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Fourier Transformed Infrared Spectroscopy (Ft-Ir) and Secondary Metabolites Production of *Zizyphus mauritiana*

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Abstract

Ziziphus mauritiana is highly valuable shrub or small tree having thorny and evergreen which grow up to 15 m height with stem diameter 40 cm or greater in thickness. Mostly it have spread crown with many hanging branches. Fourier-transform infrared spectroscopy (FT-IR) is a technique which is used to give an infrared spectrum of absorption or emission of a solid, liquid or gas. The data was collected from Distract Attock which is consider as suitable area for Z*iziphus mauritana* due to climatic and edaphic condition. The area lies in scrub forest in which main species are Zizyphus, Acacia, Olea and Dedonia. Different functional groups were analyzed and differentiate, while the result has been collected in the form of interferogram (graphs) through (FT-IR). The Plant secondary metabolites was extracted from *ziziphus mauritana.* The methanolic solvent showed positive result for the presence saponins, phenols, lignin, glycoside and tannins, the negative response was observed in flavoinds. The peak values 2917.7, 1021.62, 557.69 538.60, 571.05, 523.27 cm^{1} showed the presence of alkanes, alcohol, aliphatic amines, alkyl halide and halogen. The basic purpose of this research work was to interlink (FT-IR) and secondary metabolites by which we can enhance the commercial and industrial importance of these metabolites.

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1. INTRODUCTION

Fourier-transform infrared spectroscopy (FT-IR) is a tool used to take an infrared spectrum of absorption or emission of a liquid, gas, or solid. This provide a vital information about spectra, which have the ability to measure the intensity of spectra over a wide range of wavelengths at a time. An (FT-IR) spectrometer concurrently collects a high spectral resolution data over a wide range of spectral. The term (FT-IR) spectroscopy has been taken from the fact that a Fourier transform (a mathematical process) is compulsory to change the raw data into the real spectrum or real data (Griffiths & De Haseth, 2007).

The term "infrared" usually refers to any electromagnetic radiation which lies in the region from 0.7 mm to 1000 mm. But, the region between 2.5-25 mm (4000 to 400 cm) is most suitable for biochemical examination. FT-IR is a technique that is use now a days for measuring and calculating the intensity of infrared radiation as a function of frequency or wavelength. (Kamble & Gaikwad, 2016).

Secondary metabolites have limited role in the normal growth but are too important for the development of an organism. While primary metabolites are important for respiration and photosynthesis in the absence of secondary metabolites the plant did not died immediately, but it is important for the organism's survivability and plant defense. Plant secondary metabolites are extraordinary source for medications, food additives, taste, and other manufacturing and non- manufacturing ingredients. Systematized cultures, and particularly root cultures, can make a substantial role in the creation of secondary metabolites**.** (Jian Zhao, Davis, & Verpoorte, 2005).

Plants provide enormous diversity of secondary compounds which acts as natural protection against microorganism and pest. Most of these metabolites are also poisonous to animals, but others are too beneficial. In reality a large number of these compounds have been utilized as entire plant or extracts for foodstuff or for medicinal applications in modern era. The capability of basic oils and saponins as advantageous feed added substances in ruminant generation will be utilized here as an illustration of the potential benefits of plant compounds (John, 2004).

During (FT-IR) analysis Kamble (2016) confirmed the presence of aliphatic amines, amino acids, alkanes, alcohols, esters, ethers, aromatics, phenols, aldehyde, amide, ketones, fluorides, halogen, alkyl halides, carboxylic acids, and nitro compound. The finding of his result shows the importance of these function groups. It also designates the medical property of E. ribes that can be applied for different pharmacological purposes.

Tiwari & Rana., (2015) carried a work on plant secondary metabolites while plants secondary metabolites are irreplaceable source for antibiotics, food additives, flavors and also have commercial values. In recent years with the help of tissue culture it enhances its commercial value. The formation of various secondary metabolites is also connected to the induction of morphological changes and it seems that the cells undergo morphological differentiation and develop during plant growth advancement. In the comparison among differentiation and non-differentiation tissue in in-Vitro it is observed that the production of secondary metabolites is much higher. Metabolic engineering is possibly a great step forward if proper work has been carried out on it.

