BIOINFOLET 19 (1) : 46 - 50, 2022

FT-IR ANALYSIS OF *IN-VIVO* AND *IN-VITRO* STEM, LEAF AND CALLUS OF *CEROPEGIA BULBOSA* ROXB. *VAR. BULBOSA*

Pooja Sawant and Arvind S. Dhabe

Department of Botany, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad -431004 Maharashtra, India.

ABSTRACT

Ceropegia bulbosa Roxb. var. bulbosa (family-Asclepiadaceae), generally known as lantern flowers, is categorized as a 'threatened plant'. *Ceropegia* contain various bioactive compounds, such as phenolics, saponins, alkaloids, flavonoids, tannins, triterpenes etc. Present study was undertaken to evaluate functional groups by Fourier Transform Infrared Spectrometry (FT-IR) analysis. For this purpose, plants of *C. bulbosa* were grown in *in-vivo* and *in-vitro*. The differences in functional groups have been observed in the samples of stem, leaf, callus and tuber of *C*eropegia *bulbosa* var. bulbosa grown *in-vivo* and *in-vitro*

Key words: in-vitro, FT-IR, *Ceropegia bulbosa*, functional groups

Introduction:

Ceropegia bulbosa Roxb. var. bulbosa is one of the important medicinal plant belonging to family Asclepiadaceae. In India the genus *Ceropegia* comprises of 57 species, 3 varieties and 2 subspecies, out of which the Western Ghats alone possess about 40 species (Karthikeyan et al., 2009). *Ceropegia* contains various bioactive compounds, such as flavonoids, alkaloids, phenolic, triterpanes, saponins, tannins, etc. The tuber of ceropegia is used as folk medicine (Khan and Pradhan, 2012).

Ceropegia contain important alkaloid, Ceropegin (1, 1- dimethyl-5H-furo [3, 4-c] pyridine-3, 4-dione), along with other components. As a result of this, it can be included as an important ingredient in several conventional drug preparations (Arora and Meena, 2017). In addition, this plant contains antioxidants belonging to phenolic acids, and flavonoids, alkaloids, cyanogenic glycosides, amines, non-protein amino acids, carotenoids and ascorbic acid (Dhir and Shekhawat, 2014). *Ceropegia bulbosa* is considered as threatened species due to habitat destruction and over exploitation. However, modern biotechnological strategies such as culturing plant cells and tissues is one of the vital tool for production of medicinally valuable metabolites and conservation and mass propagation of threatened plant species (Nikam and Savant, 2009).

Present study was undertaken to evaluate functional groups available in *C. bulbosa* plants grown in *in-vivo* and *in-vitro*. It has been observed that *in-vitro* plant tissue cultures contain greater quantities of secondary metabolites, than that in mother plants. Muthukrishnan et al., (2018) revealed that, in-vitro culture accumulate and synthesize secondary metabolites, depending on type of the growth medium used, concentration of the medium and the plant species grown *in vitro*. During present study FT-IR technique was exploited to identify the functional groups and to develop FT-IR spectrum profile.

Fourier Transform Infrared Spectrometry (FT-IR) is a technique used to detect the structure of unknown chemical

compound. It is also useful to measure intensity of the absorption spectra, which depends on molecular composition of the chemical group (Griffiths and de Haseth, 1986; Bobby et al., 2012). In short, FT-IR spectroscopy is recognized, time saving tool to characterize and identify functional groups (Ganie and Yadav, 2015).

Material and methods:

The plants of *Ceropegia bulbosa* var. bulbosa were obtained procured from Goga-baba hill, Aurangabad and cultivated in the Botanical garden of Dr. Babasaheb Ambedkar Marathwada University, Aurangabad, (M. S.) Authenticity of the specimen was confirmed with the help of voucher specimen (Accession no. 0705) and it was deposited in herbarium of the Department of Botany. Dr. Babasaheb Ambedkar Marathwada University, Aurangabad.

FT-IR spectroscopic analysis:

Dried *in vivo* leaf, stem, tuber and in vitro leaf, stem, callus were crushed in mortar with pestle and sieved to obtain coarse powder. Ethanol solvent was used for extraction. The FT- IR spectra of in-vivo and in-vitro *Ceropegia bulbosa* Roxb. var. bulbosa was recorded in FT- IR instrument (Bruker, Germany 3000 Hyperion, Microscope with vertex 80 FT-IR system), with PC based software controlled instrument operation and data processing.

