J. Pharm. Res. 2024;23(2):111–118

Print ISSN: 0973-7200
Online ISSN: 2454-8405

Journal of Pharmaceutical Research

ORIGINAL ARTICLE

Antibacterial Activity of *Lactuca virosa* with an *In silico* Approach

Darshan Neelagund[1], Rajesh Kumar Rawri[2], C K Prasanna[1], Paramita Das[2,*]

[1] *Research Scholar, Department of Pharmaceutical Chemistry, Krupanidhi College of Pharmacy, Bangalore, 560035, Karnataka, India*
[2] *Professor, Department of Pharmaceutical Chemistry, Krupanidhi College of Pharmacy, Bangalore, 560035, Karnataka, India*

ARTICLE INFO

Article history:
Received 08.06.2024
Accepted 02.07.2024
Published 06.08.2024

* *Corresponding author.*
Paramita Das
paramitadas04@gmail.com

https://doi.org/
10.18579/jopcr/v23.2.51

ABSTRACT

The current work aims to screen the phytoconstitutes of the *Lactuca virosa* leaves ethanolic extract by using GC-MS analysis and investigate its antibacterial activity. GC-MS analysis was conducted to identify the various phytochemical constituents within the ethanolic extracts of *Lactuca virosa*. Subsequently, protein-ligand docking was performed using proteins PDBID: 6AHT and 5C5H, revealing a strong affinity between the bioactive compounds and the proteins, indicating potent inhibitory action. Furthermore, each concentration of *Lactuca virosa* was assessed for antibacterial activity using Minimum Inhibitory Concentration (MIC) against bacterial strains including *Bacillus cereus, Escherichia coli, Pseudomonas aeruginosa* and *Streptococcus mutans*. Preliminary phytochemical testing revealed the presence of alkaloids, coumarins, flavonoids, glycosides, phenols, terpenoids, oils, and resins. GC-MS showed the presence of many bioactive compounds in extract. Docking results highlighted that two compounds exhibited the most favourable binding energies of approximately -8.1 kcal/mol and -8.5 kcal/mol with 6AHT, and -8.8 kcal/mol with 5C5H. The ethanolic extract of 0.4mg concentration has shown good antibacterial activity against gram positive bacteria. The study identifies a new source of antibacterial compounds, which could lead to the development of new drugs, particularly effective against gram positive strains like *Bacillus cereus* and *Streptococcus mutans*.

Keywords: *Lactuca virosa*; GC-MS; Docking; ADMET; Antibacterial Activity

INTRODUCTION

Bacterial infections remain a pressing concern for public health globally, with antibiotic resistance compounding the challenge. Despite the advent of antibiotics, bacterial pathogens have evolved various mechanisms to evade the effects of these drugs, leading to the rise and spread of resistant strains. Understanding the dynamics of bacterial infections and antibiotic resistance is crucial for developing effective strategies to combat these threats[1]. Recent studies have highlighted the escalating burden of antibiotic resistance and its implications for global health. The World Health Organization (WHO) reports that antibiotic resistance is a growing concern, with multidrug-resistant bacteria causing infections that are increasingly difficult to treat[2].

The future of antimicrobial medication use remains uncertain due to the growing issue of microorganism resistance. Thus, measures to mitigate this issue are required, such as regulating the use of antibiotics, advancing research to comprehend resistance mechanisms at the genetic level, and conducting ongoing investigations to create novel pharmaceuticals, both synthetic and natural. The ultimate goal is to deliver suitable and effective antimicrobial medications to patients[3].

Lactuca virosa, a member of the Asteraceae family, is a biennial herbaceous plant indigenous to Europe and Asia[4,5]. With a rich historical presence in traditional medicine, it has been employed for diverse purposes such as pain relief, sedation, and the treatment of respiratory conditions[6–8]. Several bioactive compounds including lactucin, lactucopicrin and lactucinamide have been identified in *L. virosa*, contributing to its pharmacological effects. Among these compounds, lactucin and lactucopicrin have been reported to possess antimicrobial properties, prompting interest in exploring the antibacterial activity of *L. virosa*[9].

© 2024 Published by Krupanidhi College of Pharmacy. This is an open access article under the CC BY-NC-ND license (https://creativecommons.org/licenses/by-nc-nd/4.0/)

KRUPANIDHI COLLEGE OF PHARMACY

The molecular docking tool serves as a pivotal instrument in designing drug structures and predicting binding interactions between ligands and proteins in their three-dimensional structures, thereby elucidating specific activity. Leveraging ligand-based drug design with phytochemicals helps mitigate complexity and streamline the process. By assessing the microbial effects through binding energies of proteins with phytochemicals sourced from *L. virosa*, novel generation drugs targeting the infection and proliferation of diverse pathogens can be developed[10]. The current work aims to screen the phytoconstituents of the *L. virosa* leaves ethanolic extract using GC-MS analysis and investigate its antibacterial activity.

MATERIALS AND METHODS

Plant Collection

The raw material of freshly harvested *Lactuca virosa* leaves, collected from North Karnataka, underwent authentication by Central Ayurveda Research Institute, Bengaluru.

