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Original Article

PHYTOCHEMICAL STUDIES AND GC-MS ANALYSIS OF *SPERMADICTYON SUAVEOLENS ROXB*

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ABSTRACT

Objective: The present study was performed to identify the phytochemical constituents of leaves and flowers of a plant *Spermadictyon suaveolens* extracted with four different solvents.

Methods: Dried and powdered samples were subjected to soxhlation based on the polarity of the solvents. The extracts were scanned using Ultra Violet-visible (UV-Vis) spectrophotometry with the wavelength ranging from 200–800 nm by comparing the absorption spectrum with the spectra of known compounds, Fourier Transform Infrared (FT-IR) spectrometry was used to find out the functional groups of the compounds and GC-MS system consisting of a Perkin Elmer Technologies Model Clarus 680 GC equipped with Clarus 600 (EI) was used to identify the metabolites by matching their recorded mass spectra with the standard mass spectra from National Institute of Standards and Technology (NIST05. LIB) libraries provided by the software of the GCMS system (Turbo Mass version 5.4.2).

Results: The phytochemical tests indicated the presence of carbohydrates, alkaloids, flavonoids, phenols, tannins, saponins and terpenoids from the chloroform extract of leaves and flowers. UV-visible spectrophotometer results indicated a wavelength range between 230–660 nm for the flower and leaf extracts for major peaks. FT-IR analysis indicated major functional groups such as aromatic, primary, secondary and aliphatic amines, alkanes, carboxylic acids and amides. GC-MS analysis results revealed major bioactive compounds in the crude extracts.

Conclusion: Presence of secondary metabolites has been identified from the phytochemical studies. Many phyto-compounds have been identified from the leaves and flowers of using GC-MS analysis. Hence, this medicinal plant may be used as a source for treating many diseases.

Keywords: Medicinal plant, Phytochemical screening, UV-visible spectrophotometry, FTIR, GC-MS

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INTRODUCTION

Medicinal plants besides being used as a source of therapeutic agents also contain an extensive range of chemical constituents which could be developed as drugs with few selective compounds. They are the pools of useful chemical compounds which may serve as pointer and evidence for modern drug design [1]. Naturally, present phytochemicals in leaves, fruits, seeds, barks and roots of medicinal plants have a protective and resistant mechanism for various diseases. Phytochemicals contain both primary and secondary compounds. Chlorophyll, proteins and common sugars are considered as primary compounds and terpenoids, alkaloids, flavonoids, saponins and phenolic compounds are appraised as secondary compounds [2]. Terpenoids reveals significant pharmacological action against inflammation, cancer, malaria, viral and bacterial infections, [3]. Alkaloids are considered as anaesthetic agents, inhibitors of micro-organisms, antihypertensive effects and possess antimalarial activities which are mainly found in medicinal plants [4].

Spermadictyon suaveolens belongs to the family of Rubiaceae. It is commonly known as "Forest Champa" 'Van-champa', 'Gidesa', 'Jitsaya'. It is branched shrub, 1-2m tall, branches divaricate. Leaves are dark green in colour opposite, elliptic, lanceolate, narrowed at base. The stems are gray in colour, circular in shape. Flowers are small, white, and fragrant. Seeds are few, triquetrous, surrounded by a loose lace-like covering. Capsules 5 valved [5]. It is dispersed commonly in Maharashtra. It shows the wide distribution in tropical dry/moist deciduous forests. It is also distributed in the Himalayas. China cultivates *S. suaveolens* for its fragrant flowers. Roots are useful for production of oil which can be used to treat wounds and found to have wound healing property. The roots are also used in the treatment of diabetes, rheumatoid arthritis and bloody dysentery in veterinary medicine [6, 7]. The traditional healers of the Maharashtra use roots and stem for curing the diseases associated to the bone, wound healing, diabetes, Herpes, etc. There are 30 bioactive phyto-compounds identified in the extracts of *S. suaveolens* reported from the root of this plant. The stem powder of this plant is used by herbal medicinal practitioners for control of viral infections like herpes as well as to diabetes [8]. Several researchers are paying attention towards this plant and its drug in recent days due to its broad activity. Insufficient pharmacognostic assessment of this astonishing plant has been done so far, The rationale and the key objective of the present study is to identify the phytochemical constituents present in leaves and flowers of the plant *Spermadictyon suaveolens* extracted with four different solvents based on the polarity.