A small amount of powdered leaf samples were made into pellets using KBr for FT-IR analysis and a thin film was prepared by applying pressure. The data of infrared transmittance was collected over a wave number ranging from 4000 cm-1 to 500 cm-1. All the samples were analyzed in triplicates with plain KBr pellets as blank. The spectral data were compared with the reference data to identify the functional groups existing in the sample (SAIF, IIT Bombay).

Results and Discussion:

In the present study, the FT-IR spectroscopy was used to identify the functional groups based on the peak values in *in-vivo* and *in-vitro* samples of *Ceropegia bulbosa* Roxb. var. bulbosa. In-vivo and In-vitro powders were subjected to FT-IR analysis and the functional groups of the components were separated based on it's peaks. The results obtained indicated the presence of following functional groups viz., free alcohol; inter- and intra- molecular bonded alcohol, alkane, aromatic compounds, imine or oxime or ketone or alkene, phenol and amine stretching.

The FT-IR spectra of stem, leaf, callus and tuber of plant Ceropegia bulbosa Roxb. var. bulbosa in-vivo and in-vitro study has been shown in figures 1-6. The absorption bands and wave number (cm-1) of dominant peak obtained from absorption spectra are presented in Table 1.

Figure 1 shows the spectra in-vitro stem (P1) of *Ceropegia bulbosa* var. bulbosa. The peak at 3412.83 cm-1 revealed presence of alcohol, phenol (O-H stretch, H-bonded). The peak at 2920.39 and 2851.94 cm-1 refers to the presence of alkanes (C-H stretch). The peak at 1638.01 cm-1 corresponds to carboxylic acid group (C=O stretch). A peak at 1384.50 cm-1 denotes the presence aromatic amines (C-N stretch). A peak of 1036.31 cm-1 indicate the alcohols, carboxylic acids, esters (C-O stretch).

Figure 2 shows the spectra of in-vivo stem (P2) of *Ceropegia bulbosa* var. bulbosa. The broad peaks at 3409.63, 1432.80, 1024.08 cm-1 represent presence of functional groups such as alcohols. Phenols (O-H stretch, H-bonded), carboxylic acids (O-H stretch) aromatic (C-C stretch) and alcohol, carboxylic acids, esters ethers (C-O stretch). The peak at 2919.31 cm-1 correspond to lipids, alkanes compounds and the peak at 1624.36 cm-1 show the presence of ester carbonyl group (C=O stretch). The weak absorption bands at 3409.63, 2919.31,

1624.36, 1432.80 and 1024.08 cm-1 in in-vivo stem (P2) of Ceropegia bulbosa var. bulbosa decreasing value due to C-H/CH3/N-H/O-H stretching of amines and acids, esters etc

Functional groups	In-vitro	In-vivo	In- vitro	In-vivo	In-vitro callus P5	In-vivo tuber P6
	Stem		Leaf			
	P1	P2	P3	P4		
Free alcohol –OH	3412.83	3409.63	3408.76	3410.52	3410.84	3408.32
–OH stretching	2920.39	2919.31	2924.56	2919.57	2920.51	2923.02
C-H stretching alkane	2851.94	-----	----	2850.78	---	----
C≡C, C≡N stretching	----	-----	-----	----	---	----
-COOH protein	----	----	----	----	---	---
C=N or C=O	1638.01	1624.36	1638.55	1618.63	1637.41	1637.20
-C=C-	----	----	----	1539.00	---	----
-CH_3	1384.50	1432.80	1384.71	1434.39	---	1416.55
-CH_3, amide	----	----	----	----	1384.85	---
Alcohols C-O stretch	1036.31	1024.08	1052.82	1078.27	1053.19	1018.26

Figure 3 shows the spectra of in-vitro leaf (P3) of *Ceropegia bulbosa* Roxb. var bulbosa. The broad peak at 3408.76, 2924.56, 1638.55, 1384.71, 1052.82 cm-1 represents the presence of functional groups such as alcohols. Phenols (O-H stretch, H-bonded), carboxylic acids (O-H stretch), alkanes (C-H stretch), amines (N-H bond), aromatic (C-C stretch in ring) and alcohol, carboxylic acids, esters ethers (C-O stretch).