Plant Extract Preparation

The process began by harvesting fresh leaves of *Lactuca virosa*, which were thinly sliced and air-dried. Subsequently, approximately 25 grams of the dried leaves were subjected to extraction using ethanol as a solvent in a Soxhlet apparatus operating at a temperature of 78°C. The resulting fraction was then concentrated under reduced pressure utilizing a rotary evaporator, yielding a concentrated extract. Following concentration, the extract underwent analysis using Gas Chromatography-Mass Spectrometry (GC-MS) to identify its phytochemical constituents[11].

Preliminary Phytochemical Analysis

The extract underwent preliminary phytochemical testing to determine the presence of various chemical compounds. Air-dried and powdered plant materials were meticulously screened for the existence of an array of constituents including saponins, tannins, alkaloids, flavonoids, triterpenoids, steroids, glycosides, anthraquinones, coumarin, gum, mucilage, carbohydrates, reducing sugars, starch, protein, and amino acids. These screenings were conducted in accordance with established literature and standard procedures[12–14].

GC-MS Analysis of ethanolic extract

GC-MS analysis was conducted utilizing an Elite-1 column (100% dimethyl polysiloxane) measuring 30 × 0.25 mm inner diameter with a film thickness of 1 micron. The oven temperature ramped up to 300°C at a rate of 10°C per minute and was held for 6 minutes. The sample was injected in split mode with a ratio of 10:1, and helium served as the carrier gas with a flow rate of 1 ml/min. Mass spectrometry was performed in a 70 eV mass range with a scan time and range set from 45 to 450 Da. Solvent delay was set from 0 to 2 minutes, and the total run time for GC-MS was 36 minutes. Mass spectral data were acquired using Turbo Mass Gold-Perkin Elmer version 5.2. Compound identification was carried out based on comparison with available mass spectral libraries[15,16].

Molecular Docking

A 3D structure of a protein with the PDB IDs 6AHT (Gram+ve) and 5C5H (Gram-ve) was downloaded from RSPDB with the required resolution for this work. The receptor's active site was described in the protein study[17] and utilized to examine the results of the docking evaluation. The bioactive compounds obtained from GCMS analysis were selected as ligand. The ACD/ChemSketch tool was used to create the ligands two-dimensional structures. The data is converted, saved in mol format, and utilized for docking analysis[18]. Molecular docking analyses were carried out utilizing Auto Dock 4.2 software to predict the binding interactions between proteins and ligands, aiming to elucidate specific activities. These interactions delineate fundamental biochemical processes based on the ligand's behaviour at the protein site. Docking scores obtained were compared with the standard compound tetracycline[19].

ADMET Studies

In the present study, the bioactive compounds extracted from *L. Virosa* were subjected to *in silico* ADME screening using the Swiss ADME website. This screening aims to evaluate the individual ADMET behaviour of the compounds and interpret the results, providing insights into their potential pharmacological properties and drug-likeness[20]. The Lipinski's Rule of Five serves as a guideline to assess the drug-likeness of molecules based on physicochemical parameters. For a molecule to qualify as a ligand, it should meet specific criteria: log P < 5, molecular weight < 500 Da, hydrogen bond acceptors < 10, and hydrogen bond donors < 5. Compounds violating two or more of these criteria may be considered unsuitable for further consideration. Furthermore, Protox II is a web server utilized for predicting the toxicity of bioactive compounds. This tool aids in assessing the safety profile of potential drug candidates by predicting their toxicological properties.

Evaluation of Antibacterial activity

The extract was evaluated for its Minimum Inhibitory Concentration (MIC) against bacterial strains such as *Bacillus cereus, Escherichia coli, Pseudomonas aeruginosa* and *Streptococcus mutans* using the Agar Well Diffusion Method.

Sample Preparation

A 10mg sample was dissolved in 1mL of Dimethyl sulfoxide (DMSO). Subsequently, various aliquots of the sample were prepared by pipetting 10μL (0.1mg), 20μL (0.2mg), 30μL (0.3mg), and 40μL (0.4mg), respectively. The final volume of each aliquot was adjusted to 50μL by adding DMSO.

Standard Preparation

Tetracycline, weighing 10mg, was dissolved in 1mL of DMSO. Subsequently, various aliquots of the standard were prepared by pipetting 10μL (0.1mg), 20μL (0.2mg), 30μL (0.3mg), and 40μL (0.4mg), respectively. Each aliquot's final volume was adjusted to 50μL by adding DMSO.

