Evaluation of phytoconstituents of three plants *Acorus calamus* linn. *Artemisia absinthium* Linn and *Bergenia himalaica* boriss by FTIR spectroscopic analysis

Nadeem Mohani*[1], Mansoor Ahmad[2], Mehjabeen[3] and Noor Jahan[4]
[1]Sarhad University of Science & Information Technology, Peshawar, Pakistan
[2]Research Institute of Pharmaceutical Sciences, Faculty of Pharmacy, University of Karachi, Karachi, Pakistan.
[3]Department of Pharmacology, Federal Urdu University of Arts, Science & Technology, Karachi, Pakistan
[4]Department of Pharmacology, Dow College of Pharmacy, Dow University of Health Sciences, Karachi, Pakistan

Abstract: Qualitative and quantitative analysis of plant extracts can be achieved by using different spectroscopic techniques. In current research work we deal with the nature of the absorption and spectra of extract of *Acorus calamus*, *Artemisia absinthium* and *Bergenia himalaica* using FTIR spectroscopic technique. The present study was focused on standardization of crude extracts by utilization of infrared light. The spectra of crude extracts (*A. calamus, A. absinthium* and *B. himalaica*) displayed very clear diagnostic peaks of functional groups i.e. O-H alcoholic/acid, C-H alkyl & aromatic ring, carbonyl, and C-O-C groups. The spectra of all the three plants did not show any peak at 2220-2260cm^{-1}, which is indicative of the absence of nitrogen containing groups. These results exhibited that these plants does not contain any toxic substances.

Keywords: *Acorus calamus*, *A. absinthium*, *B. himalaica*, FTIR spectrum.

INTRODUCTION

Medicinal plants are the richest resource of drugs for traditional system of medicine; therefore, human beings have been utilizing plant extracts to guard themselves against several diseases and also to maintain health. Medicinal plants contain several chemical constituents such as flavonoids, alkaloids, phenol and tannins, carboxylic acids, terpenes and amino acids and several other inorganic acids. These phytochemical constituents gave definite individuality and properties to plants (Parekh *et al*., 2007).

Consequently, the analysis of these chemical constituents would help in determining various biological behaviors of plants. A variety of techniques can be used to determine and estimate the presences of such phytochemical constituents in medicinal plants. Chromatography and spectroscopic techniques are the most practical and accepted tools used for this purpose. Analysis of a relevant amount of compositional and structural information in plants can be done by FTIR spectroscopy. It is an established time saving method to characterize and identify functional groups (Grube *et al*., 2008).

*Acorus calamus*is an important medicinal plant with wide range of biological activities and diverse chemical components. In Ayurvedic medicine, it is used for the treatment of skin eruptions, epilepsy, mental ailments, chronic diarrhea, dysentery, rheumatic pains, neuralgia, cancer, dyspepsia, bronchial catarrh and intermittent fevers (Sabitha *et al*., 2003). Crude methanolic plant extract is mainly used for different pharmacological potential like, anti-inflammatory and anticonvulsant (Jayaraman *et al*., 2010), anti diabetic (Lee *et al*., 2010), anti microbial and antifungal (Devi *et al*., 2009), anti-diarrhoeal (Gilani *et al*., 2006), antihepatotoxic and antioxidant activities (Palani *et al*., 2009). Anticancer, antimutagenic (Aqil *et al*., 2008), anti-oxidative, anti-inflammatory and neuroprotective activities were also reported (Arunachalam & Singh, 2011). With this knowledge, the present research work was aimed to produce the FTIR spectrum profile of *A. calamus* plant extract as identification tool. *Artemisia absinthium* is a yellow-flowering, perennial, aromatic, herbaceous plant (Aberham *et al*., 2010). Traditionally it is used as anthelmintic, antibacterial, antifungal, insects repellent, in diphtheria, epilepsy, as narcotic and in anaemia (Howes *et al.,* 2003). *Bergenia himalaica* mainly distributed in the temperate Himalayas ranging from Asia, involved in East Asia, the southeastern regions of Central Asia and northern regions of South Asia between high-altitude of 900 and 3000m (Siddqui *et al*., 2014). In Pakistan, this plant species is widely distributed in Muree Hills and Nathia Gali at an altitude of about 8000 feet (Hassan *et al*., 2005). Traditionally *B. himalaica* used as antiulcer, antihepatotoxic, anti-HIV, antiarrhythmic, neuro-preotective, antifungal, anti-inflammatory, immuno-modulatory and burn wound healing effects (Nazir *et al*., 2011).

