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QUALITATIVE PREMILINARY PHYTOCHEMICAL SCREENING AND GC-MS ANALYSIS OF ROOT OF *SARCOSTEMMA VIMINALE* (L.) R. BR., AN ENDANGERED PLANT

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Abstract: The present investigation was carried out to assess the qualitative phytochemical analysis of root of *Sarcostemma viminale* (L.) R.Br. an endangered & threatened medicinal plant by using methanol, chloroform and hexane as solvents. The extract of the root of *Sarcostemma viminale* (L.) R.Br. with different solvent was investigated using Perkin-Elmer gas chromatography mass spectrometry, while the mass spectra of the compounds found in the extract was matched with the National Institute of Standard and Technology (NIST) library. Maximum % area is found for Methyl Commate B is present in maximum amount (35.35%) with RT= 46.510 min, followed by .beta.- Amyrin (13.39%) with RT= 44.277 min in the methanolic extract; LUP-20(29)-EN-3-YL Acetate is present in maximum amount (48.88%) with RT= 47.689 min, followed by 4,4,6a,6b,8a,11,11,14b-Octamethyl-1,4,4a,5,6,6a,7,8,8a,9 (13.27%) with RT= 44.770 min in the chloroform extract; Methyl Commate D is present in maximum amount (57.69%) with RT= 47.373 min, followed by 4,4,6a,6b,8a,11,11,14b- Octamethyl-1,4,4a,5,6,6a,6b,7,8,8a,9 (14.50%) with RT= 44.560 min in the hexane extracts of root of *Sarcostemma viminale* (L.) R.Br. The present study revealed that *Sarcostemma viminale* (L.) R.Br. is an important plant with many therapeutically and pharmacologically active constituents justifying the use of this plant to treat many ailments.

Keywords: GC-MS, Methanol, Chloroform, Hexane, Phytochemicals, Pharmaceutical, Retention time (RT)

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INTRODUCTION

Medicinal plants are a group of species that accumulate different active principles useful in treating various human or animal diseases. Nature has been a source of medicinal agents for thousands of years and generally produces many secondary metabolites which constitute important leads for the development of new environment friendly microbicides, pesticides, herbicides and many pharmaceutical drugs [1, 2]. Many of indigenous medicinal plants are also used as spices and food plants. They are also sometimes added to food meant for pregnant woman and nursing mother for medicinal purposes [3-6].

Phytochemicals are responsible for medicinal activity of plants[7,8]. These are non-nutritive chemicals that have protected from various diseases. Phytochemicals are basically divided into two groups on the basis of the functioning in plant metabolism that is primary and secondary metabolites. Primary metabolites are commonly carbohydrates, amino acids, proteins, chlorophylls and secondary metabolites consist of alkaloids, saponins, steroids, flavanoides, tannins etc. [9]. *Sarcostemma viminale* (L.) R.Br. is an important endangered medicinal plant belonging to the family Asclepiadaceae, a family of 130 genera and 2000 species, often twining or perennial herbs. Members of *Sarcostemma* genus are also known as "climbing milkweeds". The leaves, roots, latex of *Sarcostemma* species are employed in treating many diseases like mouth ulcer, sour throat, jaundice, ulcers [10-13]. Milky latex is used as lotion [14]. Dry stem is used as emetic and root is used to cure snake bite and taken as an infusion in dog bite cases in Thar Desert [15] and stem juice mixed with water is given in rheumatism, arthritis, joints pain [16] and stem is used to cure bone fracture [17]. Medicinal plants are believed to be with healing powers and people have used them for various centuries. The research of such plants and their medicinal uses is one of the most primary human concerns and has been practiced in the planet [18].

The study investigates on the qualitative phytochemical screening of roots of *Sarcostemma viminale* (L.) R.Br. prepared in three different extracts i.e. methanol, chloroform, hexane to identify the phytochemical constituents with the aid of GC-MS technique.

MATERIAL AND METHODS

Collection of the Plant material

The whole plant material of *Sarcostemma viminale* (L.) R.Br. was collected from Jodhpur and Udaipur region of Rajasthan state of India in the month of August – September, 2015. The specimen authentication and Botanical identification was done by Botanical Survey of India (BSI) Jodhpur, Rajasthan.

