

Harnessing the Antibacterial Potential of *Sphagneticola trilobata* Combat Human Bacterial Infections

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Abstract

This study investigates the antibacterial potential of *Sphagneticola trilobata* leaf extract against various clinically important human pathogens. The methanolic extract exhibited notable antibacterial activity, with the highest zone of inhibition measuring 21 mm against *Salmonella typhi* at a concentration of 100 µg/mL. The minimum inhibitory concentration (MIC) values for pathogens, including *S. typhi*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Vibrio cholerae*, *Klebsiella oxytoca*, *Escherichia coli*, *Salmonella Paratyphi*, *Proteus mirabilis*, and *Vibrio parahaemolyticus*, were found to range from 40 to 100 µg/mL. Correspondingly, the minimum bactericidal concentration (MBC) values varied between 20 and 100 µg/mL. Phytochemical screening, supported by FT-IR and GC-MS analyses, revealed the presence of several bioactive functional compounds within the extract. The extract of broad-spectrum antibacterial activity underscores its potential as a valuable candidate for the development of novel antibacterial agents. Further research is necessary to clarify its mechanisms of action and assess its applicability in therapeutic contexts.

Keywords: *Sphagneticola trilobata*, antibacterial activity, methanolic extract, human pathogens, bioactive compounds.

Introduction

Bacterial infections continue to present major public health concerns across the globe. Diseases such as typhoid fever and diarrhea are predominantly caused by *Salmonella* species and *Vibrio cholerae*, with the latter being the causative agent of

cholera. In addition, a broad spectrum of other pathogenic bacteria, including *Staphylococcus aureus*, *Serratia* spp., *Streptococcus pyogenes*, *Escherichia coli*, and *Pseudomonas aeruginosa*, are responsible for a range of severe infections in humans [1]. The impact of these infections is particularly severe in developing countries, where inadequate primary healthcare systems and poor sanitation hinder effective disease prevention, making treatment both challenging and costly [2]. The widespread occurrence of bacterial diseases has also led to an increased reliance on antibiotics [3]. However, various plant species have been found to contain bioactive compounds with significant medicinal properties. Numerous plantparts are known to produce secondary metabolites exhibiting diverse physiological and therapeutic effects. In this context, *Sphagneticola trilobata* (formerly known as *Wedelia trilobata*) has been extensively studied for its antibacterial activity. Its leaves contain bioactive chemicals that researchers have extracted using solvents like methanol, ethanol, and ethyl acetate. These compounds have shown significant antibacterial benefits against clinical infections. Although reclassified as *S. trilobata*, the plant remains widely recognized in traditional medicine under the name *Wedelia*, and is commonly referred to as *picão-da-Praia*, *vedalia*, and *malmequer* in Brazil[4]. This herbaceous plant, a member of the *Asteraceae* family, is native to Brazil and is known for its rapid growth and ability to form dense ground cover. It thrives in a variety of soil conditions and is often found in shaded environments, coastal areas, and abandoned sites due to its adaptability[5]. The plant is a rich source of

secondary metabolites, making it highly valuable for pharmacological research [6]. Traditional Indian medicine has long employed *W. trilobata* to treat a wide range of ailments, including fever, malaria, rheumatism, chronic wounds, inflammation, arthritis, ulcers, sore throat, varicose veins, epilepsy, amenorrhea, snakebites, kidney disorders, hepatitis, colds, and indigestion, as reported by Meena et al. (2011)[7][8]. Further studies by Shanmuganathan et al. (2016) have confirmed its pharmacological potential, noting its wound healing, anthelmintic, antibacterial, antioxidant, anti-inflammatory, and anticancer properties[9]. Because of this, scientists are becoming more interested in assessing medicinal plants for a variety of biological activities, especially those that have potent antibacterial and antifungal properties.[10–13]. According to Igbinosa et al. (2009), approximately 80% of individuals in developing nations rely on traditional medicine that incorporates plant-derived compounds[14]. The rise of antibiotic-resistant bacteria has further intensified interest in plant-based alternatives with potent antibacterial capabilities.

