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Analysis of *Tacca leontopetaloides* (L.) Kuntze by using fourier transform-infrared spectrophotometer (FT-IR)

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Abstract
The present study taken in to consideration to analyze presence of functional group from *in- vivo* and *in- vitro* plant samples of ethanol extracts of *Tacca leontopetaloides* through FTIR. Analysis of the *in-vivo* and *in-vitro* plant samples was carried out through the potassium bromide (KBr) pellet (FTIR grade) method. The FT-IR spectrum showed the presence of Free alcohol-OH stretching, Intra-molecular –OH stretching, C-H stretching alkane, -COOH protein, C=N stretching or C=O, -C=C-, -CH3, -CH3, amide, Alcohols C-O stretch.

Keywords: *Tacca leontopetaloides*, FT-IR spectrum, *in -vivo*, *in -vitro* plant samples

Introduction
Tacca leontopetaloides (L.) Kuntze is a wild perennial herb belonging to the family Taccaceae. *Tacca* (L.) Kuntze is the only genus in the family Taccaceae, a newly-developed plant family carved out of the Dioscoreaceae, however, both families still share a close taxonomic relationship. Its native range extends from Western Africa to Northern Australia to Southern Asia (Borokini, 2014) [2]. *Tacca leontopetaloides* is used as a traditional medicine. Tubers used in stomach disorders, diarrhoea, and dysentery, also used in worm infection, hepatitis and snake bite. It has an ability to make alcohol (Borokini and Ayodele, 2012) [3].

The chemical constituents of *Tacca* include steroids, diarylheptanoids and their glucosides, terpenoids, flavonoids, and some other compounds (Jiang *et al*., 2014) [5]. The analysis of these phytoconstituents is an important task for researchers. A variety of sophisticated techniques are available for screening of compounds. Fourier Transform Infrared Spectrometry (FT-IR) is a physico-chemical analytical technique employed to determine the structure of unknown constituents. (Griffiths and de Haseth, 1986; Bobby *et al*., 2012) [4, 1]. The object of this study is to provide the visual demonstration of fractionation, and isolation of biological active functional groups and chemical structures from plant part *viz*.,-stem, leaf, tuber, peel, seed, root (*in-vivo*) and leaf, stem, tuber and callus (*in-vitro*) of *Tacca leontopetaloides.*

Materials and Methods
1. Plant collection and preparation, Extraction for FT-IR:
Tacca leontopetaloides plant was collected from growing localities from Ambabarwa forest. For *in-vivo* samples the plant parts like stem, leaf, tuber, callus, peel, seed and root were taken and washed with running tap water. These processes were repeated twice. Then these samples washed with sterile distilled water. For *in-vitro* samples the apical meristems of collected plant material were cultivated by micropropogation technique at "Tissue culture Laboratory, at Department of Botany, Dr. Babasaheb Ambedkar Marathwada University Aurangabad". Each plant part for *in-vivo* and *in-vitro* analysis was kept in shade for drying. After drying a fine powder was made with the help of mortar and pestle. 100 gm Fine dried powder of each plant part of *Tacca leontopetaloides* were extracted with ethanol by using Soxhlet extractor for 08 hrs. The samples then kept for evaporation and used for further analysis.

Equipment and conditions
The FT-IR spectra of *in-vivo* and *in-vitro* plant samples of *Tacca leontopetaloides* was carried out at SAIF, IIT, Powai, Mumbi. Samples were analysed on a FT-IR (Bruker, 3000 Hyperion microscope with vertex 80system), with PC based software controlled instrument operation and data processing. A small amount of powdered samples were made in to 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 ranged 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 a reference to identify the functional groups existing in the sample.

Results and Discussion
FT-IR analysis
In the present study, the FT-IR spectroscopy was used to identify the functional groups based on the peak values in the FT-IR present *in-vivo* and *In-vitro* samples of *Tacca leontopetaloides*. The *In-vivo* and *In-vitro* powders were subjected to FT-IR analysis and the functional groups of the components were separated based on their 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 (Table No. 1).

