FTIR SPECTROSCOPIC STUDIES ON *CLEOME GYNANDRA* – COMPARATIVE ANALYSIS OF FUNCTIONAL GROUP BEFORE AND AFTER EXTRACTION

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Abstract. FTIR spectra of *Cleome gynandra* leaf powder before and after extraction with methanol were recorded. The frequency range and intensities were obtained from absorption spectra. Compression of the constituents present before and after extraction was made. The results have shown that the biomolecules were rich in the sample before extraction as compared to after extraction. The result indicates that the sample before extraction was rich in certain constituents than after extraction. This analysis showed the presence of hydroxyl compounds, carbonyl compounds, amines, halogens compounds in both samples. The analysis also revealed the presence of different types of biomolecules in both extracts. The sample prior to extraction contains aromatic compounds, halogen compounds, amides, whereas the sample after extraction showed the presence of functional groups like carboxyl acid, alkyl amine, alkyl halides.

Key words: *Cleome gynandra,* FTIR spectra, before and after extraction, functional groups.

INTRODUCTION

Traditional medicines are receiving a renewed interest in recent years, due to adverse effects of synthetic medicines. About 40% or more of pharmaceuticals of western countries are relied on natural medicines. Many medicinal plants and their purified constituents have proven the beneficial therapeutic potential [5]. A natural medicine which consists of plants contains a variety of chemical components [15]. These are responsible for defense mechanism of plants and possess medicinal properties. In order to promote the therapeutic compounds of medicinal plants for

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utilization, it is necessary to investigate their composition and activity [12]. The functional groups present in these chemical constituents of plants are usually identified by FTIR. This helps in structure elucidation with other methods and gained importance to identify medicines in pharmacopoeia of many countries [7]. Initially, FTIR was used to elucidate the structure of isolated compounds. Identification and comparison of biomolecules of *Tephrosia tinctoria* and *Atylosia albicans* were done using FTIR [6]. The presence of phenols, alkanes, alcohol, alkyl halides, carboxyl acid and aromatic compounds in ethanol extracts of *Hybanthus enneaspermus* was also studied by FTIR spectroscopy method [1]. FTIR analyses of *Aerva lanata* showed the presence of amino acids, organic hydrocarbons, halogens and amines [14]. Thus, FTIR spectroscopy method has become one of the avenues for the identification of compounds. Here in the present study an attempt was made to understand the difference of functional group present in the sample before and after extraction process. This also helps to know the influence of solvents or water on functional group present in the plant. Hence, in the present study the dried powder as well as methanol extract of *Cleome gynandra* were compared for their changes in functional group. *Cleome gynandra* is a well known medicinal plant known to possess antioxidant, antidiabetic, anti-inflammatory and immunomodulatory activity [9].

MATERIALS AND METHODS

COLLECTION AND PREPARATION OF PLANT MATERIAL

The leaves of *Cleome gynandra* were collected from the natural stands of the species in and around Mysore, Karnataka, India. The excised leaves from the healthy plant were washed with running tap water. The leaves were shade dried at room temperature in a clean environment and powdered. Cold extraction process of the sample with methanol was carried out. The powdered leaf sample was stored in air tight bottles. The powdered samples as well as the methanol extract were subjected to FTIR analysis.

SAMPLE PREPARATION

The powdered sample was kept in a lyophilizer to remove the water contents. The samples were again ground in an agate mortar and pestle in order to obtain fine powder. The samples were mixed with naphthalene (at a ratio of 1/100), and the mixture was subjected to a pressure of 5×10^6 Pa in evacuated die to produce a naphthalene pellet, which was used to determine the functional group.

CHEMICALS

The Analytical grade alcohol and naphthalene were obtained from Sigma Aldrich Company Bangalore, India and were used without further purification for the experiments.

SPECTROSCOPIC ANALYSIS

FTIR 460 plus Jasco was used to record FTIR spectra. Naphthalene and the powdered samples were mixed to prepare as a pellet and scanned at room temperature at 4000 to 400 cm^{-1} spectral range. To improve the signal to noise ratio for each spectrum, 100 interferograms with spectral resolution of ±4 cm^{-1} were averaged. Background spectra which were collected under identical conditions were subtracted from sample spectra. Six different pellets were used to scan each sample under similar conditions. Pellets of 13 mm diameter and 1 mm thickness of sample were made and subject to the same pressure.

