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GC-MS Profiling of *Ceropegia bulbosa* Roxb. var. *bulbosa*, an endangered plant from Thar Desert, Rajasthan

Sunita Arora and Sonam Meena

Abstract
Plants are important source of secondary metabolites they contain many bioactive constituents with interesting biological activities. *Ceropegia bulbosa* Roxb. var. *bulbosa* is an important, endangered herb of Thar Desert of Rajasthan, belongs to Asclepiadaceae (Milk Weed) family. This is a herb of high medicinal value as it is used to cure various diseases like kidney stone, dysentery and deafness. The aim of present investigation was to assess the presences of primary and secondary metabolites including bioactive fractions using certain standard preliminary phytochemical tools and Perkin-Elmer GC-MS with chloroform as a solvent. The mass spectra of the compounds found in the extract were matched with the National Institute of Standards and Technology and Willey 8 library. Maximum % area is found for Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester, is present in maximum amount (35.72%) with RT= 24.667 minutes in tuber. Tetracontane is present in maximum amount (41.82%) with RT= 26.255 minutes in stem and 28.24% with RT= 30.583 minutes in leaf of chloroform extract of *Ceropegia bulbosa* Roxb. var *bulbosa.* Application of Gas Chromatography Mass Spectrometry (GC-MS) includes environmental analysis, drug detection and identification of unknown samples. Isolation and identification of individual phytochemical constituent and subjecting it to the biological activity will definitely give fruitful results and will open a new area of investigation of pharmacological importance.

Keywords: Asclepiadaceae, *Ceropegia bulbosa* Roxb. var. *bulbosa*, Chloroform, GC-MS, Pharmacology, Secondary metabolites

Introduction
Medicinal plants posses therapeutic potential and are used in the discovery of novel drug against various ailments. The traditional medicines in the last few decades emerged to have immense acknowledgements and it is estimated that 80% of community depend on traditional medicine for their primary healthcare (Kalaisezhiyen and Vadivukkarasi, 2012) [6]. 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 (Dhivya and Manimegalai, 2013) [3] as they do not cause any side effect; hence, they are more protective and safe.

The genus *Ceropegia* as a whole is under threat, owing to either destructive collection or habitat degradation. In India, approximately 50 species are present (Surveswaran, 2009) [18]. Among different species, *Ceropegia bulbosa* is one of the widely distributed species but still threatened (Yadav and Kamble, 2008) [21]. *Ceropegia bulbosa* Roxb. is a twining herb that belongs to Asclepiadeceae family of angiosperms. It is a herb of sandy substratum, bearing tubers, needs support of other xerophytic bushes/ shrubs (Arora and Meena, 2016) [1]. The plant generally grows in comparatively drier parts of the country, shows slight succulence and features of C4 photosynthesis. It is locally known as Khappar-kaddu, Bhuu-tumbi, Paataal-tumbi, Gilothi, Galot (Punjab) (Khare, 2007) [9]. It is known as Khadulo in Rajasthan (Katewa *et al.*, 2003) [7]. Tuber of this plant contain cerpegin and other components that form important ingredients of several conventional drug preparations (Indian Ayurveda) which provide active defence against many diseases especially diarrhea and dysentery (Nadkarni, 1976). Cerpegin is known to posses analgesic properties (Sukumar *et al.*, 1996) [17]. Recent studies report the antiurolithic activity of *Ceropegia* (Khan and Pradhan, 2012) [8]. Parts of the plant like leaves, tuber and seeds are used for the treatment of diseases like kidney stone, urinary bladder stone, stomach pain, diarrhea, dysentery, deafness and in promoting fertility and vitality (Jain *et al.*, 2004; Swarnkar and Katewa, 2008) [19].

