WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 7.523
Volume 6, Issue 4, 1513-1528. Research Article ISSN 2277– 7105

PRELIMINARY PHYTOCHEMICAL SCREENING, INORGANIC ELEMENTS, ANTIOXIDANT ACTIVITIES, PROXIMATE ANALYSIS , FREE RADICAL SCAVENGING ACTIVITY AND GC-MS ANALYSIS OF METHANOL EXTRACT OF *CEROPEGIA WOODII*

Kiran Kumar S J[1] and Prabha S J [2]

[1]Department of PG Studies and Research in Biotechnology, Government Science College, Bengaluru- 560 001.

[2]Department of Environmental Science, Bangalore University, Bengaluru- - 560 056.

Article Received on
16 Feb. 2017,

Revised on 11 March 2017,
Accepted on 31 March 2017

DOI:10.20959/wjpr20174-8254

***Corresponding Author**
Kiran Kumar S. J.
Department of PG Studies and Research in Biotechnology, Government Science College, Bengaluru-560 001.,

ABSTRACT

Phytochemical constituents are responsible for medicinal activity of plant species. Hence in the present study preliminary phytochemical screening , inorganic elements, antioxidant activities, proximate analysis and free radical scavenging activity of *Ceropegia woodii* a medicinal plant was carried out. Qualitative phytochemical analysis of these plants confirm the presence of various secondary metabolites like saponins, triterpenoids, steroids, tannins, lignins, alkaloids, glycosides, flavonoids and phenols. Additionally, inorganic elements like iron, chloride and sulphate were identified by total ash analysis. The same extract was used for quantitative determination of total flavonoid content (88.89mg/g), total phenolic content (77.98mg/g) and total triterpenoids content (23.22 mg/g). The results suggest that the phytochemical properties are used for curing various ailments and possess potential anti-inflammatory, antimicrobial and antioxidant properties. This leads to the isolation of new and novel compounds. GC-MS analysis showed the existence of various compounds with different chemical structures. The presence of various bioactive compounds confirms the application of *Ceropegia woodii* for various ailments by traditional practitioners. However, isolation of individual phytochemical constituents may proceed to find a novel drug.

KEYWORDS: *Ceropegia woodii* , Indian Medicinal Plants, Phytochemical Screening

INTRODUCTION

Medicinal plants are the richest bio-resources of folk medicines and traditional systems of medicine; food supplements, nutraceuticals, pharmaceutical industries and chemical entities for synthetic drugs (Ncube *et al.* 2008). Modern medicine has evolved from folk medicine and traditional system only after chemical and pharmaceutical screening (Boopathi and Sivakumar, 2011). India is the birth place of renewed system of indigenous medicine such as Siddha, Ayurvedha and Unani. Traditional systems of medicines are prepared from a single plant or combinations of number of plants. 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 (Vinoth *et al.* 2011; Savithramma *et al.* 2010). There is growing awareness in correlating the phytochemical constituents of a medicinal plant with its pharmacological activity. The study of Turger and Usta (2008) on screening active compounds from plants has lead to the invention of new medicinal drugs which have efficient protection and treatment roles against various diseases, including cancer (Sheeja and Kuttan 2007) and Alzhemir's diseases (Muherjee and Kumar 2007).

Phytochemicals are responsible for medicinal activity of plants (Savithramma *et al.* 2011). These are non-nutritive chemicals that have protected human from various diseases. Phytochemicals are basically divided into two groups that is primary and secondary metabolites based on the function in plant metabolism. Primary metabolites comprise common carbohydrates, amino acids, proteins and chlorophylls while secondary metabolites consist of alkaloids, saponins, steroids, flavonoids, tannins, etc (Jigna and Sumitra, 2007; Kumar *et al.* 2009). Phytochemical constituents are the basic source for the establishment of several pharmaceutical industries. The constituents are playing a significant role in the identification of crude drugs (Savithramma *et al.* 2011).

The genus *Ceropegia* L. is the largest genus of the tribe Ceropegiaeae with about 200 species distributed only in tropical and sub tropical regions of the Old World, ranging from the Spanish Canary Islands in the west, through Central, Southern, and Northern Africa, Madagascar, Arabia, India, South Asia to Northern Australia in the East (Good, 1952; Anonymous, 1992; Bruyns, 2003). The maximum diversity of *Ceropegia* species occurs in South Africa followed by Kenya and Madagascar. Its species diversity eastwards diminishes in Arabia where only 10 species were recorded and only one species was found in Pakistan. The species of *Ceropegia* as a whole are under threat, owing to either destructive collection

or habitat degradation. They are not only genetically depleted but also are scarcely available. Ansari (1984) revised the Indian, *Ceropegia* and reported 44 species, of which 28 are said to be endemic to India. Many species of the genus *Ceropegia* have now been added to the list of Indian endangered plants (BSI, 2002). These species are placed under the categories of rare, endangered, vulnerable, extinct, and threatened plants (Nayar and Sastry, 1987; Goyal and Bhadauria, 2006; Madhav Gadgil, 2004)

