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ANTIOXIDANT ACTIVITY STUDY AND GC-MS PROFILING OF LEAVES, STEM AND ROOT EXTRACTS OF *Spermadictyon suaveolens* Roxb.

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ABSTRACT

The characterization of bioactive components in the methanolic and isopropyl alcoholic plant extracts of *Spermadictyon suaveolens* Roxb. (Rubiaceae) was undertaken with the help of GC-MS technique, followed by the study of free-radical scavenging capabilities of the plant hydroalcoholic extracts using DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) test technique with ascorbic acid as the standard. There's a constant formation of free radicals in the human body tissues originating due to the oxidation of specific chemical components, whereas the antioxidant molecules prevent or inhibit this free radical formation that may lead to lifelong or terminal diseases. The comparison of the antioxidant capacity of the plant extracts with that of the standard revealed that the leaf extracts showed maximum inhibition of DPPH, or radical scavenging activity. The EC_{50} values of ascorbic acid, leaves, stem, and root were found to be 18.62 µg/mL, 44.668 µg/mL, 89.125 µg/mL, and 97.723 µg/mL, respectively. The different peaks in the GC-MS analysis spectrum determined 24, 19, and 26 phytochemicals in leaves, stems, and roots, respectively. Out of all the phytoconstituents found, the major ones were n-Hexadecanoic acid or palmitic acid, squalene, 1.4-tert-Butylcalix[4]arene, and 1.3,5-Dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one in leaves. 11-Bromoundecanoic acid, Ethylhexanol, Tetratetracontane, 2-Decanol, Propanoate in Stem, and n-Hexadecanoic acid 9,12-Octadecadienoic acid (Z,Z), 4,6-Bis(4-fluoro-3-(trifluoromethyl) phenoxy)-2-pyrimidinol, squalene in roots. Thus, the different bioactive constituents found to be present in the plant under study prove that the plant has the capacity to exhibit good antioxidant and other pharmacological properties.

Keywords: *Spermadictyon suaveolens*, antioxidant property, phytochemical analysis, hydroalcoholic plant extract, GC-MS analysis, EC_{50}.

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INTRODUCTION

The world is collectively searching for plant alternative medicine to treat and prevent diseases. Indigenous ethnobotanical plant research is an indispensable tool for innovative and novel plant-based medical formulations. The plant *Spermadictyon suaveolens* Roxb. is a 1–2 meter tall woody and branched shrub with glabberous leaves and fragrant small white flowers, found in different parts of the Indian subcontinent, including Kedarnath forests, Tundi hills, and parts of Maharashtra (Papitha et al., 2017; Singh et al., 2015). It goes by the common names 'Padera', 'Jitsaya', Forest Champa, Van Champa, etc. (Ranjan, 2016). From the madder family 'Rubiaceae', the plant shows remarkable properties like anti-oxidant, anti-microbial, anti-diabetic, and is used as a wound healer and more (Alande et al., 2016; Muhammad Ajaib et al., 2014). The comparative study of the leaves stem, and root together has not yet been conducted for this plant for its pharmacological effects and phytochemical screening. The main purpose of this study was to investigate the comparative analysis of the phytocomponents and antioxidant properties in the leaves, stems, and roots of *S. suaveolens* with the help of GC-MS spectroscopy and the DPPH method.

MATERIALS AND METHODS

Plant materials and chemicals

The plant was procured from the outskirts of the Panvel region near Karnala Bird Sanctuary in Raigarh district, Maharashtra. It was then authenticated from St. Xavier's Blatter Herbarium, Mumbai, with the accession number 23598 of H. Santapau. The plants were segregated into leaves, stems, and roots after drying for 10 days in the shade, then they were separately pulverized and stored in airtight glass containers. All of the chemicals and solvents utilized in this study were of the analytical grade, and those were purchased from Sigma Aldrich and E. Merck in Germany.

Antioxidant study

In the antioxidant assay, 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) is a chemical agent used, which causes the electron transfer, creating many stable free radicals that can be visually seen as violet solutions in any alcohol. These stable free radicals can now be reduced by an antioxidant molecule (in the sample) that changes the color from violet shades to colorless due to the pairing of the DPPH odd electron with an antioxidant hydrogen atom, producing reduced DPPH-H and giving a distinct absorption peak at 517 nm (Hasan et al., 2024). A standard spectrophotometer was used to detect the antioxidant capacity of the plant extracts.

Preparation of plant samples

Distilled water and ethanol in the ratio of 70:30 were taken as hydroalcoholic solvents and 50 g of dried leaf, stem, and root of *S. suaveolens*. Plant powders were taken and extracted separately through the process of soxhlation for 48 hours with a minimum of 10 siphon cycles. The extract was then filtered and then evaporated in a water bath with a temperature not exceeding 45 ^{o}C to obtain the hydroalcoholic extracts. The plant samples of leaf, stem, and root extracts were prepared by taking 10 mg of each extract powder in 10 mL of ethanol and then further diluted to obtain 10 to 100 µg/mL solutions.

Preparation of standard and DPPH

Ascorbic acid was used as the standard, and the solution was prepared in the concentration range of 10 to 100 µg/mL. The DPPH solution was prepared by mixing 7.89 mg in 100 mL ethanol (Xiao et al., 2020) and kept in the dark for 2 hours.

Methodology

1.5 mL of the DPPH solution along with 1.5 mL of ethanol were used to take the initial absorbance. 1.5 mL of plant sample or the standard was further added to the DPPH solution in volumetric flasks, and the volume was finally adjusted to 3 mL. This setup was then incubated in the dark for 15 min. The absorbance of the leaf, stem, and root sample as well as the standard was measured at 517 nm after the incubation period. For the control reading, only the DPPH and ethanol

were taken immediately after incorporation, while the sample and ethanol served as blank. Three of the same test samples were reacted with the DPPH and processed in a similar way, and the average was calculated finally for each plant part used. The plant leaf, stem, and root extract's ability to scavenge the DPPH radical was then calculated.

$$\text{Radical Scavenging Activity } (\%\text{RSA}) \text{ or } \%\text{ Inhibition DPPH} = \left(A_{control} - A_{test}\right)/A_{control} \times 100$$

Where: $A_{control}$ is the absorption (without extract) of the control and where A_{test} is the absorption in the presence of the extract/standard (Thuy Dung et al., 2018). EC_{50} values were calculated by plotting the percentage of maximum control response (considering the highest response as maximum) of the percentage Radical Scavenging Capacity (%RSA) against the log of the dose concentration to give the log concentration on the graph for 50% dose, which was then converted to antilog to give the EC_{50} value for ascorbic acid and the leaf, stem, and root concentrations (Alexander et al., 1999) (Figs. 1, 2; Tables 1–3).

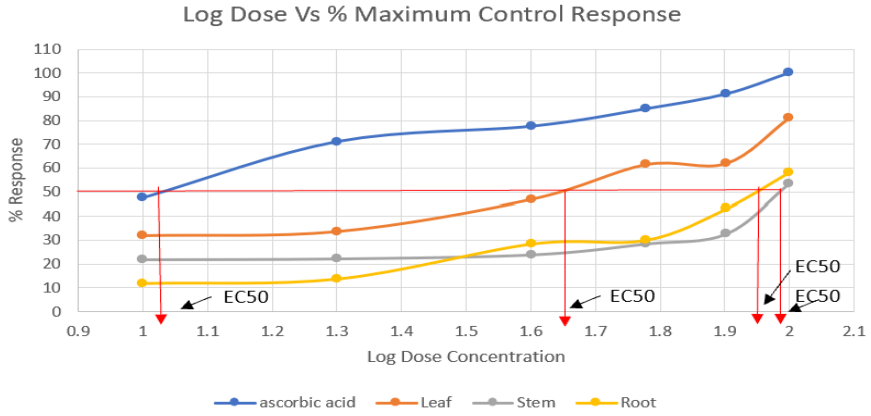

Figure 1. Log dose vs percentage radical scavenging capacity of standard ascorbic acid and *Spermadictyon suaveolens* Roxb. leave, stem and root samples using DPPH method showing EC_{50} values

