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Screening of phytoconstituents, UV-VIS Spectrum and FTIR analysis of *Micrococca mercurialis* (L.) Benth

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Abstract
The present investigation was focused on the preliminary phytochemical, UV-VIS spectrum and Fourier Transform Infrared Spectral analysis of *Micrococca mercurialis.* The aqueous and organic solvent extracts (petroleum ether, acetone, chloroform, ethanol and aqueous) from the whole plant of *Micrococca mercurialis* (Euphorbiaceae) were tested for the availability of alkaloids, glycosides, flavonoids, phenols, saponins, steroids, amino acids, tannins, terpenoids, quinones, anthraquinones and coumarin. The UV-VIS spectrum showed the peaks at 214, 446 and 472 nm with the absorption of 0.599, 0.655, and 0.550 respectively. The FT-IR spectrum showed the presence of alcohols, phenols, alkanes, alkynes, alkyl halides, aldehydes, aromatics, nitro compounds and amines. The results confirm the fact that this plant posses' important bioactive constituents useful for our health, so further scientific investigation is needed.

Keywords: *Micrococca mercurialis*, UV-VIS, FTIR, amine, aldehydes and alkanes

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. The different phytocontituents present in medicinal plants are flavonoid, alkaloid, phenol and tannins, carboxylic acids, terpenes and amino acids and inorganic acids. These phytoconstituents give specific distinctiveness and properties to plants [1]. Therefore, the analysis of these chemical constituents would help in determining various biological activities of plants. A variety of techniques can be used to determine and estimate the presences of such phytocontituents in medicinal plants. Chromatography and spectroscopic techniques are the most useful and popular tools used for this purpose. The Fourier Transform Infrared Spectrophotometer (FT-IR) was perhaps the most powerful tool for identifying the types of chemical bonds/functional groups present in the phytochemicals. The wavelength of light absorbed was the salient feature of chemical bond as can be seen in the annotated spectrum. By interpreting the infrared absorption spectrum, the chemical bonds in a compound can be determined. Moreover, FTIR spectroscopy is an established time-saving method to characterize and identified functional groups [2]. UV-VIS spectroscopic is simple, cost-effective and rapid tests for detecting phytocomponents. UV-visible spectroscopy uses light in the visible ranges or its adjacent ranges. The colour of the chemicals involved is directly affects the absorption in the visible ranges. Molecules undergo electronic transitions in these ranges of the electromagnetic spectrum [3].
Micrococca mercurialis is an important medicinal plant with wide range of biological activities and interesting phytochemical constituents. In aurvedic medicine, it is used for the treatment of sores, rheumatic pain and constipation and used to treat fever in children. Leaves are used as purgative and treat skin diseases. Plant sap is instilled in to the nose, eyes or ears to treat headache, filariasis of the eye or otitis. Ash of plant mixed with oil and is used in skin diseases. The present research work was aimed to produce the preliminary phytochemical analysis, UV-VIS and FTIR spectrum profile of *Micrococca mercurialis.*

2. Materials and Methods
2.1 Preparation of extract
Micrococca mercurialis was collected for local area of Thindal, Erode, Tamil Nadu, India. The whole plants were washed under running tap water, shade dried at room temperature, and powdered. The powdered plant sample (50 g/250 ml) was extracted successively with petroleum ether, acetone, chloroform, ethanol and water using Soxhlet apparatus at 55-850°C for 8-10 hrs to extract the polar and non-polar compounds [4]. For each solvent extraction, the powdered pack material was air dried and then used.

The solvents of the respective extracts were reduced under room temperature and stored at 4°C for further use. The dried plant extracts were then redissolved in dimethyl sulfoxide to get the solution of 10 mg/10 ml for each extract which was subjected to analysis of phytochemicals

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2.2 Phytochemical screening

Preliminary qualitative phytochemical analysis was carried out to identify the secondary metabolites present in petroleum ether, acetone, chloroform, ethanol and aqueous extract of whole plant of test plant.

