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Determination of Bioactive Compounds of *Boerhavia diffusa* Linn. Leaf Using IR Irradiation

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Abstract
Plants possess a lot of beneficial effects in traditional systems of medicine. *Boerhavia diffusa* Linn., a potential medicinal plant used in the system of Ayurvedha, belongs to the family Nyctaginaceae. The bioactive compounds, Alkane, CO_2, Alkene, Sulfate, phenol, Alkyl aryl ether and Alkyl halide are found to be present in the leaves of *B. diffusa* through IR irradiation studies. The comparison of FTIR spectrum of raw plant leaf with aqueous extract and ethanol extract in the combined form shows that the effect of *B. diffusa* is same in any extract form since the bioactive components determined are identical in all the three studies. The possible medicinal values of the active groups detected are discussed in the context of pharmaceutical interest.

Keywords
Boerhavia diffusa Linn*, Bioactive Compound, Aqueous extract, Ethanol extract, FT-IR Analysis.*

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INTRODUCTION
Boerhavia is a genus of family Nyctaginaceae, consisting of 40 species [1] distributed all over the world. In India six species are found, namely *B. diffusa, B. erecta, B. rependa, B. chinensis, B. hirsuta* and *B. rubicunda*. The genus '*Boerhavia'* is so named to honour Hermann Boerhave who was a famous 18th century Dutch physician and the species is named '*diffusa'* due to the typical diffuse branching of the plant [2,3]. In fact, *Boerhavia diffusa are found in two different varieties, said to* be white and Red. In this study the red variety is taken. It is a perennial herb and a long used drug in indigenous system of medicine. In India it is known as, Punarnava, Biskhafra (Hindi), Mukkirattai (Tamil), Thazhuthama (Malayalam), Gadhapurna (Bengali), Satodi (Gujarati), ltsit (Punjabi), etc. This plant is being used as a cooking vegetable also [4].

Many bioactive compounds like tannins, flavonoids, alkaloids, glycosides, steroids, terpenoids, phenolic compounds, rotenoids etc. are reported in *B. diffusa* plant parts [5-7]. The various Ayurvedic formulations made are used to treat numerous ailments in humans like heart disease, sciatica, inflammation, diseases of abdomen, spleen and liver disorders, arthritis etc. [2]. Umamaheswari *et al.* [8] analyzed ethyl acetate, diethyl ether, ethanol, aqueous chloroform and methanol extracts of *B. diffusa* leaves by the method of Harborne to test the presence of different phytochemicals and revealed the presence of phenols, quinones, proteins, amino acids, saponins, carbohydrates, sterols, furanoids, alkaloids, glycosides, triterpenoids, flavonoids and tannins. Gadekar *et al.* [9] studied the anxiolytic activity of hydro alcoholic extract of *B. diffusa* leaves in rats. Anti-inflammatory effect of *B. diffusa* was

evaluated in rats from the aqueous extracts of the plant leaves by Sudhamadhuri and Kalasker [10]. Devi and Jyothi [11] investigated the healing effect of *B. diffusa* on gastric ulcers. Antibacterial activity of ethanolic extracts prepared from roots, stem and leaves of *B. diffusa* was evaluated by Majgaine and Verma [12]. In vitro experiments were performed by Ramachandra *et al.* [13] to study its antibacterial potential. Manu *et al.* [14] has detected the radio-protective nature of B.*diffusa* against damage induced by exposure to radiations on mice using hydro alcoholic extracts. Role of *B. diffusa* in curing hepatotoxicity induced by antituberculosis drug rifampicin in male albino Wistar rats was investigated by Muthulingam [15]. Anti-diabetic activity of *B. diffusa* has been investigated by *Chude et al.* [16] and Pari and Satheesh [17]. Antihyperglycaemic and Reno protective effects of ethanolic extracts of *B. diffusa* were evaluated by Singh *et al.* [18] in diabetic rats. Indigenous system of medicine in India has recommended *B. diffusa* for curing diabetes mellitus [19]. Pareta et al. [20] studied the antioxidant potential of *B. diffusa* extract in urinary stones by means of inhibition of oxidative trauma and kidney cell damage and observed decrease in calcium oxalate deposition. Yasir et al. [21] reported the ability of ethanolic extract of *B. diffusa* in shrinking crystal size and promoting calcium oxalate dihydrate (COD) crystals formation more than monohydrate (COM) crystals. Olaleye et al. [22] evaluated the aqueous and ethanolic extracts of fresh leaves for antioxidant components and activity by *in vitro* and *in vivo* assays. Venkatalakshmi et al. [23] accounted for protection against paracetamol induced hepatotoxicity for *B. diffusa* extracts.

