Available online on www.ijppr.com
International Journal of Pharmacognosy and Phytochemical Research 2017; 9(7); 914-920

ISSN: 0975-4873

Research Article

Phytochemical Analysis, Antibacterial Activity, FTIR and GCMS Analysis of *Ceropegia juncea* Roxb.

Visveshwari M*, Subbaiyan B, Thangapandian V

Dept. of Botany Kongunadu arts and Science College, Coimbatore, Tamilnadu India

Received 25th March, 17; Revised 12th June, 17, Accepted 12th July, 17; Available Online 25th July, 2017

ABSTRACT

The present study was focused on the preliminary phytochemical, antibacterial activity, FTIR and GC-MS analysis of aerial parts of *C. juncea*. Phytochemical analysis of three extracts showed that the presence of alkaloids, tannins and flavonoids. The methanol extract of aerial parts were found to be exhibit highest zone of inhibition against *S. aureus* (19.3mm), *P. florescence* (17.6mm) and *K. pneumonia* (17.0mm). FTIR analysis of the methanol extract given the major peak observed was at wavenumber 3354.08cm-1 that indicates the presence of O-H Alcohol functional group. 29 components were identified through the GCMS analysis of methanol extract. From results to confirm the fact that *C. juncea* possesses potential of bioactive compounds which are responsible for the biological activities that is useful for natural health.

Keywords: *Ceropegia*, FT-IR, GC-MS, alkaloids, antibacterial activity.

INTRODUCTION

The medicinal plants are not only used as medicines to maintain their health care, also consumed as food by several Tribes of Indian subcontinent[1]. Phytochemicals are responsible for medicinal activities of the plants. Based on this fundamental knowledge several pharmaceutical industries are established. The phytochemical constituents that are playing a significant role in medicines can be identified using crude extracts/drugs of the plants[2]. These are non-nutritive chemicals that protect human beings from various diseases. Phytochemicals are basically divided into two major groups, there are primary and secondary metabolites that are categorized 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 and tannins[3,4].

The phytochemicals of the plants are chemical compounds formed during the normal metabolic processes. These chemicals are often referred to as "Secondary metabolites". The determination of phytoconstituents is largely performed by relatively expensive and often laborious techniques that are gas chromatography (GC) and liquid chromatography (LC) combined with specific detection schemes[5,6]. Analysis of chemicals has become easier and more cost-effective owing to the development of hyphenated chromatographic techniques, which are GC or LC-MS. GC-MS analysis can identify nature of compounds even less than 1mg present in the crude plant extract[7]. However, simple, cost-effective and rapid tests for detecting phytocomponents are necessary. In recent years Gas Chromatography–Mass Spectrum (GC-MS) technique has been increasingly employed to analyze the secondary metabolites present in the medicinal plants, as this technique has been proved to be a best valuable method for the analysis of essential oil, alcohols, acids, esters, alkaloids, steroids, amino and nitro compounds[8,9]. Plants derived natural products such alkaloids, flavonoids, terpenoids have received considerable attention in recent years due to their diverse pharmacological properties including antimicrobial, antioxidant and anticancer activities[10]. Plant crude extracts were containing large amount of natural antioxidants, which are used as folkloric medicines[11]. *C. juncea* is a twining leafless tuberous succulent herb, distributed in drier parts of Peninsular India and is well known medicinal plant since the Vedic period, which is reported to be the source of an Ayurvedic drug 'Soma' used for a variety of disorders[12,13].

MATERIALS AND METHODS

Plant material

Ceropegia juncea Roxb. was collected from the foothills of Madukkarai, the Western Ghats of Coimbatore district, Southern India. The plant was identified by Dr. V. Balasubramaniam, The Systemic Botanist and Associate Professor of Botany, Kongunadu Arts and Science College, Coimbatore, Tamilnadu, India.

Extraction of plant material

Plant materials were thoroughly washed and shade dried at room temperature. The dried materials were ground well using pulveriser into powder and packed with No.1 Whatman filter paper. Package was placed in a Soxhlet apparatus and running with different solvents. The crude extracts were collected and dried at room temperature,

*Author for Correspondence: junceavisu728@gmail.com

30˚C after which yield was weighed and taken for further analysis.

Aqueous extract

25g of whole plant part powder were dissolved in 100ml hot distilled water containing conical flask that was kept on a rotary shaker for 12 hours and allowed to run at 80rpm. The residues were filtered using No. 1 Whatman filter paper. Then the collected residues were dried first on a hot water bath to remove wetness and then kept at the oven. After drying, the residues were scraped to weigh 5mg and dissolved in 5ml sterile distilled water to prepare aqueous extract and it was stored in a refrigerator at 4°C for further use.

