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A FT-IR SPECTROSCOPIC STUDY ON HYDROALCOHALIC EXTRACT OF AMALAKYADI-GANA (COMPOUND FORMULATION)

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ABSTRACT

Fourier transform infrared spectroscopy (FT–IR) technique became applied to recognize the composition, chemical shape and discrimination of bio-molecules in hydroalcohalic extract of Amalakyadi – Gana (compound formulation). 50% hydroalcohalic extract of Amalakyadi – Gana have been scanned in mid infrared vicinity (4000–400cm–1) for IR spectroscopic studies. IR spectra for that reason acquired to discriminating and identifying various functional groups. FT-IR study indicates the presence of flavonoids, tannins, methyl, polysaccharides, and amide groups. Flavonoids have anti inflammatory and antioxidant activity; tannins have anticancer, anti-diarrhoeal activities and improve intestinal disorder. Presence of methyl groups have activity on DNA and switch off to the cancer causing oncogenes. Polysaccharides have immune modulating activity, anticoagulant, hypoglycaemic and antiviral activity. The effects of present study proven the conventional claims made by way of Ayurvedic practitioner. However, the chemical parts accountable for the pharmacological activities stay to be investigated.

KEYWARDS: Amalakyadi –Gana, FT–IR, Flavonoids, Tannins.

INTRODUCTION

There are good number of single and compound dosage forms are prescribed for different kinds of Jvara in original text of Ayurveda but there is one compound formulation found in Sushruta Samhita 38th chapter of Sutra Sthana, which is said as Sarvajvarahara (to alleviate all kind of fever). It is also said Cakshushya (Beneficial to eye), Dipana (enhances the Agni), Vrishya (Aphrodisiac) and Kapharocakan (Eversion of food due to Kapha). In this chapter the

Gana (group) is named as Amlakyadi Gana, which consists of Amalaki, Haritaki, Pippali and Citraka.[1] All the four drugs of this group are vegetable in origin. The fruit of Amalaki, Haritaki and Pippali and the root of Citraka are the ingredients of this Gana. Haritaki (*Terminalia Chebula* Retz.), Amalaki (*Emblica Officinalis* Gaertn.), Pippali (*Piper Longum* Linn), Citraka (*Plumbagozeylanica* Linn.) is well known plant in Ayurveda.

The principle of IR spectroscopy is the measurement of the amount of IR radiation, which is absorbed (or emitted) by a sample as a function of the wave length.[2] The IR measurement can be carried out in the modality of transmission or reflectance, with the primary being the fore most wide spread.IR spectroscopy features a high potential for the elucidation of molecular structures. The IR spectrum of poly-atomic molecules relies on molecular vibrations, every specificallydependent on atomic masses, bond strengths, and intra- or intermolecular interactions. As a consequence, the whole IR spectrum of an organic compound provides a special fingerprint, which can be readily distinguished from the IR absorption pattern of different compounds including isomers. In different words, once reference spectra are present, most compounds can be unambiguously identified on the premise of their IR spectra.The IR spectrumincorporates ample structural information. IR spectroscopy has beenthe classic analytical approach for organic compound structure. Currently, FTIR spectroscopyhas developed quickly due to its low noise, fast speed, excessive repeatability, smooth operation, low expense, and so on. Associated with different sciences similar to mathematics or computers, or with different techniques such as two-dimensional correlation analysis (2D-IR), FTIR hasemerge as increasingly more useful in the field of evaluating herbal qualities. The huge majority of molecules exhibit infrared bands within the mid-infrared regionbetween 4000 and 400 cm−1. The location and intensity of a vibrational band are characteristicof the underlying molecular motion and therefore of the atoms taking part insidethe chemical bond, their conformation, and their immediate environment. Thus, a certain sub molecular group produces bands in a characteristic spectral region. These characteristicbands form the empirical basis for the interpretation of vibrational spectra. Furthermore, characteristic absorption bands can be used for compound-specific detection. FTIR spectrometers have almost entirely replaced dispersive instruments because of their higher overall performance in nearly all respects. The application of this technique has improved the acquisition of IR spectra dramatically. Since early 1987 the finger print spectra (FPS) has been used in identification of herbal Medicines.[3] The components are extracted from the herbwith certain solvents of differentpolarities on the order of petroleum

ether (or cyclohexane), chloroform, ethanol, and water and their UV/FTIR finger print spectra are measured. Three methods are implemented for collecting IR FPS for quality control of herbal medicines.[4] Within first method, solvents of different polarities are used to extract components. After evaporating the solvent; the components are mixed with KBr powder and pressured into a pellet, and then the IR FPS of samples is collected. The samples enable us to distinguish different herbal medicines effectively. Within second method, powders of herbalmedicine are blended with KBr and pressured into a pellet after which the FT-IR spectra of the samples are collected. In the last method, the reflectance spectra are obtained directly from herbal materials. Although the last two methods are convenient, they provide lower resolution ability in identification of herbal medicines as compared with first method. FT-IR is a technique which is used to obtain an infrared spectrum of a solid, liquid or gas sample. FT-IR is in mid infrared region 4000-400 cm-1 and used to discriminate and identify various functional groups present in Hydroalcohalic extract of Amalakyadi Gana.