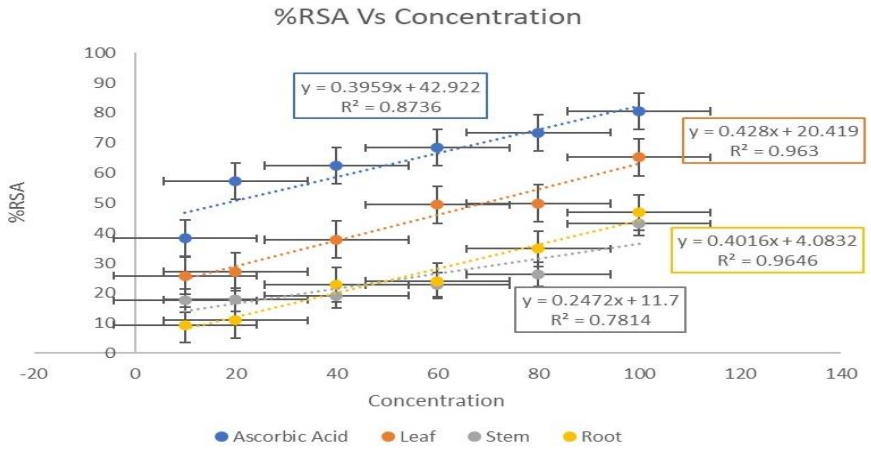

Figure 2. Standard ascorbic acid and *Spermadictyon suaveolens* Roxb. leaves, stem and root samples graphs using the DPPH method showing the relation of % RSA to concentration along with value of R^2

Table 1. Absorbance of Standard Ascorbic acid and *Spermadictyon suaveolens* Roxb. samples using the DPPH method

Sr No.	Concen tration (µg/mL)	Absorbance			
		Ascorbic acid	Leaves extract	Stem extract	Root extract
1	10	0.451	0.542	0.602	0.661
2	20	0.312	0.532	0.600	0.65
3	40	0.274	0.454	0.590	0.563
4	60	0.231	0.369	0.563	0.554
5	80	0.194	0.366	0.538	0.476
6	100	0.142	0.254	0.415	0.388
	Control	0.730			

Table 2. Percentage Radical Scavenging Capacity of Standard Ascorbic acid and *Spermadictyon suaveolens* Roxb.

Sr No.	Concen tration (µg/mL)	% Radical Scavenging Capacity			
		Ascorbic acid	Leaves extract	Stem extract	Root extract
1	10	38.21	25.75	17.53	9.45
2	20	57.26	27.12	17.80	10.95
3	40	62.46	37.8	19.17	22.87
4	60	68.35	49.45	22.87	24.1
5	80	73.42	49.86	26.30	34.79
6	100	80.54	65.2	43.15	46.84

Table 3. Effective concentration EC_{50} of standard ascorbic acid and *Spermadictyon suaveolens* Roxb. leaves, stem and root samples using the DPPH method

Sr No.	Log Dose Concentration (µg/mL)	% of Maximum Control Response			
		Ascorbic acid	Leaves extract	Stem extract	Root extract
1	1	47.44226	31.97169	21.76558	11.7333
2	1.30103	71.09511	33.67271	22.10082	13.59573
3	1.60206	77.55153	46.9332	23.80184	28.39583
4	1.778151	84.86466	61.39806	28.39583	29.92302
5	1.90309	91.15967	61.90713	32.65458	43.19593
6	2	100	80.95356	53.57586	58.15744
Dose at 50% Calculated from Graph		1.27	1.65	1.95	1.99
EC_{50} Value (antilog)		18.62	44.668	89.125	97.723

GC-MS study

GC-MS, i.e., gas chromatography and mass spectrometric analysis, is conducted to identify the biologically active constituents and the chemical composition of the plant extracts. Methanolic and isopropyl alcohol extracts of *S. suaveolens* were analyzed with GC-Agilent: 7890 and MS-Jeol Model: Accu TOF GCV with a mass resolution of 6000, Polar Columns (DB-WAX) and HP-5 MS UI, and a mass range of 10–1,000 amu with a FID detector, a mass filter of heated monolithic hyperbolic quadrupole, and the ionization

method used was Electron Impact (EI). The detection was carried out in full scan mode, and identification of the phytoconstituents was derived by the comparison of mass spectra obtained to the NIST (National Institute of Standards and Technology).

Preparation of plant extracts

The powdered plant leaves, stem, and root parts (50 g) were used to prepare methanolic as well as isopropyl alcohol extracts through the process of exhaustive soxhlation by taking 250 mL of both solvents with different samples separately in a Soxhlet apparatus for 7–8 reflux cycles. The methanolic and isopropyl alcohol extracts were further filtered with the Whatman filter paper (No. 1), and both extracts were used for GC-MS analysis. Methanol was selected as a solvent because maximum phytochemicals were found to be present in this extract in preliminary phytochemical tests. Isopropyl alcohol was selected as another solvent for GC-MS as not many studies have been done with this solvent.

RESULTS

Antioxidant study

The standard norm for expressing the result is to use the EC_{50} value, which is the antioxidant concentration at which the DPPH absorbance decreases by 50% (Chen et al., 2013). The EC_{50} is the concentration of a drug that gives the half-maximal response, in other words the EC_{50} value is defined as the half-maximal effective concentration. The EC_{50} values were formulated from the graph and were found to be 18.62 µg/mL, 44.668 µg/mL, 89.125 µg/mL, and 97.723 µg/mL in ascorbic acid, leaves, stem, and root hydroalcoholic extract of *Spermadictyon suaveolens* Roxb. (Table 2; Figs. 1, 2). The lower the EC_{50} value, the higher the free radical scavenging potency, as it indirectly means that less amount of substance is required to attain a 50% level of effect, and the exact opposite is true for higher values (Gancar et al., 2023).

GC-MS analysis

All the phytoconstituents characterized by the methanolic extracts and isopropyl alcohol extracts of leaves, stems, and roots of the plant *S. suaveolens* have been elaborated along with the peak area, retention time, relative peak, molecular formula, and name of the compound (Tables 4–9). The GCMS chromatograms of the methanolic and isopropyl alcohol extracts have been shown in Figure 3.

The *S. suaveolens* GC/MS chromatograms of the methanolic extract of leaves show 3 notable peaks with relative peak areas greater than 3% (Table 4; Fig. 3) and their mass spectra with the tentative match from the NIST library has been tabulated in Figure 4. They are 1.4-tert-Butylcalix[4]arene ($C_{44}H_{56}O_4$) with a retention time of 39.93 and a relative area of 42.326%; 1.3,5-Dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one ($C_6H_8O_4$) with a retention time of 9.79 and a relative area of 29.875%; and 2-Cyclohexen-1-one, 4-hydroxy-3,5,5-trimethyl-4-(3-oxo-1-butenyl)-($C_{13}H_{18}O_3$) with a retention time of 22.95 and a relative area of 21.932%.

The chromatograms of isopropyl alcohol extract of leaves showed 8 prominent peaks (Table 5; Fig. 3) and their mass spectra with the tentative match from the NIST library has been given in Figure 5. The phytocompounds that matched from the NIST library are tridecanoic acid and n-Hexadecanoic acid/palmitic acid ($C_{16}H_{32}O_2$) with retention times of 22.23 and 25.83 with relative areas of 12.393 and 34.244, respectively. Squalene ($C_{30}H_{50}$) with retention time 41.72 and relative area of 14.584, 9-n-Hexylheptadecane ($C_{23}H_{48}$) with a retention time of 46.16 and a relative area of 5.908%; Vitamin E ($C_{29}H_{50}O_2$) with a retention time of 46.99 and a relative peak area of 5.786%; Phytol ($C_{20}H_{40}O$) with a retention time of 29.08 and a relative area of 5.664%; Cholesta-8,24-dien-3-ol, 4-methyl-, (3,4)-($C_{28}H_{46}O$) with a relative area of 3.994% and a retention time of 51.69; Z,Z,Z-4,6,9-Nonadecatriene ($C_{19}H_{34}$) with a relative area of 3.255% and a retention time of 29.82 min.

