WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.990
Volume 4, Issue 8, 2754-2763. Research Article ISSN 2277– 7105

A FT-IR SPECTROSCOPIC STUDY OF PHYTOCONSTITUENTS OF *ASPARAGUS RACEMOSUS* WILLD ROOT TUBER

Anurag Mishra[1]*, Vijay Kumar Mishra[2], Divya Dwivedi[3], K. N. Dwivedi[1]

[1]Department of Dravyaguna, Faculty of Ayurveda, Institute of Medical Sciences, Banaras Hindu University, Varanasi–221005, India.

[2]Department of Physics, Centre of Advanced Studies, Faculty of Science, Banaras Hindu University, Varanasi–221005, India.

[3]Department of Obs. & Gynae. Guwahati Medical College & Hospital Guwahati–781032, India.

Article Received on
19 June 2015,

Revised on 10 July 2015,
Accepted on 01 Aug 2015

***Correspondence for Author**
Anurag Mishra
(Research Scholar)
Department of Dravyaguna, Faculty of Ayurveda, Institute of Medical Sciences, Banaras Hindu University, Varanasi–221005, India

ABSTRACT

Two samples of root tuber of *Asparagus racemosus* Willd. were prepared in two different forms; powder and liquid (juice). The Fourier Transform Infrared (FT–IR) technique was employed to understand the composition, chemical structure and discrimination of biomolecules in *Asparagus racemosus* Willd. root tuber samples. The prepared samples were scanned in mid infrared region (4000–400cm^{-1}) for IR spectroscopic studies. IR spectra thus obtained were analyzed to discriminating and identifying various functional groups present in both the forms of root tuber. The appearance of bending/ stretching modes of various functional groups *viz.* alkyl, methyl, phenol, alcohols, ethers and carboxylic acids revealed the presence of several biomolecules, amino acids, tannins, flavonoids, amides, lignin, cellulose, hemicelluloses and polysaccharides comprising these groups. These polysaccharides are well known to have antioxidant, antitumor and immunity modulator actions. These polysaccharides also resist the damages due to harmful radiations. FT–IR spectroscopy has been proved an effective and sensitive assay for the detection of biomolecules contained in two forms of root tuber samples. FT–IR spectra of *Asparagus racemosus* Willd. root tuber prepared in two forms powder and juice uncovered the superiority of powder over the juice due to presence of a couple of additional peaks of polysaccharides in spectrum of powder sample. This reveals that the IR active

phytoconstituents of Asparagus are more concentrated in powder form than the juice. However, it is not fully supported by classic Ayurveda.

KEYWORDS: Phytoconstituents; *Asparagus racemosus*; Dry Root; Fresh Root; FT–IR Analysis.

INTRODUCTION

Asparagus racemosus Willd. (Shatavari) is a well known Ayurvedic drug. It is a woody climber growing to 1–2 m length.[1, 2] It's leaves are reduced to minute chaffy scales, spines; cladodes. The root tuber of Shatavari, whose study is the matter of our interest looks finger–like and clustered succulent tuberous (30–100 cm long and 1–2 cm thick) appearing in silvery white/ash colored externally and white internally. However, its inflorescence has tiny white flowers in small spikes as reported by Hussain et al.[3] These roots are the vital part of plant which finds use in various medicinal preparations.[4–7] In Ayurveda, this amazing herb is also known as the "Queen of Herbs" due to its main action as Ayurvedic rejuvenative tonic. Shatavari is however, used for sexual debility and infertility in both sexes. Some extensive applications are in male genital dysfunctions, oligospermia, spermatogenic irregularities and other male disorders such as painful micturition. Shatavari is also used as brain tonic in epilepsy and vata disorders. Inspite of these applications, it also regulates cardiac disorders and hypertension. It is also used for menopausal symptoms and to increase lactation.[3, 7–9] It is also reported that Shatavari has found valuable applications in immunochemical industry to obtain more sustained and efficient immunostimulation activity and to improve immunogenicity.[10–11]

