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Antioxidant and Antibacterial Activities of the Methanolic Extract from *Sphagneticola trilobata*

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The objective of this study is to assess the phytochemical activity of the methanolic extract of *Sphagneticolatrilobata* (L.) and to evaluate the bioactive compounds present using gas chromatography-mass spectrometry (GC-MS). The objective of this study is to investigate the potential anticancer, antibacterial, and antioxidant properties of the methanolic extract derived from *S. trilobata*. The bioactive compounds of *S. trilobata* were identified through the use of Gas Chromatography-Mass Spectrometry (GC-MS). The antioxidant properties of *S. trilobata* were assessed through DPPH (1,1-diphenyl-2-picrylhydrazyl),ABTS (2,2'-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid), H_2O_2 (Hydrogen peroxide) and Superoxide dismutase (SOD) activity. The

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antibacterial properties of *S. trilobata* extract against *Pseudomonas aeruginosa* and *Staphylococcus aureus* were assessed using the disc diffusion technique, Minimum inhibitory concentrations, and growth curve analysis. The methanolic *S. trilobata* plant was contain 40 bioactive compounds. Among them amounts of oleic acid (100%), abietic acid (98.25%), and n-hexadecanoic acid (94.45%). The antioxidants revealed that dose dependent activity, the highest antioxidant activity was DPPH (53.5%), ABTS (54.7%), H_2O_2 (54.1%) and SOD (43.2%) at 1.5mg/mL.

The antibacterial activity revealed that high antibacterial properties against *P.aeruginosa*and *S. aureus* inhibition zones of *P. aeruginosa* (14 ±0.8 mm) and *S. aureus*, both (12± 0.4 mm).The preliminary examination of the volatile compounds indicates the existence of novel bioactive constituents that have not been previously documented in *S. trilobata* from different geographic regions. The present study investigates the biological activities of the methanolic extract derived from *S. trilobata* leaves, with a focus on its potential applications in the pharmaceutical and nutraceutical fields.

Keywords: Sphagneticola trilobata; Pseudomonas aeruginosa; Staphylococcus aureus; antibacterial; antioxidant.

1. INTRODUCTION

The term "medicinal plant" refers specifically to the use of plants in herbalism and other forms of alternative medicine. Traditional medicine relied heavily on plants. Herbal medicine encompasses both conventional and alternative medicines that use herbs and plants. In contrast, the effectiveness, efficiency, and safety of herbal medications are what are taken into account during their assessments [1] Therefore, extensive study and analysis are required before an extracted herb may be made available to the general population. The hazardous effects of chemical medications are well documented, and the long-term effects of chemotherapeutic treatments may have devastating consequences for patients [2,3]. It's important to discover and study alternative therapies since several strains of bacteria have shown signs of resistance to synthetic antibiotics. Herbal and other plant-based medicines are the most effective and safe forms of treatment available. Any plant with one or more chemicals in its organs that are used therapeutically or are being studied for use in the manufacturing of valuable medicines is considered a medicinal plant [4].

The global market for herbal medicine, including herbal products and raw materials, is expanding on a daily basis. Bioactive compounds derived from natural products have been extensively researched for use in medical practices. The antibacterial properties of plant extracts and phytochemicals make them attractive therapeutic options. Several studies in different countries over the last several years have shown evidence of its effectiveness. The secondary metabolism of plants is largely responsible for their antibacterial qualities, which have been used in medicine for centuries [5,6]

S. trilobata (L.) or known as *Wedeliatrilobata*, *Wedelia* was used to treat infected, intractable wounds, rheumatism, muscle spasms, sores, and swellings [7]. Modern pharmacological studies [8] show that Wedelia extract has anti-microbial, anti-inflammatory, antioxidant, anti-tumor, menstrual pain, problems in women, wound-healing, diabetes, and uterine contraction, [9-11], and hepatoprotective [12] properties. Thus, the efficacy and antibacterial activity of *S. trilobata* against numerous hazardous pathogens were demonstrated [13]. Microorganisms were used in a disc diffusion assay to determine the extracts' zones of inhibition for antibacterial activity. Herbal remedies pose less of a health risk than synthetic pharmaceuticals [14].

Antibacterial activity has been shown in several pharmacological activity studies of *S. trilobata* aerial parts. Water extract contains antimicrobial, antioxidant, and anti-inflammatory properties, whereas ethanol flower extract has a significant inhibitory effect on S. aureus and X. oryzaepv. Oryzae, as well as molluscicidal, antibacterial, and antimycobacterial properties from the fresh entire plant. *W. trilobata* has the potential to be used as an anti-inflammatory, anti-oxidant, anti-microbial, hepatoprotective, and anti-diabetic natural medicine source [15,16]. *S. trilibata* is a generally harmless plant that may be used as

traditional medicine or as a dietary supplement without altering hepatic or renal function. However, just a few research have looked into the beneficial benefits of methanol extract obtained from *S. trilobata* [17,18] The purpose of this research was to assess the antioxidant and antibacterial characteristics of an methanolic extract of *Sphagneticolatrilobata* (L.) Pruski.