Qualitative Screenings of Phytochemicals

Mature plant roots of *Sarcostemma viminale* (L.) R.Br. were collected from nature. The root sample were washed with tap water to remove dust, shade dried and prepare to powder in a mechanical grinder. Required root powder of 4g was transferred to round bottom flask each containing 200ml of different selected solvents like methanol, chloroform and hexane; boiled at 60º-70ºC for 16 hours on water bath; extracts were filtered using whatman filter paper No. 1. The extract was collected and evaporated to dryness, the final residue obtained was then subjected to GC-MS analysis and stored at 4ºC for further use.

Preliminary Phytochemical Screening

The root extract of *Sarcostemma viminale* (L.) R.Br. of different selected solvents methanol, chloroform and hexane were subjected to preliminary phytochemical tests to determine the presence of the following metabolites:-

- Alkaloids
- Carbohydrates
- Glycosides
- Phenolic compounds
- Flavonoids
- Protein and free amino acid
- Saponins
- Sterols
- Acidic compounds
- Terpenoids

Qualitative Phytochemical Screening Assays

The different qualitative chemical tests for the screening and identification of phytoconstituents are as follows:-

Test for Alkaloids Wagner's Test	**2 ml of extract +3-4 drops of wagners reagent (I_2+KI solution) produce reddish brown coloured precipitate indicating the presence of alkaloids.**
Test for Carbohydrates Molish test-	2 ml of extract + 3-4 drops of α- naphthol (20% in ethanol) + 1 ml of concentrated sulphuric acid along the side of the test tube. Indicates the reddish violet ring at the junction of the two layer - presence of carbohydrates.
Test for Glycosides Borntrager's test-	2 ml of extract + 1 ml of benzene and 0.5 ml of dilute ammonia solution, reddish pink colour indicating presence of glycosides.
Test for Phenolic Compounds Lead Acetate test-	1 ml of extract + 3-4 drops of lead acetate solution (5%) yellow precipitates were obtained indicating the presence of phenolic compounds.
Test for Flavonoids Alkaline teat-	2 ml extract treated with sodium hydroxide solution, shows increase in the intensity of yellow colour which would turn colorless on addition of few drops of dilute Hydrochloric acid, indicating the presence of flavonoids.
Test for Proteins & Amino Acid Xanthoprotein test-	2 ml of extract+ 3-4 drops of nitric acid by the side of the test tube. Presence of yellow colouration indicating the presence of protein & amino acid.
Teat for Saponins Foam test-	1 ml of extract was diluted with 5 ml of distilled water and shaken well. The presence of layer of foam indicating the presence of saponins.
Test for Sterols Salkowski test-	2 ml of extract+2 ml of concentrated sulphuric acid (H_2SO_4). The presence of a yellow ring at the junction which finally turned red colour after one minute, indicating the presence of sterols.
Test for Acidic Compounds	2 ml of extract+ 1 ml of sodium bicarbonate solution. The effervescence produced indicating the presence of acidic compounds.
Test for Terpenoids Salkowski test-	2 ml of extract, added 2 ml of chloroform and 3 ml of concentrared H_2SO_4, form a layer,a reddish browinsh colouration on the inner face is formed indicating presence of terpenoids.

RESULT AND DISCUSSION

The preliminary phytochemical screening of root extract of *Sarcostemma Viminale* (L.) R.Br. was carried out using methanol, chloroform and hexane as solvents. The plant showed the presence of various secondary metabolites like Alkaloids, Carbohydrate, Glycosides, Phenolic compounds, Flavonoids, Protein & Amino acid, Saponins, Sterols, Acidic compounds and Terpenoids (Table 1). The secondary metabolites have shown strong positive results with methanol as compared to chloroform and hexane. The components present in the methanol, chloroform and hexane

extract of root of *S.Viminale* (L.) R.Br. were identified by GC-MS analysis (Fig.1- 3) showed 94, 99 and 77 peaks indicating the presence of 89, 83 and 61 compounds in respective extracts.