Preliminary phytochemical investigation

The petroleum ether, methanol and aqueous extracts were subjected to phytochemical screening to identify the presence of alkaloid, tannins, saponin, flavonoid, terpenoid, glycosides and phenol. Presence of bioactive compounds was determined using the standard methods[14-16].

Antibacterial activity

Three gram positive bacterial pathogens, *Staphylococcus aureus, Streptococcus faecalis* and *Proteus florescence* and three gram negative bacterial pathogens, *Pseudomonas aeruginosa, Klebsiella pneumonia* and *E. coli*, were used in this study. All the extracts were impregnated with inoculated agar nutrient medium to study the antibacterial activities. The inoculation of bacterial cultures was incubated over night at 28˚C for 24 hr. Well diffusion method [17] for assessing the antibacterial activity was applied using Muller Hinton agar medium. Inhibition of each pathogen was observed and recorded using the triplicates. After the incubation period, the diameter of the inhibition zones was measured[18].

FT-IR Spectroscopic Analysis

Fourier Transform Infrared Spectrophotometer (FTIR) is perhaps the most powerful tool for identifying the types of chemical bonds/functional groups present in the phytochemicals. The wavelength of light absorbed is the salient feature of the chemical bonds seen in the annotated spectrum. By interpreting the infrared absorption spectrum, the chemical bonds in a compound can be determined. Dried powder of methanol solvent extracts of *C. juncea* was used for FTIR analysis. 10mg of the dried extract powder was encapsulated in 100mg of KBr pellet, in order to prepare translucent sample discs. The powdered sample of each extract was loaded in FTIR spectroscope (Shimadzu, Japan), with a Scan range from 400 to 4000 cm^1 with a resolution of 4 cm^{-1}.

GC-MS analysis

Gas chromatography study includes the important optimization process such as i) introduction of sample extract onto the GC column, ii) separation of its components on an analytical column and iii) detection of target analysis by using mass spectrometry (MS) detector. 5ml of ethanol extract was evaporated to dryness and reconstituted into 2ml methanol. The extracts were then subjected to GC-MS analysis. Chromatographic separation was carried out with instrument GC-MS-QP 2010 (SHIMADZU instrument) with Db 30.0 column (0.25 µm diameter × 0.25 µm thickness). The oven temperature was programmed from 70°C (isothermal for 5 minutes), with an increase of 10°C/min. up to 200°C, then 5°C/min. up to 280°C and ending with 35 minutes isothermal at 280°C. Mass spectra were taken at 70 eV; scan interval of 0.5 seconds and scan range from 40–1000 m/z. Helium was used as the carrier gas at 99.99 % pressure with flow rate of 1.0 ml/min. and electronic pressure control on Samples were dissolved in methanol and injected automatically.

RESULTS

Preliminary phytochemical analysis

The results of qualitative analysis of the crude methanol extract of *C. juncea* shown in Table-1. It revealed the presence of alkaloid, tannins, flavonoids, terpenoids, saponins. Methanol extracts were found to contain more phytochemical constituents compared to aqueous and petroleum ether extracts. As the methanol extract has maximum numbers of phytochemical constituents that are taken for further studies.

Antibacterial activity

Based on the above observations, the methanol, PET and aqueous extracts were evaluated for the effect of antibacterial activity against six pathogenic bacterial strains include 3 gram positive and 3 gram negative strains. Streptomycin was used as the standard. All the above said extracts were used against all the six pathogenic bacteria in this study by employing the disc diffusion technique (Table- 2). The results revealed that the extracts of this plant was effective against both gram positive and gram negative bacteria.

Methanol extract exhibited highest zone of inhibition against gram positive bacteria, *S. aureus* (19.3±0.5 mm) followed by *P. florescence* (17.6 ±0.52 mm) and *Streptococcus faecalis* (16.0±1.24 mm). Whereas inhibitory zones developed against gram negative bacteria were 17.0±0.81 mm in *K. pneumonia*, 15.33±0.94 mm in *E. coli* and 11.0±1.00 mm in *P. aeruginosa*. Gram positive bacteria were effectively controlled by the methanol extract compared to gram negative bacteria. Among the three extracts tested, methanol extract inhibited both the strains than other two extracts. Aqueous extract inhibited both the strains effectively than the PET extract, except against the bacteria *P. aeruginosa* and *K. pneumonia* (Table- 2). Thus lowest zone of inhibition was exhibited by the PET extract.

