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Research Article

PHARMAGNOSTIC POTENTIALITIES OF *CEROPEGIA BULBOSA* ROXB. VAR. *LUSHII* (GRAH.) HOOK.F.: AN ENDANGERED PLANT FROM THAR DESERT, RAJASTHAN, INDIA

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ABSTRACT

Plants are important source of medicine and drugs, they contain many bioactive constituents that produce definite physiological and biochemical actions in the human body. Medicinal plants are great importance to the health of individuals and play a significant role in traditional and modern drug system. *Ceropegia bulbosa* Roxb.var. *lushii* (Grah.) Hook.f. is one of the medicinally important and endangered plant (Asclepiadaceae), reported from some places of Rajasthan. The aim of present study was to determine the preliminary phytochemicals and bioactive components using standard analytical procedures and Perkin-Elmer Gas Chromatography-Mass Spectrometry of the whole plant in three extracts i.e. methanol, chloroform and hexane. The mass spectra of the compounds found in the extract was matched and compared with the National Institute of Standards and Technology (NIST) library. Methanol and chloroform extract shows better results as compared to hexane extract. This investigation concludes that extract of this plant contains various biologically active constituents and justifies its use for noval drug development.

Keywords: *Ceropegia bulbosa* Roxb.var. *lushii* (Grah.) Hook.f., Phytoconstituents, methanol, chloroform, hexane, GC-MS, Biological activity.

INTRODUCTION

Management and treatment of diseases has attracted many researchers to exploit plants as they are rich sources of primary and secondary metabolic products that show remarkable biological activities. The potential bioactive compounds are source for drug discovery. Natural products had been indispensably used by many cultures and traditions for thousands of years[1]. Plant and their derived products are always an exemplary source of drugs to treat various diseases[2]. According to world health organization (WHO), about three-quarters of the world population rely upon traditional remedies (manily herbs) for their health care[3]. The secondary metabolites show variety of structural arrangements and properties[4]. Bioactive compounds are used for curing various human diseases and also play an important role in healing[5]. Phytochemicals naturally occur in various plant parts and are involved in strengthening defense mechanism against various pathogens[6].

Several scientific investigations have proved and high-lighted the importance and contributions of many plant families. Asclepidaceae, now ranked as subfamily of Apocynaceae, is a large group of many plant species with wide therapeutical properties. The genus *Ceropegia* as a whole is under threat, owing to either destructive collection or habitat degradation. In India, approximately 50 species are present[7]. Among different species, *Ceropegia bulbosa* is one of the widely distributed species but still threatened[8]. *Ceropegia bulbosa* is used to improve defense mechanism, tubers are used in the treatment of kidney stone, urinary tracts diseases and they are eaten by ladies to enhance fertility and viability. Two varieties are found locally, the broad leaved variety is known as *Ceropegia bulbosa* Roxb. var. *bulbosa* and the variety having thin and long leaves is known as *Ceropegia bulbosa* Roxb. var. *lushii*[9]. Tuberous roots of the plant are edible and contains an alkaloid cerpegin (1, 1-dimethyl-5H-furo[3,4-c]pyridine-3,4-dione) and other components that form important ingredients in several conventional drug preparations[10]. Cerpegin ($C_9H_9NO_3$) is known to possess analgesic properties[11]. Recent studies report the antiurolithic activity of *Ceropegia*[12].

The present investigation was carried out to isolate, investigate and characterize bioactive compounds in three extracts i.e. methanol, chloroform & hexane by using GC-MS analysis for vegetative plant of *Ceropegia bulbosa* Roxb. var. *lushii* (Grah.) Hook.f.

MATERIALS AND METHODS

Collection of plant material

Ceropegia bulbosa Roxb. var. *lushii* (Grah.) Hook.f. was collected freshly from Jaipur, Udaipur, Chittorgarh, Bhilwara and Karauli districts of Rajasthan during July-September. "The Flora of Indian Desert" was consulted for identification[13], 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 each solvent under study were added simultaneously in separate flask. Crude extracts of different plant parts were prepared with methanol, chloroform and hexane by using hot extraction method[14] in soxhlet assembly. Mixture was then boiled at 60-70° C for 18 hours on water bath, filtered, evaporated to dryness, & final residue was then subjected to GC-MS analysis. The extract was further subjected to preliminary standard phytochemical studies

