FUNCTIONAL GROUPS OF TREE FERNS (*CYATHEA*) USING FT-IR: CHEMOTAXONOMIC IMPLICATIONS

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Abstract. Fourier Transform – Infra Red (FT-IR) spectra of pure compounds provide unique chemical "fingerprint" with absorption peaks which correspond to the frequencies of vibrations between the bonds of the atoms making up the material. In the present study, FT-IR analysis was used to study the functional groups present in *Cyathea nilgirensis* Holttum, *Cyathea gigantea* (Wall. ex. Hook.) Holttum and *Cyathea crinita* (Hook.) Copel. About 1.0 mg of crude extracts of selected *Cyathea* species were separately made into thin discs with 10–100 mg of potassium bromide using a mould and pressed under anhydrous conditions. The pellets were measured in an automatic recording IR spectrophotometer in the range of 400 to 4000 cm^{-1}. The percentage of transmissions was recorded against the wavenumber. The analytical evaluation of the FT-IR spectra revealed significant differences in band position and absorbance intensities. The comparative FT-IR spectra showed that there is an apparent change in relative intensity of the bands. The results of the present study have proven FT-IR spectroscopy as a valuable tool and chemotaxonomic parameter to distinguish *Cyathea* species based on the functional groups.

Key words: Spectrophotometer, *Cyathea*, FT-IR, frequency, absorbance.

INTRODUCTION

The Indian sub-continent is bestowed with a wide range of climatic and altitudinal variations. The Western Ghats is one of the 34 global biodiversity hotspots and are one of the important centres of plant diversity and richness of fern flora of the world. It covers a distance of about 1600 km from the South of the river Tapti in Gujarat to the tip of South India, Kanyakumari in Tamil Nadu. It has perennial streams, evergreen forests, grasslands and many other habitats harbouring about 320 species of ferns and fern allies with more species diversity in the southern part [9]. The unique physiography with mountainous terrain, narrow

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gorges and valleys with heavy rainfall has blessed this region with an environment most congenial to luxuriant plant growth [11].

Chemotaxonomy had a considerable impact on plant systematics and new systems of classification were being developed which took account of the distribution of secondary metabolites [3]. Phytochemistry is one of the rapidly expanding areas of plant taxonomy (chemosystematics) which utilizes chemical information to improve the classification of plants. The origin of chemotaxonomy may date back to thousands of years *i.e.* from the time of using wild plants as a source of medicine [15]. In the earlier days, only morphological characters were used to identify the drug. But now, identification of plants based on morphological parameters is very tenuous. To overcome this, molecular markers are used as an important tool to better characterize such species. Another form of accomplishing variability studies is the development of analytical techniques that can quantify chemical markers with medicinal activity in the species in study. Phytochemical characters could also be used as markers to identify and differentiate the species. These markers can identify chemotypes and correlate them with the existent genetic variability [14]. Hence, the present study was intended to study the functional groups present in the selected *Cyathea* species using FT-IR analysis.

MATERIALS AND METHODS

COLLECTION OF PLANT MATERIALS

Specimens for the present study were collected from different parts of Tamil Nadu, South India. *Cyathea nilgirensis* Holttum were harvested in and around Kakkachi stream, Tirunelveli hills, *Cyathea gigantea* (Wall. ex. Hook.) Holttum from the road sides near Nadugani, Nilgiris hills and *Cyathea crinita* (Hook.) Copel. from the Anglade Institute of Natural History, Shenbaganur, Palni hills, Western Ghats, South India. The plants were identified based on the “Pteridophyte Flora of the Western Ghats, South India” [8]. Herbarium specimens were deposited in the St. Xavier’s College Herbarium (XCH), Palayamkottai (*C. nilgirensis* – XCH 25423; *C. gigantea* – XCH 25422 and *C. crinita* – XCH 25424).