The FT-IR spectra of stem, leaf, tuber, callus, peel, seed, root of *in-vivo* and *in-vitro* plant *Tacca leontopetaloides* study shown in figure 1-10. The absorption bands, the wave number (cm^{-1}) of dominant peak obtained from absorption

spectra are presented in table No. 1. Figure No.1 show the spectra of *in-vivo* stem (T_1) of *Tacca leontopetaloides*. The peak at 3382.42 cm^{-1} revealed the presence of alcohol, phenol (O-H stretch, H-bonded). The peak at 2920.48 cm^{-1} refers to the presence of alkanes (C-H stretch). The peak at 1738.52 and 1627.82 cm^{-1} corresponds the carboxylic acid group (C=O stretch). A peak at 1427.22, 1254.69 cm^{-1} denotes the presence aromatic amines (C-N stretch). A peak of 1036.73 cm^{-1} indicates the alcohols, carboxylic acids, esters (C-O stretch).

Figure No. 2 shows the spectra of *in-vitro* stem (T_2) of *Tacca leontopetaloides*. The broad peak at 3384.55, 1061.62 cm^{-1} represents the 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 observed peak at 2922.21 cm^{-1} which correspond to lipids, alkanes compounds and the peak at 1637.62 cm^{-1} shows the presence of ester carbonyl group (C=O stretch). The weak absorption bands at 2922.21, 2852.43, 1384.52 and 1105.54 cm^{-1} *in vitro* stem (T_2) of *Tacca leontopetaloides*. Decreeing value due to C-H/CH_3/N-H/O-H stretching of amines and acids, esters etc.

Table 1: FT-IR peak assignment table compared with standard chart:

Functional groups	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	T_9	T_{10}
Free alcohol –OH stretching	3382.42	3384.55	3377.04	3384.37	3386.88	3384.74	3385.38	3393.88	3428.02	3434.21
Intra-molecular –OH stretching	2920.48	2922.21	2921.27	2921.22	2930.51	2920.94	2920.52	2919.97	2920.06	2925.11
C-H stretching alkane	----	2852.43	2851.65	2851.71	---	2852.29	2851.92	----	2851.13	2854.23
C≡C, C≡N stretching	----	-----	-----	----	2059.93	2427.20	2426.85	----	----	-----
-COOH protein	1738.52	1727.85	1736.82	1734.46	---	---	1735.54	1735.04	1742.64	1746.31
C=N stretching or C=O	1627.82	1637.62	1655.17	1646.10	1648.86	1642.53	1635.13	1627.65	1641.81	1654.27
-C=C-	----	----	1417.57	1543.62	1543.47	1527.58	1524.77	1511.37	1598.86	1544.26
-CH_3	1427.22	1384.52	1320.81	1385.14	1419.65	1384.67	1384.50	1382.33	1384.97	1462.45
-CH_3, amide	1254.69	1105.54	1255.57	1151.25	1382.57	1257.82	1149.14	1251.03	1157.85	1161.77
Alcohols C-O stretch	1036.73	1061.62	1052.51	1026.20	1016.15	1059.88	1103.46	1056.18	1020.35	1095.86

Figure No. 3 shows the spectra of *in-vivo* leaf (T_3) of *Tacca leontopetaloides*. The broad peak at 3377.04, 2921.27, 2851.65, 1655.17, 1320.81, 1052.51 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). Figure No. 4 shows the spectra of *in-vitro* leaf (T_4) of *Tacca leontopetaloides*. The broad peak at 3384.37, 2921.22, 2851.71, 1646.10, 1543.62, 1385.14, 1026.20 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 3384.37, 2921.22, 2851.71, 1646.10, 1543.62, 1385.14, 1026.20 cm^{-1} in *in-vitro* leaf (T_4) increasing value due to very intense bands occurring at above its corresponding to C-H/CH_3/N-H/O-H stretching / bending vibrations respectively indicate the presence of amines and acids, esters, alkenes, organic compounds etc.

Fig 1-10: FT-IR spectrum of vegetative parts of *in-vivo* and *in-vitro* (stem, leaf, tuber, callus, peel, seed, root) *Taccaleontopetaloides* (L.) kuntze.

