



ISSN: 2075-6240

Phytochemical analysis and antimicrobial potential of *Bauhinia tomentosa* leaf extracts

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ABSTRACT

Herbal medications have high demand in both advanced and budding nations because of their increased bioavailability and minimal side effects. In the present study, the ethanolic and acetone extracts from *Bauhinia tomentosa* leaf were investigated for their antibacterial potential against Gram-positive (*Staphylococcus aureus*), Gram-negative bacteria (*Klebsiella pneumoniae*, *Escherichia coli*, and *Pseudomonas aeruginosa*) and yeast (*Candida albicans*). Phytochemical examination revealed the presence of diverse secondary metabolites, such as flavonoids, alkaloids, phenolic compounds, tannins, and saponins in leaf extracts. GC-MS analysis detected 15 chemical constituents in the extracts, with the major compounds such as 2-Phenyl-1-3- Oxazol, Caryophyllene, dodecanoic acid, d-glycero-d-galacto-haptose, Phytol, Tetradecanoic acid, 1-Hexacosanol, Isophytol, Oleic acid, 7H-Purine-2-amine, 7-methyl, and eicosane. Antibiotics study have been used to explore drug resistance in pathogens. These extracts exhibited concentration-dependent antibacterial activity against the tested bacterial strains. The acetone displayed higher antibacterial activity than the ethanol extract, which could be attributed to the efficiency of the solvent extract in extracting the bioactive compounds. The findings of this study offer valuable information regarding the phytochemical composition and antibacterial potential of *B. tomentosa* leaf extract. The bioactive compounds identified through GC-MS analysis may be responsible for the observed antibacterial activity. Furthermore, the leaf extracts were non-toxic, and their potent antibacterial effects may be attributed to the presence of bioactive phytoconstituents. Future studies may contribute to the development of *B. tomentosa* based antimicrobial agents with potential therapeutic applications.

KEYWORDS: *Bauhinia tomentosa*, Phytochemicals, GCMS, Ethanol, Acetone extracts, Human health

Received: June 26, 2023
Revised: February 20, 2024
Accepted: February 24, 2024
Published: March 23, 2024

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INTRODUCTION

Multidrug Resistance (MDR) presents a considerable hurdle in healthcare, with the emergence of drug-resistant microorganisms limiting the effectiveness of conventional medications (Kabeerdass *et al.*, 2021). In the search for alternative solutions, plant-based medicines have gained attention for their potential to combat MDR. Traditional medical systems in the worldwide have long relied on plants for disease prevention and treatment (Balabhaskar & Vijayalakshmi, 2021). Phytochemicals possessing antimicrobial properties are

important resources for the discovery of new medicines (Zhang *et al.*, 2015). Medicinal plants have played an essential role in drug development, with many plant-derived compounds and derivatives showing clinical applications in various diseases in recent decades (Janaki *et al.*, 2018).

B. tomentosa, commonly known as the yellow bell orchid tree, is a small tree with multiple stems and slender twigs belonging to the *Caesalpinaceae* family. Although it is primarily valued for its ornamental qualities, some traditional medicinal uses have been associated with *B. tomentosa*. The root bark is used

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internally to address large intestinal conditions, while the flower has been used as a remedy for dysentery and diarrhea. The leaves have been recognized for their antidiabetic properties, and various parts of the plant, in combination with other drugs, are recommended for the treatment of scorpion stings and snake bites (Gautam *et al.*, 2012).

In traditional medicine, various parts of the plant, such as the leaves, bark, and flowers, have been used to alleviate inflammation and associated symptoms. Traditional remedies include the use of plant extracts to treat arthritis, joint pain, and skin inflammation. Some studies have identified bioactive compounds in *B. tomentosa* extract that exhibit antimicrobial effects and inhibit the growth of bacteria, fungi, and viruses. The plant contains flavonoids and other phenolic compounds, which are known for their antioxidant properties. In addition, this plant exhibits hepatoprotective (liver-protecting) effects, in which plant extracts may help to maintain liver health and protect it from certain toxins. In traditional medicine, this plant extract has been typically used for wound healing. Leaves or extracts are sometimes applied to wounds or injuries to aid the healing process (Renganathan *et al.*, 2021). Hence, the present study aimed to investigate the antibacterial and antifungal potential of ethanolic and acetone extracts from *B. tomentosa* leaves, as well as to detect the phytochemical constituents of this plant.

