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A comparative study of bioactive compounds in raw and extract forms of *Ipomoea pes-caprae* (L.) R. Br. (leaf) detected by FT-IR spectroscopy

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Abstract
Medicinal plants have always played a pivotal role as sources for drug lead compounds. Around 50% of pharmaceuticals are derived from compounds first identified or isolated from herbs/plants. Synthesizing drug from natural product needs the knowledge of presence of active groups. Hence identification of bioactive groups of plants is vital for effective drug discovery by pharmaceutical industry. In this context we have undertaken the FTIR study of *Ipomoea pes-caprae* (L.) R. Br, (leaf), creeper found in the salty sand of Sea beach, for identifying the active groups and hence 10 active groups were detected. Comparison with various leaf extracts the plant *Ipomoea pes-caprae* (L.) shows a potential use in drug discovery either in the form of raw leaf or any other extract form. The medicinal uses of various bioactive groups detected are also addressed.

Keywords: Ipomoea pes-caprae (L.) R. Br. bioactive compound, extracts, FT-IR analysis

Introduction

Medicinal plants research stimulated the development of novel medicines. It is exhibited by the fact that between 1981 and 2014 approximately 40% of all new approved drugs were based on natural products [1]. It is therefore enlightening to describe the biological activity of medicinal plants, which are the best suited candidates for natural drugs. Early humans treated their illnesses using plants around them driven by their instinct, taste, and experience and hence, the history of medicinal plants is as long as the history of humans. Over the past few decades, researchers have focused on drug discovery from herbal medicines or botanical sources, an important group of complementary and alternative medicine (CAM) therapy [2]. The direct approach in drug discovery from herbal medicines is to isolate active ingredient(s) from the respective herbs or plant source. Approximately 80% of antimicrobial, cardiovascular, immunosuppressive, and anticancer drugs are of plant origin [3]. It is widely accepted that more than 80% of drug substances are either directly derived from natural products or developed from a natural compound [4]. And, in fact, around 50% of pharmaceuticals are derived from compounds first identified or isolated from herbs/plants, including organisms, animals, and insects, as active ingredients [5].

Since Ayurveda is one among the traditional practice originated in India and routed all over the globe it is ideal to follow the procedures for the identification of medicinal plants. Hence identifying the active ingredients from plants, especially based on the traditional practice of Ayurveda, will have its own effectiveness in drug discovery. In this context we have undertaken the FTIR study of *Ipomoea pes-caprae* (L.) R. Br, belongs to the family (Convolvulacea) which is a valuable medicinal plant according to the traditional practice, distributed in the tropics and subtropics regions and used in folk and tribal medicines. In Malayalam it is called with different names such as *Aattadambu, Adambu, Adumbuvalli, Adambuvalli, Atampa, Chunvanna-adambu* and *Kuthirakulamban*. All these names refer the structure of the leaf such as either the goat foot or horse foot. It is a perennial creeping vine with milky sap commonly called as 'Beach morning glory' [6, 7]. The Roots of the plant are produced at the nodes, so that it can spread over a vast area and provide support to sand binding. Leaves are alternate, oval-shaped and notched at the end, resembling the footprint of a goat. Its flowers are attractive, bell-shaped, pink, purple or violet with deeper colour at the centre. Growing with other plant species, *I. pes-caprae* is a useful sand-binder, thriving under conditions of sand blast and salt spray [8]. For the identification of the plant it is photographed at the Paradise beach of Pondicherry, and is given in Fig.1. *I. pes-caprae* at this Paradise beach of Pondicherry attracts every visitor due to its half-merged pattern of growth in the salty sand.

Fig 1: The photograph of *Ipomoea pes-caprae*(L.) R. Br (for identification).

In accordance to the traditional practice of different parts of the world the usage of plants differs in medical remedy. A clear review about the usage of *I. pes-caprae* was given by Chan *et al.* [8]. In China, the leaves of *I. pes-caprae* are

topically applied to treat pain, boils and bedsores [9]. The local fishermen in Thailand, prepare a paste by mixing the leaves with distilled vinegar and use this as antidote to jellyfish stings to relieve pain, inflammation and allergic reactions [10, 12]. In Papua New Guinea, leaves are chewed to relieve stomach ache and young leaves are heated over fire and applied to sores [13]. In Mauritius, this plant has been traditionally used to treat stone fish stings and hemorrhoid infections [14]. With diuretic and laxative properties, leaves are used as stomachic and tonic and for treating rheumatism. The sap from the stem is used to treat sore eyelids, boils and earache. In India, leaves have been used in ritual baths said to be to dispel evil spirits [15, 16]. Manigauha *et al*., [15] explored the *in vivo* antitumor activity of *I. pe*‑*caprae* against mice melanoma (B16F10) cancer cells.

