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Analysis of bioactive constituents from *Ceropegia bulbosa* Roxb. Var. *Bulbosa*: An endangered medicinal plant from Thar Desert of Rajasthan, India

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Abstract
Plants are valuable resources for herbal drug extraction. Present analysis is based on most trusted analytical procedure to detect bioactive phytoconstituents from whole vegetative plant of *Ceropegia bulbosa* Roxb. var. *bulbosa*. Phytochemical compounds were 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 Standards and Technology (NIST), and Willey 2008 library. Leaf showed highest number of compounds followed by stem and tuber. Methanol as well as hexane could extract highest number of compounds from leaf anyhow methanolic extract shows more potential as compare to hexane. Maximum % area is covered by 2H-Azepin-2-one, 3-(dimethylamino) hexahydro (stem) in methanolic extract but it does not reveal any biological activity. Biological activity of compounds is not directly or indirectly related to the area they covered. In hexane extract maximum % area is covered by Tetracontane (stem) and it also showed some biological activities. Qualitative phytochemical study makes a way in the standardization and quality assurance of the medicinal plant. The present study provides evidence that methanol and hexane extract of *Ceropegia bulbosa* Roxb. var. *bulbosa* could extract medicinally important bioactive compounds, significant in drug development.

Keywords: asclepiadaceae, *Ceropegia bulbosa,* GC-MS, hexane, methanol, secondary metabolites

Introduction
Plants are employed for medicinal purpose in different countries and are the richest bioresources of potential and powerful drugs. The medicinal value of plants lies in some chemical substances that produce a definite physiological action on human body [1]. Research in medicinal plants reflects the recognition of the validity of many herbal products [2]. Modern medicine has evolved from folk medicine and traditional system only after through chemical and pharmaceutical screening [3]. Nearly 80% of the world population relies on traditional medicines for primary health care, most of which involve the use of plant extracts [4]. The study of plants continues principally for the discovery of novel secondary metabolites. The efficacy depends on the use of proper plant part and its biological potency which in turn depends upon the presence of required quantity and nature of secondary metabolite in a raw drug [5, 6].

Ceropegia bulbosa Roxb. var. *bulbosa* is one of the important endangered plant of family Asclepiadaceae, now ranked as Asclepiadoideae a subfamily belongs to family Apocynaceae having different species of plant with wide therapeutical properties. Asclepiadoideae is the largest cosmopolitan family having approximately 177 genera and nearly 3000 species [7] and are well known for their ethnobotanical and ethnomedicinal importance [8].

In the present study an attempt was made to isolate the phytochemical constituents present in methanol and hexane extract of plant (vegetative) of *Ceropegia bulbosa* Roxb.var. *bulbosa* by using GC-MS technique. Phytochemicals are chemical compounds synthesized during normal metabolic processes, responsible for important medicinal activity and utilized by pharmaceutical industries for identification and synthesis of useful drugs.

Materials and Methods
Plant Material
Ceropegia bulbosa Roxb. var. *bulbosa* was collected from Jaipur, Udaipur, Chittorgarh, Bhilwara and Karolli district of Rajasthan state of India during the month of June - August. "The Flora of Indian Desert" [9] was consulted for identification. The voucher specimen were finally authenticated by Botanical Survey of India (BSI) Jodhpur, Rajasthan.