**Corresponding author:* e-mail: nadeem.fls@suit.edu.pk

MATERIALS AND METHODS

Collection and preparation of plant material

Rhizomes of *A. calamus*, whole plant of *A. absinthium*, and roots of *B. himalaica* were collected from hilly areas of Muree and upper Dir during the months of June-August. Plant samples were washed carefully in running tap water to remove soil particles and adhered debris followed by sterile distilled water. The washed plants were blotted on the blotting paper and spread out at room temperature in shade dry for three week. The dry plant materials were chopped into small pieces then macerated with methanol for 15 days at room temperature for percolation. The methanol extract was then filtered. After filtration once again methanol was added in the remaining material and kept for 15 days at room temperature for further percolation. Later same procedure was repeated for other plants. The methanol extract was evaporated under reduced pressure at controlled temperature in a rotary evaporator to obtain the residues of all plants respectively. Three residues were combined and used for experiments.

Spectroscopic analysis

All spectra were obtained with the aid of an OMNI-sampler attenuated total reflectance (ATR) accessory on a Nicolet FTIR spectrophotometer (Thermo-Scientific Nicolet10, USA), which was used to detect the characteristic peaks and their functional groups. The peaks values of FTIR were recorded. Small amounts of crude extract of *A. calamus*, *A. absinthium* and *B. himalaica* were respectively placed directly on the germanium piece of the infrared spectrometer with constant pressure applied and data of infrared absorbance, collected over the wave number ranged from 4000cm^{-1} to 500 cm^{-1} and computerized for analysis by using the Omnic software (version 5.2).

RESULT

Functional groups identification

The FTIR spectrum was used to identify the functional groups of the active components present in plant extracts based on the peaks values in the region of IR radiation. When the plant extract was analyzed into the FTIR, the functional groups of the compounds were appeared on different wave's length. The results of analysis of crude extract *A. calamus, A. absinthium* and *B. himalaica* are given in tables 1-3 and figs. 1-3. Reported HPLC chromatograms of compounds of *A. calamus* and *A. absinthium*are given below (fig. 1a and 2a).

Reported chemical compounds in Acoruscalamus

Calamenone, α-pinene, Calamine, Calamol, Azulene, Isoeugenol and camphor 4. Palmitic and butyric acids, Asaronic acid, Eugenol, eugenolmethylether, Asarylic acid, calamine, Calamenol, calamenone, Heptylic acid Iso-calamendiol, pre-isocalamendiol, Aliphatic and oxygenated mono terpenes, *n*-heptanic acid, Dehydroabietic acid, acetic acid,linolenic acid, nonanoic acid, α-Ursolic acid, Furylethylketone, galagravin, retusin, Dehydro-diisoeugenol, sakuranin Elimicin, epidesminlysidine, Borneol, borynl acetate, Methyl-eugenol, cis-methyl eugenol, geranyl acetate, Shyobunone, isoshyobunone and epi-iso-shyobunone, Asoranaldehyde, acorenone, calamendiol, Z-3-[2-.4,5-trimethoxy phenyl]-2 propenal, Phenyl indane, Phenyl propane, carbonyls, phenols, aliphatic compounds, alkaloids, carbohydrates and resins, Calamusenone and its isomer, Asarone and its isomer, Acorgaermacrone, Elemene, caryophyllene, cadalene, calamenene, Acolamone and isoacolamone (Mythili *et al*., 2013).