2. MATERIALS AND METHODS

2.1 Collection of Plant and Extraction

Sphagneticolatrilobata leaves were obtained from the Bharathiar University, Coimbatore campus. The Southern Regional Centre of the Botanical Survey of India assisted in identifying the collected *S. trilobata*. To eliminate any unwanted particles or debris, the *S. trilobata* specimens were carefully washed with both freshwater and deionized water. Subsequently, the specimens were dissected into smaller fragments and subsequently subjected to a process of air drying. Subsequently, the desiccated leaves were pulverized into a fine powder.

The powdered material was subjected to extraction using a Soxhlet apparatus, with successive extraction performed using methanol. A total of 25g of powder was used for the extraction process. The collected extracts were concentrated using a rotary evaporator and subsequently stored in airtight containers at a temperature of -20°C in a refrigerator for future applications.

2.2 Qualitative Phytochemical Analysis

The qualitative phytochemical (Tannins, Anthro quinones, Flavonoids, Terpenoids, Saponins, Glycosides, Reducing Sugars, Phlobatanins, Steroids, Phenols, Amino acids, Proteins and Alkaloids) test performed by standard method [19,20].

2.3 Gas Chromatography-Mass Spectro-copy Analysis

Gas chromatography (Agilent 7890A) using a capillary column (HP Innowax Capillary; 60.0 m x 0.25 mm x 0.25 m) was linked to a flame ionization detector and mass spectrometry (Agilent 5975C) to determine the volatile composition. The hexane extraction ratio was 2:1. A split mode of 40:1 was used in the GC-MS/FID study. We settled on 1 mL for the injection volume and 250 C for the injection temperature. The carrier gas was helium (99.9% purity), and the flow rate was maintained at 0.8 ml/min. The oven was set at 60 degrees Celsius for 5 minutes, then raised by 5 degrees Celsius every minute until it reached 220 degrees Celsius, where it remained for 8 minutes. We employed electronic impact ionization at 70 eV to track MS spectra between 35 and 450 amu. Components were identified using the NIST library, and their relative proportions were determined using GC-FID peak regions.

2.4 Analysis of Antioxidant Activity

The in-vitro antioxidant activity of methanolic extract *S. trilobata* was evaluated using the DPPH [21], ABTS [22], H2O2 and SOD [23] scavenging tests.

2.5 Collection of Bacterial Strain and Antibacterial Assays

The bacterial strains such as *Pseudomonas aeruginosa,* and *Staphylococcus aureus*, were obtained from PSG Institute of medical sciences and research, Coimbatore, Tamil Nadu.

The antibacterial efficacy against *P. aeruginosa* and *S. aureus* was assessed through the utilization of agar well diffusion assay, Minimum Bactericidal Concentration (MBC) determination, and Minimal Inhibitory Concentration (MIC) analysis.

2.5.1 Agar well diffusion assay

S. trilobata leaf extract was tested for antibacterial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa* using the agar-well diffusion technique on Muller Hinton Agar (MHA). The overnight incubated bacterial culture was placed onto Mueller-Hinton agar (MHA) plates, and wells were made using a cork borer. *S. trilobata* leaf extract was added to MHA plates at dosages ranging from 0.5 mg/mL to 1.5 mg/mL. After that, the plates were incubated at 37°C incubator for 24 hours. The inhibitory zone measurement in millimeters (mm).

2.6 Antioxidant Activity of Bacterial Pathogens

2.6.1 Superoxide dismutase assay

SOD activity was measured using the method pro-posed by Beauchamp [24], using nitroblue tetrazolium (NBT) inthe presence of riboflavin. 20

lL of supernatantfrom each sample were used. The specific activity was expressed as U mg_1of protein, where U wasdefined as a 0.001 increase in absorbance at560 nm min_1.

2.6.2 Peroxidase assay

Peroxidase activity was quantified and calibrated against a peroxidase activity standard curve made with radish peroxidase (Heber Biotecsa, Cuba). Peroxidase assays were performed in flat-bottom, 96-well, polyethylene plates (Costar_, USA) by adding 20 lL of supernatant from each sample and 50 lL of reaction buffer (Na2 HPO 0.05 M; citric acid 0.02 M; pH 5) containing H24O (0.15% [V/V]) and o-phenylenediamine (0.10% [p/V]) [25]. The plates were placed 30 min at room temperature and then the reaction was stopped with 50 lLsulphuric acids (2 M). The absorbance was read at 492 nm, using a screen plate (TitertekMultiskan MCC/340). The results were expressed in U lg of protein, where U was defined as the formation of 1 lmol of 2.3-diaminophenazine, by the oxidation of o-phenylenediamine, per min of reaction per µg of protein.

2.6.3 ROS measurement

The ROS production was measured by using a fluorescent probe H2 DCF-DA [26]. The bacterial cultures (1 × 10 CFU/ml) were treated with 100 µL of different concentrations of ZnO NPs (20–100 µg/mL) for 2 h. After treatment with NPs, the bacterial strains were collected, washed, and resuspended in phosphate buffer saline (PBS). The bacterial suspension was then exposed to 100 µL of 1 mM H2 DCFDA solution and incubated at 37ºC for 30 mins in the dark. Consequently, the cultured bacteria were lysed with lysis buffer and centrifuged at 3000 rpm for 5 min, followed by the spectroscopic measurement of the supernatant at520 nm. The experiment was conducted in triplicates. The ROS level was determined using a spectrofluorometer (Ex/Em = 488/525 nm) and quantified the mean fluorescent intensity of the control and treated groups.