Phytochemical screening

Fresh plants were collected from nature, shade dried and powdered in a mechanical grinder. 6g of crude powder was transferred to round bottom flask and extracted with 200ml methanol and hexane as solvents. The crude extract was boiled at 55º-70º C for 24 hours on water bath, filtered using whatman filter paper No. 1 and evaporated to dryness. The final residue obtained was then subjected to GC-MS analysis and stored at 4ºC for further use. 1µl of this solution was employed for GC-MS analysis [10]. The test sample was injected in the injection port of the GC equipment, were the temperature was mainted at 260ºC and the detector temperature was set at 280 ºC. The components eluted from the column as per their boiling point and m/z ratio.The eluted components were detected in the mass detector. The spectrum of the unknown components was compared with the spectrum of known one stored in the NIST library [11]. The gas chromatogram shows 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 extracts. The mass spectrometer analyzes the compounds eluted at different time to identify the nature and structure of the compounds. The larger amount fragments into smaller components, producing peaks at different m/z ratio. These mass spectra are fingerprint of that compounds which can be identified from the data library. The gas chromatography- mass spectrometry (GC-MS) analysis was performed at Advanced Instrumentation Research facility (AIRF) JNU Delhi. Syringe insertion and injection speed was high and was pumped for five times. Temperature of injection port was 260^0 C, Column oven temp was maintained at 80^0 C. For GC, pressure was maintained at 81.9 kPa, split ratio was 50.0 and ion source was maintained at 230^0 C.

Preliminary Phytochemical Screening

Methanolic and hexane extracts of *Ceropegia bulbosa* Roxb. var. *bulbosa* were subjected to preliminary phytochemical screening to determine the presence primary phytoconstituents. For this purpose standard qualitative phytochemical screening assay was done 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 flavonoids, Xanthoprotein test for proteins & amino acid, Foam test for saponins, Sodium bicarbonate test for acidic compounds, Salkowski test for sterols and terpenoids (Table 1).

Results

Medicinal plants are gift from nature, their uses should be encouraged, especially in the production of drugs for alternative medicine practice [12]. Medicinal value of plants lies in some chemical substances that have a definite physiological action on the human body [13]. In recent years, use of traditional medicinal knowledge in drug discovery seems so promising that even large pharmaceutical companies have begun to show interest in this field [14, 15]. Phytochemicals are chemical formed during the plants normal metabolic processes.

The preliminary phytochemical screening of *Ceropegia bulbosa* Roxb. var. *bulbosa* was carried out using two solvents (methanol and hexane) and we could analyze many primary as well as secondary metabolites(alkaloids, carbohydrates, glycosides, phenolic compounds, flavonoids, proteins & amino acid, saponins, sterols, acidic compounds and terpenoids) with important biological activities (Table 1).

GC-MS chromatogram of the methanolic extract of tuber, stem, leaf showed 42, 56 and 65 peaks indicating the presence of 26, 49 and 57 compounds respectively. The hexne extract of tuber, stem and leaf showed 42, 37 and 49 peaks indicating the presence of 27, 29 and 40 compounds respectively (Fig. 1-2). This compound were identified on the basis of their retention time (RT), peak area, molecular formula, molecular weight, concentration (%) in methanolic extract, chloroform extract, hexane extract of *Ceropegia bulbosa* Roxb.var. *bulbosa* (Table 2-3). cis-Vaccenic acid is present in maximum amount (19.43%), followed by 2H-Azepin-2-one, 3-(dimethylamino) hexahydro- (12.84%) in the methanolic extract. 9,12-Octadecadienoic acid is present in maximum amount (33.96%), followed by Pentadecanoic acid (19.30%) in the hexane extract of tuber of *Ceropegia bulbosa* Roxb.var. *bulbosa.*

2H-Azepin-2-one, 3-(dimethylamino) hexahydro- is present in maximum amount (32.50%), followed by 9,12-Octadecadienoic acid (z,z)- (23.81%) in the methanolic extract.; Tetracontane is present in maximum amount (49.15%), followed by 9,12-Octadecadienoic acid (z,z)- (19.92%) in the hexane extract of stem of *Ceropegia bulbosa* Roxb.var. *bulbosa.* 03027205002 Flavone 4'-oh, 5-oh, 7-di-o-glucoside is present in maximum amount (15.93%), followed by cis-9-Hexadecenal (13.88%) in the methanolic extract.; Tetracontane is present in maximum amount (46.95%), followed by 03027205002 Flavone 4'-oh, 5-oh, 7-di-o-glucoside (14.49%)in the hexane extract of leaf of *Ceropegia bulbosa* Roxb.var. *bulbosa.* Mass spectrum of common compounds present in methanolic and hexane extract is shown in Fig.3.