Fourier Transform Infrared Spectroscopic Analysis

The FTIR spectrum of methanol extract of *C. juncea* is presented in Table- 3: figure 1. The data on the peak values and the probable functional groups (obtained by FTIR analysis) present in the methanol extracts of *C. juncea* are represented in Table-. The region of IR radiation helps to identify the functional groups of the active components present in extract based on the peaks values of the FTIR spectrum. When the extract was passed into the FTIR, the functional groups of the components were separated based on its peaks ratio. The results of FTIR analysis confirmed the presence of

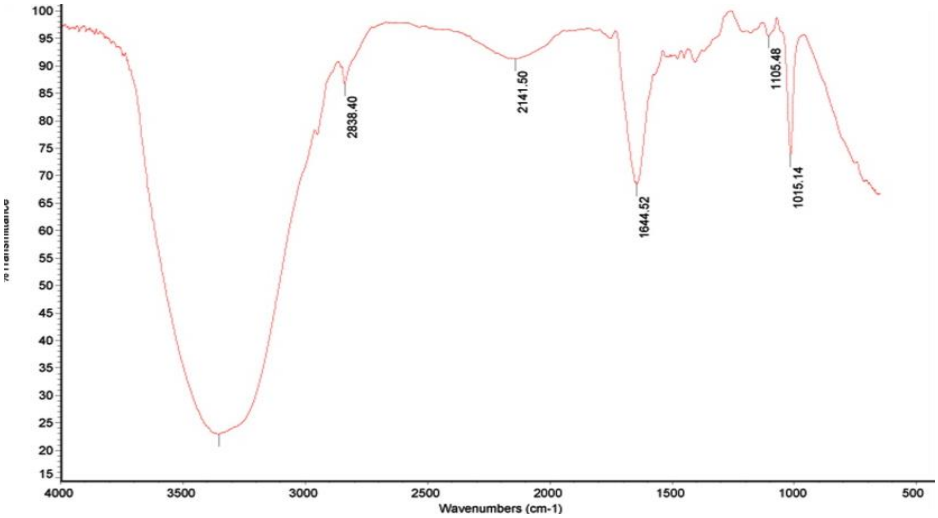

Figure 1: FTIR- Spectrum wave numbers of methanol extract of *C. juncea*.

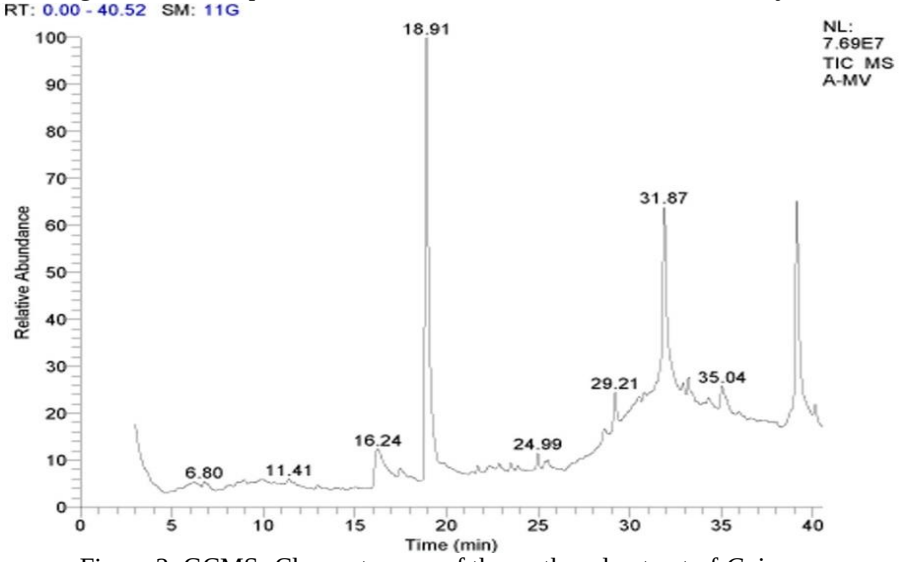

Figure 2: GCMS- Chromatogram of the methanol extract of *C. juncea*.

Table 1: Phytochemical constituents present in different solvent extracts of the aerial parts of *C. juncea*.

S. No.	Phytochemical Constituents	Aerial parts PET	Methanol	Aqueous
1	Alkaloids	+	++	+
2	Flavonoids	+	++	+
3	Phenol	-	+	+
4	Terpenoids	+	++	+
5	Tannins	+	+	-
6	Glycosides	+	+	+
7	Saponins	-	-	+
8	Coumarin	-	+	+
9	Reducing sugar	-	+	+
10	Cardio glycosides	-	+	-

+ Present, - absent

Alcohol, Aldehyde, Alkyne, Alkene, Amines and Ester. The absorbance bands analyses in bioreduction process are observed in the region between 400–4000 cm^{1} are 1015.14, 1105.48, 1644.52, 2141.50, 2838.40 and 3354.08. Major peaks were observed at 3354.08 cm^{1} that could be assigned to the 0-H stretching vibrations of O-H Alcohol. So the present study results indicate that the primary functional group present in *C. juncea* is O-H Alcohol. Other functional groups present in the methanol extracts of *C. juncea* are shown in Table- 4.