3. RESULTS AND DISCUSSION

3.1 Phytochemical Properties of *S. trilobata*

The phytochemical profile of *S. trilobata* leaf extract in methanol revealed [27] positive findings of Alkaloids, Amino acids, Terpenoids, Flavonoids, Saponins, Glycosides, Reducing Sugars, Phlobatanins, Steroids, Phenols, and Reducing Sugars are all examples of reducing sugars. Anaquinones were shown to be negative (Table 1).

Glycosides, flavonoids, steroids, alkaloids, and tannins were all discovered, but phenolic compounds, saponins, terpenoids, and triterpenoids were only detected in minor quantities. Our results back up the reported[27], Tannins, flavonoids, terpenoids, steroids, oils, and glycosides chemicals were found in 90% methanolic extracts of *S. trilobata* leaf. Recently phytochemical analysis were reported in *Beilschmiedia roxburghiana*[28] *Solanum sisymbriifolium*[29].

Table 1. Phytochemical prescreening of *S. trilobata* leaf extracts from different solvents. The marks '++' ,'+'and ' − 'indicate 'strongly detected' moderately detected, 'absent', respectively

S. No.	Phytochemicals	Methanol extract
1.	Alkaloids	++
2.	Aminoacids	+
3.	Tannins	++
4.	Terpenoids	+
5.	Flavanoids	++
6.	Saponins	+
7.	Glycosides	++
8.	Reducing Sugars	+
9.	Phlobatanins	+
10.	Steroids	++
11.	Phenols	+
12.	Proteins	+
13.	Anthroquinones	-

3.2 GC-MS Analysis

Table 2 shows the GC–MS chromatogram of methanol extract of *S.trilobata* leaf. The major components present in *S.trilobata* were N-[4-(4-Chlorophenyl)isothiazol-5-yl)-1-methylpiperidin-2-imine, 6-Aminohexanamide,N-methyl-N-[4-(1-pyrrolidinyl)-2-butynyl]-N'-[2-aminobutanoyl]- ,Undecane, (-)-Carvone, Dodecanoic acid, methyl ester, Dodecanoic acid, Methyl tetradecanoate, Tetradecanoic acid, Neophytadiene, Phthalic acid, 2-cyclohexylethyl isobutyl ester, Hexadecanoic acid, methyl ester, Oleic Acid, Dibutyl phthalate, n-Hexadecanoic acid, Estra-1,3,5(10)-trien-17á-ol, Androst-2, 16-diene, Androst-5,7-dien-3-ol-17-one, 9-Octadecenoic acid (Z)-, methyl ester, Phytol, Heptadecanoic acid, 16-methyl-, methyl ester,

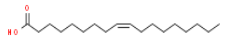
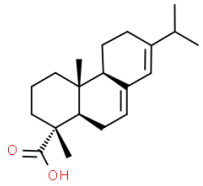
Table 2. Phytochemical constituents identified in the methanol extracts of *Sphagneticola trilobata* leaves using gas chromatography-mass spectrometry. *Sl. No*. serial number

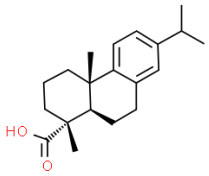
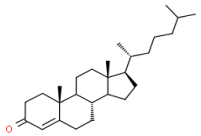
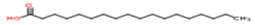
S. No.	Name of the Compound in Methanol Extract	Molecular Formula	Molecular Weight	Retention time	Height	Area	Norm %
1	N-[4-(4-Chlorophenyl)isothiazol-5-yl)-1-methylpiperidin-2-imine	C15H16ClN3S	305.82g/mol	3.208	12,221,209	3,415,393.0	5.64
2	6-Aminohexanamide, N-methyl-N-[4-(1-pyrrolidinyl)-2-butynyl]-N'-[2-aminobutanoyl]-	$C_{18}H_{32}N_4O_2$	336.472 g/mol.	3.434	10,514,006	3,000,334.2	4.96
3	Undecane	C11H24	156.31 g/mol	4.194	16,174,781	1,514,598.5	2.50
4	(-)-Carvone	C10H14O	150.22 g/mol	6.270	40,719,400	1,767,055.1	2.92
5	Dodecanoic acid, methyl ester	C17H34O2	214.349g/mol	9.896	69,480,424	3,256,161.0	5.38
6.	Dodecanoic acid	C12H24O2	200.317g/mol	10.501	17,458,608	1,993,166.8	3.29
7	Methyl tetradecanoate	C15H30O2	242.397g/mol	13.683	46,910,284	2,768,429.5	4.57
8	Tetradecanoic acid	C14H28O2	228.37 g/mol	14.453	60,481,904	7,705,302.0	12.73
9	Neophytadiene	C20H38	278.52 g/mol	15.934	35,683,724	2,380,920.5	3.93
10	Phthalic acid, 2-cyclohexylethyl isobutyl ester	C19H26O4	308.41g/mol	17.204	22,028,720	1,728,177.2	2.86
11	Hexadecanoic acid, methyl ester	C17H34O2	270.45 g/mol	17.709	158,772,992	9,532,439.0	15.75
12	Oleic Acid	C18H34O2	282.47 g/mol	18.014	18,090,864	1,733,930.1	2.87
13	Dibutyl phthalate	$C_6H_4(CO_2C_4H_9)_2$	278.34 g/mol	18.145	23,544,750	1,469,228.8	2.43
14	n-Hexadecanoic acid	C16H32O2	256.4 g/mol	18.420	702,851,776	57,156,224.0	94.45
15	Estra-1,3,5(10)-trien-17á-ol	C18H24O2	272.4 g/mol	18.820	25,313,320	1,801,216.5	2.98
16	Androst-2,16-diene	C19H28	256.426	18.965	43,750,736	4,220,518.0	6.97
17	Androst-5,7-dien-3-ol-17-one	C19H26O2	286.4085 g/mol	19.110	25,047,488	1,851,858.0	3.06
18	9-Octadecenoic acid (Z)-, methyl ester	C19H36O2	296.50g/mol	20.931	86,754,648	4,904,895.5	8.11
19	Phytol	$C_{20}H_{40}O$	296.53 g/mol	21.086	101,917,552	5,981,906.0	9.89
20	Heptadecanoic acid, 16-methyl-, methyl ester	C19H38O2	298.5g/mol	21.461	40,998,200	1,657,840.4	2.74
21	9,12-Octadecadienoic acid (Z,Z)-	C18H32O2	280.4 g/mol	21.516	37,146,392	1,947,087.0	3.22
22	Oleic Acid	C18H34O2	282.47 g/mol	21.631	544,534,208	60,514,056.0	100.00
23	Octadecanoic acid	C18H36O2	284.48 g/mol	22.096	359,381,696	28,912,898.0	47.78
24	9-Octadecenoic acid, (2-phenyl-1,3-dioxolan-4-yl)methyl ester, cis-	C28H44O4	396.65 g/mol	22.291	36,324,772	1,362,134.5	2.25

