Research Article

Screening of Functional Groups and Related Antibacterial Activity of *Mesua ferrea* L

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ABSTRACT

Plants act as treasure house for several bioactive compounds. These bioactive compounds vary in their structural arrangements and properties. The Fourier Transform Infrared (FT-IR) Spectroscopy is an established way to characterize as well as to identify the functional groups. The aim of this research work is to identify the major functional groups vis-à-vis bioactive compounds, present in different plant parts (leaf and stem) of *Mesua ferrea* L. at different (vegetative and mature) growth stages. The results confirmed the presence of Phenolics, Amines, Sulfonic acid, Sulfones, Aromatic compounds, Ketones, Carboxylic acid and many others similar functional groups in stretched forms. The methanolic extracts of these plant parts were also found to be more effective against procured MTCC (Microbial Type Culture Collection and Gene Bank) gram positive bacterial cultures, namely, *Staphylococcus aureus*, *S. epidermidis*, *Bacillus cereus, B. subtilis* and negligibly effective against gram negative *Pseudomonas aeruginosa*.

Keywords: Amines, Antibacterial, Aromatic compounds, bioactive, Carboxylic acid, FT-IR, Ketones, *Mesua ferrea,* Methanolic, Phenolics and Sulfonic acid.

INTRODUCTION

Human use of plants as medicinal agents predates recorded history.[1] As per the given reports of WHO 80% of the world's population depends upon the traditional medicine for their primary health.[2] *Mesua ferrea* L. belonging to the family Clusiaceae, is locally known by several names like- Cobra saffron, Nagakeshara, Nagasampige, Nageshwar, Nagachampakam, is found throughout Southeast Asia.[3] The plant is also well known for its medicinal value like- decoction of seeds is used for the treatment of gastritis, bronchitis and to cure snake bite,[4] whereas leaves are used as antidote against scorpion sting. Extracts, using different solvents, also reveal its potency against ulcer, venom, protozoans, cancer and as anti-oxidant.[5] The plant parts are also found to be effective against inflammation and sepsis.[6] The ash of the leaf is used for the treatment of sore eyes whereas the kernels are used as poultice on the wounds and the skin eruptions.[7] There is paucity of literature on its functional group identification against bacteria (antibacterial activity). Hence, FT-IR Spectroscopy has been used during the present studies, as a simple, cost effective and time saving method.

FT-IR is one of the most powerful tools for the identification of functional groups. The FT-IR spectrum of different plant parts reflects the presence of particular functional groups which help in tracing out the major bioactive compounds present in a specific plant part of the selected plant.

Since long, medicinal plants have contributed a lot towards pharmaceutical and scientific communities as a source of antimicrobial constituents and day by day the scope is enhancing.[8] Although, numerous synthetic antibacterial drugs are available, but several factors like- drug toxicity, emergence of resistant bacterial strains and limited effective span are a major concern.[9] Phytochemical screening, based on ethno-medical data is considered to be an effective approach for the discovery of new therapeutic agents.[10] So the present research work aims to find out the major functional groups present in different plant parts of *Mesua ferrea* L. using FT-IR Spectroscopy and to determine the antibacterial efficacy of *Mesua ferrea* L. methanolic extracts against MTCC procured bacterial cultures (*Staphylococcus aureus*, *S. epidermidis*, *Bacillus cereus, B. subtilis and Pseudomonas aeruginosa*), being maintained by the Microbiology Department of C.C.S University.

The present investigation is divided into 2 parts-

(I) FT-IR analysis of different vegetative and mature plant parts (leaf and stem) to screen major functional groups present.

(II) To correlate the antibacterial efficacy of vegetative and mature plant parts (leaf and stem) in methanolic extracts against procured bacterial cultures *Staphylococcus aureus*, *S. epidermidis*, *Bacillus cereus, B. subtilis and Pseudomonas aeruginosa* using Sulphaphenazole 200 mcg as positive control.