2.3 Preparation of plant extract for UV-VIS spectrum and FT-IR analysis

The shade dried leaves of *Micrococca mercurialis* (at 25°C) were powdered in mechanical grinder. 20 g of whole plant powder was weighed; 150 ml of solvent was added and kept for 3 days. The extract was filtered using Whatman No.1 filter paper and the supernatant was collected. The residue was again extracted two times (with 3 days of interval for each extraction) and supernatants were collected. The supernatants were pooled and evaporated (at room temperature, 28 ± 1°C) until the volume was reduced to 150 ml. Extract of the whole plant powder with petroleum ether (PE) was prepared and stored in air tight bottles for subsequent analysis.

2.4 UV-VIS Spectrum analysis

The extract was centrifuged at 3000rpm for 10min and filtered through Whatmann No.1 filter paper. The sample is diluted to 1:10 with the same solvent. The extract was scanned at wave length ranging from 200 to 1100 nm using Perkin Elmer Spectrophotometer and the characteristic peaks were detected. The peak values of the UV-VIS were recorded.

2.5 FT-IR analysis

Dried powder of *Micrococca mercurialis* was used for FTIR analysis. 1 mg of the dried extract powder was encapsulated in 10 mg of KBr pellet, in order to prepare translucent sample discs. The powdered sample of the extract was loaded in FTIR spectroscope (Shimadzu, Japan), with a Scan range from 400 to 4000 cm^{-1} with a resolution of 4 cm^{-1}.

3 Results

The present study revealed that the various solvent extracts (petroleum ether, acetone, chloroform, ethanol and aqueous) of whole plant of *Micrococca mercurialis* revealed the presence of alkaloids, glycosides, flavonoids, phenols, saponins, steroids, amino acids, tannins, terpenoids, quinones, anthraquinones and coumarin (Table 1). However alkaloids were detected only in ethanolic and aqueous extracts of test plant and the glycosides were found in chloroform and aqueous extracts. Next to aqueous extract, ethanol extract showed the presence of rich variety of secondary metabolites. Petroleum ether, chloroform and acetone extract showed less variety of these secondary matebolites. Compared to all other solvent extracts, water had higher number of secondary metabolites with high degree of precipitation (+++), Triterpenoids and resins were present with lesser amount (+) in all the extracts. The presence or absence of constituents are expressed in (+) or (-) symbol.

The qualitative UV-VIS spectrum profile of petroleum ether extract of *Micrococca mercuralis* was selected at wavelength from 200 to 700 nm due to sharpness of the peaks and proper baseline. The profile showed the peaks at 214, 446 and 472 nm with the absorption of 0.599, 0.655, and 0.550 respectively (Fig.1; Table 2).

The results of FT-IR peak values and functional groups were represented in Table -3. The FT-IR spectrum profile was illustrated in Fig. 2. The FT-IR gave broad peak at 3175.82 cm^{-1} which indicated the presence of N-H stretching. It gave a strong peak at 2.730.26, 2864.8 and 2963.65 cm^{-1} which indicated the presence of C-H stretching. The peaks obtained at 1249.88, 1299.07 and 1461.57cm^{-1} indicated the presence of O-H bending. The peak obtained at 811.56 cm^{-1} indicated the presence of C-H Bend out of plane. The peak obtained at 890.16 cm^{-1} indicated the presence of C-C stretching. The peaks obtained at 1377.19 and 984.19 cm^{-1} indicated the presence of nitrates and silicates. The FT-IR spectrum confirmed the presence of alcohols, phenols, alkanes, alkynes, alkyl halides, aldehydes, aromatics, nitro compounds and amines in petroleum ether extract.

