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Comparative analysis of n-hexane and ethyl acetate extracts of *Arisaema propinquum via* Fourier Transform Infrared Spectroscopy (FTIR)

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Abstract

The present study is the first time investigation of major functional groups *via* Fourier transform infrared spectrometer (FTIR) in n-hexane and Ethyl acetate leaf/stem extracts of an unexplored medicinal herb *Arisaema propinquum* of North-western Kashmir Himalayas. In ethyl acetate extract, the major absorption peaks were 3377 cm^{-1}(corresponding to **O-H** stretching),2918 cm^{-1} and 2847 (corresponding to **C-H** stretching in alkane),1703 cm^{-1}(corresponding to **C=O** stretching), thus reflecting presence of polyphenols.Similarly, in the n-hexane extracts, the prominent absorption peaks were 3649 cm^{-1}(corresponding to **N-H** stretching),3116 cm^{-1}(corresponding to **C-H** stretching in alkene),2914 cm^{-1},2847 cm^{-1}(corresponding to **C-H** stretching in alkane), reflecting presence of non-polar compounds like terpenoids and flavonoids. Dietary polyphenols and flavonoids are potent free radical suppressor's important life saving molecules against cardiovascular diseases, ageing, anti inflammatory signalling pathways and are thus leading in pharmacology and cosmetic industries.

Keywords: FT IR, Functional groups, secondary metabolites etc.

Introduction

Terpenes, flavonoids, alkaloids, glycosides are important secondary metabolites present in medicinal plants with phenomenal functions in physiology like defence, stress tolerance, microbial resistance, free radical neutralizing capacities and much more. Till date, more than 200,000 secondary molecules have been characterized from various plants but their concentration varies with taxa, altitude, biotic and abiotic stress conditions and still a huge number of important medicinal plants are unidentified for their pharmacological and medicinal properties (Yeshi et al., 2022). Although more than 8,000 different polyphenols have been discovered so far, the short- and long-term health impacts of these compounds are still poorly understood (Lecour et al., 2011). Numerous polyphenols have antioxidant and anti-inflammatory characteristics, according to reports in animals and epidemiology and they may act as a preventative measure or treatment for diseases including obesity, cancer, and neurological illnesses as well as cardiovascular diseases (Pérez-Jiménez et al., 2010 and Singh et al., 2011). Another important class is the carboxylic acid and their substitutes which holds wider applicability in pharmaceutical drugs, biopolymers, food additives and many more. Nowdays, the cosmetic industry is booming due to usage of α- hydroxyl acids (AHAs), derivatives of Vitamin A (retinols, retinoic acids) which belong to the class of synthesized organic acids. However naturally occurring glycolic acids, citrus acids, malic acid and lactic acid are commercialised at large scale in pharmacy and cosmetic industries. In order to identify and quantify the levels of various acids contained in medicinal plants, food and beverages, analytical methods must be continuously developed and used. In this context, we attempted to get the spectral analysis of the aerial plant extracts of unexplored medicinal plants using Fourier Transform Infrared Spectroscopic technique (FTIR), which is highly sophisticated, precise with advanced sensitivity authorizing for research applicability.

In this regards, *Arisaema propinquum*, an unexplored medicinal and ornamental herb that is known for its massive potential for anti-cancer, anti-microbial, anti-helminthic and anti-oxidative activity which have been chosen to carry out this study. The plant usually grows at elevations of 2400 to 3600 amsl with most prominence around Pir Panjal Range of North-western Himalayas and flowers from May to July. It is preferably found under shrub canopy around rocks and is exactly a resemblance to a cobra hood.

2. Methodology

Sample collection and authentication

The plant samples of *Arisaema propinquum* were collected from Pir Panjal mountain ranges of Yusmarg, Budgam district of 2294 masl with a point geographic location of 34^0 40 57.56369" N and $74^0$19' 33.8033" E. Plant specimens of *A. propinquum* bearing Voucher No.2681-(KASH) were submitted to KASH herbarium of Centre for Biodiversity and Taxonomy (CBT), University of Kashmir, India in order to get authentication for the plant specimen.

Chemicals required

Legitimate companies like Sigma Aldrich, Merck, and Hi-med4a Mumbai Pvt. Ltd. procured the chemicals, including solvents like n-Hexane and Ethyl acetate (EtOAC).

Plant Extract preparation

Weighed quantity of dried powdered stem/leaf samples of plant sample were soaked in 500ml of each of Ethyl acetate and n-hexane solvents by following cold extraction method at 4^oC in glass vials in order to get bioactive rich extract of 10-5 grams for FTIR analysis.

