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QUALITATIVE SCREENINGS OF PHYTOCHEMICAL AND GC-MS ANALYSIS OF *CEROPEGIA BULBOSA*- AN ENDANGERED TUBEROUS PLANT

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ABSTRACT

Ceropegia bulbosa is a medicinal herb, this is useful in curing many disease like kidney stone and deafness. Ethno botanical study showed that the plant has good medicinal value for tribe of Rajasthan The preliminary phytochemical studies of *Ceropegia bulbosa* tuber and leaf extracts revealed the presence of steroids, glycosides, flavonoids alkaloids, saponins, tannins, terpenoids and potassium salt. Gas chromatography mass spectroscopic investigation of methanolic extract of *Ceropegia bulbosa* – a annual land plant is investigated by GCMS technique while the mass spectra of the compounds found in the extract are matched with the standard library of NIST. Maximum % area are found for 2H-Azepin-2-one, 3-(dimethylamino) hexahydro is present in maximum amount (9.09.%) with RT=8.913min, followed by 9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z) (8.16%)with RT=16.165min in the methanolic extract of leaves of *C.bulbosa.* 2H-Azepin-2-one, 3-(dimethylamino)hexahydro is present in maximum amount (43.58%) with RT=8.955min, followed by 2-Amino-9-(3,4-Dihydroxy-5-Hydroxymethyl-(16.08%) with RT=9.543min in the methanolic extract of tuber of *C.bulbosa*.

Key words: GCMS, NIST, Methyl ester.

INTRODUCTION

The plant species *Ceropegia bulbosa* known vernacularly as khadula, tilori and patal- tumbi belongs to the family to the Asclepiadoideae (milkweed) sub-family within the family Apocynaceae. (Bruyns 2000). Of the 44 species of *Ceropegia* found in India, 27 species are endemic to the Peninsular India. [1] which is distributed mainly in Western Ghats and most of them are enlisted under endangered category.[2] There is an increasing interest in the phytochemical compounds, which could be relevant to their nutritional incidence and their role in health and disease.[3] In the recent years, the interest for the study of the organic compounds from plants and their activity has increased. A lot of extraction methods and analytical methods have been developed for the study of plant active compounds .[4] The combination of an ideal separation technique (GC) with the best identification technique (MS) made GC–MS an ideal technique for analysis for volatile and semi-volatile compounds is more accurate for identification of compounds. The tuberous roots of many *Ceropegia* species are edible. [5] and many others are of medicinal value.[6] The root tubers contain starch, sugar, gum, albuminoids, fats and crude fiber and are valuable constituents in many traditional medicinal systems in India. [7] Active principle of tuberous roots contains an alkaloid cero-pegine which is active against diarrhoea and dysentery. [8] There are no previous reports on the phytochemical information of *Ceropegia bulbosa* by GC-MS. In this paper we report the chemical composition of the methanolic extracts of *Ceropegia bulbosa* a tuberous plant of Rajasthan by using GC-MS analysis.

MATERIALS AND METHODS

Experimental details

***Collection and authentication of the plant material*:** The whole plant material of *Ceropegia bulbosa* was

collected from Jaipur and Ajmer region of Rajasthan state of India, in the month of July -August 2012. Botanical identification and authentication was done by herbarium in charge Department of botany University of Rajasthan Jaipur, where a voucher specimen was deposited with the herbarium file number RUBL21160.

***Qualitative screenings of Photochemical*:** The qualitative screenings of powdered crude drugs for their active ingredients were carried out using the following standard procedures

***Plant parts*:** Various mature plant parts (leaves, stem, tuber) of *Ceropegia bulbosa* were collected from Jaipur and Ajmer regions of Rajasthan. These were washed with tap water to remove dust and dried in shade and prepare its powder.