Figure 4 shows the spectra of in-vivo leaf (P4) of *Ceropegia bulbosa* var. bulbosa . The broad peak at 3410.52, 2919.57, 2850.78, 1618.63, 1539.00, 1434.39 and 1078.27 cm-1 represents the presence of functional groups such as alcohols. Phenols (O-H stretch, H-bonded), carboxylic acids (O-H stretch), alkanes (C-H stretch), amines (N-H bend), aromatic (C-C stretch in ring) and alcohol, carboxylic acids, esters ethers (C-O stretch), alkenes (-C=C- stretch), primary, secondary amines. The strong's absorption bands at 3410.52, 2919.57, 2850.78, 1618.63, 1539.00, 1434.39 and 1078.27cm-1 in *in-vivo* leaf (P4) increasing value due to very intense bands occurring at above its corresponding to C-H/CH3/N-H/O-H stretching/bending vibrations respectively indicate the presence of amines and acids, esters, alkenes, organic compounds etc.

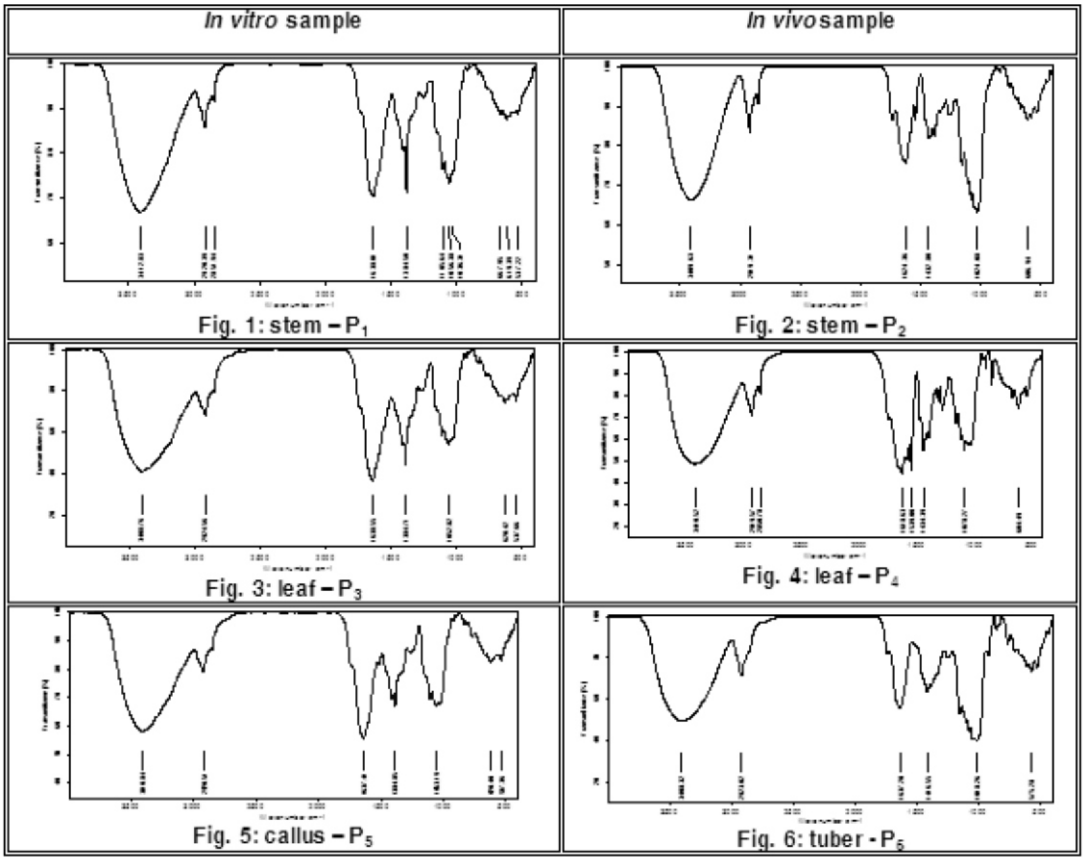

Figure 1-6: FT-IR spectrum of vegetative parts of in-vitro and in-vivo (stem, leaf, callus, tuber) Ceropegia bulbosa Roxb. var bulbosa

