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Evaluation of Phytochemical compounds by TLC and FT-IR analysis of *Cipadessa baccifera* (Roth) Miq. and *Elytraria acaulis* (L.f) Lindau

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ABSTRACT

The present study was carried out to investigate the medicinal bioactive substances present in the petroleum ether, toluene and methanolic extracts *Cipadessa baccifera* and petroleum ether and methanolic leaf extracts of *Elytraria acaulis*. In the present investigation, chromatographic techniques such as Thin Layer Chromatography (TLC) analysis was used to separate the bioactive compound from the crude leaf extracts of *Cipadessa baccifera* and *Elytraria acaulis*. The solvent system of TLC was Methanol: Chloroform (1:1, 6:4, 7:3 and 9:1) used and R_f value was recorded. TLC chromatogram was subjected to bioautography to check the antibacterial activity against Staphylococcus aureus. For FTIR analysis the leaf extract of *Cipadessa baccifera* and *Elytraria acaulis* was focused in the transmittance ranging from 400-4000cm^{-1} on a Perkin Elmer Spectrum and the characteristic peak values and their functional groups were detected. From TLC analysis spots were identified with R_f values 0.76 and 0.83 was compared with literature data showed that the presence of flavonoid compound as quercetin. Bioautography showed zone of clearance indicates the presence of active components inhibits the growth of Staphylococcus aureus when the chromatogram is sprayed with p–iodonitrotetrazolium violet (INT) spray.

Key words: Thin Layer Chromatography, FT-IR, *Cipadessa baccifera*, *Elytraria acaulis*.

INTRODUCTION

The importance of medicinal plants becomes more patent at the present time in developing countries. India is a rich country in terms of flora and medicinal plants; especially plant extracts and oils are commonly used for a variety of purposes. Since synthetic drugs have many side effects, the use of plants is encouraged as a source of alternative medicine. Plant produces a wide variety of secondary metabolites which are used either directly as precursors or as lead compounds in the pharmaceutical industry. It is

expected that plant extracts showing target sites other than those used by antibiotics will be active against drug resistant microbial pathogens. However, very little information is available on such activity of medicinal plants and out of the 4, 00,000 plant species on earth, only a small number has been systematically investigated for their antimicrobial activities (Anjana *et al*., 2009).

Bioactive compounds are normally accumulated as secondary metabolites in all plant cells but their concentration varies according to the plant parts, season climate and particular growth phase. Leaf is one of the highest accumulated plant part of such compounds and people are generally preferred it for therapeutic, purposes some of the active compounds inhibit the growth of disease causing microbes either singly or in combination (Dhia *et al*., 2006).

Scientific investigations of medicinal plants have been initiated in many countries because of their contributions to health care. The primary benefits of using plant-derived medicines are relatively safer than synthetic alternatives, offering profound therapeutic benefits and more affordable treatment (Ahamed *et al*.,2001).

Thin layer chromatography (TLC) is used to separate the components of a mixture using a thin stationary phase supported by an inert backing. It may be performed on the analytical scale as a means of monitoring the progress of a reaction, or on the preparative scale to purify small amounts of a compound. TLC is an analytical tool widely used because of its simplicity, relative low cost, high sensitivity, and speed of separation. TLC functions on the same principle as all chromatography: a compound will have different affinities for the mobile and stationary phases, and this affects the speed at which it migrates. The goal of TLC is to obtain well defined, well separated spots. After a separation is complete, individual compounds appear as spots separated vertically. Each spot has a retention factor (R_f) which is equal to the distance migrated over the total distance covered by the solvent.
The R_f formula is

R_f=distance travelled by sample/distance travelled by solvent

The R_f value can be used to identify compounds due to their uniqueness to each compound. When comparing two different compounds under the same conditions, the compound with the larger R_f value is less polar because it does not stick to the stationary phase as long as the polar compound, which would have a lower R_f value.

