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Research Article

GAS CHROMATOGRAPHY-MASS SPECTROMETRY ANALYSIS OF AN ENDANGERED MEDICINAL PLANT, *SARCOSTEMMA VIMINALE* (L.) R.BR. FROM THAR DESERT, RAJASTHAN (INDIA)

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ABSTRACT

Objective: *Sarcostemma viminale* (L.) R. Br. is one of the important endangered medicinal plants belonging to the family *Asclepiadaceae*. The aim of the present investigation was to determine the possible bioactive phytochemicals from stem of *S. viminale* (L.) R. Br. using methanol, chloroform, and hexane as solvents.

Methods: Plant material was collected from typical conditions of Indian Thar Desert in the month of July-September, 2016. This plant always grows in association with the congeneric plant, *Euphorbia caducifolia*. The phytochemical compounds were investigated using Perkin-Elmer gas chromatography-mass spectrometry, while the mass spectra of the compounds found in the extract were matched with the National Institute of Standards and Technology library.

Results: Maximum % area is found for Lup-20-(29)-en-3-yl acetate is present maximum amount (40.85%) with reaction time (RT)=43.787 minutes, followed by 4, 4, 6A, 6B, 8A, 11, 11, 14B-octamethyl-1, 4, 4A, 5, 6, 6A, 6B, 7, 8, 8A, 9, 10, 11, 12, 12A, 14, 14A, 14B-octadecahydro-2H-picen-3-one$$olean-12-en-3-one# (13.74%) with RT=44.420 minutes in the methanolic extract; acetic acid 4, 4, 6A, 8A, 11, 12, 14B-octamethyl-1, 2, 3, 4, 4A, 5, 6, 6A, 6B, 7, 8, 8A, 9, 10, 11, 12, 12A, 14, 14A, 14B-eicosahydro-picen-3-yl ester $$ urs-12-en-3-yl acetate is present maximum amount (44.98%) with RT=48.265 minutes, followed by. beta.-amyrin (18.51%) with RT=40.580 minutes in the chloroform extract; acetic acid 4, 4, 6A, 8A, 11, 12, 14B-octamethyl-1, 2, 3, 4, 4A, 5, 6, 6A, 6B, 7, 8, 8A, 9, 10, 11, 12, 12A, 14, 14A, 14B-eicosahydro-picen-3-yl ester $$ urs-12-en-3-yl acetate is present maximum amount (45.47%) with RT=48.514 minutes, followed by. beta.-amyrin (19.21%) with RT=40.555 minutes in the hexane extract of stem of *S. viminale* (L.) R. Br.

Conclusion: Medicinal plants contain one or more substances that can be used for therapeutic purpose; they are used by the world population for their basic health needs. The importance of the study is to investigate the pinpoint biological activity of some of these compounds so that they can be used by pharma or some other drug designing industry to find a novel drug.

Keywords: *Sarcostemma viminale*, Phytocomponents, Methanol, Chloroform, Hexane, Gas chromatography-mass spectrometry, Retention time.

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INTRODUCTION

Plants are man's friend in survival; providing us food, fuel, and medicine from the days beyond the dawn of civilization [1]. According to the World Health Organization, about 80% of the world population depends on the natural product for their health due to minimal side effect and cost-effectiveness [2]. The secondary metabolites are a significant source with a variety of structural arrangements and properties [3]. Medicinal plants, as a source of remedies, are widely used as alternative therapeutic tool for the prevention or treatment of many diseases [4]. Natural products which come out from medicinal plants are important for pharmaceutical research and drug development as a source of therapeutic agents. At present the demand for herbal plant products has increased significantly [5] as they do not cause any side effect; hence, they are more protective and safe. *Sarcostemma viminale* (L.) R. Br. is an important endangered medicinal plant (*Asclepiadaceae*) with twining or perennial herbs [6]. *Sarcostemma* is commonly known as moon plant and in veda it is known as "soma." It is a remarkably glabrous, vigorously tangling scrambler and climber, which can produce voluminous plants. The inflorescences are mostly lateral and sessile, rarely on short laterals (peduncles) or terminal [7]. The root is used to cure snake bite and taken as an infusion in dog bite cases in Thar Desert [8]. The dry stem is used as emetic and stem juice mixed with water is given in rheumatism, arthritis, joints pain [9], and stem is also used to cure bone fracture [10].