Fourier Transform Infrared Spectrometer

FTIR spectrometer (Agilent Technologies, Carry 630 FTIR) was used to obtain FTIR spectra. This instrument was set up as a Windows-based device and connected to OPUS operating system software (**OriginPro 8.5**). The graph was plotted between % transmittance and wave number (cm^{-1}) in order to confirm the presence of major absorption peaks.

Results

The ethyl acetate and n-hexane extract of the aerial part of the said plant was subjected to FT IR analysis in order to confirm the presence of major functional groups in these extracts. The obtained spectral corresponding data was plotted between % transmittance and wavenumber using the software **OriginPro 8.5**. It was found that in ethyl acetate extract, the major absorption peaks were 3377 cm^{-1}(corresponding to **O-H** stretching),2918 cm^{-1} and 2847 (corresponding to **C-H** stretching in alkane),1703 cm^{-1}(corresponding to **C=O** stretching). The presence of O-H peak reflects that polyphenols are present in this extract in which the O-H group interacts with ethyl acetate solvent through the hydrogen bonding . Similarly, the importance of C=O peak suggests the presence of those compounds which contain oxo

functional groups in their structure like aldehydes and ketones. The other peaks which constitute the fingerprint region were 1334 cm^{-1},1505 cm^{-1},1438 cm^{-1},1379 cm^{-1},1274 cm^{-1},1207 cm^{-1},1129 cm^{-1},1073 cm^{-1},1013 cm^{-1}.

Similar FT IR analysis was done to crude hexane extract of the plant material and it was found that the prominent absorption peaks were 3649 cm^{-1}(corresponding to **N-H** stretching),3116 cm^{-1}(corresponding to **C-H** stretching in alkene),2914 cm^{-1},2847 cm^{-1}(corresponding to **C-H** stretching in alkane). The other prominent peaks in low frequency region in this extract are 1707 cm^{-1}(**C=O** stretching).1617 cm^{-1}(**C=C** stretching). The fingerprint region of this extract consists of peaks 1584 cm^{-1},1401 cm^{-1},1274 cm^{-1},1215 cm^{-1},1177 cm^{-1},1148 cm^{-1},1021 cm^{-1},1095 cm^{-1},995 cm^{-1},961 cm^{-1}. All these peaks are indicating the presence of flavonoids, amines, carboxylic acids, ketones, alkenes and terpenoids which are non polar in nature. The FT IR absorption spectrums for comparison of both ethyl acetate extract and hexane extracts of this plant is summed up in Table 2 & Table 2 along with two respective spectra as shown in Figure 1 and Figure 2.

Table 1. FT IR analysis, assigned peaks and corresponding functional moieties present in ethyl acetate extract of stem/leaf of plant *A. propinquum.*

S.No	Absorption frequency(cm^{-1})	Appearance	Functional Group	Compound class
1	3746	medium, sharp	O-H stretching	alcohol
2	3671	strong, broad	O-H stretching	alcohol
3	3377	strong	O-H stretching	Alcohol(intermolecular bonded)
4	2918	medium	C-H stretching	alkane
6	2366	Strong, broad	N=C=O stretching	isocyanate
7	2117	weak	C ≡C	Alkyne (mono-substituted)
8	1703	strong	C=O stretching	Conjugated acid(dimer)
9	1617	medium	C=C stretching	Conjugated alkene
10	1334	medium	O-H bending	alcohol
11	1505	strong	N-O stretching	Nitro compound
12	1438	medium	O-H bending	Carboxylic acid

13	1379	Strong	S=O stretching	Sulfonic acid
14	1274	strong	C=O stretching	alkyl aryl ether
15	1207	strong	C=O stretching	ester
16	1129	strong	C=O stretching	aliphatic ester
17	1037	strong	S=O stretching	sulfoxide
18	1013	Strong, broad	CO-O-CO stretching	anhydride
19	823	medium	C=C stretching	Alkene(tri-substituted)