MATERIALS AND METHODS

Medicinal plants were collected from the Foundation for Revitalization of Local Health Traditions (FRLHT), Jarakbande Kaval, (13 ° 6' 30.6936'' N latitude and 77 ° 32' 10.1256'' E longtitude) Bengaluru, Karnataka, India. The plant materials were identified based on the morphology and taxonomy on the field by plant taxonomist N. M. Ganesh Babu PhD, Bengaluru, Karnataka, India. Plant materials were cleaned and shade dried at room temperature (37 °C) for ten days. The dried plant materials were powdered and the sample was subjected to sequential soxhlation extraction using petroleum ether, chloroform, ethyl acetate and methanol (Merck India Limited, Bangalore). The extracts were filtered in 1 µm pore size Whatman filter paper and purely refined extracts were used in this study. The plant extracts were tested for the presence and absence of secondary metabolites using phytochemical analysis by standard methods [9, 10].

a. UV-visible spectrophotometry

The absorbance and wavelength of the peaks were determined for the methanolic and chloroform plant extracts by a wavelength scan between 200 and 800 nm [11]. The UV-visible spectra were recorded on a (Shimadzu UVd-1800 PC, Japan) UV-Vis spectrophotometer.

b. FT-IR analysis

FTIR analysis was performed using Magna 750 FTIR spectrometer equipped with a DTGS (Deuterated Triglycine Sulfate) detector, Ni-Chrome source and KBr beam splitter. The spectrum of the solvent extracted plant samples were observed at the range of 4000-500 cm^{-1} with a resolution of 4 cm^{-1}. The spectrum details were collected and processed using Omnic software version 7.3

c. Gas column-mass spectrophotometry analysis

Five milligrams of the solvent extracted sample was weighed, powdered and transferred to a sterile clean test tube and dissolved with the desired solvent. The sample solution was filtered using 0.2 µm nylon membrane and the filtered sample solution was injected into the column for running GC-MS. The analysis was carried out on a Perkin-Elmer workstation, with model Clarus 600 GC coupled to a mass spectrometer (Perkin Elmer Technologies, Inc., Wilmington, DE). Elite-5MS (30m x 0.25 mm width film depth of 250µm capillary tube was used under the following condition. The instrument has an oven with an initial temperature of 55 °C for 3 min and a ramp program which elevates from 6 °C/min up to 310 °C, further 3 min isothermal hold. Helium (He) carrier gas was used, with flow rate split ratios of 10:1. Two µl volumes of samples were injected and temperature of the injector was maintained to 250 °C. An individual component was recognised with typical mass spectra from National Institute of Standards and Technology (NIST-LIB 0.5) libraries which is inbuilt by the software of the GCMS system (Wiley GC-MS-2007) and literature data. The individual phytochemicals present in the crude extract were separated by the gas chromatography column.

An individual compound separated by GC enters the Mass Spectrum (MS) and gets ionized. The MS ionizing spectrum was recorded and compared to the MS spectrum of known compounds in the NIST library. Each compound was compared with a percentage score of reverse and forward spectrum. The MS spectrum displays the molecular weight of individual molecules accurately.

RESULTS AND DISCUSSION

The leaves and flowers were subjected for phytochemical analysis to identify the presence and absence of carbohydrates, alkaloids, flavonoids, phenols, tannins, saponins, and terpenoids in *Spermadictyon suaveolens* (table 1). Almost all the phyto-compounds were present in the leaves and flowers of the plant based on the analysis with four different extracts.

