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ORIGINAL ARTICLE

PHYTOCHEMICAL INVESTIGATION AND FUNCTIONAL GROUP SCREENING OF *CARDIOSPERMUM HALICACABUM* AND *PISONIA ALBA* BY FT-IR SPECTROSCOPIC ANALYSIS

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ABSTRACT

The present study was carried out to investigate the methanol extracts of leaves of *P. alba* and *C. halicacabum* through FT-IR spectroscopy method. The FT-IR spectroscopic studies revealed different characteristic peak values with various functional compounds in the extracts. The FTIR analysis of methanol leaf extracts of *C. halicacabum* and *P. alba* confirmed the presence of amide, amino acids, protein, lipids, carbonyl groups, nitro compounds, sulpur compounds, nitrosamine, monofluorinated compounds, sulphinic acid group, thiol group, bromo compounds, iodo compounds, lactams and alkanes which showed major peaks. The FTIR method was performed on a spectrophotometer system, which was used to detect the characteristic peak values and their functional groups.

Key words: FT-IR, *Cardiospermum halicacabum*, *Pisonia alba*, Functional group, Bioactive compounds

1.INTRODUCTION

Medicinal plants are the richest bioresource of drugs for traditional systems of medicine; therefore man has been using plant extracts to protect himself against several diseases and also to improve his health and life-style. Plants generally contain both primary metabolites as well as secondary metabolites. The different phytoconstituents present in medicinal plants such as flavonoid, alkaloid, phenol, tannins, carboxylic acids, terpenes, amino acids and inorganic acids. These phytoconstituents present specific distinctiveness and properties to plants (Parekh *et al*., 2007). A large number of medicinal plants and their purified constituents have shown beneficial therapeutic potentials (Khalaf *et al*., 2007). The World Health Organization (WHO) estimates that 80 percent of the world's population presently uses herbal medicine for some aspect of primary health care. Green medicines are healthier, safer and harmless than synthetic ones (Thenmozhi *et al*., 2011). In recent years there has been renewed interest in natural medicines that are obtained from plant parts or plant extracts. India has a rich tradition of plant-based knowledge on healthcare. The medicinal properties of various plant material and extracts have been recognized since the beginning of the 5 century (Egwaikhide *et al*., 2007). The FT-IR 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 plants extract (Hazra *et al*., 2007). In addition, FT- IR spectra of pure compounds are usually so unique that they are like a molecular "fingerprint".

Cardiospermum halicacabum commonly known as Mudakkathan in Tamil. The whole plant has been used for several centuries in the treatment of rheumatism, stiffness of limbs, snake bite (Chopra and Chopra, 1986); its roots for nervous diseases, as a diaphoretic, diuretic,emetic, laxative, refrigerant, stomachic and sudorific ; its leaves and stalks are used in the treatment of diarrhoea, dysentery and headache (Kurian, 1995) and as a poultice for swellings (Chopra and Chopra, 1986); Phytochemical constituents such as flavones, aglycones, triterpenoids, glycosides and a range of fatty acids and volatile ester have been reported from the various extracts of this plant (Srinivas *et al*., 1998).

Pisonia grandis (Synmyn: *Pisonia alba*, *Pisonia morindifolia*) commonly known as Leechikottai kerai in Tamil, (Khare, 2007). In the alternative system of medicine *Pisonia grandis* leaves are used as analgesic, antiinflammatory, diuretic (Radha *et al*., 2008) hypoglycemic agent (Sunil *et al*., 2009), antifungal (Shubashini and Poongothai, 2010). It is also used in the treatment of ulcer, dysentery and snake bite. The leaves are edible and mostly used to treat wound healing (Prabu *et al*., 2008), rheumatism and arthritis (Kim *et al*., 2002). Hence, the present research was conducted to investigate the phytochemical constituents and functional groups of *C. halicacabum* and *P. alba* using FT-IR spectrum.

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2.MATERIALS AND METHODOLOGY

Collection and Preparation of Plant samples

Healthy disease free leaves of *Cardiospermum halicacabum* and *Pisonia alba* were collected from in and around Chidambaram and Thiruvenkadu, plant was identified. The live plants collected were washed thoroughly under running tap water and then were rinsed in distilled water; they were allowed to dry for some time. Then these plants were shade dried without any contamination for about 3 to 4 weeks. The dried plant sample was powdered in blender and was stored in airtight containers.