A common screen for plant antimicrobial compounds consists of separating plant extracts by paper or thin-layer chromatography (PC or TLC), exposing the chromatograms to bacterial suspensions which allows time for the microbes to grow in a humid environment, and visualizing zones with no microbial growth. The effectiveness of this screening method, known as bioautography, depends on both the quality of the chromatographic separation and the care taken with microbial culture conditions. Bioautography is an effective and inexpensive technique for the phytochemical analysis of plant extracts to identify bioactive scaffolds. It can thus be performed both in highly developed laboratories as well as in small research laboratories which have minimum access to sophisticated equipment. (Marston *et al.,* 1997).

MATERIAL AND METHODS

Plant Material

Mature and healthy plants of *Elytraria acaulis* (L.f) Lindau belonging to the family Acanthaceae was collected from Thiruvannamalai district of Tamil Nadu, India. The mature and healthy leaves of *Cipadesssa baccifera* belongs to the family Meliaceae collected from Jnanabharathi Campus, Bangalore University, Bangalore. The plants were identified by Prof. Seetharam from Biological Science Department, Bangalore University, Bangalore.

Preparation of Solvent Extract

Leaves of *Cipadessa baccifera* and *Elytraria acaulis* was collected and washed thoroughly, shade dried, pulverized mechanically and sieved. 50g of each shade dried and finely powdered of *Cipadessa bacciferra* and *Elytraria acaulis* were filled in the thimble separately and extracted successively with 200-300 ml of petroleum ether, toluene, chloroform, methanol and ethanol using a Soxhlet extractor until colorless extract obtained on the top of the extractor. Each of the solvents extracts were concentrated separately under reduced pressure using rotary flash evaporator (Mohana *et al.,* 2009). The concentrated extracts were subsequently dried at room temperature under a steam of cold air and kept in air-tight containers at 4^0C until tested. The dried organic plant extracts were re-suspended in DMSO for

the different concentration and filtered through 0.45µm membrane filter for sterilization and stored for further experiments.

Thin Layer Chromatography:

The petroleum ether, toluene and methanolic extracts of *Cipadessa baccifera* and petroleum ether and methanolic extract of *Elytraria acaulis* were subjected to thin layer chromatography.

Each solvent extract was subjected to thin layer chromatography (TLC) as per conventional one-dimensional ascending method. Commercially available TLC plate (Merck) silica gel 60F254, 7X6 cm (Merck) were cut with ordinary household scissors. Plate markings were made with soft pencil. Glass capillaries were used to spot the sample for TLC applied sample volume 1-micro liter by using capillary at distance of 1 cm at 3 tracks. In the twin trough chamber with different solvent systems Methanol: Chloroform (1:1, 6:4, 7:3 and 9:1) in different solvent system. When the solvent front moved up to 3/4th area on the TLC plate, the plate was taken out from the solvent system and allowed to air-dry. The separated bands were identified under UV light at 365 and 254 nm followed by Dragendorff's spary and R_f value of each band was determined. The single band which appeared on pTLC in different gradient solvent system was considered as the sample contains bioactive compound.

Bioautography:

To screen the antibacterial activity of the bioactive substances, direct bioautography was performed. Bioactive were separated on TLC plate and dried to remove the residual solvent.

Developed TLC plates or chromatogram were placed in a sterile Petri dish (150 mm). The culture was added to the melted Mueller-Hinton agar and a thin layer was poured over the TLC plate. After the solidification of the medium, TLC plate was incubated for 24 hours at 35 ± 2°C. The TLC-bioautography plates were sprayed with 2mg/ml salt solution of *p*–iodonitrotetrazolium violet (INT). Clear zone of inhibition was observed against a reddish pink background.

The extract is separated on flexible (aluminum-backed) silica TLC plates, and bands are visualized under ultraviolet (UV) light. Zones are cut out and incubated face down onto agar inoculated with the test microorganism. Inhibitory bands are visualized by staining the agar plates with tetrazolium red and their screening for activity against *S. aureus*.

