

Research Article

PHYTOCHEMICAL, GC-MS ANALYSIS AND ANTIBACTERIAL ACTIVITY OF LEAF EXTRACT OF *SOLANUM TRILOBATUM* L.

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ABSTRACT

The study objective was to use GC-MS analysis to extract the phytochemical components from *Solanum trilobatum* leaves. For phytochemical investigation, *S. trilobatum* leaves were evaluated for possible antibacterial action against *Bacillus subtilis* and *Staphylococcus aureus*. The ethanolic leaf extract of *S. trilobatum* included 30 distinct phytochemical components, according to the results of the GC-MS analysis. The major phytoconstituents were n-Hexadecanoic acid (14.07), 1-(2-methylsulfanyl-ethyl)-2,8,9-trioxo (12.12), 9,12,15-Octadecatrienoic acid, (Z, Z, Z)- (11.97), Pregn-5-en-3-ol, 20-methyl-21-[3-methyl- (11.88) and Phytol (10.04). Using the disc diffusion technique, the plant extracts were made in ethanolic extracts to test their antibacterial efficacy against *B. subtilis* and *S. aureus* bacterial pathogens. *Staphylococcus aureus* had the highest zone of inhibition and the greatest activity among the *S. trilobatum* leaves employed for antibacterial study. Therefore, the plant's medicinal properties may be due to the presence of these phytochemicals.

Keywords: GC-MS chromatogram, Leaves sample, Phytochemicals, Mass spectrum, *S. trilobatum* L.

INTRODUCTION

Plants have been an important source of medicine and mainly on traditional remedies. Ferns have been used as popular folk medicine. The medicinal importance of the Pteridophyte is due to the presence of some special compounds like alkaloids, flavonoids, phenols, tannins and saponins. These active principles usually remain concentrated in the storage organs of the plants *viz.*, roots, fronds, rhizome *etc.* In general, these secondary metabolites are an important source of drug with a variety of structural arrangements and properties (De Fatima *et al.*, 2006). Medicinal plants are still major parts of traditional system medicine in developing countries many infectious disease are known to be treated with herbal remedies throughout the history of mankind. Even today plant materials continue to play a major role in primary healthcare as therapeutic remedies in many developing countries (Sukanya *et al.*, 2009). Medicinal plants which form the backbone of traditional medicine have in the last few decades been the subject of very intense pharmacological studies. This has been brought about by the acknowledgement of the value of medicinal plant as potential source of new compounds of

therapeutic value and as source of new compounds in drug development.

Natural antioxidants in the form of their chemical constituents or raw plant extracts are very effective in preventing harmful conditions instigated by oxidative stress (Zengin *et al.*, 2011). These antioxidants have the ability to scavenge free radicals, which are usually in the form of reactive oxygen or nitrogen species (ROS/RNS). Oxidative stress may be associated with the appearance of various ailments such as neurological and cardiovascular disorders, as well as cancer (Park *et al.*, 2011). Many studies have reported the positive potential of certain antioxidants to help prevent these diseases (Marrelli *et al.*, 2012). Demand for therapeutic natural products with antioxidant activities capable of lessening the detrimental effects of free radicals has increased (Sofi *et al.*, 2016). Herbal medicines have been known to human for centuries. Practitioners of traditional medicine have described therapeutic efficacy of many indigenous plants for several disorders. This is due to the fact that plants contain many biologically active compounds which have potential for development as medicinal agents (De and Ifeoma, 2002). Herbal medicines

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already form the basis of therapeutic use in the developing countries, but of recent, there has been an increase in the use of herbal medicines in the developed world too (El-Mahmood *et al.*, 2010). It is likely that plants will continue to be a valuable source of new molecules which may, after possible chemical manipulation and provide new and improved drugs (Shah *et al.*, 2006).

Plants are among the most important and common sources of potentially valuable new drugs. For that reason, there is a need to investigate the biological properties of medicinal plants in order to isolate new drugs. So, the aim of the present research was to study the phytochemicals screening, GC-MS analysis and antibacterial activity of leaves of *Solanum trilobatum*.

MATERIAL AND METHODS

Preparation of leaf extract

Solanum trilobatum (L.) (Figure 1) plant leaves were collected in the campus of K.K.Government Arts College, Tiruvannamalai and its surrounding area. The leaves were washed cleanly with tap water and powdered after shade dried. The powder was filled in amber coloured bottle and stored in dark condition until it was used for analysis. Leaf powder (20g) was subjected to successive solvent extraction using different solvents (chloroform, ethanol, petroleum ether, aqueous) soaked in 1000mL of methanol solvent (1:5) and incubated for 24h under dark conditions with occasional stirring. Solvent extract obtained by muslin cloth filtration (700mL), was sub-filtered with non-absorbent cotton and then final filtration was done with Whatman No.1 filter paper. The obtained filtrate was concentrated in rotary evaporator method; rotary evaporation of filtrate was done at 50–55°C of internal temperature in the round bottom flask of rotary evaporator instrument (Raaman 2006).