i.e. Wagner's test for alkaloids (2 ml of extract, add 3-4 drops of Wagners reagent (I_2+KI solution) produced reddish brown coloured precipitate indicates the presence of alkaloids); Molish test for carbohydrates (2 ml of extract, add 3-4 drops of α-naphthol (20% in ethanol) then added 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); Borntrager's test for glycosides (2 ml of extract add 1 ml of benzene and 0.5 ml of dilute ammonia solution, reddish pink colour indicated presence of glycosides); Lead Acetate test for phenolic compounds (1 ml of extract the addition of 3-4 drops of lead acetate solution (5%) yellow precipitates were obtained indicated the presence of phenolic compounds); Alkaline test for flavanoids (2 ml extract treated with sodium hydroxide solution, shows increase in the intensity of yellow colour which would colourless on addition of few drops of dilute Hydrochloric acid, indicates the presence of flavonoids); Xanthoprotein test for protein and amino acid (2 ml of extract, add 3-4 drops of nitric acid by the side of the test tube. Presence of yellow colouration indicated the presence of protein & amino acid); Foam test for saponins (1 ml of extract was diluted with 5 ml of distilled water and shaken well. The presence of layer of foam indicated the presence of saponins); Salkowski test for steroids (2 ml of extract, add 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, indicated the presence of sterols) and Salkowski test for terpenoids (2 ml of extract, added 2 ml of chloroform and 3 ml of concentrated H_2SO_4, form a layer. A reddish brownish colouration on the inner face is formed, it is indicated presence of terpenoids)[15] to determine the presence of various metabolic products. Powdered material was stored at 4 ºC for further use.

RESULTS AND DISCUSSION

Medicinal plants besides being therapeutic agents are also a large source of information for a wide variety of chemical constituents which could be developed as drugs with precise selectivity. These are the reservoirs of potentially useful chemical compounds which could serve as newer leads and clues for modern drug design[16]. The screening of plant extracts has been of great interest to scientists in the search for new drugs for greater effective treatment of several diseases[17]. The preliminary phytochemical screening tests may be separation of pharmacologically active chemical compounds[18]. Secondary metabolites are economically important as drugs, flavor and fragrances, dye and pigments, pesticides, and food additives[19,20]. The plant under study showed presence of various secondary metabolites with important biological activities (Table 1).

GC-MS chromatogram of the methanolic extract of tuber, stem and leaf showed 43, 47 and 34 peaks indicating the presence of 38, 45 and 33 compounds respectively. The chloroform extract of tuber, stem and leaf showed 47, 47 and 55 peaks indicating the presence of 38, 38 and 47 compounds respectively & the hexane extract of tuber, stem and leaf showed 46, 37 and 37 peaks indicating the presence of 37, 30 and 29 compounds respectively (Figure 1-3). These pytoconstituents may contribute the medicinal quality of this plant. These compounds were confirmed on the basis of their retention time (RT), peak area, molecular formula, molecular weight and concentration (%) in various extract under study (Table 2-4).

2H-Azepin-2-one,3-(dimethylamino) hexahydro - is present in maximum amount (20.96%), followed by cis-Vaccenic acid (10.96%) in the methanolic extract; D:B-Friedo-B':A'-Neogammacer-5-En-3-One is present in maximum amount (12.41%), followed by 2H-Azepin-2-one, 3-(dimethylamino) hexahydro- (11.77%) in the chloroform extract and D:B-Friedo-B':A'-Neogammacer-5-En-3-One is present in maximum amount (15.22%), followed by Pentadecanoic acid (12.99%) in the hexane extract of tuber.

2H-Azepin-2-one, 3-(dimethylamino) hexahydro- is present in maximum amount (25.78%), followed by cis-9-Hexadecenal (11.26%) in the methanolic extract; Tetracontane is present in maximum amount (52.06%) followed by 2H-Azepin-2-one, 3-(dimethylamino) hexahydro- (9.89%) in the chloroform extract and Tetracontane is present in maximum amount (61.84%), followed by cis-Vaccenic acid (5.59%) in the hexane extract of stem.

Beta-Amyrin is present in maximum amount (17.40%), followed by cis-Vaccenic acid (16.92%) in the methanolic extract; Tetracontane is present in maximum amount (53.14%), followed by 03027205002 Flavone 4'-OH, 5-OH, 7-Di-O-Glucoside (8.17%) in the chloroform extract and Tetracontane is present in maximum amount (54.43%) followed by 03027205002 Flavone 4'-OH, 5-OH, 7-Di-O-Glucoside (9.58%)in the hexane extract of leaf.