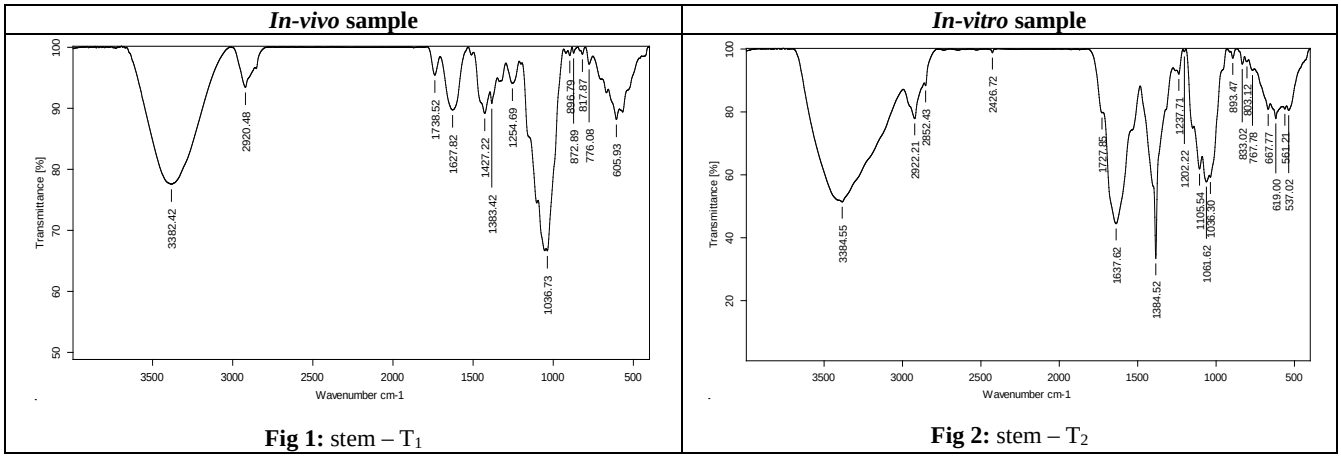

Fig 1: stem – T_1

Fig 2: stem – T_2

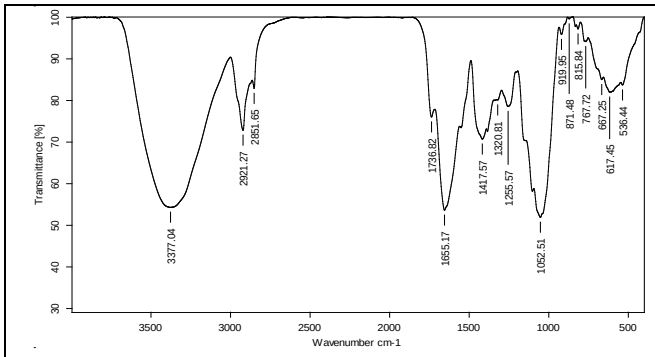

Fig 3: leaf – T_3

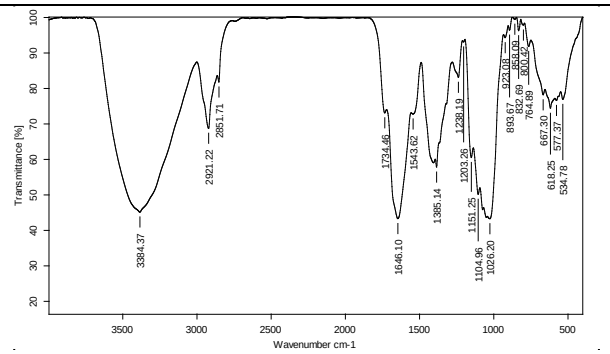

Fig 4: leaf – T_4

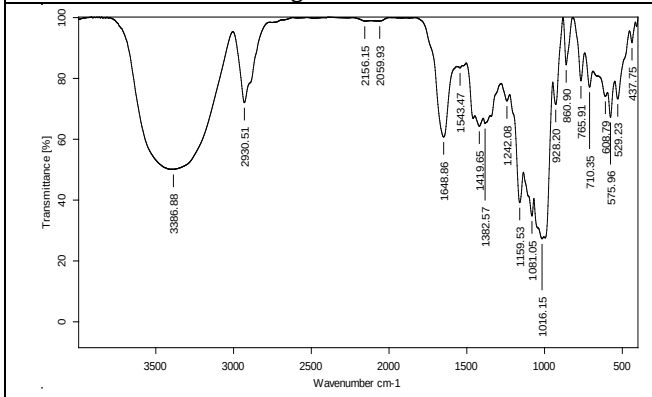

Fig 5: tuber – T_5

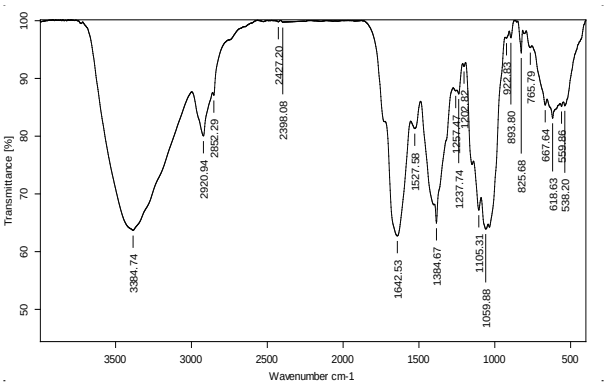

Fig 6: tuber - T_6

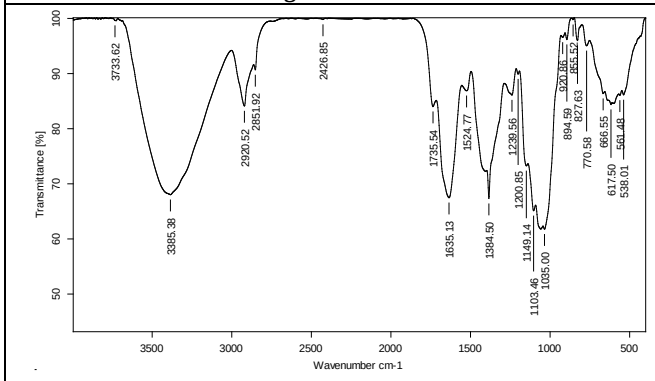

Fig 7: callus - T_6

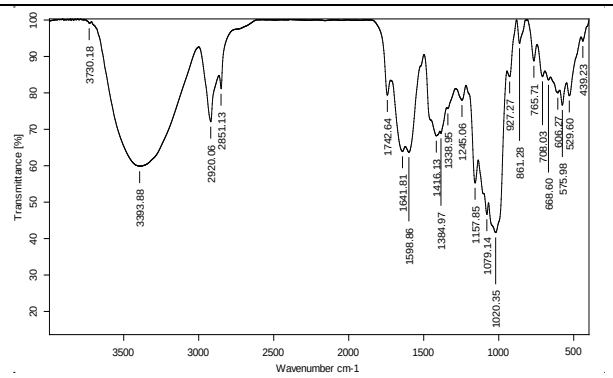

Fig 8: peel - T_6

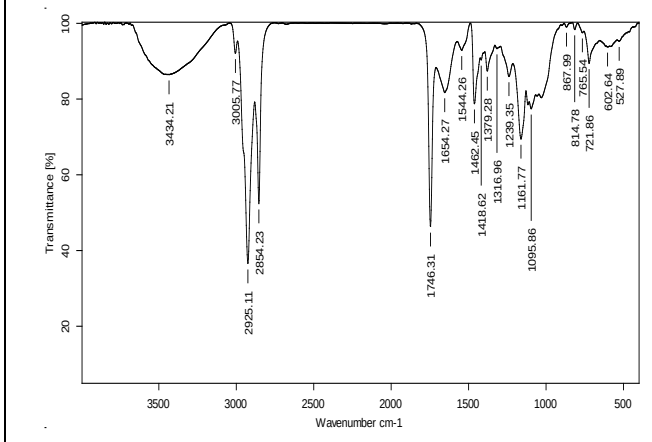

Fig 9: seed - T_6

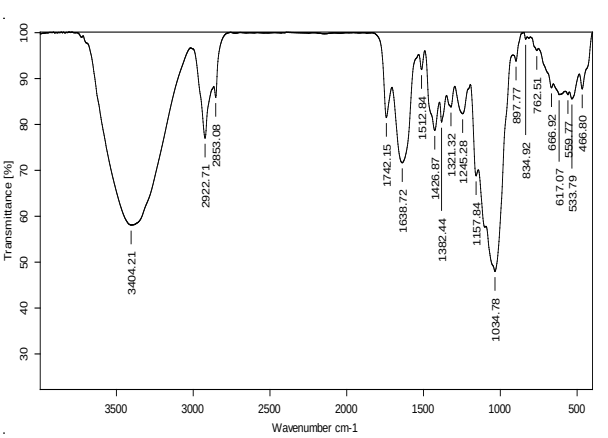

Fig 10: root - T_6

Figure No. 5 shows the spectra *in-vivo* tuber (T_5) of *Tacca leontopetaloides*. The very strong absorption band observed peak at 3386.88 cm^{-1} revealed may be due to presence of bonded N-H/C-H/O-H. The peak at 2930.51 cm^{-1} refers to the presence of alkanes (C-H stretch) methylene group appears in aliphatic compounds. The peak at 1648.86 cm^{-1} corresponds the carboxylic acid group (C=O stretch). A peak at 1382.57 cm^{-1} denotes the presence aromatic amines (C-N stretch). A peak of 1016.15 cm^{-1} indicates the alcohols, carboxylic acids, esters (C-O stretch).

In figure No. 6 shows the spectra *in-vitro* tuber (T_6) of *Tacca leontopetaloides*. The very strong absorption band

