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Comparative studies of functional groups present in invasive and economically important plant leaf methanolic extracts by using FTIR spectroscopic analysis

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Abstract

Low molecular weight substances known as secondary metabolites (SM) are essential for plants' interaction with their surroundings but are not necessary for plants to survive. Plants extracts have traditional uses against many pathogens. These substances frequently play a role in plants' defense against biotic (bacteria, fungi, nematodes, insects, or animal grazing) or abiotic (increased temperature and moisture, shade, damage, or heavy metal presence) stresses. The FTIR approach was applied with an FTIR spectroscopy instrument, which is used to identify functional groups and characteristic peak values of phytoconstituents. The chemical properties of these plants can be used to understand their therapeutic potential, which could be a creative way to develop affordable, safe, and effective herbal remedies to treat a variety of ailments. The current study focuses on functional group screening using FTIR. The analysis found that the extract has various typical peak values associated with various functional Groups like alcohol, phenol, alkanes, amino acids, aldehyde, aromatic compound, secondary alcohol, phenol, and carboxylic acids. When compared to the leaf extracts of *Callistemon subulatus*, *Centella asiatica*, *Catharanthus roseus*, *Moringa oleifera*, and *Carica papaya*, the leaf extracts of *Catharanthu roses*, *Centella asiatica* had the highest number of functional groups. This functional group could positively act as modulating metabolic processes and may act as receptor and enzyme inhibitors.

Keywords: Secondary metabolite; Phytochemical; FTIR Spectroscopy; Functional group

1. Introduction

Phytochemicals naturally occur in medicinal plants that have defense mechanisms and protect the plant from various diseases [1]. Evaluation of the phytochemical constituents of a medicinal plant is considered to be the main step in the medicinal plant is considered to be the main step in medicinal plant research [2]. Approximately 80% of the world's population relies on traditional medicine for health care, and most therapies use plant extracts and their active compounds [3], suggesting that two-thirds of all plant species have medicinal value [4]. Many plants contain natural antibacterial compounds used to treat most bacterial infections as natural medicine [5]. Today several chemicals obtained from plants are used as vital drugs in more countries in the world [6]. WHO has stated that herbal or medicinal plants are the best source to obtain a variety of drugs.[7] *Centella asiatica* is a small edible, herbaceous medicinal plant belonging to the *Apiaceae* family native to India, Sri Lanka, Iran, New Guinea, Australia, Indonesia southern and central Africa [8]. In 2006, it was ranked third position in a priority list of most essential Indian medicinal plants based on their pharmaceutical and economic importance and also the demand for its raw material is constantly rising throughout the world [9]. *Catharanthus roseus L.* (G.) Don, is an important medicinal plant belonging to the *Apocynaceae* family; this plant is a dicotyledonous angiosperm and synthesizes two terpene indole alkaloids: vinblastine and vincristine that are used to fight cancer [10]. *M. oleifera* Lam. (syn. *Moringa pteridosperms Gaerthn*, *Moringa moringa Millsp.*) is called Morunga in the Dravidian language (India), which means "generic root". Other regional names are Kelor, Marango, Moonga, Mlonge, Mulangay, Nébéday, Saijhan, and Sajna or Benzolive [11]. English names are Horseradish tree, Drumstick tree, Never Die tree, West Indian Ben tree, or Radish tree.[12] *Callistemon citrinus L.*, commonly known as

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'red bottle brush', is one of those medicinal plants with great medicinal importance. The name of the plant, Callistemon, is derived from the Greek kalos meaning beautiful stem meaning stamens and citcitrustin the train-us meaning lemon, referring to the scent of the leaves [13]. It is a beautiful evergreen tree belonging to the family *Myrtaceae* [14]. *Carica papaya* Linn belonging to the family *Caricaceae* is commonly known as papaya in English, Papita in Hindi, and Erandakarkati in Sanskrit. Papaya is a powerhouse of nutrients and is available throughout the year [15]. It is a rich source of three powerful antioxidant vitamins (C, A & E); the minerals (magnesium and potassium;) the B vitamin pantothenic acid, and folate and fiber [16]. Identification of the chemical nature of the constituents present in medicinal plants will provide valuable information related to functional groups responsible for the therapeutic reactions [17]. The characteristics cs functional group that are responsible for the medicinal properties of plants are confirmed by FTIR analysis [18]. During the decade near-infrared spectroscopy has become one of the most important tools for the following reasons: it represents a non-invasive analytical tool allowing fast and simultaneous qualitative as well as quantitative determination of natural products and their constituents. The infrared spectrum is formed due to the absorption of electromagnetic radiation at frequencies that co-relates to the co-relation of specific sets of chemical bonds from within a molecule [19]. The present study is therefore aimed at identifying the functional groups present in methanol extracts of different plants by FTIR spectroscopic analysis that could logically enable its applied aspects in present-day herbal medicines [19,20].

