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Functional group analysis of *Cleome viscosa* L. and *C. burmanni* W. & A. (Cleomaceae) extracts by FT-IR

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ABSTRACT

The present study attempts to establish the FT-IR profile and identify the functional components of the methanol extracts of two species of *Cleome*, *C. viscosa* and *C. burmanni*. Similar absorption peaks were exhibited by the two species at different transmission percentages. Also, most of the functional groups observed as per their peaks are similar in the two species with a difference only in their wave numbers. An effort was made to link the observed bands to the probable components in the samples. The two species could be distinguished based on the presence of certain marker compounds. Aromatic amines and alkynes were observed only in *C. viscosa* while *C. burmanni* contained phenols. The possible bioactive properties of the detected compounds are also discussed.

Keywords: FT-IR, spectroscopy, *Cleome viscosa*, *Cleome burmanni*, wavenumbers, amines, alkynes, phenols

1. Introduction

The present generation is witnessing an unanticipated revival in the popularity of natural foods and medicines. The non-availability of data regarding the qualitative and quantitative phytochemical content and scientific validation studies however, prevents the use of herbal drugs among the educated public.

In nature, plants are bestowed with chemical compounds to help provide defense against predators. However, some of the secondary metabolites in plants have also been identified to act as pharmaceutical intermediates or drug precursors which may be utilized for the production of the more potent synthetic drugs. Plant natural product chemistry has played an active role in generating a significant number of candidate compounds in drug discovery programs [1]. However, the climate and other ecological conditions may affect secondary metabolite production in medicinal plants and need to be incessantly monitored to maintain the potency of the plant drugs [2].

Fingerprinting (marker compound analysis) by chemical and validated chromatographic and spectroscopic techniques are gaining importance for standardization in the herbal medicinal formulations. The evaluation of an herbal product by metabolomic fingerprinting can be accomplished by appropriate methods, including HPLC with UV (DAD), ELSD, GC-MS, HPTLC, FT-IR, NIR, NMR or a combination of these techniques [3-5]. Such techniques also provide useful information about qualitative and quantitative composition of herbal medicines and their pattern recognition by chemometry [6].

It is well known that medicinal materials comprise hundreds of components, and produce their curative effects through mutual effects of many ingredients, so the limited number of specific components cannot availablely reflect the real qualities of herbal medicines. Therefore, an inexpensive and effective method to entirely monitor all the constituents of the medicinal materials and their corresponding extract products is required [7].

Infrared spectroscopy (IR) has the potential to provide biochemical information without disturbing the biological sample. It is a powerful method for the study of molecular structure and intermolecular interaction in samples. Fourier transform infrared spectrometers, with their high signal-to-noise ratio and high precision in absorbance and wave number measurements have caused a resurgence of interest in the use of infrared spectra for identification of biomolecules [8].

Fourier transform infrared (FT-IR) spectrometry is a rapid, non-invasive, physico-chemical analytical, time saving technique that does not determine the concentration of individual metabolites but possibly provides a snapshot of the metabolic composition of a tissue at a given time^[9]. The analysis can be performed both on pure compounds and complex mixtures, without separation into individual components. It is more sensitive and selective than colorimetric methods and has played a vital role in pharmaceutical analysis in recent years^[10]. This technique determines the structure of unknown composition and the intensity of absorption spectra associated with molecular composition or content of the chemical group^[11]. It measures predominantly 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. By attaining IR spectra from plant samples, it might be possible to detect the minor changes of primary and secondary metabolites^[11, 12]. At present, particularly in phytochemistry, FT-IR has been exercised to identify concrete structures of certain plant secondary metabolites and offers a rapid and non-destructive investigation to fingerprint herbal extracts or powders^[13, 14].

In the field of pharmacognosy, FT-IR is still considered as a new tool to characterize and identify commercial components from the adulterants. This method has been successively utilized in differentiating, classifying and discriminating closely related microbial strains, plant species, other organisms and monitoring biotechnological processes^[15, 16]. It is one of the most widely used methods to identify the chemical constituents and elucidate the compound structure and has been used as a mandatory method to identify medicines in Pharmacopoeia of many countries^[7].