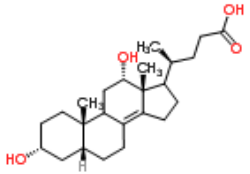
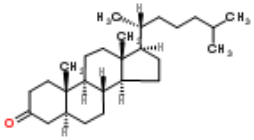
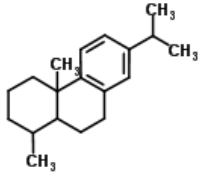
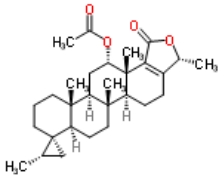
S. No.	Name of the Compound in Methanol Extract	Molecular Formula	Molecular Weight	Retention time	Height	Area	Norm %
25	Hexadecanoic acid, 1-(1-methylethyl)-1,2-ethanediyl ester	C34H66O	538.885g/mol	22.486	29,283,798	2,254,640.2	3.73
26	(1R,4aR,4bS,7S,10aR)-1,4a,7-Trimethyl-7-vinyl-1,2,3,4,4a,4b,5,6,7,8, 10,10a-dodecahydrophenanthrene-1-carbaldehyde	C20H30O	286.45 g/mol	23.442	76,194,912	4,322,462.0	7.14
27	Dehydroabietic acid	C20H30O2	302.458 g/mol	24.317	640,751,680	45,761,444.0	75.62
28	4-Norlanosta-17(20),24-diene-11,16-diol-21-oic acid, 3-oxo-16,21-lactone	C29H42O4	454.6g/mol	24.467	85,563,344	13,016,812.0	21.51
29	4H-Cyclopropa[5',6']benz[1',2':7,8]azuleno[5,6-b]oxiren-4-one, 8-(acetyloxy)-1,1a,1b,1c,2a,3,3a,6a,6b,7,8,8a-dodecahydro-3a,6b,8atrihydroxy-2a-(hydroxymethyl)-1,1,5,7-tetramethyl-, (1aà,1bá,1cá, 2aá,3aá,6aà,6bà,7à,8á,8aà)-	$C_{26}H_{34}O_{11}$	522.542 g/mol	24.697	57,510,572	2,895,040.0	4.78
30	Oleic acid, eicosyl ester	C38H74O2	562.57g/mol	24.742	52,538,024	2,084,424.9	3.44
31	Ethyl iso-allocholate	C26H44O5	436.633g/mol	24.847	48,782,640	4,317,548.5	7.13
32	Decanoic acid, 1,1a,1b,4,4a,5,7a,7b,8,9-decahydro-4a,7b-dihydroxy-3-(hydroxymethyl)-1,1,6,8-tetramethyl-5-oxo-9aH-cyclopropa[3, 4]benz[1,2-e]azulene-9,9a-diyl ester, [1aR-(1aà,1bá,4aá,7aà,7bà,8à, 9á,9aà)]-	C32H50O8	562.7g/mol	24.917	42,759,332	1,813,082.1	3.00
33	Retinoyl-á-glucuronide 6',3'-lactone	C26H34O7	458.54 g/mol	24.992	41,370,904	2,742,623.5	4.53
34	Abietic acid	$C_{20}H_{30}O_2$	302.45 g/mol	25.878	677,912,832	59,454,684.0	98.25
35	Androst-2,16-diene	C19H28	256.4256	26.183	151,894,960	13,873,561.0	22.93
36	Cholesterol	C27H46O	386.65 g/mol	27.573	240,350,176	27,816,754.0	45.97
37	17-(1,5-Dimethylhexyl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,	$C_{27}H_{44}O$	384.64g/mol	27.708	248,166,784	42,073,104.0	69.53