2. Material and methods

2.1. Plant material collection and identification

The samples of plants were gathered in Thakurgaoen. Initial taxonomic identities were determined by the Flora app, and additional identification was completed by Dr. S. Prabha, a taxonomist from Ranchi University's PG Department of Botany. Fully developed, fresh leaves were gathered, properly cleaned under the tap water, followed by distilled water, shade dried, homogenized to a fine powder, and then kept in airtight bottles.

2.2. Extraction process

A beaker containing 150ml of methanol and 10g of dried, finely ground plant material was used. For 48 hours, the mixture was incubated at room temperature while being continuously stirred on an orbital shaker. The methanol extract was then separated via filter paper, the filtrate was concentrated, dried, and used for further phytochemical investigation. When not in use, the methanol extracts were stored in a refrigerator at 4 °C.

2.3. FTIR spectrum examination

The dry powder extracts of leaves were mixed with KBr salt, to prepare a translucent sample disc. The samples were loaded onto an FTIR spectroscope and the spectroscopic results were recorded, the scan range was between 4,000-400 cm^{-1}. The FTIR spectrum of all samples was analyzed based on peak values in the region of infrared radiation.

3. Results and discussion

Centella asiatica absorption spectra in (fig 1 and Table 1). Due to O-H stretching, the band at 3289.27cm-1 was assigned to the hydroxy compound. Due to asymmetric stretching of the C-H vibration, the band at 2919.72 cm-1 is assigned to saturated aliphatic compounds. Because of the synthetic stretching of the C-H vibration, the band at 2847.3 cm-1 indicates lipids and proteins. Due to C=O stretching, the peak at 1731.8cm-1 indicates the aldehyde compound ketone. Because of the appearance of an absorption peak at 1605.68cm-1, C=O stretching was determined to be a ketone compound. The peak at 1307.1 demonstrates CN stretch, indicating aromatic primary amine. The peak at 1214.7 cm-1 was caused by a C-H in-plane blend, indicating an aromatic group. Because of the C-F stretch, the peak at 1020.77cm-1 represents an aliphatic fluro compound.

Catharanthus roseus absorption spectra are shown in (fig 2 and Table 2). Due to C-H stretching, the band at 3288.77cm-1 was assigned to the hydroxy compound. Methylene is assigned to the bands at 2922.62cm-1 and 2854.33cm-1 due to C-h stretching. The peak at 1731.31cm-1 indicates the presence of an aldehyde compound as a result of C=O stretching. The C=O stretching peak at 1603.89cm-1 indicates ketone compounds. Because of aromatic nitro compounds, CN stretching was discovered to be at 1510.33 cm-1. Because of the C-H bond in a plane bend, the band at 1377.12cm-1 represents organic sulfate. Because of the appearance of absorption pea at 1299.01cm-1, P=O stretching was determined to be organic phosphate. Due to C-I stretching, the band at 520.16 cm-1 indicates aliphatic iodocompounds.

Moringa olifera absorption spectra in (fig 3 and Table 3). Because of C-H stretching, the band at 3273.2cm-1 has been assigned to the hydroxy compound. Because of C-H stretching, the band at 2917.29cm-1 represents alkane. The peak at 2844cm-1 indicates the presence of alkanes as a result of C=C stretching. Because of the appearance of an absorption peak at 1613.22cm-1, C=O stretching was determined to be an unsaturated ketone. Due to S=o stretching, the peak at 1404cm-1 indicates sulphonyl chloride. Due to C-O stretching, the band at 1231.5cm-1 represents all aryl ether. The 1026.57cm-1 band represents amine due to C-N stretching. Due to C-I stretching, the band at 596.06cm-1 and 521.7cm-1 represents an aliphatic iodo compound.