The present paper highlights the FT-IR spectrum of the methanol extracts of two species of the genus *Cleome* (Cleomaceae) namely, *Cleome viscosa*, and *C. burmanni*. It is assumed that the FTIR data, can be considered along with other parameters for the chemotaxonomic identification between species of the genus *Cleome*.

2. Materials and methods

The plant samples, *Cleome viscosa* and *C. burmanni* were collected from Kariavattom, Thiruvananthapuram and authenticated by the

Curator, Dept. of Botany, University of Kerala and voucher specimens deposited in the herbarium (KUBH 5806, KUBH 5807).

2.1 Preparation of plant material

The aerial parts from both species were shade dried at room temperature in a clean environment to avoid contamination and powdered in a domestic grinder. The powdered samples were stored in air tight glass bottles at room temperature for further analysis.

2.2 Preparation of extract

Methanol extracts of *Cleome viscosa* and *C. burmanni* were prepared from shade-dried plant parts. About 20 g of the powdered plant material from each sample was subjected to soxhlet extraction with 300 ml of methanol. The extracts were then concentrated under reduced pressure and kept at 4 °C until further use.

2.3 Spectroscopic analysis

FTIR analysis was performed using the spectrophotometer system (Shimadzu IR prestige 21, Japan). The characteristic peaks values were recorded and their functional groups were detected in the region 4000-400 cm⁻¹ by employing standard KBr pellet technique^[17]. The analysis was repeated twice for the spectrum confirmation.

3. Results

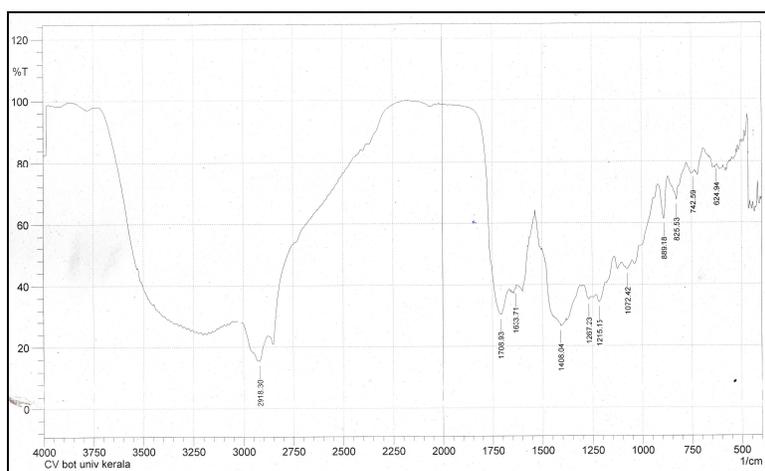
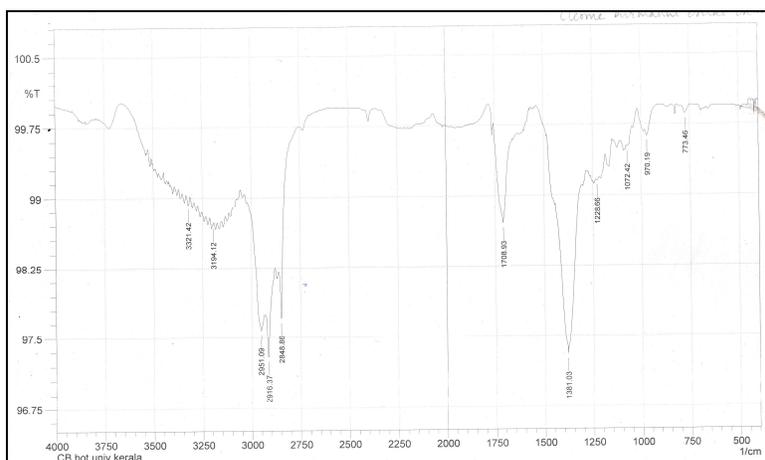
The FT-IR spectrum was used to identify the functional groups of the active components in *Cleome viscosa* and *C. burmanni* based on the peak values in the region of infrared radiation. The FT-IR spectra (4000-400 cm⁻¹) of *Cleome viscosa* and *C. burmanni* methanol extracts were registered and specific wave numbers and intensities considered. The results of FT-IR peak values and functional groups are represented (Tables 1 & 2) and the FT-IR spectra profiles illustrated (Figs. 1 & 2). The FT-IR spectrum of *Cleome viscosa* and *C. burmanni* each consisted of 11 major peaks at the range of 2918.30 - 624.94 cm⁻¹ and 3321.42 - 773.46 cm⁻¹ respectively. The observed peaks mainly corresponded to phenols, alkanes, aldehydes, ketones, amines, amides, alkenes, carboxylic acids, sulphur compounds, alcohols, alkynes and alkyl halides. Apart from that, *Cleome viscosa* consisted specifically of aromatic amines and alkynes while *C. burmanni* of phenols.