FTIR Analysis:

Fourier Transform Infrared Spectroscopy was performed to assess the functional groups of the leaf extracts. Perkin Elmer spectroscopy was used to identify the different functional groups of petroleum ether, toluene and methanolic extracts of *Cipadessa baccifera* and petroleum ether and methanolic extract of *Elytraria acaulis*. The spectrum was focus in the middle of IR region of 400-4000 cm^{-1} by the KBr pellet technique. The spectrum was recorded using Attenuated Total Reflectance (ATR) technique beach measurement.

RESULTS

TLC analysis of all the fractions using different solvent systems revealed the presence of promising spots. Phytochemical constituents give different R_f values in different solvent system. This variation in R_f values provides a very important clue in understanding of their polarity and also helps in selection of appropriate solvent system for separation of pure compounds incorporated in different fractions by column chromatography. Mixture of solvents with variable polarity in different ratio can be used for separation of pure compound from plant extract. The selection of appropriate solvent system for a particular plant extract can only be achieved by analyzing the R_f values of compounds in different solvent system.

From TLC analysis it is confirmed that the bioactive compounds bands were present when the chromatogram was subjected under UV-light at 365 and 254 nm followed by Dragendorff's spray and R_f values were determined. From TLC analysis spots were identified with R_f values 0.76 and 0.83 was compared with literature data showed that the presence of flavonoid compound as quercetin. Bioautography was carried by agar overlay method, the chromatogram was overlayed by the culture mixed agar and kept for incubation. After the incubation period the plates were sprayed by *p*–iodonitrotetrazolium violet (INT) which showed the zone of clearance against reddish pink colored background. FTIR method was validated as a fine analytical tool to investigate the finger print and to predict the composition of different solvent extracts of *Cipadessa baccifera* and *Elytraria acaulis.*