There are limited work has been done on Forest secondary metabolites in Pakistan. FT-IR is also a new tool which can be utilized to separate different function groups so the purpose of this research study is to enhance and aware people about the forest secondary metabolites which can be used in future for different purposes. The main purpose of this research study was extraction and identification of different secondary metabolites from *Ziziphus mauritiana* with comparison of function groups through FT-IT.

2. MATERIALS AND METHODS

Collection of Plant Material

A detailed survey was carried out in Bissal Village Attock District which is located in Pothohar Plateau of the Punjab Province in Pakistan. The Latitude and longitude coordinates are 33°4627.77420 N, 72°2137.64916 E with elevation of 525 m (1722 ft). The attock is a city situated in north of Punjab Province in Pakistan close to the capital Islamabad. The leaves of *Ziziphus mauritiana* collected and placed in laboratory for further analysis.

Preparation of Sample

The leaves were washed with tap water for the purpose to remove dust. Then the leaves were placed in a large bowl and treated with distalled water to remove minute dust and unwanted materials. After that the leaves were dried at room temperature in shade for almost 20 days. Using electric grinder, it was converted into powder form and placed in zipper bag.

Preparation of Extract

The extract was collected using methanol as solvent with powder of *Zizyphus mauratiana*. For the collection of methanolic extract Soxhlet apparatus was used. 25 gm of leaves powder was placed in thimble of soxhlet apparatus with 250 ml of methanol. The temperature was set at 65 C^0. The thimble sample was continue to boil in soxhlet apparatus for about 12 hours since the solution becomes clear and the green colored extract was gathered in the flask at the bottom of apparatus. The extract was filter through whatsman filter paper no 1. Then the final extract was gathered and stored in petri dishes and left to dry for 24 hours. The dried extract having a pasty form was put into petri dishes at temperatures at 4 ºC in the fridge for further usage.

Phytochemical Analysis

For screening and identification of different secondary metabolites the crude extract was experienced for the presence or absences of phenol, tannin, glycoside, lignin and flavonoid. All the standard procedures were used as prescribed by Harborne (1973), Trease & Evans (1989) and Sofowara (1993) were followed.

Test for Phenol

5 ml methanol was mixed with 2 gm of solid ferric chloride. The ferric chloride solution was formed which was placed in separate beaker. 1 ml extract was treated with 2 ml ferric chloride solution a yellowish color observed which shows positive response towards phenol presence (Gibbs R.D., 1974).

Tannin Test

0.5 gm powder of Zizyphus mixed with 10 ml of distalled water. It was boiled for 5 minutes and then filtered with whatsman filter paper no 40. 0.1 % ferric chloride was treated with filtrate extract the brownish color indicate response tannin presence (Treare GE, Evans WC. 1985).

Glycoside Test

Keller- Killani Test

1 ml extract was mixed with 0.2 % of glacial acid. The tube was boiled for 30 seconds after cooling for a minute 0.2 % ferric chloride solution was added. In another tube 2 ml conc. Sulfuric acid was taken and mixed with glacial acid and ferric chloride acid a reddish brown colure was detected at the junction of two layers which clearly show existence of glycoside (Kokate C. K. *et. al;* 2001).

Lignin Test

1 ml extract was treated with 0.2 % of glacial acid in test tube. An olive green colure observed at bottom of test tube shows presence of lignin (Gibbs R.D., 1974).

Test For Flavonoid

Pew,s Test

2 gm of zinc was treated with 2 ml extract. In another test tube conc. Sulphuric acid was taken and mixed with it so no changes were observed in color which indicates the absence of flavonoid (Kokate C. K. *et. al;* 2001).

Test for Saponin

Foam Test:

Foam test was selected for the presence of saponin. The foam test was performed by standard etiquette. 1ml of plant extract was taken and shake well in the test tube containing 5 ml distilled water. After shaking for 30 seconds a formation of honey comb like foam which indicate the presence of saponin (Kokate C. K. *et. al;* 2001).