Despite the widespread use of antibiotics, many are associated with adverse side effects, including nausea, bone marrow suppression, thrombocytopenic purpura, and agranulocytosis, which may occasionally lead to serious health complications[15, 16]. These restrictions have accelerated the global quest for new antimicrobial agents from natural sources, especially medicinal plants, as well as the rising prevalence of antibiotic resistance[17]. While advances in medical science have improved disease management, the ability of pathogens to adapt and develop resistance has significantly reduced the effectiveness of many existing antibiotics. Unnikrishnan et al. (2023) emphasized that current pharmaceutical solutions are often inadequate to meet the therapeutic demands of a growing population[18]. The escalating resistance of microbial pathogens to conventional antimicrobial drugs has, in turn, led to the increased use of combination

therapies involving multiple antibiotics for single treatments, as reported by Fair et al. (2014)[19]. This trend is particularly concerning for immunocompromised individuals, who are more susceptible to resistant infections. Moreover, the discovery and development of new synthetic drugs remain time-consuming, labor-intensive, and costly. Consequently, medicinal plants continue to garner significant research interest, as they have long served as reservoirs of bioactive compounds with therapeutic potential. Although not every plant used in traditional healing systems has been scientifically validated, many are believed to contain promising chemical constituents that may lead to the development of new drugs. Considering its wide use in traditional medicine and reported healing properties, *Sphagneticola trilobata* was selected for this study. The focus is on assessing the antibacterial activity of its methanolic leaf extract against common clinical pathogens. This approach aims to uncover natural compounds with effective antimicrobial action. With antibiotic resistance on the rise, plant-based alternatives are becoming increasingly important. The study aims to reconcile scientific validation with traditional wisdom. Overall, it shows that *S. trilobata* has the potential to be a viable source of new antibacterial compounds.

Materials and Methods

Collection and classifying plants

The plant *Sphagneticola trilobata* was collected from Saveetha Institute of Medical and Technical Sciences, Saveetha Nagar, Thandalam, Chennai- 602105.

Preparation of Plant Extract

The *Sphagneticola trilobata* leaves were cleaned using both distilled and tap water. After being shade-dried, the plant leaf was ground into a powder. After soaking for 48 hours, they ground 250 millilitres of methanol and 100 milligrammes of leaves. Following filtering, the solutions were utilised once more to assess their antibacterial properties.

Phytochemical analysis

The phytochemical analysis aimed at identifying various bioactive compounds present in the leaf extracts of *Sphagneticola trilobata*. In accordance with established protocols outlined by Leite, *et al.*[20], as well as Harborne[21], standard procedures were diligently followed. These methods are widely recognized and respected in the field for their reliability and comprehensiveness.

Alkaloids Test (Wagner's reagent)

Wagner's reagent, which consists of 1.27g of iodine and 2g of potassium iodide dissolved in 100ml of water, was applied to a portion of the extract. The goal of the observation was to look for the development of a reddish-brown precipitate or colouration, which would indicate the presence of alkaloids.

Flavonoids test

2ml of the extract underwent treatment with a small amount of 20% sodium hydroxide solution. Flavonoids were identified by the appearance of a bright yellow colour that intensified during interaction and then turned colourless following the addition of diluted hydrochloric acid.

Saponins (Foam Test)

To assess the presence of saponins, a method involving combination creation was devised. One of the test procedures involved observing the stability of foam over a 15-minute period. This approach served as an indicator of saponin content, as saponins are known for their ability to produce stable foam when agitated. Thus, the longevity of the foam served as a qualitative measure for detecting the presence of saponins in the tested samples.

Terpenoids (Salkowski's test)

A reddish-brown precipitate quickly formed when 1 millilitre of chloroform and a few drops of strong sulphuric acid were added to 2 millilitres of each extract, confirming the presence of terpenoids.

Steroids (Liebermann Burchard test)

Following the process of boiling and subsequent cooling, a small quantity of acetic anhydride was added to the crude extract. Concentrated sulfuric acid was then carefully introduced into the test tube, causing it to coat the sides. Observations were made at the interface between the two layers for the appearance of browning. The manifestation of a green hue in the upper layer signified the presence of steroids.

Tannins (Braymer's test)

10% alcoholic ferric chloride solution was applied to 2 milliliters of extract. Tannins are present when a solution takes on a blue or greenish hue. Examine Quinones A little quantity of extract was subjected to intense HCL treatment, and the production of a yellow precipitate or colouring was monitored.

Phenols (Ferric chloride test)

Three millilitres of distilled water were combined with one millilitre of the solvent extract, and then a few drops of a neutral 5% FeCl₃ solution were added. Phenolic chemicals were present because a dark green colour formed.

