Trop J Nat Prod Res, October 2025; 9(10): 5042 - 5050 **ISSN 2616-0684 (Print)**
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Metabolites Profiling and Assessment of Antibacterial Potential of *Sauropus androgynus* Extracts Through *in Vitro* and *in Silico* Methods

Nurina R. Ramadhania[1]*, Sapti Puspitarini[2], First A. Wati[1]

[1]*Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Negeri Surabaya, Jl. Ketintang Gayungan, Surabaya 60231, East Java, Indonesia*
[2]*Department of Science Education, Faculty of Mathematics and Natural Sciences, Universitas Negeri Surabaya, Jl. Ketintang Gayungan, Surabaya 60231, East Java, Indonesia*

ARTICLE INFO

Article history:
Received 24 August 2025
Revised 15 September 2025
Accepted 22 September 2025
Published online 01 November 2025

ABSTRACT

Sauropus androgynus is generally known as a cultivated plant with diverse pharmacological properties due to its various active chemical compounds. However, scientific reports on the phytochemical content and bioactivity screening of *S. androgynus* extracts are still limited. This study aims to determine the diversity of compounds in various *S. androgynus* leaf extracts, as well as their antibacterial activity. The extraction process uses solvents with different polarities (methanol, ethyl acetate, and *n*-hexane) through the maceration method. The metabolites profile of the extracts was performed through comprehensive descriptive-quantitative liquid chromatography-mass spectrometry (LC-MS). Evaluation of antibacterial activity using microdilution test was carried out on *Escherichia coli* (ATCC 11229), *Staphylococcus aureus* (ATCC 6538), and *Salmonella typhimurium* (ATCC 14028) to obtain the Minimum Inhibitory Concentration (MIC), followed by *in silico* molecular docking of selected compounds with bacterial proteins. The results of LC-MS analysis showed that there were 187 metabolites in various of *S. androgynus* leaf extracts. These compounds included phenolics, flavonoids, polyphenols, terpenoids, alkaloids, saccharides, and glycosides. The polarity of the extraction solvent was found to influence the diversity and abundance of the extracted compounds. Although, all extracts showed weak antibacterial activity *in vitro*, molecular docking analysis showed that the compounds chlorogenic acid and hypophyllanthin had strong binding affinity to bacteria target proteins in *E. coli*. These findings demonstrate the importance of selecting the solvent used in extracting bioactive compounds and support the potential of *S. androgynus* as a source of natural antibacterial drug candidates.

Keywords: Antibacterial, Metabolites, Molecular Docking, *Sauropus androgynus*.

Introduction

The potential of biodiversity in Indonesia can be harnessed in the development of herbal medicines based on indegenous tropical plants. Various ethnomedicines have been discovered from the search for sources of promising bioactive compounds. The urgency in combating bacteria resistance, particularly in individuals with impaired immune system, has intensified the search for diversity of bioactive compounds from plant extracts. Therefore, many discoveries of therapeutically effective natural drugs are considered as a valuable source of antibacterial agents.[1] Compared to synthetic antibiotics, natural products are seen as a viable alternative due to their chemical diversity, availability, and fewer side effects.[2] Bioactive compounds from plants, such as *Sauropus androgynus* have an important role in inhibiting microbial growth and their mechanisms of action include disruption of bacteria membranes, suppression of enzyme activity, and interference with protein synthesis.

*Corresponding author.Email: nurinaramadhania@unesa.ac.id
Tel: +6283849678290

Citation: Ramadhania NR, Puspitarini S, Wati FA. Metabolites Profiling and Assessment of Antibacterial Potential of *Sauropus androgynus* Extracts Through *in Vitro* and *in Silico* Methods. Trop J Nat Prod Res. 2025; 9(10): 5042 – 5050 https://doi.org/10.26538/tjnpr/v9i10.46

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

S. androgynus is traditionally used across Southeast Asia to treat fever, allergies, earache, urinary tract infections, diabetes, and cancer.[3] Phytochemical analysis of *S. androgynus* extract has identified the presence of alkaloids, flavonoids, phenols, and tannin compounds, which are characterized by various pharmacological properties.[3] However, studies investigating the comprehensive diversity of metabolites and correlation with antibacterial activity remain limited. To close this gap, advanced analytical methods such as liquid chromatography–mass spectrometry (LC-MS) have been used for profiling secondary metabolites to explore metabolites more efficiently.[4] This method provides high sensitivity and resolution with simple sample preparation that requires a short analysis time. LC-MS enhances the identification of secondary metabolites, including non-volatile compounds,[5,6] serving as an initial step to provide information in predicting active components with potential pharmacological activity.[7]

In this study, metabolite profiling and selection of effective extraction methods were employed as critical steps in identifying compounds with potential pharmacological applications. The use of different solvent polarities is expected to provide coverage of secondary metabolites in *S. androgynus* to ensure a comprehensive metabolite profiling and accurate evaluation of antibacterial potential.[8,9] By combining ethnobotanical knowledge with modern analytical instruments, *in vitro* antibacterial assays, and *in silico* methods, it is expected that the discovery of plant-based therapies can be accelerated. *In silico* molecular docking also provides a deeper understanding of how bioactive molecules interact with bacterial target proteins at the molecular level. The comprehensive method can improve the screening process, allowing the identification of key compounds for further development as plant-based antibacterial agents. The strategy supports

the use of Indonesia rich plant biodiversity and contributes to global efforts in developing sustainable alternative medicines.
Therefore, this study aimed to determine the metabolite profile and antibacterial activity of *S. androgynus* leaf extract obtained using solvents of different polarities, supported by LC-MS, *in vitro* assays, and *in silico* analysis. This is the first comprehensive study to combine LC-MS profiling, antibacterial assays, and molecular docking studies to elucidate the antibacterial potential of *S. androgynus*, demonstrating its novelty and relevance for the future development of natural antibacterial agents.

Materials and Methods

Plant collection and identification
S. androgynus leaf samples (2 kg) from Merapi Farma Herbal located in Hargobinangun, Sleman, Yogyakarta, Indonesia (7°40'11"S 110°24'30"E) were collected in March 2024. The Identification and authentication of the plant were carried out by Dr. apt. Marisca G. Evalina of the Department of Pharmacy, Surabaya University, Indonesia where a voucher: 1636/D.T/VIII/2025 was assigned.

Extraction of plant material
The fresh leaf samples were washed and dried, and then ground into a fine powder. The powdered plant (350 g each) was macerated separately with methanol, ethyl acetate, and *n*-hexane (3 L each) for 72 hours at room temperature. The extracts was then concentrated using a rotary vacuum evaporator (BUCHI Rotavapor R-215) at temperature of 40-50°C, 80 mbar, 60 rpm, until the solvents were completely evaporated.

