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A FT-IR SPECTROSCOPIC STUDY ON HEARTWOOD OF HOLOPTELEA INTEGRIFOLIA PLANCH.

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ABSTRACT

Holoptelea integrifolia Planch of Ulmaceae family is an important tree having medicinal properties, commonly known as Chirabilva in India. It is a large, spreading, glabrous, deciduous tree having foul smell in leaves and bark. Heartwood is light yellow, lustrous, interlocked-grained, even-textured, moderately heavy and strong which contains biomolecules like tannins, flavonoids, pigments and phenolic compounds. Chirabilva is described in Lekhaniya and Bhedaniya Mahakashaya of Charaka Samhita indicating its laxative and purgative action. It has anti-inflammatory, anti-bacterial, anti-oxidant, wound healing and hypolipidemic activities. It is useful in inflammations, colic, intestinal worms, vomiting, wounds, skin diseases, filariasis, obesity and diabetes mellitus. Fourier Transform Infrared Spectroscopy (FT-IR) is an absorption spectrum commonly used to detect various functional groups of the sample having specific vibrational frequencies which are like molecular fingerprints. FT-IR analysis revealed absorption bands at 3270 cm^{-1} may be present due to stretching mode of O-H of water / alcohol/flavonoids or tannins. The absorption band at 1646 cm^{-1} may be due to C=O stretching indicating the presence of carbonyl compounds (aldehyde, ketones, carboxylic acid and esters). The strongest band at 1042 cm^{-1} is due to CO-O-CO stretching suggesting the presence of compounds having acid anhydride as functional group. A weak absorption peak appearing at 631 cm^{-1} is due to C-Br stretching showing the presence of halo compounds. Tannins and flavonoids may also be present in the heartwood of *Holoptelea integrifolia* Planch.

KEYWORDS: *Holoptelea integrifolia* Planch; Chirabilva; Heartwood; FT-IR Analysis; Tannins; flavonoids.

I-INTRODUCTION

Holoptelea integrifolia Planch is an important tree having medicinal properties. It is commonly known as Chirabilva or Chilbil in India. Different parts of Chirabilva are used since ages to alleviate many diseases as described in the traditional system of medicine, Ayurveda, popular in Indian subcontinent.[1] Synonyms are Putika, Putikakaranja,Prakirya etc.[2] It'strade name is Indian Elm or Kanju.[3] It has anti-inflammatory, anti-bacterial, anti-oxidant, wound healing and hypolipidemic activities.[4] It is useful in inflammations, colic, intestinal worms, vomiting, wounds, skin diseases, filariasis, obesity and diabetes mellitus.

Taxonomical classification- Based on Bentham and Hooker system of Classification.[5]-

Phylum- Phanerogams
Class- Dicotyleydons
Subclass- Polypetalae
Series- Calyciflorae
Order- Rosales
Family- Ulmaceae
Genus- *Holoptelea*
Species- *integrifolia*

Fig.1 *Holoptelea integrifolia* Planch (Chirabilva tree).

Botanical Description

It is a large, spreading, glabrous, deciduous tree as illustrated in Fig.1. Bark 6-8 mm thick, grey, pustular, exfoliating in somewhat corky scales. Leaves elliptic-ovate, acuminate, base rounded or sub-cordate. Flowers greenish-yellow, polygamous in short racemes or fascicles on the leafless branches. Fruit sub-orbicular samara with two membranous wings. Seed one,

flat. Unpleasant odour appears on cutting the bark and crushing the leaves. The wood is light yellow, lustrous, interlocked-grained, medium and even-textured, moderately heavy and strong. Flowering occurs in February-march and fruiting march onwards.[6]

Heartwood- It is the central tough part of stem found in large old trees.The heartwood (also called *duramen*) is composed of dead cells with their walls heavily impregnated with various compounds such as resins, gums, tannins, pigments or phenolic compounds and hence become unsuitable for conduction but medicinally useful. As the growth process continues the rings of sapwood (also called *alburnum*) bordering the heartwood keeps on converting into heartwood. Distinction between sapwood and heartwood is not sharp as in case of *Holoptelea integrifolia* Planch which is the part under study.[7]

Distribution- The plant species originated from Pacific Island. Throughout the greater part of India up to an altitude of 660 m and also on the roadside, in lower ranges of Himalaya from Jammu to Awadh, Ruhelkhand, forests of Dehradun, Saharanpur, Orissa, Chota Nagpur, Bihar, West Bengal, hills of Deccan, eastern slopes of Western Ghats and North Circars. Also found in tropical and subtropical region of Asia and Africa.