FT-IR Spectroscopic analysis of methanol extract

Infrared spectroscopy involves the absorption of electromagnetic radiation within the infrared portion of the spectrum, which subsequently alters the vibrational energy of molecules. This alteration occurs because molecules commonly vibrate through processes like stretching and bonding. The methanol extract was analyzed using a thermos scientific. For further spectrum analysis. Spectra were then obtained between wave numbers 4000 and 500 cm⁻¹. Bećet *al.*, [22].

GC-MS analysis of extract

The compounds in the ethanolic extract were analysed using a Parkinson-Elmer Clarus 500 gas chromatograph-mass spectrometer and an Elite-5MS

capillary column (5% phenyl, 95% dimethylpolysiloxane). The injector temperature in the oven program was 280°C. The temperature programs were originally set at 50°C, ramped up to 220°C for two minutes at a rate of 8°C per minute, and then increased to 280°C for ten minutes at a rate of 7°C per minute. Helium, the experiment's carrier gas, flowed at a rate of millilitres per minute. The compounds were identified by comparing them with the NIST 2005 collection after the sample was injected.

Antimicrobial Activity

Bacterial strains

The Microbial Culture Maintenance Laboratory in the Department of Medical Microbiology at Saveetha Medical College, Saveetha University, Tamil Nadu, is where the bacterial strains used in this investigation were obtained. Among these strains were *Klebsiella oxytoca*, *Vibrio cholerae*, *Proteus mirabilis*, *Vibrio parahaemolyticus*, *Escherichia coli*, and *Staphylococcus aureus*.

Inoculum preparation

The nutritional broth was prepared in a test tube and autoclaved for 15 minutes at 15 pounds of pressure. Each bacterial strain was separately inoculated into the sterile nutrient broth, and the mixture was then grown for a full day at 37°C.

Antibacterial activity

The *Sphagneticola trilobata* methanol extract's antibacterial capability was evaluated using the standard agar disc diffusion technique and the minimum inhibitory concentration (MIC) assay, in accordance with the guidelines set out by the Clinical and Laboratory Standards Institute (CLSI, 2015). The Hannan (2000) method was used to test for *Mycoplasma gallisepticum*.

Agar well diffusion method

According to Seedevi et al. (2022), the agar well diffusion method was used to measure the antibacterial activity[23]. The cultures that had been cultivating for twenty-

four hours were aseptically swabbed in nutrient agar plates using a sterile cotton swab. Using a sterile 5-mm well cutter, the swabbed plates were perforated. A stock solution in 10% dimethyl sulfoxide was made by mixing methanolic extract with 1 mg/ml polysaccharide. Four distinct concentrations were used: 25, 50, 75, and 100 g/ml. One control was put into each of the matching wells.

Minimal Inhibitory Concentration (MIC)

The methanol extract was made at quantities ranging from 21mm 100 µg/ml using two-fold serial dilutions. Nine milliliters of Muller Hinton agar were combined with one milliliter of each dilution. The plates with different concentrations of the extract were spotted with ten microliters of each standardized broth culture (1.5×10^8 CFU/mL). After that, the plates were incubated in accordance with each organism's development needs, and any apparent bacterial growth was noted. The MIC was the extract concentration at which there was no discernible growth on the agar's surface.

Minimum bactericidal concentration (MBC)

For the purpose of determining the MBC, blocks of agar plates that did not develop following MIC testing were inoculated with new nutrient broth, which served as the recovery medium. The broths were incubated in accordance with each organism's requirements for growth. The recovery medium's lack of turbidity served as proof that the bacteria had been killed.

Statistical Analysis

The averages $\pm$ SD were used to represent the calculated inhibition zones. To ascertain the significance, an analysis of variance (ANOVA) was performed using Microsoft Excel. There were notable differences in the data at the 5% level of significance.

Antioxidant activity

The antioxidant assay using the *S. trilobata* methanolic extract was effectiveness of The DPPH 2,2-diphenyl-1-picrylhydrazyl

free-radical scavenging activity. In this procedure, 1 mL of 0.135 mM DPPH in methanol was combined with 2 mL of the test solution, which was either the *S. trilobata* extract at concentrations ranging from 200 to 1000 µg/mL. The mixture was incubated for 30 minutes at 37°C in the dark. Following incubation, a UV-visible spectrophotometer was used to measure the absorbance values