FT-IR SPECTROSCOPIC ANALYSIS

About 1.0 mg of crude extracts of selected *Cyathea* species were separately made into thin discs with 10–100 mg of potassium bromide using a mould and pressed under anhydrous conditions. The pellets were measured in an automatic recording IR spectrophotometer (Shimadzu 8400S) in the range of 400 to 4000 cm^{-1}. The percentage of transmissions was recorded against the wavenumber. The peak values of FT-IR were recorded and the functional groups were predicted [10].

RESULTS

The analytical evaluation of the FT-IR spectra in terms of functional groups corresponding to absorption of certain frequencies of *C. nilgirensis*, *C. gigantea* and *C. crinita* exhibits the characteristic fingerprint spectral features. It also revealed significant differences in band position and absorbance intensities. The comparative FT-IR spectra showed that there is an apparent change in relative intensity of the bands.

Table 1

FT-IR peak values with functional groups of *C. nilgirensis*

Functional groups with bond stretching	**Petroleum ether**	**Chloroform**	**Acetone**	**Ethanol**
Alcohols (O–H stretch)	–	–	–	3402.43
Primary amines (N–H stretch)	–	–	3394.72	–
Alkanes (C–H stretch)	2924.09	2924.0	2931.80	2924.09
Carboxylic acids (C=O stretch)	–	–	–	1797.66
Esters (C=O stretch)	1735.93	1735.93	–	–
Aldehydes (C=O stretch)	–	1705.07	1705.07	1720.50
Primary amines (N–H bend)	–	1620.21	1620.21	1620.21 1581.63
Nitro compounds (N–O asymmetric stretch)	1543.05 1381.03	1496.76 1373.32	1543.05 1373.32	1373.32
Alkanes (C–H bend)	1465.90	1458.18	–	1458.18
Aromatics (C=C stretch)	–	–	1442.75	–
Alkyl halides (C–F stretch)	1288.45	1165.00	–	–
Aromatic amines (C–N stretch)	–	–	1280.73	1280.73
Alcohols (C–O stretch)	1172.72	–	–	–
Aliphatic amines (C–N stretch)	–	1033.85	1072.42	1072.42
Alkenes (=C–H bend)	956.69	–	–	–
Carboxylic acids (O–H bend)	–	–	918.12	925.83
Alkyl halides (C–Cl stretch)	–	–	825.53	–
Alkyl halides (C–Br stretch)	725.23	725.23	–	–

The FT-IR peak values for various extracts of *C. nilgirensis* were displayed in Figs. 1–4; Table 1. The broad intensity band occurring at 3402 cm^{-1} and 1172 cm^{-1} may be due to O–H and C–O stretching vibration of alcohols. N–H stretch vibration at 3394 cm^{-1} and N–H band at 1620 cm^{-1} and 1581 cm^{-1} determined the presence of primary amines in medium intensity. The strong band occurring at 2924 cm^{-1}, 2931 cm^{-1} and 2854 cm^{-1} related to C–H stretching vibration and the variable band at 1465 cm^{-1} and 1458 cm^{-1} indicates the occurrence of alkanes. The vibration of C=O stretch bands at 1797 cm^{-1}, 1735 cm^{-1} and 1720 cm^{-1} showed the existence of carboxylic acids, esters and aldehydes respectively. The frequency of bands occurring at 1543 cm^{-1}, 1496 cm^{-1}, 1381 cm^{-1} and 1373 cm^{-1} corresponding to N–O asymmetrical stretching revealed the presence of nitro compounds in all the four extracts of *C. nilgirensis*. Aromatics are absorbed at the region 1442 cm^{-1} only in acetone extract of *C. nilgirensis*. Strong intensity bands at 1288 cm^{-1}, 1165 cm^{-1}, 825 cm^{-1} and 725 cm^{-1} are representative for the presence of alkyl halides. The weak absorption band at 1280 cm^{-1} corresponds to aromatic amines and the characteristic absorption at 1072 cm^{-1} and 1033 cm^{-1} showed the presence of aliphatic amines. In petroleum ether extract, the strong band 956 cm^{-1} corresponding to =C–H band indicates the presence of alkenes. O–H band appearing at 925 cm^{-1} and 918 cm^{-1} represents the presence of carboxylic acids.