Figure 5 show the spectra in-vitro callus (P5) of *Ceropegia bulbosa* var. bulbosa. Very strong absorption peak was observed at 3412.83 cm-1 revealed may be due to presence of bonded N-H/C-H/O-H. The peak at 2920.51 cm-1 refers to the presence of alkanes (C-H stretch) methylene group appears in aliphatic compounds. The peak at 1637.41 cm-1 corresponds the carboxylic acid group (C=O stretch). A peak at 1384.85 cm-1 denotes the presence aromatic amines (C-N stretch). A peak of 1053.19 cm-1 indicate the alcohols, carboxylic acids, esters (C-O stretch).

Figure 6 shows the spectra of in-vivo tuber (P6) of *Ceropegia bulbosa* var. bulbosa. The very strong absorption band observed peak at 3408.32 cm-1 revealed may be due to presence of bonded N-H/C-H/O-H. The peak at 2923.02 cm-1 refers to the presence of alkanes (C-H stretch) methylene group appears in aliphatic compounds. The peak at 1637.20 cm-1 corresponds the carboxylic acid group (C=O stretch). A peak at 1416.55 cm-1 denotes the presence aromatic amines (C-N stretch). A peak of 1018.26 cm-1 indicates the alcohols, carboxylic acids, esters (C-O stretch).

In the present investigation, differences in functional groups have been observed in the sample stem, leaf, callus and tuber of *in-vivo* and *in-vitro Ceropegia bulbosa*

Roxb. var. bulbosa. C-H stretching alkane group was observed in *in-vivo* leaf which was also observed in *in-vitro* stem. Only in-vivo leaf sample shows presence of -C=C- group. Amide group was observed in-vitro callus which was not observed in other samples. It indicates the presence of free alcohol; inter- and intra-molecular bonded alcohol, alkane, aromatic compounds, imine or oxime or ketone or alkene, phenol and amine stretching. By using FT-IR spectrum, we can confirm the functional constituents present in the plant part of Ceropegia bulbosa Roxb. var. bulbosa.

Acknowledgements :

The authors are thankful to the SAIF, IIT Bombay for providing FTIR facility. One of the authors, Pooja Sawant would like to thank CSIR NET-JRF for financial suppor

References :

Arora, S. and Meena, S. (2017). *The Pharma Innovation Journal,* **6** (11): 568.

Bobby, M. N., Wesely, E. G. and Johnson, M. (2012). *Int J Pharm Pharm Sci,* **4** (3), 293.

Dhir, R. and Shekhawat, G. S. (2014). *Acta Physiologiae Plantarum,* **36** (6): 1335.

Ganie, S. A. and Yadav, S. S. (2015). *Research Journal of Medicinal Plant,* **9** (8): 417.

Griffiths, P. and De Haseth, J. (1986). "*Fourier Transform Infrared Spectrometry*" John Wiley and Sons. 340.

Karthikeyan, S., Sanjappa, M. and Moorthy, S. (2009). "*Flowering Plants of India, Dicotyledons* ". Vol. I, (Acanthaceae-Avicenniaceae). pp. 160-164.

Khan, M. A. and Pradhan, D. (2012). *Der Pharmacia Sinica,* **3** (1): 148.

Muthukrishnan, S., Kumar, T. S., Gangaprasad, A., Maggi, F. and Rao, M. V. (2018). *Journal of Genetic Engineering and Biotechnology,* **16** (2): 621.

Nadkarni, K.M. (1976). "*Indian Materia Medica*". Popular Prakashan, Mumbai, India, pp. 303-304.

Nikam, T. D., and Savant, R. S. (2009). *Physiology and Molecular Biology of plants,* **15** (1) : 71.

Sahayaraj, P. A., Gowri, J., Dharmalingam, V., Shobana, R., and Prema, A. A. (2015). *Int J Nano Corros Sci Eng,* **2** (5) : 322.