Callistemon absorption spectra are shown in (fig 4 and Table 4). Because of N-H stretching, the band at 3320.6 cm-1 was assigned to a secondary amine. Because of C-h stretching, the band between 2921.7cm-1 and 2850.7cm-1 represents alkane. Because of C=O stretching, the band at 1728.5 cm-1 is assigned to aldehyde compounds. Because of C=C stretching, the peak at 1613.5 cm-1 represents unsaturated ketone. The bands at 1370.1cm-1 and 1157.2cm-1 are assigned to phenol or tertiary alcohol and aromatic amino, respectively, representing O-H bend, alcoholic group, and tertiary amine, CN stretching. The peak at 775.21cm-1 represents the 1,4- Distribution (para) as a result of the 1,4-distribution.

Carica papaya absorption spectra are shown in (fig 5 and Table 5). Because of O-H stretching, the band at 3283.4 cm-1 was assigned to carboxylic acid. Due to C-H stretching, the peak at 2919.90cm-1 represents alkane. The band at 1731.8cm-1 exhibits C=O stretching, indicating an aldehyde compound. Due to -C=N- stretching, the band at 1621.96cm-1 represents open chain imino. Because of O-H stretching, the band at 1539.2cm-1 is assigned to aromatic nitro compounds. Because of the appearance of an absorption peak at 1390.4cm-1, C-I stretching was determined to be phenol. Due to stretching, the peak at 12388.3 cm-1 represents aromatic phosphate. Due to S=O stretching, the band at 514.93cm-1 is assigned for the halo compound.

Table 1 FTIR spectrum peak values and functional groups of leaf methanolic extract of *Centella asiatica*

Sno.	Wave number cm^{-1} [Test sample]	Functional group assignment	Phyto compounds identified
1.	3289.27	O-H stretch Polymeric OH	Hydroxy compound
2.	2919.72	Asymmetric stretching of–CH (CH_2) vibration	Saturated aliphatic compounds –lipids
3.	2847.3	Synthetic stretching of –CH (CH_2) vibration,	Lipids and Proteins
4.	1731.8	C=O Stretch	Aldehyde compound
5.	1605.68	C=O Stretch	Ketone compound
6.	1307.1	CN stretch	Aromatic primary amine
7.	1214.7	C-H in-plane bend	Aromatic groups
8.	1020.77	C-F stretch	Aliphatic fluoro compounds
9.	521.7	C-I stretch	Aliphatic iodo compounds
10.	470.99	S-S stretch	Aryl disulfides