Table 1: Qualitative phytochemical screening of the plant powder extracts of *Micrococca mercurialis*

S. No.	Metabolites	Presence/Absence				
		Petroleum ether	Acetone	Chloroform	Ethanol	Water
1.	Carbohydrate Test 1.Molisch's Test	+	+	+++	+	–
	2.Fehling Test	+	+	++	+	–
2.	Protein & Amino acid 1.Biuret Test	++	+	++	+	+
	2.Ninhydrin Test	–	–	–	+	++
3.	Alkaloid 1.Mayer's Test	–	+	–	+	+++
	2.Wagner's Test	+	–	++	–	+
4.	Tannin & Phenolic Compounds Ferric Chloride Test	+	–	–	++	+++
5.	Flavonoids 1.Alkaline reagent Test	+	–	++	+	+
	2.Ferric chloride Test	++	–	+	++	+
6.	Terpenoid Test	+	+	+	–	+
7.	Triterpenoids Libermann-Burchard Test	+	+	+	+	–
8.	Steroid Test Salkowski's Test	+	+	+++	+	–
9.	Saponin Test Froth formation Test	+	+	++	–	++
10.	Glycoside Test	+	++	–	+	+

	Borntrager's Test					
11.	Anthraquinone Test	+	_	+	_	++
12.	Quinone Test	+	+	+	+	+
13.	Coumarin Test	+	_	+	_	+
14.	Gum Test	_	+	+	+	_
15.	Starch Test	_	_	+	_	+
16.	Fixed oil Test	_	+	+	+	++

Note: '(+)' Present, '(-)' Absent

Table 2: UV-VIS Spectrum Peak values of Petroleum ether extract of *Micrococca mercurialis*

S. No.	Wavelength nm.	Abs.
1.	472.00	0.550
2.	446.00	0.655
3.	335.00	0.116
4.	268.00	0.163
5.	214.00	0.599
6.	464.00	0.537
7.	359.00	0.089
8.	308.00	0.068
9.	243.00	0.133

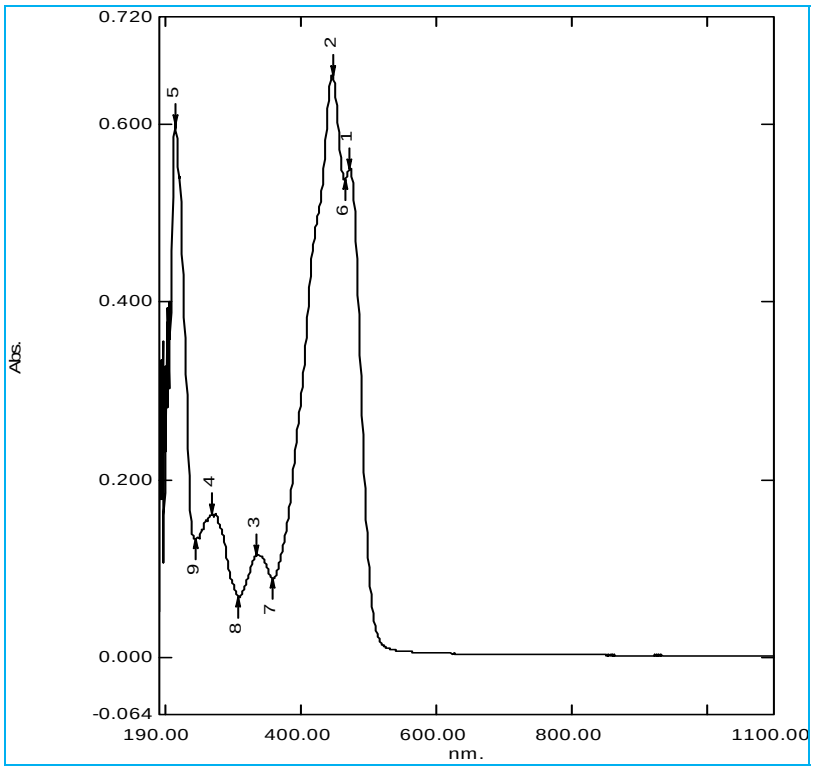

Fig 1: Ultra Violet-Visible Spectroscopy Analysis of Petroleum ether extract of *Micrococca mercurialis*

Table 3: FTIR peak values and functional groups of Petroleum ether extract of *Micrococca mercurialis*