Part Used- Bark, leaf, seed, heartwood.

Chemical constituents- Two triterpenoid fatty acid esters Holoptelin-A and B, 2-amino naphthaquinone, fiedelin, epifriedelinol, β-sitosterol and its β-D-glucose (stem bark); β-sitosterol-2α, 3α-dihydroxyelan-12-en-28-oic acid and hederagenin (heartwood); hexacosanol, octacosanol, β-sitosterol and β-amyrin (leaves); carbohydrates, pigments, oils, acids, glycosides, sterols, tannins, proteins, free amino acids, major fatty acids- palmitic acid, oleic acid, myristic, stearic, linoleic and linolenic acids; and steroids-β-sitosterol and stigmasterol (dried seeds); histamine and 5- hydroxtryptamine (pollens).

Action and uses- The bark and leaves are bitter, astringent, acrid, thermiogenic, anti-inflammatory, digestive, carminative, laxative, anti-helminthic, depurative, revulsive and urinary astringent. They are useful in inflammations, acid gastritis, dyspepsia, flatulence, colic, intestinal worms, vomiting, wounds, skin diseases, vitiligo, leprosy, filariasis, obesity, diabetes mellitus, hemorrhoids and rheumatism. Seeds are useful in infected ulcers and as a deodorant for foul smell of body.[8] Chirabilva is described in Lekhaniya and Bhedaniya

Mahakashaya of Charaka Samhita indicating its laxative and purgative action.[9] It is also described in Salasaradi Gana of Sushruta which contains woody plants.[10]

Hypolipidemic activity- The methanolic extract of leaf and bark of H. integrifolia exert hypolipidemic effect by markedly lowering body weight, serum lipid, HMGR activity and apo-B as well as increasing high-density-lipoprotein-cholesterol and apo-A1 concentration. The fecal analysis indicates the ability of the extract to prevent intestinal fat absorption due to presence of 3-(7ethoxy-4-methyl-2-oxo-2H-chromen-3-yl) compound.[11] Bark contains tannin, lignin, cellulose and hemicellulose having hypolipidemic activity.[12]

Ayurvedic Properties

Rasa - Tikta, Kashaya

Guna - Laghu, Ruksha

Virya - Usna

Vipaka - Katu

Doshakarma - Kaphapittashamaka

Rogaghnata - Kaphapaittikavikara, Shotha, Agnimandya, Gulma, Arsha, Krimi, Raktavikara, Prameha, Kustha, Medoroga.

Karma - Shothahara, Dipana, Anulomana, Pittasaraka, Bhedana, Krimighna, Raktashodhaka, Pramehaghna, Kusthaghna, Lekhana.

Dosage - Decoction - 50 to 100 ml, Bark powder -1-3 g.[13]

Need of Characterization

The importance of quality control and standardization of botanical products is of utmost concern for global acceptability of these drugs. Quality control is a major issue to ensure safety and efficacy of herbal drugs. Adulterants and substitutes are often used in herbal products which are harmful. Various methods are used for quantitative characterization based on specific compounds present in them which incorporates phyto-chemistry, chromatography and spectroscopy.[14] Spectroscopy is the study of interaction between matter and electro-magnetic radiation. It may be an emission or absorption spectrum which is used to detect various compounds as each one has unique spectrum. Infra-red (IR) Spectroscopy cause changes in vibrational and rotational movement of the molecules. Fourier Transform Infrared Spectroscopy (FT-IR) is an absorption spectrum commonly used to detect various functional groups of the sample having specific vibrational frequencies.[15,16]

Fourier Transform Infrared Spectroscopy (FT-IR)

FT-IR is one of the most widely used methods to identify the chemical constituents and molecular bonds present in the materials. In IR spectroscopy, IR radiation is passed through a sample. Some of the IR radiation is absorbed while some of it gets transmitted. The resulting spectrum represents the molecular absorption and transmission, creating a molecular fingerprint of the sample with absorption peaks which corresponds to the frequency of vibrations between the bonds of atoms. No two unique molecular structures produce the same IR spectrum like a fingerprint. This makes IR spectroscopy useful for identification of unknown material. It can determine the quality or consistency of a sample and also the amount of components in a mixture.