Test for Alkaloids: Extract 2g of powdered drug by warming for 2 min. with 20ml 1% sulphuric acid in a 50ml conical flask on a water bath, with intermittent shaking, centrifuge; pipette off the supernatant into a small conical flask. Make an initial test for alkaloids by adding to 0.1 ml extract in a semi-micro tube, one drop of Meyer's reagent. It gives a cream precipitate with alkaloids.

Test for Essential oil / Volatile oil

Test 1: Crush a small sample of the crude drug between the thumb and forefinger, and examine for the presence of an odour.

Observation: Drug containing volatile oils have a strong odour.

Test 2: Extract 1g of the powdered drug by warming with 10ml petroleum sprit (boiling point range 40-60 C.) in a boiling tube heated on a water bath. Do not let the solvent boil dry. Filter the mixture into an evaporating dish and concentrate the filtrate to about 1ml on a water bath. Using a pipette apply one drop of the extract to a filter paper. Expose the paper to a current of warm air and note the occurrence of any translucent area. If this observed, then oils are present.

Observation 1: Place the paper in an oven at 105^{o} C for 15min. and if the translucent spot can still be observed after that time, then a fixed oil is present.

Observation 2: The presence of a volatile oil is detected by the disappearance or diminution of the translucent area.

Test for Flavonoids (flavone)

Test 1: Prepare an aqueous filtrate of powdered drug, and take a portion of filtrate in a test tube, add 5 ml of dilute ammonia followed by add few drops of concentrated sulphuric acid. A yellow coloration is appears. Upon further standing, the yellow coloration disappears.

Test 2: Take a small amount of powdered drug in a test tube, add 10 ml ethyl acetate and heat it over a steam bath for 3min. then filter the mixture, take 4 ml of the filtrate with 1ml of dilute ammonia solution. Observe the formation of yellow colouration. It is the indication of flavanoids compounds of drug.

Test for Glycosides

Test 1: Extract 200 mg of the sample by warming in a test tube with 5ml of dilute (10%) sulphuric acid (Test with pH paper) on a water bath at 100C for 2min. centrifuge or filter, pipette off the supernatant or filtrate. Neutralize the acid extract with 5% solution of NaOH (Noting the volume of NaOH added). Add 0.1ml of Fehling's solution 'A" and then Fehling's solution 'B' until alkaline (Test with pH paper) and heat on the water bath for 2min. Note the quantity of red precipitate formed and compare with that formed in Test 2.

Test 2: Extract 200mg of the sample using 5ml of water instead of sulphuric acid. After boiling add a volume of water equivalent to the volume of NaoH used in Test 1. Add 0.1ml of Fehling's solution A and then Fehling's solution B until alkaline (test with PH paper) and heat on the water bath for 2min. and note the quantity of red precipitate formed (Test.2.) Compare the quantity of precipitate formed in Test 2 with that formed in Test 1. If the precipitate in Test 1 is greater than that in Test 2, then glycosides may be present, since Test 2 represents the amount of free reducing sugars already present in the crude drug, whereas Test 1 represents free reducing sugars plus those released on acid hydrolysis of any glycosides in the crude drug.

Tests for Potassium Salts: Dissolve 0.1g of substance being examined in 2 ml of water. Heat the solution with 1ml of sodium carbonate solution (10.6% w/v), no precipitate is formed. Add 0.05 ml of sodium sulphite solution (10%), no precipitate is formed, cool in ice , add 2 ml of a 15% w/v solution of tartaric acid and allow to stand, a white crystalline precipitate is produced.

Test for Saponin: Take 2g of the powdered sample and boil with 20 ml of distilled water in a water bath and filter it, 10 ml of filtrate is mix with 5 ml of distilled water and shake vigorously for a stable persistent froth. To this froth mix 3 drops of olive oil and shake vigorously, then observe for the formation of emulsion.

Tests for Starch: Take 1g of dry powder in 50 ml of water boil for one minute and cool, a thin and cloudly mucilage is produced, which gives thick and more transparent mucilage. To 10 ml of the mucilage add 0.05 ml of 0.01M Iodine, a dark blue colour is produced, which disappears on heating and reappears on cooling.