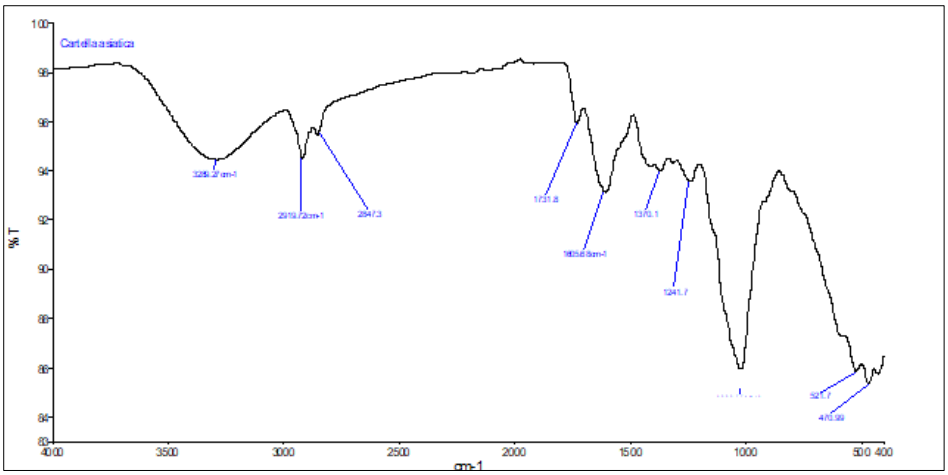

Figure 1 FTIR spectrum of leaf methanolic extract of *Centella asiatica*

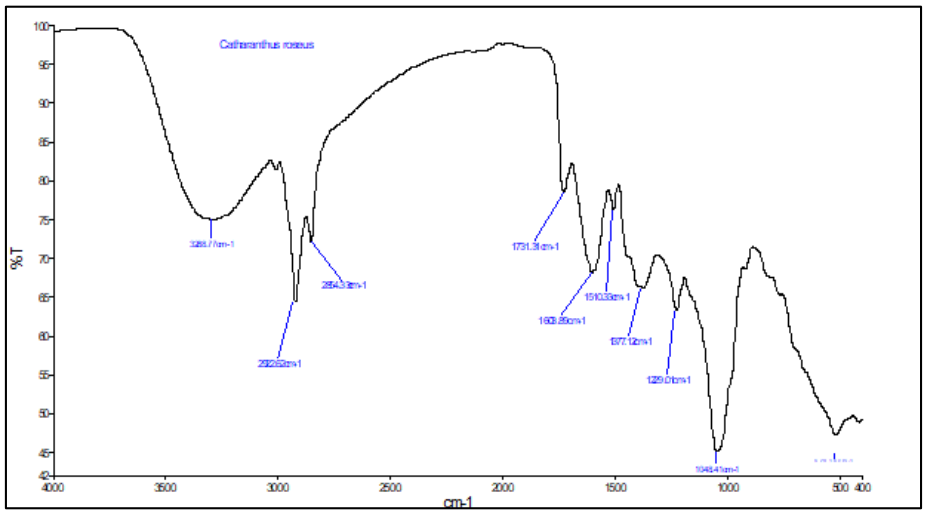

Figure 2 FTIR spectrum of leaf methanolic extract of *Catharanthus roses*

Table 2 FTIR spectrum peak values and functional groups of leaf methanolic extract of *Catharanthus roseus*

Sno.	Wave number cm^{-1} [Test sample]	Functional group assignment	Phyto compounds identified
1.	3288.77	C-H stretch	Hydroxy compound
2.	2922.62	C-H stretch	Methylene
3.	2854.33	C-H stretch	Methylene
4.	1731.31	C=O Stretch	Aldehyde compound
5.	1603.89	C=O Stretch	Ketone compound
6.	1510.33	CN stretch	Aromatic nitro compound
7.	1377.12	C-H in-plane bend	Organic sulfate
8.	1299.01	P=O stretch	Organic phosphate
9.	520.16	C-I stretch	Aliphatic iodo compounds

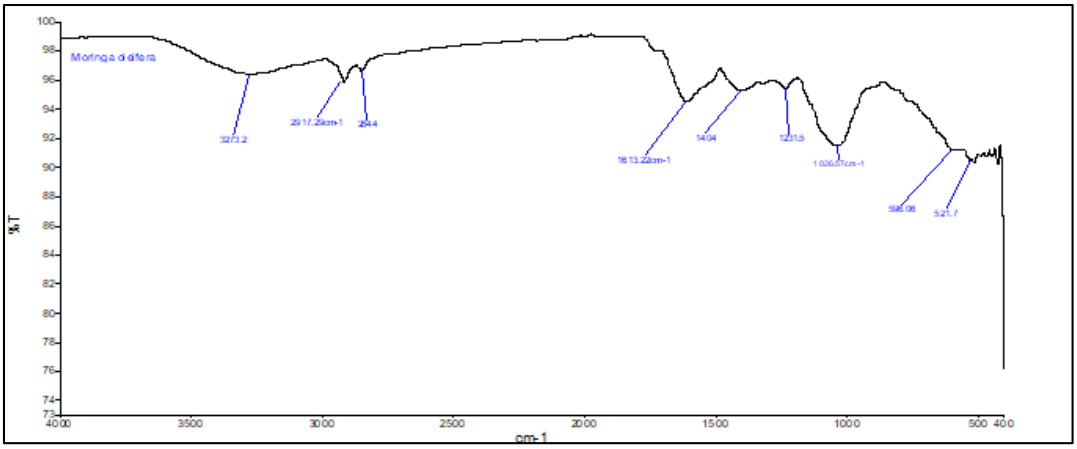

Figure 3 FTIR spectrum of methanolic extract of *Moringa olifera*

Table 3 FTIR spectrum peak values and functional groups of leaf methanolic extract of *Moringa olifera*