MATERIALS AND METHODS

For the present work the vegetative and mature parts (leaf and stem) of *Mesua ferrea* L. were collected from the garden of Department of Botany, C.C.S. University, Meerut during the vegetative phase (Feb.- March) and mature phase (Dec.–Jan.). The collected plant parts were washed with distilled water and further oven dried for about 72 hrs

at 65^{0}C. The oven dried plant parts were then grinded to fine powder.

(I) **For FT-IR analysis:** For preparation of sample pellets to analyse the functional groups through FT-IR, 2mg of each plant part sample was mixed with 200mg KBr (Sigma FT-IR Grade). The prepared pellet was kept in the sample holder and FT-IR Spectra were recorded in the range between 4000- 500cm^{-1}for all the samples. All the investigations were carried out using IR- Affinity-1 (Shimadzu) Model, FT-IR Spectrophotometer. The graphic peak values are presented in figures 1 and 2 and the identified functional groups are given in tables 1 and 2.

(II) **For antibacterial screening:** One gram of each plant part sample was extracted using 10ml methanol in water bath for 18hrs at 55^{0}C. Extracts were subsequently filtered and concentrated to 1ml. The MTCC procured bacterial cultures (*Staphylococcus aureus*, *Staphylococcus epidermidis*, *Bacillus cereus, Bacillus subtilis and Pseudomonas aeruginosa)* were revived in Nutrient broth by placing it on shaker at 120 rpm for 24hrs at 37^{0}C. Antibacterial screening was carried out by Agar well Diffusion Method[11] using the Muller Hinton Agar plates swabbed with sterile cotton swabs of respective bacterial cultures and incubated for 24hrs.

Using sterile cork-borer, 5mm diameter Agar wells were made in each of these inoculated Petriplates. About 25µl of the methanolic extract from different plant parts were added using micro pipettes into the marked wells. The plates were incubated in an upright position at 37^0±2^{0}C. After 24hrs the zones of inhibition were measured using Zone measuring scale in mm. given in Table 3 and Fig 3.