Table 1: Phytochemical profiles of four different solvent extracts of *Spermadictyon suaveolens* flowers and leaves

S. No.	Solvents	Petroleum ether		Chloroform		Ethyl acetate		Methanol	
	Phytochemical tests	Flowers	Leaves	Flowers	Leaves	Flowers	Leaves	Flowers	Leaves
1	Carbohydrates	--	--	++	++	--	++	++	++
2	Alkaloids	++	--	++	--	++	++	++	++
3	Flavonoids	++	++	++	++	--	++	--	--
4	Phenolics	++	++	++	++	++	++	--	++
5	Tannins	++	++	++	++	++	++	--	--
6	Saponins	++	--	++	++	--	--	++	--
7	Terpenoids	--	++	++	++	++	++	++	++

+Indicates; presence;-indicates; absence

UV-visible spectrophotometer readings of *S. suaveolens* flower (chloroform) extract showed four peaks. The UV spectrum peaks are at 270 nm, 320 nm, 430 nm and 660 nm with the absorption values of 0.37, 0.27, 0.13 and 0.05 considered individually. Chloroform extract of *S. suaveolens* leaves showed two peaks at 230 nm and 370 nm with the absorption of 3.5 and 3.0. Methanolic leaf extract of has two spectral peaks at 240 nm and 330 nm with the absorption of 1.7 and 0.7 respectively. The UV-Vis spectrum of above-mentioned extracts was illustrated in fig. 1.

FTIR was used to analyze and identify functional groups of active compounds based on major peak values [12]. Results were compared using the infrared chart. Flower extract has aromatic, primary, secondary and aliphatic amines and amides were found (fig. 2). Methanolic and chloroform leaf extracts showed the aromatic functional group as a major peak followed by primary and secondary amines, amides, and alkanes except for carboxylic acids which were found only in methanolic leaf extract (fig. 3 and 4). The overall details of the FTIR analysis were given in table 2.

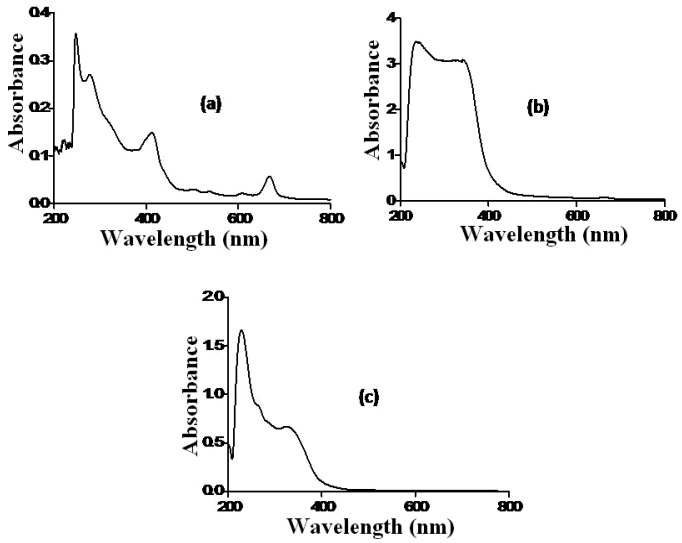

Fig. 1: UV-VIS spectrum of *S. suaveolens.* (a) Chloroform extract of *S. suaveolens* flowers; (b) chloroform extract of *S. suaveolens* flowers; (c) Methanolic extract of *S. suaveolens* leaves