RESULTS AND DISCUSSION

FTIR analysis for functional groups revealed the presence of various characteristic functional groups in both the samples (dried plant powder and methanol extract of *Cleome gynandra*). The frequency range and functional group obtained from absorption spectra are presented in Table 1.

Table 1

FTIR spectra of *Cleome gynandra* leaves samples before and after extraction

SL. No.	FREQUENCY RANGE (cm^{-1})	FUNCTIONAL GROUP
1	3550–3450	Hydroxyl compound
2	3400–3200	Hydroxyl compound
3	2854–2926	Methyl group.
4	2855–2975	Cyclo alkane
5	1725–1745	Carbonyl compounds
6	1458–1591	Phenol ring
7	1432–1621	Aromatic ring
8	1150–911	C–O–C group
9	858–733	C–H
10	600–700	C–S linkage
11	550–690	Halogen compound [Bromo-compounds] (C–Br)

The sample prior to extraction was found to contain the aromatic and halogen compounds (Table 2). However, alkanes, silicon compounds are found to be present in the sample after extraction as shown in Table 3.

Table 2

FTIR frequency range and functional groups present in the sample before extraction process

SL. No.	FREQUENCY RANGE (cm^{-1})	FUNCTIONAL GROUP
1	3675.36	Amide
2	2353	C–O bond
3	2260–2100	C=C Stretching bond of alkynes molecule
4	1535–1640	Diketones
5	1440–1470	Nitrosamine
6	1368	Iso propyl group
7	1270–1150	Ester carbonyl
8	1045, 1048	C–O bond
9	1035–1149	Polysaccharide
10	835–805	Aromatic compound
11	500–730	Halogen compound [Chloro-compound] (C-Cl)
12	490–620	Halogen compound [Iodo-compound](C–I)

Table 3

FTIR frequency range and functional groups present in the sample after extraction process

SL. No.	FREQUENCY RANGE (cm^{-1})	FUNCTIONAL GROUP
1	3854	O–H stretching vibration
2	3587.12	Phenols
3	3373–3422	Bonded N–H/C–H/O–H stretching of amines and amides
4	2918.2–2954	C–H
5	2500–3300	Carboxyl acid
6	2322.8–2138.1	C–N
7	2047.30	Silicon compounds
8	1733.59	Ketones
9	1405–1445	Alkanes
10	1421–1415	C–O/C–H bending
11	1382–1036	C–O
12	1215–1325	Alkyl ketone
13	1020–1220	Alkyl amine
14	1026	Vibration of C–O in alcohol hydroxyl group
15	469	Alkyl halides

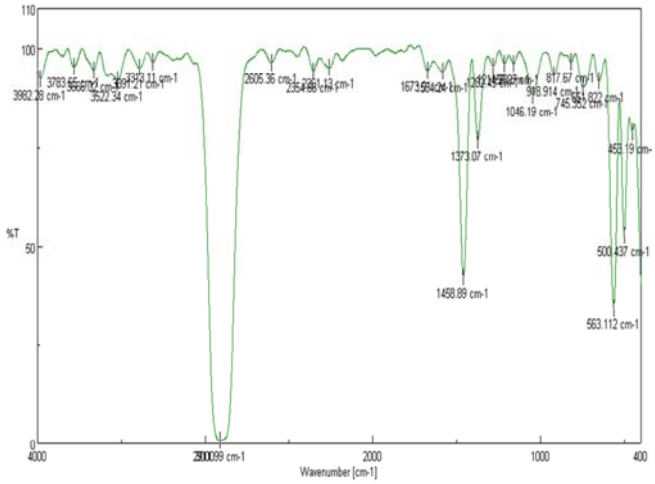

Fig. 1. The absorption spectra of sample before extraction.