MATERIALS AND METHODS

Tradition 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 the leaves of this plant and so a well suitable procedure, the FTIR analysis, is carried out in this work. Since the studied plant leaf is a common home remedy for many ailments, used in India, it is highly important to have a detailed analysis of the raw plant leaf. Hence the plant leaves are collected from the campus garden of Kanchi Mamunivar Government Institute for Post Graduate Studies and Research, located in the Union Territory of Puducherry, India, (11.9628° N, 79.8111° 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 results obtained are compared with available literatures especially with aqueous extract of *B. diffusa* leaf from Nigeria [24] and the mixture of ethanolic extracts of *Tridax procumbens* and *Boerhavia diffusa* in the ratio of 1:1 [25] from India. The functional groups identified in the raw powder of the plant leaf are addressed in the context of pharmaceutical interest. The photograph of the plant is given in Fig.1. for identification.

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 [26]. 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 [27]. 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 [28]. By attaining FTIR spectra from plant samples, it might possible to detect the minor changes of primary and secondary metabolites [29]. At present, particularly in phytochemistry, FTIR has been exercised to identify the concrete structure of certain plant secondary metabolites [30]. But, on pharmacognosy FTIR is still a new tool to characterize and identify the commercial components from the adulterant [31, 32]. FTIR method has been successfully utilized in the characterization of bacterial, fungal and higher plant [33]. FT-IR is one of the most widely used methods to identify the chemical constituents and elucidate the compounds structures [34] and has been used as a requisite method to identify medicines in Pharmacopoeia of many countries [35, 36].

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 (Fig.2) shows 10 major Bio-active compounds, shown

against the absorption peaks in the spectrum. The major active compounds obtained are, Alkane (2921.63 and 2852.20cm^{-1}) as the group C-H stretching, CO_2 as O=C=O stretching at 2358.50cm^{-1} and Alkene at 1643.05cm^{-1} as C=C stretching. Sulfate (S=O stretching), phenol (O-H bending), Alkyl aryl ether (C-O Stretching) & Alkyl halide and 1,3 di-substituted (C-H bending) are the other active groups obtained at 1415.49cm^{-1}, 1321.00cm^{-1}, 1238.08 & 1072.23cm^{-1}, and 781.029cm^{-1} respectively.

Firstly, it is very interesting to see the results obtained for raw leaf (Col.4 of Table.1) when compared with the sample in aqueous extract (Col.2. of Table.1) [24]. All the active compounds detected from the raw leaf is present in the aqueous extract except the C-O Stretching Alkyl aryl group, even though the samples were collected from different parts of the world, Nigeria and India. It is quite natural that the presence of O-H stretching alcohol group in the aqueous extract (*Arishta*) whereas it is not necessarily in raw plant leaf. And thus the FTIR spectrum of raw leaf shows an exact coincidence with the said traditional drug form called *Arishta*. Similarly, C=O stretching carbonyl group is not detected in the raw leaf. So according to the present study there is no vast difference in the active compounds when the leaf is used as raw one and the form of aqueous extract.