Plants are sources of bioactive compounds that play an important role in maintaining human health (Sermakkani and Thangapandian, 2012) [16]. Phytochemicals remain present in a variety of plants and are utilized as important components of both human and animals diets. These include leaves, barks, roots, fruits, seeds and vegetables (Okwu, 2005) [15]. These biologically important compounds are formed during the normal metabolic processes and are often referred to as secondary metabolites (Mithraja *et al.*, 2012) [12]. GC-MS analysis can identify compounds with purity even when they are present in least amount i.e. less than 1 mg (Liebler *et al.*, 1996) [11]. In recent years Gas Chromatography- Mass Spectrum (GC-MS) tool is being increasingly applied to analyze secondary metabolites from plants of medicinal importance (Muthulakshmi *et al.*, 2012; Konovalova *et al.*, 2013) [13, 10].

The present investigation was carried out to isolate, investigate and characterize bioactive compounds in chloroform extract by using GC-MS analysis for vegetative plant of *Ceropegia bulbosa* Roxb. var. *bulbosa.*

Materials and Methods

The plant specimen were collected freshly from Udaipur, Chittorgarh, Bhilwara and Karauli districts of Rajasthan during July-September. "The Flora of Indian Desert" was referred for identification at primary level (Bhandari, 1978) [12] and then the specimens were finally authenticated by Botanical Survey of India (BSI) Jodhpur, Rajasthan.

Preparation of plant extracts

The whole plant was shade dried and prepared to powder in a mechanical grinder. 4g of vegetative coarse powder was transferred to round bottom flask. 200 ml of solvent was added to each flask containing crude powerded plant material. Hot extraction method using soxhlet apperatus was followed, for this procedure crude extract of various vegetative parts was prepared using chloroform as solvent (Harborne, 1984). The solution was then boiled at 60-70º C for 18 hours on water bath, filtered, evaporated to dryness, & final residue was then subjected to GC-MS analysis. The extract was then subjected to standered phytochemical analytical tests i.e. Wagner's test for alkaloids, Molish test for carbohydrates, Borntrager's test for glycosides, Lead Acetate test for phenolic compounds, Alkaline test for flavanoids, Xanthoprotein test for protein and amino acid, Foam test for saponins, Salkowski test for steroids and terpenoids for isolation and characterization of primary products.

The gas chromatography-mass spectroscopy (GC-MS) analysis was performed at Advanced Instrumentation Research Facility (AIRF), Jawaharlal Nehru University, New Delhi, India. For GC-MS detection and electron ionization system with ionizing energy of 70ev was used. Helium gas (99.99%) was used as the carrier gas at constant flow rate 1ml/min in the split mode (10:1) and an injection volume of 2 µl of chloroform solution of different plant part sample was injected into the column with the injector temperature 260ºC, ion-source temperature 230ºC. colum oven temperature was maintained at 80 ºC, Pressure was maintained at 81.9 kPa. Equilibrium time was 0.5 min. start m/z ratio was 40.00 and it ended at 650.00.

Results and Discussion

The preliminary phytochemical screening tests may be useful in the detection of the bioactive principles and subsequently may lead to the drug discovery and development. Further, these tests facilitate their quantitative estimation and qualitative separation of pharmacologically active chemical compounds (Varadarajan *et al.*, 2008) [20]. The preliminary phytochemical screening revealed the presence of various secondary metabolites (Table 1).

Table 1: Phytochemical constituents in chloroform extract of *Ceropegia bulbosa*

S. No.	Phytoconstituents	Tests	Tuber	Stem	Leaf
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.	Terpenoids	Salkowski test	+ +	+	-

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

GC-MS analysis of tuber, stem and leaf of *Ceropegia bulbosa* Roxb. showed 43, 49 and 44 peaks respectively (Figs. 1-3) indicating the presence of 41, 37 and 37 compounds in chloroform extract. Confirmation of their presence was based on retention time (RT), peak area, molecular formula, concentration (%), and molecular weight (Tables 2-4).

Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester is present in maximum amount (35.72%), followed by Octadecanoic acid, 2,3- dihydroxypropyl ester (22.50%), Dodecanoic acid (0.12%), Undecane (0.16%) in chloroform extract of tuber. Tetracontane is present in maximum amount (41.82%), followed by 2H-Azepin-2-one, 3- (dimethylamino) hexahydro- (14.14%), Diisodecyl ether (0.04%) and Pentadecane (0.05%) in chloroform extract of stem. Tetracontane is present in maximum amount (28.24%) followed by 9, 12- octadecadienoic acid (Z, Z)- (15.25%), Pentadecane (0.07%) and Benzene, (1-methylnonadecyl)- (0.08%) in minimum amount in chloroform extracts of leaf of *Ceropegia bulbosa* Roxb.

Table 2: Bioactivity of phytocomponents identified in chloroform extract of tuber of *Ceropegia bulbosa*

S. No	R. Time	Name of Compound	% area	M.F.	M.W.	Biological Activity
1.	10.480	Dodecane	0.29	$C_{12}H_{26}$	170	Enhance antifungal activity
2.	13.313	Tetradecane	0.32	$C_{14}H_{30}$	198	Antifungal, Antibacterial, Nematicidal
3.	15.312	Dodecanoic acid	0.12	$C_{12}H_{24}O_2$	200	Antibacterial activity
4.	15.700	Diethyl phthalate	0.20	$C_{12}H_{14}O_4$	222	Estrogenic activity, Antimicrobial, Plasticizer, Antioxidant
5.	17.608	Tetradecanoic acid	0.47	$C_{14}H_{28}O_2$	228	Antioxidant, Nematicidal, Hypocholesterolemic, Anticancer
6.	18.674	Pentadecanoic acid	0.16	$C_{15}H_{30}O_2$	242	Lubricant, Adhesive agents
7.	18.722	1,2-Benzenedicarboxilic acid,bis(2-methylpropyl) ester	0.28	$C_{16}H_{22}O_4$	278	Antimicrobial, Antifouling
8.	19.225	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	0.54	$C_{17}H_{24}O_3$	276	Antimicrobial
9.	19.759	n-Hexadecanoic acid	6.52	$C_{16}H_{32}O_2$	256	Antifungal, Antioxidant, Hypocholesterolemic Nematicide, Anti-Androgenic Flavour, Haemolytic 5-Alphareductase Inhibitor, Potent Antimicrobial Agent, Antimalarial And Antifungal
10.	20.678	Heptadecanoic acid	0.19	$C_{17}H_{34}O$	270	Antioxidant, anti fungal, surfactant
11.	21.386	9,12-Octadecadienoic acid (Z,Z)-	0.42	$C_{18}H_{32}O_2$	280	Anti-inflammatory, Antibacterial, Antiarthritic, Hepatoprotective, Anti-histaminic, Anticoronary
12.	21.439	Cis-9-Hexadecenal	0.39	C16H30O	238	Antimicrobial
13.	21.643	Octadecanoic acid	0.31	$C_{18}H_{36}O_2$	284	Antifungal, Antitumor, Antibacterial
14.	22.804	1-Nonadecene	0.42	$C_{19}H_{38}$	270	Anti-fungal activity
15.	24.667	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	35.72	$C_{19}H_{38}O_4$	330	Antioxidant, Hemolytic, pesticide, flavor,
16.	25.902	1H-indole-3-ethanamine	1.32	$C_{10}H_{12}N_2$	160	Used in Neurotransmitter & psychedelics
17.	31.360	Tetracontane	1.18	$C_{40}H_{82}$	562	Anti-inflammatory, Analgesic
18.	34.165	Ergost-5-en-3-ol, 3.beta.,24R)-	3.02	$C_{28}H_{48}O$	400	Liver disease, jaundice, Artherosclerosis
19.	34.777	Stigmasterol	2.68	$C_{29}H_{48}O$	412	Semi synthetic progesterone, synthesis of cortisone
20.	36.383	Stigmast-5-en-3-ol, (3.beta.)-	4.20	$C_{29}H_{50}O$	414	Anti-inflammatory, Anti- arthritic, anti-pyretic, Anti-ulcer