The active principle with their retention time (RT), molecular formula, molecular weight, concentration (%) in methanolic extract, chloroform extract, hexane and extract of root of *S. Viminale* (L.) R.Br. (Table 2-4). GC-MS analysis revealed the presence of triterpene, linoleic acid, terpene, fatty acid, phenolic compound, saponin, alkene, steroid, steric acid, diterpene alcohol, alcoholic compound, ketone compound, palmitic acid, aliphatic hydrocarban, ester compound, oxygenated monoterpen, aldehyde etc.

Methyl Commate B is present in maximum amount (35.35%), followed by .beta.-Amyrin (13.39%) and Squalene (0.09%), 7-Cyclohexylnonadecane (0.09%), 2-Methoxy-4-Vinylphenol (0.08%) were present in minimum amount, 9,12-Octadecadienoic acid (Z,Z)- (0.08%), Estra-1,3,5 (10)-triene-7,17-dione,3-[(trimethylsilyl)oxy] (0.08%) in the methanolic extract of root of *S.Viminale* (L.) R.Br. LUP-20-(29)-EN-3-YL Acetate is present in maximum amount (48.88%), followed by 4,4,6a,6b,8a,11,11,14b- Octamethyl-1,4, 4a, 5, 6, 6a, 7, 8, 8a, 9 (13.27%) and 3-(2,3-Dimethoxyphenyl)-2-(1-Naphthyl) propen (0.08%), Cubedol (0.07%), 2-Propanone, 4-Hydroxy-3-Methoxyphenyl (0.07%) were present in minimum amount in the chloroform extract of root of *S. Viminale* (L.) R.Br. Methyl Commate D is present in maximum amount (57.69%), followed by 4,4,6a,6b,8a,11,11,14b-Octamethyl-1,4,4a,5,6,6a,6b,7,8,8a,9 (14.50%) and Platambin (0.08%), s9- Hexadecenoic Acid, Methyl Ester, (Z)- (0.08%), 6, ALPHA.- CADINA-4,9- DIENE, (-)- (0.09%) were present in minimum amount in the hexane extract of root of *S. Viminale* (L.) R.Br.

The gas chromatogram shows the relative concentrations of various compounds getting eluted as a function of retention time. The height of the peak indicate the relative concentrations of the components present in plants. The mass spectrometer analyzes the compounds eluted at different times to identify the nature and structure of the compounds. The larger amount fragments into small compounds going rise to appearance of peaks at differents m/z ratio. These mass spectra are fingerprint of that compound which can be identified from the data library. The result of the GC-MS profile can be used as pharmacognostical tool for the identification of the plant compound with different chemical structures. Application of Sarcostemma Viminale (L.) R.Br. in presence of various bioactive compounds confirms various ailments by traditional practitioners and individual phytochemical constituents may proceed to find a noval drug.

Table. 1 Phytochemical constituents of the root extract of *Sarcostemma vimilale* (L.) R.Br.

S.No.	Phytoconstituents	Tests	Methanol	Chloroform	Hexane
1.	Alkaloids	Wagner's Test	+++	+++	-
2.	Carbohydrates	Molish test	+++	++	+
3.	Glycosides	Borntrager's test	-	-	-
4.	Phenolic compounds	Lead Acetate test	++	-	-
5.	Flavanoids	Alkaline teat	+++	-	-
6.	Protein & Amino acid	Xanthoprotein test	+++	++	+
7.	Saponins	Foam test	+++	+	+
8.	Steroids	Salkowski test	+++	++	+
9.	Acidic compounds		++	-	-
10.	Terpenoids	Salkowski test	+++	+	+

Key: - (-) absent, (+) present, (++) moderately present, (+++) abundantly present

Table. 2 Phytocomponents identified in various extract of the root of *Sarcostemma vimilale* (L.) R.Br.

