



E-ISSN: 2278-4136

P-ISSN: 2349-8234

<https://www.phytojournal.com>

JPP 2023; 12(2): 40-43

Received: 16-01-2023

Accepted: 19-02-2023

Pooja S SawantDepartment of Botany,
Dr. Babasaheb Ambedkar
Marathwada University
Aurangabad, Maharashtra, India**Arvind S Dhabe**Department of Botany,
Dr. Babasaheb Ambedkar
Marathwada University
Aurangabad, Maharashtra, India**Corresponding Author:****Pooja S Sawant**Department of Botany,
Dr. Babasaheb Ambedkar
Marathwada University
Aurangabad, Maharashtra, India

HR-LCMS analysis of tuber and callus of *Ceropegia bulbosa roxb. var. bulbosa*

Pooja S Sawant and Arvind S DhabeDOI: <https://doi.org/10.22271/phyto.2023.v12.i2a.14628>**Abstract**

Ceropegia bulbosa var. bulbosa is threatened medicinal plant belongs to family Asclepiadaceae. Present analysis is based on most reliable analytical procedure, High Resolution- Liquid Chromatography Mass Spectrometry (HR-LCMS) to detect bioactive phyto-constituents from *in vivo* tuber and *in vitro* callus of *Ceropegia bulbosa*. The present study provides evidence that ethanol extract of *Ceropegia bulbosa* Roxb. var. *bulbosa* tuber showed presence of 1, 4-Dimethylpyrrolo [1, 2-a] pyrazine, Gabapentin, Americine, Oseltamivir, 4-Methylphenyl dodecanoate, 17 beta-Methylestra-1,3,5(10)-trien-3-ol, Citronellyl hexanoate, Limonoate, 1-Monopalmitin, Mitoxantrone, Butyl dodecanoate compound. *In vitro* callus showed presence of Elaeokanine C, Validamycin A, Flurazepam, Isopentenyl adenosine, Mitoxantrone, Phytosphingosine, Oxidized dinoflagellate Luciferin, Harderoporphyrinogen, 6-Deoxocathasterone, Irinotecan, Mycinamicin VII compound. It is observed that, the compounds present in *in vivo* tuber were also present in *in vitro* callus. Some variation was observed in some compounds like Validamycin A, Flurazepam found in callus which were absent in tuber.

Keywords: *Ceropegia bulbosa var. bulbosa*, Phytochemicals, HR-LCMS, Callus**Introduction**

Ceropegia genus comprises 200 species which are distributed throughout the world. In India 55 species have been reported, out of them, 28 are endemic to peninsular India and 16 species are included in the Red Data Book (Nayar and Sastry, 1987) [7]. *Ceropegia bulbosa var. bulbosa* is threatened medicinal plant belongs to family Asclepiadaceae. It is botanically curious genus with largest number of species native to Africa, Southern Asia and Australia. In India these species are mainly distributed in the Western Ghats (Jagtap and Singh, 1999) [3]. Most of the species of *Ceropegia* produce tuberous root which has numerous ethnomedicinal values and hence it is an important component in several indigenous drug preparations (Ayurveda). The existing reports on *Ceropegia* species show that they were used as in traditional medical system. They also play a vital role in the Ayurvedic field (Binish T 2018) [7]. Kambale *et al.*, 2008 reported that, in Northwest Maharashtra region Bhilla tribal group uses *Ceropegia hirsuta* fresh root for treatment of stomach disorders. The pharmacological importance of the genus *Ceropegia* is mainly due to the presence of 'cerpegin', a pyridine alkaloid. Aqueous extract of *C. bulbosa* contains steroids, polyphenols, sugars and potassium. Tuber paste of *Ceropegia bulbosa var. bulbosa* is applied on the inflammation of skin and decoction taken to get rid of urinary bladder stone (Khare, 2007) [5]. In the present study an attempt was made to isolate the phytochemical constituents present in ethanol extract of plant *in-vivo* tuber and in *in vitro* callus of *Ceropegia bulbosa* Roxb. var. *bulbosa* by using HR-LCMS technique. S. Muthukrishnan *et al.*, 2018 [6] put forth that *in vitro* callus accumulate secondary metabolites in greater quantities than mother plants, in many circumstances researchers have used various approaches such as use of PGRs, elicitors and different additives for enhancement of secondary metabolite. In *Ceropegia* species the accumulation and production of secondary metabolites in *in vitro* callus was affected by the media types and PGRs. A survey of literature revealed that the HR-LCMS exploration was not done so far with the medicinal species *Ceropegia bulbosa*. This manuscript focuses on the comparative HR-LCMS analysis of *in vitro* callus and *in vivo*-tuber of *Ceropegia bulbosa var. bulbosa*.