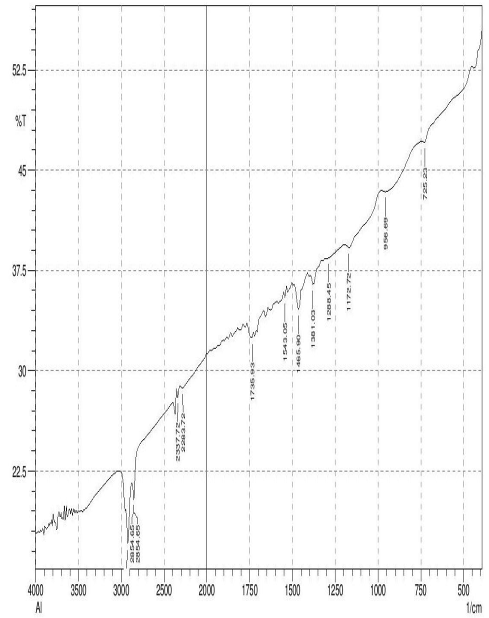

Fig. 1. FT-IR spectrum of *C. nilgirensis* chloroform.

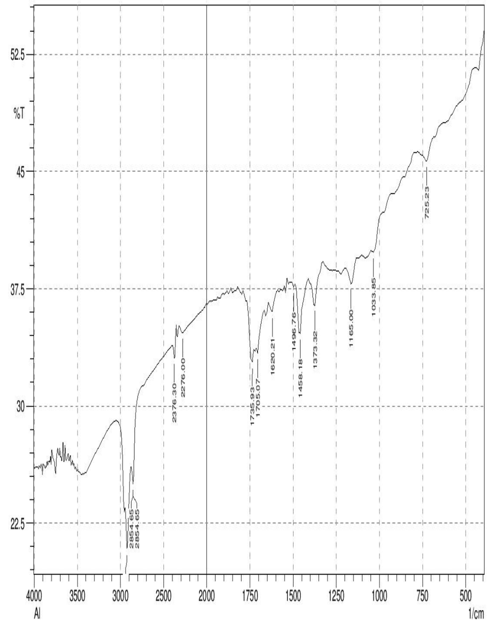

Fig. 2. FT-IR spectrum of *C. nilgirensis* petroleum ether.

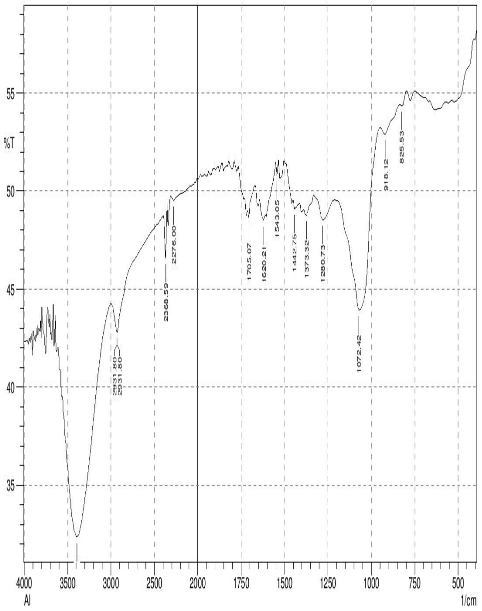

Fig. 3. FT-IR spectrum of *C. nilgirensis* acetone.