RESULTS AND DISCUSSION

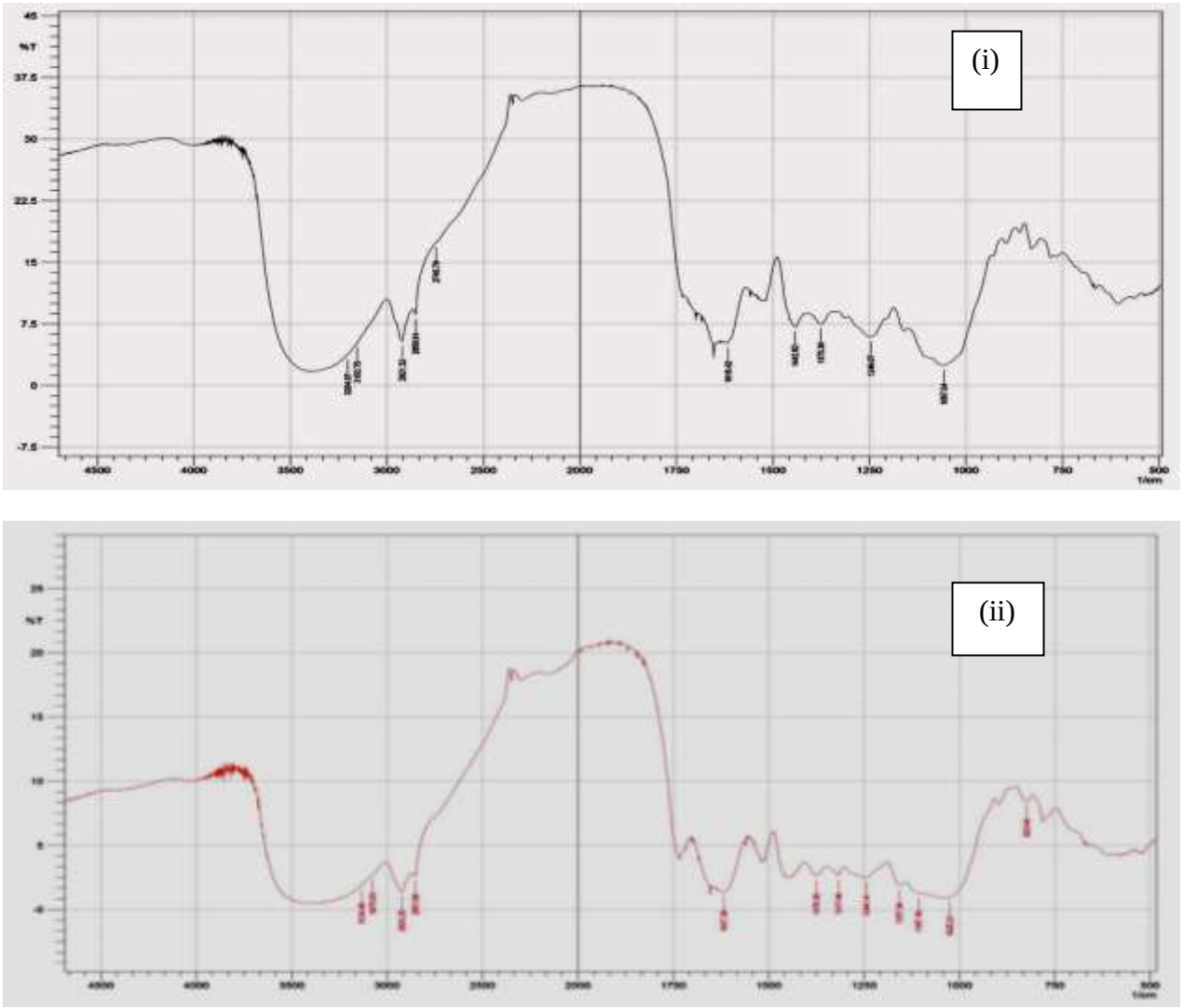

Figure 1: FTIR Spectrum of *Mesua ferrea* L. (i) vegetative and (ii) mature leaf

The FTIR spectral data exhibited the presence of several bioactive functional groups (Phenolics, Amines, Sulfonic acid, Aromatic compounds, Sulfones, Ketones, Carboxylic acid) in leaf and stem of *Mesua ferrea* L. at different stages (vegetative and mature).