The gas chromatogram shows the relative concentrations of various compounds getting eluted as a function of retention time. The peak indicates relative concentration of the components present in the extract. The mass spectrometer analyzes the compounds eluted at different time that identify the nature and structure of the compounds. The large fraction gets dissected into smaller compounds giving rise to appearance of peaks at different m/z (mass to charge) ratio. These mass spectra are fingerprint of that compounds which can be identified from the data library. The phytochemical analysis is important and has commercial interest in research institutes as the products can be used in pharmaceutical industries or by traditional practitioners for producing potent drugs against various ailments and disorders.

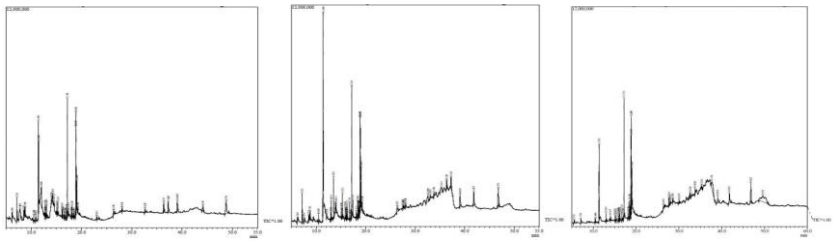

Figure 1: GC-MS chromatogram of the methanol extract of various parts of *Ceropegia bulbosa* Roxb. var. *lushii* (Grah.) Hook.f

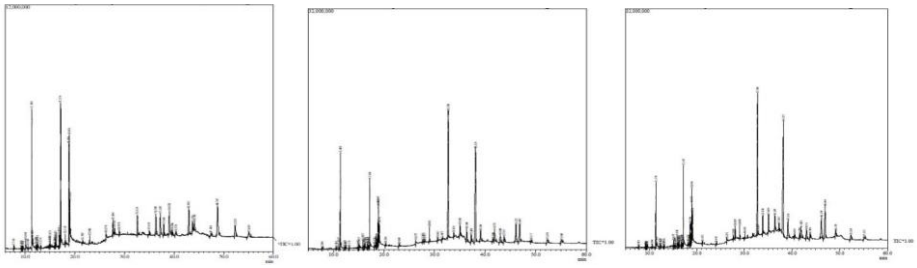

Figure 2: GC-MS chromatogram of the chloroform extract of various parts of *Ceropegia bulbosa* Roxb. var. *lushii* (Grah.) Hook.f

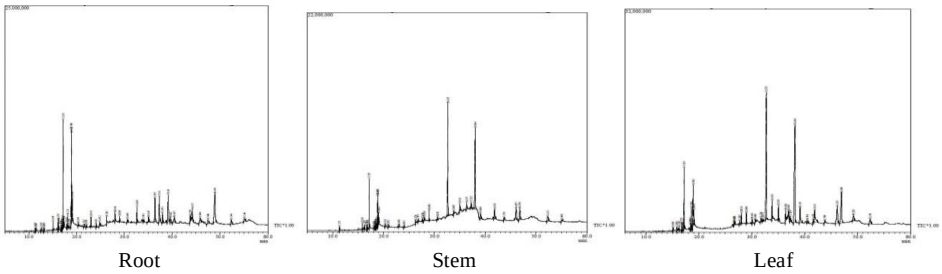

Figure 3: GC-MS chromatogram of the hexane extract of various parts of *Ceropegia bulbosa* Roxb. var. *lushii* (Grah.) Hook.f.

Table 1: Preliminary phytochemical analysis of *Ceropegia bulbosa*

S. No	Phytoconstituents	Tests	Methanol			Chloroform			Hexane		
			Root	Stem	Leaf	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.	Flavonoids	Alkaline teat	-	++	++	-	-	-	-	-	-
6.	Protein & Amino acid	Xanthoprotein test	+	+++	+++	++	++	+	-	-	-
7.	Saponins	Foam test	-	-	-	-	-	-	++	-	-
8.	Steroids	Salkowski test	++	+++	++	++	-	-	-	-	-
9.	Terpenoids	Salkowski test	+	++	-	++	-	-	-	++	+