Table 1: Phytoconstituents in Methanol & Hexane Extract of *Ceropegia bulbosa*

S. No	Phytoconstituents	Tests	Methanol			Hexane		
			Root	Stem	Leaf	Root	Stem	Leaf
1.	Alkaloids	Wagner's test	++	++	+++	-	+	++
2.	Carbohydrates	Molish test	+	++	+++	++	-	+
3.	Glycosides	Borntrager's test	-	+	+	-	-	-
4.	Phenolic compounds	Lead Acetate test	-	+	+	-	++	+
5.	Flavanoids	Alkaline test	+	++	++	-	-	-
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: Bioactive Compounds in Methanolic Extract of *Ceropegia bulbosa*

S. No.	Plant parts	Retention time (min)	Compound Name	% of Peak Area	Mol formula	Mol weight	Biological Activity
1.	Root Stem Leaf	7.156 7.159 7.121	2,3-dihydro-3,5-dihydroxy-6-methyl-4H-Pyran	5.91 2.43 0.59	$C_6H_8O_4$	144	Antimicrobial, anti-inflammatory
2.	Root Stem Leaf	15.065 15.063 15.049	Tetradecanoic acid	0.26 0.40 0.42	$C_{14}H_{28}O_2$	228	Antioxidant, Cancer preventive, Nematicide, Lybricant, Hypocholesterolemic
3.	Root Stem Leaf	15.305 15.307 15.297	1-Octadecene	0.41 0.16 0.16	$C_{18}H_{36}$	252	Finishing agent, Intermediates, Lubricants and Lubricant additives
4.	Root Stem Leaf	16.123 17.169 16.105	Pentadecanoic acid	8.40 9.10 10.09	$C_{15}H_{30}O_2$	242	Lubricants and Adhesive agents
5.	Root Stem Leaf	18.107 18.106 18.093	Heptadecanoic acid	0.47 0.32 0.33	$C_{17}H_{34}O_2$	270	Antioxidant, Antifungal, Surfactant
6.	Root Stem Leaf	19.062 19.059 19.048	Octadecanoic acid	1.47 1.95 2.34	$C_{18}H_{36}O_2$	284	Antibacterial action, Cosmetic, Flavor, Hypocholestrolemic, Lubricant, perfumery, Propecic, Suppository
7.	Root Stem Leaf	36.270 36.275 36.227	Ergost-5-en-3-ol, (3.beta.,24R)-	1.19 2.15 2.73	$C_{28}H_{48}O$	400	Liver disease, jaundice, Artherosclerosis
8.	Root Stem Leaf	38.966 38.970 38.919	Stigmast-5-en-3-ol, (3.Beta.)-	1.94 2.95 4.25	$C_{29}H_{50}O$	414	Anti-inflammatory, Anti-pyretic, Anti-ulcer, Antiarthritic