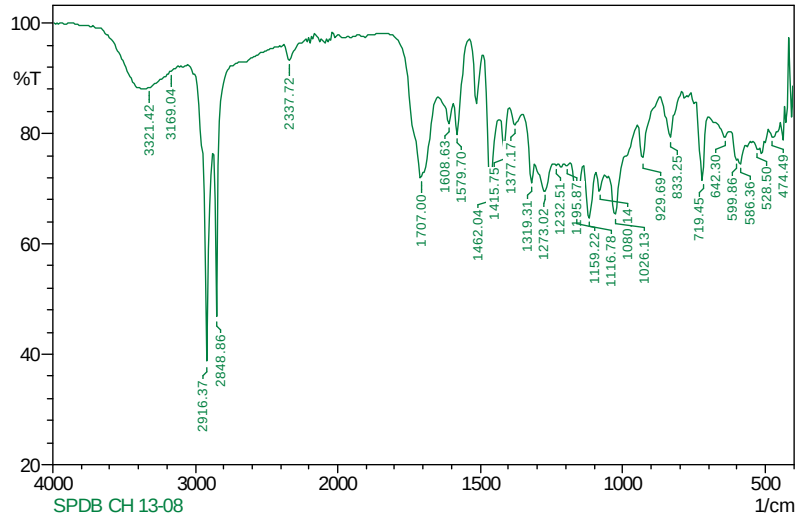

Fig. 2: FT-IR spectrum of chloroform extract *S. suaveolens* flower

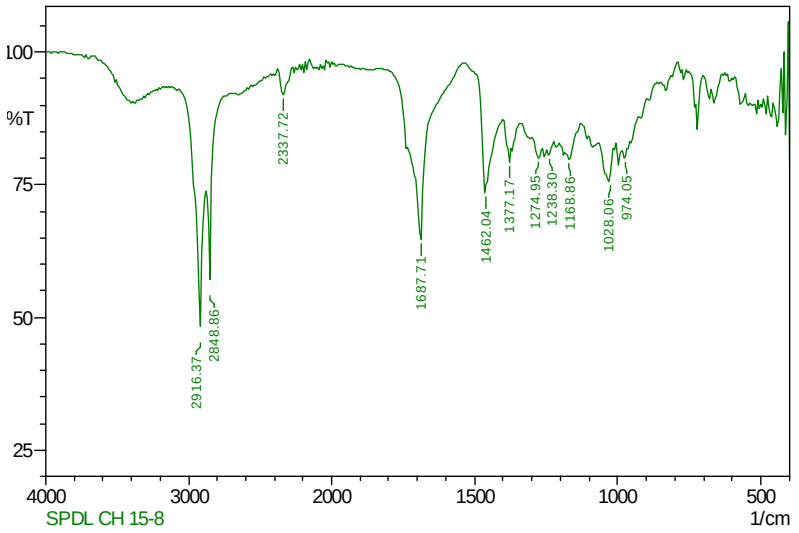

Fig. 3: FT-IR spectrum of chloroform extract *S. suaveolens* leaf

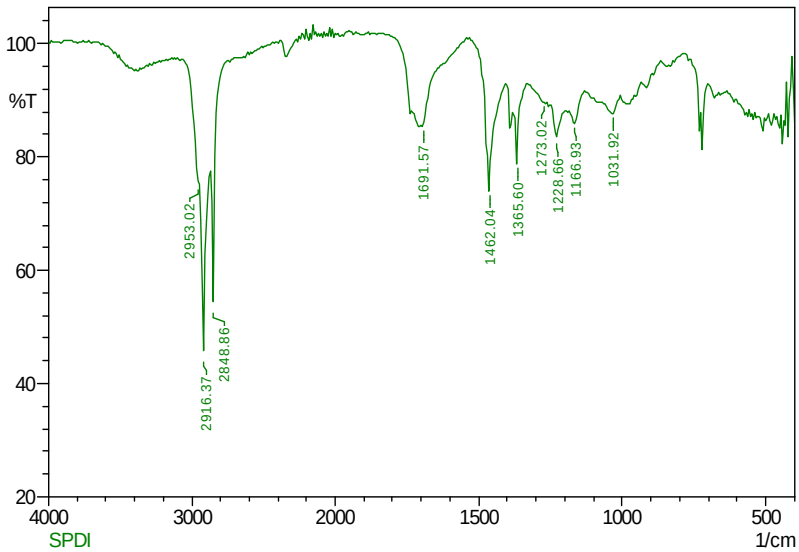

Fig. 4: FT-IR spectrum of methanolic extract *S. suaveolens* leaf

Table 2: Functional group analysis of FT-IR spectrum for *S. suaveolens* leaf and flower extract