Extracts	Peak Value	Funtional Group	Funtional Group Name	Vibrations
Petroleum Ether ***Micrococca mercurialis***	471.6	-	-	-
	730.06	C-Cl	Haloalkane	-
	811.56	C-H	Alkane	Bend out- of- plane
	890.16	C-C	-	Stretch
	984.19	Silicates	-	-
	1155.85	C-F	Haloalkane	-
	1249.88	O-H	Hydroxyl	Bending
	1299.07	O-H	Hydroxyl	Bending
	1377.19	Nitrates	-	-
	1461.57	O-H	Hydroxyl	Bending
	2614.05	-	-	-
	2730.26	C-H	Alkyl	Stretch
	2864.8	C-H	Alkyl	Stretch
	2963.65	C-H	Alkyl	Stretch
	3175.82	N-H	Amine	Stretch

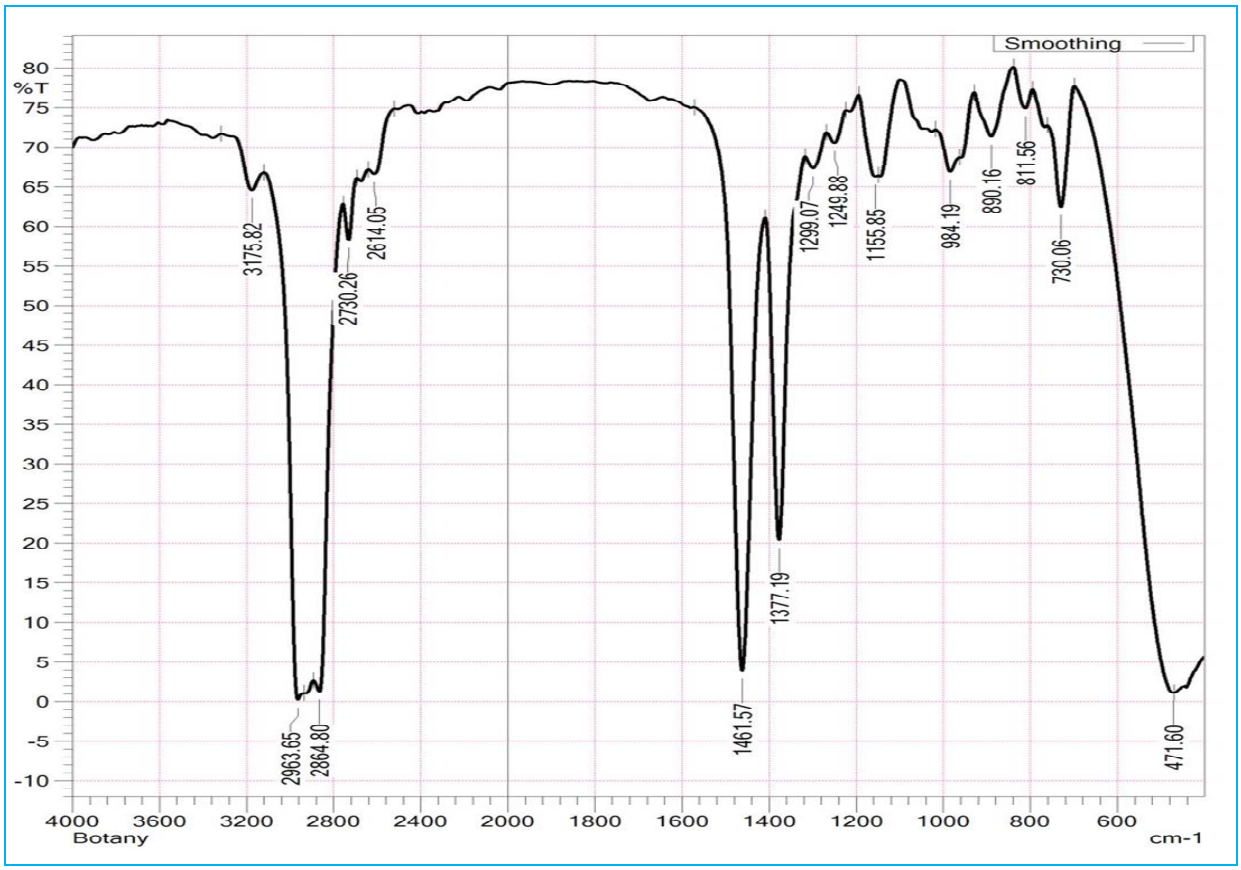

Fig 2: Fourier-Transform Infrared Spectroscopy analysis of petroleum ether extract of *Micrococca mercurialis*

