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RESEARCH ARTICLE

QUALITATIVE PHYTOCHEMICAL SCREENING AND FTIR SPECTROSCOPIC ANALYSIS OF *MITRACARPUS HIRTUS* LEAF EXTRACTS

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Abstract

The present study aim is to analyze the phytochemicals present in *Mitracarpus hirtus* leaf extracts by using Qualitative phytochemical analysis, Fourier-transform infrared spectroscopy (FTIR). The leaf extracts were prepared using three different solvents. The phytochemical analysis Fourier transform infrared spectroscopy (FTIR) analysis were performed using standard methods. The FTIR spectroscopic studies revealed different characteristic peak values with various functional groups present in the compounds of respective extracts. The FT-IR analysis shows the presence of different functional groups such as alcohols, phenols, alkanes, carboxylic acids, aldehydes, ketones, alkenes, primary amines, aromatics, esters, ethers, alkyl halides and aliphatic amine compounds, which showed major compounds present in the leaf extracts. The present study generated the FTIR spectrum profile for the medicinally important plant *Mitracarpus hirtus*. The present study provides evidences that different extracts of *Mitracarpus hirtus* leaf is useful to cure many serious diseases which remained still problematic and for further isolation of bioactive compounds from the plant which could be of interest for the development of the new drug.

Keywords: *Mitracarpus hirtus*, Phytochemicals, Fourier-transform infrared spectroscopy (FTIR)

1. INTRODUCTION

Regarding their cultural and economic benefits, plants play a key part in Indian traditional medicine. Phytocompounds are substances derived from plants that have protective or disease-prevention effects (1). In clinical practice, more than 50% of plant components and their derivatives are employed as natural medicines (2). People have recently expressed interest in natural products because they are affordable, safe, and natural without side effects (3). Knowing the chemical makeup of the phytochemicals found in medicinal plants can help us understand the various functional groups behind those compounds' therapeutic effects.

The chemical components that medicinal plants produce are what give them their therapeutic qualities. Due to the fact that the plants that synthesize them may not have a great need for them, they are regarded as secondary metabolites. All of the plant's body components, including the bark, leaves, stem, root, flower, fruit, and seeds, are where they are synthesized. Many phytochemicals can shield people from disease, according to current research. Plants create these compounds to defend themselves against herbivores. Flavonoids, carotenoids, alkaloids, anthocyanidins, phenolics and tannins, carboxylic acids, terpenes, amino acids, and inorganic acids are among the phytoconstituents found in therapeutic plants.

A high-resolution analytical technique called Fourier-transform infrared spectroscopy (FTIR) can be used to both expose the structure of the compounds and identify the bioactive chemicals based on the functional groups that are present in them (4). In FTIR, or Fourier-transform infrared spectroscopy, compounds exhibit absorption over a certain frequency range. The identification and characterization of the substances found in the various extracts depend heavily on the organic molecules, which are largely absorbed in the region of 4000-400 cm^{-1}.

Annual plants, such as *Mitracarpus hirtus*, can be either simple or heavily branched. They are often erect. The fairly thick stems can range in height from 30 to 60 cm. For local medical purposes, the herb is occasionally harvested from the wild. It is reported that the dried leaves quickly cure old sores. Arrow poison can be treated using the herb (5). In light of the medicinal properties of *M. hirtus* based on the aforementioned traditional

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knowledge, the current study concentrated on the phytochemical screening and quantitative estimation of secondary metabolites of the chosen plant sample to support the traditional claims with scientific evidence.

2. MATERIALS AND METHODS

2.1. Plant material collection

Mitracarpus hirtus Leaves was collected from Keezhariyur, Kozhikode district, Kerala. The authenticity of the selected plant materials were duly identified and confirmed by Botanical Survey of India, Coimbatore. (Vide No: BSI/SRC/5/23/2021/Tech./343, dated 16.12.2021). Fresh and healthy plant leaf of *M. hirtus* was harvested shade dried and coarsely powdered for extraction.

Figure 1: a) Habit of the plant b) leaf, c)Stem and trichome

2.2. Preparation of Plant Material

The leaves were air dried in shade at room temperature. Dried leaves pieces are cut into small pieces. These pieces are then grinded in mechanical grinder. The powdered plant material was stored in an air tight container prior to extraction. The powdered sample is used for study

2.3. Preparation of Plant Extracts

The powdered plant samples (40 g/200 ml) were extracted successively with hexane, acetone and ethanol using Soxhlet apparatus at 55-86℃ for 9-10 hr in order to extract the polar and nonpolar compounds (6). The powder was air dried and packed then used for each solvent extraction. The solvent of respective extracts were reduced under room temperature and stored at 5°C for further use.