Results and Discussion

Yield of methanolic extracts

The study of methanolic plant extracts shows that these extracts have the potential to be more potent antibacterial agents than the store-bought ones that were employed. The current study found that the methanolic extract yield from *Sphagneticola trilobata* was 0.76% (w/w) on a dry weight basis. It was outperformed by the methanolic extract of the Man plant, which yielded 23.6%. Additionally, the yields of leaf extracts in the presence of ethanol, water, and chloroform were measured and were 0.83 g/50 g, 1.46 g/50 g, and 1.64 g/50 g, respectively; similarly, the yields were 0.39 g/50 g, 0.47 g/50 g, and 0.96 g/50 g. The antibacterial effectiveness of methanolic extract against clinically isolated human pathogenic bacteria was reported in Ganesa et al. (2022) and Mierza et al. (2020), much like that of *Orchis mascula*. [24][25] recorded that the methanol extract from simplicial powder had a starting weight of 500 g, yielding an extract weight of 90.1756 g, which corresponds to a yield of 18.04%. Although *Sphagneticola trilobata* is sometimes seen as an invasive weed, it is utilised in traditional medicine. The quantity of bioactive chemicals that may be extracted via methanol extraction is usually the main focus of research on the yield of methanol extracts from *Sphagneticola trilobata*.

Phytochemical analysis

The phytochemical screening of the methanolic extract, as illustrated in (Fig. 1), confirmed the presence of several bioactive constituents. The Following a phytochemical study, total phenols 36.9 mg/g, flavonoids

21.3 mg/g, alkaloids 6.9 mg/g, tannins 9.6 mg/g, saponins 7.8 mg/g, triterpenoids 17.5 mg/g, were found in the crude methanolic extract obtained from the leaves of *Sphagneticola trilobata*. Ana Greice Borba Leite et al., 2019 [26]. Nevertheless, the leaves' methanolic extract contains particular anthraquinones, glycosides, and phenolic chemicals. Furthermore, in the ethanolic extract of *Spathodea maculate* leaves and flowers, Kowtiet al., 2020 [27] found alkaloids, saponins, steroids, flavonoids, terpenoids, and tannins. have all been found in *Sarcanthuspauciflorus* orchids, according to Kiranet al., 2022 [28]. Numerous investigations have revealed the potential antibacterial activities alkaloids, glycosides, tannins, terpenoids, flavonoids, and saponins Okeke and Rahman et al., 2010 [29, 30].

FT-IR Spectroscopic analysis of methanol extract

Sphagneticola trilobata leaf extract's FT-IR spectra, as seen in (Fig. 2), shows

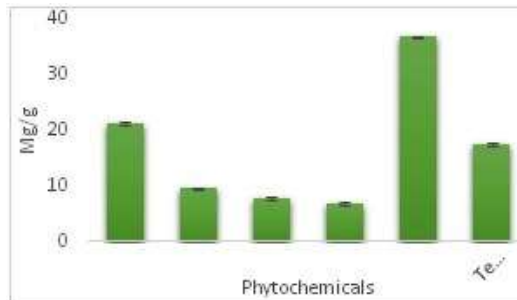


Fig. 1: Phytochemical analysis in methanolic extract

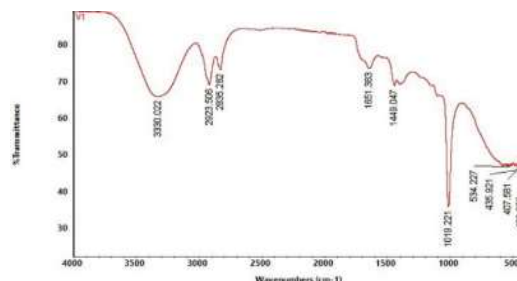


Fig. 2: FT-IR of *Sphagneticola trilobata* leaves extract

several absorption bands that represent different functional groups. A wide peak at 3300–3500 cm^{-1} showed O–H stretching vibrations, which are characteristic of hydroxyl groups in phenols and alcohols. The C–H stretching vibrations corresponding to absorption bands at 2923–3000, 2835.282 cm^{-1} were identified as being indicative of alkane. The existence of (C=N) was indicated by peaks in the 1651.383 cm^{-1} range, which may have come from esters, aldehydes, or minerals. O–H stretching vibrations, characteristic of carboxylic acid's hydroxyl groups, are present at 1449.047 cm^{-1} . Bands

ranging from 1000 to 1300 cm^{-1} were indicative of C–O stretching vibrations, which are frequently present in phenols, alcohols, and ethers. These main peaks' analysis indicated the existence of flavonoids, which are known to have antibacterial and antioxidant qualities, as well as phenolic compounds [31]. Sandip Nirwan et al., 2022. Furthermore, the presence of aliphatic hydrocarbons may suggest the presence of fatty acids or lipids, which may be involved in the plant's therapeutic qualities. The findings of the FT-IR study for the leaves of *Sphagneticola trilobata*, showing the presence of several functional groups and their possible effects on antibacterial activity.