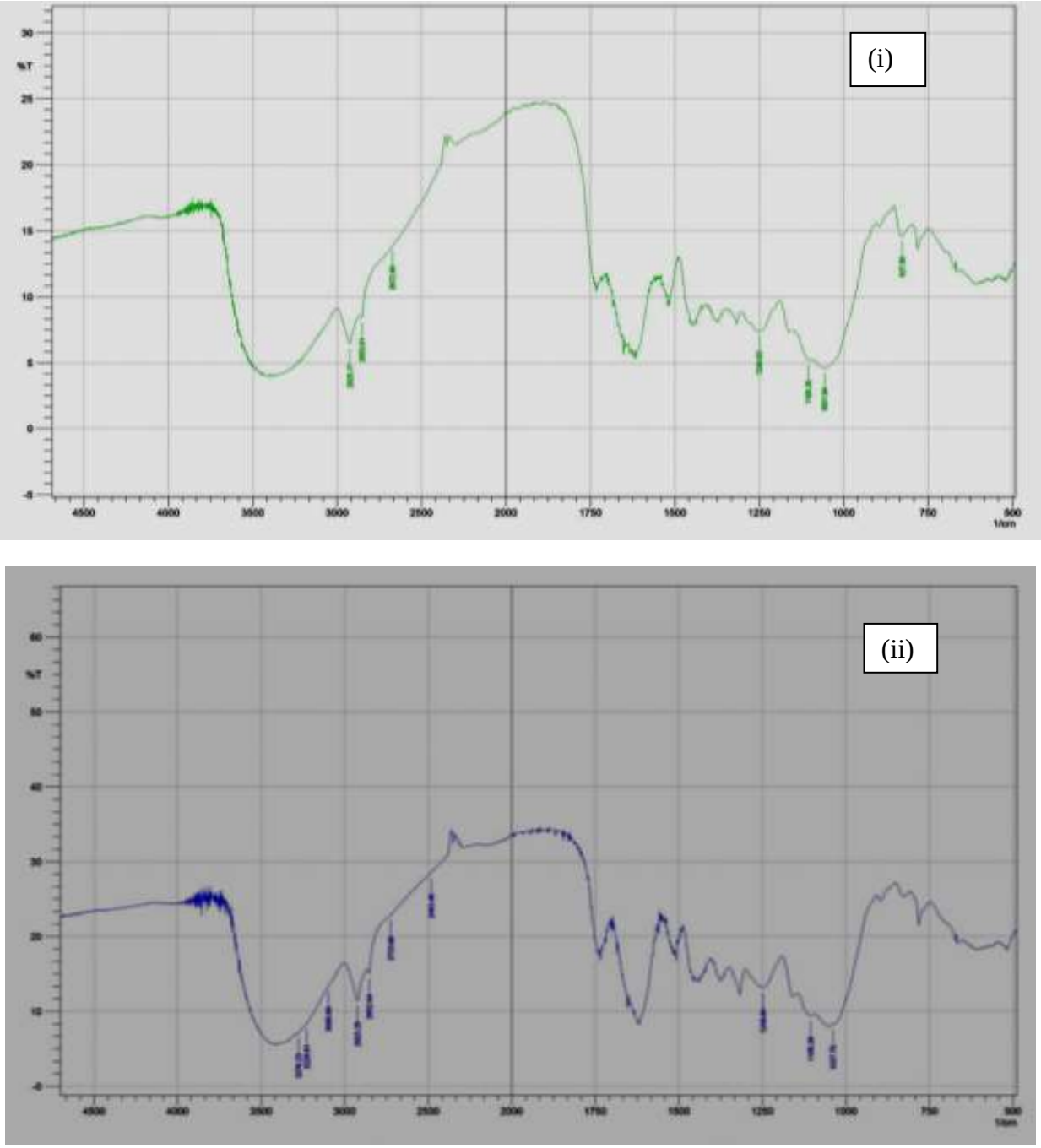

Figure 2: FTIR Spectrum of *Mesua ferrea* L. (i) vegetative and (ii) mature stem

Table 1: FTIR Spectral peak values and functional groups identified in *Mesua ferrea* L. vegetative and mature leaf

Sample	Peaks	Functional groups	Sample	Peaks	Functional groups
M. ferrea (Vegetative Leaf)	1057.04	Si-O-C	*M. ferrea* (Mature Leaf)	823.64	C-O-C
	1246.07	Sulfonic acid		1025.21	Si-O-C
	1375.30	C-CH_3		1107.19	Sulfone
	1442.82	CH_2		1157.34	Sulfone
	1616.42	Aromatic Compounds		1244.14	Sulfonic Acid
	2745.79	Aldehyde		1317.44	Carboxylate Salt
	2850.91	O-CH_3		1375.30	C-CH_3
	2921.32	CH_2		1617.38	Carboxylic acid
	3153.75	Amines		2851.88	C-CH_3
	3204.87	Phenol		2923.25	CH_2

Table 2: FTIR Spectral peak values and functional groups identified in *Mesua ferrea* L. vegetative and mature stem

Sample	Peaks	Functional groups	Sample	Peaks	Functional groups
M. ferrea (Vegetative Stem)	827.50	C-O-C	*M. ferrea* (Mature Stem)	1037.75	Si-O-C
	1057.04	Si-O-C		1105.26	Aromatic Ring
	1105.26	Aromatic Ring		1248.00	Sulfonic Acid
	1249.93	Sulfonic Acid		2483.46	P-H
	2672.49	Aldehyde		2723.60	Aldehyde
	2873.81	$C\text{-}CH_3$		2852.84	$O\text{-}CH_3$
	2925.17	$C\text{-}CH_3$		2923.25	CH_2
				3095.88	CH_2
				3228.01	Phenol
				3276.23	Alkyne