The aim of this work was to isolate, investigate, and characterize the phytochemical constituents in three different extract, i.e., methanol, chloroform and hexane by using gas chromatography-mass spectrometry (GC-MS) from stem of *S. viminale* (L.) R. Br. Traditionally used medicinal plants have recently attracted the attention of scientific communities, it has resulted in the isolation and identification of secondary metabolites produced by plants and their use as active principles in medicinal preparations [11]. 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 [12].

METHODS

Collection and processing of the plant material

S. viminale (L.) R. Br. was collected from hilly and stony regions of Jodhpur and Udaipur districts of Rajasthan (India) in the month of June-August 2015. The specimen authentication and botanical identification were done by Botanical Survey of India, Jodhpur, Rajasthan.

Qualitative screenings of phytochemicals

The stem extract of *S. viminale* (L.) R.Br. was subjected to preliminary phytochemical screening to determine the presence of the various metabolites. Standard analytical procedures were adopted for screening and identification of various phytoconstituents (Table 1).

Fresh stem of *S. viminale* (L.) R. Br. was collected from nature. The stem was shade dried and prepared to powder in a mechanical grinder. Required powder of 5 g was weighed separately using an electronic balance and transferred to round bottom flask and extracted with

200 ml of selected solvents such as methanol, chloroform, and hexane; boiled at 60-70°C for 16 hrs on water bath; filtered, collected and evaporated to dryness, the final residue obtained was then subjected to GC-MS analysis and stored at 4°C for further use.

RESULT AND DISCUSSION

Herbal medicine represents one of the most important fields of traditional medicine worldwide. Various extracts from traditional medicinal plants have been tested to identify the source of the therapeutic effects. Significance of employing bioactive compounds in pharmacy to produce drugs for the treatment of many diseases requires purification of compounds [13]. The preliminary phytochemical screening of stem extract of *S. viminale* (L.) R. Br. was carried out using three solvent, i.e., methanol, chloroform and hexane. The analysis revealed the presence of various secondary metabolites, i.e., alkaloids, carbohydrates, glycosides, phenolic compounds, flavonoids, proteins, amino acid, saponins, sterols, acidic compounds, and terpenoids with important biological activities (Table 1). Secondary metabolites by chromatography and spectroscopy provide valuable information about the qualitative and quantitative formulation of plant species [14].

The combination of the best separation technique (GC) with the best identification technique (MS) made GC-MS an ideal technique for qualitative analysis for volatile and semivolatile bioactive compounds [15]. GC-MS analysis of the stem of *S. viminale* (L.) R. Br. in different solvent such as methanol, chloroform, and hexane showed 70, 72 and 61 peaks (Figs. 1-3) indicating the presence of 64, 56 and 47 compounds in respective extracts. Confirmation of the presence was based on retention time (RT), peak area, molecular formula, concentration (%), and molecular weight (Tables 2-4).

LUP-20-(29)-en-3-YL acetate is present in maximum amount (40.85%), followed by 4, 4, 6A, 6B, 8A, 11, 11, 14B-octamethyl-1, 4, 4A, 5, 6, 6A, 6B, 7, 8, 8A, 9, 10, 11, 12, 12A, 14, 14A, 14B-octadecahydro-2H-picen-3-one$$olean-12-en-3-one# (13.74%) and hexadecanoic acid, methyl ester (0.03%), oxalic acid, cyclohexylmethyl tridecyl ester (0.04%), 13-docosenamide, (Z-) (0.05%) were present in minimum amount in the methanolic extract; acetic acid 4, 4, 6A, 8A, 11, 12, 14B-octamethyl-1, 2, 3A, 5, 6, 6A, 6B, 7, 8, 8A, 9, 10, 11, 12, 12A, 14, 14A, 14B-eicosahydro-picen-3-YL ester $$ URS-12-en-3-YL acetate is present maximum amount (44.98%), followed by. beta.-