Table 3: Bioactive Compounds in Hexane Extract of *Ceropegia bulbosa*

S. N.	Plant parts	Retention time (min)	Compound Name	% of Peak Area	Mol formula	Mol weight	Biological Activity
1.	Root Stem Leaf	15.062 15.039 15.052	Tetradecanoic acid	0.44 0.20 0.24	$C_{14}H_{28}O_2$	228	Antioxidant, Cancer preventive, Nematicide, Lybricant, Hypocholesterolemic
2.	Root Stem Leaf	16.121 16.097 16.106	Pentadecanoic acid	19.30 6.45 5.06	$C_{15}H_{30}O_2$	242	Lubricants and Adhesive agents
3.	Root Stem Leaf	16.827 16.812 16.814	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	0.19 0.21 0.05	$C_{17}H_{24}O_3$	276	Antimicrobial activity
4.	Root Stem Leaf	18.116 18.084 18.100	Heptadecanoic acid	1.19 0.18 0.21	$C_{17}H_{34}O_2$	270	Antimicrobial
5.	Root Stem Leaf	19.083 19.036 19.068	Octadecanoic acid	2.32 1.83 0.78	$C_{18}H_{36}O_2$	284	Antibacterial action, Cosmetic, Flavor, Hypocholestrolemic, Lubricant, perfumery, Propecic, Suppository
6.	Root Stem Leaf	22.988 20.289 28.928	Pentacosane	1.15 1.16 1.26	$C_{25}H_{52}$	352	Antibacterial
7.	Root Stem Leaf	26.250 26.220 26.232	Tetracontane	1.35 49.15 47.12	C_4H_{82}	562	Anti-inflammatory and Analgesic activity
8.	Root Stem Leaf	27.999 27.968 27.985	Squalene	0.23 0.09 1.17	$C_{30}H_{50}$	410	Antibacterial, Antioxidant, Antitumor, Anti-inflammatory, Antinociceptive, Potential antiplatelet components, Hypoglycemic, Hypolipidemic effects, Sedative action, Antihistaminic, Hepatoprotective activites Cancer preventing, Immunostimulant
9.	Root Stem Leaf	33.719 33.658 33.771	Vitamin E	0.23 0.49 3.49	$C_{29}H_{50}O_2$	430	Antiaging, analgesic, antidiabatic**,** Anti-inflammatory, antioxidant, antidermatitic, antileukemia, antitumor, anticancer, hepatoprotective, hypocholesterolemic, Antiulcerogenic, vasodilator, antispasmodic, antibronchitic, anticoronary
10.	Root Stem Leaf	39.009 38.869 39.038	Stigmast-5-en-3-ol, (3.Beta.)-	8.78 1.87 3.86	$C_{29}H_{50}O$	414	Anti-inflammatory, Anti-pyretic, Anti-ulcer, Antiarthritic

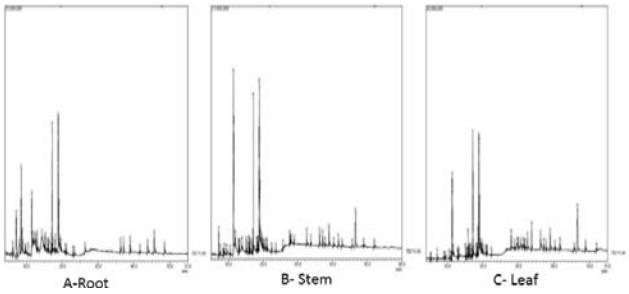


Fig 1: GC-MS Chromatogram of the Methanol Extract of Root, Stem and Leaf of *Ceropegia bulbosa*