S.No.	Presence of solvents	R.Time	Name of Compound	% area	M.F.	M.W.	Biological Activity
1.	Methanol Chloroform	10.856 10.858	Vanillin	0.19 0.12	$C_8H_8O_3$	152	Hypolipidemic activity, Antioxidant, Animicrobial activity
2.	Methanol Chloroform Hexane	11.101 11.102 11.100	Caryophyllene	0.06 0.04 0.04	$C_{15}H_{24}$	204	Antioxidant, Anti-inflammatory, Antibacterial, Analgesic, Antitumor activity
3.	Methanol Chloroform	12.792 12.800	DODECANOIC ACID	0.05 0.03	$C_{12}H_{24}O_2$	200	Pharmaceuticals activity
4.	Methanol Chloroform Hexane	14.050 14.056 14.048	.alpha.-Cadinol	0.20 0.17 0.16	$C_{15}H_{26}O$	222	Anti-fungal, Drug-resistant tuberculosis properties
5.	Methanol Chloroform	15.036 15.043	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol	1.33 0.54	$C_{10}H_{12}O_3$	180	Antimicrobial, Antioxidant, Anticancer, Anti-inflammatory activity
6.	Methanol Hexane	15.819 15.813	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	0.12 0.04	$C_{20}H_{40}O$	296	Antimicrobial, Anti-inflammatory activity
7.	Methanol Hexane	19.650 18.853	9,12-Octadecadienoic acid (Z,Z)-	0.08 0.51	$C_{18}H_{32}O_2$	280	Cancer preventive, Insectifuge, Anti-inflammatory, Nematicide, Hepatoprotective, Antihistaminic, Anticane, Antiarthritic, Antieczemic
8.	Methanol Chloroform	21.177 21.192	Eicosanoic acid	0.05 0.03	$C_{20}H_{40}O_2$	312	Anticancer, Anti-inflammatory activity
9.	Methanol Chloroform	22.991 23.014	PENTACOSANE	0.17 2.18	$C_{25}H_{52}$	352	Antibacterial activity

	Hexane	20.313		0.06			
10.	Methanol Chloroform Hexane	28.006 28.019 28.006	Squalene	0.09 0.06 0.11	$C_{30}H_{50}$	410	Antibacterial, Antioxidant, Antitumor, Anti-inflammatory, Antinociceptive, Potential antiplatelet components, Hypoglycemic, Hypolipidemic effects, Sedative action, Antihistaminic,Hepatoprotective activites Cancer preventing, Immunostimulant
11.	Methanol Hexane	36.326 36.326	Ergost-5-en-3-ol, (3.beta.)-	0.25 0.84	$C_{28}H_{48}O$	400	Antimicrobial and Anti-inflammatory effects
12.	Methanol Hexane	37.266 37.228	Stigmasterol	0.72 0.47	$C_{29}H_{48}O$	412	Antimicrobial activity
13.	Methanol Hexane	39.182 39.194	STIGMAST-5-EN-3-OL, (3.BETA.)-	2.55 1.79	$C_{29}H_{50}O$	414	Anti-inflammatory, Anti-pyretic, Anti-ulcer, Antiarthritic
14.	Methanol Chloroform Hexane	40.337 40.546 40.371	.beta.-Amyrin	18.85 2.75 3.40	$C_{30}H_{50}O$	426	Antibacterial, Antioxidant, Anti-inflammatory, Antinociceptive, Potential antiplatelet components, Hypoglycemic, Hypolipidemic effects, Sedative action, Hepatoprotective activites
15.	Methanol Hexane	40.930 47.373	METHYL COMMATE D	5.73 62.25	$C_{31}H_{50}O_4$	486	Antimicrobial, Anti-inflammatory activity

Fig. 1 GC-MS chromatogram of the methanolic extract of root of *Sarcostemma vimilale* (L.) R.Br.

