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Biological activity and chemical characterization of ethanol extract from the leaf of *Sphagneticola trilobata*

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Abstract

The study's objective was to examine the antimicrobial activity of *Sphagneticola trilobata* opposition to therapeutically separated from human hazardous pathogens and to define its morphological characteristics. The extract was tested for its photochemical substance that was identified by Gas Chromatography-Mass Spectroscopy. The ethanolic extract's working properties were examined using FT-IR. In addition, the extract of ethanol demonstrated antibacterial activity against eight of the ten pathogens examined. At the maximum dose (100 g/ml), the ethanolic extract inhibited *S.paratyphi* with a maximal zone of 18 mm. The ethanolic extract had MIC values of 100, 80, 60, and 100 µg/ml against bacteria's including *K.oxytoca*, *V.cholerae, S.typhi*, *S. paratyphi*, *S.aureus* , *E.coli*, and *P. mirabilis*. The MBC values of the ethanolic extract against bacterial pathogens demonstrated 80, 100, 100, 80, 60, 60, 40 and 60 µg/ml. *S.trilobata's* effective bacterial inhibition rate shows that it could be used as an antibiotic.

Keywords: *S. trilobata*; Antibacterial; Chemical characterization; MBC

Introduction

Sphagneticola trilobata is a plant it also known as *Wedelia trilobata, Wedelia paludosa* and *Acmella spilanthoides*. It comes from the tribe Heliantheae of the family *Asteraceae.* With a multitude of phytochemicals, the plant may perform a variety of pharmacological functions. Numerous researchers have reported using it in conventional medicine for the treatment, the leaves are included in a cough, sores, swelling, gout, cramps and cold remedy (1). The plant has also been traditionally used to clear the placenta after birth. This plant is an old traditional medicine in Chinese, South America, India, and Caribbean and also *S.trilobata* native to Central America, South America, West Indies and the Mexico (2,3). It has been discovered extensively in Indonesia, India, China, Myanmar, Bangladesh, Cambodia, Vietnam, and Malaysia. It grows well in metropolitan areas, ditches, valleys, wet roads, crops, organic forests, and pastures (4,5). For six decades ago, some species of this family have been great interest in the pharmaceutical therapy since they exhibited antibacterial activity, antifungal activity, antidiabetic activity, central nervous system depressant activity, anti-tumour activity and anticancer activities (6). Further studies in this plant will reveal more pharmacological activities.

Worldwide, bacterial illnesses have led to major health issues for people. Approximately 80,000 plant species are used in various Indian medical systems to cure a variety of illnesses since the 1990s (17) . In every year 8.3 million deaths around the world were found linked to bacterial infection (7). Antibiotics that either kill or stop the growth of germs are widely accessible in the market and play a crucial role in controlling infection-causing bacteria. However, the issue is that despite the fact that many of the microorganisms are multidrug resistant, they are growing increasingly resistant to these medications every day. Therefore, treating these disease-causing, multidrug-resistant bacteria with a variety of antibiotics has a significant impact on public health (19,20). For example, *bacillus subtilis* (8,9,10) is

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responsible for wound and burn infections; *Staphylococcus aureus* is responsible for skin and soft tissue infections (11) *Pseudomonas aeruginosa* and *Escherichia coli* cause abortion, healthcare-associated and hospital-acquired invasive infections in adults (12,13) and *Salmonella sp.* causes typhoid fever, stomach pain and headache (14).

Herein, we used the ethanolic extract for chemical characterization through FT-IR and GC-MS and Phytochemical analysis to evaluate their biological activities. The finding of the study is expected to contribute in the pharmacological to develop *S. trilobata* as an antibacterial drug.

Materials and Methods

Collection of Plant: *S. trilobata* is perennial herb, gathered from the SIMATS Engineering campus, Chennai. The leaves were let too dry in the shade. Then, using an electric blender, dried aerial portions were finely ground.

Extract Preparation: 75g of leaves sample was added in 400 ml of ethanol to a period of 24 hours in a Soxhlet extractor at 75℃ (15). The extracted substance was allowed to evaporate the solvent, generating in a slurry form that can be used for analysis and diluted as required.

Analysis of Phytochemical: Phytochemicals study of some phenolic compounds like favonoids, saponins, tannins, terpenoids and total alkaloids were performed (29).