Gas Chromatography-Mass Spectroscopy analysis

The results pertaining to GC-MS analysis led to the identification of 29 compounds from the GC fractions of the methanol extract of *C. juncea*. These compounds were identified through Mass Spectrometry attached with GC. The results were tabulated (Table- 4 & fig. 2).The gas chromatogram shows that the relative concentration of various compounds getting fractionated at their specific retention time. The heights of the percentage of peak area indicate the relative concentrations of the components present in the methanol extract of *C. juncea*. The maximum amount of bioactive compound found in the methanol extract of *C. juncea*is p-(Dimethylamino) benzaldehydeoxime. The compound is recognized by its peak area percentage (39.89%) on comparing with the data library. The compounds identified by GC-MS from

Table 2: Antibacterial activities exhibited by different solvent extracts of *C. juncea*.

S.No.	Bacterial pathogens	Plant extracts		
		MeoH	PET	Aqueous
1	*Pseudomonas aeruginosa*	11±1.00	14±0.81	13.0±0.81
2	*Streptococcus faecalis*	16.3±1.24	12.3±0.84	12.5±1.69
3	*Proteus florescence*	17.6±0.52	11±0.81	10.6±1.24
4	*Klebsiella pneumonia*	17±0.81	10.6±0.47	11.5±0.40
5	*Staphylococcus aureus*	19.3±0.57	15.2±1.7	15.2±0.81
6	*E. coli*	15.3±0.94	9.3±1.24	14.3±0.94

Table 3: FTIR spectral wavenumber's values and functional groups obtained from the aerial parts extract of *C. juncea*.

S. No.	Wavenumbers	Functional groups
1	3354.08	O-H Alcohol
2	2838.40	=C-H Aldehyde
3	2141.50	-C=C- Alkyne
4	1644.52	C=C Alkene
5	1105.48	C-H Amines
6	1015.14	C-O Ester

the methanol extract of *C. juncea* are medicinally valuable and possess various pharmaceutical applications.

DISCUSSIONS

Traditional healers and local tribal people are generally used water as the solvent, though several organic solvents are available. Methanol extract of *C. juncea* revealed the presence of alkaloid, flavonoids, terpenoids, tannins and glycosides. Saponins are absent in methanol extract but it is found in the aqueous extract of *C. juncea*. Alkaloid exhibited promising antidiarrheal, antiinflammatory, anticancer and antidiabetic activities and cure urinary disorders[19-20]. Flavonoids are also known as a vitamin p elicit a wide range of therapeutic activities such as antihypertensive, antirheumatism, antidiuretic, antioxidant, antimicrobial and anticancer properties[21-22]. Tannin and phenols are acting as antimicrobial agents[23]. The present study results confirmed the presence of these components in the methanol extracts of *C. juncea*. Hence the usage of this plant in traditional systems of medicine is in accordance with the results of the above workers.

The activities of zone of inhibition values are helpful in estimating the potential of antibacterial activities by comparing their respective controls. Pampaloma-Roger[24] reported that plant extracts containing chemicals with antibacterial properties have been useful in treating bacterial and fungal infections. Similar types of antimicrobial activities were found in *Ceropegia pusilla*[25]. Similarly antibacterial activities of the methanol extracts of *Ceropegia bulbosa* were tested against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Streptococcus faecalis*, *Klebsiella pneumonia* and *E. coli* by Subbaiyan *et al*.[26].

Previously the preliminary phytochemical analysis and antimicrobial properties of some *Ceropegia* species; *Ceropegia pusilla*[25], *Ceropegia spiralis*, *Ceropegia candelabrum*[27] and *Ceropegia juncea*[28] have been reported by many workers against some human pathogenic microorganisms. The results of this present study are coincided with the results of these researchers. Because the crude methanol extract of *C. juncea* could inhibit the growth of all pathogenic bacteria. The level of inhibitions varies between the bacteria. Certain well known urinary tract infection and urinary disorder causing bacteria, *Escherichia coli* and *Staphylococcus aureus*, were predominantly controlled by this crude methanol extract of *C. juncea*. Hence, the crude methanol and aqueous extracts can be used as natural antibacterial compounds to prevent the infection of these bacteria.