Metabolite profiling
Metabolite profiling of the plant extracts was performed qualitatively using Shimadzu LC-MS - 8040. Sample (1 µL) was injected into a Shim Pack FC-ODS column (2 mm × 150 mm particle size, 3 µm) using isocratic elution at 35°C with mobile phase (flow rate 0.5 mL/min). The analysis was performed in positive ion mode with a source maintained of 100°C, desolvation temperature of 350°C, and desolvation gas flow rate of 60 mL/h. The mass spectra were detected in ESI positive ionization mode between 10 – 1000 m/z with a scan duration of 80 minutes (0.6 seconds/scan). Quantitative analysis was calculated based on the peak area of the identified compounds.[10]

Antibacterial assay
The microdilution assay was employed to determine the antibacterial effects of the plant extracts on *Escherichia coli* (ATCC 11229), *Staphylococcus aureus* (ATCC 6538), and *Salmonella typhimurium* (ATCC 14028). The concentration of extracts ranged from 500 to 0.24 mg/mL, with bacteria suspensions prepared at 5 x 10^5 CFU/mL. Chloramphenicol (50 – 0.024 ppm for *E. coli*, and 5 – 0.003 ppm for *S. aureus* and *S. typhimurium*) was employed as the positive control, while 2% DMSO served as the negative control. The minimum Inhibitory Concentration (MIC) was measured using a 96-well microplate reader through gradual dilution to obtain concentration variations. Liquid media (100 µL) was put into the microplate, followed by the addition of bacterial suspension (10 µL) to the sample, then incubated for 16 – 20 hours at 37°C. This method was performed in duplicate and MIC values were obtained as the lowest concentration that did not show visible bacteria growth (clear solution).

Druglikeness and ADMET prediction
Druglikeness prediction was performed using the web-based ADME (Absorption, Distribution, Metabolism, and Excretion) method, following Lipinski's Rule of Five. The SMILES codes of compounds obtained from PubChem (National Center for Biotechnology Information, Bethesda, USA, release 2023) were used for druglikeness screening through SwissADME web server (Swiss Institute of Bioinformatics, Switzerland, release 2017), as previously described by Riyadi *et al*. (2021).[11] ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) prediction were performed to analyze the physicochemical and pharmacokinetic properties of candidate drug compounds. The pkCSM web tool (University of Cambridge, UK, release 2015) was used by inputting the SMILES codes of compounds

Molecular docking study
In silico molecular docking analysis was conducted to evaluate five selected compounds identified in the plant extracts, namely; caffeic acid (**4**), 1-Acetoxychavicol acetate (**8**), chlorogenic acid (**17**), phyllanthin (**23**), and hypophyllanthin (**24**). The selection of these compounds was based on their relative abundance in LC-MS metabolite profile, reported pharmacological relevance in the literature, and compliance with druglikeness parameters predicted by SwissADME. Docking targets included *E. coli* ATCC 11229 DNA Gyrase B (PDB: 5L3J), *S. typhimurium* ATCC 14028 RamR protein (PDB: 6IE8), and *S. aureus* ATCC 6538 DNA Gyrase (PDB: 4URO), which were downloaded from the Protein Data Bank (Research Collaboratory for Structural Bioinformatics, New Brunswick, USA). Prepared receptors and ligands were subjected to molecular docking using AutoDockTools-1.5.7 (The Scripps Research Institute, USA, release 2019). The 2D structures were drawn with MarvinSketch version 24.1.2 (ChemAxon, Budapest, Hungary, release 2024).[12-13]
The structure was optimized using the MMFF94 method and docking pose validation was assessed by Root Mean Square Deviation (RMSD). The optimization was conducted to show reliable docking accuracy with an acceptable value <2Å. Redocking experiments were performed by adding the native ligand back to the active site of the corresponding receptor using AutoDockTools-1.5.7, with grid boxes and grid centers defined for each receptor. Comparison of binding energy with control (amoxicillin) was used to assess the strength of interaction. When the binding energy was lower compared to the control it indicated a stronger interaction. Subsequently, ligand-receptor interactions were presented using BIOVIA Discovery Studio Visualizer 2024 Client.

Statistical analysis
Each antibacterial assay was conducted in duplicate (n = 2). Quantitative results (MIC values) were expressed as the mean ± standard deviation (SD). To compare MICs between extracts and positive control for each bacterial strain, data analysis was performed using a descriptive method based on turbidity observations, in accordance with CLSI/NCCLS standards. For *in silico* docking, results were reported descriptively as binding energies and interaction profiles; protocol validation was performed by redocking the native ligand with acceptable poses defined as those having an RMSD ≤ 2Å.

Results and Discussion

Solvent extraction yields
Extraction was carried out for a comprehensive exploration of compounds in of *S. androgynus* leaves, and to make a comparisons across different solvent extracts. *S. androgynus* leaves were performed via single-solvent maceration, employing solvents of increasing polarity: methanol, ethyl acetate, and *n*-hexane. The highest crude extract yield was obtained with methanol (19.93%), followed by ethyl acetate (8.13%) and *n*-hexane (1.36%). The concentrated extract obtained was dark in colour, odourless, and bitter. The results showed that methanol extract produced the highest yield due to its high polarity.[14] Methanol solvent is very effective in extracting both polar and nonpolar secondary metabolites compared to nonpolar solvents.[15-16]

Metabolites profile of S. androgynus extracts
The identification of active compounds in plants provides concrete scientific evidence of their potential as drug candidates and supports their application as antibacterial agents, particularly in the case of *S. androgynus* extract. A total of 187 metabolites with a similarity index of 92% to the database were identified from the LC-MS analysis of *S. androgynus* leaf extracts (Figure 1). LC-MS has been used to effectively identify complex and diverse plant extract with the influence of solvents of varying polarities.[17] The compounds identified from *S. androgynus* leaf extracts included flavonoids, polyphenols, phenolics, terpenoids, alkaloids, aromatic benzenes, saccharides, carboxylic acid derivatives,