Test for Tannins: Take 0.5g of the dried powdered sample in 20ml of water, boil on a water bath and filter it in a test tube. Add few drops of 0.1% ferric chloride and observe for brownish green or a blue-black colouration.

Test for Terpenoids (Salkowski test): Take five ml of extract, mixed with 2 ml of chloroform, and concentrated H_2SO_4 (3ml) is added to form a layer. A reddish brown coloration on the inner face is formed. It is indicates the presence of terpenoids.

Test for Vitamin C or Ascorbic Acid: To 2ml of 2% w/v solution, add 2ml of water, 0.1g of sodium bicarbonate and about 20mg of ferrous sulphate, shake and allow stand; a deep violet colour is produced. Add 5ml of 1M sulphuric acid, the colour disappears

GC-MS Description: GC-MS analysis was carried out on a GC clarus 500 Perkin Elmer system comprising a AOC-20i autosampler and gas chromatograph interfaced to a mass spectrometer (GC-MS) instrument employing the following conditions: column Elite-1 fused silica capillary column (30 x 0.25 mm ID x 1µM df, composed of 100% Dimethyl poly diloxane, operating in electron impact mode at 70eV; helium (99.999%) was used as carrier gas at a constant flow of 1 ml /min and an injection volume of 0.5µl was employed (split ratio of 10:1) injector temperature 250 ºC; ion-source temperature 280 ºC. The oven temperature was programmed from 110ºC (isothermal for 2 min), with an increase of 10ºC/min, to 200ºC, then 5ºC/min to 280ºC, ending with a 9 min isothermal at 280ºC. Mass spectra were taken at 70eV; a scan interval of 0.5 seconds and fragments from 40 to 450 Da. Total GC running time is 45 minutes.

Plant material and extraction: Aerial parts of *Ceropegia bulbosa* were collected during the month of July-august from the forest regions of Jaipur and Ajmer areas. The plant materials (stem and leaves) were air dried at room temperature and under shade, Leaves and tuber were shade dried, powdered and extracted with methanol for 6-8 hours using soxhlet apparatus. The extract was then filtered through muslin, evaporated under reduced pressure and vacuum dried to get the viscous residue. 1µl of the methanolic leaves and tuber extract of *Ceropegia bulbosa* was employed for GC-MS analysis.

Identification of the compound: The identification of the compounds present in the methanolic extracts were based on the direct comparison of the peaks by retention times and mass spectral data with those for standard compounds, and by computer matching with the online standard library of NIST.

RESULT AND DISCUSSION

Methanolic extract of the leaf and tuber of *Ceropegia bulbosa* and *Ceropegia attenauta(removed from here)* were screened for the presence of steroids, reducing sugar , alkaloids, saponins, tannins, flavonoids, terpenoids, glycosides and vitamins C /ascorbic acid results are presented in Table I.

Ceropegia bulbosa leaves: GC-MS chromatogram of the methanolic extract of the leaves of *Ceropegia bulbosa* (Fig.I) showed 53 peaks indicating the presence of fifty three compounds. The chemical compounds identified in the methanolic extract of the leaves of *Ceropegia bulbosa* are presented in Table II. The active principles with their retention time (RT), molecular formula, area %, compound name, RI are presented in Table II. The total ion chromatograph (TIC) showing the peak identities of the compounds identified have been givens in Fig. I. GC MS analysis revealed the presence of aromatic hydrocarbons, linolenic acid, paraffins, sterol, palmitic acid, Oleic acid, ketons, diterpenes and sisqusterpenes etc. 2H-Azepin-2-one, 3-(dimethylamino) hexahydro- is present in maximum amount (9.09.%), followed by 9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)- (8.16%) in the methanolic extract of leaves of *C.bulbosa*. Steroids like Ergost-5-en-3-ol-(4.00%), Stigmast-5-en-3-ol, (3.Beta.,24s) (6.47%), Triterpenoids like Lupeol (4.43%), A-Friedooleanan-28-al, 3-oxo,(1.58%) Flavanoids like Flavone 4'-oh,5-oh,7-di-o-glucoside (9.97%) were also detected. Vitamin E was also present in small amount. GC-MS analysis revealed that the minimum presence of Globulol (.71%), 9-Tricosene, (Z)-(0.45%), Phytol (1.59%) and Gamma tocopherol. The GC-MS analyses revealed that the methanolic extract is mainly composed of alkenes, phenolics and steroids. 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 leaves *C. bulbosa*. 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 fingerprint of that compound which can be identified from the data library.