Secondly the comparison is in between a single raw plant and combined form of ethanolic extract of two plants collected in India but at different locations. Data of samples given in Col.3. of Table 1. are collected from Trichirapalli and the Col.4. of Table 1. are collected from Pondicherry. Shanmugapriya and Maneemegalai [25] carried out the experiment (FTIR analysis) by combining the ethanol extract of *Tridax procumbens* and *Boerhavia diffusa* in the ratio of 1:1. Comparing our results obtained from the raw plant, it is detected that O-H stretching alcohol group is present in the ethanol extract and Alkyl halide group is absent. All other groups are present in both the samples. From this result it is obvious that the ethanolic extract and raw leaf of the plant does not make much difference and importantly the mixing of *Tridax procumbens* with *Boerhavia diffusa* did not affect the presence of bioactive groups in the leaves of *Boerhavia diffusa*.

Fig.1. The photograph of *Boerhaavia diffusa* (L.) (for identification).

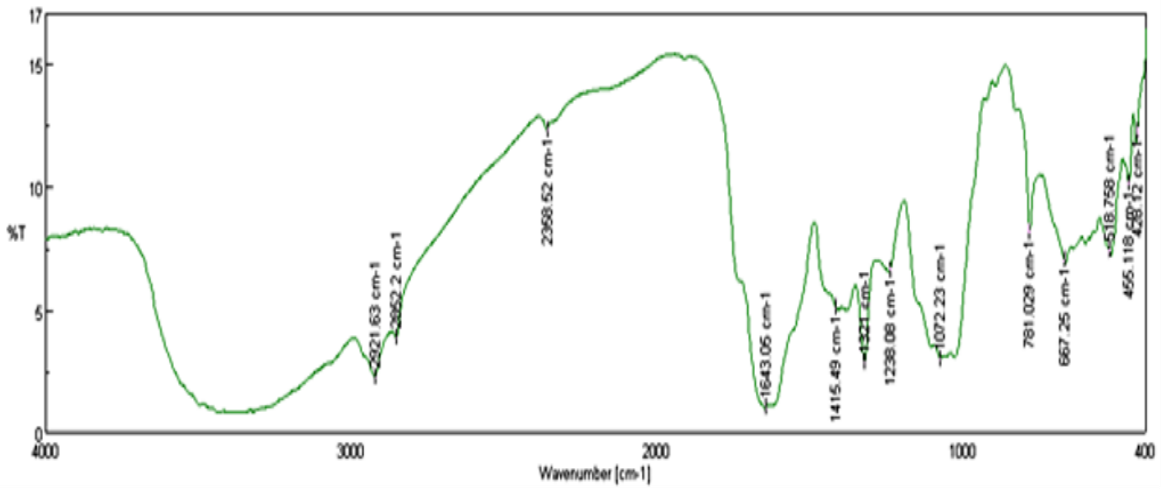

Fig.2. The FTIR spectrum of *Boerhavia diffusa* (L.) (leaf)

Table. 1. The active functional groups obtained at the respective absorption frequencies of *B. diffusa* L. and identification of common functional groups in comparison with Aqueous Extract [24] and (Ethanol extract of combined *B. diffusa* and *Tridax procumbens*) [25].

Sl.No.	Absorption (cm^{-1})			Compound Class
	Aqueous Extract[a)]	Ethanol extract of BD+TP combined[b)]	Raw plant leaf[c)]	
1.	3439.10	3363.97	--	alcohol
2.	2924.18	2975.61	2921.63	Alkane
3.	2854.74	2542.73	2852.20	Alkane
4.	2362.88	2256.98	2358.50	Carbon dioxide
5.	1701.21	--	--	carbonyl
6.	1608.69	1657.18	1643.05	Alkene
7.	1456.30	1407.38	1415.49	sulfate
8.	1377.22	1333.57	1321.00	phenol
9.	--	1230.63	1238.08	Alkyl aryl ether
10.	1031.95	--	1072.23	Alkyl halide
11.	871.85	881.54	781.029	1,3 di-substituted
12.	746.48	--	--	Mono substituted
13.	--	666.72	667.25	Halo compounds

[a)] (Akintelu et al. 2019)
[b)] (Shanmugapriya and Maneemegalai 2017).
[c)] Current study

MEDICINAL USES OF BIOACTIVE COMPOUNDS

The Pharmacological interest of the bioactive compounds present in the raw leaf of the plant is discussed below.

