**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*). 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$$ urs-12-en-3-yl acetate is present maximum amount (44.98%) with RT=48.265 minutes, followed by beta-amyrin (18.51%) with RT=40.580 minutes in the chloroform extract; acetic acid 4, 4, 6A, 6B, 7, 8, 8A, 9, 10, 11, 12, 12A, 14, 14A, 14B-eicosahydro-picen-3-yl ester $$ 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.

© 2017 The Authors. Published by Innovare Academic Sciences Pvt Ltd. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/) DOI: http://dx.doi.org/10.22159/ajpcr.2017.v10i9.18707
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, 4, 6A, 6B, 8A, 9, 10, 11, 12, 12A, 14, 14A, 14B-octadecahydro-2H-picolin-3-one$\cdot$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, 7, 8, 8A, 9, 10, 11, 12, 12A, 14, 14A, 14B-icosahydro-picolin-3-YL ester$\cdot$ 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$_{10}$H$_{14}$O | 144              | Antimicrobial, anti-inflammatory                                 |
|       |              |                                                                                  | 1.91   | C$_{10}$H$_{12}$O | 122              | Used as an expectorant and fungal skin diseases, analgesic, food industry, antifungal properties |
| 3.    | 9.631        | 2-methoxy-4-ethylphenol11-hexadecene                                             | 0.05   | C$_{10}$H$_{12}$O | 150              | Antibacterial                                                   |
|       | 13.033       | Vinylphenol11-hexadecene                                                         | 0.09   | C$_{10}$H$_{12}$  | 224              | Antibacterial, antifungal, antioxidant activity                 |
| 5.    | 13.857       | 1,3,4,5-tetrahydroxy-cyclohexancarboxyl                                           | 0.32   | C$_{10}$H$_{12}$O | 192              | Antimicrobial, anti-inflammatory, antioxidant activity          |
| 6.    | 15.035       | 4-[(1E)-3-hydroxy-1-propenyl]-2-methoxyphenol                                    | 0.12   | C$_{10}$H$_{12}$O | 180              | Antimicrobial, antioxidant, anticancer, anti-inflammatory activity |
| 7.    | 15.826       | 2-hexadecen-1-ol-3,7,11,15-tetramethyl-[[R-[R,R]^*,(E)]]-Hexadecanoic acid, methyl ester | 0.11   | C$_{10}$H$_{21}$O | 296              | Cancer preventive                                              |
| 8.    | 16.717       | 4-[(1E)-3-hydroxy-1-propenyl]-2-methoxyphenol                                    | 0.03   | C$_{10}$H$_{12}$O | 180              | Antimicrobial and antifungal, antioxidant hypcholesterolemic, nematicide, insecticide lubricant, antiandrogenic flavor, hemolytic Antioxidant, antifungal, surfactant |
| 9.    | 18.123       | Heptadecanoic acid                                                             | 0.13   | C$_{10}$H$_{21}$O | 270              | Antimicrobial activity                                          |
| 10.   | 27.483       | 13-docosenamide, (Z)-                                                          | 0.05   | C$_{10}$H$_{21}$O | 337              | Anti-inflammatory, anti-hyperetec, anti-uler, antithratic         |
| 11.   | 39.249       | Stigmast-5-en-3-ol, (3.beta.)-                                                   | 3.03   | C$_{10}$H$_{21}$O | 414              | Antioxidant, antimicrobial, antioxidant, anticancer              |
| 12.   | 40.459       | Alpha-amyrin                                                                     | 7.32   | C$_{10}$H$_{21}$O | 426              | Antioxidant, antimicrobial, antioxidant, 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, antibiotic, analgesic, antitumor activity |
| 2.    | 14.061       | Alpha.-cadinol   | 0.03   | **C_{15}H_{26}O** | 222              | Antifungal, drug-resistant tuberculosis properties |
| 3.    | 15.830       | 2, 6, 10, trimethyl 1,4-ethylen-1,4-pentadecene | 0.04 | **C_{17}H_{30}O** | 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_{30}O** | 276              | Antimicrobial activity |
| 5.    | 17.945       | 9-octadecanoic acid (Z)- | 0.01 | **C_{18}H_{38}O** | 282              | Antihypertensive, increases HDL and decrease LDL |
| 6.    | 21.532       | 2-methyltetrasosane | 0.01  | **C_{18}H_{36}O** | 352              | Free radical scavenging |
| 7.    | 23.423       | Hexadecanoic acid, | 0.02  | **C_{18}H_{36}O** | 330              | Antioxidant |
| 8.    | 27.575       | 2-hydroxy-1-(hydroxymethyl) ethyl ester | 0.09  | **C_{19}H_{38}** | 618              | Antioxidant, antioxidant activity |
| 9.    | 33.846       | Vitamin E        | 0.04  | **C_{20}H_{40}O_2** | 430              | Hypoglycemic, antioxidant activity |
| 10.   | 37.555       | Stigmasta-5, 22-dien-3-ol | 0.32  | **C_{25}H_{40}O_2** | 412              | Antimicrobial activity |