The FTIR analysis of methanol and aqueous leaf extracts of *Bauhinia racemosa* revealed the presence of protein, oil, fats, phenolic compounds, flavonoids, saponins, tannins and carbohydrate as major functional groups[29]. Starlin *et al*.[30] analyzed the ethanol extracts of *Ichnocarpus frutescens* using FTIR analysis that revealed functional group components of amino acids, amides, amines, carboxylic acid, carbonyl compounds, organic hydrocarbons and halogens. Nithyadevi and Sivakumar[31] also worked in the methanol leaf extracts of *Solanum torvum*to confirm the presence of alcohol, alkanes, aromatic carboxylic acid, halogen compound, alkyl halide through the FTIR analysis. The characterization and antibacterial effect of plant-mediated silver nanoparticles using *Ceropegia thwaitesii* was carried out by Muthukrishnan *et al*[32] and the presence of triterpenoids and methoxy groups played an important reduction role in the synthesis process was also authorized by them using FTIR. The absorbance bands analysis in bioreduction is observed in the region of 400–4000 cm^1 are 1024.02, 1383.68, 1629.55, 2921.63 and 3449.30 cm^1. Major peaks were observed at 2921 cm^1 that could be assigned to the C-H stretching vibrations of methyl, methylene and methoxy groups[32-33]. But major peaks observed in the crude methanol extract of *C. juncea* are 3354.08 and 2838.40 that are indicating the presence of O-H Alcohol and =C-H Aldehyde groups. The result of the FTIR analysis is contradictory to the results of Muthukrishnan *et al*.[32] and Feng *et al*.[33].

The FTIR spectroscopic studies revealed the presences of alcohol, phenols, alkanes, alkynes, alkyl halides, aldehydes, carboxylic acid, aromatics, nitro compounds and amines were observed from ethanol leaf extract of *Gmelina asiatica* by Florence and Jeeva[34].FTIR analysis of the crude methanol extract of *C. juncea* revealed the presence of the functional groups of alcohol, aldehyde, alkyne, alkene and amines, except ester. The results of the present study are in accordance with the study of Florence and Jeeva[34]. FTIR analysis of various parts of *Sageretia thea* showed different functional groups like

Table 4: GC-MS analysis revealed the presence of bioactive compounds in the aerial parts of methanol extract of *C. juncea*.