Preparation of Plant extracts (Methanolic extract)

Air dried powder of 10g was placed in a conical flask containing 100 ml of organic solvent, (Methanol) plugged with cotton and then kept on a rotary shaker at 190-220 rpm for 24 h. Later, it was filtered through 8 layers of muslin cloth and centrifuged at 5000 rpm for 15 min. The supernatant was collected and the solvent was evaporated to make the volume one-fourth of its original volume. Whatman No.1 filter paper was used to separate the extract of both plants. The filtrates were used for further phytochemical analysis.

FT-IR analysis

The FT-IR studies have been followed by the method described by Jagmohn (2005). The powered sample were mixed with dry potassium bromide (KBr pellet) and subjected to a pressure of about 5×106 Pa in an evacuated die to produce a clear transparent disc of diameter 13 mm and thickness 1mm. IR spectra in frequency region 4000-1000 cm $^{-1}$ were recorded at room temperature on a perkin- Elmer fourier transform spectrometer equipped an air cooled DTGs (deuterated triglycine sulfate) detector. For each spectrum, 100 scans were co- added at a spectral resolution of 4cm^{-1}. The frequencies for all sharp bands were accurate to 0.01 cm^{-1}.

3.RESULTS

The crude extract of *C. halicacabum* and *P. alba* and was passed into the FTIR and the functional groups of the components were separated based on its peak ratio. The FTIR spectrum of leaf extracts (prepared in methanol solvents) of *C. halicacabum* and *P. alba* are given in table 1 and 2 and fig 1 to 2. In the present results of FTIR analysis of *C. halicacabum* was confirmed the presence of amines, lipids, amino acids, protein, halogen derivatives, lactams, sulpur compounds, alkanes and iodo compounds which shows major peaks at 3416.87, 2923.33, 2855.19, 1649.87, 583.56, 1545.21, 1463.01, 1407.68, 1243.84, 1112.33, 1023.56, 668.49, 613.20 and 465.39 respectively (Table- 1 and Fig- 1). The results of FTIR analysis of *P. alba* was confirmed the presence of amines, lipids, amino acids, protein, carbonyl compound, nitro compounds, sulpur compounds, nitrosamine, monofluorinated compounds, sulphinic acid group, thiol group, bromo compounds and iodo compounds which shows major peaks at 3411.43, 2922.69, 2854.79, 1643.50, 1736.99, 1377.26, 1320.88, 1112.33, 1068.38, 1030.14, 827.40, 780.09, 667.91 and 526.03 respectively (Table- 2 and Fig- 2). The data on the peak values and the probable functional groups (obtained by FTIR analysis) present in this leaf extracts.

Table 1. Show the FT-IR frequency range and functional groups present in the *Cardiospermum halicacabum*

S. No.	Frequency ranges	Functional groups
1	3416.87	N-H stretching of amines
2	2923.33	C-H stretching mainly lipids
3	2855.19	CH_2 symmetric stretching of amino acid
4	1649.87	N-H-in-plane bending mainly proteins
5	583.56	C-Br stretching of halogen derivatives
6	1545.21	C=O stretching mainly proteins
7	1463.01	N-H in plane of lactams
8	1407.68	C=S stretching mainly sulphur compounds
9	1243.84	CH_2 out –of-plane bending of alkanes
10	1112.33	Symmetric stretching of amino acids
11	1023.56	SO_3 symmetric stretching mainly sulphur compounds
12	668.49	C-Br stretching of halogen derivatives
13	613.20	C-I stretching of iodo compounds
14	465.39	S-S stretching mainly sulphur compounds

Table 2. Show the FT-IR frequency range and functional groups present in the *Pisonia alba*

S. No.	Frequency ranges	Functional groups
1	3411.43	N-H stretching of amines
2	2922.69	C-H stretching of lipids
3	2854.79	CH_2 symmetric stretching of amino acid
4	1643.50	N-H in plane bending mainly in proteins
5	1736.99	C=O stretching of carbonyl compounds
6	1377.26	NO_2 symmetric stretching of nitro compounds
7	1320.88	SO_2 symmetric stretching of sulphones (Sulphur compounds)
8	1112.33	N-N stretching of nitrosamine
9	1068.38	C-F stretching of monofluorinated compound
10	1030.14	S=O stretching of sulphinic acid group
11	827.40	S-O stretching of sulphinic acid group
12	780.09	C-S stretching of thiol group
13	667.91	C-Br stretching of alicyclic axial(Bromo compounds)
14	526.03	C-I stretching iodo compounds