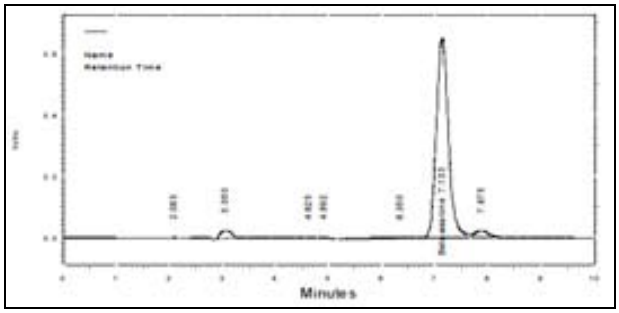

Fig 1a: HPLC of β-Asarone in Medicinal plant *Acorus calamus* (*Int. J. Pharm. Sci. Rev. Res*. 22(2), Sep-Oct 2013, No.15: 73-78)

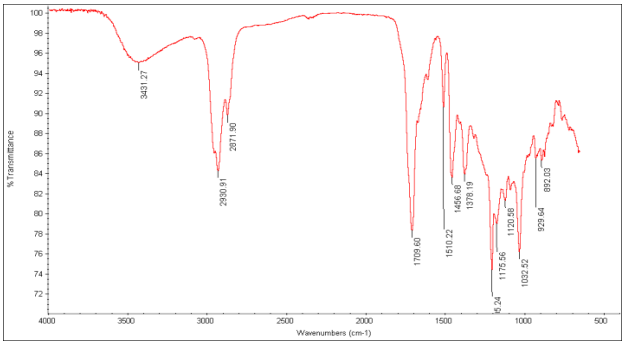

Fig. 1: FT-IR Spectra of *A. calamus.*

DISCUSSION

Spectroscopic technique has become a powerful and analytical tool for the qualitative and quantitative analysis of pharmaceuticals, biological materials and crude plant extracts. The previous researches show the main constituents of *A. calamus* are monoterpenes, sesquiterpenes, phenyl-pro-panoids, flavonoids and quinine. Acorenone was the major constituent in the rhizomes, whereas β-asarone was dominant in the leaves (Paphonngaml *et al.,* 2011). Besides monoterpene hydrocarbons, choline, flavone, acoradin, galangin, acolamone, andisocolamone were also identified (Singh *et al.,* 2011). The presence of OH group (3431.27cm^{-1}) in the IR spectra of *A. calamus* extract is characteristic for

glycosides and its derivatives whereas two values (2930.91 & 2871.90cm^{-1}) of C-H stretching indicated the occurrence of aromatic ring and alkyl group attachment. This value (1709.60cm^{-1}) indicates the presence of carbonyl group (C=O) and 1510.22 & 1456.68cm^{-1} confirm the presence of aromatic ring.1032.52 cm^{-1} value indicates the presence of ether linkage (C-O-C). Actually IR spectra of extracts reveal structural information about major and minor constituents. This information first can be used for identification (authentication) of crude extract and standardization too. The reported chemicals compounds also confirm our spectra authenticity (Mythili *et al*., 2013; Pino *et al.,* 1997; Zhang *et al.*, 2011: Gopalakrishnan *et al*., 2012). The reported HPLC chromatograms of compounds of *A. calamus* and *A. absinthium* also helped us in the determination of functional groups present in crude extracts.