MATERIALS AND METHODS

Collection and Preparation of Plant Extract

Fresh leaves of *Bauhinia tomentosa* were collected and subsequently cleaned with tap water before being left to air-dry for three–four weeks (Figure 1). Once dried, plant parts were powdered using a mixer grinder and stored in a tightly sealed container. A total of 70 g of powdered leaf material was successively extracted using ethanol and acetone in a Soxhlet apparatus for 8 h. The resulting extracts were concentrated using a rotary flash evaporator and preserved in an airtight bottle at 4 °C until further use (Janaki *et al.*, 2018).

Phytochemical Analysis

The concentrated extract of the *B. tomentosa* leaves was subjected to phytochemical analysis to examine its antimicrobial properties. The investigation involved studying

the phytochemicals obtained from plants. Qualitative phytochemical analysis plays a crucial role in determining the composition and chemical profile of the extracts (Abirami & Maghima, 2019). This analysis was employed as common precipitation and coloration reactions to identify major natural phytochemical groups, such as flavonoids, alkaloids, phenols, proteins, saponins, terpenoids, tannins, starch, and steroids, present in the crude extracts. By observing the reactions during this analysis, the presence or absence of these compounds in the plant extracts was determined. Qualitative phytochemical screening was conducted according to established procedures (Harbourne, 1998).

Test for Alkaloids

Mayer's test

To prepare the reagents, 1.36 g of HgCl_2 and 5 g of KI were separately dissolved in 60 mL and 10 mL of distilled water, respectively. The solvent solutions were then combined and diluted with purified water to a total volume of 100 mL. A small amount of the reagent was added to one ml of the leaf extract, resulting in the formation of a colorless precipitate, which indicated the presence of alkaloids (Abirami & Maghima, 2019).

Test for flavonoids

A few drops of diluted sodium hydroxide were introduced to one ml of the leaf extract. This caused the crude plant extract to exhibit a vibrant yellow color, which turned colorless upon the subsequent addition of a few drops of diluted acid. This color change indicated the presence of flavonoids in the extract (Kancherla *et al.*, 2019).

Test for phenols

For the phenol test, 1 mL of the extract was mixed with 2 mL of purified water, and then a few drops of a 10% aqueous FeCl_3 solution were added. The presence of phenols is indicated by the formation of blue or green precipitates (Abirami & Maghima, 2019).

Test for proteins

In the protein test (biuret test), 2 mL of leaf extract was mixed with two drops of 3% copper sulfate and a few drops of 10%



Figure 1: *Bauhinia tomentosa* leaf

sodium hydroxide. The presence of proteins was confirmed by the development of a violet or red color (María *et al.*, 2018).

Test for saponins

For the saponins test, 5 mL of purified water was placed in a test tube, and added 0.2 g of leaf extract in it. After blending the mixture and allowing it to stand for 3 min, the presence of saponins was indicated by the formation of a froth resembling a honeycomb (Evans *et al.*, 2014).

Test for terpenoids

For the terpenoid test (Salkowski test), 5 mL of the extract obtained from various solvents was combined with 2 mL of chloroform. Subsequently, 3 mL of concentrated H₂SO₄ was carefully added. The presence of terpenoids was confirmed by the appearance of a reddish-brown layer at the boundary (Abirami & Maghimaa, 2019).

Test for tannins

In the tannin test (lead acetate test), 5 mL of leaf extract was mixed with a small amount of 1% lead acetate. The presence of tannins was suggested by the formation of a yellow or red precipitate.

