic studies, and testing against antibiotic-resistant bacterial strains to confirm the effectiveness and safety of the identified compounds.

Author's Contribution

H.Z. Firdausi: conducted the research, including data collection, analysis, and preparation of the manuscript draft. Tukiran: provided supervision, guidance, and evaluation throughout the research process, as well as reviewed and revised the manuscript.

Acknowledgements

The author would like to thank the laboratory and all parties who have provided support, assistance, and facilities during this research. The authors also express their gratitude to Unesa for the opportunity and facilities provided to conduct this study.

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