Platting for MIC against organisms

Luria Bertani (LB) agar media was prepared by mixing tryptone (10g), sodium chloride (10g), yeast extract (6g), agar (20g), and distilled water (1000mL) to yield a total volume of 300mL. The media was then autoclaved at 121°C for 15 minutes. Subsequently, approximately 25mL of the media was dispensed into sterilized petri plates and allowed to solidify. For inoculation, 200μL of prepared inoculum containing bacteria such as *B. cereus, E. coli, P. aeruginosa*, and *S. mutans* was evenly spread on separate agar plates using a plate spreader. Subsequently, five wells, each measuring 0.6cm in diameter, were made in each plate using a borer. Following well formation, 50μL of prepared samples were loaded into the respective wells of each plate. The plates were then incubated at 37°C for 24 hours. After incubation, the Minimum Inhibitory Concentration (MIC) was recorded in millimetres (mm)[21].

RESULTS

Phytochemical screening of L virosa leaves ethanolic extract

The crude extract from the leaves of *Lactuca virosa* underwent various tests to identify its phytoconstituents. The results obtained are given in Table 1.

GC-MS Analysis of ethanolic extract

The GC-MS chromatogram analysis (Figure 1) reveals that the ethanolic extract of *L. virosa* contains thirteen phytochemical constituents. The bioactive phytocompounds identified in the ethanolic leaf extract demonstrate a range of biological activities, as detailed in Table 2.

Docking studies

In our current investigation, we conducted docking studies on five compounds using PDB IDs 6AHT for Gram-positive and 5C5H for Gram-negative proteins. The primary objective was to determine the binding energies associated with the formation of complexes and to elucidate the molecular interactions responsible for target-specific inhibition. The docking results are presented in Table 3. The molecular interactions of compounds and the protein-ligand complex are shown in Figures 2 and 3.

Table 1: Phytochemical Screening Results of Plant Extract

Phytochemical constituents	Test	Ethanol
Alkaloids	Mayer's test	+
	Hager's test	+
Flavonoids	Alkaline reagent test	+
	FeCl3 test	+
Glycosides	Keller-Killani test	+
Tannis	Gelatin test	-
Phenols	Ferric chloride test	+
Sterols	Salkowski test	-
Terpenoids	Salkowski test	+
Saponins	Foam test	-
Protein detection	Biuret test	-
Oils and resins	Precipitate test	+
Coumarin detection	Sodium Hydroxide test	+

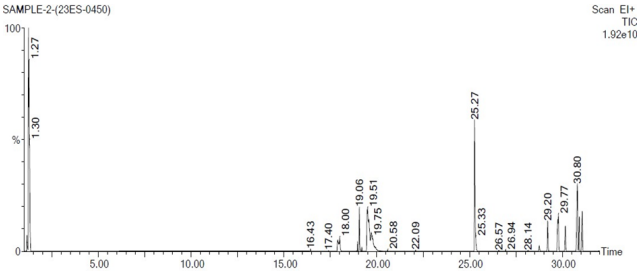

Fig. 1: GCMS graph of *Luctuca virosa* leaves ethanolic extract

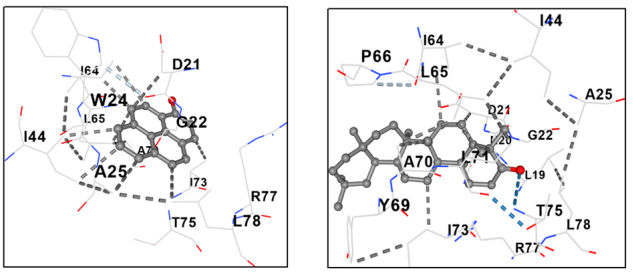

Fig. 2: 3D structure of the Protein ligand complex and Molecular interactions of 1-Hydroxypyrene and 4,4,6A,6B,8A,11,11,14B-Octamethyl-1,4,4A,5,6,6A,6B,7,8,8A,9,10,11,12,12A,14,14A,14B-Octadecahydro-2 with 6AHT

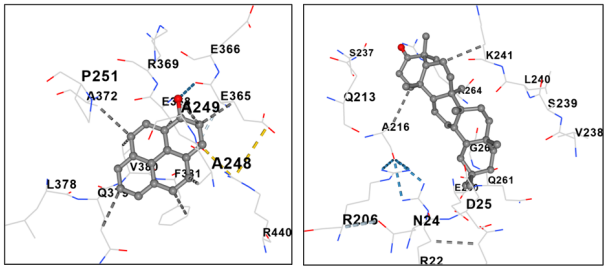

Fig. 3: 3D structure of the Protein ligand complex and Molecular interactions of 1-Hydroxypyrene and 4,4,6A,6B,8A,11,11,14B-Octamethyl-1,4,4A,5,6,6A,6B,7,8,8A,9,10,11,12,12A,14,14A,14B-Octadecahydro-2 with 5C5H

ADMET Studies

The evaluation of ADME properties was conducted using Swiss ADME for the bioactive compounds identified through GCMS analysis. It's worth noting that all compounds comply with the Lipinski rule, as demonstrated in Table 4. The Protox II server was utilized to estimate the toxicity of the primary bioactive compounds from *L. Virosa*. Most of these selected bioactive molecules surpassed all toxicity barriers, demonstrating favourable binding energy or drug-likeness activity (refer to Table 5).