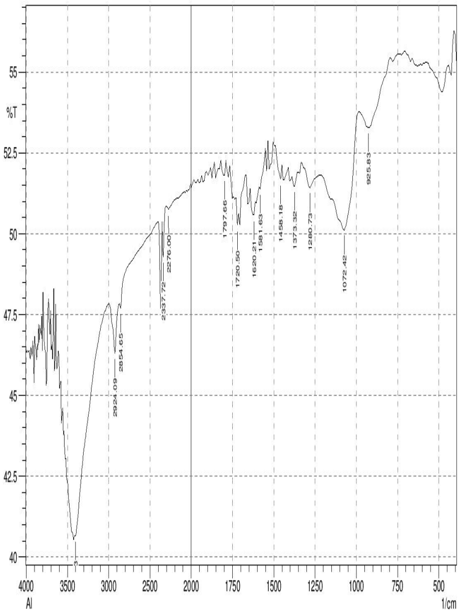

Fig. 4. FT-IR spectrum of *C. nilgirensis* ethanol.

Table 2

FT-IR peak values with functional groups of *C. gigantea*

Functional groups with bond stretching	**Petroleum ether**	**Chloroform**	**Acetone**	**Ethanol**
Alcohols (O–H stretch)	3402.43	–	3448.72	3402.43
Alkanes (C–H stretch)	2924.09	2947.23	2924.09	2924.09
Carboxylic acids (O–H stretch)	–	2839.22	–	–
Anhydride (C=O stretch)	1774.51	–	–	–
Carboxylic acids (C=O stretch)	1751.36 1705.07	1705.07	1797.66 1720.50	1735.93 1720.50
Alkenes (C=C stretch)	1658.78	1651.07	–	1658.78
Primary amines (N–H bend)	1627.92	–	1620.21 1581.63	1620.21
Amide (N–H bend)	1566.20	–	–	–
Nitro compounds (N–O asymmetric stretch)	1527.62 1373.32	–	1496.76	1527.62 1381.03
Alkanes (C–H bend)	1465.90	1458.18	1458.18 1396.46	1458.18
Alkyl halides (C–F stretch)	1157.29	–	1157.29	1165.00
Alcohols (C–O stretch)	1111.00	1111.00	–	–

Table 2

(continued)

Functional groups with bond stretching	**Petroleum ether**	**Chloroform**	**Acetone**	**Ethanol**
Aliphatic amines (C–N stretch)	–	1026.13	1072.42	1080.14
Carboxylic acids (O–H bend)	933.55	–	925.83	918.12
Alkyl halides (C–Br stretch)	–	–	–	725.23

FT-IR spectra of *C. gigantea* showed varied peak values with the presence of different functional groups (Figs. 5–8; Table 2). The broad peak intensity at 3448 cm^{-1} and 3402 cm^{-1} with O–H stretching and strong peak at 1111 cm^{-1} corresponds to C–O stretching determined the presence of alcohols. Alkanes were found to be present with C–H stretch at 2947 cm^{-1}, 2924 cm^{-1} and 2854 cm^{-1} and C–H band at 1465 cm^{-1}, 1458 cm^{-1} and 1396 cm^{-1}. The peaks at 2839 cm^{-1}, 1797 cm^{-1}, 1751 cm^{-1}, 1735 cm^{-1}, 1720 cm^{-1}, 1705 cm^{-1}, 933 cm^{-1}, 925 cm^{-1} and 918 cm^{-1} indicates the presence of carboxylic acids with O–H stretch, C=O stretch and O–H band type of vibrations. The wave numbers 1774 cm^{-1} and 1566 cm^{-1} present in petroleum ether extract of *C. gigantea* matching to C=O stretch and N–H band represent the presence of anhydride and amide respectively. Alkene bands 1658 cm^{-1} and 1651 cm^{-1} with variable intensity correspond to C=C stretching. The medium peaks obtained at 1627 cm^{-1}, 1620 cm^{-1} and 1581 cm^{-1} with N–H bending showed the presence of primary amines. Nitro compounds are absorbed at 1527 cm^{-1}, 1496 cm^{-1}, 1381 cm^{-1} and 1373 cm^{-1} with N–O asymmetrical stretching. The frequencies 1165 cm^{-1}, 1157 cm^{-1} and 725 cm^{-1} showed strong vibrations for the presence of alkyl halides. C–N stretching of aliphatic amines is located at 1080 cm^{-1}, 1072 cm^{-1} and 1026 cm^{-1} respectively.