Economic Importance of the Genus *Ceropegia*

The sweet-sour leaves are edible and are considered to be tonic and digestive. (Kirtikar and Basu 1935). It is used as an Antidote for snake bite (Duraisamy Suresh and Paulsamy 2010**)**.The tubers are edible (Mabberly, 1987) and contain starch, sugars, gum, albuminoids, carbohydrates, fats, crude fibre, and the medicinally important alkaloid Ceropegin (Kirtikar and Basu, 1935; Nadkarni, 1976; Anonymous, 1980; Jain and Defillips, 1991). The root tubers also contain starch, sugars, gum, albuminoids, fats, crude fiber and valuable constituents in many traditional Indian Ayurvedic drug preparations that are active against ulcers, inflammation etc., (Adibatti *et al.* 1991). The boiled or roosted tubers are edible and a rich source of carbohydrates (Nikam and Savanth, 2007). These species are of economic importance (Jagtap and Singh, 1999) due to their starchy edible tubers with medicinal value. The fresh tubers of these species are usually boiled before they are eaten to remove the bitterness. The active compound of tuberous roots is the alkaloid Ceropegin which is active against diarrhea and dysentery inflammation of gums and delirious fevers of parturition (Nadkarni, 1976). The tubers then again are a kind of energy source, among other things they are used to suppress fatigue The alkaloid Ceropegin from the tubers of

C. bulbosa was used in Bihar to cure cold, sneezing and eye diseases (Kirtikar and Basu, 1935). The crude extracts of

Ceropegia tuberose were active against different bacterial strains (Vijayakumar *et al*. 2013).

MATERIALS AND METHODS

Collection of Plant Material

Plant was collected from the Krishnendra Nursery near Lalbagh, Bangalore. Plants with tuber and follicle was removed from soil and deposited in polythene bag.

Sampling of Plant Material

Fresh plant tuber free from diseases was collected. The tubers were washed thoroughly 2-3 times with running tap water, tuber was cut into small pieces then air dried under shade. The plant tuber was grinded and powders were kept in air tight plastic bags with paper labeling for future uses.

Phytochemical Studies

Preparation of plant extracts for preliminary phytochemical studies

The grinded tuber materials of 5g weighed separately using an electronic balance were crushed in 25 ml of sterile water, boiled at 50-60^{0}C for 30 minutes onwater bath and was filtered through Whatman No.1 filter paper. Then filtrate was centrifuged at 2500 rpm for 15 minutes and filtrate was stored in sterile bottles at 5^{0}C for further use (Harbone, 1973).

Preliminary Phytochemical Studies

The extract was subjected to preliminary phytochemical tests to determine the group of secondary metabolites present in the plant material. Condensed extracts were used for preliminary screening of phytochemicals such as alkaloids, steroids, and phenols (Gibbs, 1974); glycosides, triterpenoids and saponins (Ayoola *et al.* 2008); tannins, (Treare and Evan, 1985); flavonoids (Peach and Tracey, 1956).

Qualitative phytochemical analysis

Qualitative phytochemical analysis of methanol extracts of pods was conducted by following standard procedures (Khandelwal, 2006). Elemental analysis of Ash for detection of inorganic elements (Calcium, Iron, Magnesium, Potassium, Sulphate, phosphate, chloride, carbonate and Nitrate) was performed by specific tests as per (Khandelwal, 2006)

Proximate Analysis

The quantitative parameters of *Ceropegia woodii* samples were used to determined the Moisture content (water loss on drying), total ash, acid insoluble ash, water soluble ash, alcohol soluble extractive value, and water soluble extractive value using standard methods as per (Stalh ,1973).

.

Free Radical Scavenging Activity

The free-radical scavenging effect of crude extracts and fractions of J. multifida was estimated using DPPH scavenging method(Jain ,2008). Exactly 1.0 mL of 0.1 mM DPPH

was mixed with 3.0 mL of extract/fraction in methanol of concentrations 0.01-0.2 mg/mL.The reaction was vortexed thoroughly and left in the dark at room temperature for 30 minutes.The absorbance of the mixture was measured spectrophotometrically at 517 nm with ascorbic acid as a reference.

Determination of total phenolic content

To determine total phenolic content from the methanolic extract of *Ceropegia woodii*, calibration curve of standard Gallic acid of 20, 40, 60, 80 and 100 mg/ml was prepared in water and 1 mg/ml of methanolic extract of *Ceropegia woodii was* prepared simultaneously. Each sample was mixed with 0.25 ml of Folinciocalteu reagent and 1.25 ml of sodium carbonate solution. The mixtures were allowed to react for 40 minutes at room temperature. After the reaction period , the blue color was measured at 725 nm on UV-visible spectrophotometer of LABINDIA 3000+ and calculated the amount of total phenolic content from calibration curve as Gallic acid (Khadbadi ,2011).

Determination of total flavonoid content

An aliquot (l ml) of standard solution of quercetin (20, 40, 60, 80 and 100 µg/ml) was added to 10 ml volumetric flask containing 4 ml of 5% NaNO2 into it. After 5 minutes 0.3 ml of 10% AlCl3 was added. Then 2 ml of 1 M NaOH was added and the total volume was made up to 10 ml with distilled water. Same dilutions were also prepared for the test solution. Blank determination was done by using methanol in place of test or standard solutions and mixed well and the absorbance were observed at 358 nm against blank. From the obtained standard curve of quercetin the total flavonoids content of methanolic extract of *Ceropegia woodii* was determined (Khadbadi ,2011).