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

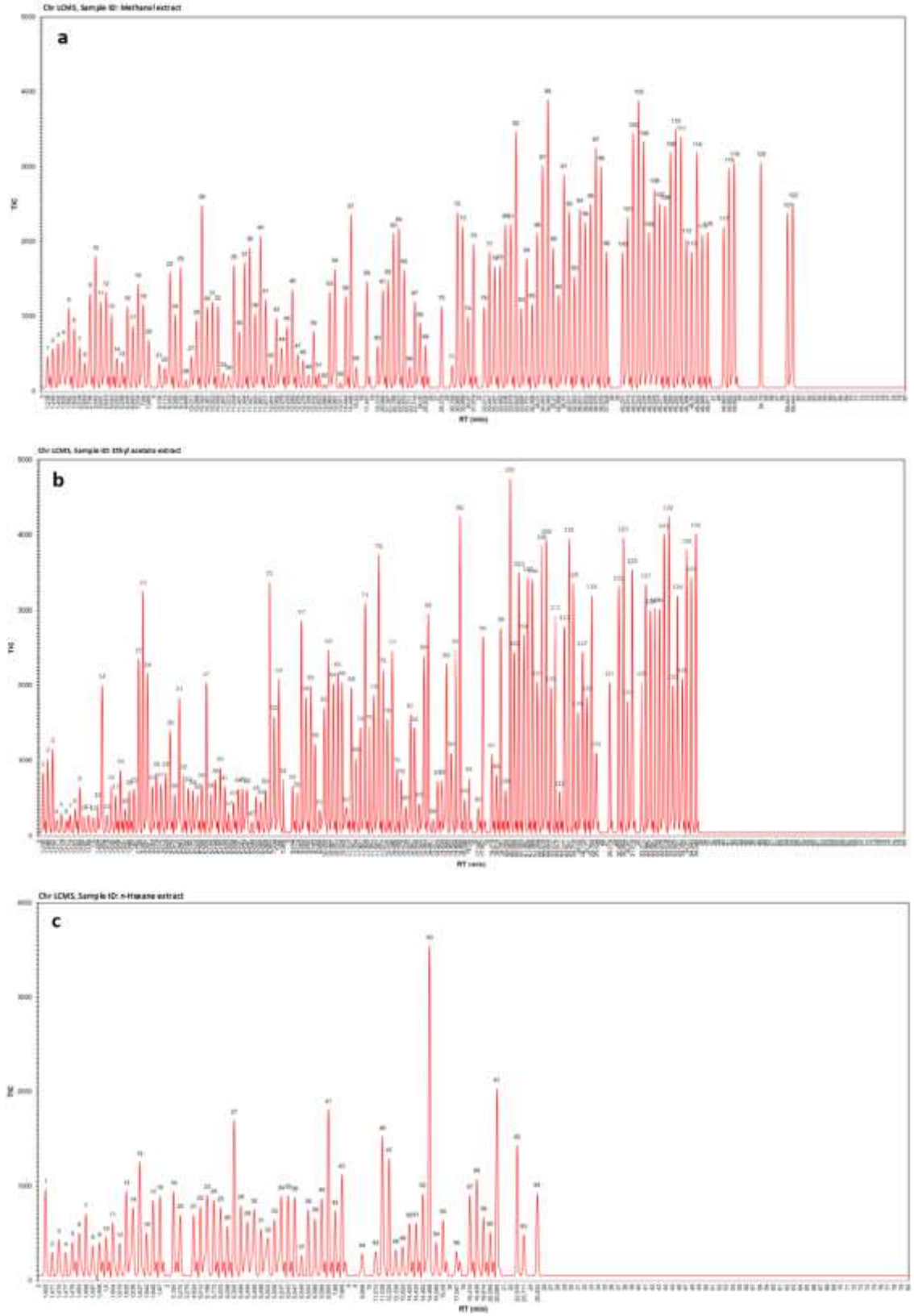

Figure 1: LC-MS chromatogram of *S. androgynus* leaf extracts (a) methanol extract, (b) ethyl acetate extract, (c) *n*-hexane extract

and glycosides, indicating the rich chemical diversity of *S. androgynus* influenced by solvent polarity.

Compound abundance was determined from the relative percentage of each identified metabolite. LC-MS analysis of *S. androgynus* leaf extracts indicated that metabolite solubility and yield were greatly affected by the polarity of the solvent used. As shown in Figure 2, increasing solvent polarity could decrease the percentage of terpenoid content, which is consistent with previous reports that showed that *n*-hexane extract was rich in terpenoids (53.42%).[18-20] This is because *n*-hexane extracts fewer phenolics due to low polarity and predominantly

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

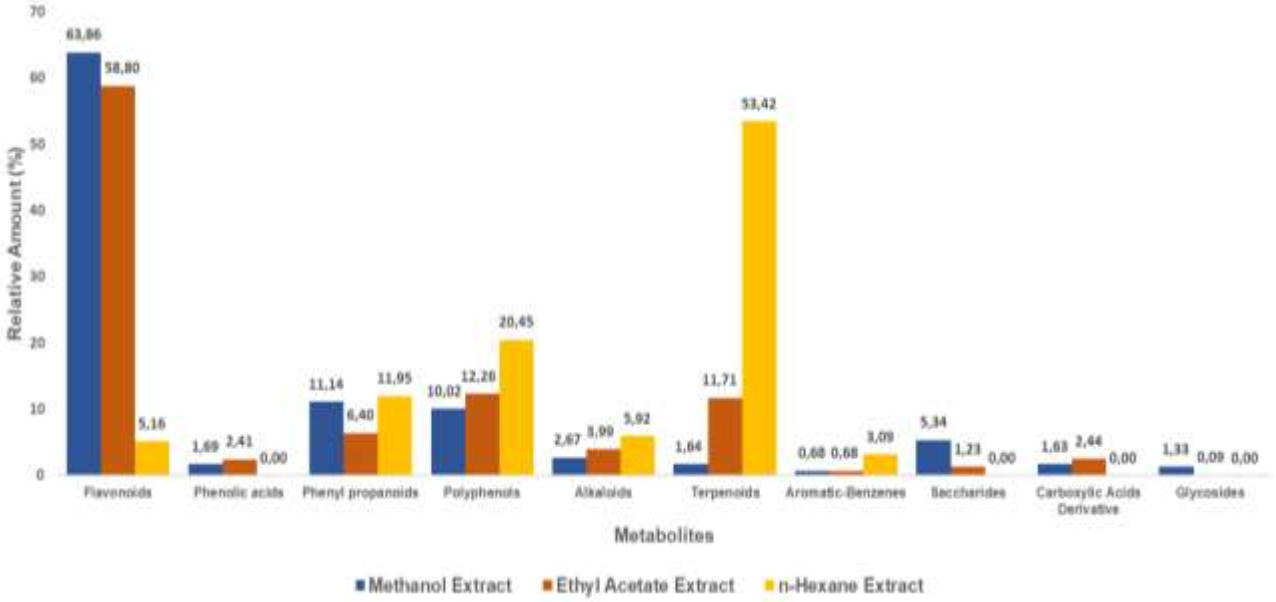

Figure 2: Metabolites profile of *S. androgynus* leaf extracts

extract non-polar terpenoids.[21] The major compounds identified in *S. androgynus* leaf extracts are shown in Table 1. In this study, ethyl acetate demonstrated the highest efficiency as a solvent, extracting a broad spectrum of metabolites ranging from polar to non-polar (73.79%), including phenolics, flavonoids, alkaloids and terpenoids. Among all extracts, flavonoids and phenolic acids were the most prevalent compounds. Additionally, coumarin and chromone groups were identified in the ethyl acetate, *n*-hexane and methanol extracts. This information supports the importance of the influence of the use of solvent variations in identifying the metabolite content of *S. androgynus*.[6,22-25]