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

Table 2: Bio-active compounds in methanol extract of *Ceropegia bulbosa*

S. No.	Plant parts	Retention time (min)	Compounds	% of Peak Area	Molecular formula	Molecular weight	Biological Activity
1.	Root Stem Leaf	14.020 14.011 14.017	8-Pentadecanone	0.17 0.58 0.24	$C_{15}H_{30}O$	226	Heptatoxic, Demyelination, Conjunctivitis activity
2.	Root Stem Leaf	15.315 15.311 15.313	1-Octadecene	0.62 1.07 0.32	$C_{18}H_{36}$	252	Finishing agent, Intermediates, Lubricants and Lubricant additives
3.	Root Stem Leaf	16.134 17.175 17.173	Pentadecanoic acid	0.39 9.95 11.57	$C_{15}H_{30}O_2$	242	Lubricants and Adhesive agents
4.	Root Stem Leaf	16.719 16.714 16.716	Hexadecanoic acid, methyl ester	0.20 0.71 0.54	$C_{17}H_{34}O_2$	270	Antibacterial and Antifungal, Antioxidant Hypocholesterolemic, Nematicide, Insecticide Lubricant, Antiandrogenic Flavor, Hemolytic
5.	Root Stem Leaf	18.120 18.114 18.118	Heptadecanoic acid	0.64 0.36 0.30	$C_{17}H_{34}O_2$	270	Antioxidant, Antifungal, Surfactant
6.	Root Stem Leaf	18.853 18.847 18.854	9,12-Octadecadienoic acid (Z,Z)-	7.73 8.66 8.79	$C_{18}H_{32}O_2$	280	Cancer preventive, Insectifuge, Anti-inflammatory, Nematicide, Hepatoprotective, Antihistaminic, Anticane, Antiarthritic, Antieczemic
7.	Root Stem Leaf	19.066 19.063 19.066	Octadecanoic acid	1.75 1.94 2.56	$C_{18}H_{36}O_2$	284	Antibacterial action, Cosmetic, Flavor, Hypocholestrolemic, Lubricant, perfumery, Propecic, Suppository
8.	Root Stem Leaf	28.034 28.026 28.032	Squalene	0.24 0.23 0.14	$C_{30}H_{50}$	410	Antibacterial, Antioxidant, Antitumor, Anti-inflammatory, Antinociceptive, Potential

							antiplatelet components, Hypoglycemic, Hypolipidemic effects, Sedative action, Antihistaminic,Hepatoprotective, Cancer preventing, Immunostimulant
9.	Root Stem Leaf	39.067 39.053 39.054	Stigmast-5-En-3-Ol, (3.Beta.)-	2.89 2.76 1.87	$C_{29}H_{50}O$	414	Anti-inflammatory, Anti- arthritic, anti-pyretic, Anti-ulcer

Table 3: Bio-active compounds in chloroform extract of *Ceropegia bulbosa*

S. No.	Plant parts	Retention time (min)	Compound Name	% of Peak Area	Molecular formula	Molecular weight	Biological Activity
1.	Root Stem Leaf	7.766 7.766 7.763	Naphthalene	0.29 0.13 0.08	$C_{10}H_8$	128	Antiseptic, Carcinogenic
2.	Root Stem Leaf	10.585 10.585 10.584	Pentadecane	0.11 0.04 0.04	$C_{15}H_{32}$	212	Sugar-phosphatase inhibitor,chymosin inhibitor, Antibacterial
3.	Root Stem Leaf	15.073 15.077 15.077	Tetradecanoic acid	0.41 0.25 0.21	$C_{14}H_{28}O_2$	228	Antioxidant, Cancer preventive, Hypocholesterolemic
4.	Root Stem Leaf	16.133 16.240 17.197	Pentadecanoic acid	10.39 0.11 4.80	$C_{15}H_{30}O_2$	242	Lubricants and Adhesive agent
5.	Root Stem Leaf	16.238 16.841 16.239	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	0.24 0.18 0.08	$C_{16}H_{22}O_4$	278	Antimicrobial activity, alpha-Glucosidase inhibition and the in vitro hypoglycemic effect
6.	Root Stem Leaf	16.837 17.190 16.840	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	0.55 4.95 0.16	$C_{17}H_{24}O_3$	276	Antimicrobial activity
7.	Root Stem Leaf	18.118 18.123 18.122	Heptadecanoic acid	0.56 0.13 0.14	$C_{17}H_{34}O_2$	270	Antimicrobial
8.	Root Stem Leaf	18.854 18.865 18.871	9,12-Octadecadienoic acid (Z,Z)-	7.62 3.66 2.64	$C_{18}H_{32}O_2$	280	Cancer preventive, Anti-inflammatory, Hepatoprotective, Antihistaminic, Anticane, Antiarthritic, Antieczemic
9.	Root Stem Leaf	19.066 19.076 19.078	Octadecanoic acid	1.54 0.83 0.93	$C_{18}H_{36}O_2$	284	Antibacterial, Hypocholestrolemic
10.	Root Stem Leaf	23.006 26.277 26.281	Tetracontane	4.54 52.06 53.14	C_4H_{82}	562	Anti-inflammatory and Analgesic activity
11.	Root Stem Leaf	28.027 28.027 28.029	Squalene	0.40 0.14 0.68	$C_{30}H_{50}$	410	Antibacterial, Antioxidant, Antitumor, Anti-inflammatory, Hypoglycemic, Hypolipidemic effects, Immunostimulant
12.	Root Stem Leaf	36.349 31.814 36.385	Ergost-5-en-3-ol, (3.beta.)-	4.05 2.56 2.21	$C_{28}H_{48}O$	400	Antimicrobial and Anti-inflammatory effects
13.	Root Stem Leaf	37.247 36.368 37.253	Stigmasterol	4.73 2.27 1.06	$C_{29}H_{48}O$	412	Antimicrobial activity
14.	Root Stem Leaf	39.070 37.260 39.136	Stigmast-5-En-3-Ol, (3.Beta.)-	6.35 1.49 3.49	$C_{29}H_{50}O$	414	Anti-inflammatory, Anti-pyretic, Anti-ulcer, Antiarthritic