FT-IR is advancement over the older dispersive techniques. It is simple, non-destructive with greater speed, sensitivity and optical throughput which require no external calibration. It measures all the IR frequencies simultaneously quickly with the help of interferometer.[17]

II-MATERIALS AND METHODS

Collection and Identification of Plant Material

Lower portion of trunk of old trees of *Holoptelea integrifolia* Planch was collected from the forest of Mirzapur near Varanasi, U.P. (India) in January, 2016. Heartwood was obtained after removing most of the bark. Winter season is ideal for collection of Sara (Heartwood).[18] Botanical identification of the heartwood was confirmed by Prof. A. K. Singh, Ex-HOD, Dept. of Dravyaguna, Faculty of Ayurveda, IMS, BHU, Varanasi. Voucher specimen of the collected raw drug is deposited in the crude drug museum of the department of Dravyaguna, Faculty of Ayurveda, IMS, BHU, with specimen accession no. DG/17-18/140.

Sample Preparation: After removing the bark, heartwood was washed thoroughly under running tap water to remove soil particles and other impurities. It was, then, kept in the sun for 2-3 days until its complete internal drying. Now, this heartwood was comminuted mechanically using bladesto obtain the coarse powder of the size of Barleycorn (Yavakuta Churna). The size of Barleycorn is 0.85×0.24 cm approximately. The size of Barleycorn was decided as per instructions given in Ayurvedic Formulary of India-

Length of Barleycorn- 3 Barleycorn = 1inch = 2.54 cm

1 Barleycorn = 1/3 inch = 0.85 cm[19]

Breadth of Barleycorn- 8 Yavodara = 1 Angula = 3/4 inch = 1.95 cm

1 Yavodara = 1/8 Angula = 1/8× 3/4 inch = 0.24 cm.[20]

The air dried coarse powder was subjected to continuous heat extraction using Soxhlet's apparatus. 100 g of coarse powder of heartwood was extracted with 450 ml of Distilled water and Ethanol (50:50) each, by running the apparatus for 72 hours till the disappearance of color of coarse powder. The hydro-alcoholicextract was, then, filtered and concentrated to get the dry residue and stored in the desiccator at 55^0C. This extract was then subjected for FT-IR studies. Fig. 2(a-d) shows samples of Heartwood, Coarse powder, Soxhlet's apparatus being run and dried hydro-alcoholic extract.

Fig. 2 (In clockwise direction) shows samples of Heartwood, Coarse powder, Soxhlet's apparatus being run and dried hydro-alcoholic extract.

III Sample characterization

For sample characterization, we used Fourier Transform Infrared (FT–IR) spectrometer (Bruker: Alpha Eco ATR). FT-IR in mid infrared region 4000-400cm^{-1} was used to discriminate and to identify various functional groups present in heartwood of *Holoptelea integrifolia* Planch.The dried extract was diluted by KBr (materials: KBr = 1:100) to form pellets for scanning through IR radiation at room temperature (25± 2˚C). To improve the signal to noise ratio of the spectrum, 100 interferograms with a spectral resolution of ± 4cm–1 was averaged. Background spectrum collected under identical conditions was subtracted

from the sample spectrum.[21] Therefore, in the present study, it is possible to directly relate the intensities of the absorption bands to the concentration of the corresponding functional groups.

IV OBSERVATION AND RESULTS

Fig.3 exhibit the FT-IR spectrum of sample of hydro-alcoholic extract of *Holoptelea integrifolia* Planch prepared as already discussed in sample preparation section of this paper. The results obtained in FT–IR spectrum indicate the presence of various characteristic functional groups of different phyto-constituents present in *Holoptelea integrifolia* Planch. The strongest absorption peak appears at 1042cm^{-1}. A weak absorption peak appears at 631cm^{-1}. Other three absorption bands appear at 3270, 2337, 1646cm^{-1}respectively. IR-active absorption bands of *Holoptelea integrifolia* Planch are enlisted along with their assignments in Table. 1.