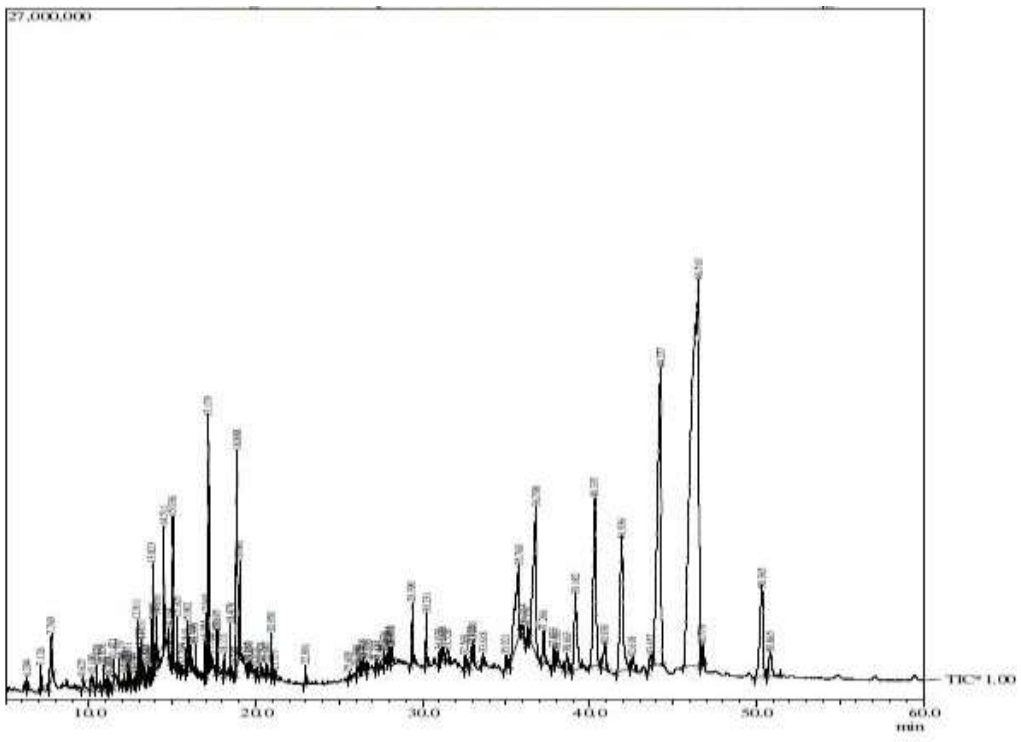

Table. 3 Bioactivity of Phytocomponents identified in the methanol extract of root of *Sarcostemma viminale* (L.) R.Br. by GC-MS analysis

S.No.	R.Time	Name of Compound	% area	M.F.	M.W.	Biological Activity
1.	6.294	1,6-OCTADIEN-3-OL, 3,7-DIMETHYL-	0.07	$C_{10}H_{18}O$	154	Anti-inflammatory and Anti-cancer properties
2.	7.769	BENZOIC ACID	0.80	$C_7H_6O_2$	122	used as an expectorant and fungal skin diseases, Analgesic, Food Industry, Antifungal properties
3.	9.625	2-Methoxy-4-vinylphenol	0.08	$C_9H_{10}O_2$	150	Antioxidant, Antimicrobial, Anti-inflammatory, Perfumery, Insecticide, Fungicide activity
4.	27.443	9-OCTADECENAMIDE	0.02	$C_{18}H_{35}NO$	281	Good therapeutic agent for the treatment of sleep disorders and pain
5.	46.779	Lanosta-8, 24-dien-3-ol, acetate, (3.beta.)-	0.41	$C_{32}H_{52}O_2$	468	Anti-amylase inhibitor, Antimicrobial, Anti-diabetic properties

Fig. 2 GC-MS chromatogram of the chloroform extract of root of *Sarcostemma viminale* (L.) R.Br.