Sno.	Wave number cm^{-1} [Test sample]	Functional group assignment	Phyto compounds identified
1.	3273.2	C-H stretch	Hydroxy compound
2.	2917.29	C-H stretch	Alkane
3.	2844	C=C stretch	Alkane
4.	1613.22	C=O Stretch	Unsaturated ketone
5.	1404	S=O Stretch	Sulponyl chloride
6.	1231.5	C-O stretch	Alkyl aryl ether
7.	1026.57	C-N stretch	Amine
8.	596.06	C-I stretch	Aliphatic iodo compounds
9.	521.7	C-I stretch	Aliphatic iodo compounds

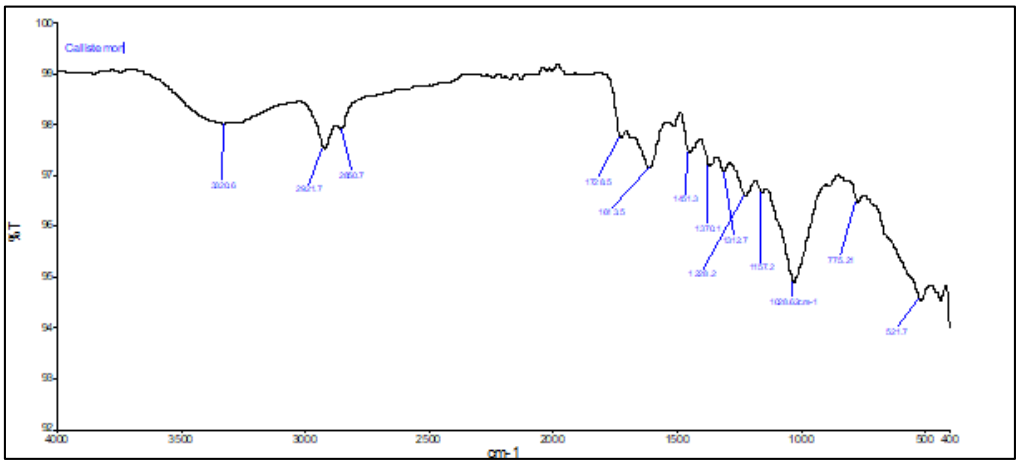

Figure 4 FTIR spectrum of methanolic extract of *Callistemon*

Table 4 FTIR spectrum peak values and functional groups of leaf methanolic extract of *Callistemon*

Sno.	Wave number cm^{-1} [Test sample]	Functional group assignment	Phyto compounds identified
1.	3320.6	N-H stretch	Secondary amine
2.	2921.7	C-H stretch	Alkane
3.	2850.7	C-H stretch	Alkane
4.	1728.5	C=O Stretch	Aldehyde compound
5.	1613.5	C=C Stretch	Unsaturated ketone
6.	1370.1	O-H bend, Alcoholic group	Phenol or tertiary alcohol
7.	1157.2	Tertiary amine, CN stretch	Aromatic amino
8.	775.21	1,4- Distribution	1,4- Distribution (para)
9.	521.7	C-I stretch	Aliphatic iodo compounds