Ceropegia bulbosa tuber : GC-MS chromatogram of the methanolic extract of the tuber of *Ceropegia bulbosa* (Fig. II) showed 28 peaks indicating the presence of twenty eight compounds. The chemical compounds identified in the methanolic extract of the Tuber of *Ceropegia bulbosa* are presented in Table III. The active principles with their retention time (RT), molecular formula, area %, compound name, RI are presented in Table III. The total ion chromatograph (TIC) showing the peak identities of the compounds identified have been givens in Fig. II. GC MS analysis revealed the presence of aromatic hydrocarbons, paraffins, sterol, Oleic acid, and diterpenes etc. 2H-Azepin-2-one, 3-(dimethylamino)hexahydro- is present in maximum amount (43.58%), followed by 2-Amino-9-(3,4-Dihydroxy-5-Hydroxymethyl-(16.08%) in the methanolic extract of tuber of *C.bulbosa*. Steroids like Ergost-5-en-3-ol-(1.67%), stigmast-5-dien-3-ol, (3.beta.) (1.69%), hexadecane and nonadecane were also detected. 1,6-Anhydro-.beta.-D-glucopyranose (levoglucosan) (7.06%) was also present in considerable amount GC-MS analysis revealed that the minimum presence of Benzophenone, 2-Acetyl-2-Hydroxy-.Gamma.-Butyrolactone, and methyl dihydromalvalate . The GC-MS analyses revealed that the methanolic extract is mainly composed of organic compound, and steroids. The total alkaloid fraction exhibited promising hepatoprotective, antipyretic, analgesic, local anesthetic, anti-ulcer, and mast-cell stabilizing, tranquilsing and hypotensive activities and was devoid of side effects as noted out by the sub-acute toxicity studies.[9]. Sekar and Francis [10] reported Ceropegia spp. was used as alternative source for renewable energy due to presence of polyphenol, oil and hydrocarbon, 2Guimarenol and lup-18-en-3beta-ol from *C. dichotoma.*[11]. *Ceropegia spp* has revealed the presence of volatile oil and terpenes, in C. *woodii*, 41 peaks were isolated, of which 24 compounds were identified. Of the total volatile matter, 70.73% was terpenes, 5.82% was taxanes, and 1.52% was ketones.[12] 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 tuber of *C. bulbosa*. 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 fingerprint of that compound which can be identified from the data library.

CONCLUSION

Gas chromatography mass spectrometry (GC-MS) is a method that combines the features of gas liquid Chromatography and mass spectrometry to identify different substances within a test sample. Applications of GC-MS include drug detection, environmental analysis, explosives investigation, and identification of unknown samples. GC-MS can also be used in airport security to detect substances in luggage or on human beings All the results concerning the present investigation revealed the following conclusions:- The qualitative screenings of powder showed the presence of Carbohydrates, phenols, steroids, alkaloids, glycosides, flavonoids, tannins and saponins in stem and leaves of *Ceropegia bulbosa.* GC-MS analysis of methanolic extract of leaves and tuber showed the presence of carbohydrates, phenols steroids, alkaloids, glycosides, flavonoids, tannins and saponins. The presence of various bioactive compounds confirms the application of *Ceropegia bulbosa* for various ailments by traditional practitioners.