Methods and Material

Plants of *Ceropegia bulbosa var. bulbosa* were collected from Gogababa hill, B. A. M. U. campus of Aurangabad, Maharashtra and cultivated in the Botanic garden of Dr. Babasaheb Ambedkar Marathwada University, Aurangabad. Authenticity was confirmed with the voucher

specimen (Accession No. 0705) and it was deposited in B. A. M. U herbarium at Department of Botany. Dr. Babasaheb Ambedkar Marathwada University, Aurangabad (M. S.). *In vivo* tuber and *in vitro* callus of selected medicinal plant were finely powdered. Bioactive compounds were extracted with ethanol solvent using a Soxhlet's extractor for 72 hrs. The extracts were concentrated to remove the solvents completely by using rotary evaporator. Plant extracts were sent for qualitative analysis of their chemical constituent at SAIF, IIT, Bombay by HRLC-MS technique. The instrument used is Agilent technologies G6550A-ifunnel, Q-TOF, LC/MS. Column type is ZORBAX RRHDSBC18, with 100 mm length, 2.1 mm diameter and 1.8 pore size.

Results and Discussion

In vivo tuber of *Ceropegia bulbosa* var. *bulbosa* showed presence of 25 compound as shown in table no.1. These compounds possess medicinal properties. Pyridine compound is used to treat hypertension and congestive heart failure. Compound Gabapentin a drug which is used to treat epilepsy. It's also taken for nerve pain, which can be caused by different conditions, including diabetes and shingles. Nerve pain can also happen after an injury. In epilepsy, it's thought that gabapentin stops seizures by reducing the abnormal electrical activity in the brain (National library of medicine). Oseltamivir is an inhibitor of the influenza neuraminidase enzyme and is used as therapy and prophylaxis against influenza A and B. Oseltamivir, sold under the brand name Tamiflu, is an antiviral medication used to treat and prevent influenza A and influenza B (pub. chem). Epothilone A has a role as an antineoplastic agent it is a tubulin modulator and used as microtubule-stabilizing agent. Mitoxantrone is an

antineoplastic antibiotic it is used in the treatment of acute leukemia, lymphoma, and prostate and breast cancer.

Table no. 2 showed that compound in *in vitro* callus of *Ceropegia bulbosa* var. *bulbosa*, Validamycin is an antibiotic and fungicide produced by *Streptomyces hygroscopicus*. It is used as an inhibitor of trehalase. It is used for the control of sheath blight of rice and damping-off of cucumbers. Flurazepam is an orally available benzodiazepine used for therapy of insomnia. Flurazepam (marketed under the brand names Dalmane and Dalmadorm) a drug which is a benzodiazepine derivative. It possesses anxiolytic, anticonvulsant, hypnotic, sedative and skeletal muscle relaxant properties (Pub chem). Riboprine is an isopentenyl derivative of adenosine naturally derived from certain plants and purine nucleoside with potential antineoplastic activity. Riboprine may cause cell cycle arrest and apoptosis. Phytosphingosine is used in skin care products to treat a variety of inflammatory skin conditions, such as eczema. Dr. Joshua Zeichner (MD, the director of cosmetic and clinical research at Mount Sinai Hospital's department of dermatology) state that Phytosphingosine is considered as precursor to ceramides, which are the dominant fat that fills in the cracks between skin cells. When added to skin care products, phytosphingosine enhances skin's barrier function. A strong skin barrier protects the body from allergens and irritants and helps lock in moisture. Dinoflagellate luciferase an enzyme is found in bioluminescent dinoflagellates, it is eukaryotic protists which are found in ocean surface waters. Dinoflagellate luciferase allowed these organisms to emit blue light (max 475 nm) after stimulation. The light produced is theorized to act as a defense against predators or lure for prey.