Table 4. Chemical constituents of methanolic leaf extract of *Spermadictyon suaveolens* Roxb. by GC-MS analysis

Pk. No.	R. time min	Area	Area %	Molecular Formula and Nature	Name	Pharmacological Properties
1	9.79	2604206.5	29.87	C_6 H_8 O_4 (Isomaltol) Ketone	1.3,5-Dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one	Antioxidant, anti-arthritic, anti-inflammatory and bactericidal potentials (Zs et al., 2017)
2	19.66	107974.55	1.23	$C_{16}H_{26}O_2$ Fatty Acid	Acetic acid, 3-(2,2-dimethyl-6-methylene-cyclohexylidene)-1-methyl-butyl ester	Antioxidant potential (Arista et al., 2023)
3	20.17	194224.62	2.22	$C_9H_{20}S_2$ Organic Disulfide	n-Propyl n-hexyl disulfide	Anti-inflammatory, antioxidant and anti-skin irritation (Oršolić et al., 2014)
4	22.61	209143.23	2.39	$C_{26}H_{36}O_9$ Indole alkaloids	7,8,12-Tri-O-acetyl ingol	Anti-bacterial and anti-oxidant activities (Ayandiran Aina & Fagbemi, 2022)
5	22.95	1911828.4	21.93	C_{13} H_{18} O_3 Ketone	2-Cyclohexen-1-one, 4-hydroxy-3,5,5-trimethyl-4-(3-oxo-1-butenyl)-	Antioxidant anti-carcinogenicity, anti-aging, antibacterial, anti-irritant, antidiabetic, neuroprotective, hepato-protective, and analgesic (Naser et al., 2022)
6	39.93	3689584.8	42.32	C_{44} H_{56} O_6 Macrocyclic Calixarene compound	1.4-tert-Butylcalix[4]arene	Antiviral, antibacterial, antifungal, and anticancer Naseer et al., 2017. Anti-Bacterial (Aissaoui et al., 2019)
Total			100			

Table 5. Chemical constituents of isopropyl alcohol leaf extract - *Spermadictyon suaveolens* Roxb. by GC-MS analysis

Pk. No.	R. time min	Area	Area %	Molecular Formula and Nature	Name	Pharmacological properties
1	22.23	1592102.88	12.39	$C_{16}H_{32}O_2$ Fatty Acid	Tridecanoic acid	Anti-bacterial and anti-biofilm properties (Jin et al., 2021)
2	22.84	162057.04	1.26	$C_{28}H_{40}O_{10}$ Steroid	3,9-Epoxypregn-16-ene-14,20-diol, 7,11,18-triacetoxy-3-methoxy	It has been identified in a plant that shows antioxidant and antibacterial properties (Degfie et al., 2024).
3	24.89	146307.05	1.13	$C_{20}H_{40}O_2$ Fatty Acid	Nonadecanoic acid, methyl ester	Inhibition of proliferation of cancer cells (B. Arirudran et al., 2023)
4	25.83	4399367.14	34.24	$C_{16}H_{32}O_2$ Fatty Acid	n-Hexadecanoic acid	Treatment of Epilepsy (Warren et al., 2020)
5	26.92	130585.28	1.01	$C_{40}H_{64}O_8$ Fatty Acid	Decanoic acid	Antioxidant, hypocholesterolemic, nematicide, pesticide, antiandrogenic, flavour, hemolytic, (Mohamed Zaky Zayed et al., 2014)
6	28.59	55500.17	0.43	$C_{17}H_{31}Cl$ Alkyne	7-Heptadecyne, 1-chloro	No pharmacological activity reported yet
7	28.75	105712.49	0.82	$C_{21}H_{36}O_2$ Fatty Acid Ester	8,11,14-Eicosatrienoic acid, methyl ester, (Z,Z,Z)-	Anti-bacterial and anti-cancer properties (Goda et al., 2020)
8	29.08	727654.18	5.66	$C_{20}H_{40}O$ Terpenoid (diterpenoi)	Phytol	Apoptosis inducing, immune-modulating, and antimicrobial effects. (Islam et al., 2018)
9	29.82	418209.29	3.25	$C_{19}H_{34}$ Poly-unsaturated hydro-carbon	Z,Z,Z-4,6,9-Nonadecatriene	Increase Zinc Bioavailability (Amudha & rani 2018)
10	30.29	168056.6	1.30	$C_{40}H_{64}O_8$ Phorbol compound	4a-Phorbol 12,13-didecanoate	Treatment of Renal issues(Nakai et al., 1987)

Pk. No.	R. time min	Area	Area %	Molecular Formula and Nature	Name	Pharmacological properties
11	37.41	347018.16	2.70	$C_{24}H_{38}O_4$ Plasticizer compound	Di-n-octyl phthalate	Anti-tumor Activity(Gandhi et al., 2024)
12	39.77	68940.01	0.53	$C_{16}H_{28}O_2S_2$ Hydropyran	2-[1-(2-[1,3]Dithian-2-yl-ethyl)-pent-4-enyloxy]-tetrahydropyran	Anti-cancer potential (Lu et al., 2018)
13	40.51	112830.65	0.87	$C_{12}H_{24}O_3$ Fatty acid	β-Hydroxydodecanoic acid	Antioxidant and antibacterial activities (Gandhi et al., 2024)
14	41.72	1873629.52	14.58	$C_{30}H_{50}$ Triterpenoid	Squalene	Antioxidant property used in food, cosmetic and medicine (Selvaraj et al., 2019)
15	42.69	154822.46	1.20	$C_{26}H_{54}$ Alkane	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	Anti-microbial properties (K. Patil &Singh, 2022)
16	46.16	758995.76	5.90	$C_{23}H_{48}$ Hydro-carbon	9-n-Hexylheptadecane	Antitumor (Nasopharynx) Narcotic, Natriuretic, NCS-Depressant Nematicide (Duke, 1992)
17	46.99	743312.03	5.78	$C_{29}H_{50}O_2$ Tocopherol	Vitamin E	Anti-Inflammatory, and Metabolic Properties (Di Vincenzo et al., 2019)
18	49.16	165282.87	1.28	$C_{21}H_{32}O_3$ Steroid	17α-Hydroxypregnenolone	Used in hormone treatment during pregnancy (Včeláková et al., 2007)
19	50.04	203758.87	1.586	$C_{28}H_{46}O$ Terpenoid	1.4-Methylcholesta-8,24-dien-3-ol #	Inhibits cell proliferation(Urbina et al., 1997)
20	51.69	513127.92	3.99	$C_{28}H_{46}O$ Sterol	Cholesta-8,24-dien-3-ol, 4-methyl-, (3β,4α)-	Anti-diabetic Activity. (Bonita Wowor et al., 2022)
Total			100 %			

Table 6. Chemical constituents of methanolic stem extract - *Spermadictyon suaveolens* Roxb. by GC-MS analysis

Pk. No.	R. time min	Area	Area %	Molecular Formula and Nature	Name	Pharmacological properties
1	6.59	4606587	64.38	$C_8H_{18}O$ Alcohol	1-Hexanol, 2-ethyl (Ethylhexanol)	Used as a fragrant. Anti-cancer properties have been studied,.(Rios et al., 2019)
2	17.55	39937.03	0.55	$C_{18}H_{38}OSi$ Cyclo pentane	1-Methyl-1-(4-tridecyl)oxy-1-silacyclopentane	No pharmacological activity reported yet
3	18.53	326070.9	4.55	$C_{40}H_{64}O_8$ Fatty Acid	Decanoic acid	Anti-bacterial activity, Anti-microbial (Kitahara et al., 2004)
4	22.18	783072	10.94	$C_{11}H_{21}BrO_2$ Fatty acid	11-Bromoundecanoic acid	Anti-biofilm properties (Yasa et al., 2017)
5	24.88	180357.7	2.52	$C_{16}H_{32}O_2$ Fatty acid Ester	Tetradecanoic acid, 12-methyl-, methyl ester	Treatment of ischemic stroke.(Nagarjunakonda et al., 2017)
6	25.7	951279.7	13.29	$C_{16}H_{32}O_2$ Fatty Acid	n-Hexadecanoic acid (Palmitic Acid)	Antioxidant, hypo cholesterolemic, pesticide, antiandrogenic, hemolytic, (Mohamed Zaky Zayed et al., 2014)
7	28.59	96890.33	1.35	$C_{57}H_{98}O_6$ Triglyceride	Trilinolein	Anti-cancer (Chou et al., 2011)
8	28.73	24000.77	0.335	$C_{29}H_{42}F_5NO_3$ Amino acid ester	L-Proline, N-(pentafluorobenzoyl)-, heptadecyl ester	It has shown anti-tumor activities (Oguz et al., 2020).
9	29.32	38010.37	0.531	$C_{23}H_{46}O_2$ Fatty acid ester	Docosanoic acid, methyl ester	They come under the group of fatty acid that show antioxidant and antibacterial effects. (Jitendra et al., 2015)
10	37.41	57158.1	0.799	$C_{33}H_{50}O_5$ Fatty acid ester	Urs-9(11)-en-12-one-28-oic acid, 3-acetoxy-, methyl ester (14β,20β)	No pharmacological activity reported yet
11	39.77	51875.02	0.725	$C_{11}H_{19}N_5O_4$ Organic complex compound	1,2,5-Triazole, 1-octyl-3-nitro-4-formamido-, 2-oxide	No pharmacological activity reported yet
Total			100%			

Table 7. Chemical constituents of isopropyl alcohol stem extract - *Spermadictyon suaveolens* Roxb. by GC-MS analysis