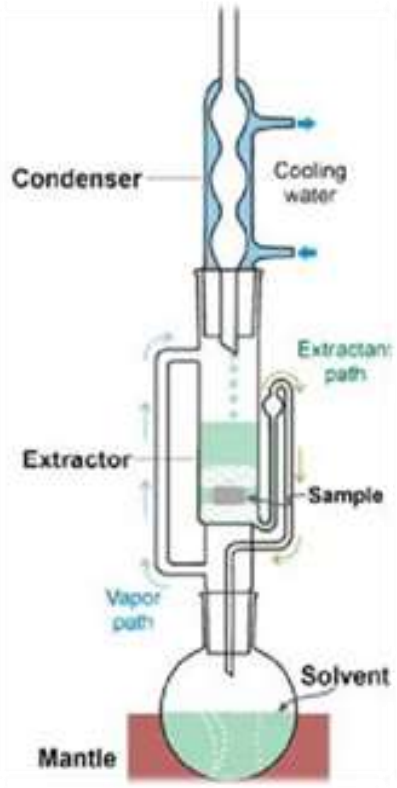

Figure 2: Soxhlet extraction method

2.4. Qualitative screening of Phytochemical Compounds

Alkaloids, terpinoids, flavanoids, saponins, coumarins, glycosides, phenolics, carboxylic acids, amino acids, carbohydrates, proteins, and many other primary and secondary metabolites are found in plants. These phytochemicals contribute to certain plant characteristics and properties and have important biological roles (7). Here using the following techniques, preliminary qualitative phytochemical screening was done In accordance with (Harborne 1984), all qualitative tests [Table 1] were performed to determine the presence of the active phytochemical elements in the defatted leaf materials (8).

Table 1. Phytochemical screening procedure:

S. No	Phytocompounds	Name of the test	Combinations of solutions	Result to be observed
1.	Carbohydrates	Molish's test	2 mL filtrate+ 2 drops of alcoholic α– naphthol + 1 mL conc. H2SO4 (along the sides of test tube)	A violet ring
2.	Proteins	Biuret test	2 ml of extract with few drops of 2% of copper sulphate solution, add 1 ml of ethanol followed by excess of potassium hydroxide pellets,	Formation of pink colour in the extract layer indicates the presence of protein.
3.	Alkaloids	Mayer's test	2 ml of extract + few drops of 1HCl. Take1ml of this mixture and add 6 drops of Mayer's reagent.	Yellow cream is precipitate.
4.	Glycosides	Keller-killani test	5 ml of extract + 2 ml of glacial acetic acid contain in gone drop of ferric chloride solution	Appearance of brown ring
5.	Saponin	Foam test	1 mL of each extract was taken in separate test tubes and to this 5mL of distilled water was added. Then this mixture was shaken vigorously	A persistent froth that lasted for at least 15min indicates the presence of saponins
6.	Phenol	Ferric chloride test	Extract aqueous solution + few drops 5% ferric chloride sol	Dark green/bluish black colour
7.	Flavonoid	Shinoda test	1 ml of extract + three pieces of magnesium chips and add few drops of concentrated HCl.	Appearance of an orange, pink or red to purple colour.

2.5. Fourier Transform Infrared Spectrophotometer

Some plant secondary metabolites have had their concrete structures determined using FTIR. An established, time-saving technique called Fourier Transform Infrared Spectroscopy (FTIR) can be used to determine a substance's structure or chemical group as well as the strength of the absorption spectra linked to its molecular makeup or chemical group content. The FT-IR approach creates a spectrum that can be used to identify a sample's biochemical or metabolic "fingerprint" by measuring the vibrations of bonds inside chemical functional groups. For the FTIR analysis, plant extracts were used. Translucent sample discs were created by encapsulating 10 mg of the dried extract powder within a 100 mg KBr pellet. Each extract's powdered sample was placed onto an FTIR spectroscope with a scan range of 400 to 4000 cm-1 and a resolution of 4 cm-1.

3. RESULT AND DISCUSSION

Table 2. The yield of sequential extract (%)

Solvent	Colour of extract	Yield (%, w/w)
Hexane	Light green	0.2
Acetone	Dark green	1.9
Ethanol	Dark yellowish brown	0.8

3.1. Phytochemical analysis

Phytochemical screening of the sequential extract of presence of various bioactive components of which phenolics, saponins, alkaloids, Glycosides, Proteins and Carbohydrates are the most prominent components and the result of phytochemical test given in the Table 3. Among these phytochemical tests, Alkaloids, were present in all solvent extracts. Whereas most of the active compound are alkaloid, flavonoid, Glycosides, Tannin, Phenols are present in the ethanolic extract of plant material.