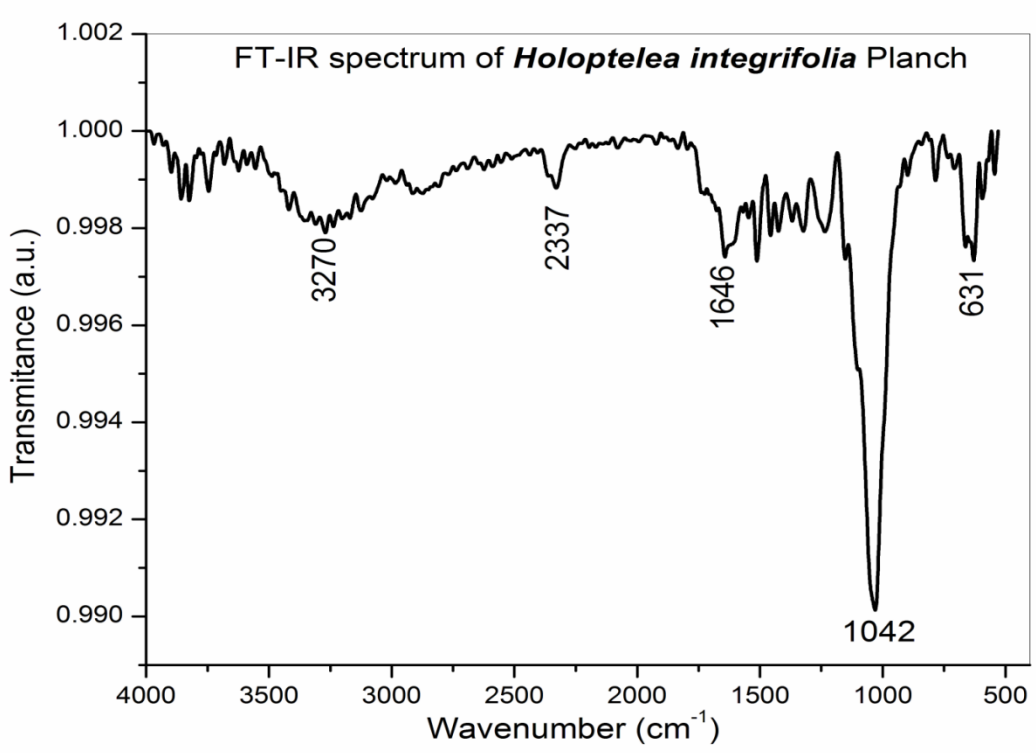

Fig. 3: FT-IR spectrum of Heartwood of *Holoptelea integrifolia* Planch.

Table 1: Showing active absorption bands of Heartwood of *Holoptelea integrifolia* Planch. with their assignments.

Peak positions in wave number (cm^{-1})	Assignments
3270	Stretching mode of O-H (Water/Alcohol/ Flavonoids/ Tannins etc.)
2337	Still remained unassigned
1646	C=O stretching
1042	CO-O-CO stretching
631	C-Br stretching (Halo compound)

DISCUSSION

Absorption bands at 3270cm^{-1} may be present due to stretching mode of O-H of water / alcohol/flavonoids or tannins. However, absorption peak at 2337cm^{-1} is still not assigned. The absorption band at 1646cm^{-1} may be due to C=O stretching indicating the presence of carbonyl compounds. This may be due to the presence of aldehyde, ketones, carboxylic acid and/or esters. The strongest band at 1042cm^{-1} is due to CO-O-CO stretching suggesting the presence of compounds having acid anhydride as functional group. A weak absorption peak appearing at 631cm^{-1} is due to C-Br stretching showing the presence of halo compound.[22] Tannins were used to convert animal hides into leather by the process called tanning, mostly glycosides in nature.[23] The tannins may be used for preventing obesity and reducing the degree of obesity by virtue of the lipase inhibiting properties preventing digestion and absorption of oils and fat.[24] Flavonoids are included under polyphenols. These compounds usually occur as glycosides in which one or more of the phenolic hydroxyl group are combined with sugar residue.[25] Flavonoids or bioflavonoids are named from the Latin word *flavus*, meaning yellow and are ubiquitous in plants; these compounds are the most abundant polyphenolic compounds in human diet.[26] A number of studies have demonstrated the potential health benefits of natural flavonoids against obesity.[27]

CONCLUSION

A careful examination of IR spectral profile of hydro-alcoholic extract of heartwood of *Holoptelea integrifolia* Planch reveals the presence of various functional groups as hydroxyl group, aldehyde, ketones, carboxylic acid, esters, acid anhydride and halogens. So, it indicates the probable presence of several compounds viz. tannins, flavonoids, glycosides, alcohols, phenols, halo compounds etc. Fourier Transform Infrared Spectrophotometry (FT–IR) was found highly advantageous to detect several compounds of medicinal value in the heartwood of *Holoptelea integrifolia* Planch.

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Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this research article.

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