Determination of total Triterpenoids

5 g of extracted powder was added to 50 ml distilled water and heated on water bath for 30 min. Then the extract was allow to cool and then filtered . 75 ml chloroform and diethyl ether was added in 1:2 concentrations by continuous stirring for 30 min. After 5 gm of sodium carboxyl methyl cellulose was added to forms lumps and sticky mass were obtained and they were separated. Further marc were subjected to extraction with 75 ml chloroform: diethyl ether (1:2) for four times. The obtained residue was dissolved in 50 ml of neutral absolute alcohol. Then the mixture were titrated with 0.1 N NaOH using phenolphthalein as an indicator. Similarly blank readings were taken without addition of sample. Percentage of

Triterpenoids content was calculated as per the given factor. Factor for the calculation: each ml of 0.1N NaOH = 48.8 mg of Triterpenoids (Khadbadi ,2011).

Gas Chromatography-Mass Spectrometry Analysis

The GC – MS analysis was carried out using a Clarus 500 Perkin – Elmer (Auto system XL) Gas Chromatograph equipped and coupled to a mass detector Turbo mass gold – Perkin Elmer Turbomass 5.2 spectrometer with an Elite – 5MS (5% Diphenyl / 95% Dimethyl poly siloxane), 30m x 0.25 µm DF of capillary column. The instrument was set to an initial temperature of 110ºC, and maintained at this temperature for 2 min. At the end of this period the oven temperature was increased to 280ºC at the rate of an increase of 5ºC/min, and maintained for 9 min. Injection port temperature was ensured to be 200 ºC and Helium flow rate as one ml/min. The ionization voltage was 70eV. The samples were injected in split mode as 10:1. Mass spectral scan range was set at 45-450 (m/z). NIST software (Version 2011) were used for MS data library access and comparing the spectrum obtained through GC – MS compounds present in the plants sample were identified.

RESULTS AND DISCUSSIONS

Phytochemical Screening Medicinal Plants

Traditional medicine also known as indigenous or folk medicine comprises medical knowledge systems that developed over generations within various societies before the era of modern medicine. Traditional medicines are prepared from a single plant or combination of more than one plant. Indian contribution to herbal market and emphasis on novel research is continuously increasing. Phytochemical constituents are responsible for medicinal activity of plant species. Hence in the present study preliminary phytochemical screening of *Ceropegia woodii* a medicinal plants was carried out, qualitative phytochemical analysis of this plant confirm the presence of various secondary metabolites like alkaloids, glycosides, tannins, saponin, flavonoids, steroid, triterpenes and phenol. The results suggest that the phytochemical properties are used for curing various ailments and possess potential anti-inflammatory, antimicrobial and antioxidant properties .Thus, leading to the isolation of new and novel compounds.

Table 1: Phytochemical Screening

Phytochemical screening		
Sl.No	**Test**	**Inference**
1	Carbohydrate	+
2	Protein	+
3	Glycoside	+
4	Saponin	+
5	Coumarin	+
6	Flavonoid	+
7	Anthraquinone glycoside	-
8	Phytosterol	-
9	Phenol	+
10	Alkaloids	+
11	Lipid	+
12	Steroids	+

(+) present (-) absent

Table 02: Detection of inorganic elements.

Phytochemical analysis of inorganic elements		
Sl.No	Test	Inference
1	Calcium	+
2	Iron	+
3	Magnesium	-
4	Potassium	+
5	Sulphate	+
6	Phosphate	+
7	Chloride	-
8	Carbonate	+
9	Nitrate	+

Table 3: Percentage (%) values of proximate analysis *Ceropegia woodii* sample

Parameter	**Value± SEM (%)**
Moisture Content	10.01 ± 1.20
Water extractive index	2.14 ± 0.03
Total Ash	9.99 ± 0.06
Acid Insoluble Ash	7.83 ± 0.01
Alcohol extractive index	0.52 ± 0.02

Table 4: The IC-50 values of the different fractions of the root extract of *Ceropegia woodii*

Sample	**IC50 value (µg/mL)**
Methanol extract	369.98
Pet – Ether fraction	188.21
Ethyl acetate fraction	98.79
Chloroform fraction	39.99
Ascorbic acid	3.01

Table 05: Quantitative analysis of phytochemicals (mg/g)

Ceropegia woodii	
Total flavonoids	88.89*
Total phenols	77.98*
Total Triterpenoids	23.22*

Alkaloids have a bitter taste while many are toxic to other organisms (Gupta *et al.* 2010). Flavonoids are a group of polyphenolic compounds which influence the radical scavenging, inhibition of hydrolytic and oxidative enzymes and also act as anti-inflammatory agent (Frankel, 1995). The flavonoids show antioxidant activity and their effects on human nutrition and health is considerable. The mechanism of action of flavonoids are through scavenging or chelating process (Kessler *et al.* 2003); Cook and Samman, 1996), they also inhibit microbes which are resistant to antibiotics (Linuma *et al.*1994). Flavonoids are free radical scavengers, super antioxidants and potent water soluble which prevent oxidative cell damage and have strong anti-cancer activity (Salah *et al.* 1995). As antioxidants, flavonoids provide anti-inflammatory actions (Okwu, 2001A; Okwu, 2001B). Glycoside compounds contain a carbohydrate and non-carbohydrates residue in the same molecule. In these compounds, the carbohydrate moiety is attached by an acetyl linkage carbon-I to the non-carbohydrate residue (aglycone). They all contain steroid as a glycone component in combination with sugar molecules. They are important in medicine because of their action on heart and are used in cardic insufficiency (Balch and Balch, 2000). Thus, cardiac glycosides are drugs that can be used in the treatment of congestive heart failure and cardiac arrhythmia. They work by inhibiting the Na^{+}/ Na^{+} pump, resulting in an increase in the levels of sodium ions in the myocytes, which then leads to a rise in the level of calcium ions. This inhibition increases the amount of Ca^{2+} ions available for concentration of the heart muscle, improves cardiac output and reduces distention of the heart (Bertorello *et al.* 1990; Clausen and Nielsan, 1994; Beltowski *et al.* 1998). However, same glycosides such as ovarian are toxic as it inhibits active transport of Na^{+} in cardiae muscle (Sodium pump inhibitor), resulting in inhibition of translocases during electron transport chain and leading to death (Beltowski *et al.* 1998).