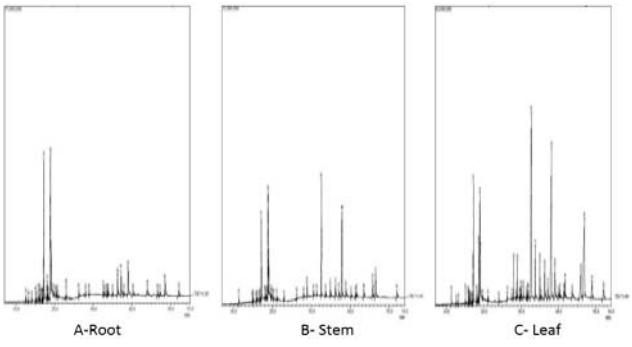
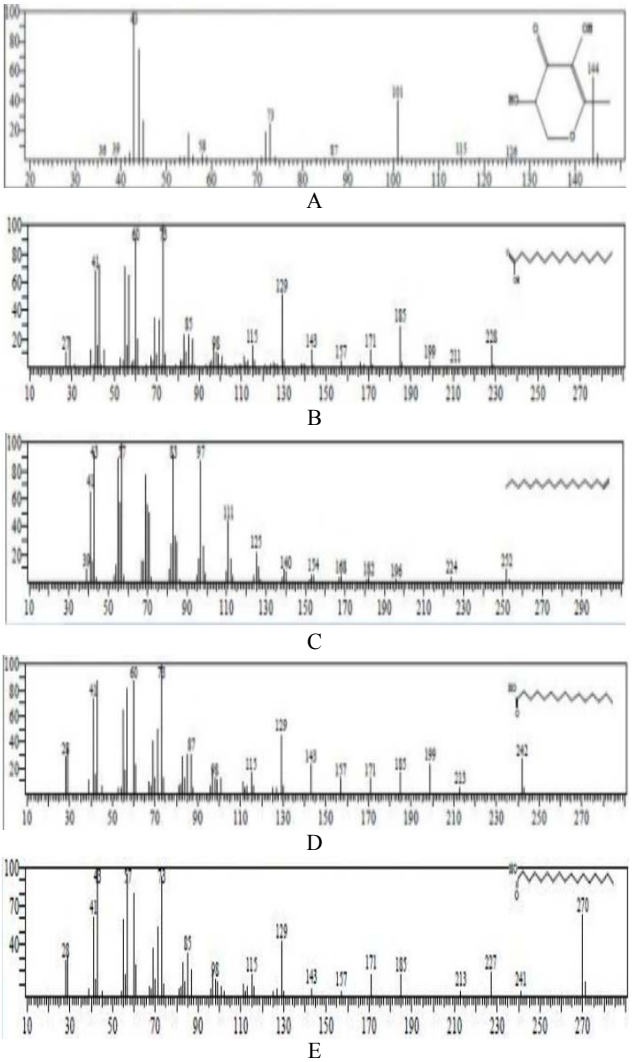


Fig 2: GC-MS chromatogram of the hexane extract of root, stem and leaf of *Ceropegia bulbosa*