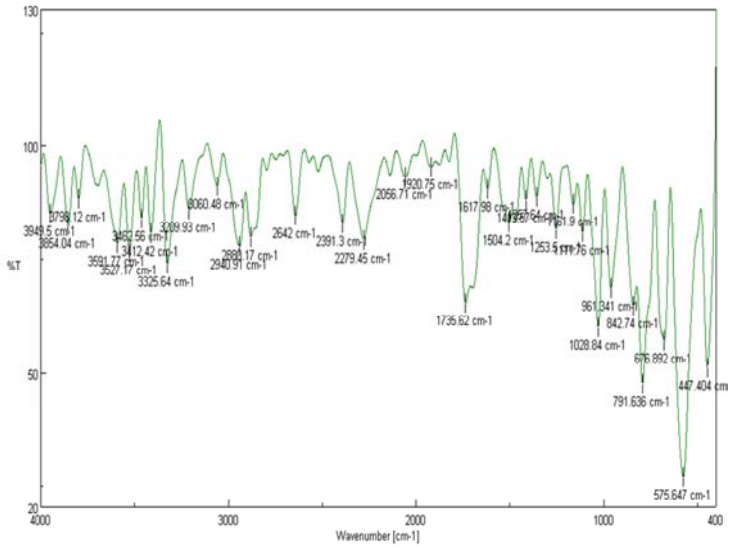

Fig. 2. The absorption spectra of sample after extraction.

The absorption spectra of sample, both before and after extraction, consist of phenol ring and nitrosamine and were indicated by the presence of a strong absorption band at 1458.89 cm^{-1} [8, 11]. The presence of hydroxyl compound was revealed due to the presence of band at 3313.11, 3522.34 and 3391.21 cm^{-1} [8]. The peak at 1584.24 resulted from presence of phenol ring [11]. The band at 918.914 cm^{-1} was attributed to C–O–C group [10]. The band at 651.822 represents C–S linkage [10]. The strong band at 563.112 cm^{-1} and 500.43 cm^{-1} was attributed

to halogen compound (C–Br) or (C–I) [8]. The band at 3527.17 cm^{-1}, 3452.56 cm^{-1}, 3412.42 cm^{-}1, 3325.64 cm^{-1} and 3209.93 cm^{-1} was due to polymeric hydroxyl compound [8]. The band at 2940.91 cm^{-1} was attributed to cyclo alkanes [2]. The band at 1111.76 cm^{-1} represents C–O bond [8]. The band at 961.341 cm^{-1} was attributed to C–O–C group [10]. The band at 676.89 and 575.64 cm^{-1} represents C–S linkage and halogen compound (C–Br) [8, 10]. The absorption band at 1504.2 cm^{-1} represents phenol ring [11].

The absorption spectra of sample before extraction are shown in Fig. 1. The band at 3666.65 cm^{-1} was attributed to amide group [7]. The band at 2354.66 cm^{-1} represents C–O bond. The band at 2261.13 cm^{-1} represents C=C stretching bond of alkynes molecules [10]. The peak at 1584.24 resulted from presence of or due to diketones and the band at 651.822 represents halogen compounds [8]. The peak at 1373.07 cm^{-1} indicated isopropyl group [3]. C–O bond was found to be present due to the appearance of strong absorption peak at 1046.19 cm^{-1} [11]. The band at 1413.57 cm^{-1} was due to alkanes.

The absorption spectra of the sample after extraction are shown in Fig. 2. The dominant band in case of extract was observed at 1735.62 cm^{-1} representing carbonyl compound [8]. The band at 3412.42 cm^{-1} was due to bonded N–H/C–H/O–H stretching of amines and amides [10]. The band at 1028.84 cm^{-1} revealed the presence of C–N stretching alkyl amine [8]. The band at 3854.04 cm^{-1} was attributed to O–H stretching vibration [13]. Phenols presence was determined by the presence of band at 3591.77 cm^{-1}. The band at 1735.62 cm^{-1} was due to ketones [7]. The bands at 2279.45 cm^{-1}, 1357.64 cm^{-1} and 791.63 cm^{-1} correspond to C–N/C–O/C–H respectively [2]. The bands at 2642 cm^{-1}, 3060 cm^{-1} and 3209 cm^{-1} were attributed to carboxyl acid [14]. The band at 447.40 cm^{-1} represents alkyl halides [1]. The band at 2056.71 cm^{-1} was attributed to silicon compounds [4].

Significant changes have been recorded in the functional groups after subjecting the sample for extraction. This includes the presence of carboxyl acid, alkyl amine, alkyl halides which were not observed in the sample prior to extraction. The occurrence of these compounds is revealed in the spectra obtained for the sample after extraction. This may be due to the solubility property of particular compounds in solvents.

CONCLUSION

In the present investigation, twelve functional groups have been observed in the sample before extraction while fifteen functional groups have been observed in the sample after extraction. Functional groups of polysaccharide seen in the sample before extraction were not present in the sample after extraction. Thus, a varied degree of functional groups were observed. The significant changes in the chemical constituents observed in the leaf sample of *Cleome gynandra* before and after extraction offer the scope for research in bio prospection of this plant.

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