Primarily phenolic compounds are of great importance as cellular support material because they form the integral part of cell wall structure by polymeric phenolics (Gupta *et al.* 2010), bioactive polyphenols have attracted special attention because they can protect the human body from the oxidative stress which may cause many diseases, including cancer,

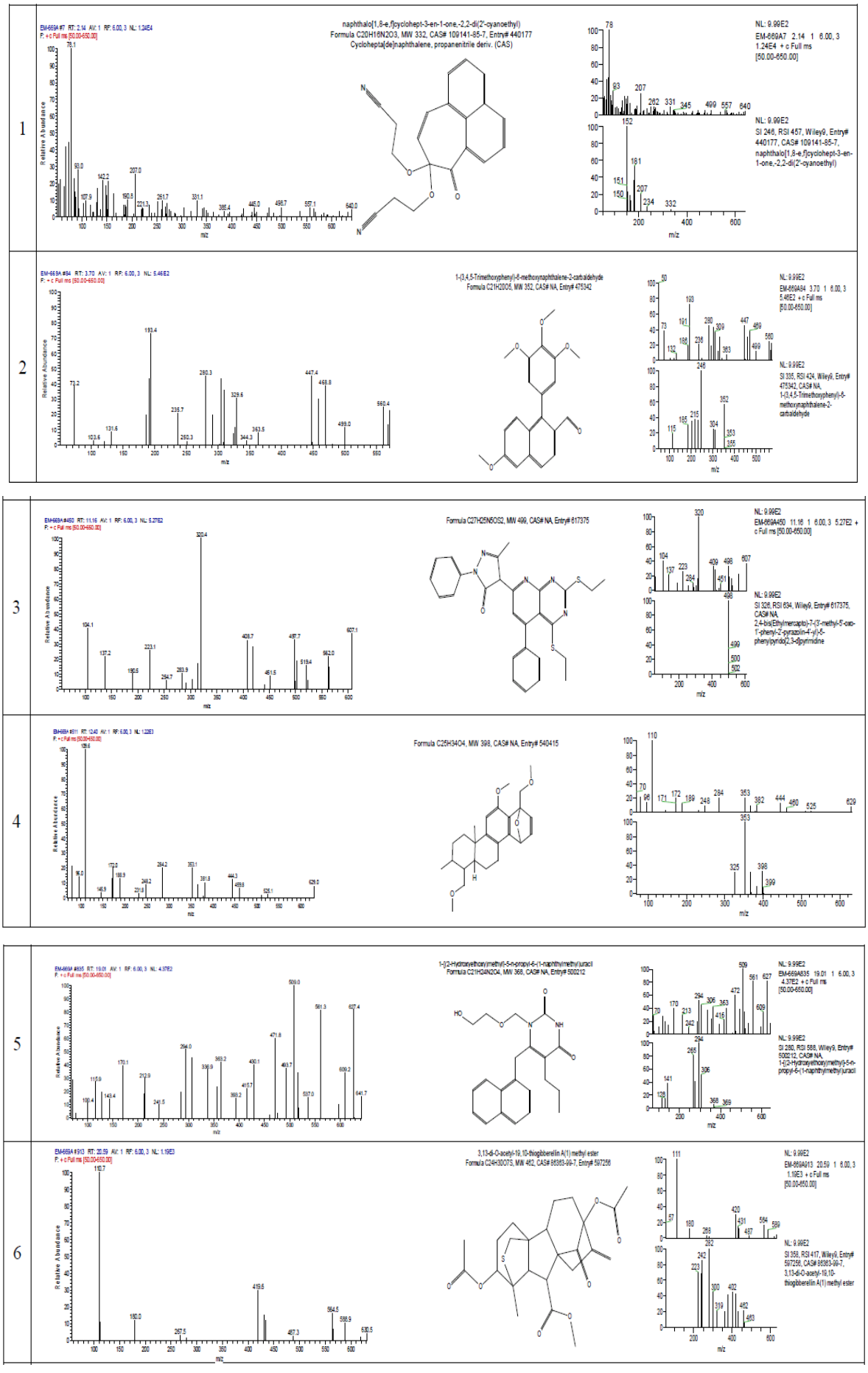
cardiovascular problems and ageing (Robards *et al.* 1999). The phenolic compounds are one of the largest and most ubiquitous group of plant metabolites. A number of studies have focused on the biological properties such as antiapoptosis, anti-ageing, anticarcinogen, anti-inflammation, anti-artherosclerosis, cardiovascular protection and improvement of the endothelial function, as well as inhibition of angiogenesis and cell proliferation activity (Han *et al.* 2007). Phenolic compounds have been extensively used in disinfections and maintain the standards with which other bacteriocides are compared (Okwu, 2001).