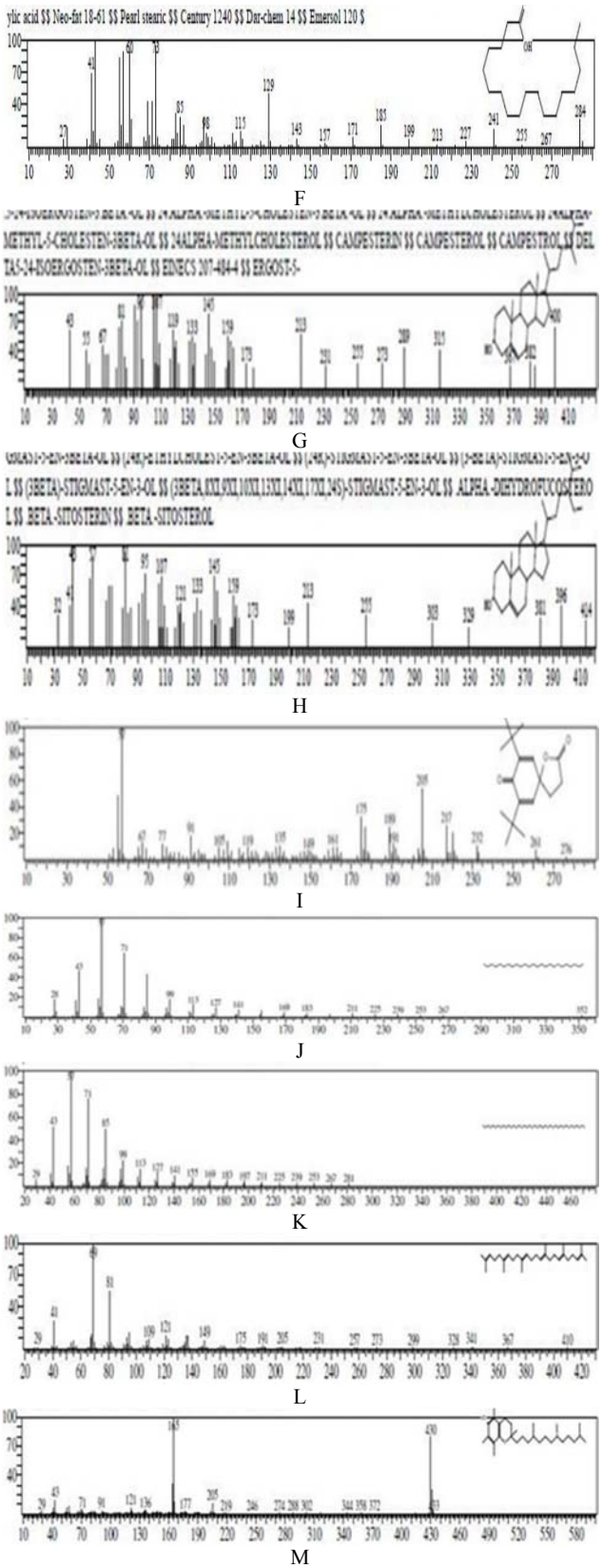


Fig 3: Mass Spectrum of (A) 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran, (B) Tetradecanoic acid, (C) 1-Octadecene, (D) Pentadecanoic acid, (E) Heptadecanoic acid, (F) Octadecanoic acid, (G) Ergost-5-en-3-ol, (3.beta.,24R), (H) Stigmast-5-en-3-ol, (3.beta.), (I) 7, 9-Di-tert-butyl-1-oxaspiro(4, 5)deca-6, 9-diene-2, 8-dione, (J) Pentacosane, (K) Tetracontane, (L) Squalene and (M) Vitamin E

Discussion

Phytosterols are cholesterol like molecules found in plants; the most common phytosterols are stigmasterol, β-sitosterol, and campesterol [16]. They have been clinically proved to reduce blood cholesterol, scientific reports suggest that they possess anticancerous & antioxidant activity [17, 18]. Stigmast-5-en-3β-ol (β-Sitosterol), a phytosterol shows anti-inflammatory, anti-pyretic, antiarthritic, anti-ulcer, insulin releasing and estrogenic effects. Beta-sitosterol is used for its cholesterol lowering property [19]. Ergost-5-en-3-ol, (3. beta., 24 R)- (Campesterol) is used in treatment for liver diseases and jaundice. Octadecanoic acid (Stearic acid) shows hypocholesterolemic property and is used in cosmetics, flavor, lubricant, perfumery and suppository [20]. Stearic acid shows antifungal, antitumor and antibacterial activity [21, 22]. Pentadecanoic acid and 1-Octadecene are used as finishing agent, Intermediates and as Lubricant. Squalene a triterpene, Vitamin E and Tetradecanoic acid show antioxidant activity and prevent the propagation of free radical reaction. Squalene is also an antitumor agent. 2, 3-dihydro-3, 5-dihydroxy-6-methyl-4H-Pyran, Heptadecanoic acid, 7, 9-Di-tert-butyl-1-oxaspiro (4, 5) deca-6, 9-diene-2, 8-dione and Pentacosane shows antimicrobial activity. Tetracontane is an alkane in nature and shows anti-inflammatory and analgesic activity. This study suggests that the phytochemical constituents from *Ceropegia bulbosa* Roxb. var. *bulbosa* show various biological activity helpful in curing various ailments and can lead to the isolation of new and novel drugs. The study on bioactive compounds provided a platform that will be helpful in drug synthesis and various formulations for pharmaceutical application.

Conclusion

GC-MS analysis is a speedy and more accurate tool for identification of important bioactive compounds from solvent based solution. *Ceropegia bulbosa* Roxb. var. *bulbosa* is medicinally more important but it is endangered. Prime need is to conserve this plant for future usage. As we could extract valuable compounds that could be used for human health care, second need is to standardize and evaluate its diagnostic features for its proper use.

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