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RESEARCH ARTICLE

PHYTOCHEMICAL SCREENING AND GC-MS ANALYSIS OF METHANOLIC EXTRACT OF *CEROPEGIA BULBOSA*

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ABSTRACT

Secondary metabolites are valuable source for drug preparation. Phytochemical analysis of methanolic extract of *Ceropegia bulbosa* showed presence of alkaloids, carbohydrates, glycosides, phenolic compounds, flavonoids, proteins and amino acids, saponins, sterols, and, terpenoids. The gas chromatography- mass spectrometry of methanolic leaf extract showed Pentadecanoic acid having highest area % of 10.09 with retention time 16.105 followed by Octadecanoic acid with area % of 2.43, retention time 18.049 followed by 2,3-dihydro-3,5-dihydroxy-6- methyl-4H-Pyran with area % of 0.55, retention time 7.125 followed by Tetradecanoic acid with area % of 0.45, retention time 15.002 followed by Heptadecanoic acid with area % of 0.31, retention time 18.097 followed by 1-Octadecene with area % of 0.16, retention time 15.297. The present study shows that the methanolic extract of *Ceropegia bulbosa* have important bioactive compounds which can play a significant role in drug development.

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INTRODUCTION

Medicinal plants are the richest source of bioactive metabolites that produce a definite physiological action on human body, also they are useful in supplements, nutraceuticals, pharmaceutical industries, and chemical entities for synthetic drugs (Okwu DE 2001). Since ages, medicinal plants are used for primary health care, which involves use of plant extracts (Sandhya T *et al*. 2006). There is an awareness growing in correlating the phytochemical constituents with their activities against many diseases. Thus, studies on medicinal plant are continuous for discovery of secondary metabolites (Vinoth S *et al*. 2011). Phytochemicals are responsible for medicinal activity of plants which are the basic source for the establishment of several pharmaceutical industries. The constituents present in medicinal plants play a significant role in the identification of crude drugs (Savithramma *et al*. 2011). *Ceropegia bulbosa* is one of the important endangered medicinal plants of family Asclepiadaceae, having various therapeutical properties. As Asclepiadoideae is the largest cosmopolitan family having approximately 177 genera and nearly 3000 species (Bhandari MM 1978) they are well known for their ethnobotanical and ethnomedicinal importance (Merlin *et al*. 2009).

The present study was carried out to screen the phytochemicals and to characterize bioactive ompounds in methanol extract of *Ceropegia bulbosa* by using GC-MS technique.

MATERIALS AND METHODS

Collection and Identification of Plant Material: *Ceropegia bulbosa* was collected from Akola district, Maharashtra, India, during the month of June-August. The specimen was authenticated from Department of Botany, Ghulam Nabi Azad College, Akola, Maharashtra.

Sampling of Plant Material: Fresh leaves were collected from nature, shade dried and powdered in a mechanical grinder. The powder was kept in airtight plastic bags with paper labeling for future uses.

Phytochemical Studies: For phytochemical study, 10g of crude powder was transferred to round bottom flask and extracted with 200 ml methanol as a solvent. It was filtered and used to determine the group of secondary metabolites present in the plant material. Condensed extracts were used for preliminary screening of phytochemicals such as alkaloids,

carbohydrates, glycosides, phenolic compounds (Gibbs RD, 1974), flavonoids, proteins & amino acid, saponins (Ayoola *et al*. 2008), sterols and terpenoids (Peach and Tracey, 1956).

Gas Chromatography-Mass Spectrometry Analysis: For GC-MS analysis, 6g of crude powder was transferred to round bottom flask and extracted with 200ml methanol as solvent. The crude extract was boiled at 55 ºC -70 ºC for 24 hours on water bath and was filtered using Whatman filter paper No. 1 and then evaporated to dryness. The final residue obtained was then subjected to GC-MS, of which 1µl was subjected to analysis and rest was stored at 4ºC for further use (Merlin *et al*. 2009). The test sample was injected in the injection port of the GC equipment, where the temperature was maintained 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 which were detected in the mass detector. The spectrum of the unknown components was compared with the spectrum of known stored in the NIST library (Amin *et al*. 2013). The mass spectrometer analyzes the compounds which get eluted at different time to identify the nature and structure of the compounds. Temperature of injection port was 260 ºC, Column oven temperature was maintained at 80 ºC. The gas chromatography- mass spectrometry (GC-MS) analysis was performed at Indian Institute of Science, Bangalore.

RESULTS

Medicinal plants are used since ages for human benefits, also used as an alternative for drug production as a medicinal practice (Osuntokun OT and Oluwafoise BO 2015). The preliminary phytochemical screening of *Ceropegia bulbosa* was carried out using methanol extract of leaf to check the presence of alkaloids, carbohydrates, glycosides, phenolic compounds, flavonoids, proteins and amino acids, saponins, sterols, and terpenoids (Table 1).