Pk. No.	R. time min	Area	Area %	Molecular Formula and Nature	Name	Pharmacological properties
1	33.74	88959.6	5.37	$C_{26}H_{54}$ Alkane	Octadecane, 3-ethyl-5-(2-ethylbutyl)	Anti-microbial, anti-fungal (Patil & Singh, 2022)
2	34.33	315977.3	19.08	$C_{24}H_{38}O_4$ Plasticizer Compound	Di-n-octylphthalate	Anti-fungal (Javed et al., 2018)
3	35.82	450184.7	27.19	$C_{44}H_{90}$ Alkane	Tetratetracontane	Anti-cancer and anti-microbial properties (Pierre Luhata et al., 2023)
4	36.36	109137.8	6.59	$C_{11}H_{11}NO_4$ Ester	4-Nitrobenzoic acid, cyclobutyl ester	No pharmacological properties have been reported yet
5	36.76	462999.2	27.96	$C_{13}H_{26}O_2$ Ester	2-Decanol, propanoate	Anti-microbial Activity (Gębarowska et al., 2017)
6	37.05	29923.63	1.80	$C_{16}H_{30}O_3$ Fatty acid Ester	Tetradecanoic acid, 2-oxo-, ethyl ester	Treatment of ischemic stroke.(Nagarjunakonda et al., 2017)
7	37.29	104607.9	6.31	$C_{25}H_{41}NO_5$ Pregnane steroids	3,9-Epoxypregnane-11β,20-diol, 3α-methoxy-18-[N-methyl-N-(2',14-epoxyethyl)amino]-	No pharmacological properties have been reported yet
8	38.13	93777.48	5.66	$C_{41}H_{66}O_8$ Complex polycyclic Compound	5H-Cyclopropa (3,4)benz(1,2-e)azulen-5-one, 1,1a-α,1b-β,4,4a,7a-α,7b,8,9,9a-decahydro-7b-α,9-β,9a-α-trihydr	No pharmacological properties have been reported yet
Total			100			

Table 8. Chemical constituents of methanolic root extract of *Spermadictyon suaveolens* Roxb by GC-MS analysis

Pk. No.	R. time min	Area	Area %	Molecular Formula	Name	Pharmacological properties
1	17.55	304569.11	0.47	$C_{12}H_{23}BrO_2$ Fatty Acid Ester	Undecanoic acid, 11-bromo-, methyl ester	Anti-microbial and biofilm inhibitory activities (Yasa et al., 2017)
2	18.6	1763044.34	2.75	$C_{11}H_{22}O_2$ Fatty Acid	Undecanoic acid	Anti-microbial and biofilm inhibitory activities (Yasa et al., 2017)
3	21.39	1873083.72	2.92	$C_{15}H_{30}O_2$ Fatty Acid Ester	Methyl tetradecanoate	Larvisidal activity (Bharathithasan et al., 2021)
4	22.34	4949832.6	7.73	$C_{14}H_{28}O_2$ Fatty Acid	Tetradecanoic acid	Anti-cancer and anti-microbial properties (Pierre Luhata et al., 2023)
5	23.17	251755.99	0.39	$C_{16}H_{32}O_2$ Fatty acid Ester	Tetradecanoic acid, 12-methyl-, methyl ester	Treatment of ischemic stroke.(Nagarjunakonda et al., 2017)
6	24.94	11396979.77	17.8	$C_{17}H_{34}O_2$ Fatty Acid Ester	Hexadecanoic acid, methyl ester	Anti-fungal properties (Abubacker & Deepalakshmi, 2013)
7	26.12	14859267.21	23.2	$C_{16}H_{32}O_2$ Fatty Acid	n-Hexadecanoic acid	Antioxidant, pesticide, antiandrogenic, flavour, hemolytic, 5-alpha reductase inhibitor (Mohamed Zaky Zayed et al., 2014)
8	28.24	4165421.15	6.51	$C_{19}H_{34}O_2$ Fatty Acid Ester	9,12-Octadecadienoic acid, methyl ester, (E,E)-	Anti-inflammatory, Anti-coronary (Akeem et al., 2020)

Pk. No.	R. time min	Area	Area %	Molecular Formula	Name	Pharmacological properties
9	28.36	5812369.43	9.08	$C_{19}H_{36}O_2$ Fatty Acid Ester	9-Octadecenoic acid (Z)-, methyl ester	Raises VLDL And Lowers HDL Cholesterol (Akeem et al., 2020)
10	28.44	335936.06	0.52	$C_{19}H_{38}O_2$ Fatty Acid	Nonadecanoic acid	Cytotoxic Properties (Jin-Cheol Yoo et al., 2002)
11	28.79	3432085.11	5.36	$C_{19}H_{38}O_2$ Fatty Acid Ester	Octadecanoic acid, methyl ester	Treatment of measles (Entigu et al., 2013)
12	29.33	7888855.48	12.3	$C_{18}H_{32}O_2$ Fatty Acid	9,12-Octadecadienoic acid (Z,Z)	Cancer preventive, Hepatoprotective, Antihistaminic, Antiarthritic, Anti-eczemic (Akeem et al., 2020)
13	33.82	555219.29	0.86	$C_{36}H_{72}O_2$ Fatty Acid Ester	Octadecanoic acid, octadecyl ester	No pharmacological properties have been reported yet
14	33.88	1313100.32	2.05	$C_{18}H_{30}O_2$ Fatty Acid	9,12,15-Octadecatrienoic acid, (Z,Z,Z)	Analgesic, Anesthetic, Allergenic, Antibacterial, Anticonvulsant, Anti-inflammatory, Antioxidant, Antipyretic, Antisalmonella, Antiseptic, Antistaphylococci (Kalaivani et al., 2012)
15	34.35	1544111.93	2.41	$C_{24}H_{38}O_4$ Plasticizer compound	Di-n-octyl phthalate	Anti-tumor Activity (Gandhi et al., 2024)

Table 9. Chemical constituents of isopropyl alcohol root extract of *Spermadictyon suaveolens* Roxb. by GC-MS analysis

Pk. No.	R. time min	Area	Area %	Molecular Formula	Name	No pharmacological properties have been reported yet
1	29.67	77103.01	15.156	$C_{24}H_{22}F_8N_2O_3Si$ Pyrimidinol	4,6-Bis(4-fluoro-3-(trifluoromethyl)phenoxy)-2-pyrimidinol tbdms	No pharmacological properties have been reported yet
2	33.74	7301.62	1.435	$C_{32}H_{68}O_5Si_4$ Fatty Acid Ester	Prost-13-en-1-oic acid, 9,11,15-tris [(trimethylsilyl)oxy]-, trimethylsilyl ester, (9α,11α,13E,15S)-	No pharmacological properties have been reported yet
3	34.33	53417.99	10.500	$C_{23}H_{29}NO_2$ Acridone ketone	4-Decyloxy-10H-acridin-9-one	No pharmacological properties have been reported yet
4	35.82	14057.77	2.763	$C_{16}H_{13}NO_3$ Amine	N-Phthaloyltyramine	No pharmacological properties have been reported yet
5	36.36	37229.52	7.318	$C_{19}H_{22}N_6O_4S$ Sulphamoyl Compound	Pirenzepine, 8-sulfamoyl	Perinzepine derivatives used in treatment of peptic ulcers (Carmine & Brogden, 1985)
6	37.28	319626.8	62.828	$C_{30}H_{50}$ Triterpenoid	Squalene	Antioxidant property (Selvaraj et al., 2019)
Total			100%			