S. No.	Name of the Compound in Methanol Extract	Molecular Formula	Molecular Weight	Retention time	Height	Area	Norm %
	16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-ol						
38	Diisooctyl phthalate	C24H38O4	390.5561 g/mol	28.074	562,491,712	28,066,392.0	46.38
39	1H-Cyclopropa[3,4]benz[1,2-e]azulene-4a,5,7b,9,9a(1aH)-pentol, 3-[(acetyloxy)methyl]-1b,4,5,7a,8,9-hexahydro-1,1,6,8-tetramethyl-, 9, 9a-diacetate, [1aR-(1aà,1bá,4aá,5à,7aà,7bà,8à,9á,9aà)]-	C36H39NO11	661.69496g/mol	28.184	45,471,468	1,588,496.2	2.63
40	Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester	C19H38O	330.5026g/mol	29.834	41,510,896	1,366,756.6	2.26

Table 3. Chemical structure of ten volatile compounds identified based on the peak area

S. No.	Compound Name	Chemical Structure	Norm%
1	Oleic Acid		100.00
2	Abietic acid		98.25

S. No.	Compound Name	Chemical Structure	Norm%
3	n-Hexadecanoic acid		94.45
4	Dehydroabietic acid		75.62
5	17-(1,5-Dimethylhexyl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-ol		69.53
6	Octadecanoic acid		47.78

S. No.	Compound Name	Chemical Structure	Norm%
7	Diisooctyl phthalate		46.38
8	Cholesterol		45.97
9	Androst-2,16-diene		22.93
10	4-Norlanosta-17(20),24-diene-11,16-diol-21-oic acid, 3-oxo-16,21- lactone		21.51