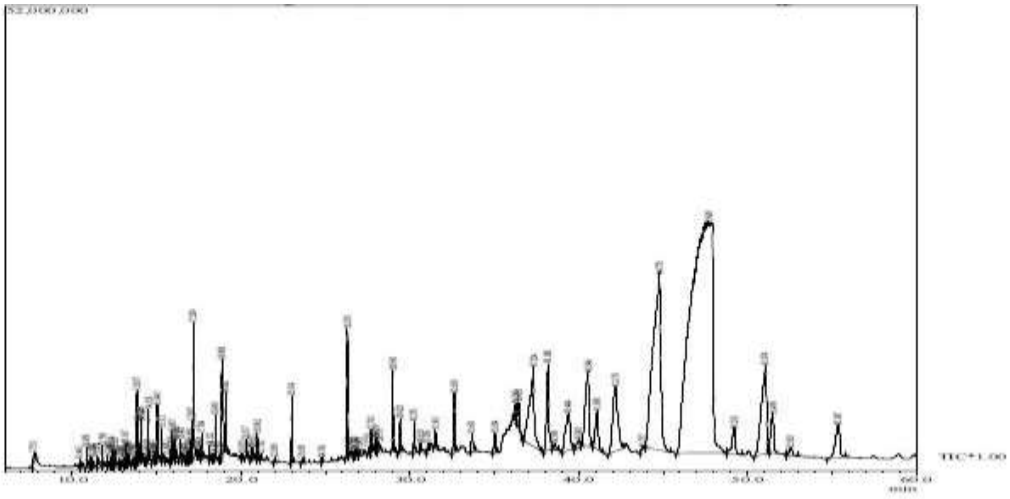

Table. 4 Bioactivity of Phytocomponents identified in the chloroform extract of root of *Sarcostemma viminale* (L.) R.Br. by GC-MS analysis

S.No.	R.Time	Name of Compound	% area	M.F.	M.W.	Biological Activity
1.	7.753	NAPHTHALENE	0.16	$C_{10}H_8$	128	Antiseptic, Carcinogenic
2.	10.465	.alfa.-Copaene	0.03	$C_{15}H_{24}$	204	Anticancer agent, Antioxidant, Antigenotoxic
3.	14.922	Benzeneacetic acid, .alpha., 4-dihydroxy-3-methoxy-,methyl	0.04	$C_{10}H_{12}O_5$	212	Antioxidant activity
4.	24.761	Tetratetracontane	0.03	$C_{44}H_{90}$	618	Hypoglycaemic, Antioxidant activity
5.	47.689	LUP-20(29)-EN-3-YL ACETATE	54.46	$C_{32}H_{52}O_2$	468	No activity reported

Fig. 3 GC-MS chromatogram of the hexane extract of root of *Sarcostemma viminale* (L.) R.Br.