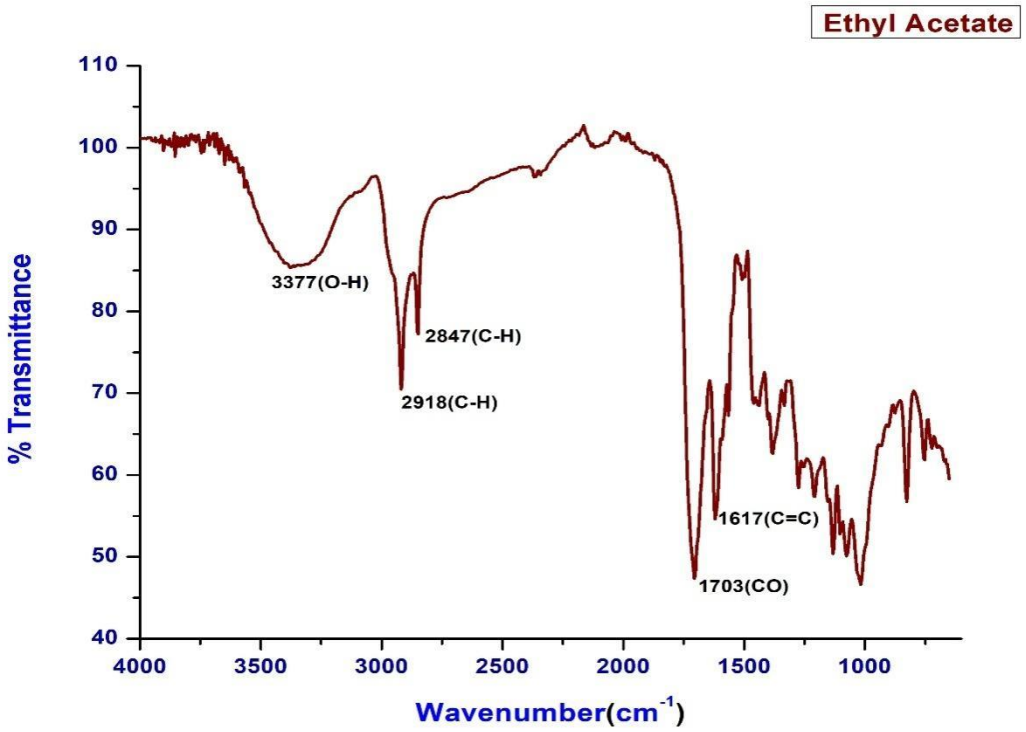

Figure 1 FT IR spectrum of Ethyl acetate stem/leaf extract of *A. propinquum*

Table 2. FT IR analysis, assigned peaks and corresponding functional moieties present in hexane extract of aerial part of plant *A. propinquum*

S.No	Absorption frequency(cm^{-1})	Appearance	Functional Group	Compound class
1	3649	medium, sharp	O-H stretching	alcohol
2	3116	strong, broad	O-H stretching	alcohol

3	2914	Strong, broad	N-H stretching	Amine(salt)
4	2847	Strong, broad	N-H stretching	amine
5	2366	Strong, broad	N=C=O stretching	isocyanate
6	1617	strong	C ≡C stretching	α,β unsaturated ketone
7	1707	strong	C=O stretching	Aliphatic ketone
8	1461	medium	C-H bending	Methyl group
9	1297	strong	C-O stretching	Aromatic ester
10	1584	medium	C=C stretching	Cyclic alkene
11	1401	medium	O-H bending	Carboxylic acid
12	1177	Strong	C=O stretching	ester
13	1148	strong	C=O stretching	alkyl aryl ether
14	1095	strong	C-O stretching	Secondary alcohol
15	995	strong	C=C stretching	Alkene(monosubstituted)
16	961	strong	C=C stretching	Alkene(monosubstituted)
17	872	medium	C-Cl stretching	Halo compound