FeCl₃ test

The presence of tannins can be determined by combining 2 mL of leaf extract with 2 mL of FeCl₃, resulting in the formation of a precipitate that appears either blue or black (Liu *et al.*, 2014).

Test for steroids

In the steroid test (Liebermannburchard reaction), a small amount of crystal was dissolved in chloroform, and concentrated H₂SO₄ was added, followed by 2-3 drops of acetic anhydride solution. Finally, the color of the solution changed from violet to green, suggesting the presence of steroids (Patil *et al.*, 2021).

Test for starch

For the starch test, 1 mL of the leaf extract was mixed with 10 mL of saturated NaCl solution. The mixture was heated and then treated with a starch reagent. The presence of starch is indicated by the development of a blue-purple color (Benmehdi *et al.*, 2012).

Test for carbohydrates

To perform Molisch's test, approximately 500 mg of the crude extract was dissolved individually in 5 mL of distilled water and subsequently filtered. A few drops of Molisch's reagent, consisting of α -naphthol at a concentration of 10% (w/v) in 90% ethanol, were introduced into the filtrates. Subsequently, 1 mL of concentrated H₂SO₄ was carefully poured along the side of the test tube, and after two minutes, 5 mL of distilled water was added to it. The presence of carbohydrates was indicated

by the development of a dull violet or red color at the boundary between the two liquid layers (Aziz, 2015).

Gas Chromatography-Mass Spectroscopy (GCMS) Analysis

The plant extract was analyzed using a GC-MS instrument (Model: GC-MS-QP 2010, Shimadzu, Tokyo, Japan) equipped with a VF 5 ms fused silica capillary column measuring 30 mm in length, 0.25 mm in diameter, and a 0.25 μ m in film thickness. The GC-MS detection employed an electron ionization system with an ionization energy of 70 eV. Helium (99.99 percent) was used as the carrier gas at a constant flow rate of 1.51 mL/min. The injector temperature at 200 °C and the mass transfer line temperature at were kept 240 °C. The oven temperature started at 70-220 °C, has increased at a rate of 10 °C/min, and was held isothermal for 1 min, followed by a final ramp to 300 °C at the same rate. Split mode infusion was conducted with 2 μ L of the sample, and the scan range for mass spectrometry was set from 40 to 1000 m/z. The total runtime for the GC-MS analysis was 35 min. The relative percentages of the extract constituents were determined by peak area normalization. Identification of the test samples involved a double-checking process using the NIST database for name, structure, and molecular weight, as well as evaluation through mass-spectrum GC-MS analysis (Vakayil *et al.*, 2021).

Collection of Specimens

Wound pathogens were collected aseptically from the Department of Microbiology, Government Mohan Kumaramangalam Hospital, Salem, via sterile cotton swabs. The collected swabs were labeled, brought to the research laboratory, and processed instantly.

Microscopic and Biochemical Characterization

In the laboratory, collected swabs were inoculated on plates containing cetrinide agar, EMB agar, CLED agar, MSA agar, and nutrient agar. The plates were incubated for overnight at 37 °C. Growth was then observed on the plates (Kabeerdass *et al.*, 2022a), and the secluded colonies were branded by phenotypic factors such as Gram reaction, motility, germ tube test, and biochemical profiling (Kabeerdass *et al.*, 2022b; Vakayil *et al.*, 2022).

Kirby Bauer Antibiotic Sensitivity Test

Antibiotic disc diffusion (Kirby Bauer) assessment was executed with Chloramphenicol (10 mg), penicillin (10 mg), streptomycin (10 mg), Cephalexin (10 mg), Vancomycin (5 mg), Tetracycline (30 mg), Erythromycin (15 mg), nitrofurantoin (10 mg), and Ampicillin (10 mg). Inoculums (100 μ L) were spread over the surface of Muller Hinton agar (MHA) and dried before application of the antibiotic disc. Antibiotic discs were then located decisively on the MHA agar plates, after which the inoculated plates has maintained for 24 h at 37 °C. The inhibition zone was measured on an electronic scale in millimeters (Baburam *et al.*, 2022).