S. No.	Area	Retention time	Compound name	Molecular formula	Molecular weight
1	1.17	68.0	(Z)-3-(2'-(2"-(9"',10"'-Anthracenedionyl))ethenyl)-10-methyl-10H-phenothiazine	$C_{29}H_sNO_3$	577
2	0.71	6.80	N-Phenyl-2,3,4,5-tetramethyl-7-thiabicyclo(2.2.1)hept-5-ene-2,3-dicarboxamide 7-oxide	$C_{18}H_{19}NO_3S$	329
3	0.33	8.04	-Acetoxy-3à,hy-Dihydroxy-3à,12à epoxy-8à,14à 5á-cholanoate de methyl	$C_{25}H_{40}O_5$	420
4	0.47	8.94	Morphinan-4,5-epoxy-3,6-di-ol,6-(7-nitrobenzofurazan-4-yl)amino-	$C_{26}H_{27}N_5O_6$	505
5	0.50	9.91	1,4-Di-tert-butyl-2-(difluorophenylsilyl)-3,3,5,5-tetramethyl-1,2,4-triaza-3,5—isilacyclopentane	$C_{18}H_{35}F_2N_3Si_3$	415
6	0.75	11.41	Ethyl 5,7-diphenyl-2-(1',3'-diphenyl-5'-hydroxypyrazol-4' -yl)-pyrazolo(3,4-b)pyridine-3-carboxylate	$C_{36}H_{27}N_5O_3$	577
7	0.38	12.98	exo-8-(Benzenesulfonyl)-9,11,11-trimethyl-2,10,12-trioxatricyclo(7.2.1.0(1,6))dodecane	$C_{18}H_{24}O_5S$	352
8	4.86	16.19	9,12,15-Octadecatrienoic acid, methyl ester (CAS) 5.99 C19H32O2 292 4.86 358 396 Manganese,	$C_{19}H_{32}O_2$	292
9	1.05	17.48	1-Benzoxiren-3-ol, 2,2,5a-trimethyl-1a-(2-(2-methyl)-1,3-dioxolan-2-y l)-1-ethenyl)perhydro	$C_{15}H_{24}O_4$	268
10	39.89	18.89	p-(Dimethylamino)benzaldehydeoxime	$C_9H_{12}N_{2O}$	164
11	0.43	21.70	Hexadecanoic acid, methyl ester (CAS)	$C_{17}H_{34}O_2$	270
12	0.97	2.35	Methyl (12E)-12-((2,4-dinitrophenyl)HYDRAZONO)dodecanoate	$C_{19}H_{27}DN_4O_6$	408
13	0.81	22.87	Phthalic acid, hex-3-yl isobutyl este	$C_{18}H_{26}O_4$	306
14	0.63	23.51	(-)-Loliolide	$C_{11}H_{16}O_3$	196
15	0.40	23.89	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione.	$C_{17}H_{24}O_3$	276
16	0.96	24.99	Dibutyl phthalate	$C_{16}H_{22}O_4$	278
17	1.03	25.52	Octadecanoic acid, methyl ester (CAS)	$C_{19}H_{38}O_2$	928
18	0.35	26.84	3,3'-bis(2-Bromo-5-(trimethylsilyl)thiophene)	$C_{14}H_{20}Br_2S_2Si_2$	466
19	1.04	28.61	4'-(4-{2-(trimethylsilyl)-1-ethynyl}phenyl)-2,2':6',2"-terpyridine	C $H_{23}N_3Si$	405
20	2.77	29.21	2,6,10,14,18,22-Tetracosahexaene,2,6,10,15,19,23-hexamethyl- (CAS)	$C_{30}H_{50}$	410
21	0.95	30.51	3,3'-bis(2-Bromo-5-(trimethylsilyl)thiophene)	$C_{14}H_{20}Br_2S_2Si_2$	466
22	0.62	30.77	1(10)-Methoxy-6-thia-1(1,8)-anthracena-4,8(1471,3)dib enzacyclodeca-2,9-diynaphane	$C_{33}H_{22}OS$	466
23	14.59	31.87	13-Docosenamide, (Z)-	$C_{22}H_{43}NO$	337
24	0.56	32.92	Dimethyl4-amino-5-oxo-5H-dibenzo(c,f)-2H-chromen-2,3-d	$C_{21}H_{15}NO_6$	377
25	1.55	33.21	1-(2-Hydroxymethoxy-6-methoxyphenyl)ethanol	$C_{10}H_{14}O_3$	182
26	1.19	34.29	5-methyl-1,2,4-triazine-6-thione	$C_4H_5N_3S$	127
27	0.56	35.96	5-(2-Bromo-4,5-dimethoxyphenyl)-4-(2-bromophenyl)-1-phenylpyrazole	$C_{23}H_{18}Br_2N_2O_2$	512
28	0.30	36.59	Epinephrine-Tetratms	$C_{21}H_{45}NO_3Si_4$	471
29	17.48	39.12	13-Docosenamide, (Z)-	$C_{22}H_{43}NO$	337

amides, alkanes, bending water, lipids, alkenes, aromatic ring and chlorides compounds, which shows peaks at range of 3305- 3325, 2920-2937, 2050-2098, 1739-1769, 1617-1639, 1440-1505 and 405-4027[35]. Similarly, the present investigation FTIR analysis revealed the presence of functional group, alkene at peak value 1644.52. But other functional groups are absent. Early studies of FTIR analyses were also reported in some medicinal plants, *Calotropis gigantea*[36]; *Tylophora pauciflora*[30]; *Caralluma geniculate*[37] and *Caralluma nilagiriana*[38]. Kalimuthu and Prabakaran[25] reported 28 compounds with different chemical structures in the methanol extract of *Ceropegia pusilla*. Palawat and Payal[39] carried out the Gas chromatography mass spectroscopic investigation of methanol extract of *Ceropegia bulbosa*, an annual land plant using GCMS technique. They compared the mass

spectra of the compounds with the standard library of NIST. Maximum peak area % found in leaf extract are 2H-Azepin-2-one, 3-(dimethylamino) hexahydro, 9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z) and followed by 2H-Azepin-2-one, 3-(dimethylamino) hexahydro, 2-Amino-9-(3,4-Dihydroxy-5-Hydroxymethyl-(16.08%) in the methanol tuber extract of *C. bulbosa*. Though 29 compounds were identified using GC-MS analysis, the maximum amount of bioactive compound found in the methanol extract of *C. juncea* is p-(Dimethylamino) benzaldehydeoxime. The compound is recognized by its peak area percentage (39.89%) on comparing with the data library.

CONCLUSION

From this study, it's concluded that the presence of phytocompounds in *Ceropegia juncea* crude extract of aerial parts suggests that the contribution of these compounds in the pharmacological activity should be evaluated. However further studies will need to be undertaken to isolate and screening of bioactive compounds from methanol extract of aerial parts and find out its biological activity.