Table 3: Bioactivity of phytocomponents identified in chloroform extract of stem of *Ceropegia bulbosa*

S. No	R. Time	Name of Compound	% area	M.F.	M.W.	Biological Activity
1.	7.754	Naphthalene	0.11	$C_{10}H_8$	128	Antiseptic, Carcinogenic
2.	10.577	Pentadecane	0.05	$C_{15}H_{32}$	212	Sugar-phosphatase inhibitor, acrocylindropepsin inhibitor, chymosin inhibitor, Antibacterial
3.	12.400	Eicosane	0.06	$C_{20}H_{42}$	282	Antifungal, antitumor antibacterial, larvicidal, antimicrobial, cytotoxic effects
4.	13.106	Heptadecane	0.08	$C_{17}H_{36}$	240	Antioxidant
5.	13.202	1,2-Benzenedicarboxilic acid, diethyl ester	0.07	$C_{12}H_{14}O_4$	222	Cosmetics, Insecticide, Aspirin
6.	15.066	Tetradecanoic acid	0.16	$C_{14}H_{28}O_2$	228	Antioxidant, Cancer preventive, Nematicide, Lybricant, Hypocholesterolemic
7.	15.819	2,6,10,trimethyl,14-ethylene-14-pentadecene	0.39	$C_{20}H_{38}$	278	Antiproliferative
8.	16.830	7,9-Di-tert-butyl-1-oxaspiro (4,5)deca-6,9-diene-2,8-dione	0.24	$C_{17}H_{24}O_3$	276	Antimicrobial activity
9.	17.170	Pentadecanoic acid	4.90	$C_{15}H_{30}O_2$	242	Lubricants and Adhesive agents
10.	18.108	Heptadecanoic acid	0.14	$C_{17}H_{34}O_2$	270	Antioxidant, Antifungal, Surfactant
11.	18.575	Phytol	0.19	$C_{20}H_{40}O$	296	Antimicrobial, anticancer, diuretic, Anti-inflammatory
12.	18.853	Octadecanoic acid	1.17	$C_{18}H_{36}O_2$	284	Antibacterial action, Cosmetic, Flavor, Hypocholestrolemic, Lubricant, perfumery, Propecic, Suppository
13.	19.652	10,12-Hexadecadien-1-ol	0.11	$C_{16}H_{30}O$	238	Sex pheromone
14.	20.309	Pentacosane	0.94	$C_{25}H_{52}$	352	Antibacterial
15.	20.903	Cyclobutanecarboxylic acid, undec-2-enyl ester	0.11	$C_{16}H_{28}O_2$	252	Antimicrobial activity
16.	22.984	Hexatriacontane	0.39	$C_{36}H_{74}$	506	Radical scavenger

17.	23.614	Octadecanal	3.90	$C_{18}H_{36}O$	268	Alkane-lyase activity
18.	26.255	Tetracontane	41.82	$C_{40}H_{82}$	562	Anti-inflammatory and Analgesic activity
19.	33.744	Vitamin E	0.39	$C_{29}H_{50}O_2$	430	Antiaging, analgesic, antidiabatic**,** Anti-inflammatory, antioxidant, antidermatitic, antileukemia, antitumor, anticancer, hepatoprotective, hypocholesterolemic, Antiulcerogenic,vasodilator, antispasmodic, antibronchitic, anticoronary
20.	36.285	Ergost-5-en-3-ol, (3.beta.)-	2.05	$C_{28}H_{48}O$	400	Antimicrobial and Anti-inflammatory effects
21.	37.164	Stigmast-5-en-3-ol, (3.beta.)-	2.05	$C_{29}H_{50}O$	414	Anti-inflammatory, Anti-pyretic, Anti-ulcer, Antiarthritic
22.	41.723	Lupeol	0.92	$C_{30}H_{50}O$	426	Antimalarial, Antioxidant, Antiflue, Antihyperglycemic, Antitumor, Antiviral, Pesticide, Cytotoxic Anti-inflammatory