Analysis of FT-IR: 10 µl extract were assessed with the use of thermo-scientific FT-IR-40 (SIMATS). Among wave numbers 4000 to 400 cm−1, the spectrum was recovered.

Analysis of GC-MS: In particular substances, the composition of extract was exposed to GC-MS analysis at department of biotechnology, periyar university, Salem. The oven program had been adjusted to 45ºC @ 7ºC per minute to 180ºC (90 seconds) @ 6ºC/min to 240ºC (8 minutes) and 240ºC for the injector. At a flow rate of one millilitre per minute, as the carrier gas, helium is utilized. Once injected the sample, compounds that was retrieved matched.

Antimicrob's activity

Bacterial strains: Eight pathogens were retrieved from Microbiology Department, Periyar University, Salem.

Inoculum preparation: After being put in test tubes, the broth had been autoclaved for 15 minutes at 130ºC. Every bacterial strain added one at a time to the sterile nutritional broth, and cultivated for whole day at room temperature.

Antibacterial activity: Antibacterial activity was done by the method of well diffusion technique. It was employed to evaluate antibacterial efficacy (16). 24-hour-cultures streaking to the agar plates with the help of inoculation loop. Using well cutter, 5-mm wells were cut thoroughly on plates. At a concentration of 1 mg/ 10% DMSO used to create the methanol extract of the stock solution in milliliters. In the concentrations (25, 50, 75 and 100 µg/ml) were employed. To deliver the results, the inhibitory zone of every well has been measured in mm.

MIC: Using extract, the MIC was ascertained by means of MIC (18). Stock solution (1mg/ml) was prepared then subsequently mixed in steps to yield different level of concentrations between 20 to 100 µg/ml. In every dilution, take 0.75ml, which included 5 ml of N.broth, was placed in 10ml test tube and mixed with 1 milliliter of the previous bacterial culture. Test tubes with only broth were used as a control. For twenty-four hours at 37 °C, every test tube as well as the control was incubated. MIC was examined by selecting the extract-containing tube with the least amount of focus that did not exhibit any discernible expansion during the incubation period.

MBC: Take a broth from MIC tubes, that was spread to NA plate then incubated at 37ºC for hole day. Concentration level at notable growth observed and recorded.

Results and Disscussion

Yield and Phytochemical study of ethanolic extract: In the present work, the

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ethanolic extract's yield from *S. trilobata* was found to be 18.5mg/g on dry weight basis. The phytochemical analysis of extract of *S. trilobata* reported 53.5 mg/g of total phenolic compound, 23.5 mg/g of flavonoids, 14.1 mg/g of tannins and 8.9 mg/g of saponins respectively (Fig. 1) (29). Mardina *et al* reported different secondary metabolites, such as Alkaloids, Flavonoids, Phenol, Saponin, Tannin from extract of *S. trilobata*. (22) Rajamalar *et al* reported phytochemicals, namely saponins anthraquinone, tannins, alkaloids, cardiac glycosides cyanogenic and flavonoids.

FT-IR: The extract of *Sphagneticola trilobata* were analyzed by FT-IR for determine the functional groups indicates notable bands at 3331.732, 1640.213, 1405.268, 1014.627, 406.363 and 417.758 cm^{-1}. Spotted peak at 3331.732 cm^{-1} is indicating hydroxyl group OH's stretching vibration. The peak at 1640.213 cm^{-1} was group of carbonyl C=O. The next peak value of 1405 cm^{-1} is indicating carboxyl group of C-H. The band at 1014.627 cm^{-1} confirms the α-type glycosidic linkage of C-H. Another peak observed at 406.363 cm^{-1} is due to presence of alkyl halides aromatic sp2 C-H bend. The peak at 417.758 cm^{-1} indicating halogen compound(C-I) respectively (Fig. 2). Similar to that, all of these functional groupings have been established secondary metabolites from *Orchis mascula* in the earlier study (22).