Tannins contribute property of astringency i.e. fasten the healing of wounds and inflamed mucous membrane and have received considerable attention in the fields of nutrition, health and medicine, largely due to their physiological activity, such as antioxidant, antimicrobial and anti-inflammatory properties. Tannins are complex moieties produced by majority of plants as protective substances, they have wide pharmacological activities. They have been used since past as tanning agents and they posses astringent, anti-inflammatory, antidiarrhoeal, antioxidant and antimicrobial activities (Killedar and More, 2010).

The results pertaining to GC-MS analysis led to the identification of number of compounds from the GC fractions of the methanolic extract of *Ceropegia woodii.* These compounds were identified through mass spectrometry attached with GC. The results of the present study were tabulated in Table 1. The gas chromatogram shows the relative concentrations of various compounds getting eluted as a function of retention time. The heights of the peak indicate the relative concentrations of the components present in the plant. The mass spectrometer analyzes the compounds eluted at different times to identify the nature and structure of the compounds. The large compound fragments into small compounds giving rise to appearance of peaks at different m/z ratios. These mass spectra are like a fingerprint of that compound which can be identified from the data library. This report is the first of its kind to analyze the chemical constituents of *Ceropegia woodii* using GC-MS. In addition to this, the results of the GC-MS profile can be used as pharmacognostical tool for the identification of the plant compounds with different chemical structures. The presence of various bioactive compounds confirms the application of *Ceropegia woodii* for various ailments by traditional practitioners. However, isolation of individual phytochemical constituents may proceed to find a novel drug.

Table 6: Activity of Phytocomponents Identified in *Ceropegia woodii* by GC-MS

S. No	RT	Compound Name	Molecular Formula	MW	Peak Area
1	2.14	naphthalo[1,8-e,f]cyclohept-3-en-1-one,-2,2-di(2'-cyanoethyl)	$^{C}20^{H}16^{N}2^{O}3$	332	4.53
2	2.65	à-[Tris(trimethylsilyl)methyl]silyl-4-methoxybenzyl propanoate	$^{C}21^{H}42^{O}3^{Si}4$	454	3.81
3	3.70	1-(3,4,5-Trimethoxyphenyl)-6-methoxynaphthalene-2-carbaldehyde	$^{C}21^{H}20^{O}5$	352	4.01
4	4.87	2,6-Dibromo-3,5-dimethoxy-4-(1,2,2,2-tetraffluoro-1-trifluoromethylethyl) pyridine	$C_{10}H_6Br_2F_7NO_2$	463	3.03
5	5.17	6-methyl-2-(4-methylphenyl)-7-(2,4,5-trimethylbenzyl) indolizine	$^{C}26^{H}27^{N}$	353	2.21
6	9.35	7-Bromo-1-(2'-methylprop-2'-en-1'-yl)-4,6-dimethoxy-2,3-diphenylindole	$C_{26}H_{24}BrNO$	461	2.42
7	9.65	GRAYANOTOXIN 111 3,6-DIPROPINATE 2,4-bis(Ethylmercapto)-7-(3'-methyl-5'-oxo-1'-	$^{C}26^{H}42^{O}8$	482	2.99
8	11.16	phenyl-2'-pyrazolin-4'-yl)-5-phenylpyrido[2,3-d]pyrimidine [6aR-(1a,4a,6ab,7a,10aa)]-1,4-epoxy-12-methoxy-1,7-bis(methoxymethyl)-7,10a-dimethyl-	$^{C}27^{H}25^{N}5^{OS}2$	499	5.10
9	12.40	1,4,5,6,6a,7,8,9,10,10a-decahydrochrysene and [6aR-(1a,4a,6aa,7b,10ab)]-1,4-epoxy-12-methoxy-1,7-bis(methoxymethyl)-7,10a-dimethyl-1,4,5,6,6a,7,8,9,10,10a-decahydrochrysene 1-(4'-Nitrophenyl)-4-(4''-N,N-	$^{C}25^{H}34^{O}4$	398	4.33
10	14.60	dimethylaminophenyl)imino-3-phenyl-4,5-dihydro-5-oxopyrazole Dimethyl 6,7-(methylenedioxy)-4-(3,4-	$^{C}23^{H}19^{N}5^{O}3$	413	2.34
11	18.56	methylenedioxyphenyl) naphthalene-2,3-dicarboxylate	$^{C}22^{H}16^{O}8$	408	2.04
12	19.01	1-[(2-Hydroxyethoxy)methyl]-5-n-propyl-6-(1-naphthylmethyl) uracil	$^{C}21^{H}24^{N}2^{O}4$	368	4.45
13	19.29	5,8-Dihydroxy-3,4',7-trimethoxyflavone	$^{C}18^{H}16^{O}7$	344	2.14
14	20.17	2,4-Dimethoxy[carboxy-13C]benzoic acid	$^{C}9^{H}10^{O}4$	182	2.79
15	20.59	13-di-O-acetyl-19,10-thiogibberellin A(1) methyl ester	$^{C}24^{H}30^{O}7^{S}$	462	6.91
16	20.86	4-Diallylaminomethyl-3-ethoxymethyl-2-methyl-3H-imidazole-4-carboxylic acid ethyl ester	$^{C}17^{H}27^{N}3^{O}3$	321	3.29
17	23.06	Ethyl ester of 2-[6-(ethylamino)-3-(ethylimino)-2,7-dimethyl-3H-xathen-9-yl]-benzoic acid	$^{C}28^{H}30^{N}2^{O}3$	442	2.60
18	24.47	16á,28-O,O-ETHYLIDENELUP-20(29)-ENE-3á,16á,28-TRIOL	$^{C}32^{H}52^{O}3$	484	4.90
19	25.36	2-[Diacetylamino]-6-(3'-methyl-5'-oxo-1'-phenyl-2'-pyrazolin-4'-yl)-4-phenylpyridine-3-carbonitrile	$^{C}26^{H}21^{N}5^{O}3$	451	4.93
20	27.85	Ethylene ketal of Methyl trans-(6-Methoxy-2-naphthyl)-1-methyl-5-oxocyclopentane-r-1-acetate	$^{C}22^{H}26^{O}5$	370	3.81
21	29.44	EXO-1,3-DIMETHYL-2,9-DIOXA-BICYCLO(3.3.1) NONANE	$^{C}9^{H}16^{O}2$	312	3.44
22	31.11	7-à-Acetoxy-(1Hà,6Hà)-bicyclo[4.4.1]undeca-2,4,8-trien-11-one	C13H14O3	218	2.18
23	31.33	Tetramethyl ester of 9-benzyl-9-azabicyclo-[4.2.1]nona-4,7-dien-1,4,7,8-tetroic acid	C23H25NO8	443	2.08
24	39.73	35.61 6à,7á-DIACETOXYDIHYDRODRIMENIN	C19H28O6	352	1.85
25	39.73	2- Phenyl-4,7-dihydro-4,7-methano-2H-isoindole	C15H13N	207	2.74
26	40,70	2,6-Diacetoxyandrosta-1,4,6-trine-3,17-dione	C23H26O	6 398	4.80
27	41.23	methyl elaiate	C19H36O2	296	2.48
28	41.93	ERIOCALYXIN C	C22H28O7	404	1.82