Table 1: Major compounds identified from the LC-MS analysis of *S. androgynus* leaf extracts

No.	RT (min)	m/z	Compounds	Relative Amount (%)		
				MeOH*	EA*	HX*
1	1.64	154.14	Linalool	-	0.38	2.47
2	1.65	154.14	α-Terpineol	-	0.26	1.67
3	3.04	170.02	Gallic acid	0.94	1.40	-
4	4.64	180.04	Caffeic acid	0.69	0.60	-
5	5.31	217.11	Virosecurinine	0.59	0.88	3.34
6	6.88	220.18	Spathulenol	0.45	1.45	3.56
7	7.66	233.11	Phyllantidine	0.60	0.90	1.45
8	7.97	234.09	1-Acetoxychavicol acetate	0.35	0.32	2.21
9	9.37	270.05	Apigenin	0.53	0.79	-
10	9.73	272.07	Naringenin	0.86	0.86	-
11	10.27	286.05	Luteolin	0.49	0.73	-
12	10.32	286.05	Kaempferol	1.29	1.07	-
13	10.36	288.06	Dihydrokaempferol	0.58	0.87	-
14	11.04	302.22	8(17),12-labdadiene-15,16-dial	0.87	0.44	3.00
15	11.43	302.04	Quercetin	1.00	0.63	-
16	11.50	306.07	Epigallocatechin	0.54	0.80	-
17	12.42	354.10	Chlorogenic acid	-	0.70	-
18	12.23	400.19	5-[(1S,2R,3R)-6,7-Dimethoxy-2,3-bis (methoxymethyl)-1,2,3,4-tetra hydro naphthalen-1-yl]-1,3-benzodioxole	0.45	0.67	2.54
19	12.34	344.16	5-Hydroxy-7-(4-hydroxyphenyl)-1-(4-hydroxy-3-methoxy phenyl)-3-heptanone	0.11	0.17	0.64

20	12.43	354.11	Hinokinin	0.41	0.62	-
21	12.62	356.16	[1,7-Bis(4-hydroxy-3-methoxyphenyl) hepten-3-one]	0.12	0.19	0.71
22	14.49	418.24	4-[(2*R*,3*R*)-3-[(3,4-Dimethoxyphenyl) methyl]-4-methoxy-2-(methoxy methyl) butyl]-1,2-dimethoxybenzene	1.23	1.83	6.97
23	17.46	418.24	Phyllanthin	0.76	1.14	-
24	19.74	430.20	Hypophyllanthin	-	1.19	-
25	20.06	432.11	Kaempferol-4'-rhamnoside	-	2.05	-
26	20.09	432.11	Apigenin-7-*O*-glucoside	-	1.51	-
27	21.39	432.11	Vitexin	0.77	1.15	-
28	21.43	432.11	Kaempferol-3-*O*-rhamnoside	-	1.48	-
29	23.58	458.19	[3-(Acetyloxymethyl)-2-(1,3-benzo dioxol-5-yl methyl)-4-(3,4-dimethoxyphenyl) butyl] acetate	0.84	1.26	2.82
30	24.00	463.09	Quercetin-3-glucoside	-	1.70	-
31	24.02	464.10	Isoquercitrin	-	1.45	-
32	25.93	490.22	3,5-Diacetoxy-1-(4-hydroxy-3,5-dimethoxy phenyl)-7-(4-hydroxy-3-methoxyphenyl) heptane	0.32	0.48	1.81
33	30.87	534.10	Kaempferol-3-*O*-(6"-malonylglucoside)	1.24	1.43	-
34	30.87	534.10	Luteolin-7-*O*-(6"-malonylglucoside)	1.14	1.71	-
35	35.52	610.15	Rutin	1.24	-	-

*: MeOH = Methanol; EA = Ethyl Acetate; HX = *n*-Hexane.

Antibacterial activity of S. androgynus leaf extracts

As presented in Table 2, the antibacterial activity of *S. androgynus* leaf extracts was determined by their MIC values against *E. coli*, *S. typimurium*, and *S. aureus*. MIC indicates the lowest concentration of each extract that have antibacterial activity. Methanol extract was the most effective against *S. aureus* and ethyl acetate extract showed similar efficacy against *E. coli*. Methanol and ethyl acetate extract showed broad-spectrum bacteriostatic activity, effectively inhibiting the growth of three bacterial strains, while *n*-hexane extract had no inhibitory effect. However, the antibacterial activity of all extracts remained relatively weak when compared with chloramphenicol, which served as the positive control. Chloramphenicol act as a bacteriostatic agent against both Gram-positive and Gram-negative bacteria, providing broad-spectrum activity. In this study, 2% DMSO was used as negative control since it does not inhibit bacterial growth.

Antibacterial activity can be influenced by bacteria type, diffusion ability, extract concentration, and bioactive compound such as alkaloids, flavonoids, phenolics, and terpenoids contents. Flavonoids such as apigenin (**9**), naringenin (**10**), luteolin (**11**), kaempferol (**12**), and quercetin (**15**) have shown inhibitory effects against both Gram-positive and Gram-negative bacteria, as well as anti-inflammatory properties.[26-29] Compound **12** (Kaempferol) exhibited activity against *S. aureus*, which was likely due to the hydroxyl group on its ring structure and the double bond adjacent to the carbonyl and hydroxyl groups. This indicate that the balance of hydrophilic/lipophilic groups is crucial for antibacterial activity.[26] Gallic acid (**3**) showed its activity against against *P. aeruginosa* and *E. coli*,[30] while caffeic acid (**4**) and chlorogenic acid (**17**) are also known for their potential as antibacterials.[31-32] 1-Acetoxychavicol acetate (ACA) (**8**) found in all three extracts have been shown to inhibit bacterial enzymes, penetrate bacterial membranes and disrupt cellular functions against methicillin-resistant Staphylococcus aureus (MRSA) with an MIC of 0.313 mg/mL.[33-34] Hinokinin (**20**) has also showed strong activity against *S. aureus*.[35] Lignan compounds such as phyllanthin (**23**) and hypophyllanthin (**24**) can inhibit the growth of *Salmonella*, while the alkaloid virosecurinine (**5**) and its isomer viroallosecurinine show bactericidal effects against several pathogens.[36-37] They also exhibit pharmacological activities, such as antioxidant, anticancer, and anti-inflammatory effects.[38]

Table 2: Antibacterial Activity of *S. androgynus* leaf extracts

Sample	**MIC (mg/mL)**		
	***E. coli* ATCC 11229**	***S. typimurium* ATCC 14028**	***S. aureus* ATCC 6538**
Methanol Extract	500.00 ± 2.50	125.00 ± 1.20	31.25 ± 0.90
Ethyl Acetate Extract	31.25 ± 0.30	62.50 ± 0.40	62.50 ± 0.50
n-Hexane Extract	-	-	-
Chloramphenicol	1.56 ± 0.06*	1.25 ± 0.04*	1.25 ± 0.03*

*: MIC in parts per million (ppm).