ACKNOWLEDGEMENTS

First author is greatly thankful to Dr. V. Thangapandian, Asso. Professor, Dept. of Botany, Kongunadu Arts and Science College [Autonomous], Coimbatore-641 029, his for valuable guidance and supervision of this work. All authors thanks to the Asso. Professor and Head, Department of Botany, Kongunadu Arts and Science College [Autonomous] for providing necessary facilities.

REFERENCES

1. Samydurai P, Thangapandian V and Aravindhan V. Wild habits of Kolli Hills being staple food of inhabitant tribes of Eastern Ghats, Tamil Nadu, India. *Indian J. of Natural Product and Resou*. 2012;3:432-437.
2. Savithramma N, Venkateswarlu P, Suhrulatha D, Basha SKM and Venkataramanadevi CH. Studies of *Boswellia ovalifoliolata* Bal. and Herny – An endemic and endangered medicinal plant. *The Biosc*. 2011; (5): 359-362.
3. Jigna P and Sumitra CV. *In vitro* antimicrobial activity and phytochemical analysis of some Indian Medicinal plants. *Turk J Biol*. 2007;31:53-58.
4. 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.
5. Uzer A, Ercag E, Apak R. Selective spectrophotometric determination of TNT in soil and water with dicyclohexylamine extraction. *Anal. Chim. Acta* 2005;(534): 307-317.
6. Eisenhauer N, Klier M, Partsch S, Sabais ACW, Scherber C, Weisser W and Scheu S. No interactive effects of pesticides and plant diversity on soil microbial biomass and respiration. *Appl. Soil Ecol*. 2009;(42):31-36.
7. Lieble DC, Burr JA, Philips L, Ham AJL. Gas chromatography - mass spectrometry analysis of vitamin E and its oxidation products. *Anal. Biochem*. 1996; 236:27-34.
8. Muthulakshmi A, Margret J and Mohan VR. GC-MS analysis of bioactive components of *Feroniaele phantumcorrea* (Rutaceae). *App. pharmac. Sci*. 2012; (2):69-74.
9. Konovalova O, Gergel E and Herhel V. GC-MS analysis of *Shpherdia argentea* (Pursh.) Nutt. From Ukraineen flora. *The Pharma Journal*. 2013;2(6):7-12.
10. Takeoka GR, Dao LT. Antioxidant constituent of almond (*Prunus dulcis* (Mill.)D.A.Webb) hulls. *J Agri Food Chem*. 2003;51:496-501.
11. Kumbhare MR, Sivakumar T, Udavant PB, Dhake AS and Surana AR. *In vitro* antioxidant activity, phytochemical screening, cytotoxicity and total phenolic content in extracts of *Caesalpinia pulcherrima* (Caesalpiniaceae) pods. *Pakistan Journal of Biological Sciences.* 2012;15(7): 325-332.
12. Asolkar LV, Kakkar KK and Chakre OJ. IInd supplement to the Glossary of Indian Medicinal plant with Active Principle. Part 1 (A-K), Publication and Information Directorate, CSIR, New Delhi. 1992;193.
13. Jagtap AP and Singh NP. Asclepiadaceae and Periplocaceae. Fascicles of Flora of India, Pb1. Botanical Survey of India, Kolkata. Fascicle 1999;(24): 211-241.
14. Harborne JB. 1973. Phytochemical methods, London Chapman and Hall, Ltd. 49-88.
15. Trease GE, Evans WC. 1989. Pharmacognosy 11th Edn, Brailliar Triidalcan Macmillian Publishers.
16. Sofowora A. Medicinal plants and traditional medicine in Africa. 2nd ed. Sunshine house, Ibadan, Nigeria: Spectrum books Ltd. Screening plants for bioactive agents 1993;134-156.
17. Collins CH, Lynes PM and Grange JM. Microbiological Methods. (7 thEdn.) Butterwort-Heinemann Ltd., Britain 1995;175-190.
18. Govindasamy C and Srinivasan R. *In vitro* antibacterial activity and phytochemical analysis of *Catharanthus roseus* (Linn) G. Don. *Asian Pacific Journal of Tropical Biomedicine*. 2012;155-158.
19. Awoyinka AO, Balogun IO, Ogunnowo AA. Phytochemical screening and *in vitro* bioactivity of *Cnidoscolus aconitiifolus* (Euphorbiaceae). *J. Med. Pla. Res.* 2007; 1(3): 63-65.
20. Monika J, Anil B, Aakanksha B, Priyanka P. Isolation, Characterization and *In vitro* Antiurolithiatic activity of Cerpegin Alkaloid from *Ceropegia bulbosa* var. *lushii* root. *Int. J. Drug Dev. and Res*. 2012;4(4): 154-160.
21. Del-Rio A, Obdulio BG, Castillo J, Marin RR and Ortuno A. Uses and properties of citrus flavonoids. J. Agric. *Food Chem.* 1977;45:4505-4515.
22. Veerachari U, Bopaiah AK. Preliminary phytochemical evaluation of the leaf extract of five Cassia species. *J. Chem. Pharm. Res.* 2011;3(5):574-583.