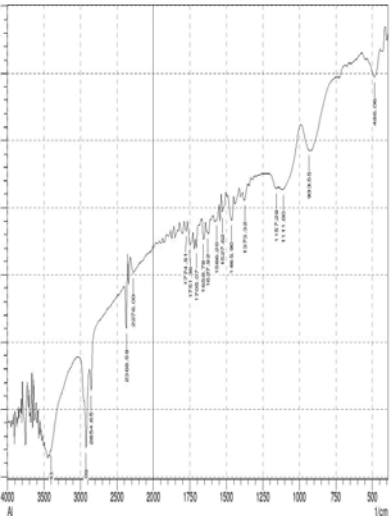

Fig. 5. FT-IR spectrum of *C. gigantea* petroleum ether.

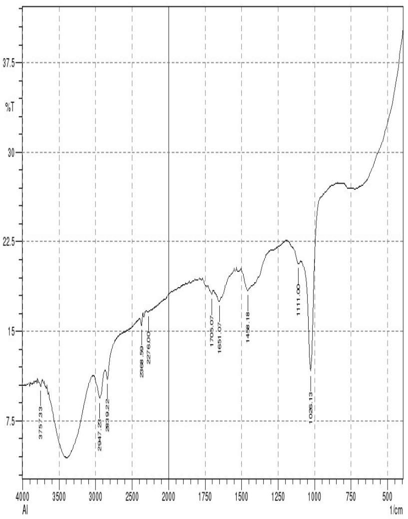

Fig. 6. FT-IR spectrum of *C. gigantea* chloroform.

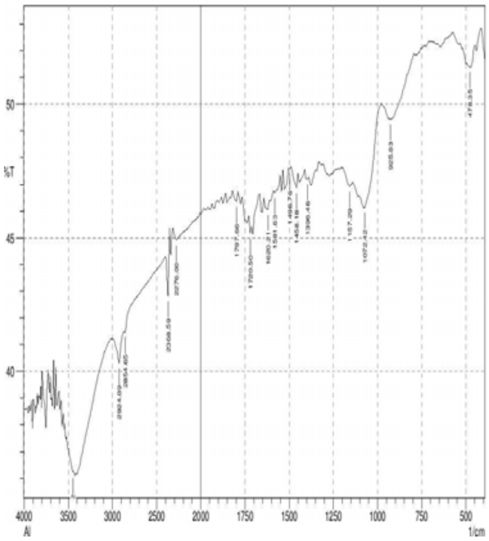

Fig. 7. FT-IR spectrum of *C. gigantea* acetone.

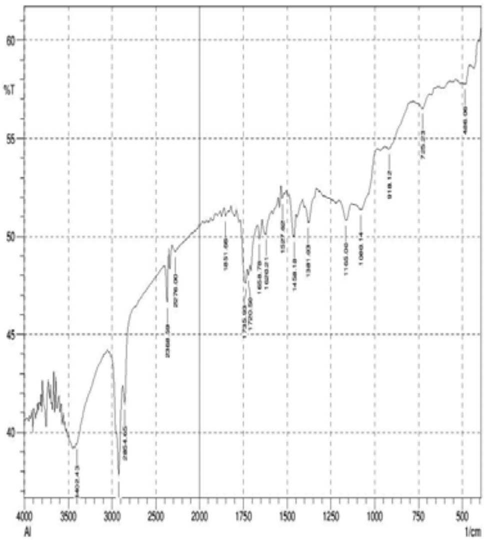

Fig. 8. FT-IR spectrum of *C. gigantea* ethanol.