4.DISCUSSION

The FT-IR spectroscoic analysis showed the presence of phytoconstituents in the plants crude extracts of *C. halicacabum* and *P. alba*. The FT-IR analyzes of *C. halicacabum* represent the following functional groups. The

Fig 1. Shows the FT-IR spectrum of *Cardiospermum halicacabum*

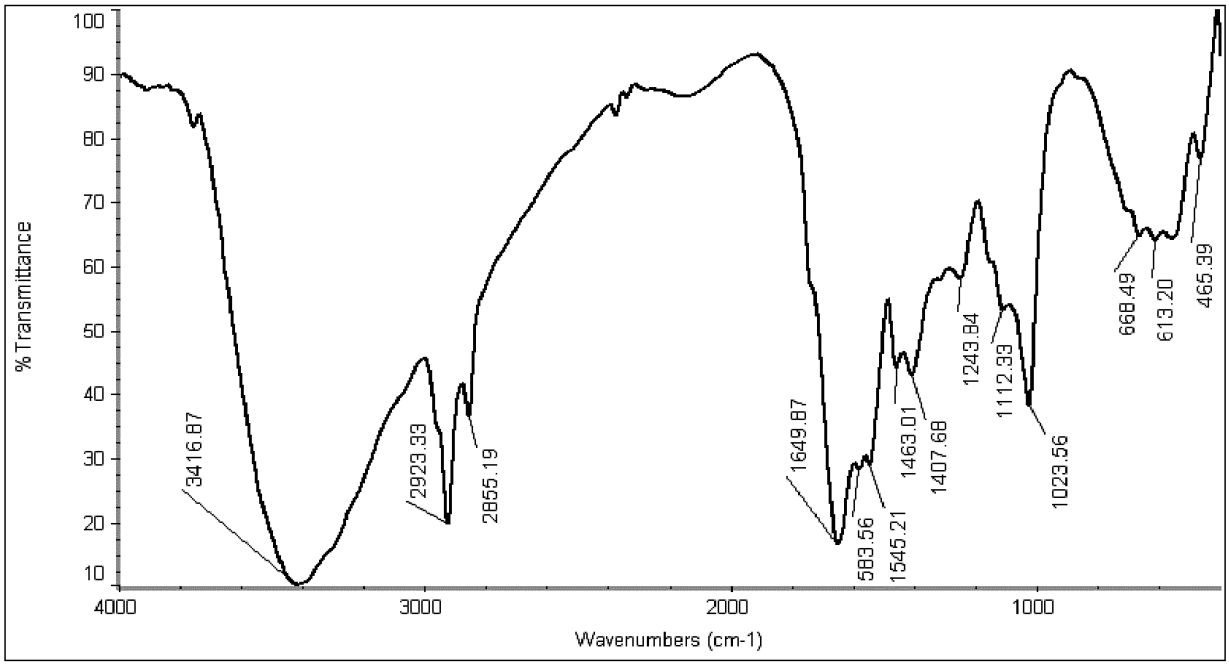
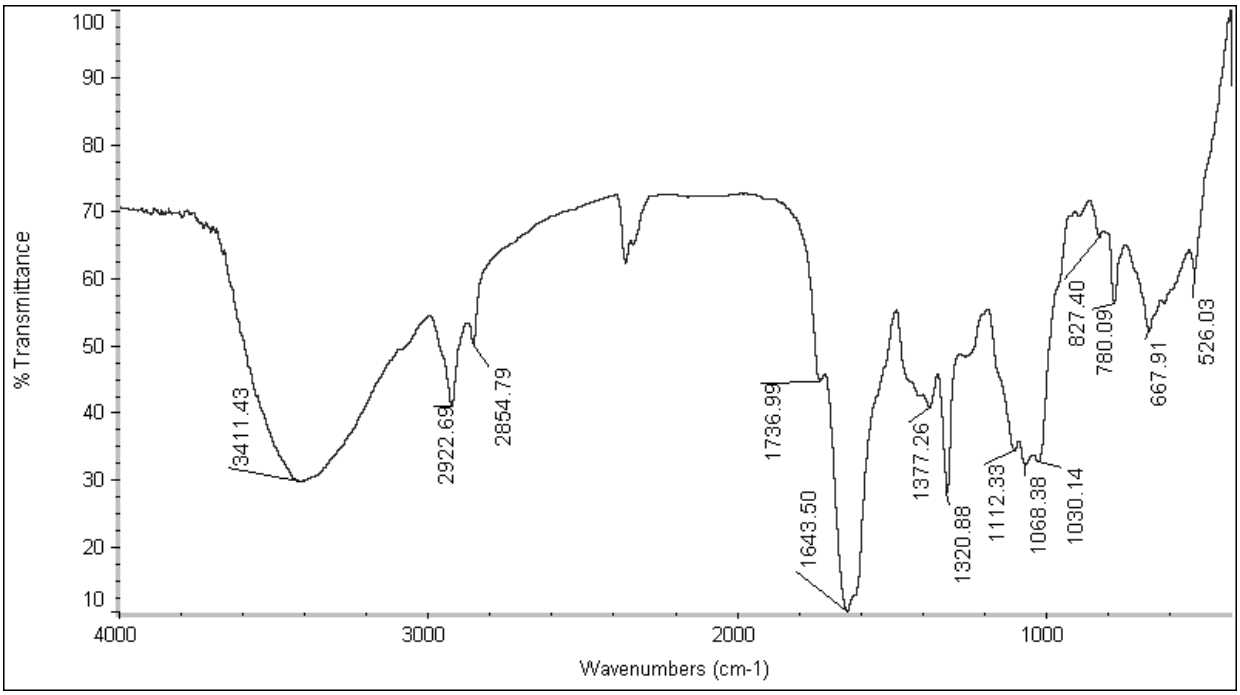