1
naphthalo[1,8-e,f]cycloheptene-3-en-1-one,-2,2-di(2'-cyanoethyl)
Formula C20H16N2O3, MW 332, CAS# 109141-85-7, Entry# 440177
Cycloheptal[de]naphthalene, propanenitrile deriv. (CAS)
2
1-(3,4,5-Trimethoxyphenyl)-6-methoxynaphthalene-2-carbaldehyde
Formula C21H20O5, MW 352, CAS# NA, Entry# 475342
3
Formula C27H25N5O2, MW 499, CAS# NA, Entry# 617375
4
Formula C25H34O4, MW 398, CAS# NA, Entry# 540415
5
1-[(2-Hydroxyethoxy)methyl]-5-n-propyl-6-(1-naphthylmethyl)uracil
Formula C21H24N2O4, MW 368, CAS# NA, Entry# 500212
6
3,13-di-O-acetyl-10,10-thiogibberellin A(1) methyl ester
Formula C24H30O7S, MW 462, CAS# 86383-99-7, Entry# 597256

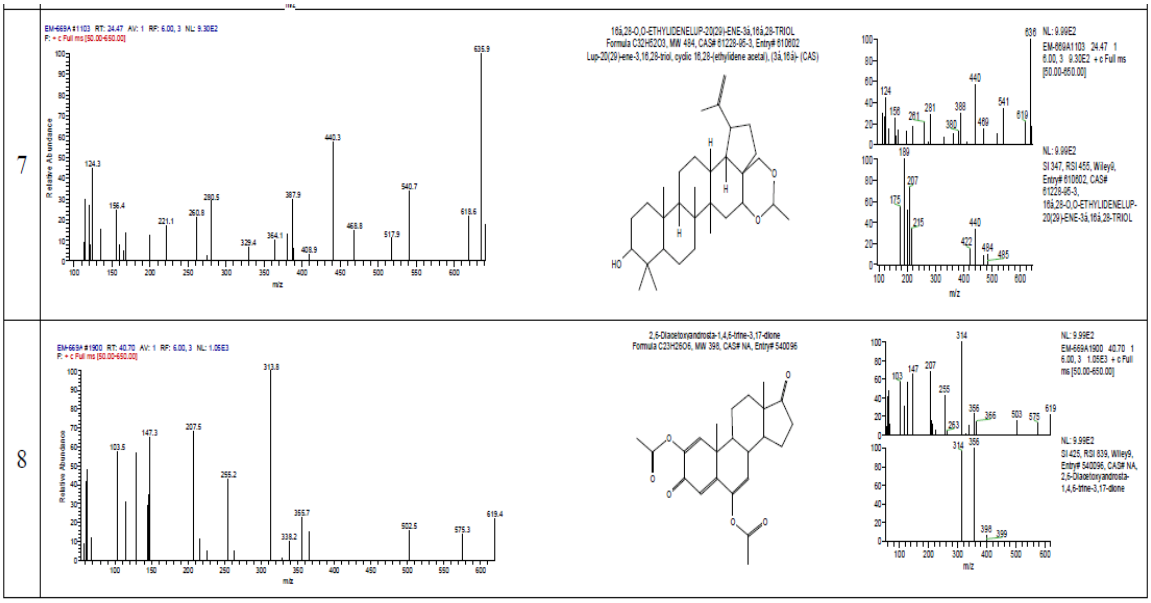


Figure 1 to 8 : GC-MS Chromatogram of Methanolic Extract of Stem of *Ceropegia woodii*.