FT-IR Fourier Transformed Infrared Spectroscopy

Fourier Transform Infrared Spectrophotometer (FT-IR) is an incredible tool for the identification of different functional groups present in compound. To detect the infrared assimilation range FT-IR spectrum was utilized for the identification of different functional groups of the various compounds based on its peak value in the region of IR spectra. The grinded leaves of *Ziziphus mauritiana* was passed into the FT-IR and the functional groups of different compounds were divided based on its peak value Bobby *et al.,* (2012).

Dried powder of *Zizyphus muriatina* was used for (FT-IR) examination. 10 mg of the dried extract powder was compressed in 100 mg of potassium Bromide (KBr) capsule, in order to arrange translucent sample discs. The Scan range for sample was from 500 to 4000 cm^{-1} with a resolution of cm^{-1}.

3. Result and Discussion

The peak values of FT-IR shows different functional groups alkanes, alcohol, aliphatic amines, halogens and alkyl halides with peak values of 2917.77, 1017.62, 571.05, 557.69, 523.27 cm-1. On the bases of its peak values we can also find its bond strength and intensity All data is present in fig 1 and table 1. The peak values was taken from the available literature which show same resemblance with Kamble *et al.,* (2016) and Bobby *et al.,* (2012).

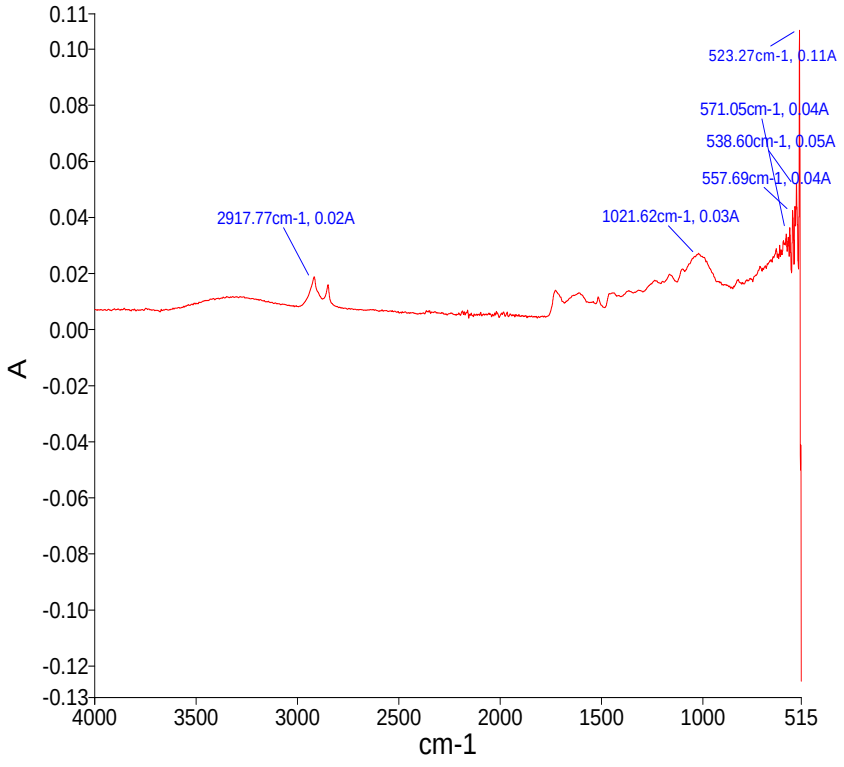


Figure No.1 Different Spectra of *Ziziphus mauritiana* Through FT-IR

The range of IR spectra was 500 to 4000 cm^{-1}. The detection of different metabolites was observed from 2900 to 520 cm^{-1}.