Asparagus racemosus Willd. belongs to family Liliaceae. It is an important medicinal plant regarded as a 'rasayana' (promoting general well being by increasing resistance and cellular vitality) of tropical and subtropical India.[3, 12] Some medicinal uses of Shatavari have been reported in the India and British pharmacopoeias and in some indigenous medicine systems. Around 300 numbers of species of genus *Asparagus* are found world-wide. However, only 22 species of *Asparagus* recorded in India. Due to the presence of steroidal saponins and sapogenins in various parts of this plant the genus is considered world-wide to be medicinally important.[13–14] Medicinal plants have been used as indispensable bioresources of drugs for traditional medicine system. Therefore, man has been using plant extracts to protect himself against several diseases and also to improve his health and life–style.[15] The plant extracts usually are occurring as a combination of various types of bioactive compounds

and/or phytochemicals having different polarities. Thus, their separation still remains a big challenge for the process of identification and characterization of bioactive compounds. Various techniques have been employed to determine and estimate the presence of such phytoconstituents in medicinal plants. Spectroscopic techniques are the most useful and popular techniques which have been used for identification and authentication of the phytoconstituents/natural drugs/plant materials/medicinal plants. It is well known fact that green medicines are always supposed to be safe with almost no or less side effect. Another merit with natural drugs is their easy availability. So, most of the natural drugs are of low cast as compared to other medicines. One of the very burdensome disadvantages with the herbal drugs is the victims of adulteration. The demand of a drug is directly dependent on its effectiveness; i.e. more effective drug is demanded more. In this situation, the chance of non-availability increases which invites some time the victim of adulteration in natural drugs with low grade material(s). In order to prevent the adulteration, the spectroscopic techniques have been found very effective and advantageous to trace the added/mixed foreign material(s) in natural material. Thus, on one hand, the present study is very fruitful in identification and authentication of the Asparagus plant materials/natural drug, on the other, this study can prevent the adulteration. The study may be also helpful in maintaining the quality, efficacy and reproducibility of Asparagus racemosus Willd. root tuber/natural drugs. The spectroscopic informations, which have been collected in the present study may provide as reference information for correct identification of Asparagus racemosus Willd. root tuber, which may also be useful in making a monograph of Asparagus racemosus Willd. plant. The Fourier transform infrared (FT–IR) is one of the most widely used spectroscopic tools to identify the chemical constituents and to elucidate the structure of compounds. It has also been employed world-wide as a requisite method to identify medicines in Pharmacopoeia[16]. This technique is a well established time–saving method to characterize and to identify the functional groups which are known to be reflecting the fingerprint of a material on the basis of vibrational energy of a particular (bending/stretching) mode of chemical bonds present in the compound/ material obtained synthetically or some natural sources.[15–18] The active constituents of plants are the major source for the development of new chemotherapeutic agents.In the present investigations, the *Asparagus* in powder and in juice forms are subjected to phytochemical screening by using FT-IR spectroscopic technique to identify phytoconstituents.

MATERIALS AND METHODS

2.1 Collection and Identification of Plant Material: Fresh root tuber was collected from the Ayurvedic garden of the Department of Dravyaguna, Faculty of Ayurveda, Institute of Medical Sciences, Banaras Hindu University (IMS-BHU) Varanasi-221005 (U. P.), India. Botanical identification of root tuber was confirmed by the help of Prof. N. K. Dubey, plant taxonomist, Department of Botany, Faculty of Science BHU, Varanasi. Voucher specimen is deposited in the herbarium of the Department of Dravyaguna, (IMS-BHU), Varanasi. Herbarium code number is DG/KND/1/2015.

2.2 Sample Preparation: Fresh plant roots were washed under running tap water to remove soil particles and adhered debris followed by sterile distilled water. The clean root tuber thus obtained was dried up under the shade. Fig. 1 (a–d) shows fresh root tuber, dry root tuber, juice (liquid) form of fresh root tuber and powder form of dry root tuber respectively. Two different routes of synthesis were employed to prepare two samples of root tuber in two different forms powder (solid) and juice (liquid) as described below.

Fig. 1 *Asparagus racemosus Willd.* roots are in form of (a) fresh root tuber, (b) dry root tuber, (c) juice of fresh root tuber and (d) powder form of dry root tuber.