Antibacterial activity

The antimicrobial activity of the ethanolic extract of *Lactuca virosa* was studied against *Bacillus cereus, Escherichia coli, Pseudomonas aeruginosa*, and *Streptococcus mutans* using the Agar Well Diffusion Method, with concentrations ranging from 0.1 to 0.4 mg/ml. The results indicated that the extract was more potent against *B. cereus* and *S. mutans* compared to the other tested pathogens. The results indicated that the extract exhibited antibacterial properties against all tested strains, with efficacy comparable to tetracycline at a concentration of 0.4 mg/ml. The MIC results are presented in Table 6.

DISCUSSION

The phytochemical constituents found in plants play a vital role in their defence mechanisms against various microorganisms, insects, and herbivores[30]. In the selected plant investigated, a variety of phytochemicals have been identified, including alkaloids, coumarins, flavonoids, glycosides, phenols, terpenoids, oils and resins. These compounds are summarized in Table 1. The presence of these bioactive compounds suggests their potential involvement in the observed antimicrobial properties of the plants. Alkaloids and phenols are known for their antimicrobial properties due to their ability to disrupt microbial cell membranes or inhibit essential enzymes[31,32]. Flavonoids possess antioxidant and antimicrobial activities[33]. The presence of these phytochemical constituents highlights the potential of the selected plant as sources of antimicrobial agents and provides insights into their mechanisms of action against microorganisms.

The ethanolic extract of *L. virosa* contains thirteen phytochemical constituents as indicated by GC-MS chromatogram analysis (Figure 1). Upon comparison with the NIST library, these constituents were characterized and identified. The bioactive phytocompounds identified in the ethanolic leaf extract exhibit diverse biological activities, as detailed in Table 2. Among the identified compounds, the most abundant compounds are 1-Hexyl-2-Nitrocyclohexane, 1-Hexyl-1-Nitrocyclohexane, 1-Heptacosanol, 2R-Acetoxymethyl-1,3,3-trimethyyl-4t-(3-Methyl-2-buten-1-yl)-1t-cyclohexanol, 1-Hydroxypyrene, and 2,4,4-Trimethyl-3-hydroxymethyl-5a-(3-Methyl-but-2-enyl)-cyclohexene. These compounds are noted for their antibacterial activity, indicating the potential of *L. virosa* as a source of natural antibacterial agents.

The ethanolic extract of *L. virosa* contains thirteen phytochemical constituents as indicated by GC-MS chromatogram analysis (Figure 1). Upon comparison with the NIST library, these constituents were characterized and identified. The bioactive phytocompounds identified in the ethanolic leaf extract exhibit diverse biological activities, as detailed in Table 2. Among the identified compounds, the most abundant compounds are 1-Hexyl-2-Nitrocyclohexane, 1-Hexyl-1-Nitrocyclohexane, 1-Heptacosanol, 2R-Acetoxymethyl-1,3,3-trimethyyl-4t-(3-Methyl-2-buten-1-yl)-1t-cyclohexanol, 1-Hydroxypyrene, and 2,4,4-Trimethyl-3-hydroxymethyl-5a-(3-Methyl-but-2-enyl)-cyclohexene. These compounds are noted for their antibacterial activity, indicating the potential of *L. virosa* as a source of natural antibacterial agents.

According to the tabulated docking results presented in Table 3, all five compounds identified through GCMS have effectively docked within the active binding site of the protein domain. Notably, the calculated binding energies for these interactions fall within the range of -6.0 to -9.0 kcal/mol. Among the five evaluated compounds, the docking results highlighted that compounds 1-Hydroxypyrene and 4,4,6A,6B,8A,11,11,14B-Octamethyl-1,4,4A,5,6,6A,6B,7,8,8A,9,10,11,12,12A,14,14A,14B-Octadecahydro-2 exhibited superior binding energies compared to the others. Specifically, these two compounds demonstrated the most favourable binding energies of approximately -8.1 and -8.5 kcal/mol with 6AHT and -8.8 and -8.8 kcal/mol with 5C5H, respectively, based on the molecular docking analyses.

It's worth noting that all compounds comply with the Lipinski rule, as demonstrated in Table 4. However, the specific molecules 2R-Acetoxymethyl-1,3,3-trimethyl-4t-(3-Methyl-2-buten-1-yl)-1t-cyclohexanol and 2,4,4-Trimethyl-3hydroxymethyl-5a-(3-Methyl-but-2-enyl)-cyclohexene