CONCLUSIONS

The medicinal plants appear to be rich in secondary metabolites, widely used in traditional medicine to combat and cure various ailments. The anti-inflammatory, antispasmodic, analgesic and diuretic properties can be attributed to their high alkaloids, phenols, tannins and flavonoids. Exploitation of these pharmacological properties requires further investigation of these active ingredients by implementation of techniques like extraction, purification, separation, crystallization and identification.

REFERENCES

1. Adibatti NA, Thirugnanasambantham P and Kuilothngan C .A pyridine alkaloid from *Ceropegia juncea.* Phytochemistry (1991) 30:2449-2450.
2. Anonymous (1992). *Ceropegia* Linn. (Asclepiadaceae). In: The wealth of India.. New Delhi: CSIR: 3: 448-449.
3. Anonymous The Wealth of India: A dictionary of Indian raw materials and Industrial products: first supplement series (Raw materials): Vol. A-Ci. New Delhi, (1980). pp. 283.
4. Ansari MY Asclepiadaceae: Genus *Ceropegia* - Fascicles of Flora of India. Botanical Survey of India, Calcutta (1984). 16: 1-34.
5. Ayoola GA, Coker HAB, Adesegun SA, Adepoju–Bello AA , Obaweya K, Ezennia EC and Atangbayila TO, Phytochemical screening and antioxidant activities of some selected

medicinal plants used for malaria therapy in South Western Nigeria. Trop. J. Pharm. Res., (2008); 7: 1019-1024.

6. Balch, JF and Balch, PA. Prescription for Nutritional Healing. New York. A very,penguin Putnam Inc. (2000); 267-270.
7. Beltowski J, Gorny D and Maeciniak A. The mechanism of Na+ - K+ - ATPase inhibition by artiral nattruiretic factor in rat and medulla. J Physio Pharma, (1998); 49: 271-283.
8. Bertorello A and Aperra A. Short term of Na+ - K+ - ATPase activity by dopamine. Ameri J Hyper, (1990); 3: 515-545.
9. Boopathi AC and Sivakumar R. Phytochemical screening studies on the leaves and stem of *Andrographis neesiana* wight – An endemic medicinal plant from India. Worl d App Sci J, (2011); 12(3): 307-311.
10. Bruyns PV Three new succulent species of Apocynaceae (Asclepiadoideae) from southern Africa. Kew Bull (2003) 58:427–435.
11. BSI (2002). Studies on Rare and Endangered Species http://www.envfor.nic.in/bsi/research.html.
12. Clausen T and Nielsan O.B. The Na+ - K+ - ATPase and muscle contractivity. Acta Physio Scander. (1994); 152: 365-375.
13. Cook NC and Samman S, flavonoids chemistry, metabolism, cardioprotective effects and dietary sources. Nutr Bioche, (1996); 7: 66-76.
14. Duraisamy Suresh and Subramaniam Paulsamy Phenological observation and population dynamics of six uncommon medicinal plants in the grasslands of Nilgiris, Western Ghats, India *Maejo Int. J. Sci. Technol.* (2010) *4* (02), 185-192.
15. Frankel E, Nutritional benefits of flavonoids, International conference on food factors: chemistry and cancer prevention, Hamamatsu, Japan, Abstracts, C-2, (1995).
16. Gibbs RD, Chemotaxonomy of Flowering Plants. Vol.1, McGill Queen's University Press, Montreal and London, (1974).
17. Good R (1952) An atlas of the Asclepiadaceae. New Phytol 51:198–209.
18. Goyal D, Bhadauria S (2006). *In vitro* propagation of *Ceropegia bulbosa* using nodal segments. Indian J Biotechnol., 5: 565-567.
19. Gupta VK, Singh GD, Singh S and Kaul A, Medicinal Plants: Phytochemistry, Pharmacolgoy and Therapeutics, Daya Publishing House, Delhi. (2010).
20. Han X, Shen T and Lou H, Dietary polyphenols and their biological significance. Int J Mol Sci, (2007); 8: 950-988.