GC–MS analysis of methanol extract

As shown in Figure 3 and compiled in (Table 1), the phytochemical composition of *Sphagneticola trilobata* leaf extract was thoroughly revealed by the GC–MS analysis. Each of the 16 unique peaks in the methanolic extract represented a different bioactive component. The chemical 1-[1-Methoxy-3,3-dimethyl-2-(3-methylbuta)] was notable since it was found with a peak area of

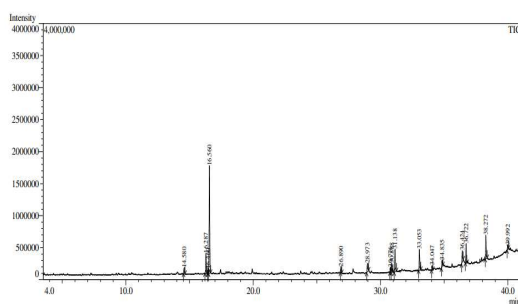


Fig. 3: GC–MS Analysis of Methanol Extract

Peak	R. Time	Area	Area%	Name
1	14.58	326266	2.19	1-[1-Methoxy-3,3-dimethyl-2-(3-methylbuta
2	16.287	1167765	7.84	1h-cycloprop[e]azulen-7-ol, decahydro
3	16.46	253483	1.7	(-)-5-oxatricyclo [8.2.0.0(4,6)] dodecane
4	16.56	5926906	39.77	Diethyl Phthalate
5	26.89	271742	1.82	Hexadecenoic acid, methyl ester
6	28.973	589480	3.96	Phenanthrene, 7-ethenyl-1,2,3,4,4a,4b,5,6,7,8,10,10a-dodecahydro
7	30.776	225052	1.51	9,12-Octadecadienoic acid (Z, Z)-, methyl ester
8	30.888	424564	2.85	9,12,15-Octadecatrienoic acid, methyl ester, (Z, Z, Z)-
9	31.138	1220133	8.19	2-hexadecen-1-ol, 3,7,11,15-tetramethyl
10	33.053	1176125	7.89	3-(4-Fluoro-butoxy)-1-iodomethyl-adamantane
11	34.047	202984	1.36	Podocarp-7-en-3-one, 13. beta. -methyl-13-vinyl-
12	34.835	351141	2.36	Kaura-9(11),16-dien-18-oic acid, (4. alpha.)-
13	36.424	581559	3.9	Kaur-16-en-18-oic acid
14	36.722	799228	5.36	Nandrolone phenpropionate
15	38.272	1157016	7.76	9,19-Cycloergost-24(28)-en-3-ol, 4,14-dimethyl-, acetate,
16	39.992	229977	1.54	Monotrimethylsilyl 3. beta. -hydroxy-5. alpha. -androst-16-ene

2.19% and a retention duration (RT) of 14.580 minutes. The decahydro of 1H-cycloprop[e]azulen-7-ol was detected at RT 16.287, accounting for 7.84% of the entire area.

Additionally, Oxatricyclo [8.2.0.0(4,6)] dodecane appeared at RT 16.460 with a peak area of 1.70%, while diethyl phthalate showed the highest abundance at RT 16.560, accounting for 39.77%. Several fatty acid derivatives and aromatic compounds were also identified, including methyl esters of hexadecenoic acid (RT 26.890; 1.82%), phenanthrene derivatives (RT 28.973; 3.96%), linoleic acid (RT 30.776; 1.51%), and linolenic acid (RT 30.888; 2.85%). 3-(4-Fluoro-butoxy)-1-iodomethyl-adamantane showed up at RT 33.053 with 7.89%, whereas 3-hexadecen-1-ol, 3,7,11,15-tetramethyl-, displayed a noteworthy peak at RT 31.138 with a considerable peak area of 8.19%. Podocarp-7-en-3-one, 13 β -methyl-13-vinyl (RT 34.047; 1.36%), and many diterpenoids, including kaura-9(11),16-dien-18-oic acid (RT 34.835; 2.36%), and kaur-16-en-18-oic acid (RT 36.424; 3.90%), were among the other chemicals found. At RT 36.722, nandrolone phenpropionate was detected with an area contribution of 5.36%. Sterol derivatives such as 9,19-Cycloergost-24(28)-en-3-ol, 4,14-dimethyl-acetate (RT 38.272; 7.76%), and Mon trimethylsilyl 3 β -hydroxy-5 α -androst-16-ene (RT 39.992; 1.54%) were among the other minor ingredients. These varied phytochemicals indicate that *Sphagneticola trilobata* has a large number of bioactive compounds that may have therapeutic use. These findings validate the plant's traditional uses and emphasise the necessity of more study to separate, describe, and assess the biological activity of the chemicals found[32].