Table 3

FT-IR peak values with functional groups of *C. crinita*

Functional groups with bond stretching	**Petroleum ether**	**Chloroform**	**Acetone**	**Ethanol**
Alcohols (O–H stretch)	–	3417.86	–	–
Alkanes (C–H stretch)	2924.09	2939.52	2947.23	2954.95
Carboxylic acids (O–H stretch)	–	–	2846.93	–
Esters (C=O stretch)	–	–	–	1735.93
Carboxylic acids (C=O stretch)	1743.65	1705.07	–	–
Alkenes (C=C stretch)	1658.78	–	–	1658.78
Primary amines (N–H bend)	–	1635.64	1635.64	–
Nitro compounds (N–O asymmetric stretch)	1381.03	1543.05 1381.03	1543.05	1381.03
Aromatics (C=C stretch)	1465.90	1442.75	1404.18	1465.90
Aromatic amines (C–N stretch)	–	1280.73	1280.73	1273.02
Alkyl halides (C–F stretch)	1172.72	–	–	1165.00
Alcohols (C–O stretch)	–	–	1103.28	–
Aliphatic amines (C–N stretch)	–	1056.99	–	1080.14
Esters (C–O stretch)	–	–	1018.41	–
Alkenes (C–H bend)	964.41	–	–	–
Alkyl halides (C–Br stretch)	725.23	–	–	725.23
Alkyl halides (C–Cl stretch)	–	–	717.52	–

The FT-IR analysis results of *C. crinita* different extracts were demonstrated in Figs. 9–12; Table 3. The FT-IR spectrum exhibited very broad absorption band at 3417 cm^{-1} with O–H stretching and strong intensity at 1103 cm^{-1} with C–O stretching alcohols. The strong C–H stretching vibration band corresponding to alkanes showed its occurrence in all the four extracts of *C. crinita* at 2954 cm^{-1}, 2947 cm^{-1}, 2939 cm^{-1}, 2924 cm^{-1} and 2854 cm^{-1} respectively. The wave numbers 2846 cm^{-1}, 1743 cm^{-1} and 1705 cm^{-1} were assigned as characteristic absorption of carboxylic acids. The fingerprint bands 1735 cm^{-1} and 1018 cm^{-1} were attributed to esters with C=O and C–O stretching respectively. The variable band 1658 cm^{-1} represents the stretching vibration of C=C and strong band 964 cm^{-1} confirmed the bending vibrations of C–H indicative of the alkenes. Medium absorption peak with N–H bending appearing in the region 1635 cm^{-1} showed the occurrence of primary amines. The strong absorption band observed around 1543 cm^{-1} and 1381 cm^{-1} was due to the N–O asymmetric stretching of nitro compounds. Medium C=C stretching vibrations were located at 1465 cm^{-1}, 1442 cm^{-1} and 1404 cm^{-1} for the presence of aromatics in *C. crinita*. The medium-weak bands in the region 1280 cm^{-1}, 1273 cm^{-1}, 1080 cm^{-1} and 1056 cm^{-1} may be due to the existence of amines. The strong regions 1172 cm^{-1}, 1165 cm^{-1}, 725 cm^{-1} and 717 cm^{-1} were assigned due to stretching vibrations of alkyl halides.

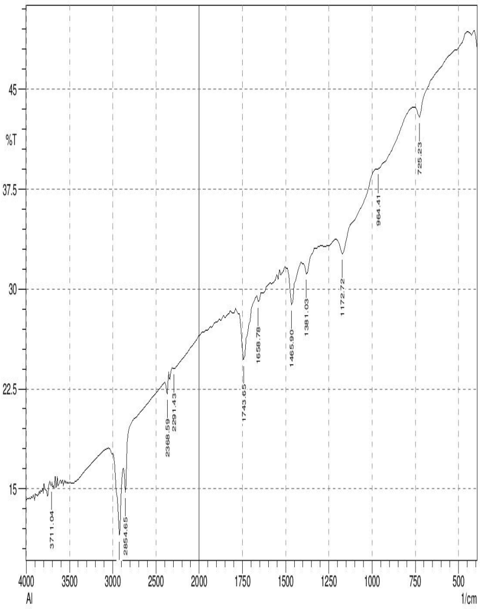

Fig. 9. FT-IR spectrum of *C. crinita* petroleum ether.