Table 2: GC-MS Analysis of Phytocompounds in *Lactuca virosa* Ethanolic Extract

Sl. No	Compound name	Molecular weight	Molecular formula	Retention time	Area %	Biological activity
1	Sulfurous acid, 2-propyl heptyl ester	222	$C_{10}H_{22}O_3S$	17.905	2.524	Antioxidant activity
2	4-Methyloctanoic acid	158	$C_9H_{18}O_2$	18.000	3.065	Used as flavor and fragrance
3	1-Hexadecen-3-ol,3,5,11,15-tetramethyl	296	$C_{20}H_{40}O$	19.061	4.566	No activity reported
4	1-Hexyl-2-Nitrocyclohexane	213	$C_{12}H_{23}O_2N$	19.506	19.622	Antimicrobial activity[22] and Neuroactive, anti-inflammatory, analgesic Property[23]
5	1-Hexyl-1-Nitrocyclohexane	213	$C_{12}H_{23}O_2N$	19.696	10.370	Antioxidant, antimicrobial, anti-inflammatory activity[24]
6	1-Heptacosanol	396	$C_{27}H_{56}O$	25.268	17.443	Antimicrobial and antioxidant activity[25]
7	Pregnan-3,11-diol-20-one	334	$C_{21}H_{34}O_3$	28.749	0.760	No activity reported
8	9,19-Cyclolanost-23-ene-3,25-diol,3-acetate,(3.Beta.,23E)	484	$C_{32}H_{52}O_3$	29.205	4.002	Anti-inflammatory and antioxidant activity
9	2R-Acetoxymethyl-1,3,3-trimethyyl-4t-(3-Methyl-2-buten-1-yl)-1t-cyclohexanol	282	$C_{17}H_{30}O_3$	29.775	9.134	Antibacterial, antioxidant, and anticancer activities[26]
10	1-Hydroxypyrene	218	$C_{16}H_{10}O$	30.150	3.687	Antimicrobial, phytotoxic, cytotoxic and mutagenic activities[27]
11	4,4,6A,6B,8A,11,11,14B-Octamethyl-1,4,4A,5,6,6A,6B,7,8,8A,9,10,11,12,12A,14,14A,14B-Octadecahydro-2	424	$C_{30}H_{48}O$	30.800	13.493	Antimicrobial activity[28]
12	2,4,4-Trimethyl-3-hydroxymethyl-5a-(3-Methyl-but-2-enyl)-cyclohexene	222	$C_{15}H_{26}O$	30.900	5.010	Antioxidant Potential and Antimicrobial Activity[29]
13	2,4,4-Trimethyl-3-hydroxymethyl-5a-(3-methyl-but-2-enyl)-cyclohexen	222	$C_{15}H_{26}O$	31.065	6.323	Antioxidant and antimicrobial activity

Table 3: Docking Results for Compounds Interacting with PDB ID: 6AHT and 5C5H

Sl. No	Ligand	Binding Energy (Kcal/mol)	
		6AHT (Gram +)	5C5H (Gram -)
1	1-Hexyl-2-Nitrocyclohexane	-6.1	-6.5
2	2R-Acetoxymethyl-1,3,3-trimethyyl-4t-(3-Methyl-2-buten-1-yl)-1t-cyclohexanol	-6.8	-7.4
3	1-Hydroxypyrene	-8.1	-8.8
4	4,4,6A,6B,8A,11,11,14B-Octamethyl-1,4,4A,5,6,6A,6B,7,8,8A,9,10,11,12,12A,14,14A,14B-Octadecahydro-2	-8.5	-8.8
5	2,4,4-Trimethyl-3-hydroxymethyl-5a-(3-Methyl-but-2-enyl)-cyclohexene	-6.4	-7.4

Table 4: *In-silico*

Sl.No	Ligand	Molecular Weight (g/mol)	Molecular Formula	Log P	H-Donors	H-acceptors	Rotatable Bond	TPSA ($Å^2$)
1	1-Hexyl-2-Nitrocyclohexane	213.32	$C_{12}H_{23}O_2N$	2.97	0	2	6	45.82
2	2R-Acetoxymethyl-1,3,3-trimethyyl-4t-(3-Methyl-2-buten-1-yl)-1t-cyclohexanol	282.42	$C_{17}H_{30}O_3$	3.48	1	3	5	46.53
3	1-Hydroxypyrene	218.25	$C_{16}H_{10}O$	2.06	1	1	0	20.23
4	4,4,6A,6B,8A,11,11,14B-Octamethyl-1,4,4A,5,6,6A,6B,7,8,8A,9,10,11,12,12A,14,14A,14B-Octadecahydro-2	424.70	$C_{30}H_{48}O$	4.53	0	1	0	17.07
5	2,4,4-Trimethyl-3-hydroxymethyl-5a-(3-Methyl-but-2-enyl)-cyclohexene	222.37	$C_{15}H_{26}O$	3.21	1	1	3	20.23

Table 5: Toxicity profile of bioactive compounds

Sl. No	Compound	Predicted Toxicity Class	Predicted LD_{50} value (mg/kg)	Hepato-toxicity	Carcino-genicity	Immuno-toxicity	Muta-genicity
1	1-Hexyl-2-Nitrocyclohexane	5	2700	Inactive	Active	Inactive	Inactive
2	2R-Acetoxymethyl-1,3,3-trimethyyl-4t-(3-Methyl-2-buten-1-yl)-1t-cyclohexanol	6	6800	Inactive	Inactive	Inactive	Inactive
3	1-Hydroxypyrene	3	98	Inactive	Inactive	Inactive	Active
4	4,4,6A,6B,8A,11,11,14B-Octamethyl-1,4,4A,5,6,6A,6B,7,8,8A,9,10,11,12,12A,14,14A,14B-Octadecahydro-2	5	5000	Inactive	Inactive	Active	Inactive
5	2,4,4-Trimethyl-3-hydroxymethyl-5a-(3-Methyl-but-2-enyl)-cyclohexene	4	1600	Inactive	Inactive	Inactive	Inactive