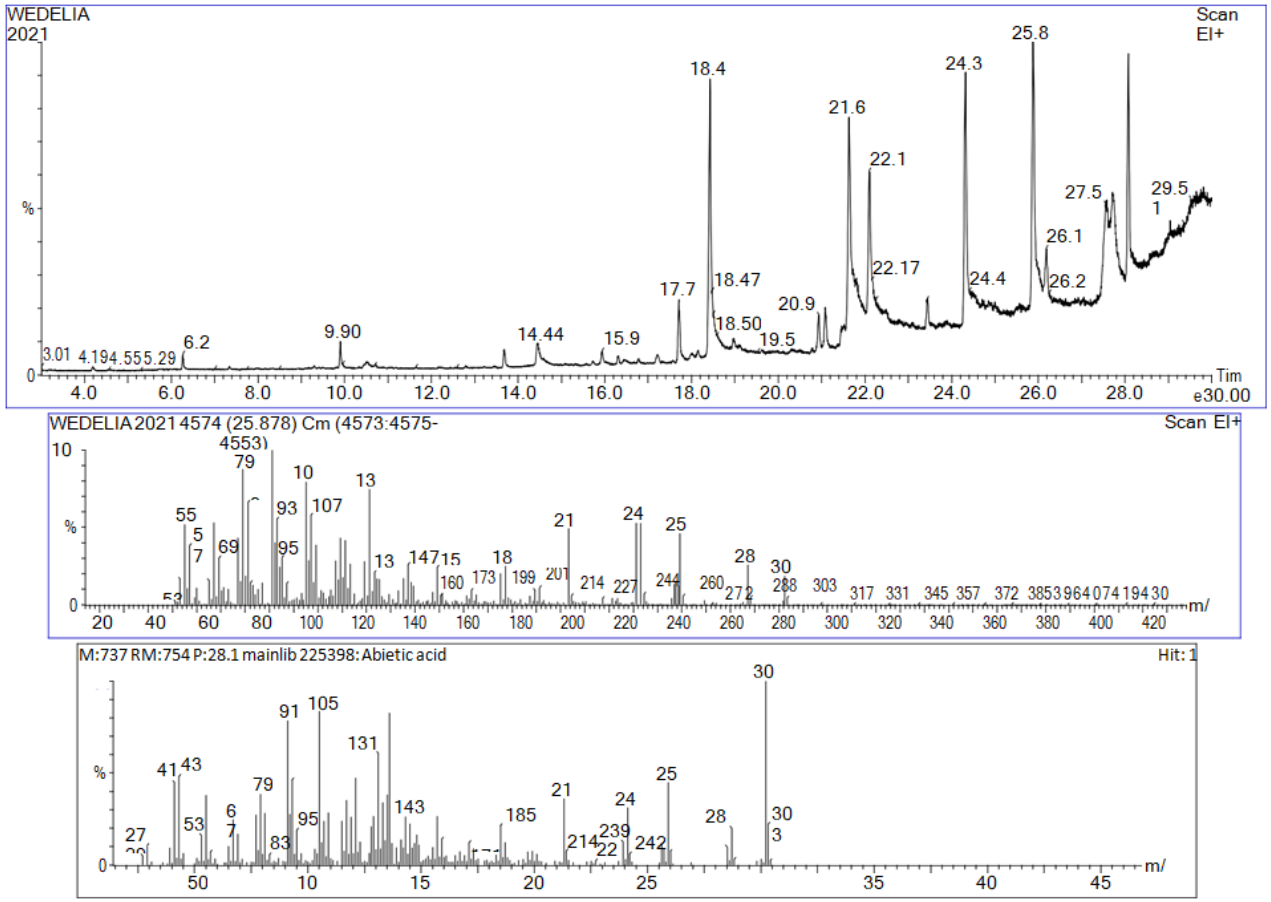


Fig. 1. GC-MS analysis of *Sphagneticola trilobata*

9,12-Octadecadienoic acid (Z,Z)-, Octadecanoic acid, 9-Octadecenoic acid, (2-phenyl-1,3-dioxolan-4-yl)methyl ester, cis-, Hexadecanoic acid, 1-(1-methylethyl)-1,2-ethanediyl ester, (1R,4aR,4bS, 7S,10aR)-1,4a,7-Trimethyl-7-vinyl-1,2,3,4,4a,4b,5,6,7,8,10,10a-dodecahydrophenan threne-1-carbaldehyde, Dehydroabietic acid, 4-Norlanosta-17(20),24-diene-11,16-diol-21-oic acid, 3-oxo-16,21-lactone, 4H-Cyclopropa [5',6']benz [1',2':7,8] azuleno[5,6-b]oxiren-4-one, 8 (acetyloxy)-1,1a,1b,1c,2a,3,3a,6a,6b,7,8,8a-dodecahydro-3a,6b,8atrihydroxy- 2a-(hydroxymethyl)-1,1,5,7-tetramethyl-, (1aà,1bá,1cá, 2aá,3aá,6aà,6bà, 7à,8á,8aà)-, Oleic acid, eicosyl ester, Ethyl iso-allocholate, Decanoic acid, 1,1a,1b,4,4a,5,7a ,7b,8,9-decahydro-4a,7b-dihydroxy- 3-(hydroxymethyl)-1,1,6,8-tetramethyl-5-oxo-9aH-cyclopropa[3, 4]benz[1,2-e]azulene-9,9a-diyl ester, [1aR-(1aà,1bá,4aá,7aà,7bà,8à, 9á,9aà)]-, Retinoyl-á-glucuronide 6',3'-lactone, Abietic acid, Androst-2,16-diene, Cholesterol, 17-(1,5-Dimethylhexyl)-10,13-dimethyl-2,3,4,7,8,9, 10,11, 12,13,14,15, 16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-ol, Diisooctyl phthalate, 1H-Cyclopropa[3,4] benz[1,2-e]azulene-4a, 5,7b,9,9a(1aH)- pentol, 3-[(acetyloxy)methyl]-1b,4,5,7a,8,9-hexahydro-1,1,6,8-tetramethyl-, 9, 9a-diacetate, [1aR-(1aà, 1bá,4aá,5à,7aà,7bà,8à,9á,9aà)]-, Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester. Table 3 shows the top ten peak percentage of compound chemical structures. Oleic Acid also present. Fig. 1 shows GC-MS analysis of *S. trilobata* leaf extract

3.3 Antioxidant Action of *S. trilobata*

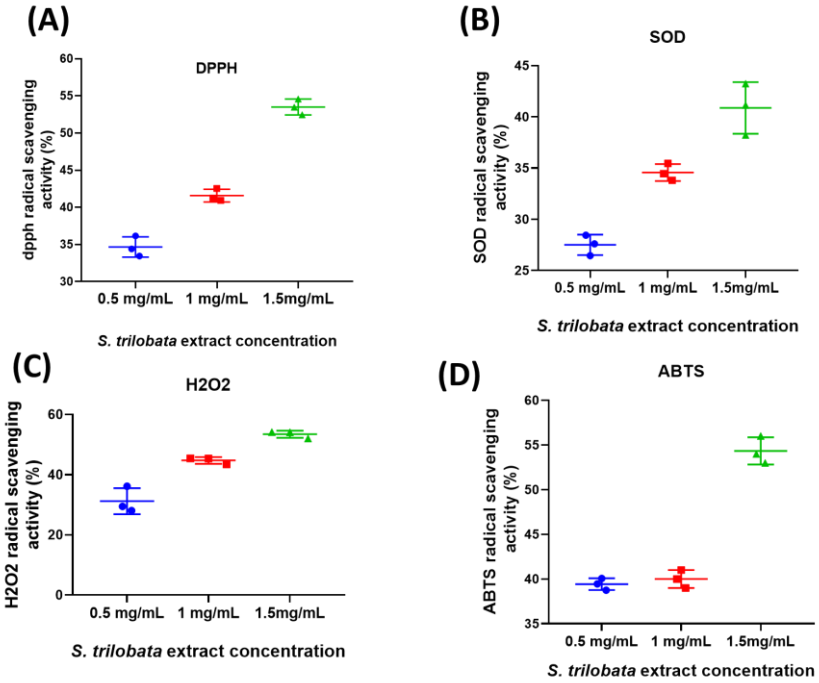
In this work, the DPPH, Hydrogen peroxide, ABTS scavenging, and Superoxide radical scavenging assays showed dose-dependent antioxidant efficacy. The activity of the DPPH, ABTS, SOD, and hydrogen peroxide scavenging enzymes were 53.5%, 54.7%, 53.4%, and 40.8%, respectively [30]. Antioxidants are essential for a healthy human body. Antioxidants are crucial for avoiding free radical damage and maintaining the integrity of cellular structures [31]. They are essential in eliminating these damaging free radicals, which stops the organism from being harmed by oxidation [32]. Numerous plant extracts have natural antioxidant properties that are not reliant on antioxidant functionalization but rather on the substance's surface properties [33]. So, we evaluated the *S. trilobata* leaf extract's in vitro antioxidant capacity. Numerous techniques can be needed to evaluate the individual compound's in vitro antioxidant efficacy entirely.According to prior studies, phenolic compounds, which are often present in plant epidermal tissue, have an impact on the free radical scavenging abilities of plant-based extracts. The ability of the intrinsic phenolics to donate atomic hydrogen or ions to trap free radicals is primarily responsible for the antioxidant action of plant and fruit extracts [34,35].