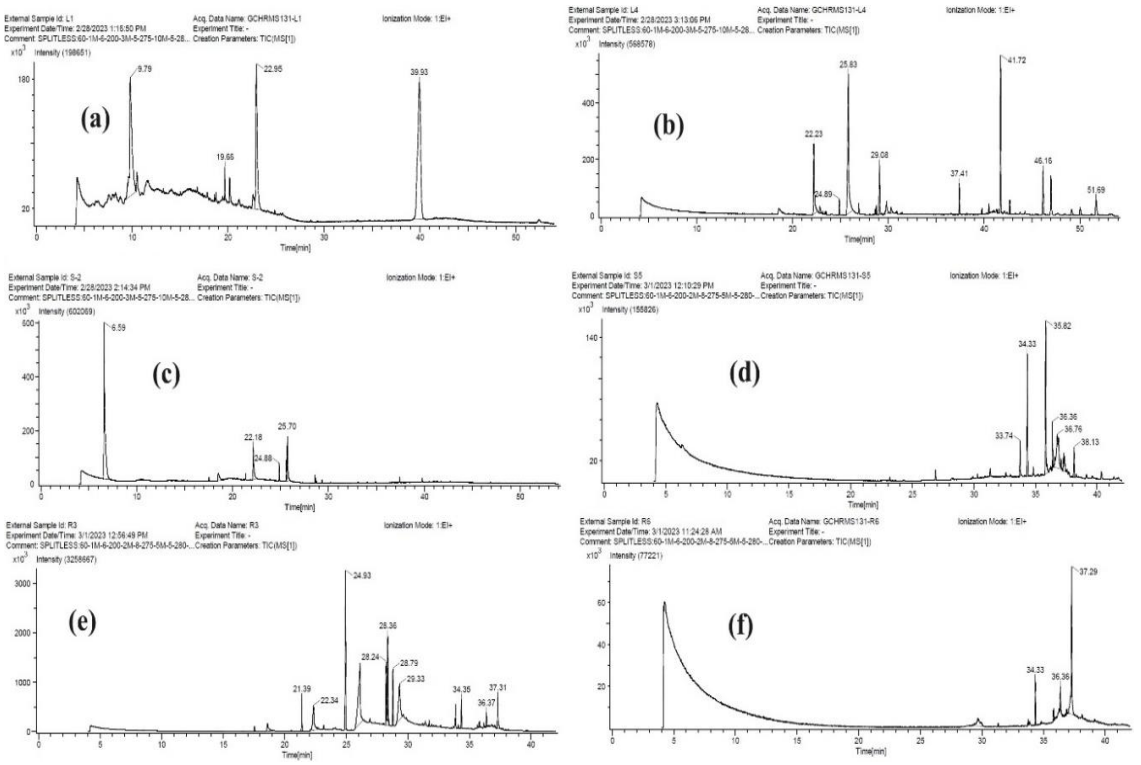

Figure 3. GC-MS chromatogram.of *Spermadictyon suaveolens* Roxb. (a) Methanolic leaf extract (b) Isopropyl alcohol leaf extract (c) Methanolic stem extract (d) Isopropyl alcohol stem extract (e) Methanolic root extract (f) Isopropyl alcohol root extract

The chromatograms of the methanolic stem extract display 4 important peaks with a relative area of more than 3 % (Table 6; Fig. 3) and their mass spectra with the tentative match from the NIST library has been tabulated in Figure 6 out of the major phytochemicals, 2 peaks are 1-Hexanol, 2-ethyl (Ethylhexanol) ($C_8H_{18}O$) with a retention time of 6.59 min and a relative peak area of 64.381%, and n-Hexadecanoic acid (Palmitic Acid) with a retention time of 25.7 min and a relative peak area of 13.295%. while the isopropyl alcohol extract chromatogram reveals 7 major peaks with more than 3% peak area (Table 7; Fig. 3) and their mass spectra with the tentative match from the NIST library has been tabulated in Figure 7. The components with the highest areas are 2-Decanol, propanoate ($C_{13}H_{26}O_2$) with a relative area of 27.966% and a retention time of 36.76 min, and tetratracontane ($C_{44}H_{90}$) with a retention time of 36.36 min and a relative area of 27.192%. The methanolic root extract chromatogram shows 8 notable peaks with more than 3% peak area (Table 8; Fig. 3) and their mass spectra with the tentative match from the NIST library has been tabulated in Figure 8, out of which the 2 peaks with the highest relative area are n-Hexadecanoic acid ($C_{16}H_{32}O_2$), also known as palmitic acid, with a retention time of 26.12 min and a relative area of 23.233, and hexadecanoic acid, methyl ester ($C_{17}H_{34}O_2$), with a retention time of 24.94 min and a relative area of 17.820%. The isopropyl alcohol root extract chromatogram reveals 4 major peaks with more than 3% peak area (Table 9; Fig. 3) and their mass spectra with the tentative match from the NIST library has been tabulated in Figure 9,

out of which the 2 components with the highest area were seen to be Squalene ($C_{30}H_{50}$) with a retention time of 37.28 min and a relative area of 62.828% and 4,6-Bis (4-fluoro-3-(trifluoromethyl) phenoxy)-2-pyrimidinol ($C_{24}H_{22}F_8N_2O_3Si$) with a retention time of 29.67 min and a relative area of 15.156%.

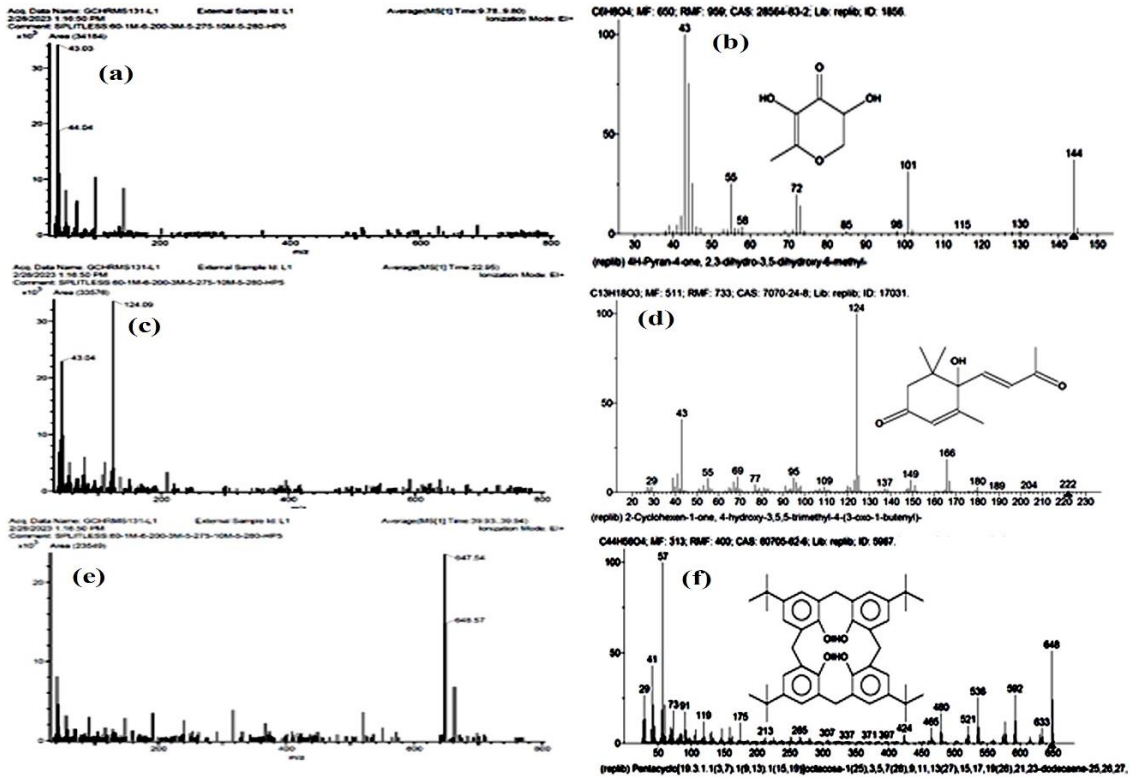

Figure 4. Methanolic leaf extract of *Spermadictyon suaveolens* Roxb. with mass spectra of compound matched with NIST Library. a, c, e- The Leaf methanolic extract GC Constituents chromatogram (> 3% Area); b, d, f- Corresponding mass spectra of compound matched with NIST Library and structure. (a)-Unknown Compound -Ret. Time (9.79). Area 29.875%; (b)-1.3,5-Dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4; (c)-Unknown Compound -Ret. Time (22.95). Area 21.932%; (d)- 2-Cyclohexen-1-one, 4-hydroxy-3,5,5-trimethyl-4-(3-oxo-1-butenyl)-; (e)-Unknown Compound -Ret. Time (39.93). Area 42.326%. (f)- 1.4-tert-Butylcalix[4]arene

DISCUSSION

The antioxidant scavenging activity has been previously worked out for the plant *Spermadictyon suaveolens* and they found that the leaves and bark in the different solvent extracts exhibited comparatively lower IC_{50} values (Inhibition Concentration which can be compared with EC_{50}) than that exhibited in this study that involved hydroalcoholic extracts (Muhammad Ajaib et al., 2014). This may be possible due to the different methods of extraction that attribute different intensities of pharmacological potential to the extracts (Olajuyigbe & Afolayan, 2011).