Table 3. Qualitative phytochemical analysis of the different extracts of *Mitracarpus hirtus* leaf

S.No	Phytoconstituents	Hex	Acet	EtoH
1	Carbohydrates	++	+	+
2	Proteins	_	_	_
3	Alkaloids	+	+	++
4	Glycosides	_	++	+
5	Saponin	-	+	+
6	Phenol	+	++	+
7	Flavonoids	+	++	+

(+++ abundant; ++ moderately present: +weakly present; -----absent

3.2. FT-IR Analysis

Based on the values of the peak values in the region of IR radiation, Fourier-transform infrared spectroscopy has been utilized to pinpoint the functional groups of the active components found in the extract. The functional groups of the components were divided based on the extracts' peak ratios before being sent via the FTIR. The findings of FTIR analysis of leaf extracts in hexane, acetone, and ethanol are displayed in Figures 1-3.

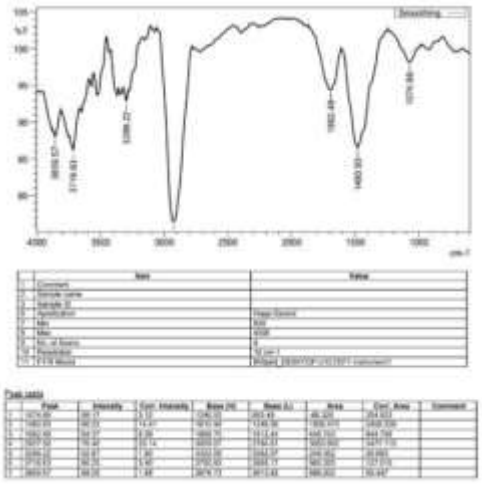

Fig. 3. FTIR of Hexane leaf extract

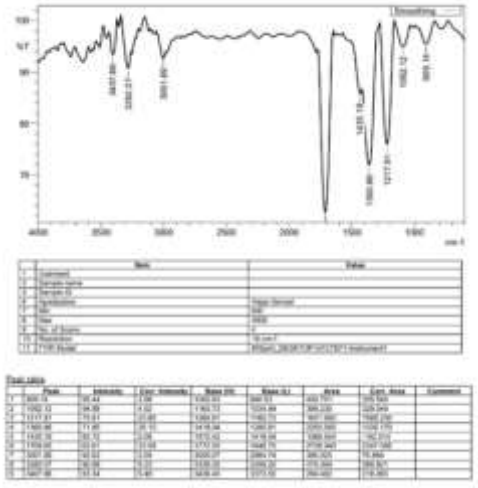

Fig. 4. FTIR of Acetone leaf extract

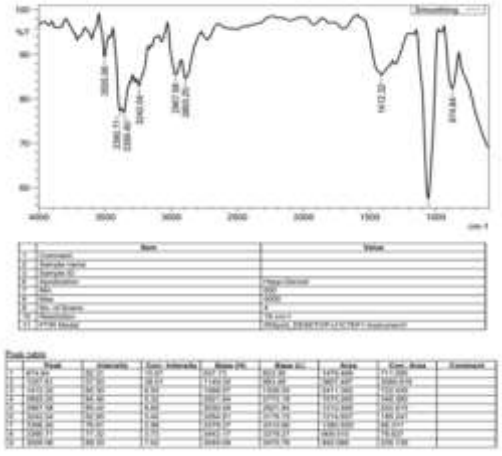

Fig. 5. FTIR of Ethanolic leaf extract

The FTIR spectroscopy has been demonstrated to be an accurate and sensitive approach for determining the composition of bio-molecular systems. Halo compounds and compounds. Alkane, sulphate, and halo chemicals are evident in the ethanol extract. Alcohol, alkene, carboxylic acid, and fluoro compounds were found in the hexane extract of leaves according to the results of FTIR analysis. Alcohol, carboxylic acid, sulphones, aromatic esters, fluoro compounds, and halo compounds can all be found in acetonic leaf extract. Alkane, sulphate, and halo chemicals are evident in the ethanol extract.

4. CONCLUSION

In the present study analysis of different extracts of *Mitracarpus hirtus* leaf was done under FTIR will act as Pharmacognostic marker to distinguish the medicinally important *Mitracarpus hirtus* species this spectrocopic technique is relatively simple, cost effective and can be useful to easily detect functional groups. The results of present study is a way to predict and compare the phytoconstituents present in this plant with other bioactive medicinally important plants. Further the bioactive compounds need to be isolated and the structure of the compounds can be determined by using advanced analytical techniques such as Mass and NMR Spectrophotometers. The FT-IR analysis shows the presence of different functional groups such as alcohols, phenols, alkanes, carboxylic acids, aldehydes, ketones, alkenes, primary amines, aromatics, esters, ethers, alkyl halides and aliphatic amine compounds, which showed major compounds present in the leaf extracts.

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