4 Discussion

The pharmacological action of crude drugs and other therapeutic uses are due to their therapeutically active constituents such as tannins, flavonoids, alkaloids and several other aromatic compounds or secondary metabolites of plants which serve as defense mechanism against predation by many microorganisms, insects and herbivores. So, the preliminary phytochemical analysis revealed pronounced importance because the crude drugs possess varied composition of secondary metabolites [5, 6]. In the present study, phytochemical screening of all five extracts showed significant indication about the presence of metabolites. Alkaloids, Saponinis, Tannins, Amino acids, Flavonoids and Terpenoids, were found to be present in the all the sequential extracts of *Micrococca mercuralis.* Similar results were also reported by (Doshi *et al.* [7] in the latex of *Calotropis procera.*

Spectroscopic technique has become a powerful and analytical tool for the qualitative and quantitative analysis of pharmaceutical and biological materials. Characterization of secondary metabolite fingerprint by chromatography and spectroscopy provide valuable information about qualitative and quantitative formulation of plant species and their pattern of recognition by chemometry. Spectroscopic (UV-VIS, FT-IR) methods together or separate can be used in this sense as well as conventional methods [8, 9]. Fourier Transform Infrared Spectroscopy (FT-IR) is a high-resolution analytical technique to identify the chemical constituents and elucidate the structural compounds [10, 11]. FT-IR offers a rapid and non destructive investigation to fingerprint plant extracts or powders. In addition, UV-VIS and FT-IR spectroscopy is proved to be a reliable and sensitive method for detection of biomolecular composition [12].

Therefore in the present study UV.VIS and FT-IR techniques are employed to evaluate the UV visible and IR finger print in petroleum ether solvent extract of *Micrococca mercurialis*. UV-VIS spectrum of this plant extracts has absorption peaks at 214, 446 and 472 nm with the absorption of 0.599, 0.655, and 0.550 respectively. These absorption bands are characteristic for flavonoids and its derivatives. The flavonoids spectra typically consist of two absorption maxima in the ranges 230-285 nm (band I) and 300-350 nm (band II). The precise position and relative intensities of these maxima give valuable information on the nature of the flavonoids. This is in accordance with the previous literature on *Acorus calamus* [13].

The FTIR anaylsis (Table 3) revealved the prescence of alkaloids due to N-H stretching, polyphenols and flavonoids due to O-H stretching, terpens due to C-H group[14]. The functional groups present in test plant are aldehydes, alkenes, amines, amides, alcohols, phenols, aromatics, carboxylic acids and anhydride, esters and lactones, ethers, quinines and organic halogen compounds. All these compounds belong to secondary plant metabolites as per researcher explanations [15, 16]. These were confirmed by FT-IR spectrophotometer study that predicted the presence of the groups: O-H, N-H, C-H, C-Cl, C=C, nitrates and silicates stretching. The presence of characteristic functional groups of carboxylic acids, anhydrides, alcohols, phenols, amines, amides, esters, ethers, sulphur derivatives, glycosides, nitrates, nitriles, isonitriles, organic halogens and carbohydrate could be responsible for the various medicinal properties of *Micrococca mercurialis* [14].

5 Conclusion

In the present study analysis of the whole plant extract of *Micrococca mercurialis* sample under FTIR and UV-VIS

spectroscopic technique showed that the presence of phenolic compound and flavonoid which can be isolated and further screened for different kind of biological activities depending their therapeutic uses. Further research will be needed to find out the structural analysis of flavonoid compound by use of different analytical methods such as NMR and Mass spectrophotometer.

6. Acknowledgement
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7. Conflict of interests
We declare that we have no conflict of interest

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