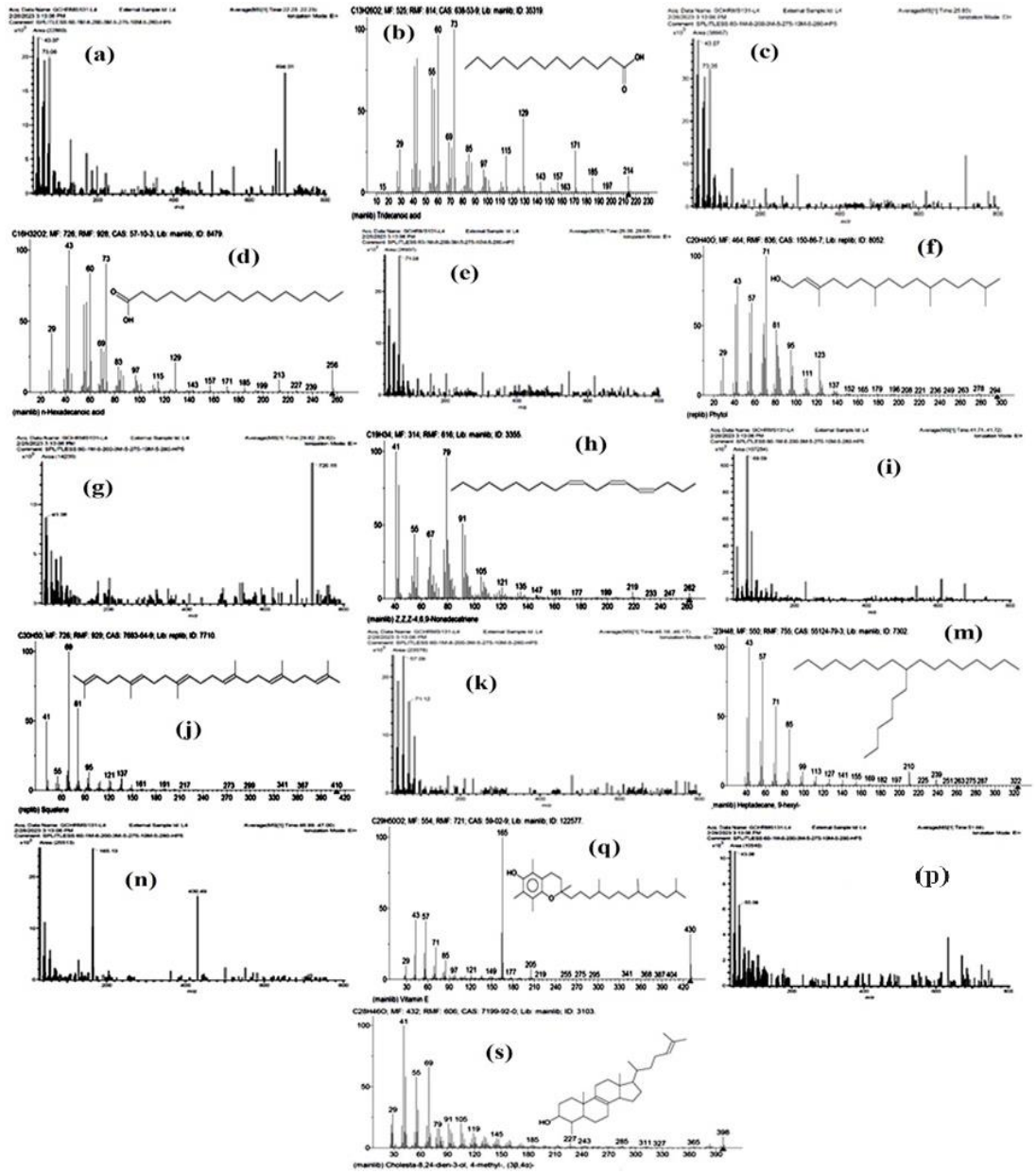

Figure 5. Isopropyl alcohol leaf extract of *Spermadictyon suaveolens* Roxb. with mass spectra of compound matched with NIST Library. a, c, e, g, i, k, n, p- The leaf isopropyl alcohol extract GC constituent's chromatogram (> 3% Area); b, d, f, h, j, m, q, s- Corresponding mass spectra of compound matched NIST Library with structure respectively. (a)- Unknown Compound -Ret. Time (22.23), Area 12.393%; (b)- Tridecanoic acid; (c)-Unknown Compound -Ret. Time (25.83), Area 34.244%; (d)-n-Hexadecanoic acid; (e)-Unknown Compound -Ret. Time (29.08), Area 5.664%; (f)-Phytol; (g)-Unknown Compound -Ret. Time (29.82), Area 3.255%; (h)- Z,Z,Z-4,6,9-Nonadecatriene; (i)-Unknown Compound -Ret. Time (41.72), Area 14.584%; (j) Squalene;(k)-Unknown Compound -Ret. Time (46.16).Area 5.908%; (m) 9-n-Hexylheptadecane; (n)-Unknown Compound -Ret. Time (46.99). Area 5.786%; (q)-Vitamin E; (p)-Unknown Compound- Ret. Time (51.69). Area 3.994%. (s)-Cholesta-8,24-dien-3-ol, 4-thyl-

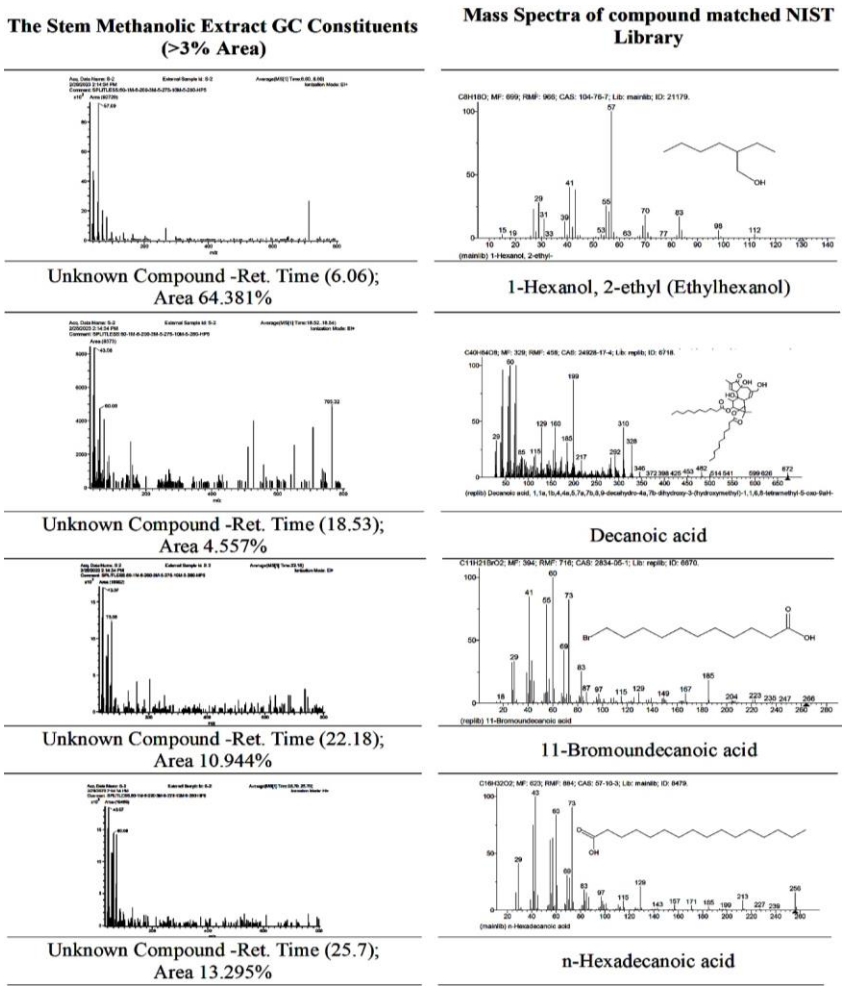

Figure 6. Methanolic stem extract of *Spermadictyon suaveolens* Roxb. with mass spectra of compound matched with NIST Library

Methanolic leaf extract of *S. suaveolens* showed 3 components with more than 3% area in the GCMS chromatogram. The structure, nature, and pharmacological effects of all the phytocompounds tentatively matched by the NIST library have been tabulated and graphically represented for the methanolic leaf extracts in Table 4 & Figures 4, 10. The isopropyl alcohol leaf extract revealed 8 constituents above 3% area. The structure, nature, and pharmacological effects of all the phytocompounds tentatively matched by the NIST library have been tabulated and graphically represented for the isopropyl alcohol leaf extracts in Table 5 & Figures 5, 10. Methanolic stem extract of *S. suaveolens* showed 4 components with more than 3% area in the GCMS chromatogram The structure, nature, and pharmacological effects of all the phytocompounds tentatively matched by the NIST library have been tabulated and graphically represented for the methanolic stem extracts in Table 6 & Figures 6, 10. Isopropyl alcohol stem extract of *Spermadictyon suaveolens* Roxb. revealed 7 constituents above 3% area. The structure, nature, and pharmacological effects of all the phytocompounds tentatively matched by the NIST library have been tabulated and graphically represented for the isopropyl alcohol stem extracts in Table 7 & Figures 7, 10. Methanolic root extracts revealed 8

phytocomponents with an area of more than 3%. The structure, nature, and pharmacological effects of all the phytocompounds tentatively matched by the NIST library have been tabulated and graphically represented for the methanolic root extracts in Table 8 & Figures 8, 10. Isopropyl alcohol root extract showed 4 constituents above 3% area The structure, nature, and pharmacological effects of all the phytocompounds tentatively matched by the NIST library have been tabulated and graphically represented for the isopropyl alcohol root extracts in Table 9 & Figures 9, 10.