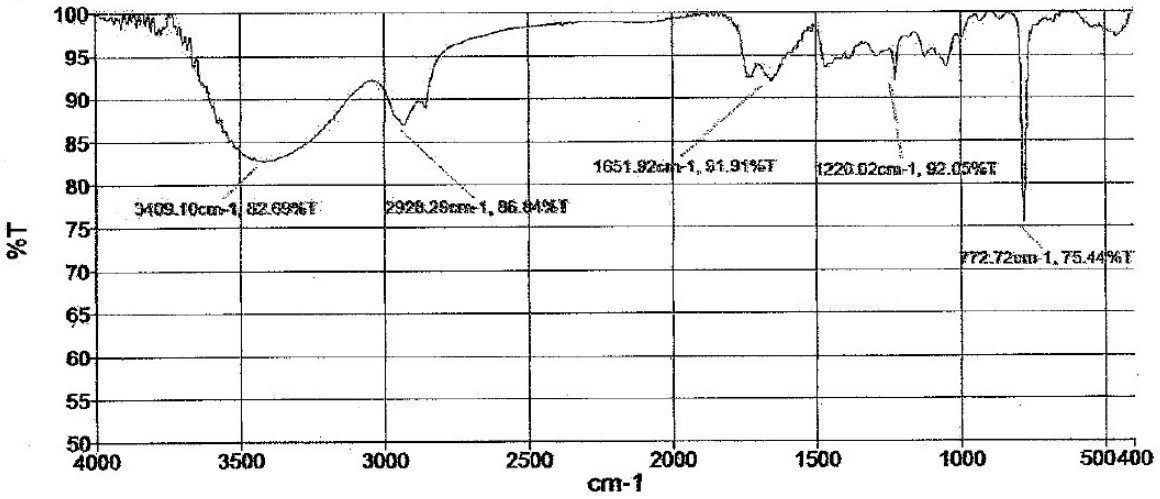

Fig. 1: FTIR Spectrum of petroleum ether extract of *Cipadessa baccifera*

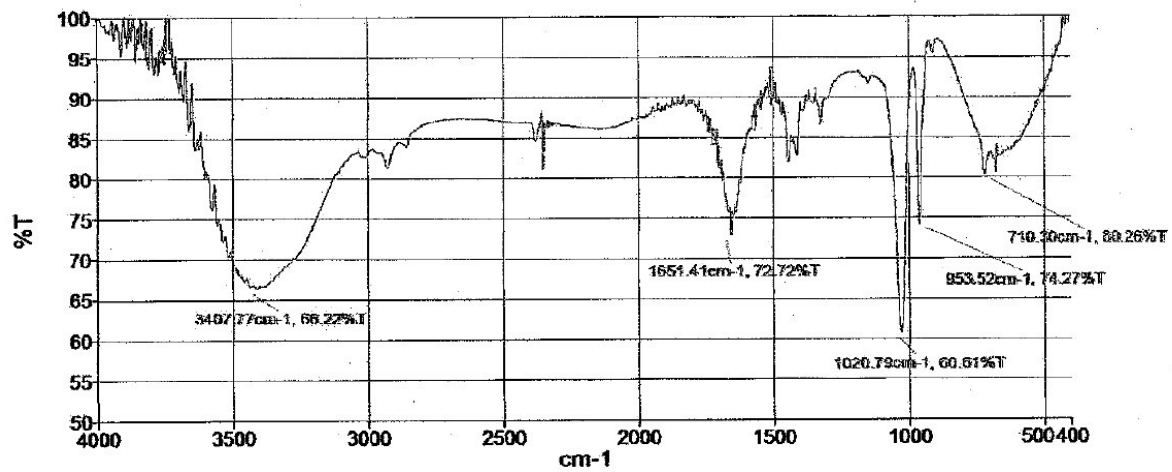

Fig. 2: FTIR Spectrum of toluene extract of *Cipadessa baccifera*

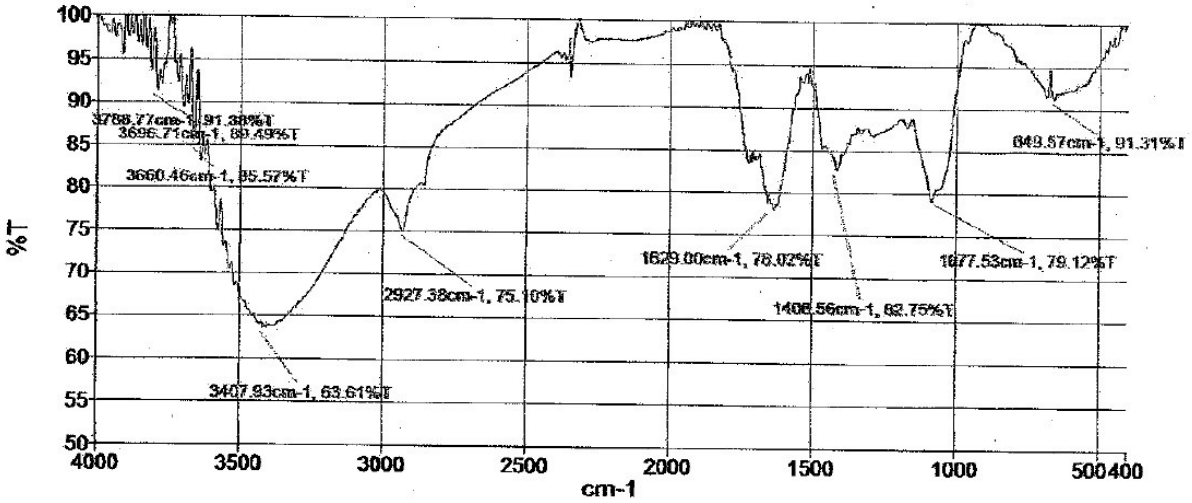

Fig. 3: FTIR Spectrum of methanol extract of *Cipadessa baccifera*

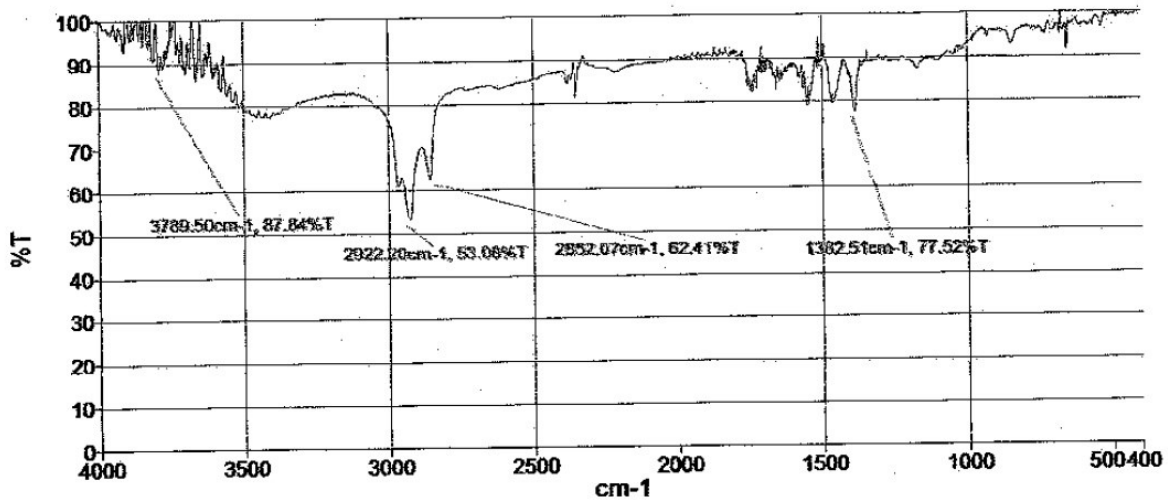

Fig. 4: FTIR Spectrum of petroleum ether extract of *Elytraria acaulis*

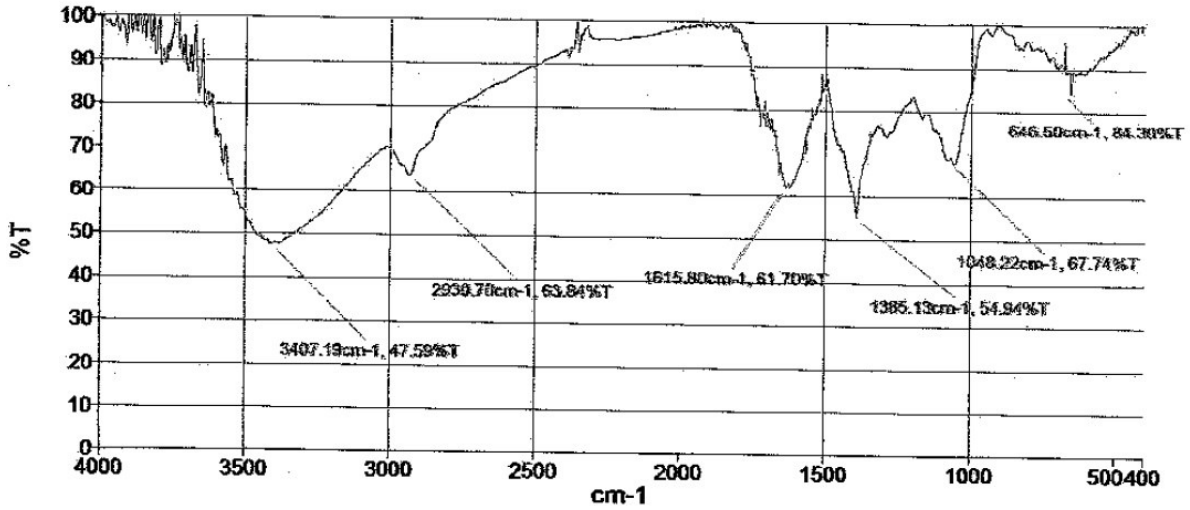

Fig. 5: FTIR Spectrum of methanol extract of *Elytraria acaulis*

Fig 1-3 shows the FTIR spectra of Petroleum ether, toluene and methanolic extract of *Cipadessa baccifera.* The peak at 3409.10cm^{-1} revealed the presence of alcohols, phenols (O-H stretch, H bonded). The peak at 2928.29 cm^{-1} refers to the presence of alkanes (C-H stretch). The peak at 1651.92 cm^{-1} correspond the carboxylic acid group (C=O stretch). A peak at 1220.02 cm^{-1} denotes the primary and secondary amines. A peak at 772.072 cm^{-1} shows the presence of meta disubstituted aromatics. Similarly the toluene extract spectrum showed at a peak 3407.77 cm^{-1}, 1651.41cm^{-1} 1020.79 cm^{-1},953.52 cm^{-1} and 710.30 cm^{-1} indicates the presence of functional groups such as alcohols, phenols ((O-H stretch, H bonded), carboxylic acids (O-H stretch), aliphatic amines (C-N stretch), primary