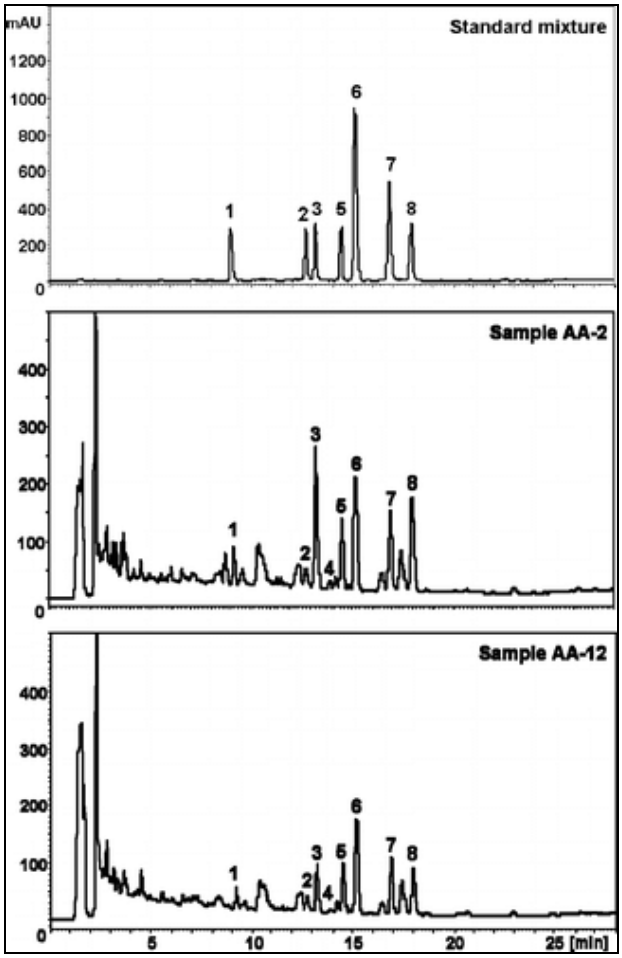

Fig. 2a: Separation of a standard mixture of compounds **1**–**8** and HPLC analysis of samples AA-2 and AA-12 extracted with methanol obtained under optimized HPLC conditions. Peak assignments: 1, anabsin; 2, ketopelenolide b; 3, absinthin; 4, 3′-hydroxyanabsinthin; 5, epiyangambin; 6, anabsinthin; 7, sesartemin; and 8, artemisetin.

The same results were also found for *A. absinthium* and *B. himalaica* but there are quite different signals patterns in diagnostic and finger print region of the spectra which shows the identity of each crude extract because of the occurrence of chemicals compounds having different natures.