3.4 Antibacterial Action of *S. trilobata* Leaf Extract

The antibacterial activity of *S. trilobata* leaf extract against *S. aureus and P. aeruginosa* was investigated in the present research. *S. trilobata* leaf extract was shown to have high antibacterial activities in the investigation. At 1.5 mg/mL, the highest efficacy was seen against *P. aeruginosa* (21 mm) compared to *S. aureus* (12.6).

S. aureus and *P. aeruginosa* growth kinetics were also investigated. *S. trilobata* leaf extract suppressed bacterial growth at doses of 0.5,1.0, and 1.5 mg/mL.The growth of bacteria is impeded by the extract derived from *S. trilobata* leaves. The leaf extract of *S. trilobata* demonstrates a more pronounced antibacterial effect against *P. aeruginosa* and *S*. *aureus*. The reduction in bacterial viability can be attributed to the synergistic activity of the leaf extract of *S. trilobata*. Prior research has provided evidence of the antibacterial efficacy of *S. trilobata* leaf extract against pathogenic bacteria [36,37]. In the course of our research, it was observed that the leaf extract derived from *S. trilobata* exhibited significant antibacterial efficacy. Previous studies demonstrated the antibacterial property of *S. trilobata* leaf extract against pathogenic bacteria [38,39]. The *S. trilobata* leaf extract showed high antibacterial activity in our study.Some of the medicinal plants significant the antibacterial effect on different kinds of baterias [40-45].

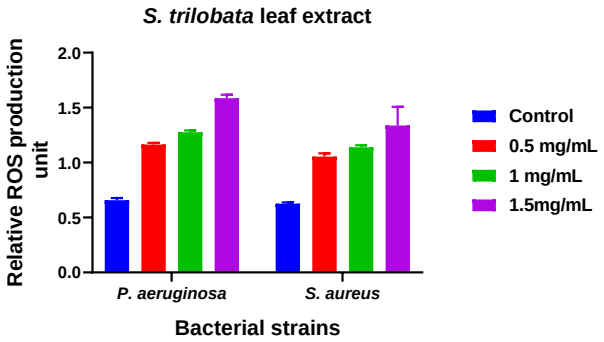
The antibacterial mechanism of *S. trilobata* leaf extract was investigated using ROS (reactive oxygen species) and POD (peroxidase associated with ROS), MDA (malondialdehyde) levels, and SOD (superoxide dismutase) activity. In order to gain further insights, the research team employed H2DCF-DA as a means to



Graph 1(A-D). Antioxidant activity of *S.trilobata* leaf *extract* on scavenging the free radicals DPPH, SOD, H_2O_2 and ABTS

investigate the impact of *S. trilobata* leaf extract on the generation of reactive oxygen species (ROS) in bacteria. Fig. 1 illustrates a positive correlation between the quantity of *S. trilobata* leaf extract and the level of reactive oxygen species (ROS) generated by bacterial cells. Based on the findings presented, it can be inferred that the leaf extract of *S. trilobata* exhibits potential antibacterial properties against *S. aureus* and *P. aeruginosa* through the stimulation of reactive oxygen species (ROS) production. In the absence of the plant extract, the control group did not exhibit any fluorescence emission, thereby suggesting the absence of reactive oxygen species (ROS) generation. Fluorescence images depicting elevated levels of reactive oxygen species (ROS) induced by pathogens exhibit pronounced fluorescent intensities across all tested doses of *S. trilobata* leaf extract. Moreover, the production of reactive oxygen species (ROS) leads to their interaction with various cellular components such as DNA, peptidoglycan layer, proteins, lipids, cytoplasmic membrane, and other physiological processes. The repulsion between the negatively charged membrane of a microbe and the positively charged nickel molecule results in the release of proteins and other intracellular components, ultimately resulting in the demise of the cell [44].