Table 4: Bioactivity of phytocomponents identified in chloroform extract of leaf of *Ceropegia bulbosa*

S. No	R. Time	Name of Compound	% area	M.F.	M.W.	Biological Activity
1.	7.757	Naphthalene	0.33	$C_{10}H_8$	128	Antiseptic, Carcinogenic
2.	10.583	Pentadecane	0.15	$C_{15}H_{32}$	212	Sugar-phosphatase inhibitor, Antibacterial, acrocylindropepsin inhibitor, chymosin inhibitor,
3.	12.403	Eicosane	0.13	$C_{20}H_{42}$	282	Antifungal, Antitumor, Antibacterial, Larvicidal, Antimicrobial and Cytotoxic effects
4.	15.056	Tetradecanoic acid	0.26	$C_{14}H_{28}O_2$	228	Antioxidant, Cancer preventive, Nematicide, Lybricant, Hypocholesterolemic
5.	15.810	2,6,10,trimethyl,14-ethylene-14-pentadecene	1.59	$C_{20}H_{38}$	278	Antiproliferative
6.	16.263	2-hexadecen-1-ol,3,7,11,15-etramethyl-,[R-[R*,R*-(E)]]	0.54	$C_{20}H_{40}O$	296	Antimicrobial, Sedatives and anesthetics
7.	16.826	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	0.38	$C_{17}H_{24}O_3$	276	Antimicrobial activity
8.	17.160	Pentadecanoic acid	7.77	$C_{15}H_{30}O_2$	242	Lubricants and Adhesive agents
9.	18.104	Heptadecanoic acid	0.15	$C_{17}H_{34}O_2$	270	Antimicrobial
10.	18.571	Phytol	0.57	$C_{20}H_{40}O$	296	Antimicrobial, anticancer, diuretic, Anti-inflammatory
11.	18.867	9,12-Octadecadienoic acid (Z,Z)-	15.25	$C_{18}H_{32}O_2$	280	Cancer preventive, Insectifuge, Anti-inflammatory, Nematicide, Hepatoprotective, Antihistaminic, Anticane, Antiarthritic, Antieczemic
12.	18.899	Cis-Vaccenic acid	12.19	$C_{18}H_{34}O_2$	282	Hypolipidaemic, Cosmetic, Antihypertensive, Anti-inflammatory
13.	19.064	Octadecanoic acid	2.81	$C_{18}H_{36}O_2$	284	Antibacterial action, Cosmetic, Flavor, Hypocholestrolemic, Lubricant, perfumery, Propecic, Suppository
14.	19.277	9,12-Octadecadienoic acid	0.42	$C_{18}H_{32}O_2$	280	Antioxidant, Anti-inflammatory, Anticarcinogenic, Antiatherogenic
15.	20.899	Cyclobutanecarboxylic acid, undec-2-enyl ester	0.31	$C_{16}H_{28}O_2$	252	Antimicrobial activity
16.	27.993	Squalene	0.59	$C_{30}H_{50}$	410	Antibacterial, Antioxidant, Antitumor, Anti-inflammatory, Antinociceptive, Potential antiplatelet components, Hypoglycemic, Hypolipidemic effects, Sedative action, Antihistaminic, Hepatoprotective activites Cancer preventing, Immunostimulant
17.	28.939	Tetratriacontane	0.62	$C_{34}H_{70}$	478	Antibacterial and Antifungal
18.	30.583	Tetracontane	28.24	$C_{40}H_{82}$	562	Anti-inflammatory and Analgesic activity
19.	31.940	.gamma.-Tocopherol	0.23	$C_{28}H_{48}O_2$	416	Antioxidant, Anticancer, Anti-inflammatory and Cardio-protective
20.	33.715	Vitamin E	1.93	$C_{29}H_{50}O_2$	430	Antiaging, analgesic, antidiabatic**,** Anti-inflammatory, antioxidant, antidermatitic, antileukemia, antitumor, anticancer, hepatoprotective, hypocholesterolemic, Antiulcerogenic,vasodilator, antispasmodic, antibronchitic, anticoronary
21.	36.247	Ergost-5-en-3-ol, (3.beta.)-	1.56	$C_{28}H_{48}O$	400	Antimicrobial and Anti-inflammatory
22.	38.927	Stigmast-5-en-3-ol, (3.beta.)-	1.98	$C_{29}H_{50}O$	414	Anti-inflammatory, Anti- arthritic, anti-pyretic, Anti-ulcer
23.	41.662	Methyl commate D	0.64	$C_{31}H_{50}O_4$	486	Antimicrobial, Anti-inflammatory
24.	43.552	Octadecanal	0.57	$C_{18}H_{36}O$	268	Alkane-lyase activity
25.	48.919	Phytol, acetate	0.81	$C_{22}H_{42}O_2$	338	Antimicrobial, Anti-inflammatory, diuretic and Anticancer