Molecular docking results

Molecular docking analysis is essential for screening drug compounds by measuring druglikeness properties to assess whether compounds have the right characteristics to be considered a potential drug candidate. In this study, based on Lipinski's Rule of Five, drug similarity prediction using SwissADME was performed for compounds **4 (**caffeic acid), **8 (**1-Acetoxychavicol acetate), **17 (**chlorogenic acid), **23 (**phyllanthin), and **24 (**hypophyllanthin)**,** and amoxicillin as the control (Figure 3). These five compounds were specifically selected, representing major and pharmacologically relevant metabolites detected in *S. androgynus* leaves, and showed potential for oral bioavailability. All the compounds met the criteria, except compound **17** which exceeded the acceptable H-bond donor limit based on Lipinski's rule.[39]

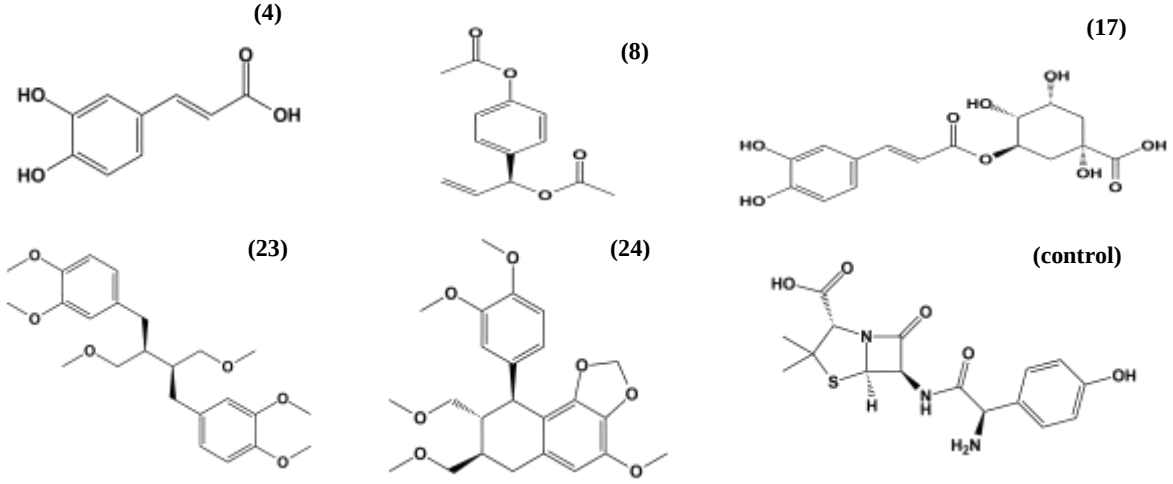

Figure 3: The 2D structures of caffeic acid (**4**), 1-Acetoxychavicol acetate (ACA) (**8**), chlorogenic acid (**17**), phyllanthin (**23**), hypophyllanthin (**24**) and amoxicillin (control), retrieved from Pubchem database

ADMET analysis using pkCSM was conducted to investigate absorption, distribution, metabolism, excretion, and toxicity profiles. This prediction helps in analyzing the physicochemical and pharmacokinetic properties of candidate drug compounds while ensuring low levels of toxicity. Molecules with higher molecular weights tend to have more complex structures, which can affect their pharmacokinetic properties. Most of the compounds investigated in this study showed good water solubility at 25°C, intestinal absorption, and moderate Caco-2 permeability (>0.90). Furthermore, distribution properties prediction was performed to categorize the compounds in various tissues and organs after entering systemic circulation. The results showed that compound **17** had high tissue distribution (VDss), while compounds **4** and **8** showed moderate permeability across the central nervous system (CNS) and blood-brain barrier (BBB).

Metabolism properties describe the biochemical transformation of compounds in the body. The parameters used are CYP2D6 and CYP3A4 inhibitors/substrates. CYP2D6 (Cytochrome P450 2D6) is a key enzyme in the metabolism of compounds, particularly in oxidizing lipophilic compounds. Furthermore, CYP2D6 substrates are metabolized by this enzyme, suggesting that the activity can change when combined with inhibitors. CYP2D6 inhibitors have the potential to slow substrate metabolism, increasing compound levels in the blood and increasing the risk of side effects or toxicity. CYP3A4 is the main enzyme in the Cytochrome P450 system, which is responsible for metabolizing more than 50% of compounds in the liver and intestines. CYP3A4 substrates are drugs broken down or activated by this enzyme. CYP3A4 inhibitors can increase the levels of CYP3A4 substrates in the blood, potentially leading to overdose or toxic side effects. In this study, none of the compounds were CYP2D6 substrates/inhibitors. However, compounds **23** and **24** acted as CYP3A4 substrates/inhibitors, which could slow drug metabolism, increase plasma concentration, and increase the risk of side effects.

Excretion analysis can evaluate how quickly compounds are eliminated from the body, which affects the duration of action and toxicity risk. In this study, compounds **4**, **8**, and **23** showed high clearance rates, leading to rapid elimination. On the other hand, compounds **17** and **24** could persist longer in the body and showed potential for increased toxicity. However, none of the compounds could be substrates for OCT2 transporter, suggesting their renal excretion was independent of OCT2. Toxicity analysis showed that all compounds were safe for AMES, non-hepatotoxic, and not inhibitors of hERG I, indicating low mutagenic, liver, and cardiac arrhythmia risks. However, compound **23** acted as an inhibitor of hERG II, which could require further cardiac safety evaluation. Compounds **17** and **24** had low maximum tolerated doses, indicating higher toxicity potential at lower concentrations.