2.2.1 Powder form of dried root tuber

The clean fresh root tuber of *Asparagus racemosus* was peeled off by sharp knife and then left in shade until its complete internal drying. These complete dried tuber thus obtained was crushed mechanically and gently by mortar pestle till the fine powder is obtained. The

powder of root tuber was filtered by mesh (size no. 80) to find more fine powder of homogeneous particles of roots. The powder sample thus obtained was stored in air tight plastic tubes at room temperature for further characterization.

2.2.2 Juice form of fresh root tuber: The upper layers of clean and neat fresh root tuber were peeled off while still fresh and cut in to small pieces. These pieces of root tuber were crushed with the help of mortar pestle to form paste like sample. The paste thus obtained was filtered under pressure to collect the juice. The obtained juice was stored in air tight plastic tubes at room temperature for characterization. This sample was prepared just before the characterization in order to avoid any bacterial growth as no preservative was added in these preparations. The Analytical R grade KBr (Potassium Bromide) of Sigma-Aldrich, Bangalore India was used without further purification for the experiment.

2.3 Sample characterization: Fourier transformed infrared (FT–IR) spectrometer (Perkin Elmer spectrum 65, FT–IR spectrometer; Perkin Elmer). The FT–IR spectra of samples were recorded in the range 4000–400 cm^{-1} to study the molecular structure of both the samples prepared in two different forms. The powder samples was diluted by KBr (materials: KBr = 1:100) to form pellets for scanning through IR radiation. While, in case of juice of fresh root tuber (liquid) sample, a drop of juice was poured on KBr pellet before scanning at room temperature (25± 2 ˚C). To improve the signal to noise ratio for each spectrum, 100 interferograms with a spectral resolution of ± 4cm^{-1} were averaged. Background spectra collected under identical conditions were subtracted from the sample spectra. 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.[17]

RESULTS

Figs. 2 and 3 exhibit the FT–IR spectra of two samples of *Asparagus racemosus* Willd. root tuber prepared in two forms powder and liquid (juice) via two different routes respectively as discussed already in sample preparation section of the manuscript. The results obtained in FT–IR spectra revealed the existence of various characteristic functional groups of different phytoconstituents contained in *Asparagus racemosus* Willd. root tuber. Absorption bands at 3402 and 3432 cm^{-1} are found to be appearing in FT-IR spectra of powder and juice samples respectively in Figs. 2 and 3. Two absorption bands at 2931 and 2882 cm^{-1} are recorded in IR of only powder sample. Strong absorption bands are noticed at 1632 and 1629 cm^{-1} in IR of powder and juice samples. A weak peak appears at 1402 and 1394 cm^{-1} in Figs.2 and 3

respectively. Fig.2 and Fig.3 also show a very weak peak at 1133 and 1108 cm^{-1} in powder and juice samples. An absorption band at 1031 cm^{-1} (strong) in powder sample and at 1048 cm^{-1} (weak) in juice sample is noticed. IR of powder sample contains two weak bands at 931 and 818 cm^{-1}. These bands are not present in IR spectrum of juice form of *Asparagus*. A broad and medium band centered around 2110 in IR of juice and at 2128 cm^{-1} in IR of powder is also observed. All the IR active absorption bands of *Asparagus racemosus* Willd. root tuber are enlisted in Table 1 along with their assignments.