Fig 2. Shows the FT-IR spectrum of *Pisonia alba*



infra red spectrum shows a frequency ranges 3416.87 cm^{-1} representing the N-H stretching and indicates the presence of amines. The peak obtained at 2923.33 cm^{-1} indicated the C-H stretching and mainly presence of lipids. The peak obtained at 2855.19 cm^{-1} indicated the CH_2 symmetric stretching conform the presence of amino acid. The peak obtained at 1649.87 cm^{-1} indicated the N-H-in plane bending mainly presence of proteins. The peak obtained at 583.56 cm^{-1} indicated the C-Br stretching of halogen derivatives. The peak obtained at 1545.21 cm^{-1} indicated the C=O stretching, mainly presence of proteins. The peak obtained at 1463.01 cm^{-1} indicated the N-H in plane and conform the presence of lactams. The peak obtained at 1407.68 cm^{-1} indicated the C=S stretching and mainly presence of sulphur compounds. The peak obtained at 1243.84 cm^{-1} indicated the CH_2 out of plane bending, presence of alkanes. The peak obtained at 613.20 cm^{-1} indicated the C-I stretching, mainly presence of iodo compounds.

The FT-IR analyzes of *P. alba* represent the following functional groups. The infra red spectrum shows a frequency ranges 3411.43 cm^{-1} representing the N-H stretching and indicates the presence of amines. The peak obtained at 2922.69 cm^{-1} indicated the C-H stretching and mainly presence of lipids. The peak obtained at 2854.79 cm^{-1} indicated the CH_2 symmetric stretching conform the presence of amino acid. The peak obtained at 1643.50 cm^{-1} indicated the N-H-in plane bending mainly presence of proteins. The peak obtained at 1736.99 cm^{-1} indicated the C=O stretching conform the presence of carbonyl compounds. The peak obtained at 1377. 26 cm^{-1} indicated the NO_2 symmetric stretching of nitro compounds. The peak obtained at 1320.88 cm^{-1} indicated the SO_2 symmetric stretching presence of sulphones (Sulphur compounds). The peak obtained at 1112.33 cm^{-1} indicated the N-N stretching presence of nitrosamine. The peak obtained at 1068.38 cm^{-1} indicated the C-F stretching presence of monofluorinated compound. The peak obtained at 1030.14 cm^{-1} indicated the S=O stretching, conform the presence of sulphinic acid group. The frequency range 827.40 cm^{-1} peak is representing S-O stretching vibration of sulphinic acid group. The peak obtained at 780.09 cm-1 indicated the C-S stretching presence of thiol group. The peak obtained at 667.91 cm^{-1} indicated the C-Br stretching conform the presence of alicyclic axial (Bromo compounds). The peak obtained at 526.03 cm^{-1} indicated the C-I stretching of iodo compounds was conformed. Muruganantham *et al*. (2009) carried out the FTIR spectral analysis of plant parts like leaf of the medicinal plants, *Eclipta alba* and *Eclipta prostrate* and reported the presence of characteristic functional groups of carboxylic acids, amines, amides, sulphur derivatives, polysaccharides are responsible for various medicinal properties of both herbal plants. Ragavendran *et al*. (2011) point out the functional groups of carboxylic acids, amines, amides, sulphur derivatives, organic hydrocarbons, halogens that are responsible for various medicinal properties of *Aerva lanata*.