Table 1: Phytochemical analysis *In vivo* tuber of *Ceropegia bulbosa* var. *bulbosa*

Sr. No.	Compound name	Formula	Mass	Rt	DB Diff
1	1,4-Dimethylpyrrolo[1,2-a]pyrazine	C ₉ H ₁₀ N ₂	146.0848	4.628	-2.47
2	Gabapentin	C ₉ H ₁₇ N O ₂	171.1258	11.527	0.73
3	Americine	C ₃₁ H ₃₉ N ₅ O ₄	545.3015	8.976	-2.4
4	Oseltamivir	C ₁₆ H ₂₈ N ₂ O ₄	312.2055	9.434	-1.75
5	4-Methylphenyl dodecanoate	C ₁₉ H ₃₀ O ₂	290.224	9.658	2.08
6	8-Geranylchrysin	C ₂₅ H ₂₆ O ₄	390.1849	9.882	-4.61
7	alpha-Amylcinnamyl isovalerate	C ₁₉ H ₂₈ O ₂	288.2081	10.157	2.85
8	17beta-Methylestra-1,3,5(10)-trien-3-ol	C ₁₉ H ₂₆ O	270.1979	10.387	1.68
9	Epothilone A	C ₂₆ H ₃₉ N O ₆ S	493.2475	10.895	4.72
10	Citronellyl hexanoate	C ₁₆ H ₃₀ O ₂	254.2239	11.161	2.68
11	Mitoxantrone	C ₂₂ H ₂₈ N ₄ O ₆	11.786	11.786	1.89
12	16-Hydroxy hexadecanoic acid	C ₁₆ H ₃₂ O ₃	272.234	12.141	4.2
13	Limonate	C ₃₅ H ₄₉ N O ₁₀	643.3372	12.565	-2.46
14	1-Monopalmitin	C ₁₉ H ₃₈ O ₄	330.2751	12.986	5.68
15	Icariside E5	C ₂₆ H ₃₄ O ₁₁	522.209	13.218	2.21
16	Butyl dodecanoate	C ₁₆ H ₃₂ O ₂	256.2395	14.119	2.71
17	(3beta,5alpha,6alpha,7alpha,2 2E,24R)-5,6-Epoxyergosta- 8,14,22-triene-3,7-diol	C ₂₈ H ₄₂ O ₃	426.3092	15.955	9.8
18	7a,12a-Dihydroxy-5bcholestan-3-one	C ₂₇ H ₄₆ O ₃	418.3421	16.178	6.29
19	5-(12-Nonadecenyl)-1,3-Benzenediol	C ₂₅ H ₄₂ O ₂	374.3164	16.325	5.67
20	Harderoporphyrinogen	C ₃₅ H ₄₂ N ₄ O ₆	614.3093	17.295	1.88
21	3-Hydroxy-1-phenyl-1-Eicosanone	C ₂₆ H ₄₄ O ₂	388.3325	18.22	4.32
22	9-chloro-10-hydroxy-hexadecanoic acid	C ₁₆ H ₃₁ ClO ₃	306.1966	9.94	-1.32
23	Lamioside	C ₁₈ H ₂₈ O ₁₁	420.1606	11.652	6.2
24	Embelin	C ₁₇ H ₂₆ O ₄	294.1825	26.657	2.01
25	15-Acetyl-4-deoxynivalenol	C ₁₇ H ₂₂ O ₇	338.1391	26.761	-7.39

Sample Name	T1	Position Inj.	P2-F1	Instrument Name	QTOF	User Name	Success
Inj. Vol.	5	Position		Sample Type	Sample	IRM Calibration Status	
Data Filename	T1.d	ACQ Method	30 min_+ESI_01112021_	Comment		Acquired Time	11/28/2021 2:38:32 AM