Figure 1. *Solanum trilobatum* L view.

Phytochemical screening of the plant material

The phytochemical tests were carried out on the chloroform, ethanol, petroleum ether, aqueous extracts using procedure to identify the phytochemicals as described by Sofowara (1993), Trease and Evans (1989) and Harborne (1978).

Test for Alkaloids

To 2ml of extract, 2ml of concentrated hydrochloric acid was added. Then few drops of Mayer's reagent were added. Presence of green color indicated the presence of alkaloids.

Test for Flavonoids

5ml of dilute ammonia solution was added to a portion of the aqueous filtrate of extract followed by addition of concentrated sulphuric acid. Appearance of yellow colouration indicated the presence of flavonoids.

Test for Glycosides

To 1 ml of the extract add few drops of HCl, allowed for 5 minutes for hydrolysis and neutralized with NaOH solution. A few drops of Fehling's solution A and B for few minutes. An orange red precipitate indicates the presence of glycosides.

Test for Phenols

To 1ml of the extract, 2ml of distilled water followed by few drops of 10% ferric chloride was added. Formation of green color indicated the presence of phenols.

Test for Terpenoids

To 0.5ml of extract, 2ml of chloroform was added and concentrated sulphuric acid was added carefully. Red brown color formation at the interface indicated the presence of terpenoids.

Test for Saponins

To 2ml of extract, 2ml of distilled water was added and shaken in a graduated cylinder for 15 minutes lengthwise. Formation of 1cm layer of foam indicated the presence of saponins.

Test for Tannins

To 1ml of extract, 2ml of 5% ferric chloride was added. Formation of greenish black color indicated the presence of tannins.

Test for Steroids

To 1ml of extract mixed with chloroform and a few drops of concentrated H_2SO_4 , shaken well and allowed to stand for some time. Red color appeared at the lower layer indicated the presence of steroids.

GC-MS Spectrometry

The identification of varied compounds was resolute by GC-MS analysis of the leaf ethanolic extracts. The study was conducted at Periyar University, Department of Microbiology, Salem, Tamil Nadu. The GC-MS analysis of the ethanolic leaf extract of *Solanum trilobatum* was performed employing a Perkin Elmer GC-MS (Model: Shimadzu QP-2020) equipped with associate degree agilent column (30m $\times$ 250 μ m $\times$ 0.25 μ m). The oven appliance temperature was programmed at 40°C for 2 min so exaggerated to 30°C for six min, at 10°C/min. Helium was used because the carrier gas at flow of 1.0 ml/min. The one μ L of the ethanolic leaf extract of *S. trilobatum* was injected with split ratio 10:1 at contrivance temperature was 280°C. The characterization of compounds was resolute supported the retention time. The spectrums of the parts were compared with the info of the spectrum of famous parts hold on within the GC-MS, National Institute of ordinary Technology (GC-MS, NIST-2017) Library.

Antibacterial activity of the extracts

The antibacterial activity of the extracts against the isolates was determined using disc diffusion method as described by Ali *et al.*, (2017) with slight modifications. The prepared bacterial suspension equivalent to 0.5 McFarland Standard

(equivalent to 1.5×10^6 CFU) was inoculated into sterile Mueller- Hinton agar medium in a sterile Petri-dish. A sterile 6 mm diameter sterile cork-borer was used to bore 5 wells into the agar medium at equidistance. The disc were then filled up with approximately 0.1mL of the extract solution at a concentration of 25, 50, 75, 100 and 200 mg/L taking care to prevent spillage onto the surface of the agar medium. The plates were allowed to stand on the laboratory bench for 1 hour to allow proper diffusion of the extract into the medium after which the plates were incubated at 37°C for 24 hours thereafter the plates were observed for zones of inhibition and measured. Ciprofloxacin 100 mg/mL was used as a positive control in the experiment. The experiment was conducted in triplicate and the average zone of inhibition was calculated.

Data Analysis

The results of the phytochemical and proximate measurements were presented as the mean standard deviation. The values are $\pm$ SD for three samples in each group.