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_{36}O_2** | 280              | Cancer preventive, insecticidal, hepatoprotective, anesthetic, anticancer, antiarthritic, antieczemic |
| 2.    | 20.920       | Cyclobutane carboxylic acid, undec-2-enyl ester | 0.01 | **C_{19}H_{38}O_2** | 252              | Antimicrobial activity |
| 3.    | 23.001       | Pentacosane      | 0.17  | **C_{20}H_{42}** | 352              | Antibacterial activity |
| 4.    | 24.043       | 1, 2-benzenedicarboxylic acid | 0.02 | **C_{20}H_{42}O_4** | 390              | Antioxidant, antimicrobial, antifouling activity |
| 5.    | 28.977       | Tetractacontane  | 0.13  | **C_{20}H_{42}** | 562              | Anti-inflammatory and analgesic activity |
| 6.    | 37.317       | Stigmasterol     | 0.57  | **C_{20}H_{42}O_4** | 412              | Antimicrobial, antihypotensive, antiviral, antioxidant, antilipidemic, hypolipidemic, sedative action, hepatoprotective activities |
| 7.    | 40.555       | Beta.-amyrin     | 4.36  | **C_{26}H_{46}O_2** | 426              | Antiinflammatory, antioxidant, antifungal, antihypertensive, antifungal, antiviral, pesticidal, cytoxic, anti-inflammatory |
| 8.    | 42.550       | Lupeol           | 7.98  | **C_{26}H_{46}O_2** | 426              | Antiinflammatory, antioxidant, antifungal, antihypertensive, antifungal, antiviral, pesticidal, cytoxic, anti-inflammatory |

amyrin (18.51%) and 2-methyltetrasosane (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-penic-3-YL ester $$ URS-12-en-3-YL acetate is present maximum amount (45.47%), followed by beta.-amyrin (19.21%) and tetractacontane (0.01%), cis-vaccenic acid (0.01%), docosane (0.01%), eicosanoic acid (0.01%), octadecanol (0.01%), cyclobutane carboxylic 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 of fragments into smaller compounds, giving rise to appearance of peak at different m/z ratio. These mass spectra are fingerprint of that nature and structure of the compounds. The relative concentrations of the compounds present in plants 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.
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.

ACKNOWLEDGMENT
The authors are thankful to AIRF, JNU, Delhi and CAS Department of Botany, JNVU, Jodhpur, Rajasthan for providing infrastructure and technical support.

REFERENCES
1. Sheela D, Uthayakumari F. GC-MS analysis of bioactive constituents from coastal sand Dune taxon--Sesuvium portulacastrum. Biosci Discov 2013;4(1):47-53.
2. Jagtap NS, Khadabadi SS, Ghorpade DS, Banarase NB, Naphade SS. Antimicrobial and antifungal activity of Centella asiatica (L.) Urban Umbelifereae. Res J Pharm Technol 2009;2(2):328-30.
3. Vanitha V, Umadevi KJ, Vijayalakshmi K. Determination of bioactive components of Anonna squamosa L leaf by GC-MS analysis. Int J Pharm Sci Drug Res 2011;3(4):309-12.
4. Nagavani V, Rao TR. Evaluation of antioxidant potential and qualitative analysis of major polyphenole by RP-HPLC in Nypahea nouchali Burm. Int J Pharm Pharm Sci 2010;2(4):98-104.
5. Dhiviya R, Manimagalai K. Preliminary phytochemical screening and GC-MS profiling of ethanolic flower extract of Calotropis gigantea Linn. (Apocynaceae). J Pharmacogn Phytochem 2013;2(3):28-32.
6. Arora S, Meena S. Qualitative preliminary phytochemical screening and GC-MS analysis of root of Sarcostemma viminale (L.) R. Br., An endangered plant. Int J Pharm Res Biosci 2016;5(2):89-100.
7. Meve U, Lied-Schumann S. Taxonomic dissolution of Sarcostemma (Apocynaceae: Asclepiadoideae). Kew Bull 2012;67(4):751-8.
8. Mohammed S, Kasera PK, Shukla JK. Unexploited plants of potential medicinal value from the Indian Thar desert. Nat Prod Rad 2004;3(2):69-74.
9. Ray S, Sheikh M, Mishra S. Ethnomedicinal plants used by tribals of East Nimar Madhya Pradesh. Indian J Tradit Knowl 2011;10(2):367-71.
10. Patil DA. Indian ethnomedicines: Origins in the perspective of doctrine of signature. Life Sci Leaf 2012;1:6-15.
11. Helen PA, Aswathy MR, Deepthi KG, Rathi RM, Joseph JJ, Sree SJ. Phytochemical analysis and anticancer activity of leaf extract of Mangifera indica (Kottukonam Varika). Int J Pharm Sci Res 2013;2(3):819-24.
12. Ruthiran P, Lokesh R, Chinnadurai RS. Phytochemical studies and GC-MS analysis of Spermadictyon suaveolens Roxb. Int J Pharm Pharm Sci 2017;9(3):143-9.
13. Hammed IH, Lena FH, Sabreen AK. Analysis of bioactive chemical compounds of Aspergillus niger by using gas chromatography-mass spectrometry and Fourier-transform infrared spectroscopy. J Pharmacogn Phytother 2015;7(8):132-63.
14. Dhiviya SM, Kalaichelvi K. UV-visible spectroscopic and FTIR analysis of Sarcostemma brevistigma, wight. and arn. Int J Curr Pharm Res 2017;9(3):46-9.
15. Grover N, Patni V. Phytochemical characterization using various solvent extracts and GC-MS analysis of methanolic extract of Woodfordia fruticosa (L.) Kurz. leaves. Int J Pharm Pharm Sci 2013;5(4):291-5.