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Phytochemical and Fourier transform infra-red spectroscopic analysis of *Rhizophora mucronata*

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Abstract
Medicinal plants are therapeutically effective and culturally acceptable, since the increase in the use of synthetic chemicals in cancer therapy has led to many side effects and undesirable hazards, which led to go back to natural resources and economically within the research of the poor people. Phytochemical analysis of *Rhizophora mucronata* has revealed that numerous compounds traditionally used for medicinal purposes have many therapeutical properties. The phytochemical screening of the leaf extract revealed that the presence of alkaloids, tannins, steroids, terpenoids and coumarins. FT-IR spectroscopic analysis of the Ethyl acetate, Butanol and Methanol extract of *Rhizophora mucronata* revealed the presence of -CH, -OH, CH-OH and $-NH_2$ bond stretching. The results suggest that the phytochemical properties demostrated by the plant extract and leads to the isolation of new and novel compounds.

Keywords: medicinal plant, phytochemical, ft-ir, novel compounds

1. Introduction

Natural products have been an important resource for the maintenance of life for ages. Natural products become increasingly important as a source of pharma cotherapeutics for the treatment of chronic diseases or as raw material from which more or less complex chemical structures with particular biological activity. During the past 20 years, a core group of marine natural products chemists from several countries, in collaboration with both academic pharmacologists and the pharmaceutical industry has reported a very large number of novel metabolites with useful and sometimes sensational pharmacological properties. Due to the uncertainty involved in predicting future pharmacological potential, any selection of bioactive marine natural products is bound to be incomplete.

The goal of highlighting compounds that are likely to become clinical candidates is also complicated by the fact that, pharmaceutical companies are naturally reluctant to talk about compounds in the early stages of development. Herbal remedies and alternative medicines are used throughout the world and in the past herbs often represented the original sources of most drugs (Cooper, 2004) [3]. The plant kingdom has provided an endless source of medicinal plants first used in their crude forms as herbal teas, syrups, infusions, ointments, liniments and powders (Tsao and Zeltzer, 2005) [14]. Indian subcontinent is a vast repository of medicinal plants that are used in traditional medical treatments (Chopra *et al.*, 1956) [2], which also forms a rich source of knowledge. The various indigenous systems such as siddha, ayurveda, unani and allopathy use several plant species to treat different ailments (Rabe and Staden, 1997) [13].

In India around 20,000 medicinal plant species have been recorded (Dev, 1997) [4], but more than 500 traditional communities use about 800 plant species for curing different diseases (Kamboj, 2000) [7]. Currently 80% of the world population depends on plant-derived medicine for the first line of primary health care for human alleviation because it has no side effects**.** Plants are important sources of medicines and presently about 25% of pharmaceutical prescriptions in the United States contain at least one plant-derived ingredient. In the last century, roughly 121 pharmaceutical products were formulated based on the traditional knowledge obtained from various sources (Perumal Samy and Gopalakrishnakone, 2007) [11].

Mangrove ecosystems provide a unique and valuable range of resources, services and to a certain extent products, but they have always been an undervalued resource. For instance, the production of “traditional products and artefacts” from mangroves can be better exploited. Mangroves are one of the easiest tropical forest types to generate. They have the ability to grow where no other vascular plants can, as shown by their existence in calm, nutrient-rich environments. They thrive under stressful and extreme tropical environmental conditions such as high concentration of moisture and high temperature. They exist in muddy, shifting saline and anaerobic conditions, soil acidity and high and low tides of brackish water (Bandaranayake, 1998). Hence, the plants that can survive in these extreme habitats have evolved special methods to survive.

Marine plants produce novel metabolites unique to the environment. It is therefore reasonable to assume that, the mangrove plants produce metabolites which in turn are unique to them and are of interest to the “curious” chemist. Studies of potentially commercial importance are needed focusing on the extraction of tannins and the use of the plants for the production of methanol, acetic acid and coal tar. The chemistry of mangrove plants is of growing importance because of their great potential as a source of novel agrochemicals and compounds of medicinal value. They may also provide a new source for many already known

biologically active compounds. Numerous mangroves and mangal associates are used in folklore medicine and have found applications as insecticides and piscicides (Bandaranayake, 1998). Even though there are some recent investigations of the chemical constituents describing several novel compounds, the exploration of mangroves for pharmacologically important compounds is in its infancy.

2. Materials and Methods

2.1 Collection and extraction of *Rhizophora mucronata*

Fresh elder leaves of *Rhizophora mucronata* plant were collected from Manakudy, Kanyakumari district, Tamilnadu, India. Leaves were washed thrice in sterile water to remove adhering soil particles and salts. All the samples were shade dried and were powdered. 500 g of powdered leaves were soaked with 1 litre of ethanol water mixture (3:1) for 10 days in an air tight clean glass container (percolation). Mixing was done every day so as to enable to enhance the maximum extraction of bioactive compounds. After that, solvent with bioactive compounds were filtered with Whatman filter paper. The filtrate was then subjected to the rotary flash evaporator and further lyophilized to remove excess organic residue. After filtration, the suspended materials were dried under room temperature and weighed. The result was expressed in percentage.