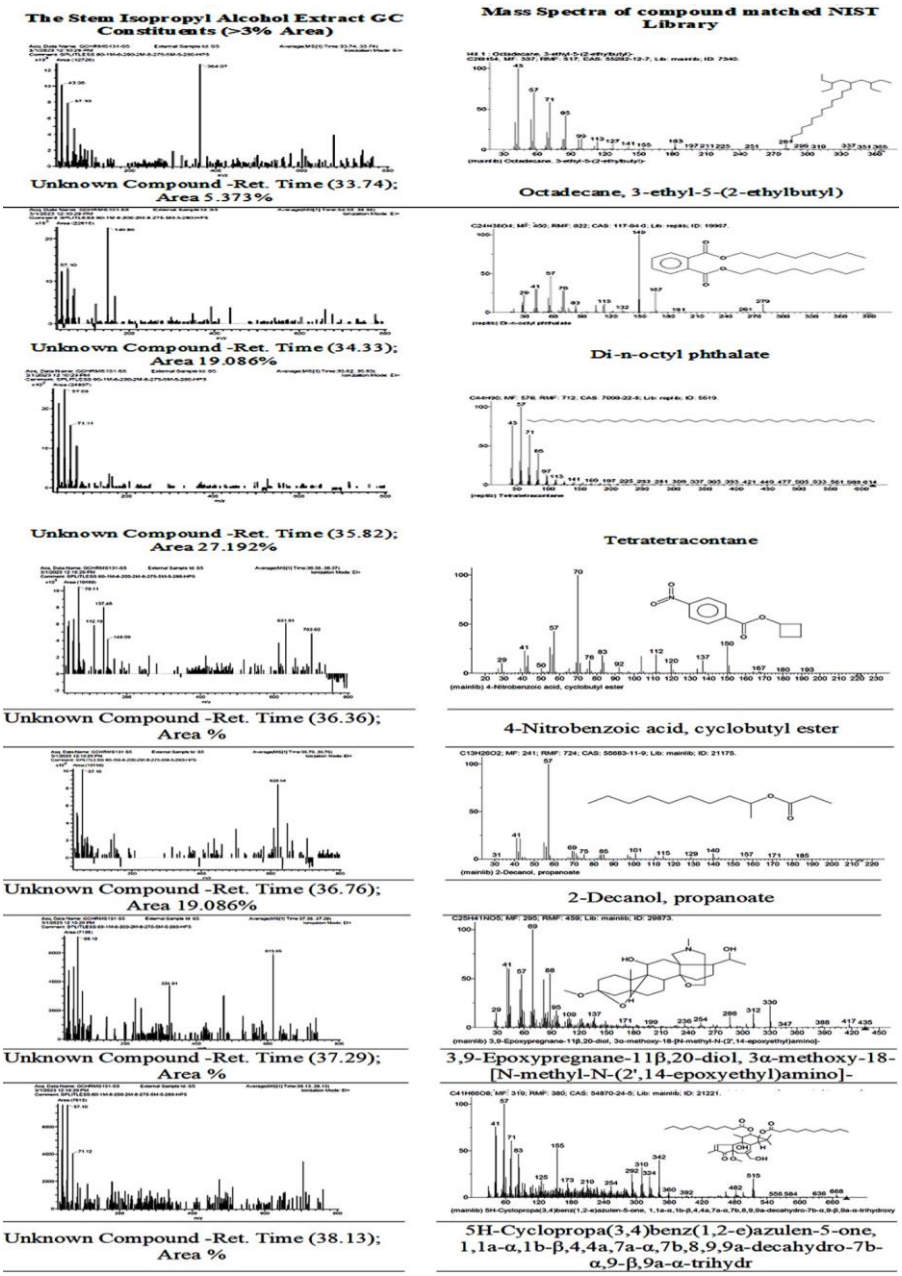

Figure 7. Isopropyl alcohol stem extract of *Spermadictyon suaveolens* Roxb. with mass spectra of compound matched with NIST Library

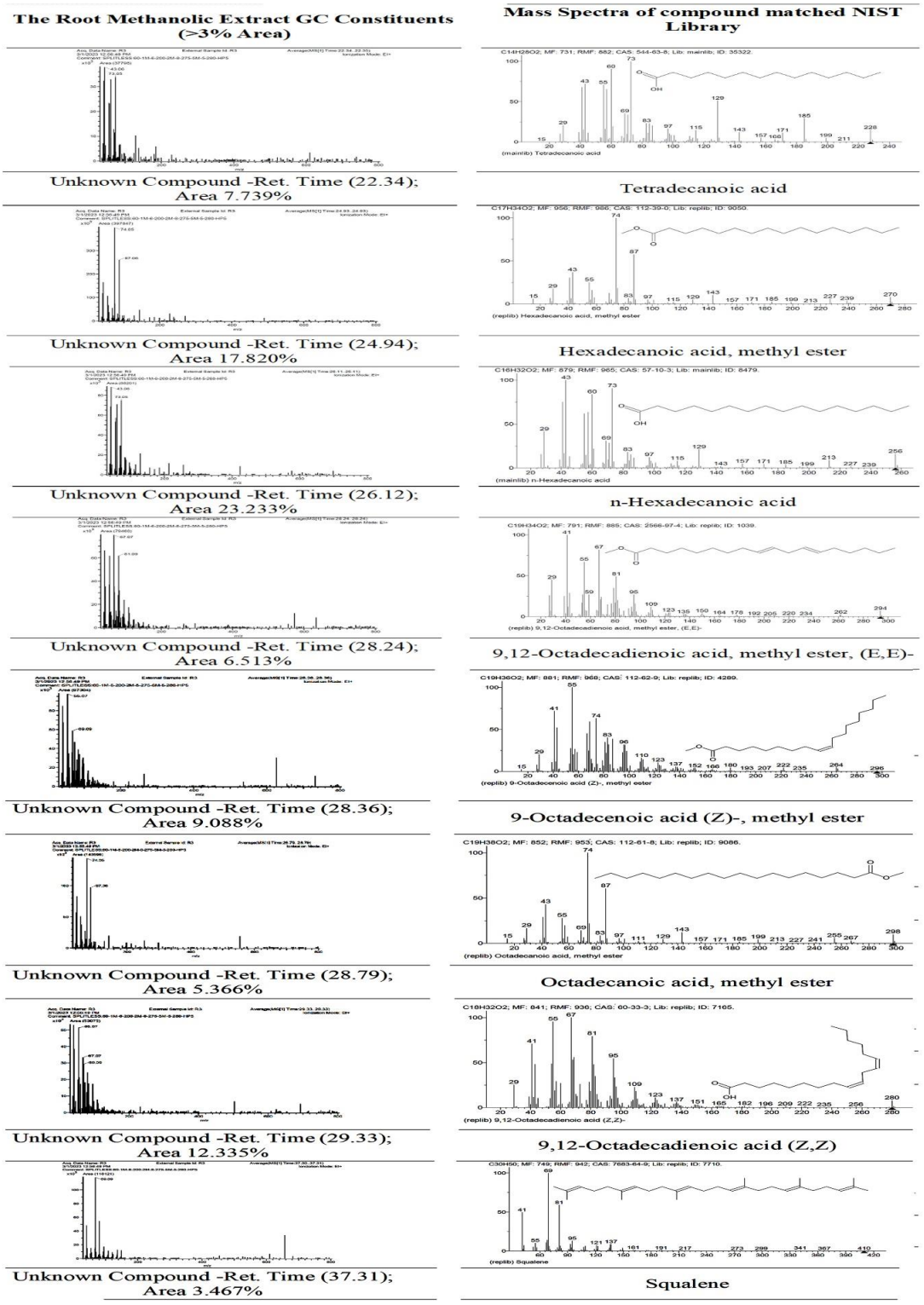

Figure 8. Methanolic root extract of *Spermadictyon suaveolens* Roxb. with mass spectra of compound matched with NIST Library