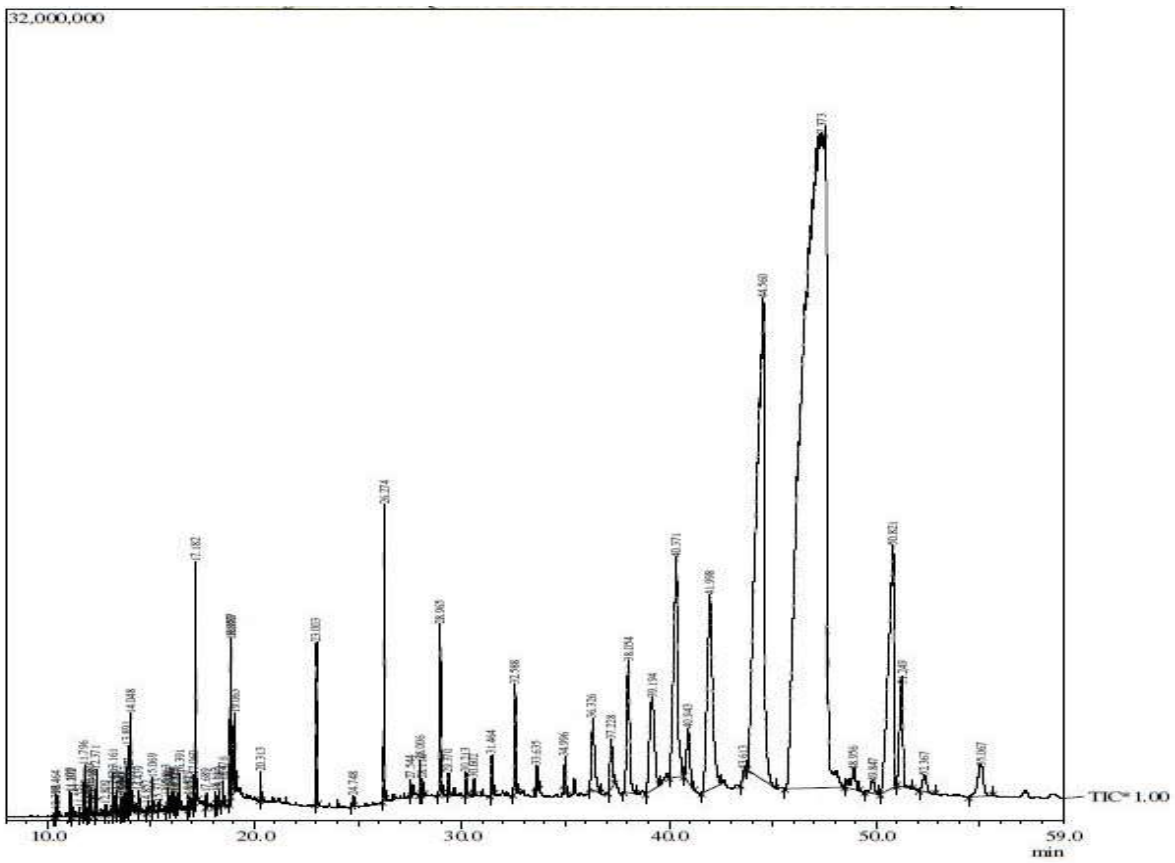

Table. 5 Bioactivity of Phytocomponents identified in the hexane extract of root of *Sarcostemma viminale* (L.) R.Br. by GC-MS analysis

S.No.	R.Time	Name of Compound	% area	M.F.	M.W.	Biological Activity
1	10.464	Copaene	0.04	$C_{15}H_{24}$	204	Antioxidant, Anticancer, Antigenotoxic activity
2.	11.202	1,3-CYCLOHEXADIENE, 5-(1,5-DIMETHYL-4-HEXENYL)-2-METHYL-, [S-(R*,S*)]-	0.04	$C_{15}H_{24}$	204	Anti-tumor, Analgesic, Antibacterial, Anti-inflammatory, Sedative, Fungicide
3.	11.983	POGOSTOL	0.01	$C_{16}H_{28}O$	236	Patchouli Essentia oil show Antidepressant activity, Reduces inflammation, prevent infection, stimulates hormone, Help metabolic system
4.	15.069	Tetradecanoic acid	0.06	$C_{14}H_{28}O_2$	228	Antioxidant, Cancer preventive, Nematicide, Lubricant, Hypocholesterolemic
5.	15.900	2-PENTADECANONE, 6,10,14-TRIMETHYL-	0.02	$C_{18}H_{36}O$	268	Allelopathic activity
6.	16.229	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	0.01	$C_{16}H_{22}O_4$	278	Antimicrobial activity, alpha- Glucosidase inhibition and the in vitro hypoglycemic effect
7.	16.275	1-OCTADECANOL	0.02	$C_{18}H_{38}O$	270	Cleaning agent and coatings, use paper and textile industries, used as antifoam
8.	16.829	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	0.03	$C_{17}H_{24}O_3$	276	Antimicrobial activity
9.	17.060	9-HEXADECENOIC ACID, METHYL ESTER,(Z)-	0.08	$C_{17}H_{32}O_2$	268	effects of the permeability and partition of ions
10.	18.282	n-Nonadecanol-1	0.03	$C_{19}H_{40}O$	284	Cytotoxic and Anti-microbial activity

CONCLUSIONS

The qualitative screening of powdered root extract showed that the Methanol, Chloroform and Hexane extracts posses different bioactive compounds like Alkaloids, Carbohydrate, Glycosides, Phenolic Compounds, Flavanoids, Protein & free Amino acid, Saponins, Sterols, Acidic Compound and Terpenoids. Methanol and Hexane solvents analyzed more compounds as compared to Chloroform.

Gas Chromatography Mass Spectrometry (GC-MS) is a method that combines the features of gas liquid chromatography and mass spectrometry to identify different substances within a test sample. Application of GC-MS includes environmental analysis, drug detection and identification of unknown sample. The presence of various bioactive compounds confirms the application of *Sarcostemma Viminale* (L.) R.Br. for treating various ailments by traditional practitioners and phytochemical constituents seemed to have the potential to act as source of useful drugs and improve the health status.

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