Table I: Preliminary phytochemical screening/characterization of leaf and tuber extract of *Ceropegia bulbosa*

SAMPEL	**Leaves**	**Tuber**
COMPOUND	***Ceropegia bulbosa***	***Ceropegia bulbosa***
Alkaloids	**+**	**+**
Tannins	**+**	**+**
Glycosides	**+**	**+**
Vitamin C	**-**	**-**
Steroids	**+**	**+**
Flavanoids	**+**	**+**
Starch	**+**	**+**
Terpenoids	**+**	**+**
Volatile Oil	**+**	**+**
Potassium Salts	**+**	**+**

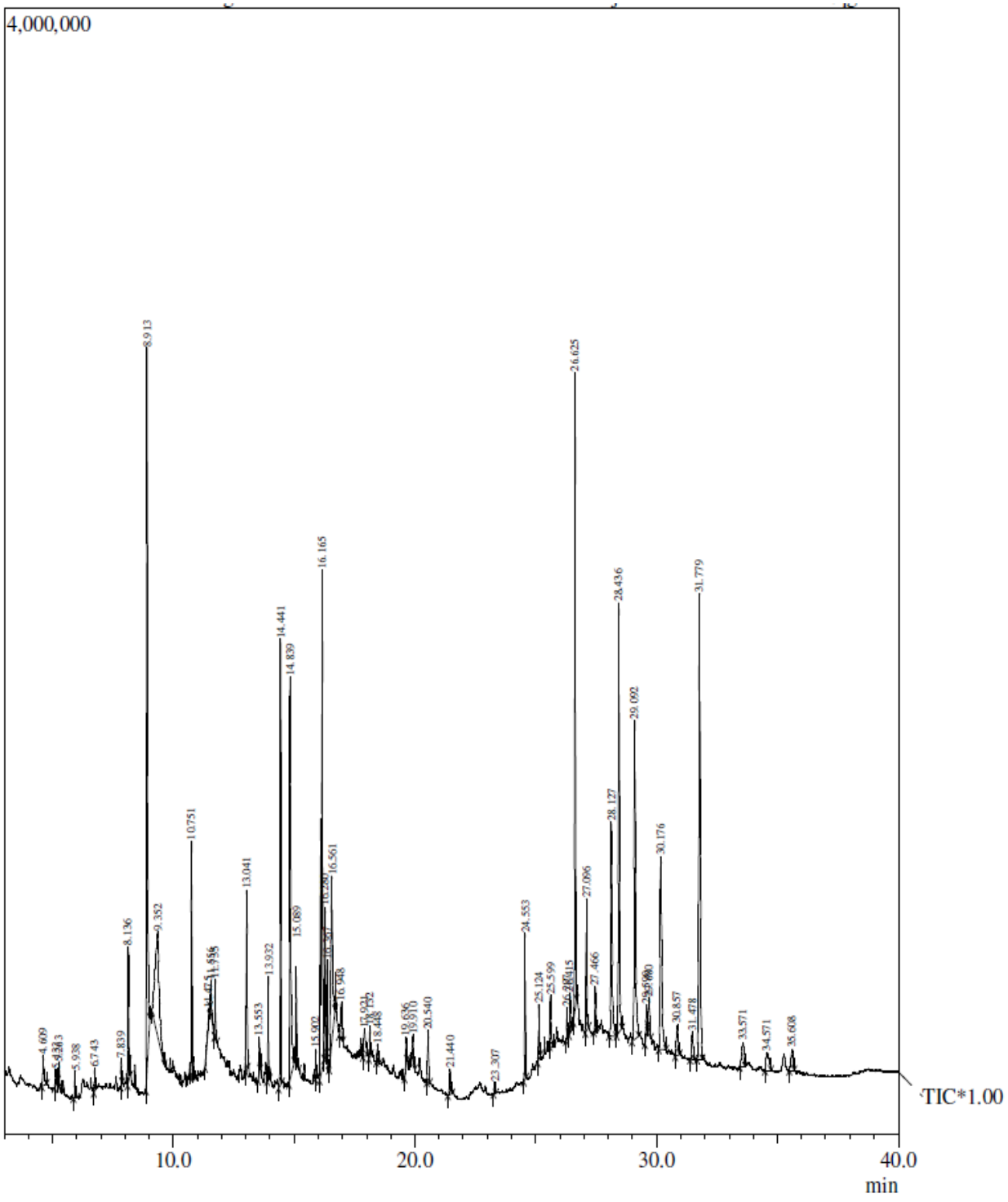

Fig. I: Chromatogram of *Ceropegia bulbosa* leaf by GCMS

Table II: GC-MS analysis of the leaves of *C. bulbosa*

Peak Report TIC

Peak#	R.Time	Area	Area%	Name
1	4.609	471836	0.63	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-
2	5.133	203888	0.27	1-DODECENE
3	5.263	142851	0.19	NAPHTHALENE
4	5.938	234446	0.31	CYCLOHEXANE, HEXYL-
5	6.743	171217	0.23	2-UNDECANONE
6	7.839	161472	0.21	Dodecane, 4-cyclohexyl-
7	8.136	936107	1.24	1-Tridecene
8	8.913	6855358	9.09	2H-Azepin-2-one, 3-(dimethylamino)hexahydro-
9	9.352	4383505	5.81	2-AMINO-9-(3,4-DIHYDROXY-5-HYDROXYMETHYL-T
10	10.751	1421555	1.88	1-Pentadecene
11	11.475	307760	0.41	18-Nonadecen-1-ol
12	11.556	257994	0.34	Cyclohexane, undecyl-
13	11.735	361945	0.48	8-PENTADECANONE
14	13.041	1116494	1.48	1-Heptadecene
15	13.553	320944	0.43	2,6,10-TRIMETHYL,14-ETHYLENE-14-PENTADECNE
16	13.932	689658	0.91	8-Octadecanone