Enormous number of natural drugs was identified from plants that were known to have antimicrobial activity. The antimicrobial properties of the leaf extracts of *I. pes-caprae* showed that the methanol extract has inhibition on Escherichia coli and Salmonella paratyphi and the acetone extract was found to inhibit Mucor sp. and Candida albicans [17]. The methanol extract, and ethyl acetate and aqueous fractions exhibited antinociceptive activity against pain and inflammation [18]. A strong antibacterial activity on human pathogens was exhibited by Methanol extract, whereas Kumar *et al*., [19] reported that hexane, dichloromethane and ethyl acetate extracts showed no antibacterial activity. Agoramoorthy *et al*., [20] reported that most of these active compounds derived from *I. pes-caprae* are having high anti-oxidant properties, and Gurudeepan *et al*., [21] reported that this plant also have potent α-glucosidase inhibitory activity, which is a key element in reducing the glucose level.

Materials and Methods

Traditional medicine in modern pharmaceutical form may reach the end users considerably and hence it is highly essential to identify the chemical functional group present in plant parts or as whole and so a well suitable procedure, the FTIR analysis, is carried out in this work for the identification of chemical functional group present in the raw plant leaf of *I. pes-caprae*. Since the studied plant has a vast usage in medical applications as per the traditional practices around the world it is highly vital to have a detailed analysis of its raw leaf. Hence the plant leaves are collected from the Paradise Beach, located in the Union Territory of Puducherry, India, (11.9139° N, 79.8145° E). The collected leaves are washed well in clear water and then in distilled water. The leaves are shadow dried in room temperature at the Central Instrumentation Room of the Institute and ground with mortar and pestle. It is further pelletized in KBr press. These pellets in triplets are subjected to IR irradiation. The spectrum obtained is fine, without noise, due to the purity of the sample. The active components detected are compared with other forms such as hexane, Dichloromethane, Ethyl acetate and methanol extract of the leaf collected within 80km distance, the nearby place Chidambaram in TamilNadu [19]. The functional groups identified in the raw powder of the plant leaf are addressed in the context of pharmaceutical interest.

FT-IR Spectroscopy

Fourier transform infrared spectrometry is a physico-chemical analytical technique that does not resolve the concentrations of individual metabolites but provides a snapshot of the metabolic composition of a tissue at a given time [22]. FTIR can be employed to determine the structure of unknown composition and the intensity of the absorption spectra associated with molecular composition or content of the functional group [23]. The FTIR method measures the vibrations of bonds within chemical functional groups and generates a spectrum that can be regarded as a biochemical or metabolic “fingerprint” of the sample [24]. By attaining FTIR spectra from plant samples, it might possible to detect the minor changes of primary and secondary metabolites [25]. At present, particularly in phytochemistry, FTIR has been exercised to identify the concrete structure of certain plant secondary metabolites [26]. But, on pharmacognosy FTIR is still a new tool to characterize and identify the commercial components from the adulterant [27, 28]. FTIR method has been successfully utilized in the characterization of bacterial, fungal and higher plant [29]. FT-IR is one of the most widely used methods to identify the chemical constituents and elucidate the compounds structures [30], and has been used as a requisite method to identify medicines in Pharmacopoeia of many countries [31, 32].

Hence FTIR analysis is being done to determine the functional group present in the sample. Functional groups are structural units within organic compounds defined by specific atom and bond arrangements. Infrared is a powerful identification tool for functional groups because of the similar absorption frequencies for those groups in different molecules. The identification of functional groups is a cornerstone of IR spectroscopy and organic chemistry.

Results and Discussion

The FTIR spectrum obtained for the raw plant leaf of *I. pes-caprae* (Fig.2) shows 10 major Bio-active compounds, shown against the absorption peaks in the spectrum. The major active compounds obtained are, Alkane (2925.48, 2850.27 and 1380.78cm^{-1}) as the group C-H stretching, CO_2 as O=C=O stretching at 2360.44cm^{-1} and carbonyl at 1729.83cm^{-1} as C=O stretching. Alkene (C=C stretching), Alkyl aryl ether(C-O Stretching) & Alkyl halide, 1,3 di-substituted (C-H bending) and halo compound are the other active groups obtained at 1629.55cm^{-1}, 1263.15cm^{-1} & 1062.59, 773.31cm^{-1}, and 619.03cm^{-1} respectively.