GC-MS chromatogram of the methanolic extract of leaf of *Ceropegia bulbosa* showed presence of six compounds. These compounds were identified based on their retention time (RT), peak area, molecular formula, molecular weight, concentration (%) in methanolic extract (Table 2). Pentadecanoic acid showed highest area % of 10.09 with retention time 16.105 followed by Octadecanoic acid with area % of 2.43, retention time 18.049 followed by 2,3-dihydro-3,5-dihydroxy-6- methyl-4H-Pyran with area % of 0.55, retention time 7.125 followed by Tetradecanoic acid with area % of 0.45, retention time 15.002 followed by Heptadecanoic acid with area % of 0.31, retention time 18.097 followed by 1-Octadecene with area % of 0.16, retention time 15.297.

DISCUSSION

Secondary metabolites are responsible for medicinal activity of plant species. In *Ceropegia bulbosa*, alkaloid, carbohydrates, flavonoids, protein and amino acids were abundantly present. Saponin and glycoside were moderately present whereas phenolic compounds, steroids and terpenoids were present in less amount. Alkaloids have a bitter taste and can be toxic to other organisms (Gupta *et al*. 2010). The flavonoids show antioxidant activity and are effective to human health at a considerable amount. As these are polyphenolic compounds which influence radical scavenging activity and helps in inhibiting hydrolytic and oxidative enzymes (Frankel, 1995). Glycosides are important in medicine because of their action on heart and are used in cardiac insufficiency (Balch and Balch, 2000). The phenolic compounds are one of the largest groups of plant metabolites and have attracted special attention since they have property to protect the human body from the oxidative stress which may lead to occurrence of many diseases, such as cancer, cardiovascular problems, and ageing (Robards *et al*. 1999). Tannins has astringent properties, hastens the healing of wounds, and inflamed mucous membrane (Killedar and More, 2010).

Table 1. **Primary screening of phytoconstituents of *Ceropegia bulbosa***

Sr.No	Phytoconstituents	Tests	Leaf Methanol Extract
1	Alkaloids	Wagner'stest	+++
2	Carbohydrates	Molishtest	+++
3	Glycosides	Borntrager'stest	++
4	Phenoliccompounds	LeadAcetatetest	+
5	Flavanoids	Alkalinetest	+++
6	Protein&Aminoacid	Xanthoproteintest	+++
7	Saponins	Foamtest	++
8	Steroids	Salkowskitest	+
9	Terpenoids	Salkowskitest	+

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

Table 2. GC-MS analysis of leaf methanolic extract of *Ceropegia bulbosa*

Sr No.	Retention time (min)	Compound Name	% Of Peak Area	Molformula	Mol weight	Biological Activity
1	7.125	2,3-dihydro-3,5-dihydroxy-6-methyl-4H-Pyran	0.55	$C_6H_8O_4$	144	Antimicrobial, anti-inflammatory
2	15.002	Tetradecanoicacid	0.45	$C_{14}H_{28}O_2$	228	Antioxidant, Cancer preventive, Nematicide, Hypocholesterolemic
3	15.297	1-Octadecene	0.16	$C_{18}H_{36}$	252	Finishingagent, Intermediates, Lubricants, andadditives
4	16.105	Pentadecanoicacid	10.09	$C_{15}H_{30}O_2$	242	Lubricants and Adhesiveagents
5	18.097	Heptadecanoicacid	0.31	$C_{17}H_{34}O_2$	270	Antioxidant, Antifungal, Surfactant, Antibacterial action, Cosmetic
6	18.049	Octadecanoicacid	2.43	$C_{18}H_{36}O_2$	284	Hypocholestrolemic,, perfumery, Propecic,

In GC-MS, 2,3-dihydro-3,5-dihydroxy-6- methyl-4H-Pyran, Heptadecanoic acid, shows antimicrobial activity. Tetradecanoic acid show antioxidant activity and prevent the propagation of free radical reaction. Octadecanoic acid (Stearic acid) shows hypocholesterolemic property and is used in cosmetics, flavor, lubricant, perfumery, and suppository (Akpuaka *et al*. 2013). Pentadecanoic acid and 1-Octadecene are used as finishing agent, Intermediates and as Lubricant. This study suggests that the phytochemical constituents from *Ceropegia bulbosa* show various biological activity helpful in curing various ailments which can lead to the isolation of new and novel metabolite or drugs. The study on bioactive compounds provided a platform that will be helpful in drug synthesis and various formulations for pharmaceutical application.

CONCLUSION

The medicinal plants appear to be rich in secondary metabolites, widely used in traditional medicine to combat various ailments. The anti-inflammatory, antispasmodic, analgesic, and diuretic can be attributed to their high alkaloids, phenols, tannins, and flavonoids. Exploitation of these pharmacological properties involves further investigation of these active ingredients by implementation of techniques like separation, extraction, purification, identification etc. *Ceropegia bulbosa* is medicinally more important but it is endangered so there is need to conserve this plant. Valuable compounds can be extracted using different solvents and evaluated for diagnostic purpose that could be used for human health care for future usage.

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