observed peak at 3384.74 cm^{-1} revealed may be due to presence of bonded N-H/C-H/O-H. The peak at 2920.94 cm^{-1} refers to the presence of alkanes (C-H stretch) methylene group appears in aliphatic compounds. The peak at 1642.53 cm^{-1} corresponds the carboxylic acid group (C=O stretch). A peak at 1384.67 cm^{-1} denotes the presence aromatic amines (C-N stretch). Peaks of 1059.88 cm^{-1} indicate the alcohols, carboxylic acids, esters (C-O stretch).

Figure No. 7 show the spectra callus (T_7) of *Tacca leontopetaloides*. The peak at 3385.38 cm^{-1} revealed the presence of alcohol, phenol (O-H stretch, H-bonded). The peak at 2920.52 and 2851.92 cm^{-1} refers to the presence of alkanes (C-H stretch). The peak at 1635.13 cm^{-1} corresponds the carboxylic acid group (C=O stretch). A peak at 1384.50 cm^{-1} denotes the presence aromatic amines (C-N stretch). A peak of 1149.14, 1103.46 cm^{-1} indicates the alcohols, carboxylic acids, esters (C-O stretch).

Figure No. 8 shows the spectra of peel (T_8) of *Tacca leontopetaloides*. The broad peak at 3393.88, 1511.37, 1056.18 cm^{-1} represents the 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 observed peak at 2919.97 cm^{-1} which correspond to lipids, alkanes compounds and the peak at 1627.65 cm^{-1} show the presence of ester carbonyl group (C=O stretch). The weak absorption bands at 3393.88, 2919.97, 1627.65, 1382.33, 1056.18 cm^{-1} peel (T_8) of *Tacca leontopetaloides*. decreeing value due to C-H/CH_3/N-H/O-H stretching of amines and acids, esters etc.

Figure No. 9 shows the spectra of seed (T_9) of *Tacca leontopetaloides*. The broad peak at 3428.02, 2920.06, 1641.81, 1384.97, 1020.35 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).

Figure No. 10 shows the spectra of root (T_{10}) of *Tacca leontopetaloides*. The broad peak at 3434.02, 2925.11, 2854.23, 1654.27, 1544.26, 1462.45 and 1095.86 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 3434.02, 2925.11, 2854.23, 1654.27, 1544.26, 1462.45 and 1095.86 cm^{-1} in root (T_{10}) increasing value due to very intense bands occurring at above its corresponding to C-H/CH_3/N-H/O-H stretching/bending vibrations respectively indicate the presence of amines and acids, esters, alkenes, organic compounds etc.

Conclusion

According to our knowledge this is the first study reporting comprehensive profiling of ethanolic vegetative parts of *in-vivo* and *in-vitro* (stem, leaf, tuber, callus, peel, seed and root) *Tacca leontopetaloides* using Fourier Transform Infrared Spectrometry (FT-IR) method. Our study suggested that *Tacca leontopetaloides* is s source of important metabolites contributing phyto-pharmacology activity i.e. phytochemical study, antioxidant and antimicrobial activity.

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