Fourier transform infrared spectroscopy (FTIR)[5] is a technique that is employed to get an infrared spectrum of a solid, liquid or gas sample. An FT-IR spectrometer simultaneously collects high spectral resolution data over a wide spectral range. This confers a big advantage over a dispersive spectrometer that measures intensity over a narrow range of wavelengths at a time. It can identify unknown materials, quality or consistency of a sample & the amount of components in a mixture.[6]

FT-IR is one amongst the foremost wide used methods to identify the chemical constituents and molecular bonds present within the material.

FT-IR is in mid infrared region 4000-400 cm-1 and used to discriminate and identify various functional groups present in Hydroalcohalic extract of Amalakyadi Gana.

MATERIAL AND METHOD

Plant material Haritaki (*Terminalia Chebula* Retz.), Amalaki (*Emblica Officinalis*gaertn.), Pippali (*Piper Longum* Linn), Citraka (*Plumbagozeylanic*a Linn.) has been identified by Prof. V.K. Joshi, Department of Dravyaguna, B.H.U. The fruit of Amalaki, Haritaki and Pippali became taken and root of Citraka was taken. The mature fruit of Amalaki and Haritaki was collected from the Ayurvedic Dravyaguna garden, B.H.U. Varanasi, Citraka root was collected from the Rajiva Gandhi south Campus Barkacha, Mirzapur. The fruit of Pippali was taken from the local crude drug market Goladinanath after ensuring that the drug is greater

than 1 year old. Sample of accrued drug were stored in the museum of the department of Dravyaguna faculty of Ayurveda IMS, BHU Varanasi as with Voucher specimen no- **DG/17/136, DG/17/137/, DG/17/138, DG/17/139.**

1. Haritaki.

2. Amalaki.

3. Pippali.

4. Citraka.

Fig. 1: Drug of Amalakyadigana.

Preparation of extract: The coarse powdered was extracted in a Soxhlet apparatus for 7 days. 100 g of coarsely powdered of each of air-dried material was accurately weighed and placed in a glass-stoppered conical flask. Powder was then macerated with 400 ml of the solvent (Water/ethanol) concerned for 6 hours, shaking frequently, and then was allowed to stand for 18 hours. It was then filtered rapidly taking care not to lose any solvent; 25 ml of this filtrate was transferred to a tarred flat-bottomed dish and was evaporated to dryness on a water-bath. It was followed by drying at 105°C for 6 hours, cooled in a desiccator for 30 minutes and was weighed without delay. The content of extractable matter in mg per g of air-dried material was then calculated.

Sample characterization: The sample became diluted with the aid of KBr (materials: KBr = 1:100) to make pellets for scanning through IR radiation after which scanning executed at room temperature (25± 2 ˚C). To enhance the signal to noise ratio for every spectrum, one hundred interferon grams with a spectral decision of ± 4cm–1 were averaged. Background spectra collected under identical conditions were subtracted from the sample spectra. Hence, in the present study it is possible to directly relate the intensities of the absorption bands to the concentration of the corresponding functional groups.

RESULTS

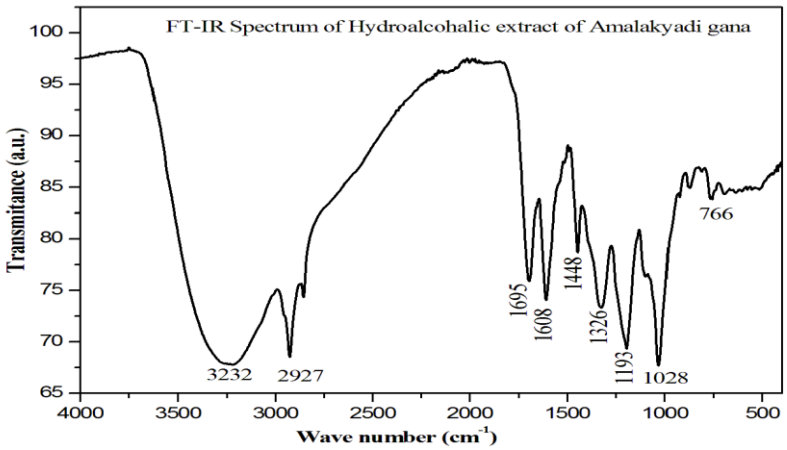

Fig. 2: exhibits the FT-IR spectrum of 50% Hydroalcohalic extract of Amalakyadi Gana.

Table. 1: FT-IR Spectrum of Hydroalcohalic extract of Amalakyadi Gana.