Antibacterial Activity of *B. tomentosa* Ethanolic and Acetone Leaf Extract

The antibacterial activities of *B. tomentosa* ethanolic and acetone leaf extracts were determined at different concentrations using the agar well diffusion protocol. Extracts were prepared at five different concentrations (25, 50, 75, 100, and 125 µg). The test sample was dissolved in dimethyl sulfoxide (DMSO). DMSO was used as a control. Muller Hinton Agar plates were prepared and swabbed the pathogens over the agar and allowed for drying. Using a cork-borer, the wells with 6 mm in size has impregnated with different concentrations (25, 50, 75, 100, 125 µg) of *B. tomentosa* ethanolic and acetone leaf extracts into the wells using a micropipette (Maghimaa & Alharbi, 2020). The plates were then incubated at 37 °C for 24 h. Finally, the antimicrobial effectiveness of the ethanolic and acetone leaf extracts of *B. tomentosa* was assessed by measuring the diameter of the inhibition zone (ZOI).

Statistical Analysis

The data from antibacterial activity are reported as mean ± standard error of the mean (SEM), whenever possible. Data were evaluated by Student's t-test using GraphPad Software, USA. Statistical significance was set at $P < 0.05$.

RESULT AND DISCUSSION

Screening of Phytochemical Analysis

In the present study, qualitative phytochemical screening of *B. tomentosa* leaves was performed using ethanol and acetone extracts. The results indicate the presence of various phytochemicals, including flavonoids, alkaloids, phenols, proteins, saponins, terpenoids, tannins, starch, and steroids. Alkaloids are widely used in medicinal applications and serve as important indicators for the development of novel synthetic drugs. Their antimicrobial properties are attributed to the inhibition of DNA topoisomerases. Tannins have been found to play a significant role in kidney protection and exhibit potential antiviral, antibacterial, and antiparasitic effects. Saponins found in plants have been suggested as potential anticarcinogens and have positive effects on blood cholesterol levels, cancer, bone health, and immune system stimulation. However, saponins can also cause hemolysis in blood cells. Flavonoids act as antioxidants and enhance the effects of Vitamin C. They are known for their biological activity against liver toxins, tumors, viruses, and other microorganisms. Phenols, predominantly found in the plant kingdom, possess antioxidant properties, which are attributed to their redox properties. The biological activities of phenolic compounds involve the scavenging of free radicals within cells (Balabhaskar & Vijayalakshmi, 2021). The presence of preliminary phytochemicals in the ethanolic and acetone extracts of *B. tomentosa* leaves are shown in Table 1.

Table 1: Phytochemical Analysis of leaf extract

S. No.	Test Name	Positive	Negative
1	Flavonoids	+	
2	Phenols	+	
3	Terpenoids	+	
4	Starch		
5	Saponins	+	-
6	Proteins		
7	Steroids	+	-
8	Tannins	+	

GC-MS Analysis of Ethanol and Acetone Leaf Extract of *B. tomentosa*

In the present study, the leaf extract exhibited a significant number of peaks with corresponding retention times and area percentages. The highest peak in the leaf sample observed at retention times of 22.763, 17.330, 16.119, 15.086, and 14.208, whereas the lowest peaks were detected at retention times of 11.131 and 8.820 (Figure 2). The major bioactive compounds identified in the leaf extract were 2-Phenyl-1-3- Oxazol, Caryophyllne, dodecanoic acid, d-glycero-d-galacto-haptose, Phytol, Tetradecanoic acid, 1-Hexacosanol, Isophytol, Oleic acid, 7H-Purine-2-amine,7-methyl, Eicosane, 2,4,4-Trimethyl-3-hydroxymethyl, Pentadecane, Pyridine-3-carboxamide, 2,6-10 Dodecatrine-1-ol, 2,6,10,14-Hexadecane (Table 2). These phytochemicals exhibit various pharmacological functions, such as antimicrobial, antioxidant, and anti-inflammatory activities. The Gas chromatogram provided information on the relative concentrations of the different compounds eluted at specific retention times. The height of each peak indicates the relative concentration of the corresponding component in the *B. tomentosa* leaf extract.