Table 4: Bio-active compounds in hexane extract of *Ceropegia bulbosa*

S. No.	Plant parts	Retention time (min)	Compound Name	% of Peak Area	Molecular formula	Molecular weight	Biological Activity
1.	Root Stem Leaf	16.139 17.170 17.195	Pentadecanoic acid	12.99 4.87 4.34	$C_{15}H_{30}O_2$	242	Lubricants & Adhesive
2.	Root Stem Leaf	16.241 16.239 16.243	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	0.25 0.14 0.10	$C_{16}H_{22}O_4$	278	Antimicrobial activity, alpha-Glucosidase inhibition and the in vitro hypoglycemic effect
3.	Root Stem	16.840 16.838	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-	0.16 0.23	$C_{17}H_{24}O_3$	276	Antimicrobial activity

	Leaf	16848	diene-2,8-dione	0.11			
4.	Root Stem Leaf	19.090 19.064 19.081	Octadecanoic acid	1.16 0.76 0.66	$C_{18}H_{36}O_2$	284	Antibacterial Hypocholestrolemic
5.	Root Stem Leaf	22.020 43.710 43.789	Octadecanal	3.04 0.77 0.33	$C_{18}H_{36}O$	268	Alkane-lyase activity
6.	Root Stem Leaf	24.812 41.838 39.145	Stigmast-5-En-3-Ol, (3.Beta.)-	9.93 2.24 3.72	$C_{29}H_{50}O$	414	Anti-inflammatory, Anti-pyretic, Anti-ulcer, Antiarthritic
7.	Root Stem Leaf	26.281 26.276 28.990	Tetracontane	5.20 61.84 0.82	C_4H_{82}	562	Anti-inflammatory and Analgesic activity
8.	Root Stem Leaf	28.034 28.026 28.036	Squalene	0.79 0.46 0.74	$C_{30}H_{50}$	410	Antioxidant, Antitumor, Hepatoprotective, Immunostimulant
9.	Root Stem Leaf	37.312 37.232 37.284	Stigmasterol	7.04 2.03 0.88	$C_{29}H_{48}O$	412	Antimicrobial activity

CONCLUSION

Medicinal plants are important sources of bioactive compounds and play a key role in maintaining human health as they are used to formulate novel drug against various diseases. Application of Gas Chromatography Mass Spectrometry (GC-MS) includes environmental analysis, drug detection and identification of unknown samples. From this study, this is the first report to conclude that this plant is of high medicinal value, but the tyranny is that it is endangered. The plant needs utmost care and advanced conservation strategies as it is yielding so many phytoconstituents that are biologically active against several disorders. All the extract under study was equally effective, as they extracted almost equal compounds from the plant. As far as the plant part is concerned tuber and leaves contain more compounds as compared to stem. Further studies on efficacies, ethico-legal aspects and conservation are required.

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