and secondary amines, alkenes and monosubstituted aromatic phenyl group in bending patterns. The methanolic extract spectrum 3788.77 cm^{-1}, 3696.71 cm^{-1}, 3660.46 cm^{-1}, peak very narrow in range because of moisture contents and 3407.93 cm^{-1} denotes the presence of carboxylic acids, alkanes (C-H stretched). 2927.38 cm^{-1} denotes the presence of alkanes (C-H stretch), 1629.00 cm^{-1} refers to alkenes aromatic ring (C=C), 1406.56 cm^{-1}, 1077.53 cm^{-1},649.57 cm^{-1} denotes the presence of ethers and esters.

Fig. 4 and 5 shows the petroleum ether and methanolic extracts spectrum of *Elytraria acaulis.* The spectrum of petroleum ether spectrum a peak 3789.50 cm^{-1} refers to the presence of carboxylic acids but in the graph it is in narrow range because of moisture, 2922.20 cm^{-1} and 2852.07 cm^{-1} indicates the presence of alkanes and 1382.51 cm^{-1} denotes the presence of alkanes thiol S-H group. The spectrum of methanolic spectrum of 3407.19 cm^{-1} refers to the presence of functional group such as alkanes, carboxylic acids, 2930.70 cm^{-1} indicates the presence of alkane, 1651.80 cm^{-1} and 1385.13 cm^{-1},

1048.22 cm^{-1}, 646.50 cm^{-1} denotes the presence of ethers, esters, carboxylic acids, amides and aromatics.

FTIR spectra showed the presence of the functional group in all the extracts which have medicinal properties and antimicrobial activities.

CONCLUSION

TLC and bioautography offers the simplest mean of bioassay guided lead discovery from natural products. For the natural product the separation process is not easy, and if separated the amount is very less in maximum cases, so it is necessary to develop a process which can detect lead in a small amount and biological activity can also be measured successively. Considering these problems, we can say that bioautographic detection technique would create a new era in separation science.

The results in the present study showed that FTIR spectroscopy is valuable techniques to fingerprint and to analyze the different biomolecules extracted with different solvent extracts of *Cipadessa baccifera* and *Elytraria acaulis* a potent exotic herbicidal application. The FTIR result the chosen plants contains functional groups such as alcohols, alkanes, phenols, carboxylic acids, primary and secondary amines, ethers and esters. In order to validate the FTIR method as a good tool to investigate the fingerprint and to predict the composition of the exotic medicinal plants and to evaluate the quality and authenticity of formulating herbicidal potential. The FTIR data will be correlated in future with the detailed HPLC, NMR and GCMS analysis of the same extracts.

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