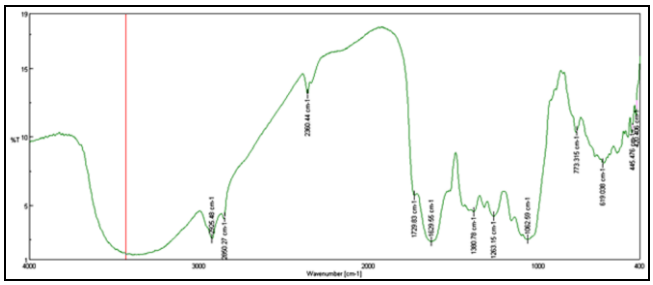

Fig 2: The FTIR spectrum of *Ipomoea pes-caprae* (L.) R. Br. (leaf)

It is very interesting to see the detected active compounds from the raw leaf (Col.2 of Table.1) are all found in different extracts such as Hexane, Dichloromethane, Ethyl Acetate and methanol (Col.3-6. of Table.1) (Kumar *et al*, 2014). The Table.1 is self-explanatory in which major FTIR absorption peaks observed by Kumar *et al*, (2014) for various extracts are only quoted since the raw leaf contain no more functional groups. It is observed that the absorption peaks are obtained more or less at same frequency (Fig.3). Now a big question arises that whether all these solvents are

capable of dissolving all the same detected compounds? Since the answer, according to the study of Kumar *et al*., (2014), is a big yes, it is highly advantageous to the pharmaceutical industry. In fact the Drug discovery from herbs may be divided into three stages, namely, pre drug stage, quasi-drug stage, and full-drug stage [2]. In simple these three stages can be defined as follows: (i) Identification of plant from the traditional practice either oral tradition or from old manuscripts of various traditional practices like Ayurveda, Unani, Sidha, etc.is the pre-drug stage, (ii) quasi-drug stage of drug discovery from herbal medicines is to search for an active herbal ingredient or lead compound from herbs or plant materials for further drug development, and (iii) the full drug stage is from the pharmaceutical industry in the form of oral, IM or IV intake. In general, this type of study provides information about the first two stages of drug discovery from herbal medicine. Since the raw leaf and the different extracts provide same bioactive groups (Table.1) the usage of the detected compounds according to the requirement of the design of the drug is comparatively easy to bring out the full drug.

Table 1: The bioactive groups obtained at the respective absorption frequencies of *Ipomoea pes-caprae*(L.) R. Br. (leaf) and identification of common functional groups in comparison with Hexane, Dichloromethane, Ethyl acetate and Methanol Extracts (Kumar *et al*, 2014) [19].

Sl. No.	Absorption (cm^{-1})					Group	Compound Class
	Raw leaf	Extract					
		Hexane	Dichloro-methane	Ethyl Acetate	Methanol		
1.	2925.48	2924.09	2922.16	2922.16	2924.09	C-H Stretching	Alkane
2.	2850.27	2860.43	2852.72	2852.72	2856.58	C-H Stretching	Alkane
3.	2360.44	2250.93	2358.94	2360.87	2355.08	O=C=O stretching	CO2
4.	1729.83	1726.29	1730.15	1726.29	1726.29	C=O stretching	Carbonyl
5.	1629.55	1627.92	1635.64	1631.78	1631.78	C=C stretching	Alkene
6.	1380.78	1384.89	1379.1	1381.03	1394.53	C-H bending	Alkane
7.	1263.15	1257.59	1259.52	1261.45	1261.45	C-O stretching	Alkyl aryl ether
8.	1062.59	1062.78	1064.71	1060.85	1060.85	C-O stretching	Alkyl halide
9.	773.31	713.66	723.31	792.74	813.96	C-H bending	1,3, disubstituted
10.	619.03	617.22	619.15	615.29	615.29	C-I stretching	Halo compound

The various bioactive compounds detected by our FTIR study of the raw leaf powder is compared with various extracts by Kumar *et al*. [19] is plotted as a bar chart for better understanding of the closeness of absorption peaks obtained (Fig.3). The peaks taken from Ref. [19] are only for those have been identified in the raw leaf powder.

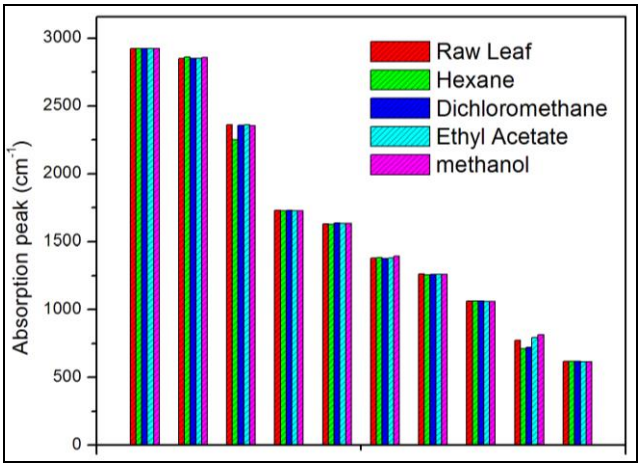

Fig 2: The comparative bar chart of the Absorption peaks obtained in the FTIR spectrum for raw leaf and different extracts (mentioned in the graph) of *Ipomoea pes-caprae* (L.) R. Br.