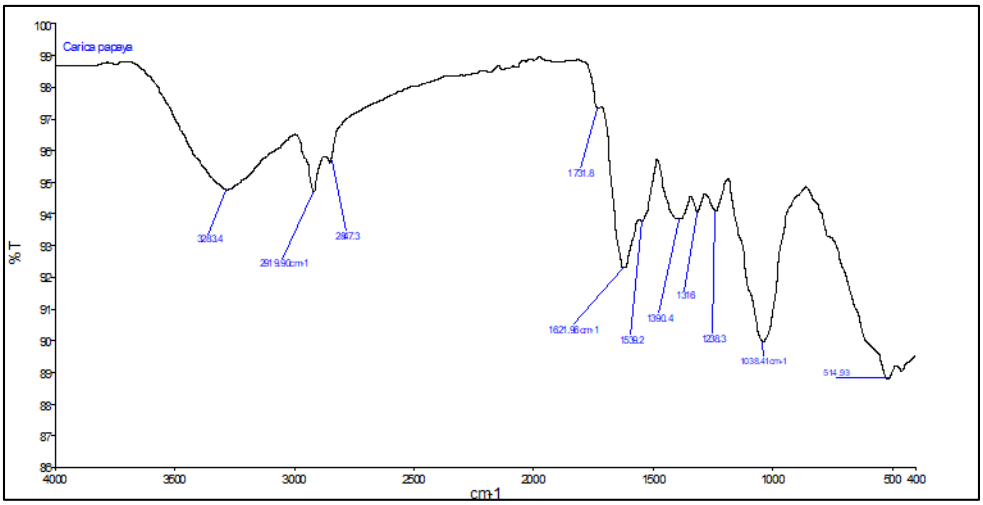

Figure 5 FTIR spectrum of methanolic extract of *Carica papaya*

Table 5 FTIR spectrum peak values and functional groups of leaf methanolic extract of *Carica papaya*

Sno.	Wave number cm^{-1} [Test sample]	Functional group assignment	Phyto compounds identified
1.	3283.4	O-H stretch	Carboxylic acid
2.	2919.90	C-H stretch	Alkane
3.	1731.8	C=O stretch	Aldehyde
4.	1621.96	-C=N- Stretch	Open chain imino
5.	1539.2	O-H Stretch	Aromatic nitro compound
6.	1390.4	C-I stretch	Phenol
7.	1238.3		Aromatic phosphate
8.	514.93	S=O stretch	Halo compounds

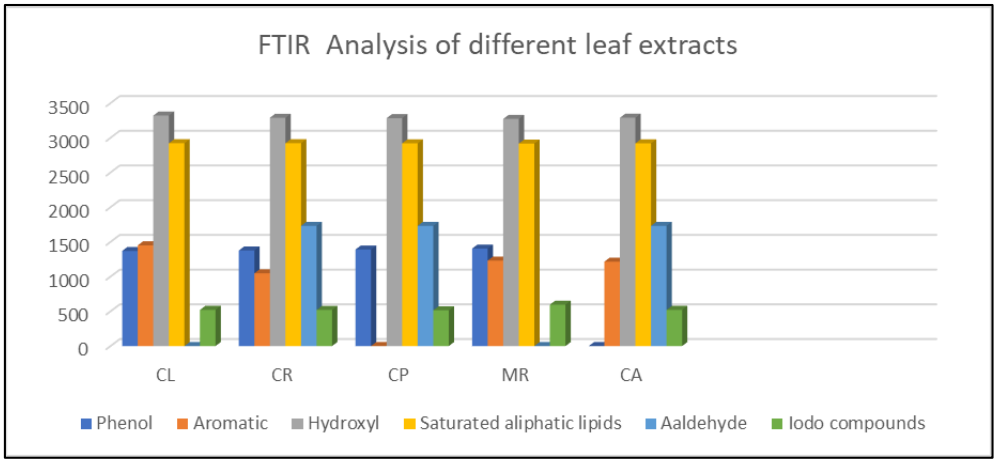

Figure 6 FTIR spectrum peak values (wave number ranges from 0 to 3500 cm^{-1}) of methanolic extract of *different plant leaf extract Callistemon subulatus (CL)*, *Centella asiatica* (CA), *Catharanthus roses (CR)s*, *Moringa oleifera (MR)*, and *Carica papaya (CP)*,

4. Conclusion

FTIR tool is a rapid technique that also allows micro sample analysis to be precise to nanogram quantity. Using this tool region of the spectrum gives information about biomolecules also. As range from 2100 cm^{-1} to 900 cm^{-1} gives information about proteins. According to the findings, all plant methanolic extracts contain potential bioactive compounds such as hydroxy compounds, phenol, alcohol, aromatic amines, and aldehyde. This could be given the protein structure and composition analysis and is used to identify biological fingerprinting. The presence of the functional group was detected and a monolayer of protein could be observed by following the Powell *et all.* algorithm in which they defined spectral subtraction of any polar solvent (including water). FTIR results could be applied in cluster analysis and practical natural networks to automate the assessment of disease state by comparing with normal cells.

Compliance with ethical standards

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Disclosure of conflict of interest

There is no conflict of interest between the authors and any other party in this publication

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