Microscopic and Biochemical Profiling

Pathogens procured from the wound specimens were identified based on phenotypic and biochemical characterization. The phenotypic characteristics of these isolates were small, circular, shiny, convex, large, opaque, swarming, smooth, dome-shaped, translucent, tiny, and mucoid colonies. Gram-negative and gram-positive bacterial pathogens were identified under microscopic evaluation. Biochemical analysis was performed using IMVIC- (Indole, MR, VP, and Citrate test), motility, catalase, oxidase, coagulase, carbohydrate and germ tube test, triple sugar iron, H₂S, and urease tests. The predominant isolates were identified based on these as *C. albicans*, *E. coli*, *P. aeruginosa*, *K. pneumonia* and *S. aureus*. In which *E. coli* and *P. aeruginosa* are motile also *S. aureus* and *K. pneumonia* are non-motile (Table 3). All the pathogens were Gram-negative except *C. albicans* and *S. aureus*, which is Gram-positive. These findings were consistent with those of Vakayil et al. (2021a).

Antibiotic Sensitivity Test

According to the antibiotic sensitivity test results, all procured organisms showed resistance to Cephalixin, Vancomycin,

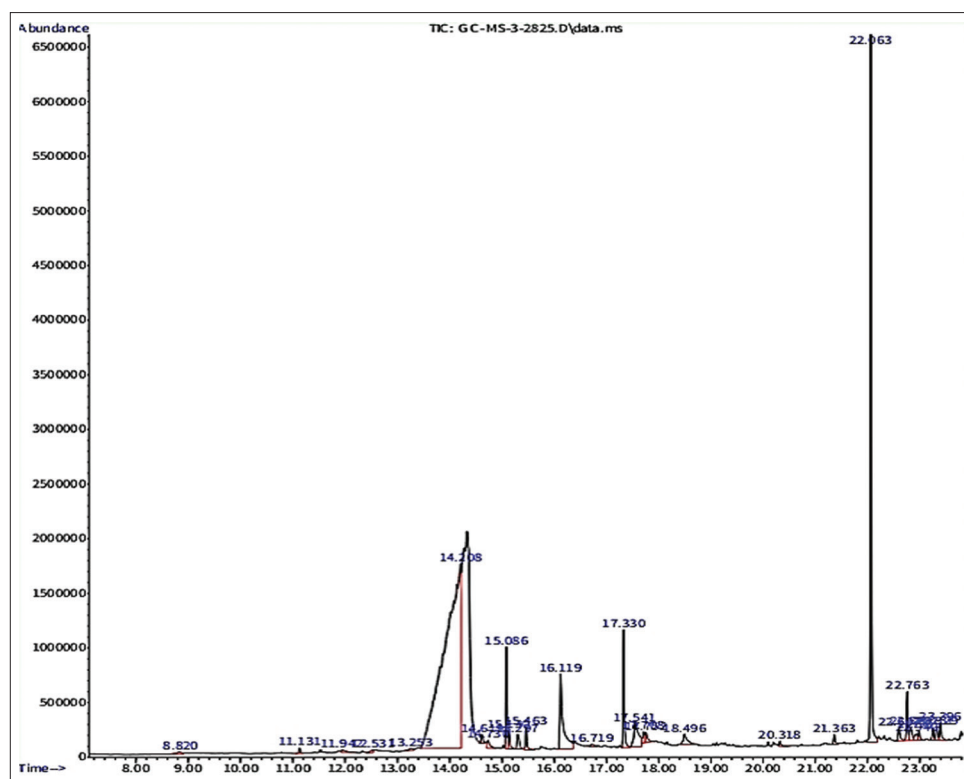


Figure 2: GC-MS analysis of *B. tomentosa* leaf extract

Table 2: Detection of Phytochemical compounds from *B. tomentosa* leaf extract

S. No.	RT	PhytoCompound	Molecular formula	Molecular Weight (g/mol)	Peak Area %
1	8.820	2-Phenyl-1-3-Oxazol	C ₈ H ₆ N ₂ O	14.15	0.19
2	11.131	Caryophyllene	C ₁₅ H ₂₄	204.36	0.19
3	12.531	Dodecanoic Acid	C ₁₂ H ₂₄ O ₂	200.317	0.18
4	13.253	d-Glycero-d-galacto-haptose	C ₇ H ₁₄ O ₇	210.18	0.18
5	15.463	Phytol	C ₂₀ H ₄₀	128.170	0.79
6	16.119	Tetradecanoic acid	C ₁₄ H ₂₈ O ₂	228.376	4.88
7	16.719	1-Hexacosanol, Isophytol	C ₂₆ H ₅₄ O	382.7	2.94
8	17.524	Oleic acid	C ₁₈ H ₃₄ O ₂	282.47	2.36
9	20.318	7H-Purine-2-amine, 7-methyl	C ₆ H ₇ N ₅	149.153	0.18
10	21.363	Eicosane	C ₂₀ H ₄₂	282.54	0.32
11	22.829	2,4,4-Trimethyl-3-hydroxymethyl	C ₁₅ H ₂₆ O	222.366	0.60
12	22.985	Pentadecane	C ₁₅ H ₃₂	212.42	0.29
13	23.252	Pyridine-3-carboxamide	C ₆ H ₈ N ₂ O ₂	122.12	0.42
14	23.329	2,6,10-Dodecatrine-1-ol	C ₁₅ H ₂₆ O	344.5	0.45
15	23.396	2,6,10,14-Hexadecane	C ₂₀ H ₄₂	282.55	0.61