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 [37]. 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 [38]. 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 [39]. 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 [40]. 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.

Sulfate as a chemical has potential application in the field of medicine in many forms, such as, Aluminum sulfate - used to prevent infections and to treat minor bleeding, Barium sulfate - A contrast agent used for CT scans of the gastrointestinal tract, Copper sulfate - used as an intravenous copper supplement for Total Parenteral Nutrition (TPN), Magnesium sulfate - used to treat convulsions during pregnancy, nephritis in children, magnesium deficiency, and tetany, and which causes direct inhibition of action potentials in myometrial muscle cells (Drug Evaluations Annual, by American Medical Association,1992, p1083).

Zinc sulfate - used to replenish low levels of zinc or prevent zinc deficiency and is also used as a topical astringent. Ferrous sulfate is a synthetic agent used in the treatment of iron deficiency causing anemia, which is a large public health concern worldwide, especially in young children, infants, and women of childbearing age [41,42]. Sodium sulfate anhydrous

is an electrolyte replenisher and is used in isosmotic solutions so that administration does not disturb normal electrolyte balance and does not lead to absorption or excretion of water and ions [43]. Potassium and Sodium are the major cations inside and outside the animal cells respectively. The difference in concentration of these charged particles causes a difference in electric potential between the inside and outside of cells, known as the membrane potential. The balance between potassium and sodium is maintained by ion pumps in the cell membrane which allows the cells to generate an action potential - a "spike" of electrical discharge and which is the critical cause for the body functions, such as, neurotransmission, muscle contraction, and heart function. Potassium is also an essential mineral needed to regulate water balance, blood pressure and levels of acidity [44].

Phenol is an antiseptic and disinfectant, active against a wide range of micro-organisms including some fungi and viruses, but is only slowly effective against spores. It has been used to disinfect skin, to relieve itching, to phenolization and is used as an oral analgesic or anesthetic in products such as Chloraseptic to treat pharyngitis. Research indicates that parental exposure to phenol and its related compounds are positively associated with spontaneous abortion [45]. During the second world war, phenol injections were used as a means of execution by the Nazis. Phenol is a toxic compound whose vapours are corrosive to the skin, eyes, and respiratory tract. Phenol is primarily indicated for minor sore throat pain, sore mouth, minor mouth irritation, and pain associated with canker sores. Additionally, phenol is indicated in the treatment of focal spasticity [46].

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. [47] 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 [48]. 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 [49].

The in vitro antiproliferative activity of the synthesized 1,3-disubstituted urea derivatives was studied by Li et al. [50] 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 [51,52] reported that 1, 3 - disubstituted urea derivatives are having antiglycating potential.

CONCLUSION

The FTIR studies of the well-studied medicinal plant *B. diffusa* show that the leaf contains the bioactive compounds like Alkane, CO_2, Alkene, Sulfate, phenol, Alkyl aryl ether and 1,3 di-substituted, etc. The presence of such bio-active compounds with the aqueous extract and the ethanol extract of the studied plant are compared with our raw plant leaf spectrum and found that there is not much difference in the form of extract to the raw plant and also the combined effect of ethanolic extract of *Tridax procumbens* with *Boerhavia diffusa* which does not alter the active group of *Boerhavia diffusa.* Hence in the traditional practice, the use of *B. diffusa* in any form will provide the complete effect of the plant leaf. But in the modern pharmaceutical interest, the various functional groups are to be analysed separately and hence the medicinal use of detected active compounds are presented. Hence this study may pave a way to the pharmaceutical industries in preparing various drugs from the single use of *B. diffusa* plant alone. However, extra care should be taken for minimizing/eliminating the adverse health effects of intake of fresh *B. diffusa* leaves.

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