Table 6: Antibacterial activity of different concentrations of ethanolic extract of *Lactuca Virosa* against tested strains

Organism	Zone of inhibition (mm)							
	Concentration (mg/ml)				Tetracycline (mg/ml)			
	0.1mg	0.2mg	0.3mg	0.4mg	0.1 mg	0.2 mg	0.3 mg	0.4 mg
Bacillus cereus	0.8	0.9	2.2	3.3	2.8	3.0	3.2	3.5
Escherichia coli	0.8	1.0	1.0	1.2	3.0	3.3	3.5	3.8
Pseudomonas aeruginosa	0.9	1.1	1.2	1.3	2.6	2.8	3.0	3.3
Streptococcus mutans	1.0	1.2	2.3	3.4	2.8	3.0	3.3	3.5

stood out as potential candidates for further investigation in drug discovery and development (Table 5). This suggests they have desirable properties that make them worth exploring as potential therapeutic agents.

The Minimum Inhibitory Concentration (MIC) of the *L. Virosa* leaf extract was assessed against *Bacillus cereus, Escherichia coli, Pseudomonas aeruginosa*, and *Streptococcus mutans*, using concentrations ranging from 0.1 to 0.4 mg/ml. The ethanolic extract of *Lactuca virosa* showed increasing antibacterial activity with higher concentrations against *Bacillus cereus*. At 0.1 mg/mL, 0.2 mg/mL and 0.3 mg/mL, the zones of inhibition were 0.8 mm, 0.9 mm and 2.2 mm, respectively. Significant antibacterial activity was observed at highest concentration with 3.3 mm at 0.4 mg/mL. Similarly, the extract showed increasing antibacterial activity with higher concentrations against *Streptococcus mutans*, with zones of inhibition from 1.0 mm at 0.1 mg/mL to 3.4 mm at 0.4 mg/mL. Antibacterial activity was observed at the highest concentration, with a zone of inhibition of 3.3 mm at 0.4 mg/mL. Tetracycline showed higher zones of inhibition at all concentrations, ranging from 2.8 mm to 3.5 mm. The findings revealed that the extract displayed antibacterial properties against all examined strains, showing efficacy comparable to tetracycline at a concentration of 0.4mg/ml (Table 6). Furthermore, the MIC values revealed that the 0.4mg/ml concentration of the crude ethanol extract effectively inhibited the growth of *S. mutans* and *B. cereus*[34]. This suggests that the extract may have potential as an antimicrobial agent, particularly against dental caries caused by *S. mutans*. The research findings suggest that the ethanolic extract of *L. virosa* exhibits stronger antibacterial activity against Gram-positive bacteria compared to Gram-negative bacteria.

CONCLUSION

The fact that it showed different antibacterial activity profiles against various strains, with *Streptococcus mutans* and *Bacillus cereus* being particularly vulnerable, suggests its potential as a source for novel antimicrobial agents. The presence of alkaloids, terpenoids, flavonoids, and phenols in the preliminary phytochemical screening indicates that these compounds might contribute to the observed antibacterial activity. However, you rightly note that further studies are necessary to fully understand the effectiveness of the crude extracts and to isolate and characterize the specific bioactive compounds responsible for the antibacterial properties. It provides a valuable foundation for further investigation into the potential therapeutic applications of *Lactuca virosa* and the discovery of new natural bioactive compounds with antibacterial properties.

ACKNOWLEDGMENTS

We would thank the Principal and management of Krupanidhi College of Pharmacy, Bangalore, for their support and encouragement.

Conflict of Interest Declaration

The author declares no conflict of interest.