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. The phytochemical analysis is important and has commercial interest in research institutes as the products can be used in pharmaceutical industries. Compounds isolated four all the parts are biologically active and in one or other way they can be used for various drug formulations. Extraction of vitamin E, squalene and phytol from stem and leaf enhances utility of this plant in future, but as it is an endangered/threatened plant first of all it needs utmost care and conservation for future use to replace synthetic formulations. Ethical and legal aspects also needs to be considered before proper application and marketing.

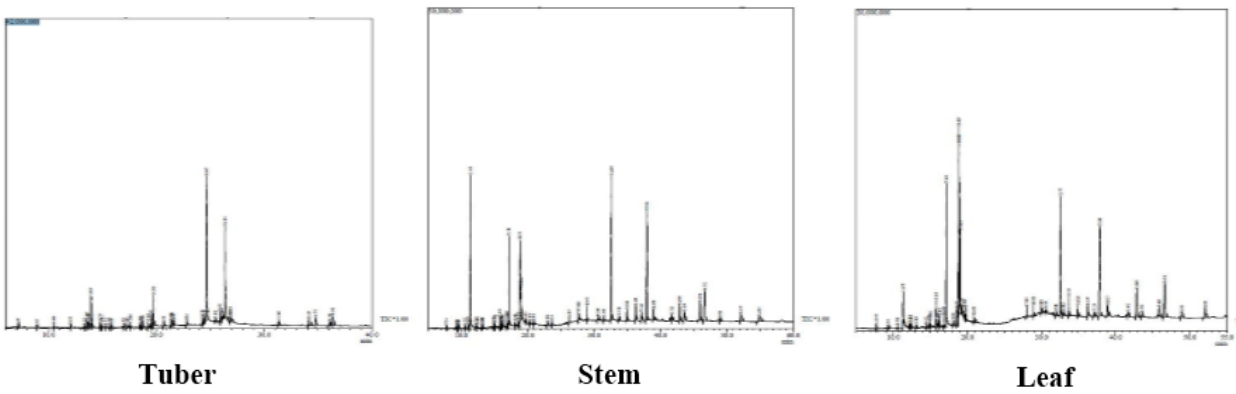

Fig 1: GC-MS chromatogram of the chloroform extract of various parts of *Ceropegia bulbosa*

Conclusion

GC-MS is a highly reliable as it can extracts compounds in their pure form. It is the power of this tool as well as solvent that can give more products. Extraction of compounds using GC-MS can open a big platform for pharmacological companies to formulate various drug from plants that can be good source of these drugs, but what we need in turn is also care and conservation for these plants.