17	14.441	2802690	3.71	HEXADECANOIC ACID, METHYL ESTER
18	14.839	4263542	5.65	n-Hexadecanoic acid
19	15.089	607648	0.81	1-OCTADECENE
20	15.902	191955	0.25	10-Nonadecanone
21	16.165	6154810	8.16	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-
22	16.280	1203227	1.59	Phytol
23	16.367	548470	0.73	Octadecanoic acid, methyl ester
24	16.561	3172993	4.21	9,12-Octadecadienoic acid (Z,Z)-
25	16.948	338468	0.45	9-TRICOSENE, (Z)-
26	17.921	462542	0.61	1-DOCOSANOL
27	18.132	214880	0.28	TETRACOSANOIC ACID, METHYL ESTER
28	18.448	175251	0.23	2H-Pyran-2-one, tetrahydro-6-tridecyl-
29	19.636	279145	0.37	9-OCTADECENAL, (Z)-
30	19.910	247874	0.33	METHYL DIHYDROMALVALATE
31	20.540	483831	0.64	1,2-BENZENEDICARBOXYLIC ACID
32	21.440	311259	0.41	9-Octadecenoic acid, 12-(acetyloxy)-, methyl ester, [R-(Z)]-
33	23.307	130727	0.17	OCTADECANOIC ACID, METHYL ESTER
34	24.553	1177980	1.56	2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexameth
35	25.124	409417	0.54	TETRATETRACONTANE
36	25.599	530388	0.70	2,8-DIMETHYL-2-(4,8,12-TRIMETHYLTRIDECYL)-6-CH
37	26.297	265036	0.35	.beta.-Tocopherol
38	26.415	352931	0.47	.gamma.-Tocopherol
39	26.625	4780762	6.34	TETRATETRACONTANE
40	27.096	1189397	1.58	Vitamin E
41	27.466	397986	0.53	CELIDONIOL, DEOXY-
42	28.127	3014589	4.00	Ergost-5-en-3-ol, (3.beta.)-
43	28.436	4828058	6.40	Tetratetracontane