REFERENCES

1. Fikri AA, Arifin S. Global antimicrobial resistance and use surveillance system. 2022. Available from: https://www.who.int/initiatives/glass.
2. Tacconelli E, Carrara E, Savoldi A, Harbarth S, Mendelson M, Monnet DL, et al. Discovery, research, and development of new antibiotics: the WHO priority list of antibiotic-resistant bacteria and tuberculosis. *The Lancet: Infectious Diseases*. 2018;18(3):318–327. Available from: https://doi.org/10.1016/S1473-3099(17)30753-3.
3. Capelo-Martínez JL, Igrejas G, editors. Antibiotic Drug Resistance. John Wiley & Sons. 2019. Available from: https://www.wiley.com/en-us/Antibiotic+Drug+Resistance-p-9781119282525.
4. Zargari A. Medicinal plants. *Tehran University Publications*. 1978;3:223–228.
5. Gharpure S, Yadwade R, Ankamwar B. Lactuca virosa leaf-mediated biosynthesis of zinc oxide nanoparticles and estimation of antimicrobial and anticancer activities. *Chemistry Letters*. 2022;51(7):739–743. Available from: https://doi.org/10.1246/cl.220071.
6. Abdul-Jalil TZ. Lactuca serriola: Short review of its phytochemical and pharmacological profiles. *International Journal of Drug Delivery Technology*. 2020;10(3):505–508. Available from: http://dx.doi.org/10.25258/ijddt.10.3.34.
7. Uwaya DO, Bello AK, Aikpitanyi I. Evaluation of Antitussive, Expectorant and Analgesic Activities of Aqueous Extracts of Di-herbal Formulation of Whole Plant of Euphobia hirta and Lactuca virosa Leaf on Rodents. *Journal of Applied Sciences and Environmental Management*. 2023;27(8):1881–1888. Available from: https://doi.org/10.4314/jasem.v27i8.35.
8. Uwaya DO, Okakwu R, Omozuwa OP. In-Vivo and In-Vitro Anti-Inflammatory Activities of the Aqueous Extract of Di-Herbal Formulation [Euphorbia Hirta and Lactuca Virosa. *Journal of Applied Sciences and Environmental Management*. 2020;24(11):1979–1985. Available from: https://dx.doi.org/10.4314/jasem.v24i11.19.
9. Häkkinen ST, Cankar K, Nohynek L, Van Arkel J, Laurel M, Oksman-Caldentey KM, et al. Cichorium intybus L. Hairy Roots as a