Table 1: Phytochemical constituents of the stem extract of *Sarcostemma viminale*

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

-: Absent, +: Present, ++: Moderately present, +++: Abundantly present

Table 2: Bioactivity of phytocomponents identified in methanol extract of stem of *Sarcostemma viminale*

S.No.	RT (minutes)	Name of compound	% area	Molecular formula	Molecular weight	Biological activity
1.	7.137	2,3-dihydro-3,5-dihydroxy-6-methyl-4H-Pyran	0.22	$C_6H_8O_4$	144	Antimicrobial, anti-inflammatory
2.	7.857	Benzoic acid	1.91	$C_7H_6O_2$	122	Used as an expectorant and fungal skin diseases, analgesic, food industry, antifungal properties
3.	9.631	2-methoxy-4-	0.05	$C_9H_{10}O_2$	150	Antibacterial
4.	13.033	Vinylphenol 1-hexadecene	0.09	$C_{16}H_{32}$	224	Antibacterial, antifungal, antioxidant activity
5.	13.857	1,3,4,5-tetrahydroxy-cyclohexanecarboxyl	0.32	$C_7H_{12}O_6$	192	Antimicrobial, anti-inflammatory, antioxidant activity
6.	15.035	4-((1E)-3-hydroxy-1-propenyl)-2-methoxyphenol	0.12	$C_{10}H_{12}O_3$	180	Antimicrobial, antioxidant, anticancer, anti-inflammatory activity
7.	15.826	2-hexadecen-1-ol-3,7,11,15-tetramethyl-[R-[R*R*,-(E)]]-	0.11	$C_{20}H_{40}O$	296	Cancer preventive
8.	16.717	Hexadecanoic acid, methyl ester	0.03	$C_{17}H_{34}O_2$	270	Antibacterial and antifungal, antioxidant hypocholesterolemic, nematicide, insecticide lubricant, antiandrogenic flavor, hemolytic
9.	18.123	Heptadecanoic acid	0.13	$C_{17}H_{34}O$	270	Antioxidant, antifungal, surfactant
10.	27.483	13-docosenamide, (Z)-	0.05	$C_{22}H_{43}NO$	337	Antimicrobial activity
11.	39.249	Stigmast-5-en-3-ol, (3.beta.)-	3.03	$C_{29}H_{50}O$	414	Anti-inflammatory, anti-pyretic, anti-ulcer, antiarthritic
12.	40.459	.Alpha.-amyrin	7.32	$C_{30}H_{50}O$	426	Antioxidant, antimicrobial, anticancer, anti-inflammatory

RT: Reaction time

Table 3: Bioactivity of phytocomponents identified in the chloroform extract of stem of *Sarcostemma viminale*

S.No.	RT (minutes)	Name of compound	% area	Molecular formula	Molecular weight	Biological activity
1.	11.109	Caryophyllene	0.02	$C_{15}H_{24}$	204	Antioxidant, anti-inflammatory, antibacterial, analgesic, antitumor activity
2.	14.061	.Alpha.-cadinol	0.03	$C_{15}H_{26}O$	222	Anti-fungal, drug-resistant tuberculosis properties
3.	15.830	2, 6, 10, trimethyl, 14-ethylene-14-pentadecene	0.04	$C_{20}H_{38}$	278	Antiproliferative
4.	16.847	7, 9-di-tert-butyl-1-oxaspiro (4,5) deca-6, 9-diene-2, 8-dione	0.02	$C_{17}H_{24}O_3$	276	Antimicrobial activity
5.	17.945	9-octadecanoic acid (Z)-	0.01	$C_{18}H_{34}O_2$	282	Antihypertensive, increases HDL and decrease LDL
6.	21.532	2-methyltetracosane	0.01	$C_{25}H_{52}$	352	Free radical scavenging
7.	23.423	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester	0.02	$C_{19}H_{38}O_4$	330	Antioxidant
8.	27.575	Tetratetracontane	0.09	$C_{44}H_{90}$	618	Hypoglycemic, antioxidant activity
9.	33.846	Vitamin E	0.04	$C_{29}H_{50}0_2$	430	Antiaging, analgesic, antidiabatic, anti-inflammatory, antioxidant, antidermatitic, antileukemia, antitumor, anticancer, vasodilator, hepatoprotective, hypocholesterolemic, antiulcerogenic, antispasmodic, antibronchitic, anticoronary
10.	37.355	Stigmasta-5, 22-dien-3-ol	0.32	$C_{29}H_{48}O$	412	Synthetic progesterone