Peak#	R.Time	Area	Area%	Name
44	29.092	4883581	6.47	STIGMAST-5-EN-3-OL, (3.BETA.,24S)-
45	29.590	336551	0.45	4,4,6A,6B,8A,11,11,14B-OCTAMETHYL-1,4,4A,5,6,6A,6E
46	29.680	319211	0.42	.ALPHA.-SELINENE
47	30.176	3343807	4.43	Lupeol
48	30.857	505342	0.67	CELIDONIOL, DEOXY-
49	31.478	406715	0.54	03027205002 FLAVONE 4'-OH,5-OH,7-DI-O-GLUCOSIDE
50	31.779	7526201	9.97	03027205002 FLAVONE 4'-OH,5-OH,7-DI-O-GLUCOSIDE
51	33.571	546274	0.72	TRICYCLO[20.8.0.0E7,16]TRIACONTAN, 1(22),7(16)-DIE
52	34.571	538550	0.71	(-)-Globulol
53	35.608	478458	0.63	E,E,Z-1,3,12-Nonadecatriene-5,14-diol
		75457576	100.00	

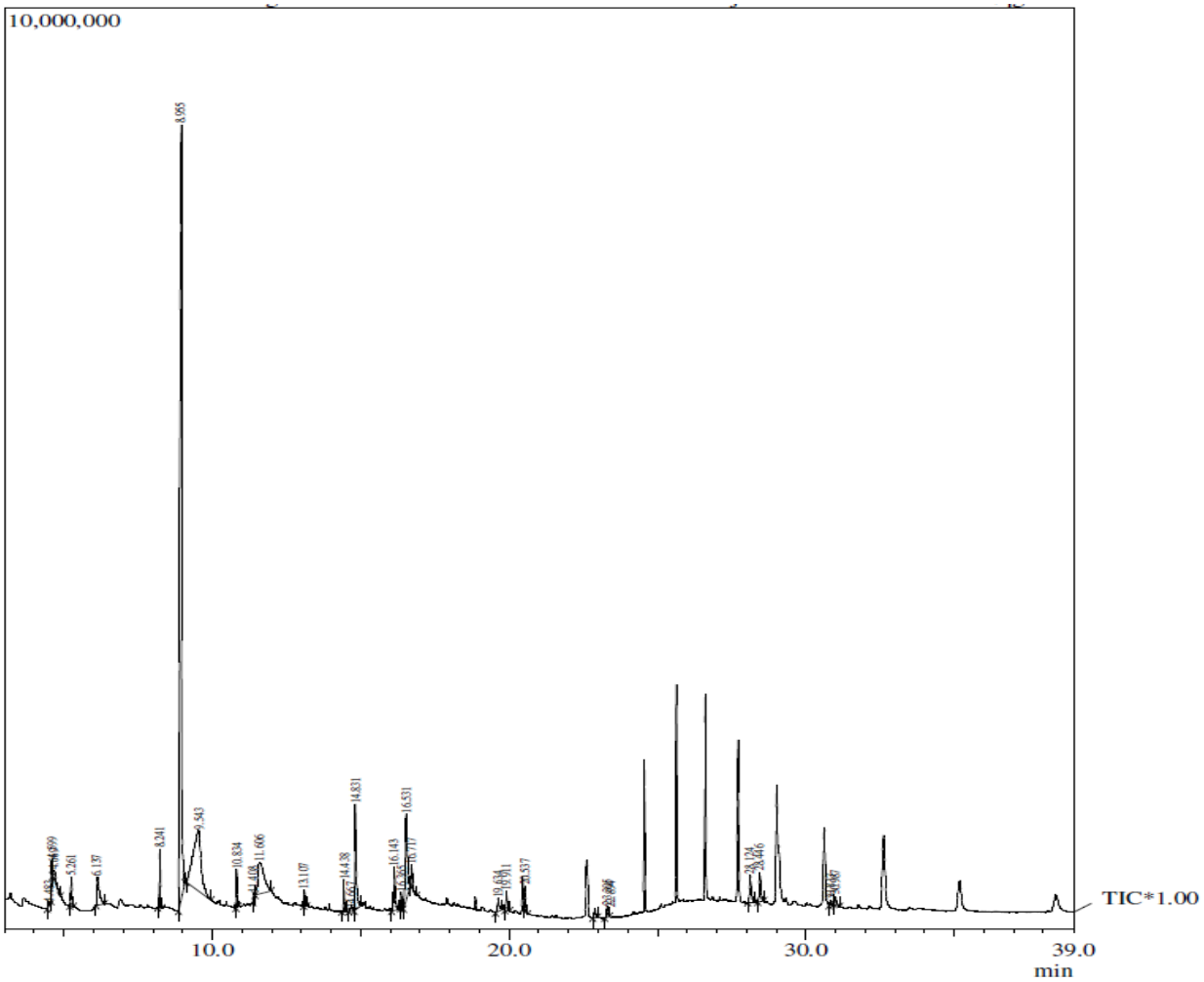

Fig. II: Chromatogram of *Ceropegia bulbosa* tuber by GCMS

Table III
GC-MS analysis of the tuber of *C. bulbosa*

Peak Report TIC

Peak#	R.Time	Area	Area%	Name
1	4.483	168815	0.24	2-ACETYL-2-HYDROXY-.GAMMA.-BUTYROLACTONE
2	4.599	786089	1.10	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-
3	4.669	655404	0.92	Glycerine
4	5.261	951516	1.33	DODECANE
5	6.137	1804526	2.52	1,2,3-Propanetriol, 1-acetate
6	8.241	1210447	1.69	HEPTADECANE
7	8.955	31215168	43.58	2H-Azepin-2-one, 3-(dimethylamino)hexahydro-