Antibacterial Activity

The antibacterial efficacy of the methanolic extract of *Sphagneticola trilobata* leaves was evaluated using the disc diffusion method, as shown in (Fig. 4 and Fig. 5). This method allowed for the preliminary screening

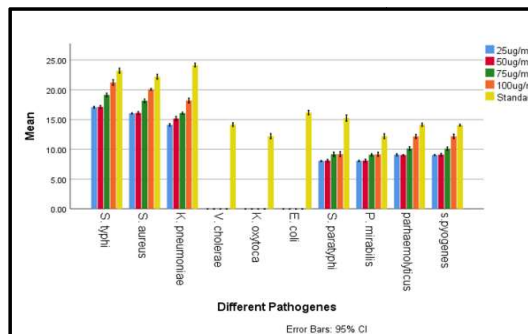


Fig. 4: Statistical Analysis

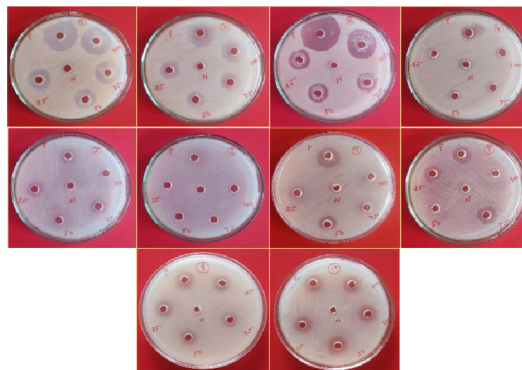


Fig. 5: Zone of inhibition

of the plant's bioactivity against a panel of Gram-positive and Gram-negative bacterial strains, commonly associated with human Hassan et al., 2022 [33]. The results are summarized in (Table 2), which details the diameter of inhibition zones (in mm) produced by the extract. Among the tested strains, the methanolic extract exhibited significant zones of inhibition against *Salmonella typhi* (23 ± 0.46 mm), *Staphylococcus aureus* (22 ± 0.58 mm), and *Klebsiella pneumoniae* (24 ± 0.28 mm). Moderate inhibitory effects were observed against *Vibrio cholerae* (14 ± 0.2 mm), *Klebsiella oxytoca* (12 ± 0.1 mm), *Escherichia coli* (16 ± 0.3 mm), *Salmonella Paratyphi* (15 ± 0.8 mm), *Proteus mirabilis* (12 ± 0.11 mm), *Vibrio parahaemolyticus* (14 ± 0.9 mm), and *Streptococcus pyogenes* (14 ± 0.21 mm). Notably, the highest antibacterial activity was recorded against *Salmonella typhi*, with a clear inhibition zone of 23 mm,

Table 2: Antibacterial Activity							
S. No	Name of the strains	Zone of inhibition (mm)					
		25 µg/ml	50 µg/ml	75 µg/ml	100 µg/ml	+ve	-ve
1	<i>Salmonella. typhi</i>	17	17	19	21	23	-
2	<i>S. aureus</i>	16	16	18	20	22	-
3	<i>K. pneumoniae</i>	14	15	16	18	24	-
4	<i>V. cholerae</i>	-	-	-	-	14	-
5	<i>K. oxytoca</i>	-	-	-	-	12	-
6	<i>E. coli</i>	-	-	-	-	16	-
7	<i>S. paratyphi</i>	8	8	9	9	15	-
8	<i>P. mirabilis</i>	8	8	9	9	12	-
9	<i>V. parahaemolyticus</i>	9	9	10	12	14	-
10	<i>S. pyogenes</i>	9	9	10	12	14	-