In silico molecular docking was conducted to predict the binding affinity and molecular interaction between the selected compounds and bacterial target proteins. The docking protocol was validated by redocking the native ligands into the prepared receptors, while determining the grid box and center for each receptor. This method was considered valid when RMSD value did not exceed 2Å, as shown in Table 3. Subsequently, all compounds were docked with the three receptors; *E. coli* (DNA Gyrase B), *S. typhimurium* (RamR), and *S. aureus* (DNA Gyrase) to analyze the lowest binding affinity and determine *in silico* activity. Based on binding affinity values in Table 4, compounds **17** (-7.07 kcal/mol) and **24** (-7.55 kcal/mol) showed stronger affinity than amoxicillin (-7.05 kcal/mol) against *E. coli*, indicating antibacterial potential. However, no significant binding was observed for other bacteria. Visualization was performed to identify the binding sites and interaction types between the receptor and the test compounds, comparing with the control compound. Furthermore, visualization through BIOVIA Discovery Studio Visualizer showed that compound **17** formed multiple interactions with active residues (HIS55, ILE94, THR165, and VAL167), while compound **24** showed Van der Waals interactions at residue GLU50, similar to amoxicillin, as shown in Figure 4 and Table 5. In summary, ADMET analysis indicated good pharmacokinetic profile for compounds **4**, **8**, **17**, **23**, and **24**, although excretion results indicated high clearance for **4**, **8**, and **23**, while **17** and **24** persisted longer in the body. All compounds were non-OCT2 substrates. Toxicity screening showed no AMES toxicity or hepatotoxicity, although **23** was a hERG II inhibitor, suggesting potential cardiac risk. Molecular docking against *E. coli* (DNA Gyrase B), *S. typhimurium* (RamR), and *S. aureus* (DNA Gyrase) validated the method (RMSD < 2Å). These results indicate that compounds **17** and **24** in *S. androgynus*, have promising *in silico* antibacterial activity, especially against *E. coli*.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Table 3: Molecular docking validation results

Receptors	Grid Box			Grid Center			RMSD (Å)
	X	Y	Z	X	Y	Z	
E. coli DNA Gyrase B (*E. coli* ATCC 11229)	24	32	40	-12.459	19.173	22.483	1.261
RamR in complex with cholic acid (*S. typhimurium* ATCC 14028)	20	38	22	-11.124	-35.927	10.769	1.190
DNA gyrase enzyme complexed with novobiocin (*S. aureus* ATCC 6538)	32	38	34	-1.581	-0.035	-12.115	1.892

Table 4: Binding affinities of test compounds against receptors

Receptors	Binding Affinity (kcal/mol)					
	Control	4	8	17	23	24
E. coli DNA Gyrase B (*E. coli* ATCC 11229)	-7.05	-6.09	-6.46	-7.07	-6.31	-7.55
RamR in complex with cholic acid (*S. typhimurium* ATCC 14028)	-6.53	-5.05	-4.92	-5.67	-5.07	-6.25
DNA gyrase enzyme complexed with novobiocin (*S. aureus* ATCC 6538)	-7.13	-6.68	-6.33	-6.81	-5.30	-6.53

Table 5: Visualization Results of Molecular Docking Interactions

Compound	Type of interaction	Amino acid residue
Amoxicillin (control)	Van Der Waals	ARG76; **GLU50**; GLY77; **HIS55**; **ILE94**; **THR165**; **VAL167**; VAL43
	Conventional Hydrogen Bond	ARG136; ASN46
	Pi-Sigma	ILE78; VAL120
	Pi-Sulfur	MET95
	Alkyl	PRO79
Chlorogenic acid (**17**)	Van Der Waals	ALA53; ARG136; ASN46; GLY75; **HIS55**; ILE78; **ILE94**; PRO79; **THR165**; VAL120; **VAL167**; VAL71
	Conventional Hydrogen Bond	ARG76; ASP73; GLU50; GLY77; VAL43
	Unfavorable Acceptor-Acceptor	GLY77
	Pi-Alkyl	ALA47
Hypophyllanthin (**24**)	Van Der Waals	ARG136; ASN46; ASP49; ASP73; **GLU50**; GLY75; MET95; PRO79
	Carbon Hydrogen Bond	GLY77; THR165; VAL71
	Amide-Pi Stacked	GLY77
	Alkyl	ALA47; ARG76; ILE78; ILE94; VAL; 43; VAL120; VAL167
	Pi-Alkyl	HIS55; ILE78; ALA47

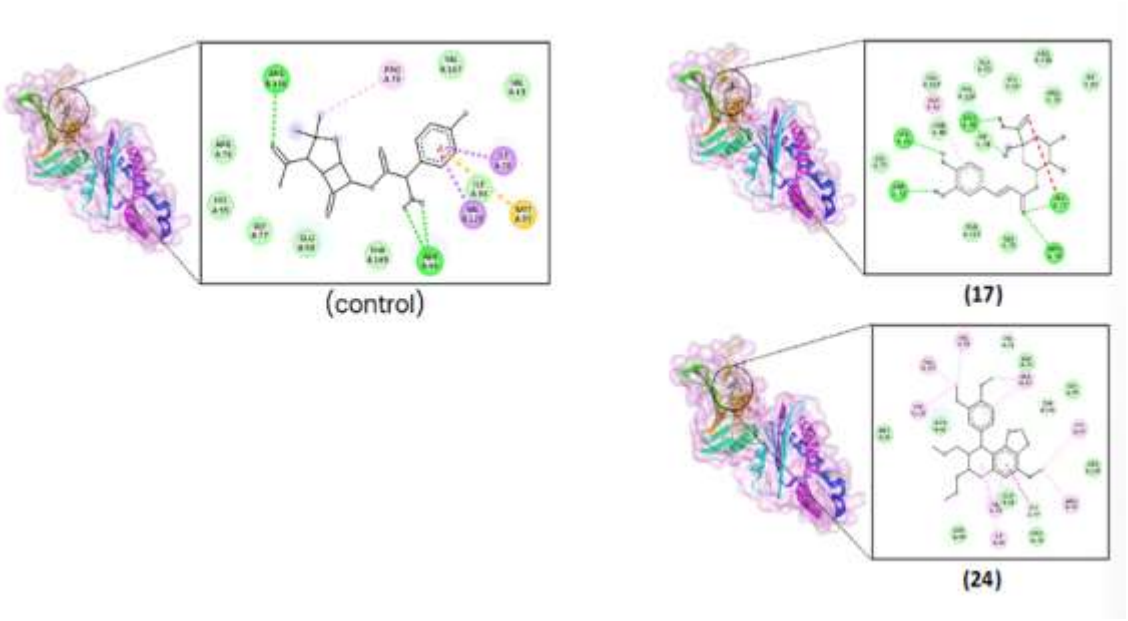

Figure 4: Molecular Docking Visualization of *E. coli* DNA Gyrase B Receptor with Ligands Amoxicillin (Control), chlorogenic acid (**17**), and hypophyllanthin (**24**).