Table No.1 FT-IT Peak values and functional group of *zizyphus muriatiana*

Frequency (cm^{-1})	Functional group	Bond	Intensity
2917.77	Alkanes	C-H stretch	Strong
1017.62	Alcohol, Aliphatic amines	O-H ,C-N	Medium
571.05	Alkyl halide, Halogen	C-Br	Strong
557.69	Alkyl halide, Halogen	C-Br, C-I	Strong
523.27	Halogen	C-I	Strong

Phytochemical Screening

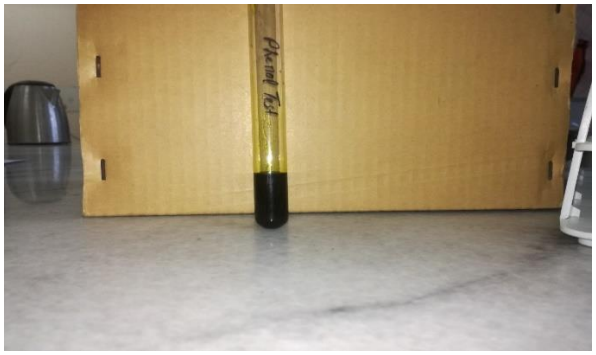
The extract was collected from successive hot extraction of plant leaves which was subjected to different phytochemical tests to reveal the presence of different phytochemicals more importantly different secondary metabolites present in the extracts. Different secondary metabolites can be obtained from any part of plant (roots, leaves, stem and bark). The data about these metabolites is vital because it play a dynamic role in syntheses of different biochemical substances. Different tests were conducted for the purpose to find out various secondary metabolites so lignin, phenol, glycoside, saponion and tannin have positive response while flavonoids have negative response. All data is given in (Table 2) and (Fig 2).

Table No.2 Details of Secondary Metabolites in Zizyphus muriatana

Different Tests	Result
Sapoin	+
Phenol	+
Tannin	+
Glycoside	+
Flavonoids	_
Lignin	+

+= shows presence, -= shows absence

Phenol Test



Saponion Test

Lignin test Glycoside Test

Flavoniods test Tannin test

Figure No.2 Presence of Different Secondary Metabolites in *Ziziphus mauritiana*

Saponin was also find by Parmar *et al.,* (2012) in *Ziziphus mauritiana* which showed positive response. According to medical field saponins can be used in anticancer, antioxidant, hypercholesterolemia, hyperglycemia, anti-inflammatory and weight loss etc. It is a bioactive antibacterial mediator of plants. (Manjunatha, 2006). There are many classes of Saponins while glycosides is one of them having soapy appearance (Fluck, 1973) while Saponins have active against antifungal agent (Sodipo *et. al.,* 1991). For researchers phenol have great interest due to its significant effects in different diseases (Zheng, 2001).

Based on the result from preliminary phytochemical screening the methanolic extract showed maximum amount of phenol. Similar observation was given by Jimoh et al., (2008). Tannins play key role in antifungal and antimicrobial activities (Rievere *et. al.*, 2009). These contents shows various types of activity against different germs and diseases causing agents. Therefore, it can be utilized in the treatment of different pathogens and diseases. . Mostly 30% of the organic matter of trees consists of lignin global commercial production of lignin is a consequence of paper making Patt et al. (2005) the same result was provided by parmar (2012).

Conclusion

As indicated by our examination, it is easy method to find different functional groups in plants through FT-IR. These functional groups provide us information about constituents present in plant. It has been concluded that various kinds of metabolites are available which have different functions. These metabolites shows an effective response against disease causing agents (Pathogens) while its leaves can be utilized in treatment of different diseases like cancer and liver. We can utilize its leaves for the purpose of growth treatment, drugs preparation, antifungal and antibacterial activities.

Recommendation

The present study stated that secondary metabolites have great importance in the life and wellbeing of human being. It can be use in the treatment of different diseases and drug use. For this purpose, FT-IR can be use for finding the functional groups of plant species. It is also predicted that near in the future its commercial and industrial importance are too high.

Especially Pakistan has a suitable market for these metabolites due to high density of population, more species (plant) composition, verities and market potential. It requires more studies on secondary metabolites.

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