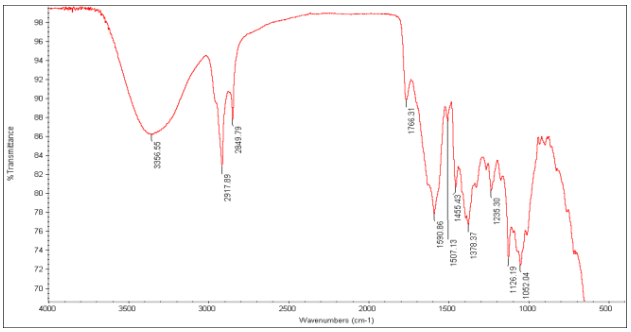

Fig. 2: FT–IR Spectra of *A. absinthium.*

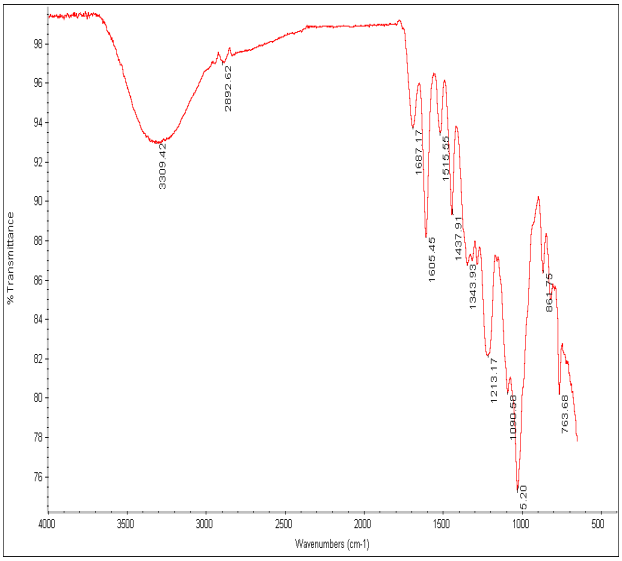

Fig. 3: FT-IR Spectra of *B. himalaica.*

Reported chemical composition of essential oil of Artemisia absinthium

Tricyclene, α-thujene, α-pinene, Camphene, Sabinene, *p*-Pinene, Myrcene, α-terpinene, *p*-Cymene, Cineole, (Z)-*p*-ocimene, (E)-*p*-ocirnene, □-terpinene,cis-sabinene hydrate,Eucatvone, α-p-dimethylstyrene, Terpinolene unidentified compound A, Linalool,*p*-thujone,Safranal (Pino, 1997).

Reported chemical composition of Bergina himalaica

Bergenin, arbutin, catechin, β-sitosterol, gallic acid, β-sitosterol-D-glucoside, tannins, (+)-afzelechin, leucoeyanidin, methylgallate, paashaanolactone, gallicacylated compounds, Kaempferol, Quer, diglucoside, mologlucoside, Aloe emodin, physicion, aloe emodin 8-O-glucoside, chrysophanein, emodin 1-O-β-D-glucopyranoside, Hydroquinone, hydro-quinonemonomethylethe, Others volatile oil,

Table 1: FT-IR peak values and functional groups of *A. calamus*

Characteristic Absorption (s) (cm^{-1})	Bond	Functional Group
3431.27	O–H stretch, H–bonded	Alcohols, phenols
2930.91	C–H stretch	Alkanes
2871.90	C–H stretch	Alkanes
1709.60	C=O stretching	α, β–unsaturated aldehydes, ketone
1510.22 1456.68	C=C	Aromatic ring
1378.19	C–H rock	Alkanes
1215.24	C–N stretch	Aliphatic amines
1175.56	C–N stretch	Aliphatic amines
1032.52	C–O-C	Ether linkage

Table 2: Peak values and functional groups of *A. absinthium* in the spectrum

Characteristic Absorption(s) (cm^{-1})	Bond	Functional Group
3356.55	N–H stretching	1^o, 2^o amines, amide
2917.89	C–H stretch	Alkanes
2849.79	C–H stretch	Alkanes
1766.31	C=O	Carboxylic acids
1590.86	C–C stretch (in-ring)	Aromatics
1507.13	N–O asymmetric stretch	Nitro compounds
1455.43	C–C stretch (in-ring)	Aromatics
1378.37	C–H rocking	Alkanes
1235.30	C–N stretch	Aliphatic amines
1126.19	C–N stretch	Aliphatic amines
1052.04	C-N stretch	Aliphatic amines

Table 3: Peak values and functional groups of *B. himalaica* in the spectrum.

Characteristic Absorption(s) (cm^{-1})	Bond	Functional Group
3356.55	O–H stretching	Alcoholic OH
2917.89	C-H	Alkyl group
2892.62	C–H stretch	Alkyl group
1766.31	C=O stretching	α, β-unsaturated aldehydes, ketone
1515.55 1437.91	C–C stretch (in-ring)	Aromatics
1343.93	N–O symmetric stretch	Nitro compounds
1213.17	C–N stretch	Aliphatic amines
1090.58	C–O-C stretch	Aliphatic amines
1005.20	=C–H bend	Alkenes

polysaccharide, amino acid, sterols, organic acid, carotenoids, daucosterol (Zhang, 2011).

CONCLUSION

The research articles on chemistry, clinical data and uses of these plants are in favour of safe use in medicine but in low dose. Our present research can be utilized for identification of crude extracts.

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