Conclusion

In conclusion, this study shows that *S. androgynus* leaf extract contains various bioactive compounds, with a total of 187 metabolites identified through LC-MS analysis. The polarities of extraction solvent significantly affected the yield and type of metabolites. Methanol extract had the highest yield, but ethyl acetate extracted the highest diversity of compounds. Although the antibacterial activity of the extract *in vitro* was generally weak, the methanol and ethyl acetate extracts showed good inhibition against *S. aureus* and *E. coli*. Molecular docking analysis showed that chlorogenic acid and hypophyllanthin have stronger binding affinity to *E. coli* target proteins compared to amoxicillin (control). Therefore, these results show the importance of solvent selection in metabolite profile identification and suggest that *S. androgynus* is a promising natural source antibacterial agent candidate for further development through experimental and computational methods.

Conflicts of Interest

The authors declare no conflict of interest.

Author's Declaration

The authors hereby declare that the work presented in this article are original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgments

The authors are grateful for the support provided by the Research and Community Service (LPPM) of Universitas Negeri Surabaya, Indonesia through Non-APBN scheme Competitive Basic Research 2024 program (Grant No. 309/UN38/HK/PP/2024). Furthermore, the authors are grateful to individuals who provided support during the fieldwork.

References

1. Dahiya P and Purkayastha S. Phytochemical Screening and Antimicrobial Activity of Some Medicinal Plants Against Multi-Drug Resistant Bacteria from Clinical Isolates. Indian J Pharm Sci. 2012; 74(5):443-450. Doi: 10.4103/0250-474x.108420.
2. Salam AM and Quave CL. Opportunities for Plant Natural Products in Infection Control. Curr Opin Microbiol. 2018; 45:189-194. Doi: 10.1016/j.mib.2018.08.004.
3. Kuttinath S, Kh H, Rammohan R. Phytochemical Screening, Antioxidant, Antimicrobial, and Antibiofilm Activity of *Sauropus androgynus* Leaf Extracts. Asian J Pharm Clin Res. 2019; 12(4):244-250. Doi: 10.22159/ajpcr.2019.v12i4.31756.
4. Liu R, Bao ZX, Zhao PJ, Li GH. Advances in the Study of Metabolomics and Metabolites in Some Species Interactions. Molecules. 2021; 26(11):3311. Doi: 10.3390/molecules26113311.
5. Gao W, Kirschbaum C, Grass J, Stalder T. LC-MS Based Analysis of Endogenous Steroid Hormones in Human Hair. J Steroid Biochem Mol Biol. 2016; 162:92-99. Doi: 10.1016/j.jsbmb.2015.12.022.
6. Syarpin, Permatasari S, Pujianto DA. Analysis of Phytochemical Constituents and Antioxidant Activity from the Fractions of *Luvunga sarmentosa* Root Extract Using LCMS/MS. Biodiversitas. 2023; 24(2):733-740. Doi: 10.13057/biodiv/d240208.
7. Akbar SA, Hasan M, Afriani S, Nuzlia C. Evaluation of Phytochemical Composition and Metabolite Profiling of Macroalgae *Caulerpa taxifolia* and *C. peltata* From the Banda Aceh Coast, Indonesia. Biodiversitas. 2023; 24(10):5283-5292. Doi: 10.13057/biodiv/d241009.
8. Theodoridis G, Gika H, Franceschi P, Caputi L, Arapitsas P, Scholz M, Masuero D, Wehrens R, Vrhovsek U, Mattivi F. LC-MS Based Global Metabolite Profiling of Grapes: Solvent Extraction Protocol Optimisation. Metabolomics. 2012; 8:175-185.
9. El Sayed AM, Basam SM, El-Naggar EMA, Marzouk HS, El-Hawary S. LC–MS/MS and GC–MS Profiling as Well as the Antimicrobial Effect of Leaves of Selected *Yucca* Species Introduced to Egypt. Sci Rep. 2020; 10:1-15.
10. Tukiran, Sururi AM, Constanty IC. LC-MS Chemical Profiling of Dichloromethane Fraction of Methanol Extract of *Syzygium samarangense* Stem Bark. Trop J Nat Prod Res. 2024; 8(4):6963-6974. Doi: 10.26538/tjnpr/v8i4.30.
11. Riyadi PH, Romadhon, Sari D, Kurniasih RA, Agustini TW, Swastawati F, Herawati VE, Tanod WA. SwissADME Predictions of Pharmacokinetics and Druglikeness Properties of Small Molecules Present in *Spirulina platensis*. IOP Conf Ser Earth Environ Sci. 2021; 890(1):1-12. Doi: 10.1088/1755-1315/890/1/012021.