Platform for Antimicrobial Activity. *Pharmaceuticals*. 2023;16(2):1–11. Available from: https://doi.org/10.3390/ph16020140.
10. Das P, Nayak A, Preethi K, Nikhil K, Kiruba AA. Antibacterial activity and molecular docking study of Coptis teeta. *Herba Polonica*. 2023;69(2):1–8. Available from: https://herbapolonica.pl/resources/html/article/details?id=610101&language=en.
11. Jo K, Kim S, Ahn Y, Suh HJ. Effects of green lettuce leaf extract on sleep disturbance control in oxidative stress-induced invertebrate and vertebrate models. *Antioxidants*. 2021;10(6):1–15. Available from: https://doi.org/10.3390/antiox10060970.
12. Pengelly A. The Constituents of Medicinal Plants: An introduction to the chemistry and therapeutics of herbal medicine. 2nd ed. Routledge. 2004. Available from: https://www.routledge.com/The-Constituents-of-Medicinal-Plants-An-introduction-to-the-chemistry-and-therapeutics-of-herbal-medicine/Pengelly/p/book/9781741140521#:~:text=This%20unique%20book%20explains%20in,structures%20and%20their%20pharmacological%20activities.
13. Sam S. Importance and effectiveness of herbal medicines. *Journal of pharmacognosy and phytochemistry*. 2019;8(2):354–357. Available from: https://www.phytojournal.com/archives/2019/vol8issue2/PartF/8-4-205-517.pdf.
14. Abidet A, Gherraf N, Kalla A, Zellagui A, Fellah O. Assessment of total phenolics and flavonoids, and evaluation of scavenging activity of the aerial parts of Verbascum thapsus L. and Lactuca virosa L. grown in Algeria. *International Journal of Chemical and Biochemical Sciences*. 2020;17:86–92. Available from: https://www.iscientific.org/wp-content/uploads/2020/11/10-IJCBS-20-17-10.pdf.
15. Unver T, Gurhan I. Chemical composition and antimicrobial activity of an apolar extract from Lactuca serriola L. leaves. *Biochemical Systematics and Ecology*. 2024;114. Available from: https://doi.org/10.1016/j.bse.2024.104832.
16. Beale DJ, Pinu FR, Kouremenos KA, Poojary MM, Narayana VK, Boughton BA. Review of recent developments in GC-MS approaches to metabolomics-based research. *Metabolomics*. 2018;14:1–31. Available from: https://doi.org/10.1007/s11306-018-1449-2.
17. Vakayil R, Anbazhagan M, Shanmugam G, Ramasamy S, Mathanmohun M. Molecular docking and in vitro analysis of phytoextracts from B. serrata for antibacterial activities. *Bioinformation*. 2021;17(7):667–672. Available from: https://doi.org/10.6026/97320630017667.
18. Stanzione F, Giangreco I, Cole JC. Use of molecular docking computational tools in drug discovery. *Progress in Medicinal Chemistry*. 2021;60:273–343. Available from: https://doi.org/10.1016/bs.pmch.2021.01.004.
19. Zeleke D, Eswaramoorthy R, Belay Z, Melaku Y. Synthesis and Antibacterial, Antioxidant, and Molecular Docking Analysis of Some Novel Quinoline Derivatives. *Journal of Chemistry*. 2020;2020:1–16. Available from: https://dx.doi.org/10.1155/2020/1324096.
20. Riyadi PH, Sari ID, Kurniasih RA, Agustini TW, Swastawati F, Herawati VE, et al. SwissADME predictions of pharmacokinetics and drug-likeness properties of small molecules present in Spirulina platensis. In: 2nd International Conference on Fisheries and Marine ;vol. 890 of IOP Conference Series: Earth and Environmental Science. IOP Publishing. 2021;p. 1–12. Available from: https://iopscience.iop.org/article/10.1088/1755-1315/890/1/012021/pdf.
21. Eloff JN. Avoiding pitfalls in determining antimicrobial activity of plant extracts and publishing the results. *BMC Complementary and Alternative Medicine*. 2019;19:1–8. Available from: https://doi.org/10.1186/s12906-019-2519-3.
22. Nabi M, Tabassum N, Ganai BA. Phytochemical screening and antibacterial activity of Skimmia anquetilia N.P. Taylor and Airy Shaw: A first study from Kashmir Himalaya. *Frontiers in Plant Science*. 2022;13:1–16. Available from: https://dx.doi.org/10.3389/fpls.2022.937946.
23. Motwani DN, Vignesh A, Raja K, Selvakumar S, Vasanth K. Exploration of phytochemicals and probing potential effects of Priva cordifolia active extract on PACAP 38 and its nociceptor in the human trigeminovascular system. *3 Biotech*. 2023;13(2). Available from: https://dx.doi.org/10.1007/s13205-023-03462-w.
24. Bhardwaj K, Sharma R, Cruz-Martins N, Valko M, Upadhyay NK, Kuča K, et al. Studies of Phytochemicals, Antioxidant, and Antibacterial Activities of Pinus gerardiana and Pinus roxburghii Seed Extracts. *BioMed Research International*. 2022;2022:1–10. Available from: https://dx.doi.org/10.1155/2022/5938610.
25. Anilan A, Silpa IS, Sona CA, Gopika KS, Haneefa MF, Jayakumar A. GC-MS analysis of ethanolic inflorescence extract. *World Journal of Pharmaceutical Research*. 2022;11(6):882–888. Available from: https://wjpr.s3.ap-south-1.amazonaws.com/article_issue/5d3d34d79363696dd6a81454fd7d0170.pdf.
26. Nabi M, Zargar MI, Tabassum N, Ganai BA, Wani SUD, Alshehri S, et al. Phytochemical Profiling and Antibacterial Activity of Methanol Leaf Extract of Skimmia anquetilia. *Plants*. 2022;11(13):1–10. Available from: https://dx.doi.org/10.3390/plants11131667.
27. Li Z, Wan J, Zhang Y, Dang C, Pan F, Fu J. Influences of petroleum hydrocarbon pyrene on the formation, stability and antibacterial activity of natural Au nanoparticles. *Science of The Total Environment*. 2021;795. Available from: https://doi.org/10.1016/j.scitotenv.2021.148813.
28. Premakumari JV, Gopinath MJ, Narmadha B. Comparative Analysis of Latex Plants by GC-MS using Methanol Extraction. *Mass Spectrometry Letters*. 2023;14(1):9–23. Available from: https://doi.org/10.5478/MSL.2023.14.1.9.
29. Arulvendhan V, Bhavan PS, Rajaganesh R. Molecular Identification and Phytochemical Analysis and Bioactivity Assessment of Catharanthus roseus Leaf Extract: Exploring Antioxidant Potential and Antimicrobial Activities. *Applied Biochemistry and Biotechnology*. 2024;p. 1–28. Available from: https://doi.org/10.1007/s12010-024-04902-w.
30. Ramírez-Gómez XS, Jiménez-García SN, Campos VB, Campos MLG. Plant Metabolites in Plant Defense Against Pathogens. In: Topolovec-Pintarić S, editor. Plant Diseases - Current Threats and Management Trends;vol. 15. IntechOpen. 2019;p. 49–68. Available from: https://www.intechopen.com/chapters/68107.
31. Wang SH, Yang KY, Sheu CC, Chen WC, Chan MC, Feng JY, et al. Efficacies of Colistin–Carbapenem versus Colistin–Tigecycline in Critically Ill Patients with CR-GNB-Associated Pneumonia: A Multicenter Observational Study. *Antibiotics*. 2021;10(9):1–11. Available from: https://doi.org/10.3390/antibiotics10091081.
32. Forconesi GV, Banfi L, Basso A, Lambruschini C, Moni L, Riva R. Synthesis of Polyoxygenated Heterocycles by Diastereoselective Functionalization of a Bio-Based Chiral Aldehyde Exploiting the Passerini Reaction. *Molecules* . 2020;25(14):1–22. Available from: https://doi.org/10.3390/molecules25143227.
33. Kaul TN, Jr MDEM, Ogra PL. Antiviral effects of flavonoids on human viruses. *Journal of Medical Virology*. 1985;15(1):71–79. Available from: https://doi.org/10.1002/jmv.1890150110.
34. Valgas C, De Souza SM, Smânia EFA, Jr AS. Screening methods to determine antibacterial activity of natural products. *Brazilian Journal of Microbiology*. 2007;38(2):369–380. Available from: https://doi.org/10.1590/S1517-83822007000200034.