RESULT AND DISCUSSION

Phytochemical screening of chloroform, ethanol, petroleum ether, aqueous extracts of leaves of *Solanum trilobatum* were analysed and the results are presented in Tables-1, Various phytochemicals, including alkaloids, flavonoids, glycosides, phenols, terpenoids, saponins, tannins, and steroids were found in *S. trilobatum* leaves. Stronger phytochemicals, including alkaloids, flavonoids, glycosides, phenols, terpenoids, saponins, tannins, and steroids were found in ethanol extracts in comparison to other extracts. In contrast, flavonoids, phenols, terpenoids and steroids were found in the petroleum ether and chloroform extract, whereas the remaining secondary metabolites were absent. Aqueous extracts had a smaller amount of terpenoids, flavonoids, phenols, and phytochemicals (Table 1). From GC-MS analysis 30 active components were detected from ethanolic leaves of *S. trilobatum* (L.). The identification of phytochemical compounds was based on retention time, molecular formula, peak area, molecular weight and medicinal activity is presented in Table 2 and Figure 1.

Table 1. Preliminary phytochemical screening of leaves of *S. trilobatum* (L.) with different solvents.

S. No	Phytochemical constituents	Chloroform	Ethanol	Petroleum ether	Aqueous
1.	Alkaloids	-	+	+	-
2.	Flavonoids	+	+	+	+
3.	Glycosides	-	+	-	-
4.	Phenols	+	+	+	+
5.	Terpenoids	-	+	+	+
6.	Saponins	-	+	-	-
7.	Tannins	-	+	+	-
8.	Steroids	+	+	+	-

Note: (+) = positive (present); (-) = negative (absent)

Five major compounds were identified, such as n-Hexadecanoic acid (14.07), 1-(2-methylsulfanyl-ethyl)-2,8,9-trioxa (12.12), 9,12,15-Octadecatrienoic acid, (Z, Z, Z)- (11.97), Pregn-5-en-3-ol, 20-methyl-21-[3-methyl- (11.88), and Phytol (10.04). In addition, the minor compounds such as eugenol (4.32), neophytadiene (3.78), octadecanoic acid (2.79), 7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-di (2.63), Pregn-5-en-3-ol, 20-methyl-21-[3-methyl- (2.51), Tetracontane (2.27) and 6-methoxy-2,7,8-trimethyl-2-(4,8,12-trimethyltridecyl (2.16).

Table 2. Phytochemical compounds identified in the ethanolic leaf extract of *S. trilobatum* by GC–MS analysis.

S. No	RT	Area %	Peak Height %	Name of the compound
1.	4.239	0.40	0.78	Silane, dimethoxydimethyl-
2.	13.788	4.98	4.32	Eugenol
3.	16.875	0.67	0.97	(-)-5-oxatricyclo [8.2.0.0(4,6)] dodecane,12
4.	18.510	0.94	1.19	(-)-spathulenol
5.	19.517	2.57	3.78	Neophytadiene
6.	19.772	0.63	0.78	Neophytadiene
7.	19.968	0.75	1.14	Neophytadiene
8.	20.311	1.65	2.63	7,9-Di-tert-butyl-1-oxaspiro (4,5) deca-6,9-diene-2,8-di
9.	20.785	11.29	14.07	n-Hexadecanoic acid
10.	22.132	0.87	1.35	9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl es
11.	22.235	6.98	10.04	Phytol
12.	22.443	8.44	12.12	1-(2-methylsulfanyl-ethyl)-2,8,9-trioxa
13.	22.498	9.76	11.97	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-
14.	22.695	2.07	2.79	Octadecanoic acid
15.	23.103	0.63	0.96	1-Hexadecanol, Acetate
16.	23.309	0.54	0.80	2-Butylamine, N-(3-methylbutyl)-
17.	23.697	1.46	1.00	25-Nor-9,19-cyclolanostan-24-one, 3-acetoxy-24-phen
18.	23.940	4.46	2.51	Pregn-5-en-3-ol, 20-methyl-21-[3-methyl-
19.	24.085	22.20	11.88	Pregn-5-en-3-ol, 20-methyl-21-[3-methyl-
20.	25.125	1.00	1.15	Phorbol
21.	25.686	0.65	0.91	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethy
22.	25.847	3.23	2.24	Stigmasterol
23.	25.899	1.78	1.96	5,8 .alpha.-epidioxycholesta-6,22(23)-dien
24.	26.185	1.05	0.64	Tetrapentacontane
25.	26.494	0.62	0.65	Cyclohexanol, 1,3,3-trimethyl-2-(3-methyl-2-methylen
26.	26.976	2.31	2.27	Tetracontane
27.	27.214	1.21	0.93	Tetrahydrofuran-2,5-diol, 3-hydroxyimino-4-methoxy-
28.	27.506	4.45	2.16	6-methoxy-2,7,8-trimethyl-2-(4,8,12-trimethyltridecyl
29.	28.395	1.08	1.17	Squalene
30.	28.638	1.33	0.84	Tetrapentacontane