GC-MS: The chemical elements of ethanoic extract formed 38.27% of 2-Hexadecen-Ol,3,7,11,15-Tetram, 12.15% of 2-

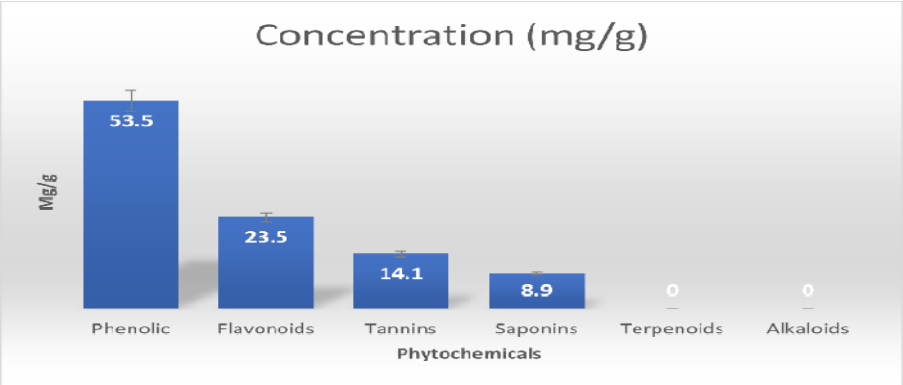

Fig 1: Screening of phytochemical compounds of the ethanolic *S. trilobata* leaves

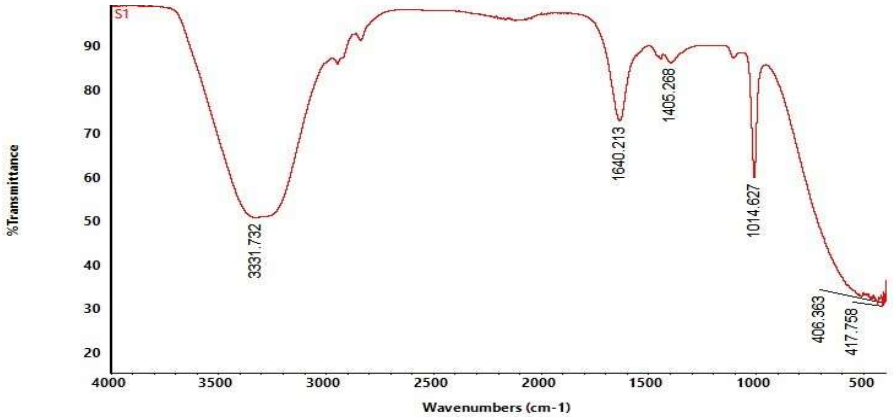

Fig. 2: FT-IR spectra for the ethanolic extract

Leaf of *Sphagneticola trilobata*

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Pentadecanone, 6,10,14-trimethyl-, 7.15% of Hexadecanoic acid, methyl ester, 6.26% of l-(+)-Ascorbic acid 2,6-dihexadecanoate, 6.20% of 2,6,10-Trimethyl, 14-Ethylee-14-P, 5. 92% of 1-Heptatriacotanol, 5.42% of 9,12,15 – Octadecatrienoic acid, Methyl ester, 4.67% of 9,12 – octadecadienoicacid (Z), Methyl ester, 3.65% of dl-Isopulegol, 2.38% of 9-Octadecenoic acid, 2.16% of 2,6,10, - Trimethyl, 14 - Etylene, 2.14% of Oleic Acid, 1.81% of (3-Fluorophenyl)carbamic acid, 2-isopropyl-, 1.81% of Hexadecanoic acid, 2-hydroxy-1-hydroxym respectively (Table 1 and Fig. 3).

Antibacterial activity: The ethanolic extract form *Sphagneticola trilobata* demonstrated antimicrobial efficacy against eight bacterial strains tested. The ethanolic