2.2 Identification of chemical classes

Phytochemical analysis was estimated by following the method of Obdoni and Ochuko (2001) [10], Kaur and Arora, (2009) [8].

2.2.1 Detection of alkaloids

A few drops of dilute HCI were separately treated with 1 ml of the extract. Then it was filtered and the filtrates were treated with one ml of Dragendoff's reagent. Formation of reddish orange precipitation indicated the presence of alkaloids.

2.2.2 Detection of carboxylic acids

One ml of extract was separately treated with a few ml of saturated solutions of sodium bicarbonate. Observation of effervescence (due to liberation of CO2) indicated the presence of carboxylic acids.

2.2.3 Detection of coumarins

One ml each of alcoholic extract was treated with alcoholic NaOH solution. Production of dark yellow colour indicated the presence of coumarins.

2.2.4 Detection of flavonoids

Five ml of extract was were separately dissolved 1 ml each of alcohol (stock solution) and then subjected of the following test. One ml each of stock alcoholic solution was added with a few drops of neutral $FeCI_3$ solution. Formation of blackish red colour indicated the presence of flavanoiods.

2.2.5 Detection of phenols

One ml of extract was dissolved in 5 ml of alcohol was treated separately with a few drops of neutral FeCI3 solution. Any change in colour indicated the presence of phenolic compounds.

2.2.6 Detection of protein and free amino acids

One ml of extract was warmed gently with 10% NaOH solution and a drop of dilute CuSO4 solution. Formation of reddish violet colour indicated the presence of proteins and free amino acids.

2.2.7 Detection of Quinones

One ml of extract was separately treated with alcoholic KOH solution. Quinones give coloration ranging from red to blue.

2.2.8 Detection of saponins

One ml of extract was separately mixed with 20 ml of distilled water and then agitated in a graduated cylinder for 15 min. Foam formation indicated the presence of saponins.

2.2.9 Detection of Steroids

Five ml of extract was dissolved in 5 ml each of chloroform separately (stock solution) and was subjected to the following test. One ml each of concentrated H_2SO_4 was added to the stock solution and allowed to stand for 5 min after shaking. Turning of golden yellow color in the lower layer indicated the presence of sterols.

2.2.10 Detection of tannins

One ml of extract was separately mixed with 20 ml of distilled water. To the filtrate, a few drops of aqueous basic lead acetate solution were added. Formation of reddish-brown precipitate indicated the presence of tannins.

2.3 Fourier Transform Infra-Red (FT-IR) spectroscopic analysis

The selected hot water extract *R. mucronata* were analyzed qualitatively for the active compounds by Fourier transform infra red (FTIR) method described by Kemp (1991). In the present study the funtional groups of most potent extract was analysed by using FT-IR spectrophotometer (Shimadzu). A small amount of powdered *R. mucronata* extract was place directly on the germanium pieces of the infra red spectrometer with constant pressure applied and data of infra red absorption were collected over the wavenumber ranged from 400 – 5000/cm. The frequency of the spectra and the vibration spectrum was recorded as graphical chart.

3. Results

3.1 *Rhizophora mucronata* extract

Many drugs are of plant origin and hence plants are a valuable source of medicine. The leaf extracts of Annona reticulata contains certain amount of secondary metabolites and were extracted using different organic solvents. The leaf extracts showed better antimicrobial activity the present study extract yield from mangrove *Rhizophora mucronata* had a crude extract yield of 35.4%.

3.2 Phytochemical analysis of mangrove plant

The present studies on the phytochemical analysis of the mangrove plant *Rhizophora mucronata* revealed that presence of flavanoids, sugars, glycosides, coumarins, phenols, proteins and free amino acids in both the plants (Table 1). In addition

to these chemical, *R. mucronata* contained alkaloids, tannins, xanthoproteins and anthracene. Both the plants tested negative for carboxylic acids, quinones, saponins anthraquinones and steroids.

3.3 FT-IR analysis of *R. mucronata*

The FT-IR spectrum was used to identify the functional group of the active components based on the peak value in the region of infra red radiation. The ethanolic extract of *R. mucronata* was passed in to the FT-IR and functional groups of the compounds were separated based on its peak ratio. The results of powdered *R. mucronata* extract FT-IR analysis confirmed the presence of functional groups such as Phenols, primary amines, aliphatic amines, alkenes and alkyl halides compounds. This shows major peak at 3447.16, 1635.39, 1387.06, 1116.53, 986.14, 538.01respectively. In addition that, biological activity of dominated peaks of functional group also searched and activities were represented (Figure 1, Table 2-3).