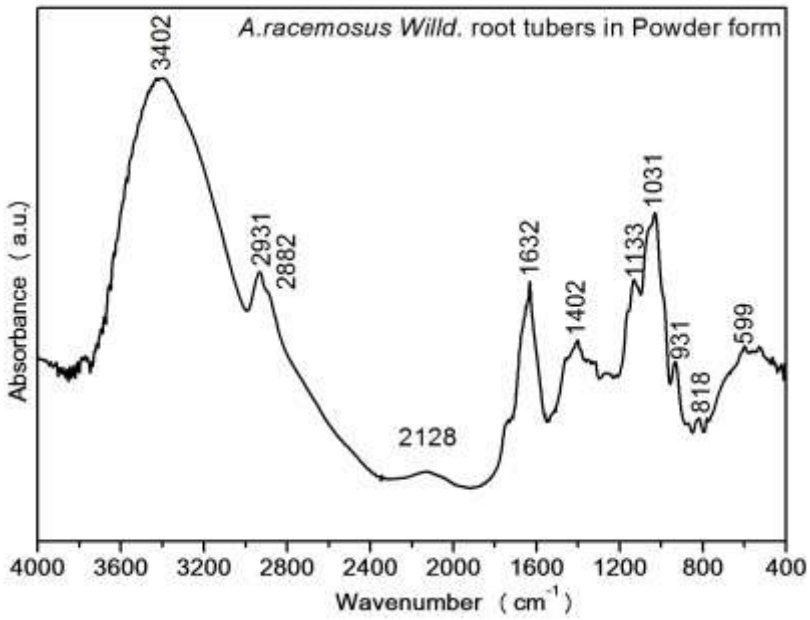

Fig. 2 exhibits the FT–IR spectrum of *Asparagus racemosus* Willd. root tuber Prepared in powder form.

Table 1: FT-IR active absorption bands of *Asparagus racemosus* Willd. Root tubers with their assignments

Peak positions in wavenumber (cm^{-1})		Assignments
Powder sample	Liquid sample	
3402	3432	stretching mode of O-H (H-bonded) of phenolic compounds (Tannins/ Flavonoids)
2931	-----	ν (C-H) stretching mode of methyl and methylene
2882	-----	ν (C-H) stretching mode of methyl and methylene
2128	2105	Still remained unassigned
1632	1630	C=O (amide I) vibration
1402	1394	C-H bending mode of lignin / Carboxylic acid
1133	1108	Absorption of polysaccharides
1031	1048	ν(C-O) in cellulose and hemicelluloses
931	-----	C-H out of plane bending vibrations
818	-----	C-H out of plane bending vibrations
599	590	Ring deformations for phenyl compounds

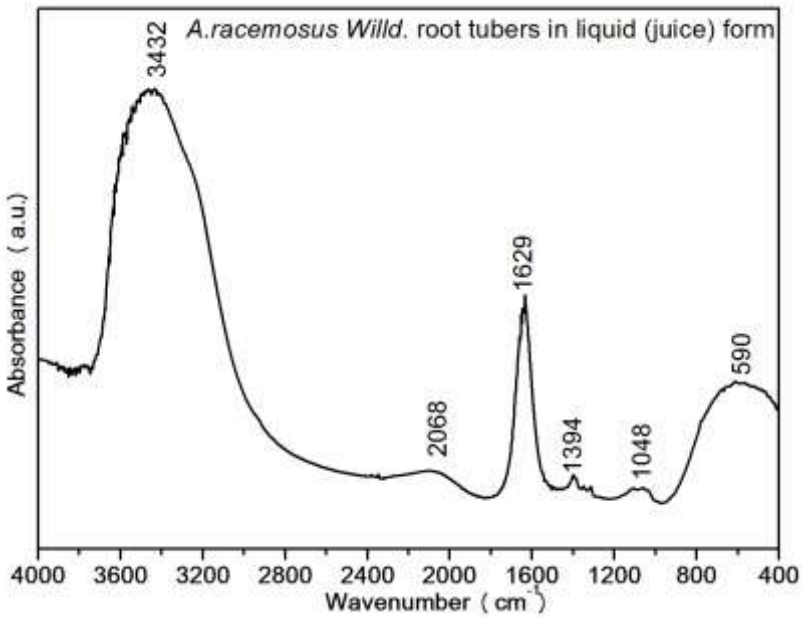

Fig. 3 exhibits the FT–IR spectrum of *Asparagus racemosus* Willd. root tuber Prepared in liquid (juice) form.