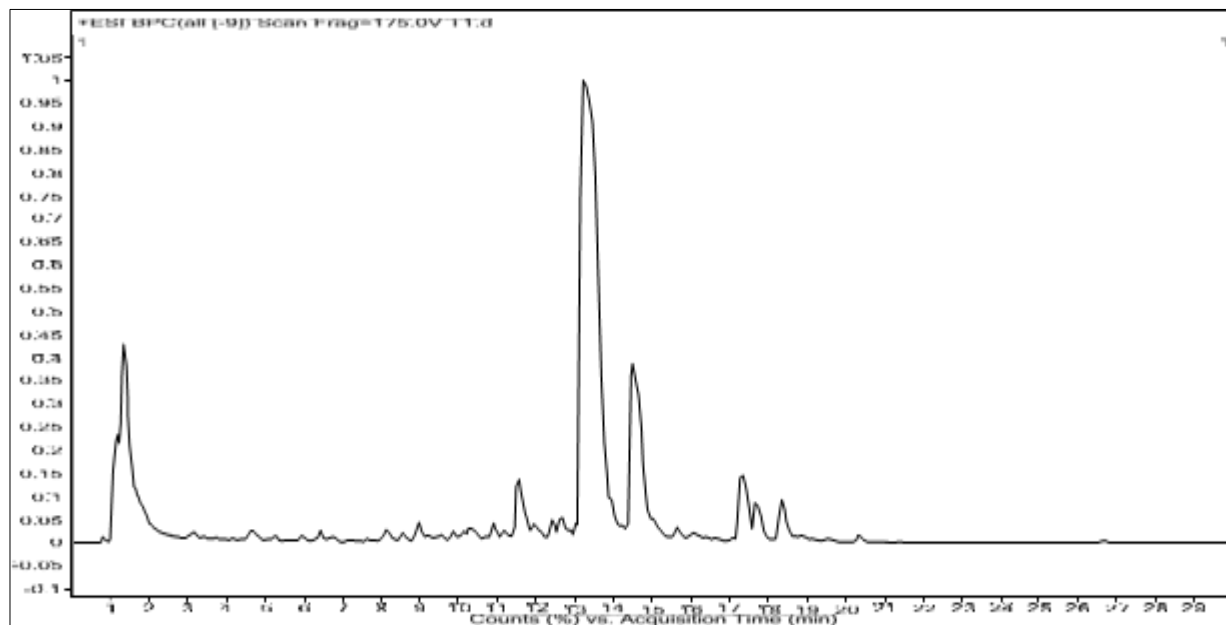


Fig 1: Counts (%) vs. Acquisition time (min)

Table 2: Phytochemical analysis *In vitro* callus of *Ceropegia bulbosa* var. *bulbosa*