12. Floresta G, Fallica AN, Salerno L, Sorrenti V, Pittalà V, Rescifina A. Growing the Molecular Architecture of Imidazole-Like Ligands in HO-1 Complexes. Bioorg Chem. 2021; 117:105428. Doi: 10.1016/j.bioorg.2021.105428.
13. Zulqurnain M, Aijijiyah NP, Wati FA, Fadlan A, Azminah A, Santoso M. Synthesis, *Mycobacterium tuberculosis* H37Rv Inhibitory Activity, and Molecular Docking Study of Pyrazinamide Analogs. J Appl Pharm Sci. 2023; 13(11):170-177. Doi: 10.7324/JAPS.2023.140149.
14. Bactiar CF and Fahami NAM. LC-MS Analysis of Phytocomponents in the Methanol Extract of *Piper sarmentosum* Leaves. Pharmacogn J. 2019; 11:1071-1076. Doi: 10.5530/pj.2019.11.167.
15. Ramadhania NR, Purnomo AS, Fatmawati S. Antibacterial Activities of *Syzygium polyanthum* Wight Leaves. AIP Conf Proc. 2018; 2049:020024. Doi: 10.1063/1.5082429.
16. Ramadhania NR, Harun F, Purnomo AS, Fatmawati S. Anti-Oxidant and Anti-Bacterial Activities of *Anthurium plowmanii* Leaves Extracts. Mal J Fund Appl Sci. 2019; 15(2):194-199. Doi: 10.11113/mjfas.v15n2.1040.
17. Septaningsih DA, Darusman LK, Afendi FM, Heryanto R. Liquid Chromatography Mass Spectrometry (LC-MS) Fingerprint Combined with Chemometrics for Identification of Metabolites Content and Biological Activities of *Curcuma aeruginosa*. Indones J Chem. 2018; 18(1):43-52. Doi: 10.22146/ijc.25456.
18. Cravotto C, Fabiano-Tixier AS, Claux O, Abert-Vian M, Tabasso S, Cravotto G, Chemat F. Towards Substitution of Hexane as Extraction Solvent of Food Products and Ingredients with No Regrets. Foods. 2022; 11(21):3412. Doi: 10.3390/foods11213412.
19. Delgado-Povedano MM, Sánchez-Carnerero CC, Priego-Capote F, Ferreiro-Vera C. Untargeted Characterization of Extracts from *Cannabis sativa* L. Cultivars by Gas and Liquid Chromatography Coupled to Mass Spectrometry in High Resolution Mode. Talanta. 2019; 208:120384. Doi: 10.1016/j.talanta.2019.120384.
20. Lee J, Krotulski AJ, Fogarty MF, Papsun DM, Logan BK. Chromatographic Separation of the Isobaric Compounds Cyclopropyl Fentanyl, Crotonyl Fentanyl, Methacryl Fentanyl, and Para-Methyl Acryl Fentanyl for Specific Confirmation by LC-MS/MS. J Chromatograph B. 2019; 1118-1119:164-170. Doi: 10.1016/j.jchromb.2019.04.033.
21. Kainama H, Fatmawati S, Santoso M, Papilaya PM, Ersam T. The Relationship of Free Radical Scavenging and Total Phenolic and Flavonoid Contents of *Garcinia lasoar* PAM. Pharm Chem J. 2020; 53:1151-1157.
22. Acquaviva A, Di Simone SC, Nilofar, Bouyahya A, Zengin G, Recinella L, Leone S, Brunetti L, Uba AI, Guler O, Balos M, Cakilcioğlu U, Menghini L, Ferrante C, Orlando G, Libero ML, Chiavaroli A. Screening for Chemical Characterization and Pharmacological Properties of Different Extracts from *Nepeta italica*. Plants (Basel). 2023; 12(15):2785. Doi: 10.3390/plants12152785.
23. Purba RAP and Paengkoum P. Exploring the Phytochemical Profiles and Antioxidant, Antidiabetic, and Antihemolytic Properties of *Sauropus androgynus* Dried Leaf Extracts for Ruminant Health and Production. Molecules. 2022; 27(23):8580. Doi: 10.3390/molecules27238580.
24. Gu D, Yang Y, Bakri M, Chen Q, Aisa HA. Biological Activity and LC-MS Profiling of Ethyl Acetate Extracts from *Nitraria sibirica* (Pall.) Fruits. Nat Prod Res. 2018; 32(17):2054-2057. Doi: 10.1080/14786419.2017.1359175.
25. Alara OW, Abdurahman NH, Ukaegbu CI. Extraction of Phenolic Compounds: A Review. Curr Res Food Sci. 2021; 4:200-214. Doi: 10.1016/j.crfs.2021.03.011.
26. Resende FA, Nogueira LG, Bauab TM, Vilegas W, Varanda EA. Antibacterial Potential of Flavonoids with Different Hydroxylation Patterns. Ecletica Quimica. 2016; 40(1):173-179. Doi: 10.26850/1678-4618eqj.v40.1.2015.p173-179.
27. Donadio G, Mensitieri F, Santoro V, Parisi V, Bellone ML, De Tommasi N, Izzo V, Dal Piaz F. Interactions with Microbial Proteins Driving the Antibacterial Activity of Flavonoids. Pharmaceutics. 2021; 13(5):1-23. Doi: 10.3390/pharmaceutics13050660.
28. Qian W, Liu M, Fu Y, Zhang J, Liu W, Li J, Li X, Li Y, Wang T. Antimicrobial Mechanism of Luteolin Against *Staphylococcus aureus* and *Listeria monocytogenes* and Its Antibiofilm Properties. Microb Pathog. 2020; 142:104056. Doi: 10.1016/j.micpath.2020.104056.
29. Alam W, Khan H, Shah MA, Cauli O, Saso L. Kaempferol as a Dietary Anti-Inflammatory Agent: Current Therapeutic Standing. Molecules. 2020; 25(18):1-12. Doi: 10.3390/molecules25184073.
30. Borges A, Saavedra MJ, Simões M. The Activity of Ferulic and Gallic Acids in Biofilm Prevention and Control of Pathogenic Bacteria. Biofouling. 2012; 28:755-767. Doi: 10.1080/08927014.2012.706751.
31. Lou Z, Wang H, Zhu S, Ma C, Wang Z. Antibacterial Activity and Mechanism of Action of Chlorogenic Acid. J Food Sci. 2011; 76(6):398-403. Doi: 10.1111/j.1750-3841.2011.02213.x.
32. Kępa M, Miklasińska-Majdanik M, Wojtyczka RD, Idzik D, Korzeniowski K, Smoleń-Dzirba J, Wąsik TJ. Antimicrobial Potential of Caffeic Acid Against *Staphylococcus aureus* Clinical Strains. Biomed Res Int. 2018; 2018:7413504. Doi: 10.1155/2018/7413504.
33. Zhang D, Zou L, Wu D, Zhuang QG, Li HB, Mavuwengmana V, Corke H, Gan RY. Discovery of 1'-Acetoxychavicol Acetate (ACA) as a Promising Antibacterial Compound from Galangal (*Alpinia galanga* (Linn.) Willd). Ind Crops Prod. 2021; 171:113883. Doi: 10.1016/j.indcrop.2021.113883.
34. Lefebvre T, Destandau E, Lesellier E. Selective Extraction of Bioactive Compounds from Plants Using Recent Extraction Techniques: A Review. J Chromatogr A. 2021; 1635:461770. Doi: 10.1016/j.chroma.2020.461770.
35. De Souza Pereira JJ, Pereira APC, Jandú JJB, da Paz JA, Crovella S, dos Santos Correia MT, de Azevêdo Silva J. *Commiphora leptophloeos* Phytochemical and Antimicrobial Characterization. Front Microbiol. 2017; 8:52. Doi: 10.3389/fmicb.2017.00052.
36. Patel JR, Tripathi P, Sharma V, Chauhan NS. *Phyllanthus amarus*: Ethnomedicinal Uses, Phytochemistry and Pharmacology: A Review. J Ethnopharmacol. 2011; 138(2):286-313. Doi: 10.1016/j.jep.2011.09.040.
37. Du GH. Natural Small Molecule Drugs from Plants. Springer; 2018. p. 325-330.
38. Omoruyi SI, Delport J, Kangwa TS, Rahman Z, Hussein A A, Lorke DE, Ekpo OE. An Update on the Bioactivities and Health Benefits of Two Plant-Derived Lignans, Phyllanthin and Hypophyllanthin. Adv Tradit Med. 2024; 24:1011-1036. Doi: 10.1007/s13596-023-00738-7.
39. Alamri MA, Afzal O, Akhtar MJ, Karim S, Husain M, Alossaimi MA, Riadi Y. Synthesis, In Silico and In Vitro Studies of Novel Quinazolinone Derivatives as Potential SARS-CoV-2 3CLpro Inhibitors. Arab J Chem. 2024; 17(1):1-19. Doi: 10.1016/j.arabjc.2023.105384.