Table 1: GC–MS analysis

Peak#	R. Time	Area	Area%	Name
1	24.525	65045	6.20	2,6,10-Trimethyl,14-Ethylene-14-P
2	24.629	127408	12.15	2-Pentadecanone, 6,10,14-Trimethyl
3	25.699	226595	2.16	2,6,10-Trimethyl,14-Ethylene-1
4	26.892	750581	7.15	Hexadecanoic Acid, Methyl Ester
5	27.860	656690	6.26	l-(+)-Ascorbic acid 2,6-dihexadecanoate
6	30.674	189769	1.81	(3-Fluorophenyl)carbamic acid, 2-isopropyl
7	30.778	489644	4.67	9,12-Octadecadienoic acid (Z,Z)-, methyl ester
8	30.890	569101	5.42	9,12,15-Octadecatrienoic acid, methyl ester
9	30.930	249739	2.38	9-Octadecenoic acid, methyl ester, (E)
10	31.138	4014519	38.27	2-Hexadecen-1-Ol, 3,7,11,15-Tetram
11	31.708	225002	2.14	Oleic Acid
12	34.939	621493	5.92	1-Heptatriacotanol
13	37.675	190152	1.81	Hexadecanoic acid, 2-hydroxy-1-
14	38.096	382537	3.65	dl-Isopulegol

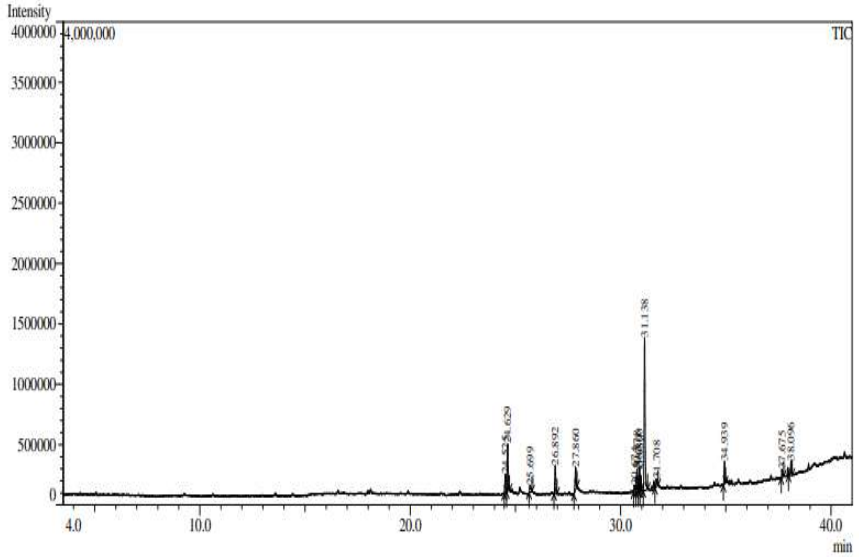

Fig. 3: GC – MS study of the ethanolic extract

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extract reveled the activity at 100 µg/ml extremely (Table 2). The extract showed a drastic zone (18 mm) opposing *Salmonella* at 100 µg/ ml concentration. The smallest zone (12mm) has been established against *Klebsiella pneumoniae*. At 75 µg/ml the extract displayed a highest zone (15mm) against *Vibrio cholerae* and a minimum (10mm) against *Klebsiella pneumoniae*. In 50 µg/ml, the extract displayed the highest zone (13mm) against *Vibrio* and *Salmonella* and a minimum (10mm) against *Klebsiella pneumonia.* At 25 µg/ml, extract displayed a highest (13mm) against *Salmonella Paratyphi* and the minimum zone (10mm) against *Klebsiella pneumoniae, Klebsiella oxytoca* and *Escherichia coli* (Fig. 4). Seedevi *et al* (21) monitered the Gladius of *S. lessoniana* polysaccharide's antibacterial properties showed 20, 10 and 10 mm of zone at 100µg/ml concentration *against S. paratyphi* and *S. aureus*. Rajamalar *et al* (22) reported the antibacterial activity of ethanolic extract from *O. mascula* was 14,14,10,10,12 and 12 at 50 µg/ ml. In the present study, ethanolic extract form the leaf of *Sphagneticola trilobata* have good antibacterial properties against selected human pathogens.

Minimum Inhibitory Concentration (MIC): The Minimum Inhibitory Concentration (MIC) values from the ethanolic extract of *Sphagneticola trilobata* leaves against bacterial strains were showed as 100, 100, 80, 100, 80, 60 and 100 µg/ml (Table 3). Harikrishnan *et al* (23) MIC of the ethanolic extract of *azdirachta indica* against *Aeromonas hydrophila, Pseudomonas aeruginosa* and *vibrio vulnificus* were 4.20, 3.68 and 6.74 mg/ml. Morteza Saki *et al* (24) MIC value of *cinnamomum zeylanicum* bark EO against *E. faecium and A. baumannii* were 0.15-2.5 and 0.31-10 µl/ml. Phuyal Nirmala *et al* (25) reported MIC value of methanolic extracts from the seeds of *zanthoxylum armatum* against *B. subtilis, S. aureus* and *S. epidermidis* were 16.28, 16.44 and 13.25 mm. In this study, when human pathogens were tested at various concentrations, the growth of the *S. paratyphi* was significantly inhibited for 60 g/ml as opposed to 0.156 µg /ml.