DISCUSSION

Strongest band noticed at 3402 and 3432 cm^{-1} in Figs. 2 and 3 (powder and juice) respectively may be due to O–H stretching mode of (H-bonded) phenolic compounds [18] *viz.* tannins, flavonoids, etc. This band reveals the presence of tannins and/or flavonoids. Peaks recorded at 2931 and 2882 cm^{-1} are assigned to ν(C–H) stretching mode of methyl and methylene. These peaks are present only in powder sample not in the sample prepared in liquid (juice) phase. Strong absorption peaks at 1630 and 1632 cm^{-1} in powder and liquid samples respectively, may be attributed to C=O (lipids) vibration. This same peak observed at 1632/1630 cm^{-1} may also be present due to ν_2 bending mode of H–O–H in H_2O. Peaks centered around 1402 and 1394 in Figs. 2 and 3 respectively are present due to C–H bending mode of lignin.[19] Absorption peaks recorded at 1133 and 1108 cm^{-1} may be attributed to polysaccharides. Polysaccharides are one of the well known functions on increasing antitumor, body immunity and resisting damages due to harmful radiations.[17–19] The peak appearing at 1031 cm^{-1} may be assigned to presence of carboxylic acid in powder sample.[17] This peak is strong in powder sample and very weak in liquid sample. This reveals that the powder sample shows more concentration of IR active phytoconstituents of Asparagus in powder form rather than juice. Carboxylic acid is found to play a vital role as a main pharmaceutical product in curing ulcer, hemicarnia, jaundice, stomatitis, headache, fever, wounds, liver pain, treatment of edema and rheumatic joint pains.[20] These also resist the

damages due to harmful radiations. This peak is strong in powder sample, however, it is very weak (almost disappearing) in juice sample. This shows that administration of *Asparagus racemosus Willd*. in powder form is more suitable and may be proved a better form of herbal medicine for oral administration route. A week absorption peak noticed at 1048 cm^{-1} in liquid sample is attributed to ν(C–O) which is a typical absorption of cellulose and hemicelluloses. This band is strong in powder sample and very weak in juice. Two weak peaks noticed at 931 and 818 cm^{-1} in IR spectrum of powder sample are attributed to C–H out of plane vibrations.[19] The broad bands centered around 599 and 590 cm^{-1} in powder and liquid samples respectively are due to ring deformations for phenyl compounds present in the samples. Broad but weak bands around 2128 and 2105 cm^{-1} in powder and liquid samples still remained unassigned. Above discussion show the superiority of powder sample over juice form which is not supported by classics.[14]

CONCLUSION

A careful examination IR spectral profiles of *Asparagus racemosus Willd*. Root tuber samples in form of powder and juice revealed the presence of various groups *viz.* phenol, alkyl group, methyl groups, alcohols, ethers, carboxylic acid which may present due to several biomolecules; tannins/flavonoids, amino acids, amides, lignin, cellulose, hemicelluloses and polysaccharides bearing these groups. Fourier transform infrared (FT–IR) spectroscopy has been found extremely advantageous and easy to identify the IR active phytoconstituents of Asparagus in powder and juice forms. Some additional peaks are noticed merely in powder sample not in juice of Asparagus racemosus Willd. root tuber and some peaks are comparatively strong in juice form of the sample which emphasize the superiority of powder form of Asparagus. However, classics say that the drugs should be used in wet (liquid extract) state. But in the dry (powder) form, the drugs show more concentration of IR active phytoconstituents rather than wet (juice) form which is not supported by Classical Ayurveda. Powder form of a drug is also more durable and convenient to store than liquid form.

ACKNOWLEDGEMENTS

Authors are cordially thankful to the In-charge, Biophysics Laboratory, (Department of Physics, Banaras Hindu University) for FT–IR measurements. Authors are also thankful to the Prof. N. K. Dubey (Department of Botany, Faculty of Science, Banaras Hindu University) for his help in plant identification.