Tetracycline, Chloramphenicol, Ampicillin, and Penicillin. *P. aeruginosa* was sensitive to streptomycin (10 mg) at a diameter of 16 mm; *S. aureus* and *E. coli* were sensitive to nitrofurantoin (10 mg) at diameters of 25 mm and 13 mm, respectively (Table 4). The emergence of drug-resistant pathogens poses a significant challenge in the healthcare setting, leading to the increased mortality and morbidity. Studies have revealed variations in drug resistance profiles among hospitals worldwide, with a high prevalence of multidrug resistance in bacterial infections.

The emergence of multidrug-resistant strains poses a significant challenge in the healthcare setting. To address this issue, it is crucial to consider recent studies that explore the prevalence of resistance and potential treatment options. Recent studies have highlighted the global rise in multidrug resistance, including resistance to *P. aeruginosa*, a common pathogen associated with nosocomial infections. In a study by Pachori *et al.* (2019), which focused on *P. aeruginosa* infections in intensive care units, high rates of multidrug resistance were observed, emphasizing the need for alternative treatment options. Regarding the sensitivity of *P. aeruginosa* to streptomycin, it is essential to consider its clinical relevance and efficacy. Breidenstein *et al.* (2020) investigated the use of aminoglycosides including streptomycin for the treatment of *Pseudomonas* infections. This highlights the potential benefits of combining aminoglycosides with other antimicrobial agents to enhance their efficacy against multidrug-resistant strains. In the case of *S. aureus* and *E. coli*, both of which are sensitive to nitrofurantoin, recent research have explored the effectiveness of this antibiotic against bacterial infections. Chanda *et al.* (2019) evaluated the susceptibility patterns of bacterial pathogens and found that nitrofurantoin is a reliable treatment option owing to its low resistance rates. To address the issue of multidrug resistance, it is important to consider alternative approaches beyond traditional antibiotics. Koo and Seo (2021) explored the potential of antimicrobial peptides as alternatives to conventional antibiotics, highlighting their diverse mechanisms of action and lower likelihood of resistance development. Antibiotic sensitivity test results indicated the presence of multidrug resistance in the tested organisms. Recent studies have emphasized the global challenge

of multidrug resistance and the need for alternative treatment strategies. Further research into novel antimicrobial agents and combination therapies can effectively combat multidrug-resistant strains.