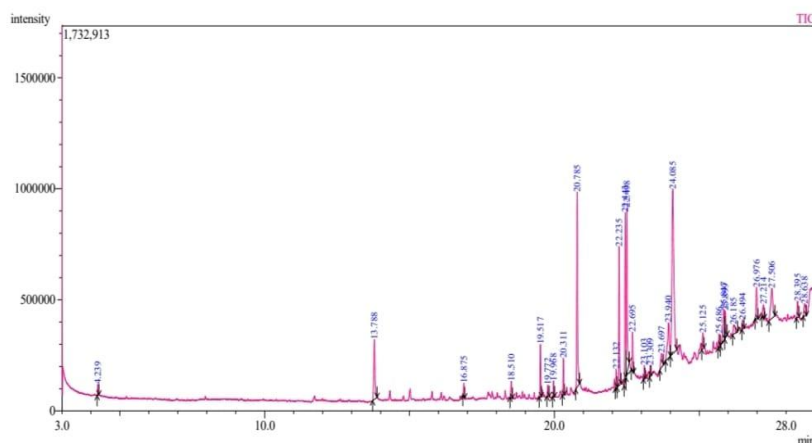


Figure 1. GC-MS chromatogram of leaf ethanolic extract of *S. trilobatum* L.

The table-3 displays the antibacterial activity of *S. trilobatum* L. aqueous extracts, petroleum ether, ethanol and chloroform against *B. subtilis* and *Staphylococcus aureus*. With a zone of inhibition of 22.10 ± 0.66 mm at 200 mg/mL of ethanol leaf extract, the results indicate that *Staphylococcus aureus* exhibited the strongest antibacterial activity. Inhibition zones for *S. aureus* and *B. subtilis* were 26.30 ± 0.78 mm and 22.60 ± 0.67 mm, respectively, as demonstrated by the control (100 mg/mL Ciprofloxacin). Similarly, various solvent extract of *S. trilobatum* leaf

shows good antibacterial activity against 3 gram positive and 5-gram negative bacterial pathogens (Doss and Dhanabalan, 2008). Previous reports showed that, four different species of Methanol extract of *Solanum* shows significant antibacterial activity (Akilan *et al.*, 2014). These plants showed significant antimicrobial activity against some bacterial pathogens such as *E.coli*, *Klebsiella pneumoniae* and *Proteus mirabilis* (Toor and Savage, 2005).

Table 3. Antibacterial activity of the extracts against *S. aureus* and *B. subtilis*.

Extracts	Concentration ($\mu\text{g/mL}$)	Zone of inhibition (mm in dm)	
		<i>Staphylococcus aureus</i>	<i>Bacillus subtilis</i>
Chloroform	25	8.60 ± 0.77	8.10 ± 0.72
	50	10.10 ± 0.90	9.70 ± 0.29
	75	13.30 ± 0.39	12.20 ± 0.36
	100	14.60 ± 0.43	14.40 ± 0.43
	200	16.60 ± 0.49	16.30 ± 0.48
Petroleum ether	25	10.10 ± 0.90	9.30 ± 0.27
	50	13.30 ± 0.39	12.40 ± 0.37
	75	15.00 ± 0.45	14.10 ± 0.42
	100	16.70 ± 0.50	15.60 ± 0.46
	200	20.10 ± 0.60	18.70 ± 0.56
Control	100	26.30 ± 0.78	22.60 ± 0.67
	25	10.70 ± 0.32	9.70 ± 0.29
Ethanol	50	13.60 ± 0.40	12.40 ± 0.37
	75	15.10 ± 0.45	14.60 ± 0.43
	100	17.40 ± 0.52	15.70 ± 0.47
	200	22.10 ± 0.66	19.00 ± 0.57
	25	8.60 ± 0.77	8.10 ± 0.72
Aqueous	50	10.10 ± 0.90	9.70 ± 0.29
	75	13.30 ± 0.39	12.20 ± 0.36
	100	14.60 ± 0.43	14.40 ± 0.43
	200	16.60 ± 0.49	16.30 ± 0.48

Mean value $\pm$ SD, n = 3; Statistical analysis data are expressed as means $\pm$ SD

CONCLUSION

The many phytochemical classes found in *S. trilobatum* (L.) leaves were better understood to the qualitative and quantitative phytochemical analysis, which also clarified the reason for their efficacy in traditional medicine. Historically, plants have been used to cure a wide range of illnesses. Herbs are a class of chemical compounds that are extracted from plants that have therapeutic and health-promoting qualities. The fact that *Solanum trilobatum* L. is a common medicine among the many ethnic groups of Siddha and Ayurvedic qualities was illustrated by screening the literature on the plant.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest

ETHICS APPROVAL

Not applicable

AI TOOL DECLARATION

The authors declares that no AI and related tools are used to write the scientific content of this manuscript.

DATA AVAILABILITY

Data will be available on request

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