CONFLICT OF INTERESTS

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

REFERENCE

1. Sharma PV, Dravyaguna Vijanana, reprint, Chaukambha Bharti Academy, Varanasi, Vol. (II) (Hindi): 2005, pp. 562.
2. Mahadevan V, Tijeri RD, Mythreyi R, Gurudeva MR, Yoganarasimhan SN. Pharmacognastical studies on the root tuber of Asparagus gonoclados Baker–Alternate source for the Ayurvedic drug Satavari. Indian Journal of natural products and resources, 2010; 1(1): 57–62.
3. Hussain A, Ahmad MP, Wahab S, Hussain MS, Ali M. A review on pharmacological and phytochemical profile of Asparagus racemosus Willd. Pahrmacologyonline, 2011; 3: 1353–1364.
4. Chopra RN, Chopra IC, Handa KL, Kapur LD. Indigenous drugs of India. Calcutta Academic Publishers: 1994, pp. 496.
5. Chawla A, Chawla P, Manglesh, Roy RC. Aspergas racemosus (Willd.): Biological activities and its principles. Indoglobal journal of pharmaceutical science, 2011; 2(1): 113–120.
6. Bopana N, Saxena S. Asparagus racemosus—Ethnopharmacological evaluation and conservation needs. Journal of Ethnopharmacology, 2007; 110: 1–15.
7. Dartsch PC. The potential of Asparagus–P to inactivate of reactive oxygen radicals. Phytother. Res. 2008; 22: 217–222.
8. Thakur RS, Puri HS, Hussain A. Major medicinal plants of India. Publication Cen. Inst. Med. Arom. Plant. Res: 1989, pp. 531.
9. Saxena G, Singh M, Bhatnagar M, Phytoestrogens of Aspergas racemosus (Willd.). Journal of Herbal medicine and Toxicology, 2010; 1(4): 15–20.
10. Gautam M, Diwanay S, Gairola S, Shinde Y, Patki P, Patwardhan B. Immunoadjuvant potential of Asparagus racemosus aqueous extract in experimental system. Journal of Ethnopharmacology, 2004; 91: 251–255.
11. Gautam M, Saha S, Bani S, Kaul A, Mishra S, Patil D, Satti N K, Suri KA, Gairola S, Suresh K, Jadhav S, Qazi GN, Patwardhan B. Immunomodulatory activity of Asparagus racemosus on systemic Th1/Th2 immunity: Implications for immunoadjuvant potential. Journal of Ethnopharmacology. 2009; 2(121): 241–247.

12. Goyal R, Singh J, Lal H. Asparagus racemosus– an update. Indian Journal of Medical Sciences, 2003; 57: 408–414.
13. Okteh–Rabah HA. Phytochemical constuents of genus Asparagus and their biological activities. Hamdard, 1998; (41): 33–43.
14. Raval PK, Nishteshwar K, Patel BR, Shukla VJ. Asparagus racemosus Willd. – A comperative phytochemical analysis of fresh dried roots of Shatavari. International Journal of Pharmaceutical and biological, Archives, 2012; 6(3):1458–1461.
15. Saxena M, Saxena J. Evalution of phytoconstuents of Acorus Calmus by FT–IR and UV–Visible Spectroscopic Analysis. Internatinal Journal of Biological and Pharmaceutical Reearch, 2012; 3(3): 498–501.
16. Grube M, Muter O, Strikauska S, Gavare M, Limana B. Application of Ft–IR Spectroscopy for controlle of the medium composition during the biodegradation of nitro aramatiic compounds. Journal of Indian Microbiology and biotechnology, 2008; 35: 1545–1549.
17. Cakmak G, Togan I, Severcan F. 17 β–esteroidal induced compositional structural and functional changes in rainbow trout liver, revealed by FT–IR spectroscopy: A comparative study with nonyphenol. Aquatic Toxicology, 2006; 77: 53–63.
18. Sumathy C, Vijay Kumar N. Anti-Inflamatory activity and FTIR analysis on the ethanolic extract of Musa sapientum on experimental peptic ulcers in rats.WJPR, 2015; 4(7): 710-721.
19. Prasad AGD, Kumar J K, Sharanappa P. Fourier transform infrared spectroscopic study of rare and endangered medicinal plants. Romanian journal of biophysics, 2011; 3(21): 221–230.
20. Ragavendran P, Sophia DC, Raj A, Gopalakrishnan VK. Group analysis of various extracts of Aerva lanta (L.,) by FT–IR spectrum. Pharmacologyonline, 2011; 1: 358–364.