Plant part used	Peak values	Structural unit	Functional groups
Flower extract (chloroform)	2916	C-H	Aromatic
	2848	N-H	1 °, 2 ° amines, amides
	1707	C=O	α,β-unsaturated aldehydes, ketones
	1026	C-N	Aliphatic amines
Leaf extract (chloroform)	2916	C-H	Aromatic
	2848	N-H	1 °, 2 ° amines, amides
	1687	C=O	Carboxylic acids
	1462	C-H	Alkanes
Leaf extract (methanol)	2916	C-H	Aromatics
	2848	N-H	1 °,2 ° amines, amides
	1365	C-H	Alkanes

The study on the active principles of *Spermadictyon suaveolens* flowers by GC-MS analysis exhibited the presence of eight major peaks in the methanolic extract (fig. 5), corresponding to compounds adamantine methylamine, alpha-methyl-with a retention time of (16.70), has antiviral activity [13]. 3,7,11,15-tetramethyl-2-hexadecen-1-ol (17.07) showed analgesic, anti-inflammatory and antipyretic activities [14]. 1-octadecyne (17.51) antibacterial [15]. Phytol (19.75) anti-inflammatory [16] Phosphine, triphenyl (20.03) was reported to have *In vivo* and *in-vitro* inhibition of rat neurotoxic esterase [17]. 1,6;3,4-dianhydro-2-deoxy-. Beta-d-lyxo-hexo-pyranose (21.66) bioactivity of a natural compound isolated from cyanobacteria[18]. Cyclotrisiloxane, hexamethyl-(30.00) showed antibacterial activity [19]. The details were given in table 3.

Spermadictyon suaveolens leaf extracted with chloroform showed ten major peaks in the GC-MS spectrum (fig. 6). Phenol, 2, 4-bis (1, 1-dimethylethyl) (13.41) was reported to possess antifungal, antimicrobial, antimalarial, UV stabilizer and an antioxidant for hydrocarbon-based products [20]. The compound 3,7,11,15-tetramethyl-2-hexadecen-1-ol (17.08) was reported to have antimicrobial, anticancer, anti-inflammatory, anti-diuretic [21], Pentatriacontene (20.05) an herbistat [22], Eicosane, 9-octyl-(22.74) with anticancer activity [23], Octadecane, 3-ethyl-5-(2-ethylbutyl (25.97) was reported to possess *In vitro* antifungal activity [24]. The details were given in table 4.

GC-MS spectrum of the methanolic extract *of S. suaveolens* leaves indicated eight major peaks (fig. 7). 4-acetoxy-3-methoxystyrene (11.92) was reported in DSC studies on hydrogen bonding and related derivatives [25], Hexadecanoic acid, ethyl ester (18.71) showed antibacterial activity [26], 1,2-benzenedicarboxylic acid, mono (2-ethylhexyl) ester (23.39) indicated antimicrobial activity [27], Tetratetracontane (27.60) antioxidant [28], Beta-sistosterol (30.75) with antimicrobial activity [29]. The details were given in table 5.