Table 1: Phytochemical analysis of mangrove *Rhizopora mucronata*

S.No	Phytochemical constituents	*Rhizopora mucronata*
1	Detection of alkaloids	+
2	Detection of carboxylic acids	-
3	Detection of coumarins	+
4	Detection of flavonoids	+
5	Detection of phenols	+
6	Detection of quinines	-
7	Detection of saponins	-
8	Detection of tannins	+
9	Detection of steroids	-
10	Detection of proteins and free amino acids	+

+ Positive - negative

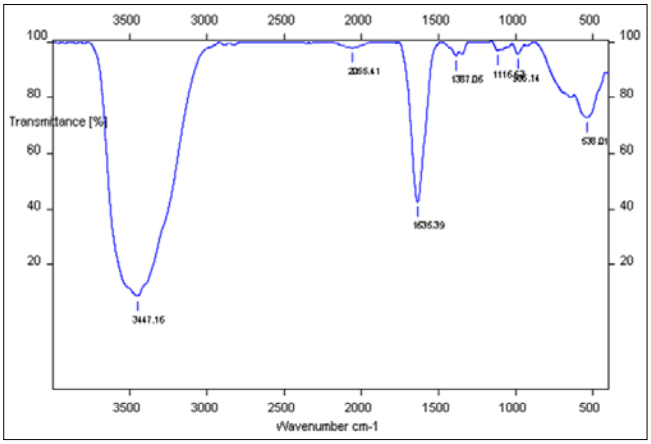
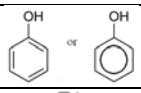
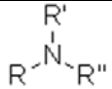
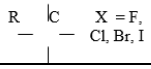
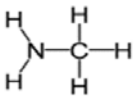
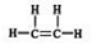


Fig 1: FT-IR - Spectrum of *Rhozophora mucronata* - Leaves

Table 2: Wave number (cm-1) of dominant peak obtained from absorption spectra from *Rhizophora mucronata*

Frequency (cm^{-1})	Bond	Functional groups	Molecular formula
3447.16	O-H stretch, H bonded	Alcohols and phenols	C_6H_50H (Carbolic acid)
2055.41	Unknown	_	_
1635.39	N-H bend	Primary amines	C_2H_7N (Ethyl amine)
1387.06	C-F stretch	Alkyl halides	-
1116.53	C-N stretch	Aliphatic amines	CH_5N (Methyl amine)
986.14	=C-H bend	Alkenes	C_2H_4 (Ethane)
538.01	C-Br stretch	Alkyl halides	-

Table 3: Biological activity of functional groups obtained by using FT-IR spectrum from *Rhizophora mucronata*

Name of the Functional group	Molecular structure of Functional groups	Biological activity
Phenol (Carbolic acid)		Anti-oxidant and antibacterial activity
Primary amines (Ethyl amine)		Anti-oxidant, Haemolytic efficiency and anti-microbial activity
Alkyle halide		Antibacterial activity
Aliphatic amine (Methyl amine)		Biocidal and Biostatic activity
Alkenes (Ethane)		Antimicrobial activity

4. Discussion

Phytochemicals from medicinal plants showing antimicrobial activities have the potential bioactive compounds, because of their structures are different from those of more studied microbial sources and therefore their mode of action may too very likely differ (Fabricant and Fansworth, 2001) [5]. There is growing interest in correlating the Phytochemical constituents of a medicinal plant with pharmacological activity (Prachayasittikul *et al*., 2008) [12]. Screening active compounds from plants have lead to the discovery of new medicinal drugs which have efficient protection and treatment roles against various diseases (Mukherjee *et al.*,2007) [9].

In recent years, the search for phytochemicals possessing anti-inflammatory properties has been on the rise due to their potential use in the therapy of various chronic and infectious diseases. Epidemiology and experimental studies have implicated oxidative cellular damage arising from an imbalance between free radical generating and scavenging systems as the primary cause of cardiovascular, diseases, cancer, aging etc (Halliwell, 1996) [6].

These activities may be due to strong occurrence of polyphenolic compounds such as alkaloids, flavonoids, tannins, steroids, phenols and Saponins. The anti-inflammatory activity was comparable with standard drugs.

As *Rhizopora mucronata* showed most potent activity the same was subjected to FT-IR analysis. The results of powdered *Rhizopora mucronata* extract FT-IR analysis confirmed the presence of functional group such as Phenols, primary amines, alkyl halides, aliphatic amines, alkenes, alkyl halides compounds. Which shows major peak at 3447.16, 1635.39, 1387.06, 1116.53, 986.14, 538.01 respectively. It was believed that the activity of functional groups could be related to branched sugar chains or aldehyde groups (Bomford *et al*., 1992) [18] or to an acyl residue bearing the aglycone (Kensil, 1996) [19]. Latter, soyasaponins and lablabosides were found to show strong activity despite lacking acyl residues and possessing only un-branched sugar chains (Oda *et al*., 2000) [20]. These activities may be due to strong occurrence of polyphenolic compounds such as alkaloids, flavonoids, tannins, steroids, phenols and Saponins. These findings provide scientific evidence to support traditional medicinal uses and indicate apromising potential for the development of anti-microbial agent from *Rhizopora mucronata* plant. This medicinal plant contains active phytochemicals as interesting and promising and may be effective as potential sources of novel drugs.

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