8	9.543	12029277	16.80	2-AMINO-9-(3,4-DIHYDROXY-5-HYDROXYMETHYL-T
9	10.834	681166	0.95	HEXADECANE
10	11.408	120063	0.17	Benzophenone
11	11.606	5058464	7.06	1,6-Anhydro-.beta.-D-glucopyranose (levoglucosan)
12	13.107	293928	0.41	NONADECANE
13	14.438	720409	1.01	HEXADECANOIC ACID, METHYL ESTER
14	14.667	245513	0.34	1-Bromodocosane
15	14.831	3345299	4.67	n-Hexadecanoic acid
16	16.143	1573130	2.20	9-Octadecenoic acid (Z)-, methyl ester
17	16.365	324375	0.45	Octadecanoic acid, methyl ester
18	16.531	4220823	5.89	Oleic Acid
19	16.717	591240	0.83	Octadecanoic acid
20	19.634	640401	0.89	9-OCTADECENAL, (Z)-
21	19.911	584844	0.82	METHYL DIHYDROMALVALATE
22	20.537	594597	0.83	1,2-BENZENEDICARBOXYLIC ACID
23	22.894	376276	0.53	15-TETRACOSENOIC ACID, METHYL ESTER, (Z)-
24	23.305	414214	0.58	Tetracosanoic acid, methyl ester
25	28.124	1197547	1.67	Ergost-5-en-3-ol, (3.beta.)-
26	28.446	1210209	1.69	STIGMASTA-5,22-DIEN-3-OL
27	30.817	165244	0.23	Cholest-4-en-3-one
28	30.987	440108	0.61	9,19-Cyclolanost-25-en-3-ol, 24-methyl-, (3.beta.,24S)-
		71619092	100.00	

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