Antimicrobial Activity of *B. tomentosa* Ethanolic and Acetone Leaf Extract

The antimicrobial activity of ethanolic and acetone extracts of *B. tomentosa* exhibited a considerable zone of inhibition of 12-23 mm for all isolates. The ethanol extract of *B. tomentosa* at 125 µg showed the highest antimicrobial activity against the tested microorganisms in the following order: *S. aureus* (23 mm), *E. coli* (20 mm), *P. aeruginosa* (20 mm), *C. albicans* (20 mm), and *K. pneumoniae* (16 mm) (Table 5). The acetone extract of *B. tomentosa* at a concentration of 125 µg showed the highest antimicrobial activity against the tested microorganisms, in the following order: *S. aureus* (19 mm), *E. coli* (19 mm), *P. aeruginosa* (19 mm), *C. albicans* (20 mm), and *K. Pneumonia* (15 mm) (Table 5). In comparison, the acetone extracts of *S. aureus*, *E. coli*, *P. aeruginosa*, and *K. pneumoniae* showed greater inhibition, and the ethanolic extract of *C. albicans* showed maximum inhibition (Table 5). *B. tomentosa* leaf extract showed a promising effect against these wound pathogens.

Recent studies have focused on exploring the antimicrobial potential of natural plant extracts, including those derived from *B. tomentosa*. These studies have provided valuable insights into the efficacy of plant-based compounds against various microorganisms. A study by Neena et al. (2018) investigated the antimicrobial activity of *B. tomentosa* extracts and found them to exhibit potent inhibitory effects against several bacterial strains, including *S. aureus*, *E. coli* and *Vibrio cholerae*. These results align with the current findings, emphasizing the broad-spectrum antimicrobial properties of the *B. tomentosa* extracts. Moreover, another study by Singh et al. (2023) evaluated the antimicrobial activity of *B. tomentosa* leaf extracts against clinical isolates, including *Enterobacillus*, *Micrococcus*, *K. pneumoniae*, *Streptococcus thermophilus* and *Haemophilus influenza*. These findings demonstrated significant inhibition of these pathogens, supporting the results observed in the current study.

The antifungal potential of *B. tomentosa* has been documented in previous research on the maximum antimicrobial activity of the ethanolic extract against *C. albicans*. Gopalakrishnan and Vadivel (2011) evaluated the antifungal activity of *B. tomentosa* leaf extracts and identified their efficacy against *Candida* species. The current findings corroborate these observations, highlighting the promising effects of *B. tomentosa* extracts against *C. albicans*. The antimicrobial properties of plant

Table 3: Identification of bacterial isolates using Microscopic and biochemical studies

S. No.	Bacteria	Grams staining	Motility	Indole	MR	VP	Citrate	Urease	H ₂ S production
1	<i>P. aeruginosa</i>	Gram Negative Rods	Motile Unipolar	-ve	-ve	-ve	+ve	-ve	-ve
2	<i>E. coli</i>	Gram Negative Rods	Motile	+ve	+ve	-ve	-ve	-ve	-ve
3	<i>K. pneumoniae</i>	Gram Negative Rods	Non-motile	-ve	-ve	+ve	+ve	+ve	-ve
4	<i>S. aureus</i>	Gram Positive Cocci in clusters	Non-motile	-ve	+ve	+ve	+ve	+ve	-ve