Minimal Bactericidal Concentration (MBC): The extract of *Sphagneticola trilobata* showed MBC values against eight pathogens were demonstrated 80,100,100,80,60,60,40 and 60 µg/ml (Table 4). Naik *et al* (26) reported the MBC value of the seed extract from *solanum surattense* showed 46 and 18 at 12.5 µg/ml concentration against *streptococcus mutans* and *aggregatibacter actinomycetemcomitans*. Gumgumjee *et al* (27) minimal bacterial concentration of the extract from the leaves of *Tamarindus indica* showed 10, 15, 10 and 5 mg/ml against four pathogens (30-32). In the results ethanolic extract thus confirm of MBC against

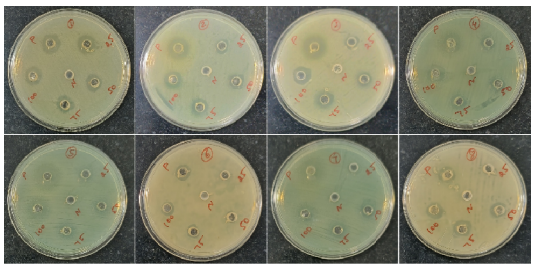

Fig. 4: Antibacterial activity of ethanolic extract

Table 2: Antibacterial activities of ethanolic extract

S. No	Name of Pathogens	25 µg/ml	50 µg/ml	75 µg/ml	100 µg/ml	+ve
1.	*S. typhi*	12	12	12	14	18
2.	*S. aureus*	12	12	12	14	18
3.	*K. pneumoniae*	10	10	10	12	16
4.	*V. cholerae*	12	13	15	17	20
5.	*K. oxytoca*	10	11	13	14	19
6.	*E. coli*	10	11	13	14	17
7.	*S. paratyphi*	13	13	14	18	19
8.	*P. mirabilis*	12	12	13	14	18

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Table 3: MIC values of the extract

S. No	Name of the strains	20 µg/ml	40 µg/ml	60 µg/ml	80 µg/ml	100 µg/ml	+ve	-ve
1	*S. typhi*	+++	+++	++	+	*	-	+++
2	*S. aureus*	+++	+++	++	+	*	-	+++
3	*K. pneumoniae*	+++	+++	+++	++	+	-	+++
4	*V. cholerae*	+++	++	+	*	-	-	+++
5	*K. oxytoca*	+++	+++	++	+	*	-	+++
6	*E. coli*	+++	++	+	*	-	-	+++
7	*S. paratyphi*	++	+	*	-	-	-	+++
8	*P. mirabilis*	+++	+++	++	+	*	-	+++

Table 4: MBC values of the extract

S. No	Name of the strains	20 µg/ml	40 µg/ml	60 µg/ml	80 µg/ml	100 µg/ml	control
1	*S. typhi*	+++	++	+	-	-	-
2	*S. aureus*	+++	++	++	+	-	-
3	*K. pneumoniae*	+++	+++	++	+	-	-
4	*V. cholerae*	+++	++	+	-	-	-
5	*K. oxytoca*	++	+	-	-	-	-
6	*E. coli*	++	+	-	-	-	-
7	*S. paratyphi*	+	-	-	-	-	-
8	*P. mirabilis*	+++	+	-	-	-	-

Fig. 5: MBC of the extract

S. paratyphi was discovered to be efficient at dilution of 40 µg /ml (Fig 5).

Conclusion

The extract of ethanol from the leaves of *s. trilobata* has showed good antibacterial activity against many human pathogens and notable concentration was examined via MIC and MBC. So, the massive bacterial inhibition amount of *s. trilobata* may provide an exciting drug against bacterial infection.

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