Table 3: Antibacterial potential of *Mesua ferrea* L. Methanolic extracts against bacterial cultures

Inhibition Zones (in mm) shown by *Mesua ferrea* L. methanolic extracts of different plant parts				Tested bacterial cultures	Zone of inhibition shown by antibiotic used as Positive control (Sulphaphenazole 200mcg)
Vegetative leaf	Mature leaf	Vegetative stem	Mature stem		
19mm	18mm	21mm	18mm	*S. epidermidis*	24mm
23mm	14mm	18mm	14mm	*S. aureus*	27mm
21mm	21mm	**27mm**	**23mm**	*B.subtilis*	34mm
18mm	**22mm**	18mm	14mm	*B. cereus*	27mm
11mm	10mm	-	-	*P. aeruginosa*	20mm

The methanolic extracts also reveal that the plant possesses maximum antibacterial potential against tested gram-positive bacterial cultures *(Staphylococcus aureus*, *S. epidermidis*, *Bacillus cereus, B. subtilis)* procured from MTCC while negligible effectiveness against gram negative *Pseudomonas aeruginosa* (Fig.3).

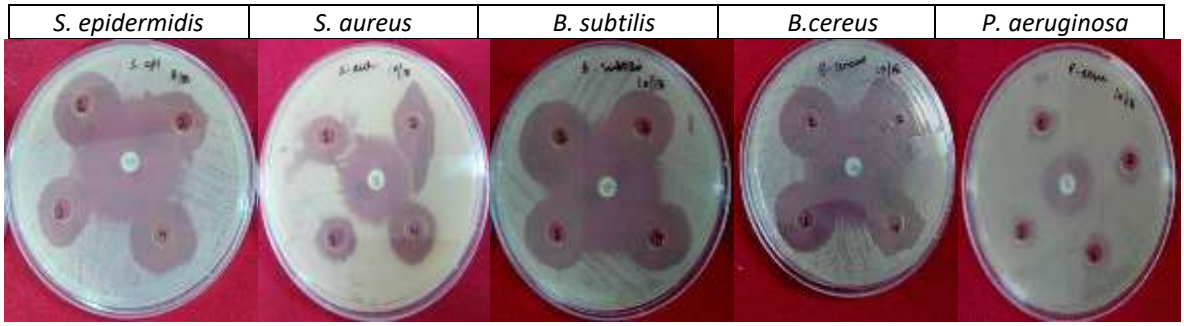

Figure 3: Screening of Antibacterial efficacy using methanolic extracts of different plant parts of *Mesua ferrea* L.

Where Antibiotic disk in centre (Sulphaphenazole 200mcg), 1. (Vegetative leaf) 2. (Mature leaf) 3. (Vegetative stem) 4. (Mature stem)

The Sulphaphenazole and sulfa compounds are reported as inhibitor of folic acid synthesis in susceptible microbes due to structural similarity with PABA (para aminobenzoic acid), essential for Nucleic acid synthesis as proposed by Hawser and co-workers.[12] In the present studies, sulphaphenazole has hence been used for positive control and the recorded sulfa compounds (sulfonic acid and sulfone) are therefore assumed to be exhibiting similar activity against gram positive bacteria (). While negligible antibacterial effectiveness against *Pseudomonas aeruginosa* might be due to the presence of Sulphonamide resistant genes SulI and SulII present on Plasmid of gram negative bacteria.[13]

CONCLUSION

The methanolic extracts of all the four samples, i.e. vegetative and mature leaf and stem, of *Mesua ferrea* synthesize sulfonic acid as an antibacterial bioactive compound effective against gram positive bacteria. Sulfones in mature leaf probably add to this effectivity. Besides, sulphonamide resistant genes of gram negative bacteria (present in their plasmids) could be responsible for negligible effectivity of sulfa compounds.

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