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References

1. Arora S, Meena S. Qualitative premilinary phytochemical screening and GC-MS analysis of root of S*arcostemma viminale* (L.) R. Br., an endangered plant. International Journal of Pharmaceutical Research and Bio-Science. 2016; 5(2):89-100.
2. Bhandari MM. Flora of Indian Desert. India; Scientific Publisher, Jodhpur, 1978.
3. Dhivya R, Manimegalai K. Preliminary phytochemical screening and GC-MS profiling of ethanolic flower extract of *Calotropis gigantea* Linn. (*Apocynaceae*). Journal of Pharmacognosy and Phytochemistry. 2013; 2(3):28-32.
4. Harborne JB. Methods of plant analysis. In: Harbone JB, editor. Phytochemical Methods. Edn 2, Chapman and Hall, London, 1984, 5-6.
5. Jain A, Katewa SS, Chaudhary BL, Galav PK. Folk herbal medicines used in birth control and sexual diseases by tribals of Southern Rajasthan, India. Journal of Ethnopharmacology. 2004; 90(1):171-177.
6. Kalaisezhiyen P, Vadivukkarasi SK. GC-MS evaluation of Chemical constituents from methanolic leaf extract of *Kedrostis foetidissima* (Jacq.) Cogn. Asian Journal of Pharmaceutical and Clinical Research. 2012; 5(4):77-81.
7. Katewa SS, Chaudhar BL, Jain A, Galav P. Traditional uses of plant biodiversity from Aravalli hills of Rajasthan. Indian Journal of Traditional Knowledge. 2003; 2(1):27-39.
8. Khan MA, Pradhan D. Antiurolithic activity of Ceropegia bulbosa extract in rats. Der Pharmacia Sinica. 2012; 3(1):148-152.
9. Khare CP. Indian medicinal plants, Springer, 2007, 139.
10. Konovalova O, Gergel E, Herhel V. GC-MS analysis of Shpherdia argentea (Pursh.) Nutt. From Ukraineen Flora. The Pharma Innovation Journal. 2013; 2(6):7-12.
11. Liebler DC, Burr JA, Philips L, Ham AJL. Gas chromatography - mass spectrometry analysis of vitamin E and its oxidation products. Analytic Biochemistry. 1996; 236(1):27-34.
12. Mithraja MJ, Johnson MA, Mahesh M, Paul ZM, Jeeva S. Chemical diversity analysis on some selected medicinally important Pteridophytes of Western Ghats, India. Asian Pacific Journal of Tropical Biomedicine. 2012; 2(1):S34-S39.
13. Muthulakshmi A, Joshibhi M, Mohan VR. GC-MS analysis of bioactive components of Feronia elephantum correa (Rutaceae). Journal of Applied Pharmaceutical Science. 2012; 2(2):69-74.
14. Nadkarni KM. Indian material medica. Edn 3, Popular Prakashan Bombay, 1976, 2.
15. Okwu DE. Phytochemicals, vitamins and mineral content of to Nigerian medicinal plants. International Journal of Molecular Medicine and Advance Sciences. 2005; 1(4):375-381.
16. Sermakkani M, Thangapandian V. GC-MS analysis of Cassia italic leaf methanol extract. Asian Journal of Pharmaceutical Clinical Research. 2012; 5(2):90-94.
17. Sukumar E, Hamsaveni GR, Bhima RR. Pharmacological actions of cerpegin, a novel pyridine alkaloid from Ceropegia juncea. Fitoterapia. 1996; 66:403-406.
18. Surveswaran S, Kamble M, Yadav SR, Sun M. Molecular phylogeny of *Ceropegia* (Asclepiadoideae, Apocynaceae)

from Indian Western Ghats. Plant Systematics and Evolution. 2009; 281(1):51-63.
19. Swarnkar S, Katewa SS. Ethnobotanical observation on tuberous plants from tribal area of Rajasthan (India), Ethnobot Leaflets. 2008; 12(1):647-666.
20. Varadarajan P, Rathinaswamy G, Rangasamy D, Asirvatahm D. Antimicrobial properties and phytochemical constituents of *Rheo discolor*. Ethnobotanical Leaflets. 2008; 12(1):841-845.
21. Yadav SR, Kamble MY. Threatened *Ceropegias* of the Western Ghats and strategies for their conservations. Edn 11, In: Rawat GS editor. In: Special Habitats and Threatened Plants of India. Deharadun (Wildlife Institute of India): Envis Bulletin of Wildlife and Protected Area. 2008; 1:123-134.