HDL: High density lipoprotein, LDL: Low density lipoprotein, RT: Reaction time

Table 4: Bioactivity of phytocomponents identified in the hexane extract of stem of *Sarcostemma viminale*

S.No.	RT (minutes)	Name of compound	% area	Molecular formula	Molecular weight	Biological activity
1.	18.881	9, 12-octadecadienoic acid (Z, Z)-	0.50	$C_{18}H_{32}O_2$	280	Cancer preventive, insectifuge, anti-inflammatory, nematicide, hepatoprotective, antihistaminic, anticancer, antiarthritic, antieczemic
2.	20.920	Cyclobutanecarboxylic acid, undec-2-enyl ester	0.01	$C_{16}H_{28}O_2$	252	Antimicrobial activity
3.	23.001	Pentacosane	0.17	$C_{25}H_{52}$	352	Antibacterial activity
4.	24.043	1, 2-benzenedicarboxylic acid	0.02	$C_{24}H_{38}O_4$	390	Antioxidant, antimicrobial, antifouling activity
5.	28.977	Tetracontane	13.12	C_4H_{82}	562	Anti-inflammatory and analgesic activity
6.	37.317	Stigmasterol	0.57	$C_{29}H_{48}O$	412	Antimicrobial, antihepatotoxic, antiviral, antioxidant, anticancer, hypocholesterolemic
7.	40.555	.Beta.-amyrin	4.36	$C_{30}H_{50}O$	426	Antibacterial, antioxidant, anti-inflammatory, antinociceptive, potential antiplatelet components, hypoglycemic, hypolipidemic effects, sedative action, hepatoprotective activities
8.	42.550	Lupeol	8.98	$C_{30}H_{50}O$	426	Antimalarial, antioxidant, antiflu, antihyperglycemic, antitumor, antiviral, pesticide, cytotoxic anti-inflammatory

RT: Reaction time

amyrin (18.51%) and 2-methyltetracosane (0.01%), eicosanoic acid (0.01%), 9-octadecenoic acid (Z)- (0.01%) were present in minimum amount in the chloroform extract; acetic acid 4, 4, 6A, 8A, 11, 12, 14B-octamethyl-1, 2, 3, 4, 4A, 5, 6, 6A, 6B, 7, 8, 8A, 9, 10, 11, 12, 12A, 14, 14A, 14B-eicosahydro-picen-3-YL ester $$ URS-12-en-3-YL acetate is present maximum amount (45.47%), followed by. beta.-amyrin (19.21%) and tetradecanal (0.01%), cis-vaccenic acid (0.01%), docosane (0.01%), eicosanoic acid (0.01%), octadecanal (0.01%), cyclobututanecarboxylic acid, undec-2-enyl ester (0.01%), and 7,9-di-tert-butyl-1-oxaspiro(4,5) deca-6,9-diene-2,8-dione (0.01%) were present in minimum amount in the hexane extract.