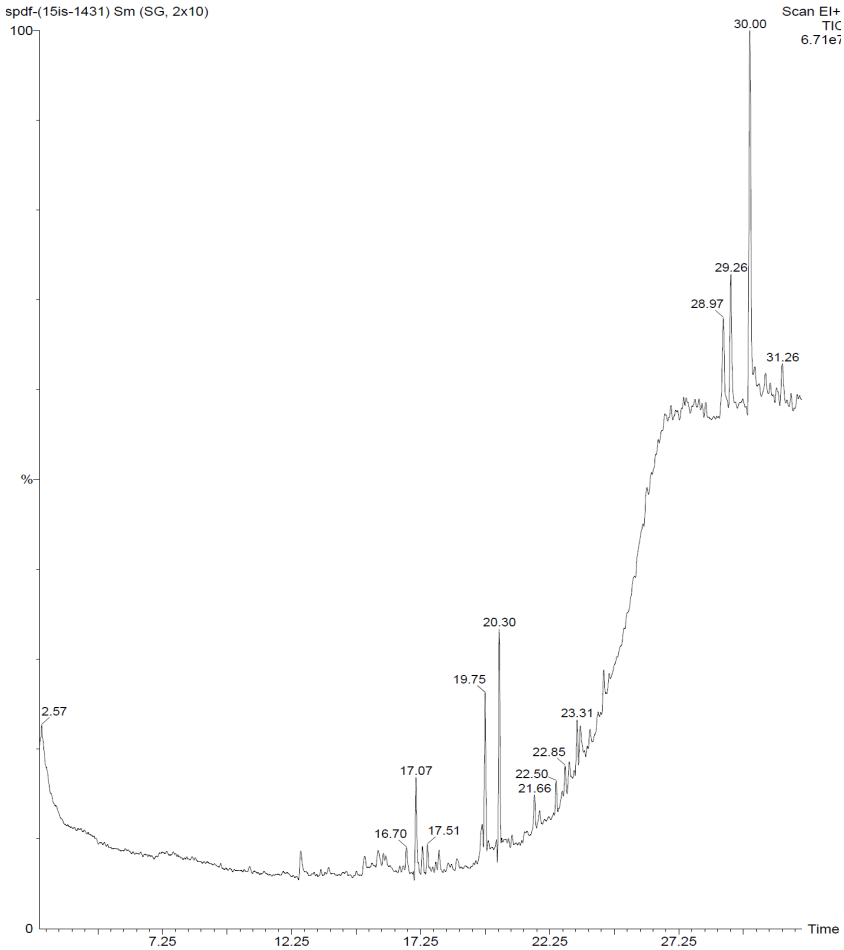

Fig. 5: GC-MS chromatogram of chloroform extract of *S. suaveolens* flower

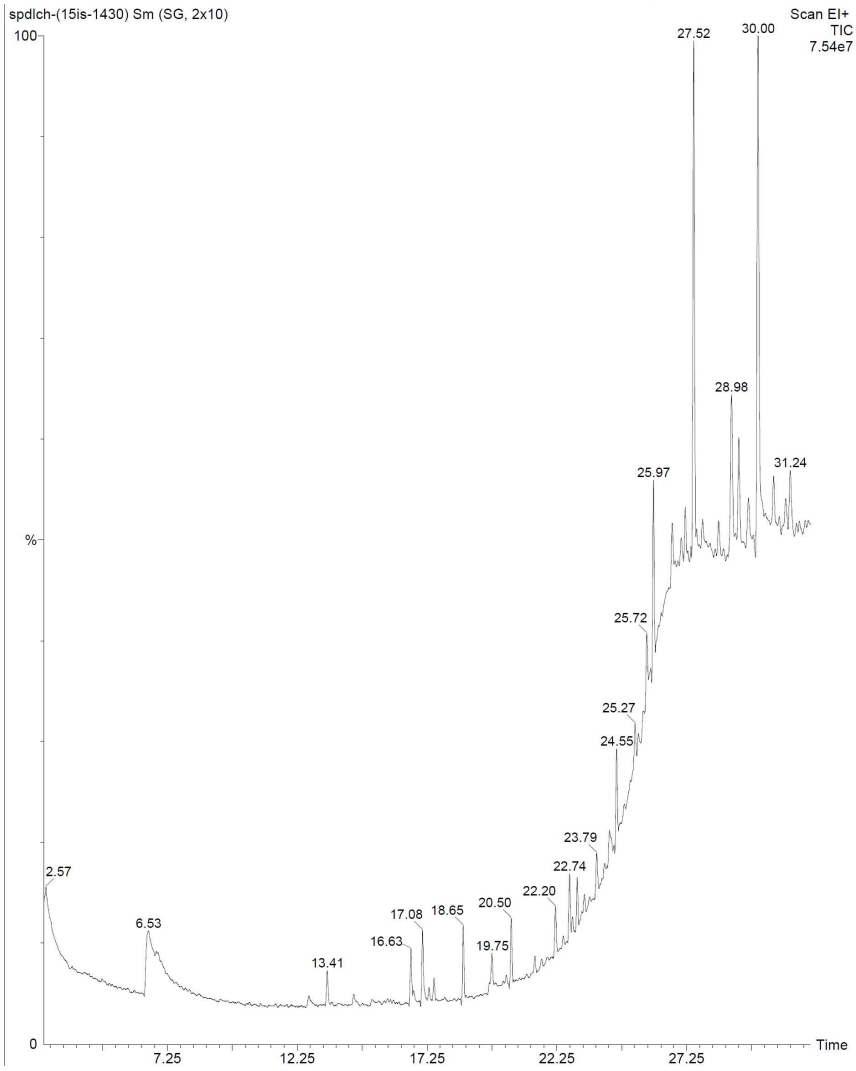

Fig. 6: GC-MS chromatogram of chloroform leaf extract of *S. suaveolens* leaf

Table 3: Chemical composition of *Spermadictyon suaveolens* flowers (chloroform extract)

S. No.	RT	IUPAC name	Chemical formula
1.	16.70	Adamantine Methylamine, Alpha.-Methyl-	$C_{12}H_{21}N$
2	17.07	3,7,11,15-Tetramethyl-2-Hexadecen-1-ol	$C_{20}H_{40}O$
3	17.51	1-Octadecyne	$C_{18}H_{34}$
4	19.75	Phytol	$C_{20}H_{40}O$
5	20.30	Phosphine, Triphenyl	$C_{18}H_{15}P$
6	21.66	1,6;3,4-Dianhydro-2-Deoxy-. beta.-d-Lyxo-Hexopyranose	$C_6H_8O_3$
7	22.50	Paredrine TMS	$C_{12}H_{21}ONSi$
8	30.00	Cyclotrisiloxane, Hexamethyl-	$C_6H_{18}O3Si_3$

Table 4: Chemical composition of *Spermadictyon suaveolens* leaves (chloroform extract)

Peak	RT	Peak name	Chemical formula
1.	2.65	Cyclobutanol	C_4H_8O
2	11.92	4-acetoxy-3-methoxy styrene	$C_6H_{18}O_3Si_3$
3	12.71	Cyclohexane, Decyl-	$C_{16}H_{32}$
4	14.41	1-Pentadecene	$C_{15}H_{30}$
5	14.74	Palmitaldehyde, Di iso penty lacetal	$C_{26}H_{54}O_2$
6	15.34	Benzene ethanamine, 3,4-benzyloxy-2,5-difluoro-. beta.-hydroxy-n-me	$C_{23}H_{23}O_3$
7	16.65	1-octadecene	$C_{18}H_{36}$
8	17.51	ethanone, 1-(3-methylene cyclo pentyl)-	$C_8H_{12}O$