23. Ebrahimzadeh MA, Pourmorad F and Bekhradnia AR. Iron chelating activity screening, phenol and flavonoid content of some medicinal plants from Iran. *Afr. J. of Biotech*. 2008;7(18): 3188-3192.
24. George D and Pamplona R. Encyclopedia of medical plants. Editional Safelize Spain. 1999;1:381.
25. Kalimuthu K and Prabakaran R. Preliminary phytochemical screening and gc-ms analysis of methanol extract of *Ceropegia pusilla*. *International Journal of Research in Applied, Natural and Social Sciences.* 2013;1(3): 49-58.
26. Subbaiyan B, Jagatheskumar S, Mahendran G and Thangapandian V. Preliminary phytochemical screening, antibacterial activity and Gas chromatography and Mass spectrum analysis of *Ceropegia bulbosa* roxb. (Asclepiadaceae). International *Journal of Recent Advances in Multidisciplinary Research.* 2015;2(10): 0841-0847.
27. Binish T, Ben CP and Paulraj K. *In vitro* plant regeneration and antibacterial activity studies on three endemic species of *Ceropegia*. *Int J Pharm Bio Sci.* 2014;5(3): 833 – 839.
28. Boomibalagan P, Eswaran S and Rathinavel S. Phytochemical screening and antimicrobial activity of *Ceropegia juncea* roxb. – A rare medicinal plant. *Int. j. of life science & pharma research*. 2015;2(5):2250-0480.
29. Gaurav Kumar L, Karthik, and Bhaskara Rao K.V. Phytochemical composition and *in vitro* Antimicrobial activity of *Bauhinia racemosa* Lamk (Caesalpiniaceae). *International Journal of Pharmaceutical Sciences and Research*. 2010;1(11): 51-58.
30. Starlin T, Raj CA, Ragavendran P and Gopalakrishnan VK. Phytochemical screening, functional groups and element analysis of *Tylophora pauciflora* Wight and Arn. *International Research Journal of Pharmacy*. 2012;3(6): 180-183.
31. Nithyadevi J and Sivakumar R. Phytochemical Screening and GC- MS, FT-IR Analysis of Methanolic Extract Leaves of *Solanum torvum* Sw. *Int. J. Res. Studies in Biosci*. 2015; 3(9): 61-66.
32. Muthukrishnan, S. Bhakyab, S. Senthil Kumarc T. and Rao. M.V. Biosynthesis, characterization and antibacterial effect of plant-mediated silver nanoparticles using *Ceropegia thwaitesii* – An endemic species. *Industrial Crops and Products*. 2015;63:119–124.
33. Feng N, Guo X and Liang S. Adsorption study of copper (II) by chemically modified orange peel. *J. Hazard. Mater.* 2009;164:1286–1292.
34. Florence AR and Jeeva S. FTIR and GC-MS spectral analysis of *Gmelina asiatica* L. Leaves. *Science Research Reporter*. 2015;5(2):125-136.
35. Shah S, Siraj-ud-Din, Rehmanullah. Pharmacognostic standardization and FTIR analysis of various parts of Sageretia thea. *In. journal of bioscience*. 2013;3(3): 108-114.
36. Ramamurthy N and Kennan S. Fourier transform infrared spectroscopic analysis of a plant (*Calotropis gigantea* Linn) from an Industrial Village, Cuddalore Dt, Tamilnadu, India. *Romanian Journal of Biophysics*. 2007;17(4): 269 276.
37. Asha V, Jeeva S, Paulraj K. Phytochemical and FT-IR spectral analysis of *Caralluma geniculata* Grev.et Myur. an endemic medicinal plant. *Journal of Chemical and Pharmaceutical Research*. 2014;6 (7): 2083-2088.
38. Renuka B, Sanjeev B and Ranganathan D. Evaluation of phytoconstituents of *Caralluma nilagirina* by FTIR and UV-Vis spectroscopic analysis. *Journal of Pharmacognosy and Phytochemistry*. 2016;5(2):105-108.
39. Palawat R and Lodha P. Qualitative screenings of phytochemical and GC-MS analysis of *Ceropegia bulbosa*- an endangered tuberous plant. *Int J Pharm.* 2014;4(1): 100-107.