The GC showed the relative concentrations of various compounds getting eluted as a function of retention time. The height of peak indicates the relative concentrations of the components present in plants. The mass spectrometer analyses the compounds eluted at different time; identify the nature and structure of the compounds. The larger amount fragments into smaller compounds, giving rise to appearance of peak at different m/z ratio. These mass spectra are fingerprint of that compounds which can be identified from the data library. The GC-MS analysis of *S. viminale* (L.) R. Br. with all the three solvents may open an innovative platform to design more herbal formulations. Methanol and chloroform were proved to be better solvents as compared to hexane.

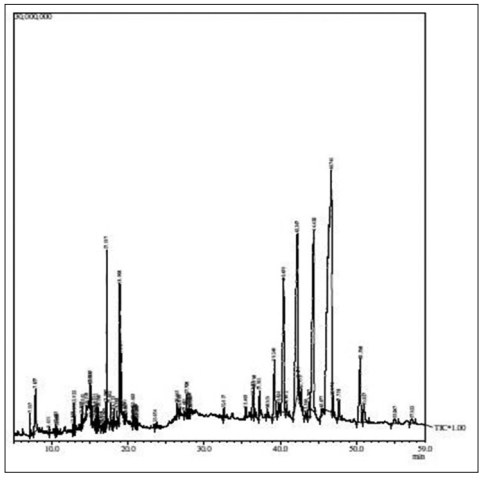

Fig. 1: Gas chromatography-mass spectrometry chromatogram of the methanolic extract of stem of *Sarcostemma viminale*

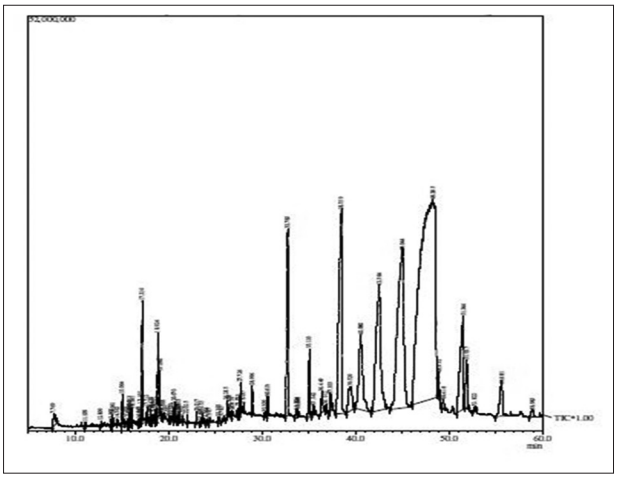

Fig. 2: Gas chromatography-mass spectrometry chromatogram of the chloroform extract of stem of *Sarcostemma viminale*

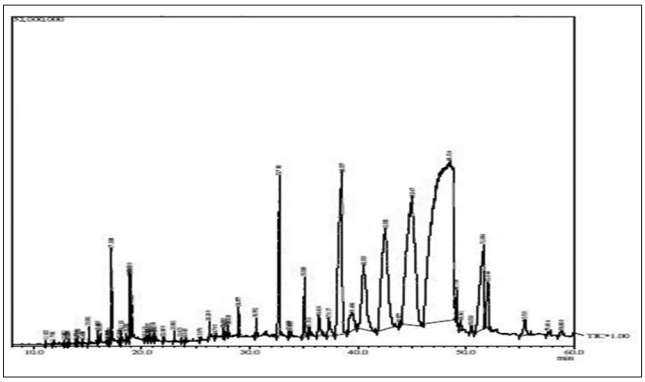

Fig. 3: Gas chromatography-mass spectrometry chromatogram of the hexane extract of stem of *Sarcostemma viminale*

CONCLUSION

This is the first report, where we have analyzed so many bioactive compounds using GC-MS analysis. The compound shows antifungal, antibacterial, antioxidant, anticancerous, antiaging, and anti-inflammatory properties. Plant-derived bioactive phytocompounds can be used for herbal drug formulations. This valuable bioactive compound justifies the use of the stem of this plant for the treatment of various ailments by traditional practitioners. As the plant is endangered, first of all, it requires proper strategies for its *in situ* as well as *ex situ* conservation. Further research is required as far as ethico-legal issues are concerned.

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