Table 1: FT-IR peak values of *Cleome viscosa* methanol extract

Peak	Area (%)	Bond	Functional group
2918.3	31.367	C-H stretch	Alkanes
1708.93	29.996	C=O	α,β-unsaturated aldehydes, ketones, esters
1633.71	2.367	N-H bend/C=C stretch	1° amines/alkenes
1408.04	13.862	C-N bend	Amides
1267.23	15.687	C-N stretch/C-O stretch/C=S stretch	Aromatic amines/carboxylic acids/sulphur compounds
1215.15	20.135	C-O stretch	Alcohols, Carboxylic acids, esters, ethers
1072.42	17.559	C-N stretch/C-O stretch	Aliphatic amines/alcohols
889.18	8.389	N-H wag/S-O stretch	1°,2° amines/sulfonates
825.53	7.993	C-Cl stretch	Alkyl halides
742.59	1.348	C-Cl stretch/=C-H bend	Alkyl halides/alkenes
624.94	0.596	C-Br stretch/C-S stretch/C-H bend	Alkyl halides/disulfides/alkynes

Table 2: FT-IR peak values of *Cleome burmanni* methanol extract

Peak	Area (%)	Bond	Functional group
3321.42	0.095	N-H stretch / O-H stretch	1°, 2° amines, amides/phenols
3194.12	0.032	=C-H stretch	Alkenes
2951.09	0.635	C-H stretch/O-H stretch	Alkanes, carboxylic acids
2916.37	0.467	C-H stretch	Alkanes
2848.86	0.368	C-H stretch	Carboxylic acids
1708.93	0.216	C=O stretch	α,β -unsaturated aldehydes, ketones, esters
1381.03	1.064	C-H bend/S=O stretch	Alkanes/sulfonyl group
1228.66	0.056	C-N stretch/C-O stretch	Aliphatic amines/alcohols, carboxylic acids
1072.42	0.055	C-N stretch/C-O stretch	Aliphatic amines/alcohols, carboxylic acids
970.19	0.059	=C-H bend	Alkenes
773.46	0.018	C-Cl stretch	Alkyl halides

**Fig 1:** FT-IR chromatogram of *Cleome viscosa***Fig 2:** FT-IR chromatogram of *Cleome burmanni*

4. Discussion

Infra-Red spectroscopy is basically a vibrational spectrum and involves the measurement of wavelength and intensity of absorption of mid infrared light by a sample. The principle value of this technique relates to the detection of the bands present in organic molecules. Since different bands have different vibrational frequency, the presence of the bands can be detected by identifying the characteristic vibrational frequencies as an absorption band in the IR spectrum.