Medicinal Value of Detected Bioactive Groups

Alkane is an anionic surfactant used to treat varicose veins of the lower extremitie, and to maintain alcohol abstinence in patients with alcohol dependence. It is also used in cosmetics and pharmaceuticals as a fat emulsifier, wetting agent, and detergent.

Medical carbon dioxide (CO_2) has various medical purposes. It is used as a pure gas or in specialised mixtures with other gases in stimulating breathing, anaesthesia and sterilisation of equipment [33]. It can be used as an insufflation gas for minimal invasive surgery, such as laparoscopy, endoscopy and arthroscopy, to enlarge and stabilise body cavities for better visibility of the surgical field. In its liquid phase, medical carbon dioxide can be used to provide temperatures down to -76° C, for cryotherapy or for local analgesia. Other applications of medical carbon dioxide include transient respiratory stimulation and encouragement of deep breathing and coughing to prevent or treat atelectasis.

Alkenes are the raw materials for the manufacture of chemicals like alcohols, aldehydes etc. A number of neurotropic agents contain a conjugated alkene group incorporated in an iminostilbene or dibenzosuberene ring system [34]. Alkenes are suitable functional groups to carry out bioorthogonal ligations because there are no naturally occurring functional groups; they have good compatibility with water and high selectivity. It was demonstrated that highly strained alkenes (electron-rich dienophile), such as transcyclooctene and norbornene, can react rapidly with tetrazines. This approach was successfully employed to functionalize thioredoxin and to label the cell surface of living cells [35]. Propylene glycol which is an alkene is used as a solvent in many pharmaceuticals, including oral, injectable and topical formulations, which are insoluble in water [36]. These are also used as general anesthesia. Ethene is a plant hormone which controls growth, seed germination and fruit development. Therefore, ethene is used for artificial ripening of fruits, flower maturation, etc.

Alkyl halides are valuable intermediates in synthetic organic chemistry, and their use as bioactive motifs in drug discovery and medicinal chemistry is rare in comparison. [37] Alkyl halides are used in the medical field as anesthetics, the agents that can cause reversible loss of consciousness, which have been used in medicine since the mid-1800s [38]. An antibiotic, Clindamycin, is used for the treatment of a variety of bacterial infections, including bone and joint

infections, strep throat, pneumonia, and endocarditis. Alkyl halides are often the drug of choice for their anti-inflammatory and immunosuppressive properties [39].

A carbonyl group is a chemically organic functional group composed of a carbon atom double-bonded to an oxygen atom (C=O). The simplest carbonyl groups are aldehydes and ketones usually attached to another carbon compound. These structures can be found in many aromatic compounds contributing to smell and taste. Another feature of the carbonyl group is its polarity. As for polarity and solubility, they are related each other such as water is polar, and carbonyl is polar, so water dissolves carbonyl. Ketosteroid, a carbonyl compound, isolated from Natural products by Girard reaction, acts as sex hormones. The mixture of Aldehyde and ketones can be converted into water soluble hydrazine derivatives by treatment with Girard reagent and the non-carbonyl compounds can be removed by extraction with non-polar solvent like ether [40]. The chemical reactivity of carbonyl functional groups can also be important in drug action.

The in vitro antiproliferative activity of the synthesized 1,3-disubstituted urea derivatives was studied by Li *et al*. [41] on a panel of one human liver cell line (L02) and two human tumor cell lines (KB, K562) by applying the MTT colorimetric assay. 1, 3 - disubstituted ureas are potent inhibitors of soluble epoxide hydrolase (sEH) that are active both in vitro and in vivo [42, 43] reported that 1, 3 - disubstituted urea derivatives are having antiglycating potential.

Conclusion

The FTIR spectrum of the raw plant leaf powder of *I. pes-caprae* shows the presence of 10 bioactive compounds in accordance to the absorption peak found at different frequencies. The FTIR absorption peaks of various extracts of the leaf are compared with the raw leaf. The similarity found in the detected groups at almost same frequency pronounces the flexibility in utilization of the either the plant leaf or its extract form according to the pharmacological interest. This study also revealed the completion of fist two stages of the drug discovery in this plant leaf is concerned. However, it's pharmaceutical utilization mainly depends on (1) the concentration of the bioactive component(s) present, (2) the degree of purification, and (3) the availability of the plant.

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