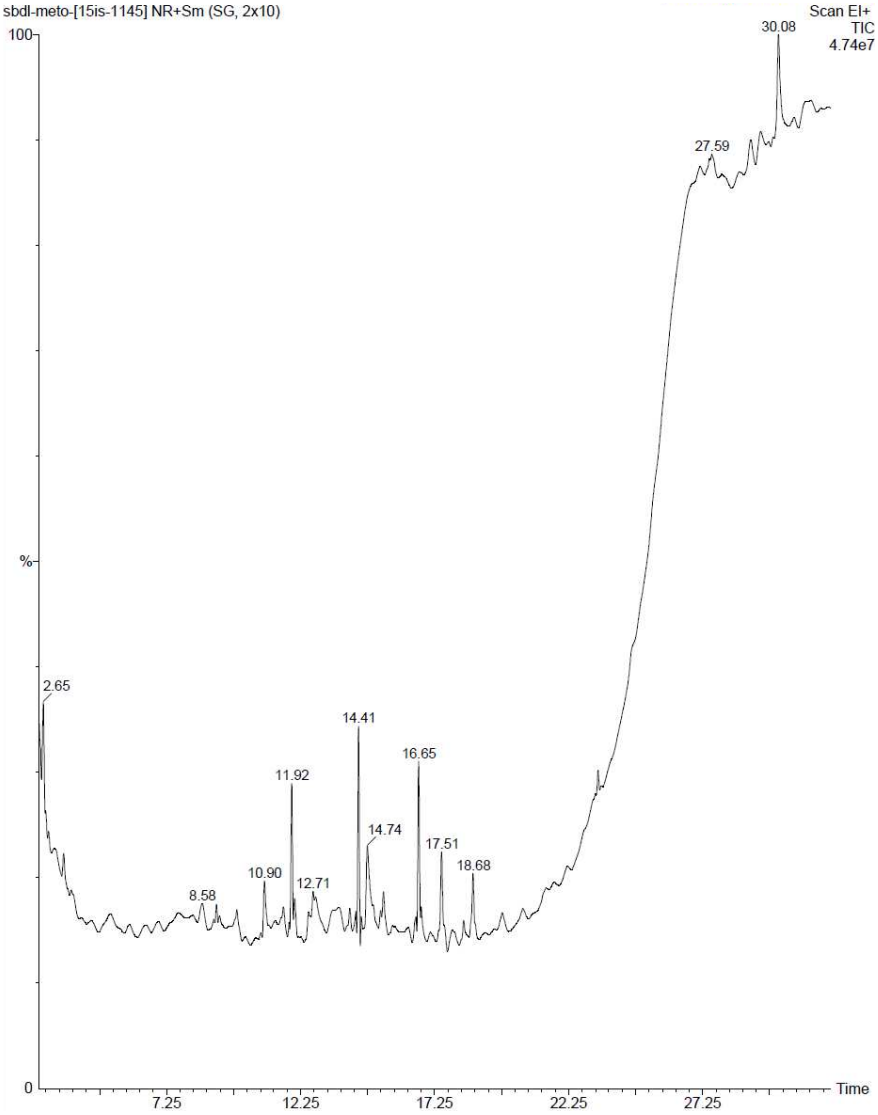

Fig. 7: GC-MS chromatogram of methanolic leaf extract of *S. suaveolens* leaf

Table 5: Chemical composition of *Spermadictyon suaveolens* leaves (methanolic extract)

Peak	RT	Peak name	Chemical formula
1.	13.41	Phenol, 2,4-bis(1,1-Dimethylethyl)	$C_{14}H_{22}O$
2	16.63	e-14-Hexadecenal	$C_{20}H_{40}O$
3	17.08	3,7,11,15-Tetramethyl-2-Hexadecen-1-ol	$C_{16}H_{30}O$
4	19.75	Phytol	$C_{20}H_{40}O$
5	20.50	Penta triacontene	$C_{35}H_{70}$
6	22.20	Bacteriochlorophyll-c-stearyl N4Mg	$C_{52}H_{72}O_4$
7	22.74	Eicosane,9-octyl-	$C_{28}H_{58}$
8	23.79	Cyclotrisiloxane, Hexamethyl-	$C_6H_{18}O_3$
9	25.72	Trimethyl[4-(1,1,3,3,-Tetra methylbutyl)phenoxy] Silane	$C_{28}H5_8$
10	25.97	Octadecane, 3-ethyl-5-(2-ethylbutyl)	$C_{17}H_{30}$

CONCLUSION

The present study was carried out to analyze of the presence of phytocompounds present in the unexplored plant *Spermadictyon suaveolens,* which belongs to the family Rubiaceae. UV-visible spectrophotometer analysis revealed absorption of the spectrum of known compounds. FT-IR analysis of functional groups of phytocompounds was analyzed with major peak values.

The individual phytochemicals present in the crude extract are identified by GC-MS. Since *Spermadictyon suaveolens* is found to have significant numbers of bioactive compounds based on GC-MS analysis; the compounds were found to have biological significance for treating various ailments as per earlier literature. Nevertheless, Isolation of unique phytocompounds from this plant and in-depth study of their biological activity will ensure best possible results in future and open new opportunity for discovery of potential drugs of therapeutic worth from this plant.

ABBREVIATION

GC-MS: Gas column-mass spectrophotometer; FTIR: Fourier transform infrared spectrometer; Ultraviolet-visible spectrophotometer; R. T-retention time.

CONFLICT OF INTERESTS

Author's reveal no conflict of interest

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