The FT-IR spectrum was used to identify the functional groups of active components based on the peak value in the region of infrared radiation. The sample was passed into the FT-IR and the functional groups of the components were separated based on their peak ratio. The spectra shows substantial overlap between the absorption spectra of various components, each band representing an overall overlap of some characteristic absorption peaks of functional groups in the samples.

Spectral differences are the objective reflection of componential differences. By using the macroscopic fingerprint characters of FT-IR spectrum, one can judge the origin of different extracts accurately and effectively, confirm the functional constituent's presence in the given parts and extract, identify medicinal materials from the adulterate and even evaluate the quality of the medicinal materials [7]. So FT-IR spectrum reflects the panorama of chemical constituents in complex system thereby validating and identifying the mix-substance systems such as traditional and herbal medicine [18, 19].

The present FT-IR results confirmed the presence of phenols, alkanes, aldehydes, ketones, amines, amides, alkenes, carboxylic acids, sulphur compounds, alcohols, alkynes and alkyl halides in the methanol extracts of *Cleome viscosa* and *C. burmanni* (Tables 1 & 2) based on previous studies conducted in other plants [8, 20-22].

Aromatic amines and alkynes were observed in *C. viscosa* only and *C. burmanni* contained phenols. Aromatic amines are used in rubber, textile and dye industries. Many amine-rich proteins are bound to DNA and some neurotransmitters are amines including epinephrine, dopamine. They are used industrially for removing carbon dioxide and hydrogen sulphide from natural gas and refinery process streams [23]. Alkynes have been isolated from a wide variety of plant species, fungi, corals, bacteria, marine sponges. Some pharmaceuticals are also alkynes such as the contraceptive norethynodrel. Some acids like tartaric acid contain alkynes. Alkynes are highly bioactive nematocides. They possess antifungal, antitumor and antiviral properties [24].

Phenols are of great importance as they protect the human body from the oxidative stress, which cause many diseases including cancer, cardiovascular problems and ageing [25]. They are of great importance as cellular support material as they form the integral part of the cell wall structure. They exhibit antimicrobial, anthelmintic, antiapoptotic and anti diarrhoeal activities [26]. They are antiseptic and reduce inflammation when taken externally. They regulate nitric oxide, decrease leukocyte immobilization and exhibit phytoestrogenic activity. They have been found to be useful in the preparation of some antimicrobial compounds such as Dettol and cresol.

The alkanes are found in the plant cuticle and epicuticular wax of many species. They protect the plant against water loss, prevent the leaching of important minerals by rain and protect against microorganisms and harmful insects [27]. Alkenes are important in the manufacture of plastics, e.g. polythene and as fuel and illuminant. They serve as raw materials for the manufacture of alcohols and aldehydes. They are used for artificial ripening of fruits, a general anaesthetic, making poisonous mustard gas and ethylene-oxygen flame. Amines and amides are the main groups of protein synthesis. Carboxylic acids are biologically very important in the formation of fat in the body and act as strong antibacterial agents. They serve as main pharmaceutical products in curing ulcers, jaundice, headache, fever, pain in liver, wound in cattle, treatment of edema and rheumatic joint pains. Aldehydes are used in the production of resins when combined with phenols [28]. Esters in combination with volatile oils produce the pleasant aroma of fruits. The sulfur compounds are present in the plant in three forms: in the amino acids of proteins, volatile oils and sulfates. They were used as disinfectants and dental creams. Halogen compounds function within the plant cell to generate chlorinated tryptophan, which is then shuttled into monoterpene indole alkaloid metabolism to yield chlorinated alkaloids [22]. Chlorates play the role of disinfectants and bromide is needed by eosinophils for generating anti-parasitic brominating compounds by the action of eosinophil peroxidase [29].

5. Conclusion

The results of the present study identified novel phytochemical markers for the detection of *Cleome viscosa* and *C. burmanni*. *Cleome viscosa* consisted specifically of aromatic amines and alkynes while *C. burmanni* of phenols. The plants may be rich sources of phytoconstituents which can be isolated and screened for different kinds of biological activities depending on their reported therapeutic uses.

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