The study aimed to assess the influence of reactive oxygen species (ROS) formation on the antioxidant defenses by monitoring the activity of peroxidase (POD) and superoxide dismutase (SOD). The experimental findings demonstrate a positive correlation between the concentration of *S. trilobata* leaf extract (0.5, 1, and 1.5 mg/mL) and the enzymatic activity of SOD and POD in bacterial cells, as depicted in Graph 1a and 1b. The assessment of membrane injury was conducted through the measurement of malondialdehyde (MDA) concentration. The concentration of MDA in bacterial pathogens showed a significant increase following exposure to the leaf extract of *S. trilobata* (Graph 1c). Microorganisms possess a variety of reactive oxygen species (ROS)-scavenging enzymes that effectively counteract ROS, even under non-stressful conditions. The scavenging mechanism of reactive oxygen species (ROS) by the bacteria was likely unable to cope with the increased production of ROS when exposed to a concentration of 200 g/mL of *S. trilobata* leaf extract. The antibacterial mechanisms are dependent on the production of reactive oxygen species (ROS), which induce damage to the DNA of bacteria, ultimately leading to their demise. The aforementioned study conducted by [45] yielded comparable results.



Graph 2. Bar graphs represent the zone of inhibition

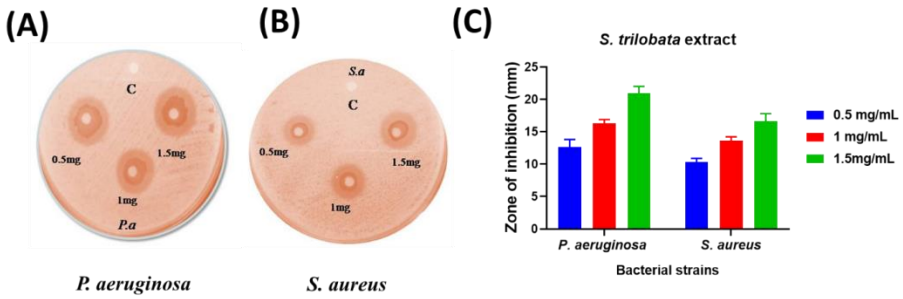
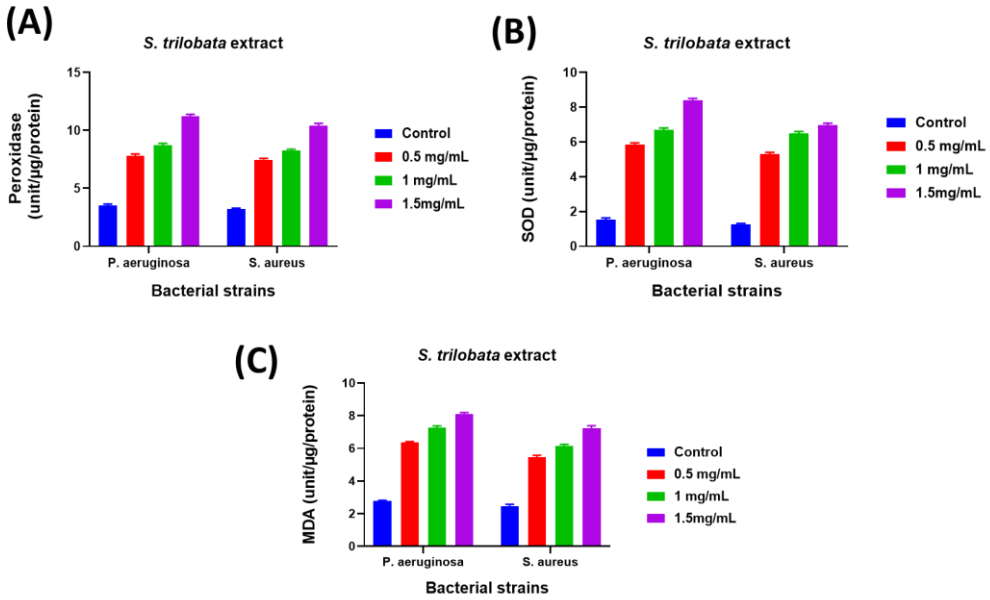


Fig. 2 (A-B). Antibacterial efficacy of *S. trilobata* leaf extractagainst *P. aeruginosa* and *S. aureus*. Graph 3 *S. trilobata* leaf extract induced ROS production in bacterial cells *S. aureus*and *P. aeruginosa*, in a concentration dependent fashion



Graph 3. Peroxide dismutase and superoxide dismutase were both elevated in response to treatment with S. trilobata leaf extract. Nanoparticles at varying doses produce membrane damage, as shown by the MDA test. The data are shown as a mean SD. Statistical significance was determined at the mean±SD.p<0.05, *p<0.01levels; ns = not significant**

4. CONCLUSION

In conclusion, this study has the potential to be utilized as a diagnostic tool for standardization purposes. The leaves of *S.trilobata* plants possess a diverse array of phytochemical compounds. The examination of the extracts is being conducted with the purpose of isolating, characterizing, and clarifying the composition of the bioactive compounds found within the extracts. These compounds are accountable for the notable pharmacological effects observed. The antimicrobial properties of certain compounds serve as a catalyst for additional investigations into novel chemical architectures that have the potential to mitigate or eradicate specific diseases. The findings of this study lend support to the hypothesis that several medicinal plants commonly utilized in India may possess potential as viable sources of antioxidants.

NOTE

The study highlights the efficacy of "Herbal" which is an ancient tradition, used in some parts of India. This ancient concept should be carefully evaluated in the light of modern medical science and can be utilized partially if found suitable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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