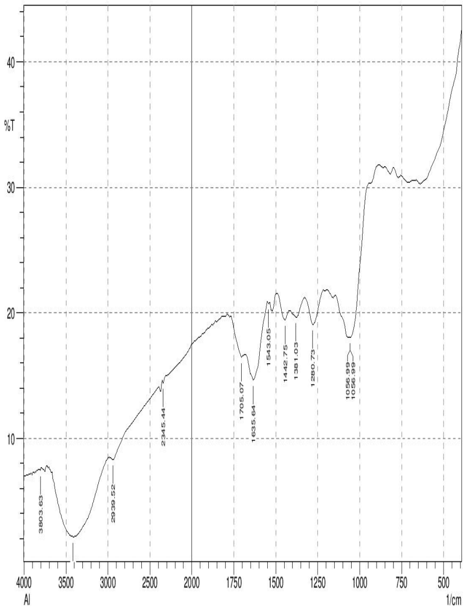

Fig. 10. FT-IR spectrum of *C. crinita* chloroform.

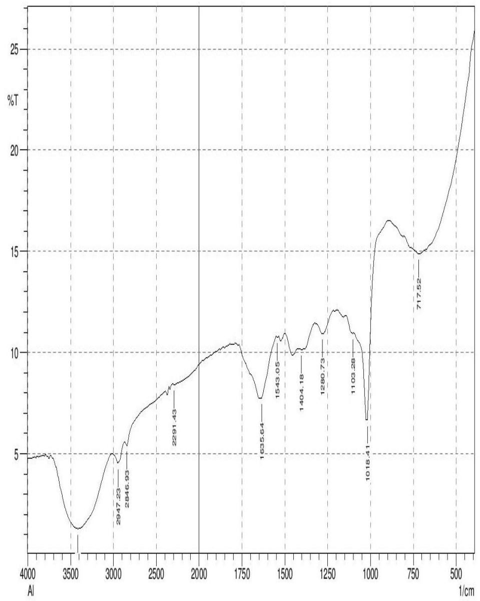

Fig. 11. FT-IR spectrum of *C. crinita* acetone.

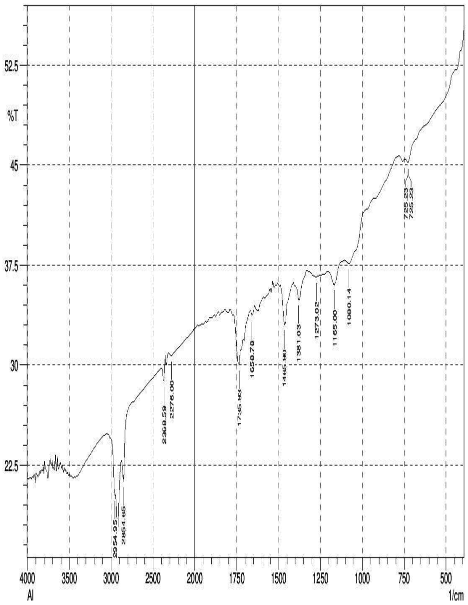

Fig. 12. FT-IR spectrum of *C. crinita* ethanol.

The similarity indices were calculated and the cladogram was constructed based on the FT-IR spectroscopic profile of *Cyathea*. The results revealed the similarities and variation among the studied *Cyathea* species (Fig. 13). The evolutionary tree constructed expressed two clusters (C_1 and C_2). The cluster C_1 includes *C. nilgirensis* and *C. gigantea* whereas cluster C_2 showed the unique presence of *C. crinita*.