Peak positions in wavenumber (cm^{-1})	Assignments
3232	Stretching mode of O-H (flavonoids/ tannins etc.)
2927	ν (C-H) stretching mode of methyl and methylene
1695	Still remained unassigned
1608	C=O (amide I) vibration
1448	C-H bending mode of lignin / Carboxylic acid
1326	Still remained unassigned
1193	Absorption of polysaccharides
1028	ν(C-O) in cellulose and hemicelluloses
766	C-H out of plane bending vibrations

The FT–IR spectrum of Hydroalcohalic extract of Amalakyadigana a as shown in previous **Fig 2.** Exhibits the presence of various characteristic functional groups of different phyto constituents. IR active absorption bands of Amalakyadi Gana are enlisted along with their assignments in previous **Table1.**

The strongest absorption bands appeared at 3232 cm^{-1} may be because of O-H stretching mode of water/alcoholic/ phenolic compounds[7] (flavonoids and tannins). The absorption bands at 2927cm^{-1} is because of C-H Stretching mode of methyl and methylene. A weak peak appeared at 1695 cm^{-1} remains unassigned. A strong peak appeared at 1608 cm^{-1} is because of C=O (amide I) vibration. A strong peak appeared at 1448 cm^{-1} is due to C-H bending mode of lignin / Carboxylic acid. There is another weak peak appeared at 1326 cm^{-1}that is also unassigned. A strong absorption bands appeared at 1193 cm^{-1} may be due to absorption of polysaccharides. A strong peak appeared at 1028 cm^{-1} may be due to ν(C-O) in cellulose and

hemicelluloses. However, an absorption peak at 766 cm^{-1} may be assigned to C-H out of plane bending, C-Cl stretching, alcohols, O-H out of plane bending, disulfides (C-S) stretching, CH2-S- C-S stretch, Mn-O stretching.

DISCUSSION

Peak of Flavonoid is found at absorption band of 3402 and 3179 cm–1. Flavonoids known for its Antioxidant and Anti-inflammatory health benefits, as well as its contribution of vibrant colour to the foods. Therefore this favours much to the healing of wound because antioxidant activity of drug scavenges the free radical and promotes the healing. Equally Anti-inflammatory activity subsides the inflammation of wound and pacifies the all five signs of inflammation i.e. rubor (redness), tumour (swelling), callor (heat), dollar (pain), loss of sense. Therefore this seems very rationale for wound healing. They are regularly consumed in the human diet and have various biological activities including anti-inflammatory, anti-cancer, and anti-viral properties. The flavonoids maybe one of the safest non-immunogenic drugs because they are small organic compounds which have been normally absorbed by the human body for long time.

Phenolic compounds have already been reportable as potential free radical scavengers[8-10] and this property is extremely useful in treatment of wound. The tannins contained plants are found to have important activity in prevention of cancer-like horrific nosogenic disease. Tannins precipitate protein and have been used traditionally as styptics and internally for the protection of inflamed surfaces. They act as Anti-diarrhoeal and are used as antidotes in poisoning by heavy metals, alkaloids and glycosides.[11] Not solely this, these plants are also used potentially in treatment of intestinal disorders.[9-10,12-15] Bands at 2946 and 2850 cm-1 is because of C-H Stretching mode of methyl and methylene. Methyl groups are vital for normal cell replication at the level of the DNA. They literally turn genes “on” or “off.” When methyl groups are depleted, bad genes like cancer causing oncogenes are turned “on” and good genes, like cancer preventing tumor-suppressor genes, are turned “off.” If there are sufficient methyl groups, good genes, like tumor suppressor genes, are turned “on”, and bad genes, like oncogenes, are turned “off.” Methyl groups are also important for phase II liver detoxification, protein methylation, homocysteine metabolism (increasing the methyl groups decreases inflammation), neurotransmitter synthesis, and nucleic acid synthesis. So these properties facilitate the wound healing.

A strong band at 1597 cm-1 is recorded because of C=O (amide I) vibration. Amide bonds play a major role in the elaboration and composition of biological systems, representing for example the main chemical bonds that link amino acid building blocks together to give proteins. Proteins are building blocks; they make new healthy tissues and therefore facilitate in wound healing. Strong band at 1383 cm-1 is recorded is because of C-H bending mode of lignin / Carboxylic acid.

A Weak peak appears at 1119 cm-1 is because of absorption of polysaccharides. Polysaccharides have Immuno - modulating, Anti-tumour, Anti-inflammatory, Anticoagulant, hypoglycaemic and antiviral properties, which are supposed to enhance cell renewal, helpful in reducing blood sugar and so simultaneously treats diabetes and wound.[15] Some skin tightening may also be produced by surface deposition of certain film-forming actives, including proteins, polysaccharides, and polymers.[16-17]

CONCLUSION

In present study of FT-IR of 50% hydroalcohalic extract of Amalkyadi Gana we came to conclusion that there is presence of various groups viz. phenol, methyl groups, carboxylic acid, polysaccharides, amide which may present due to several biomolecules; tannins/flavonoids, amino acids, amides, lignin, and polysaccharides bearing these groups. Fourier transform infrared (FT–IR) spectroscopy has been found highly advantageous and simple to identify the IR active phyto constituents of Amalkyadi Gana. They lead to Anti – inflammatory and Anti-oxidant activity, tannins have Anti-cancer, Anti-diarrhoeal activity and improves intestinal disorder. Presence of methyl groups have activity on DNA and switch off to the cancer causing oncogenes. Polysaccharides have immuno - modulating activity, anticoagulant, hypoglycaemic and antiviral activity.

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