MR-Methyl Red; VP-Voges Proskauer

Table 4: Antibiotic sensitivity analysis of the isolated pathogens

S. No.	Name of the antibiotic	Zone of inhibition (mm)				
		<i>P. aeruginosa</i>	<i>E. coli</i>	<i>K. pneumoniae</i>	<i>S. aureus</i>	<i>C. albicans</i>
1	Cephalexin (CE) 10 mg	6±0.764	6±0.764	6±0.764	6±0.764	6±0.764
2	Vancomycin (Va) 5 mg	6±0.764	6±0.764	6±0.764	6±0.764	6±0.764
3	Streptomycin (S) 10 mg	16±0.500	8±0.500	6±0.764	6±0.764	7±0.500
4	Tetracycline (T) 30 mg	6±0.764	6±0.764	6±0.764	6±0.764	6±0.764
5	Chloramphenicol (c) 10 mg	6±0.764	6±0.764	6±0.764	6±0.764	6±0.764
6	Erythromycin (e) 15 mg	6±0.764	6±0.764	6±0.764	6±0.764	10±1.000
7	Nitrofurantoin (n) 10 mg	10±1.000	13±0.300	8±0.500	25±0.320	6±0.764
8	Ampicillin (Amp) 10 mg	6±0.764	6±0.764	6±0.764	6±0.764	6±0.764
9	Pencillin G (P) 10 mg	6±0.764	6±0.764	6±0.764	6±0.764	6±0.764

Table 5: Antimicrobial activity of *B. tomentosa* leaf ethanolic and acetone extract at different concentration

S. No.	Pathogen name	Zone of inhibition (mm)									
		Ethanolic extract in µg					Acetone extract in µg				
		25	50	75	100	125	25	50	75	100	125
1	<i>S. aureus</i>	12±0.500	14±1.000	15±0.500	17±0.600	19±0.500	12±0.500	13±0.200	16±2.000	18±0.700	23±0.305
2	<i>E. coli</i>	12±0.500	14±1.000	15±0.500	17±0.600	19±0.500	12±0.500	13±0.200	16±2.000	18±0.700	20±1.000
3	<i>P. aeruginosa</i>	12±0.500	13±0.200	15±0.500	18±0.700	19±0.500	13±0.200	15±0.500	17±0.600	19±0.500	20±1.000
4	<i>K. pneumoniae</i>	10±0.500	12±0.500	13±0.200	14±1.000	15±0.500	9±0.400	10±0.500	12±0.500	14±1.000	16±2.000
5	<i>C. albicans</i>	12±0.500	13±0.200	14±1.000	18±0.700	20±1.000	10±0.500	12±0.500	13±0.200	14±1.000	18±0.700

extracts can be attributed to their phytochemical constituents, including phenolic compounds, flavonoids, and terpenoids, which possess antimicrobial activities. These compounds act through various mechanisms, such as the disruption of microbial cell membranes, inhibition of enzymatic activity, and interference with cellular processes.

CONCLUSION

In conclusion, the results demonstrated the significant antimicrobial activity of *B. tomentosa* ethanol and acetone extracts against a range of pathogenic microorganisms. Recent studies support these findings and emphasize the potential of *B. tomentosa* as a source of natural antimicrobial agents. Continued research on the isolation and characterization of active compounds from *B. tomentosa* could lead to the development of novel therapeutic agents for combating wound pathogens. Gas chromatography-mass spectrometry analysis of the ethanolic and acetone extracts of *B. tomentosa* revealed the presence of diverse bioactive compounds, including alkaloids, flavonoids, phenolics, terpenoids, and tannins. These compounds are known to have potential therapeutic properties. GC-MS analysis of the extracts identified several chemical constituents. The results showed the presence of a diverse range of compounds such as alkaloids, terpenoids, flavonoids, and other secondary metabolites. The specific compounds and their concentrations varied between ethanol and acetone extracts. The antibacterial activity of *B. tomentosa* ethanol and acetone extracts were assessed against different bacterial strains. The extracts demonstrated significant antibacterial activities at various concentrations. The efficacy of the extracts varied depending on the concentration used and the bacterial strain tested. These findings suggest that *B. tomentosa* extracts possess promising antimicrobial properties, likely due to the presence of bioactive compounds identified through phytochemical and GC-MS analyses. Further investigations are to explore the underlying mechanisms of action and potential applications of these extracts in the fields of medicine and healthcare.

ACKNOWLEDGMENT

The authors are thankful for the DST–FIST Centralized laboratory, Muthayammal College of Arts and Science, Rasipuram, Namakkal, Tamil Nadu, India for executing the instrumentation facilities.

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