Table 3: Minimum Inhibitory Concentration (MIC)						
S. No	Name of the strains	Minimum Bactericidal Concentration				
		20 µg/ml	40 µg/ml	60 µg/ml	80 µg/ml	100 µg/ml
1	<i>S. typhi</i>	-	-	-	-	-
2	<i>S. aureus</i>	+	-	-	-	-
3	<i>K. pneumoniae</i>	++	+	-	-	-
4	<i>V. cholera</i>	+++	+++	+++	+++	+++
5	<i>K. oxytoca</i>	+++	+++	++	+	-
6	<i>E. coli</i>	+++	+++	+++	+++	+++
7	<i>S. paratyphi</i>	+++	+++	++	+	-
8	<i>P. mirabilis</i>	+++	+++	++	+	-
9	<i>V. parahaemolyticus</i>	+++	+++	+++	+++	+++
10	<i>S. pyogenes</i>	+++	+++	++	+	-

- MIC concentration; - No growth; * - considerably arrest; + - Cloudy solution; ++ - Turbid solution; +++ - Highly turbid solution

indicating strong bactericidal potential. However, some bacterial strains demonstrated only mild sensitivity to the extract. The lowest inhibitory zones recorded were 8–12 mm, particularly for *Vibrio parahaemolyticus* and *Streptococcus pyogenes*, suggesting variable susceptibility among different pathogens. These findings are consistent with earlier studies by Das et al., [34] emphasized measuring inhibition zones in millimeters as a standard method for assessing antimicrobial action. Chethanet al., [35] highlighted the antibacterial activity of *Sphagneticola trilobata* flower extracts, particularly against *Staphylococcus aureus*. The current study extends this knowledge by reporting additional activity against *Pseudomonas aeruginosa* and *Salmonella typhi*, further supporting the plant's broad-spectrum antibacterial potential. In contrast to

earlier studies by, Govindappa et al., [36] which reported limited antifungal activity of various parts of the plant against *Aspergillus* and *Fusarium* species, our findings present a unique outcome, particularly regarding bacterial inhibition. These results suggest that *Sphagneticola trilobata* possesses promising antibacterial constituents, warranting further investigation into their active principles and mechanisms of action. The clear zones of inhibition observed in (Fig. 5) visually support these findings, while the statistical data in (Fig. 4) confirm the reproducibility and significance of the results.

Minimum Inhibitory Concentrations (MIC)

The Minimum Inhibitory Concentration (MIC) of the *Sphagneticola trilobata* methanolic extract, as determined against a few selected bacterial strains, is

shown in (Table 3). The methanolic leaf extracts had MIC values of 40, 60, 60, 100, and 100 µg/mL against *S. aureus*, *S. typhi*, *K. pneumoniae*, *V. cholerae*, *K. oxytoca*, *E. coli*, *S. paratyphi*, *P. mirabilis*, and *V. parahaemolyticus*, in that order. Compared to fucoidan obtained from *Sphagneticola trilobata*, the MIC results from this study are different. Balekaret al. found that the ethyl acetate fraction of *Wedelia trilobata* (L.) leaf ethanolic extract exhibited high antibacterial activity[37]. From 250 to 1,000 µg/mL, this fraction's minimum inhibitory concentration (MIC) values indicated that it would be helpful in treating microbial infections. The present result is consistent with a study conducted by Leite et al. [38] that examined the Asteraceae family of plants' antibacterial qualities. The ethyl acetate fraction may prove useful in the creation of antimicrobial drugs, according to this study. The growth of cultures derived from human and canine skin was significantly inhibited by the crude hydroalcoholic extract of *S. trilobata* leaves. These results are in line with studies conducted by Sartori (2005) that found comparable outcomes from different extract fractions. This implies that *S. trilobata*'s active ingredients may have broad-spectrum antibacterial qualities, which makes them exciting candidates for additional study and advancement in dermatological applications. were selected for additional

tests of minimum inhibitory concentration (MIC). Prior research by Budha Magar [39, 40] indicates that it is often regarded as a substitute for conventional medical procedures. According to Koura et al. and other academic research, certain phytochemical compounds, like glycosides, Alkaloids, flavonoids, terpenoid, saponin, and tannin all have antimicrobial qualities. Misskire [41].[42] Hamid et al. We used extract from samples of *S. trilobata* plants at varied doses, and the plants' responses to several diseases were observed. In order to create medicinal applications, more research is necessary to identify the precise components of *Sphagneticola trilobata* and how they interact with particular microbes.