Sr. No.	Compound name	Formula	Mass	Rt	DB Diff
1	Elaeokanine C	C ₁₂ H ₂₁ N O ₂	211.1572	4.213	0.04
2	Validamycin A	C ₂₀ H ₃₅ N O ₁₃	497.2097	4.904	2.22
3	Flurazepam	C ₂₁ H ₂₃ Cl F N ₃ O	387.1508	4.987	1.57
4	Isopentenyl adenosine	C ₁₅ H ₂₁ N ₅ O ₄	335.1582	6.054	3.56
5	Mitoxantrone	C ₂₂ H ₂₈ N ₄ O ₆	444.2004	11.595	1.19
6	Phytosphingosine	C ₁₈ H ₃₉ N O ₃	317.2898	11.597	10.07
7	(5x,6x)-5,6-Epoxyergosta-7,22-dien-3-ol	C ₂₈ H ₄₄ O ₂	412.3364	12.483	-5.48
8	Limonoate	C ₂₆ H ₃₄ O ₁₀	506.2152	13.155	-0.06
9	Butyl dodecanoate	C ₁₆ H ₃₂ O ₂	256.2396	13.897	2.57
10	Citronellyl hexanoate	C ₁₆ H ₃₀ O ₂	254.2242	14.363	1.59
11	Mycinamicin VII	C ₂₉ H ₄₇ N O ₇	521.331	14.284	8.16
12	16-Hydroxy hexadecanoic acid	C ₁₆ H ₃₂ O ₃	272.2343	14.364	3.22
13	Irinotecan	C ₃₃ H ₃₈ N ₄ O ₆	586.275	16.872	7.04
14	Oxidized dinoflagellate Luciferin	C ₃₃ H ₃₈ N ₄ O ₇	602.2701	17.06	6.53
15	Harderoporphyrinogen	C ₃₅ H ₄₂ N ₄ O ₆	614.3099	17.449	0.94
16	(3beta,5alpha,6beta,24R)-Stigmastane-3,5,6-triol	C ₂₉ H ₅₂ O ₃	448.3892	19.565	5.46
17	6-Deoxocathasterone	C ₂₈ H ₅₀ O ₂	418.3784	18.374	6.45
18	D-Lombricine	C ₆ H ₁₅ N ₄ O ₆ P	270.0714	3.046	5.53
19	Estradiol-17β 3-sulfate	C ₁₈ H ₂₄ O ₅ S	352.1339	3.323	1.49
20	2,3,4-Trioxycyclopentanone	C ₁₄ H ₂₆ O ₄ Si	286.1621	5.049	-7.08
21	Phaseolotoxin	C ₁₅ H ₃₄ N ₉ O ₈ P S	531.1957	6.057	5.9
22	Azelaic acid	C ₉ H ₁₆ O ₄	188.1039	6.651	5.22
23	Plakortin acid	C ₁₇ H ₃₀ O ₄	298.2136	6.993	2.69
24	9,10-Dihydroxy-12,13-epoxyoctadecanoate	C ₁₈ H ₃₄ O ₅	330.2409	9.154	0.87
25	9,10-Dihydroxy-12,13-epoxyoctadecanoate	C ₁₈ H ₃₄ O ₅	330.2406	9.522	0.15
26	Roxatidine acetate	C ₁₉ H ₂₈ N ₂ O ₄	348.2045	12.631	1.16
27	Matesaponin 3	C ₅₃ H ₈₆ O ₂₂	1074.5531	14.402	7.45
28	Polyporusterone G	C ₂₈ H ₄₄ O ₅	460.3197	16.146	-1.85
29	Elaeokanine C	C ₁₉ H ₂₂ O ₃	98.1595	17.406	-8.87
30	Validamycin A	C ₄₀ H ₅₂ O ₂	564.3926	20.145	7.34
31	Flurazepam	C ₂₁ H ₂₆ O ₃	326.1873	26.663	2.6

Sample Name	T2	Position	P2-F2	Instrument Name	QTOF	User Name	Success
Inj. Vol.	5	Inj. Position		Sample Type	Sample	IRM Calibration Status	
Data Filename	T2.d	ACQ Method	30min_+ESI_01112021_	Comment		Acquired Time	11/28/2021 3:39:33 AM