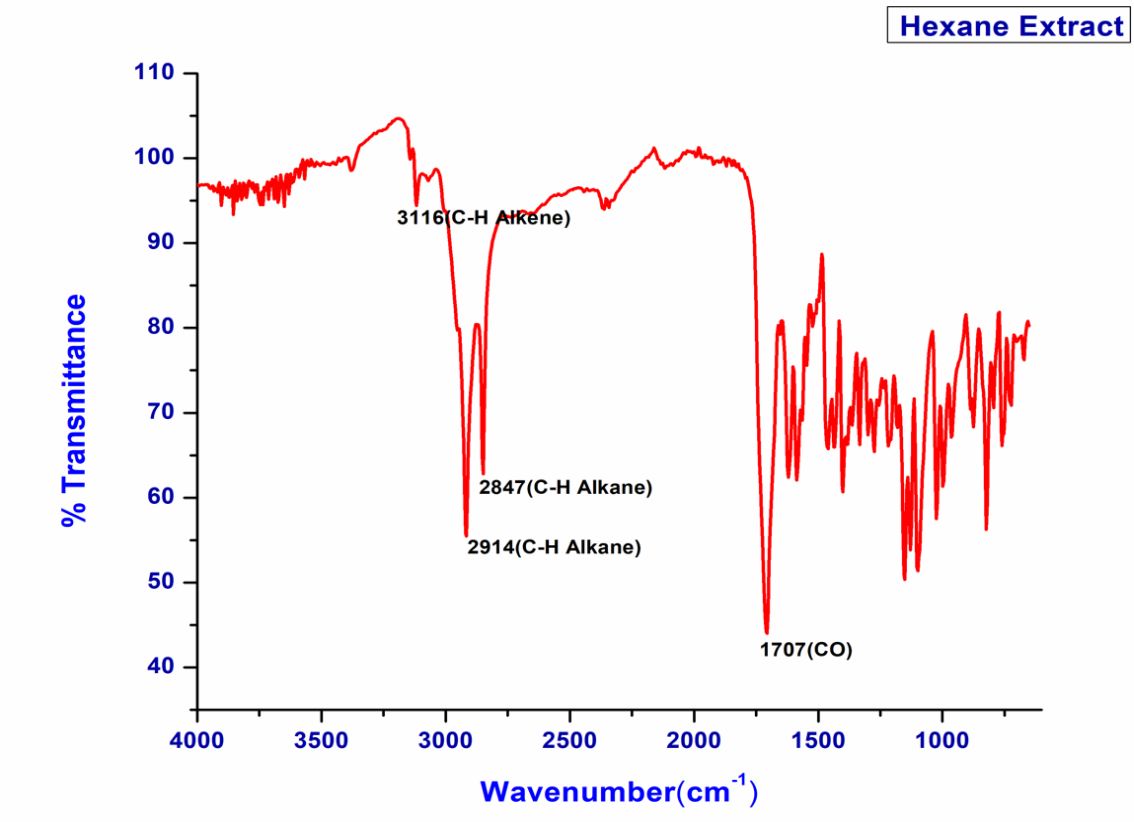

Figure 2, Absorption peaks in stem/leaf hexane extract of *A. propinquum*

Discussions

In this enclosed study, comparative FT IR analysis revealed the presence of functional groups of plant secondary metabolites including polyphenols, flavonoids, terpenoids, esters, amines, glycosides, alkanes, alkaloids, fatty acids and alcohols. Hence, both the ethyl acetate and n-hexane stem/leaf plant extracts of *A. propinquum* clearly indicated the presence of polar and non polar compounds respectively which are of immense pharmacological importance in drug discovery. 4000 to 1500 cm^{-1} were the range of absorption peaks in IR spectrum of n-hexane with prominent presence of O-H and C-H bonds while the range for ethyl acetate extract spectra were from 2500-3500 cm^{-1}. The strong O-H stretching in both the IR spectrums were found in the range of 3000 to 3800 cm^{-1} due to presence of functional groups including carboxylic acids, ketones, amines, polyphenols, terpenoids, tertiary and secondary alcohols. However our findings are in line with Abubaker and co-workers, 2021 who carried out the FTIR analysis of petroleum ether oil extract for the plant *Ziziphus spina-christi* hence, revealed the presence of alkanes, alkenes, carboxylic acids, ketones, amines, alcohols, and phenols as major ones. While examining the methanol extract of *Ceropegia juncea*, Visveshwari et al. (2017) discovered the presence of alcohols, aldehydes, alkynes, alkenes, esters, and amines groups. This justification suggests that *A. propinquum* has a variety of therapeutic phyto-constituents that have a variety of bioactivities, including antibacterial, antifungal, antioxidant, anti-inflammatory, anti-diabetic, anti-aging, anticancer, hepatoprotective, hypercholesterolemic, antihistaminic, anticoagulant, diuretic, etc. The identification of several phyto-constituents in the n-hexane and ethyl acetate aerial plant extracts of *A. propinquum*, hence demonstrates significant therapeutic implications. Moreover, there are preliminary phyto-chemical screenings of *A. propinquum* which can proceed with further analytical techniques including compound isolation and characterization.

Conclusions

The current work is the first report from Kashmir Himalaya to describe the presence of several bioactive components by FT IR analysis . The predominant bioactive constituents were polyphenols, terpenoids, flavonoids, glycosides, secondary and tertiary alcohols with bond stretching range of C-H, O-H, N-H within 3500-1500 cm^{-1} in the obtained spectra for

both the n-hexane and ethyl acetate plant extracts. Thus, these compounds can be supportive in the development of novel drugs.

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Figure 2.Absorption plots and comparison of major absorption peaks in hexane extract of plant…..