Meenambal *et al*. (2012) pointed out the phytochemical information from FTIR studies of methanol Extract of *Delonix elat* Leaves. Muthumani *et al*. (2010) reported that the chemical investigation of *Toddalia asiatica* and *Cardiospermum halicacabum*. A peak at 750-800 cm^{-1} was indicated C-H bending of aromatic compounds. In previous researches show the main constitutents of *Acorus Calamus* are monoterpenes, sequiterpenes, phenylpropenoids,

flavonoids and quinine. With the knowledge Beta-Asarone was the major constituent in the leaves, whereas acorenone was dominant in the rhizomes (Venskutonsis *et al*., 2003). Besides Monoterpene hydrocarbons, sequestrine ketones, Asarone (2,4,5-trimethoxy-1- propenylbenzene) and Beta-asarone (cis-isomer) and eugenol were also identified (Kindscher *et al*., 1992). The FT-IR spectrum of ECH confirms the presence of functional groups for phenolics and flavonoids, which are widely reported for their antioxidant potential. Flavonoids and phenolic acids have antibacterial, antifungal, antiviral, hepatoprotective, immunomodulating and anti-inflammatory properties (Havsteen, 1983).

The chemical profile of *C. halicacabum* L. is relatively complete there is some variability in the content of specific chemicals. (Broadley *et al*., 2004) reported the chemical profile: specified fatty acids 98.8 % of lipids; Oil content 31.60% by weight; Iodine value 71% by weight. However, noticed that leaves contain considerable amounts of saponins, alkaloids, (+)-pinitol, apigenium, luteolin and chrysoeriol. The major cyano lipid (49%) is a diester having two fatty acid moieties esterfied with 1-cyano-2- hydroxymethyl-prop-2ene-l-ol followed by a diester derived from 1-cyano- 2-hydroxymethyl-prop-2-ene-3-ol (6%). Of the fatty acids, 11-eicosenoic acid is the major component (42%), other chief components of the oil include oleic acid (22%), arachidic acid (10%), linolenic acid (8%), palmitic acid (3%) and stearic acid (2%) including small proportions (1- 2%) of a low-molecular weight acid, and several C22 acids. Other minerals such as Ca (1.30%), K (4.01%), Mg (0.43%), P (0.83%), Organic-N (5.19%), Total-N (7.16%), and C (48.1%) were recorded by (Vasantharaja *et al*., 2012). The preliminary phytochemical studies of *P. alba* showed the presence of Vitamin A, Vitamin C, thiamine, riboflavin, nicotinic acid (Vitamin B3), alkaloids, proteins and fats. Vitamin C is one of the four dietary antioxidants, the others being Vitamin E, Vitamin A precursor β-carotene and Selenium (Dhanasekar and Sorimuthu, 2005). These bioactive compounds present in *Cardispermum halicacabum* and *Pisonia alba*.

5.CONCLUSION

FT-IR spectroscopic technique was proved to be a rapid and sensitive method to analyze the majority of the constituents of *C. halicacabum* and *P. alba*. It paves the way for the development of several treatment regimens based on this extract. In addition, further research is needed with these plants to identify the unknown functional groups, isolate, characterize and elucidate the structure of the bioactive compounds which are responsible for the other therapeutic activity.

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