Minimum bactericidal concentration (MBC)

Figure 6 shows the Minimum Bactericidal Concentration (MBC) values of the *Sphagneticola trilobata* methanolic extract. Table 4 and Minimum Bactericidal Concentration (MBC).The MBC is the outcome of this inquiry. For a number of bacterial species, including *S. typhi*, *S. aureus*, *V. cholerae*, *K. oxytoca*, *E. coli*, *S. paratyphi*, *P. mirabilis*, and *V. parahaemolyticus*, the leaf extracts exhibit MBC values of 20, 40, 60, 80, 100, 100, 100, and 100 µg/mL. While *Sphagneticola trilobata* leaf extract in methanol had an MBC of S

Table 4: Minimum Bactericidal Concentration (MBC)

S. No	Name of the strains	Minimum Inhibitory Concentration						
		20 µg/ml	40 µg/ml	60 µg/ml	80 µg/ml	100 µg/ml	V-	V+
1	<i>Salmonella typhi</i>	+	*	-	-	-	+++	-
2	<i>Staphylococcus aureus</i>	++	+	*	-	-	+++	-
3	<i>Klebsiella pneumoniae</i>	++	+	*	-	-	+++	-
4	<i>Vibrio cholera</i>	+++	+++	+++	+++	+++	+++	-
5	<i>Klebsiella oxytoca</i>	+++	+++	++	+	*	+++	-
6	<i>Escherichia coli</i>	+++	+++	+++	+++	+++	+++	-
7	<i>Salmonella Paratyphi</i>	+++	+++	++	+	*	+++	-
8	<i>Proteus mirabilis</i>	+++	+++	++	+	*	+++	-
9	<i>Vibrio parahaemolyticus</i>	+++	+++	+++	+++	+++	+++	-
10	<i>Streptococcus pyogenes</i>	+++	+++	++	+	*	+++	-

- MBC concentration (no growth); +, slight growth; ++, medium growth: + + +, higher growth

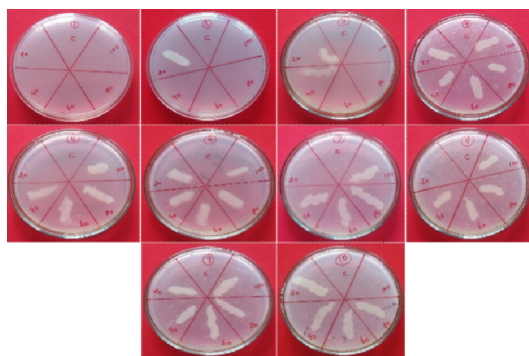


Fig. 6: Minimum Bactericidal Concentration (MBC)

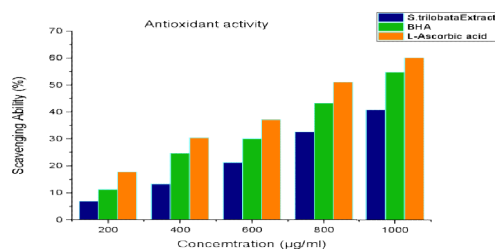


Fig. 7: Antioxidant activity of *S. trilobata* extract

typhi 16.92 ± 0.58 mm, this study's MBC values for leaf extracts were superior (Toppo et al., [43,44]). *Sphagneticola trilobata* (L.) Pruski has been shown to have antimicrobial action against a few human pathogenic bacteria.

Antioxidant activity

S. trilobata extract's antioxidant potential was evaluated. A test for DPPH free radical scavenging was performed at different ranging from 200 to 1000 µg/mL (Fig. 7). The effectiveness of the extract and the common antioxidants L-ascorbic acid and BHA were compared [45]. All three samples showed an increase in scavenging capability as concentrations rose. BHA and L-ascorbic acid showed 13% and 17% scavenging activity, respectively, at a concentration of 200 µg/mL, whilst the extract showed 10%. BHA and L-ascorbic acid demonstrated around 59% and 66%, respectively. (2022) recorded Dhavale et al., this plant sample *S. trilobata* extract

obtained 42% at the highest quantity tested (1000 µg/mL [46].

Conclusion

In conclusion, *Sphagneticola trilobata*'s methanolic leaf extract showed strong antibacterial activity against a variety of clinically significant infections, including human ones. The results point to its potential as a viable source of natural antibacterial agents and validate its historic therapeutic use. To identify the active ingredients, investigate their modes of action, and assess the extract's efficacy and safety for possible medicinal uses, more investigation is necessary.

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