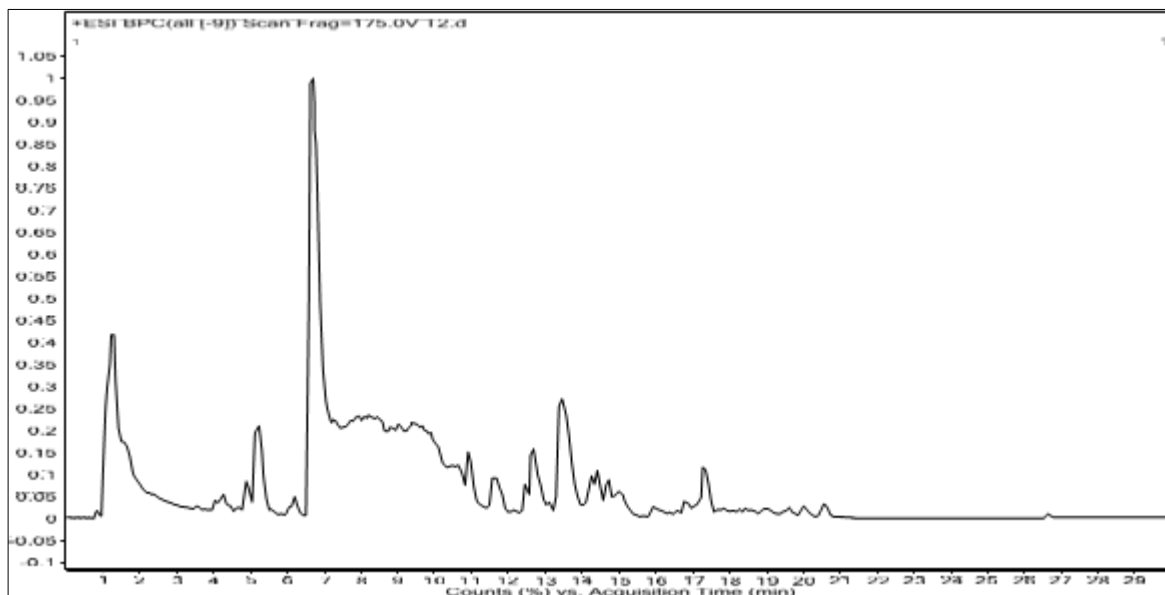


Fig 2: Counts (%) vs. Acquisition time (min)

Conclusion

The ethanolic extract of *Ceropegia bulbosa* var. *bulbosa* *in vivo* tuber revealed the presence of therapeutically important bioactive compounds like 1,4-Dimethylpyrrolo [1,2-a] pyrazine, Gabapentin, Americine, Oseltamivir, 4 - Methylphenyl dodecanoate, 17 beta-Methylestra-1,3,5(10)-trien-3-ol, Citronellyl hexanoate, Limonoate, 1-Monopalmitin, Mitoxantrone, Butyl dodecanoate compound. *In vitro* callus shows presence of Elaeokanine C, Validamycin A, Flurazepam, Isopentenyl adenosine, Mitoxantrone, Phytosphingosine, Oxidized dinoflagellate Luciferin, Harderoporphyrogen, 6-Deoxocathasterone, Irinotecan, Mycinamicin VII compound. It is observed that, the compounds present in *in vivo* tuber also present in *in vitro* callus some variation observed in some compound like Validamycin A, Flurazepam found in callus which were absent in tuber. By using HR-LCMS analysis, we can confirm the phytoconstituents present in the plant part of *Ceropegia bulbosa* Roxb. var. *bulbosa*.

Acknowledgement

The authors are thankful to Sophisticated analytical instrument facility (SAIF) IIT Powai, Mumbai for HR-LCMS analysis of the sample. The first author is thankful to CSIR-UGC for sanctioning fellowship.

References

1. Arora S, Meena S. GC-MS Profiling of *Ceropegia bulbosa* Roxb. var. *bulbosa*, an endangered plant from Thar Desert, Rajasthan. *The Pharma Innovation Journal*. 2017;6(11):568-73.
2. Binish T. Micropropagation of traditional medicinal plant *Ceropegia juncea*. *Ann Plant Sci*. 2018;7:1992-1996.
3. Jagtap AP, Singh NP, Jagtap AP. *Fascicles of Flora of India Fasc. 24*. Botanical Survey of India; c1999.
4. Kamble SY, More TN, Patil SR, Pawar SG, Bindurani R, Bodhankar SL. Plants used by the tribes of Northwest Maharashtra for the treatment of gastrointestinal disorders; c2008.
5. Khare CP. *Indian Medicinal Plants: An Illustrated Dictionary* Springer-Verlag, Berlin; 2007. p. 699-700.
6. Muthukrishnan S, Kumar TS, Gangaprasad A, Maggi F, Rao MV. Phytochemical analysis, antioxidant and

antimicrobial activity of wild and *in vitro* derived plants of *Ceropegia thwaitesii* Hook: An endemic species from Western Ghats, India. *Journal of Genetic Engineering and Biotechnology*. 2018;16(2):621-30.

7. Nayar MP, Sastry AR. *Red data book of Indian plants*; c1987.
8. Zeichner JA. *Acneiform Eruptions in Dermatology*. Springer: Berlin/Heidelberg, Germany; c2014.
9. <https://pubchem.ncbi.nlm.nih.gov/>.