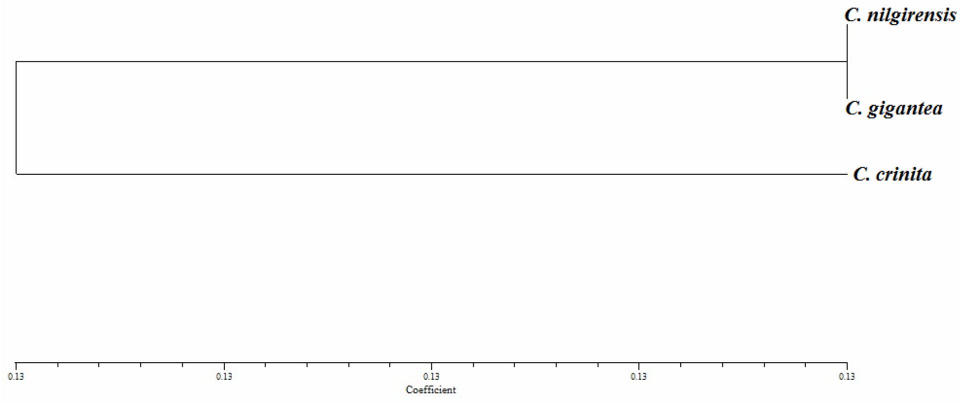

Fig. 13. Cladogram based on FT-IR spectroscopic profile of *Cyathea* species studied.

DISCUSSION

FT-IR spectroscopy has proven to be a valuable tool for the characterization and identification of compounds or functional groups (chemical bonds) present in an unknown mixture of plant extracts. It records the interaction of infra red radiation with experimental samples, measuring the frequencies at which the sample absorbs the radiation and the intensities of the absorptions. Determining these frequencies allows identification of the sample's chemical makeup since chemical functional groups are known to absorb light at specific frequencies [1, 2].

Studies based on FT-IR spectral bands analysis in conjunction with plant taxonomic classification have yielded fruitful data. Kim *et al.* [4] proposed that FT-IR fingerprinting was an excellent method for the determination of phylogenetic relationships between different groups of plants. Lu *et al.* [6] employed FT-IR spectroscopy for identifying the species *Hypericum* and *Triadenum.* Kumar and Murugan [5] used FT-IR approach in taxonomic identification of various accessions of *Solanum capsicoides.* Renisheya and Johnson [13] used UV-Vis and FT-IR spectroscopic profile as pharmacognostic criteria to distinguish the medicinally important *Plumbago* species. Previous reports on FT-IR analysis of ferns viz., *Macroneuropteris scheuchzeri, Alethopteris lesquereuxii, Neuropteris ovata* var. *simonii, Eusphenopteris neuropteroides, Oligocarpia brongniartii, Pecopteris nyranensis, Pecopteris miltonii, Pecopteris aspidioides* and *Pecopteris polypodioides* showed the presence of various functional groups with strong bands in different regions. They employed FT-IR characteristics to distinguish the ferns and used a chemotaxonomic parameter for identifying ferns [7, 12, 16].

Similarly, FT-IR analysis was carried out to identify the similarity and variation of functional groups present in *C. nilgirensis, C. gigantea* and *C. crinita.* The data for infrared spectra of *Cyathea* were shown based on the most common and characteristic group frequencies. The common functional groups present in studied *Cyathea* species include alkanes, nitro compounds, carboxylic acids and aromatics whereas alcohols, primary amines, alkyl halides, amide, anhydride, alkenes and esters were fingerprint peaks present only in a particular extract. The range of the peaks in the FT-IR spectrum is a direct indication of the amount of compounds present in the extracts.

CONCLUSION

FT-IR spectra of pure compounds provide unique chemical "fingerprint" with absorption peaks which correspond to the frequencies of vibrations between the bonds of the atoms making up the material. The results of the present study have proven FT-IR spectroscopy as a valuable tool and chemotaxonomic parameter to distinguish *Cyathea* species based on the functional groups.