21. Harbone JB, Phytochemicals methods. London. Chapman and Hill, (1973).
22. Jagtap A, Singh NP (. Fasc. Flora of India 24, Botanical Survey of India, Kolkata, 1999) pp. 211-241.
23. Jain SK, Defillips RA (1991). Asclepiadaceae. In: Medicinal plants of India. Algonac, India 1:89-94.
24. Jain A, Soni M, Deb L, Jain A, Rout S, Gupta V, Krishna K. Antioxidant and hepatoprotective activity of ethanolic and aqueous extracts of Momordica dioica Roxb. leaves. J. Ethnopharmacol. 2008; 115(1): 61-66
25. Kessler M. Ubeand G and Jung L, Anti and prooxidant activity of rutin and quercetin derivatives, J. Pharm and Pharmacol, (2003); 55: 131-142.
26. Khandelwal KR. Practical Pharmacognosy, 16th Edition: Nirali Prakashan, Pune, 2006; 155-156.
27. Khadbadi SS, Deore SL, Baviskar BA. Experimental Phytopharmacognosy, 1st edition, Pune, Nirali Prakashan, 2011; 47.
28. Killedar SG and More HN, Estimation of tannins in different parts of *Memecylon umbellatum* Burm., J Phar Res, (2010); 3(3): 554-556.
29. Kirtikar KR, Basu BD (1935). Indian Medicinal Plants Vol.3 Bishen Singh Mahendra, New Delhi, India.
30. Kumar A, Ilavarasan R, Jayachandran T, Decaraman M, Aravindhan P, Padmanaban N and Krishna MRV. Phytochemical investigation on a tropical plants. Pak J Nutri, (2009); 8: 83-85.
31. Linuma M, Tsuchiya H, Salo M, Yokoyama J, Ohyama M, Ohkawa Y et al., Flavanones with potent antibacterial activity against methicillin-resistant staphylococcus aureus. J. Pharm. Pharmacol, (1994b); 46(11): 892-895.
32. Mabberly DJ (1987). The plant book. Cambridge University Press, Cambridge, pp.114-115.
33. Madhav Gadgil (2004). ENVIS Technical Report No. 16, Environmental Information, Bangalore, http://www.ces.iisc.ernet.in/hpg/envis pp. 96-98.
34. Mukherjee PK, Kumar V, Houghton PJ. Screening of Indian medicinal plants for acetyl cholinesterase inhibitory activity. Phytother Res (2007); 21: 1142-1145.
35. Nadkarni KM) Indian MateriaMedica. Popular Prakasha, Bombay, India, ISBN: 81-7154-144-5, (1976) pp.: 303-304.

36. Nayar MP, Sastry ARK Red Data Book of Indian plants Vol. 1 Botanical Survey of India, Calcutta. pp. (1987). 170.
37. Ncube NS, Afolayan AJ and Okoh AI, Assessment techniques of antimicrobial properties of natural compounds of plant origin: Current methods and future trends. African J Biotechnol., (2008); 7: 1797-1806.
38. Nikam TD, Savanth RS Callus culture and micropropagation of *Ceropegia sahyadrica* Ans. et Kulk: An edible starchy tuberous rare asclepiad. Indian J. Plant Physiol., 12: (2007). 108-114.
39. Okwu, D.E. Evaluation of Chemical composition of indigenous species and flavouring agents. Global J. Pure Appl. Sci., (2001): 458-459.
37. Okwu, D.E. Evaluation of the chemical composition of indigenous species and flavouring agents. Global J. Pure Appl, Sci, (2001B); 8; 455-459.
38. Okwu, D.E. Improving the nutrition value of cassava tapioca meal with local species. Nutracentical, Functional and medicinal food, (2001A); 3: 43-51.
39. Parekh Jigna and Chanda V Sumitra. *In vitro* antimicrobial activity and phytochemical analysis of some Indian Medicinal plants. Turk J Biol, (2007); 31: 53-58.
40. Peach K and Tracey MV, Modern methods of plant analysis. Vol.3, Springer Verlag, Berlin, (1956).
41. Robards K, Prernzler PD, Tucker G, Swatsitang P and Glover W, Phenolic compounds and their role in oxidative processes in fruits. Food Chem., (1999); 66: 401-36.
42. Salah, W., N. Miller, G. Pagauga, G. Tybury, E. Bolwell, E. Rice and C. Evans, Polyphenolic flavonoids as scavenger of aqueous phase radicals and chain breaking antioxidants. Arch. Biochem., (1995). 2: 239-346.
43. Savithramma N, Linga Rao M and Beenaprabha, Phytochemical studies of *Dysophylla myosuroides* (Roth.) Benth. In. wall. and *Talinum cuneifolium* (Vahl.) Willd. Res J Phyto, (2011); 5(3): 163-169.
44. Savithramma N, Venkateswarlu P, Suhrulatha D, Basha SKM and Venkataramanadevi CH, Studies of *Boswellia ovalifoliolata* Bal. and Herny – An endemic and endangered medicina l plant. The Biosc., (2010); 5: 359-362.
45. Sheeja K, Kuttan G. Activation of cytotoxic T lymphocyte responses and attenuation of tumor growth in vivo by Andrographis paniculata extract and andrographolide. Immunopharmacol Immunotoxicol (2007); 29: 81-93

46. Stalh E. Drug analysis by chromatography and Microscopy. A Practical Supplement to Pharmacopoeias, 1st ed., Ann Arbor, Michigan: 1973; 219-224.
47. Treare GE and Evans WC, Pharmacognosy 17th edn, Bahive Tinal, London, (1985): 149.
48. Turker AU, Usta C. Biological screening of some Turlish medicinal plants for antimicrobial and toxicity studies. Nat Prod (2008); 22: 136-146.
49. Vijayakumar S, Chandrasekar S & Prabhu S Screening of ethnomedicinal plants for antibacterial activity International Journal of Medicine and Pharmaceutical Sciences (2013) Vol. 3, Issue 2, , 11-20.
50. Vinoth S, Rajesh Kanna P, Gurusaravanan P and Jayabalan N, Evaluation of phytochemical, antimicrobial and GC-MS analysis of extracts of *Indigofera trita* L.F. spp. Subulata (Vahl ex poir). Int J Agric Res. (2011); 6(4): 358-367.