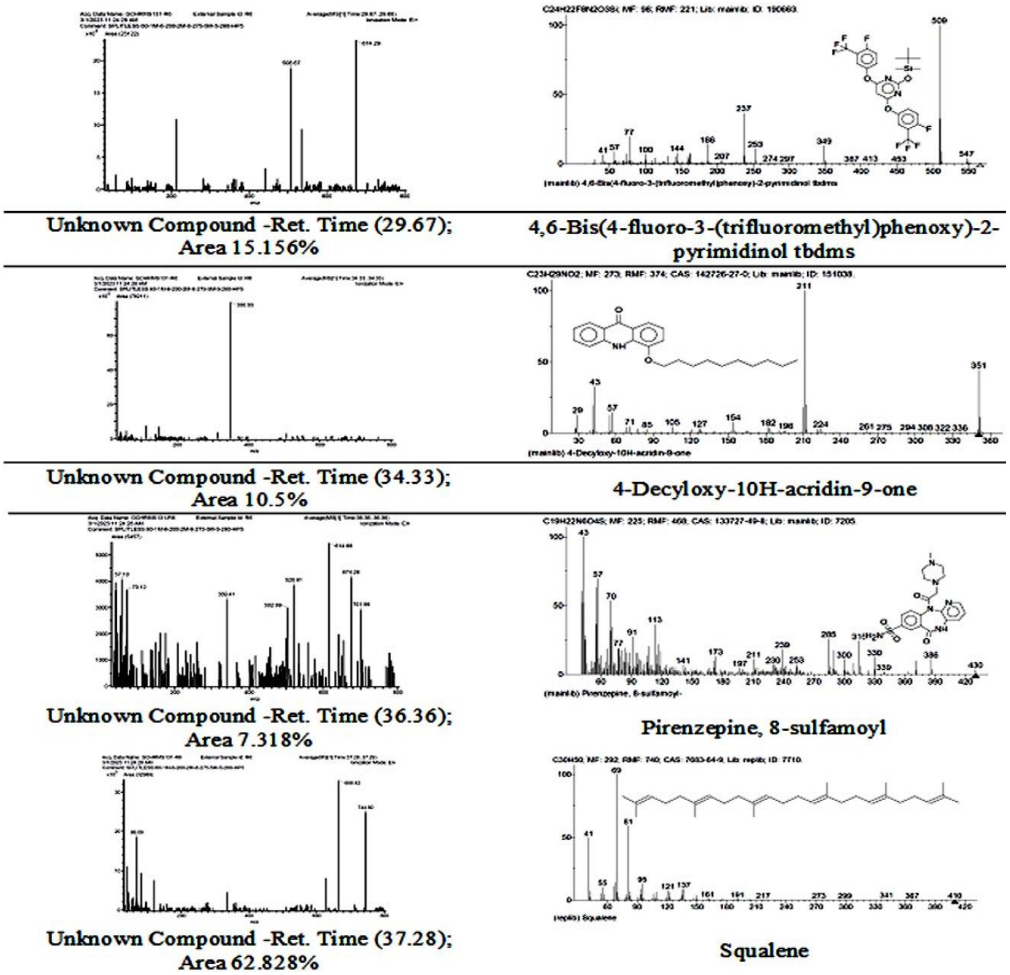

Figure 9. Isopropyl alcohol root extract of *Spermadictyon suaveolens* Roxb. with mass spectra of compound matched with NIST Library

CONCLUSION

This study used the GC-MS technique to compare the phytoconstituents found in the leaves, stems, and roots of *S. suaveolens*. The methanolic and isopropyl alcohol extracts of the plant parts were studied. 69 chemical constituents have been tentatively characterized with GC-MS analysis in the methanolic and isopropyl alcohol extracts of *S. suaveolens*, out of which 3,5-Dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one, 2-Cyclohexen-1-one, 4-hydroxy-3,5,5-trimethyl-4-(3-oxo-1-butenyl)-, n-Hexadecanoic acid, Squalene, Phytol, Z,Z,Z-4,6,9-Nonadecatriene, Vitamin E, Hexadecanoic acid methyl ester, and 9-Octadecenoic acid (Z)- methyl ester may be responsible for conferring antioxidant properties to the plant.

The EC_{50} antioxidant values of leaf, stem, and root hydroalcoholic extracts reveal that the leaf extract exhibits the best antioxidant capabilities in comparison to the stem and root. Thus, it shows promising potential as an antioxidant agent, which indirectly elaborates the potential of this plant for the synthesis of novel drugs for the betterment of humankind and animals alike. More work, however, is warranted on this plant so as to obtain greater detailed information regarding the bioactivity and identification of the phytoconstituents and their pharmacological properties.

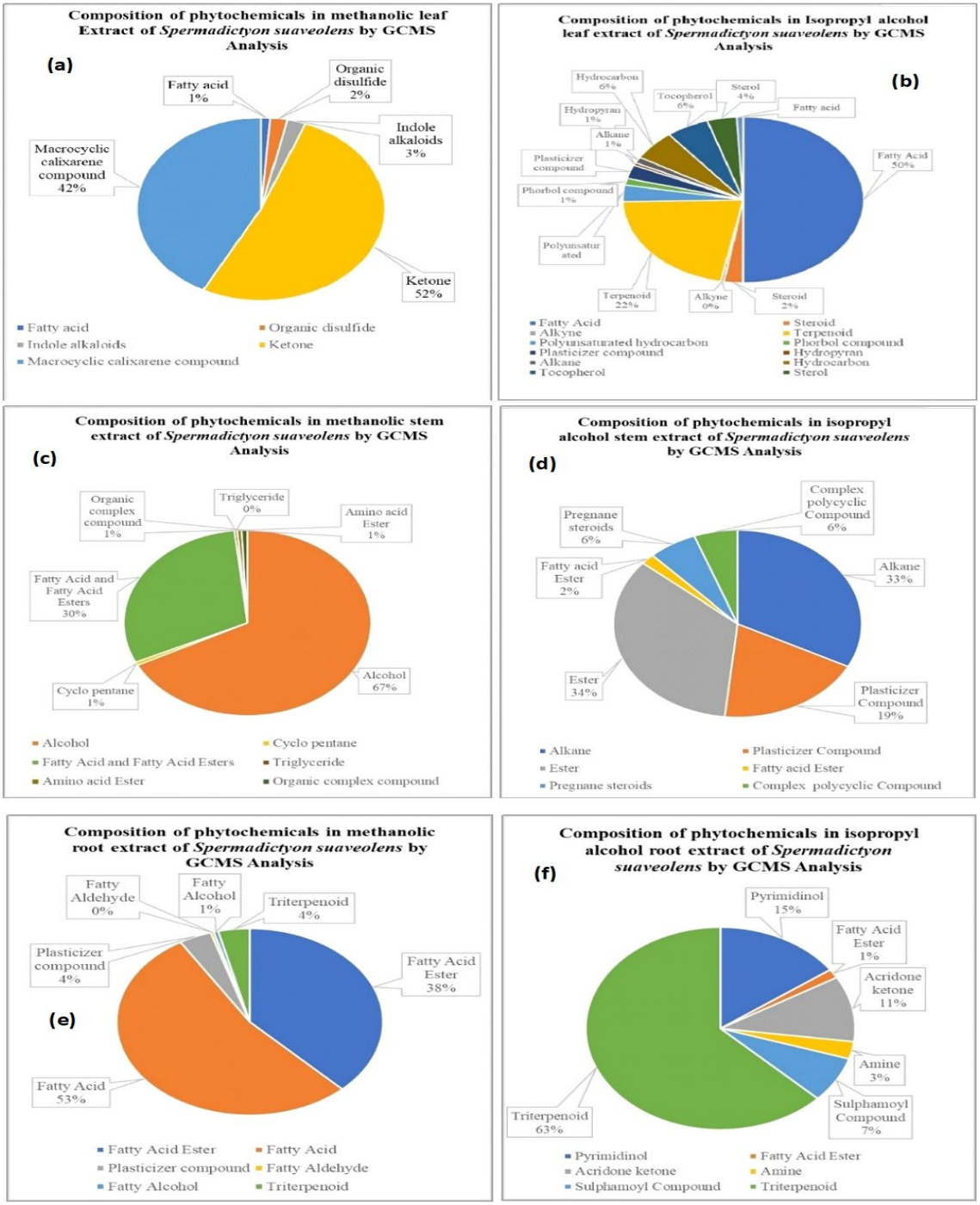

Figure 10. Composition nature of phytochemicals of *Spermadictyon suaveolens* by GCMS Analysis. (a)- methanolic leaf extract; (b)- isopropyl alcohol leaf extract; (c)- methanolic stem extract; (d)- isopropyl alcohol stem extract; (e)- methanolic root extract; (f)- isopropyl alcohol root extract

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