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Research Article

Bioactive Metabolites Identification and FTIR Analysis in *Ziziphus oenoplia* Mill

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Abstract

Medicinal Plants have inexpensive amount of bioactive phytochemicals or bionutrients. Phytochemicals are present in all medicinal plants at different levels and are useful compounds of human diet. In recent decades research studies have shown that phytochemicals are playing major role in preventing chronic diseases such as diabetes, cancer and heart disease. They are being used to control oxidation and chronic diseases associated with stress, such as cardiovascular diseases and diabetes because it possesses strong antioxidant activity. Aim of the present study was *Ziziphus oenoplia* Mill. phytochemical screening, alkaloid estimation and their FTIR analysis. This experiment provides evidence to support the existence of different biologically important medicinal bioactive compounds or phytochemicals that explain their use in conventional medicines. This medicine can be therapeutically used separately or combination with other extracts to cure any disorder and disease.

Keywords: Phytochemical Analysis; Alkaloids; FTIR; *Ziziphus oenoplia*

Abbreviation

FTIR: Fourier-transform Infrared Spectroscopy

Introduction

India has a rich history of traditional medicine systems, researchers estimated more than 70,000 plant species were used in medicines worldwide. Since ancient times, medicinal herbs discovered and used in conventional medicine practices. Plants produce several chemical compounds for defense against fungi, insects and mammals. Various phytochemicals have been associated with potential biological activity. Various therapeutic plants is utilized to endeavor health benefits, to regulate for specific condition or both regardless of whether in conventional medication or in current medication. Around a quarter of the medicine approved for patients in ongoing medicines are obtained from medicinal plants. Medicinal herbs provide about a quarter of the drugs prescribed to patients in modern medicine, and they are thoroughly tested. Medicinal plants mat offer three main types of advantages: health advantages to the folks that consume them as medicine; financial benefits to folks that harvest, method and distribute them for sale; society-wide advantages, like job opportunities, taxation financial gain, and a healthier labour force. Medicative plants are widely used in non-industrialized societies, primarily as a result of they're without delay on the market and cheaper than trendy medicines. The annual international export price of 50,000 to 70,000 plants with suspected medicative properties was calculable to be in US$2.2 billion in 2017, the potential international marketplace for biological science extracts and medicines was at many hundred billion dollars. Medicinal plants face each general threats, like temperature change and habitat destruction and specific threat of over assortment to satisfy meet market demand [1].

Ziziphus oenoplia Mill. (Family-Rhamnaceae) commonly documented as Makai in Hindi and Jackal Jujube in English, is a straggling shrub distributed everywhere the warmer regions of Pakistan, Sri Lanka, India, Malaysia, and Tropical Asia. It's often utilized in province of Uttar Pradesh (India) for liver diseases [2,3]. The roots of the plant possess antiulcer and antioxidant, antihelminthic [4,5], antiplasmodial [6] angiogenic potential, antidenaturation and antibacterial activity [7], wound healing activity [8], hepatoprotective potential against antitubercular drugs induced hepatotoxicity [9] and as an ingredient in the preparation of stomach ache pills among the Munda tribe [10]. Aim of the current study to investigate *Ziziphus oenoplia* Mill preliminary phytochemical analysis, estimate the number of alkaloids present and identification of functional group present within the extracts using FTIR analysis.

Materials and Methods

Sample preparation and extraction

Ziziphus oenoplia Mill. was collected from Perungulathur forest (Figure 1) Chennai, Tamilnadu. Air dried Leaf and fruit (along with seed) of genus *Ziziphus oenoplia* Mill were aseptically grinded and any accustomed for the study. 5 gm of each leaf and fruit samples of genus *Ziziphus oenoplia* Mill were additionally 50 ml of ethanol and hexane respectively. The extracts were carried out by maceration technique for 12 hrs. After 12 hrs the extracts were removed and dried. The samples were any used for the study.

Figure 1

Preliminary qualitative analysis

Alkaloids, amino acids, carbohydrates, fixed oils and fats, glycosides, Phyto sterols, proteins, Saponins, Mucilages, volatile oil phenolic compounds and tannins were analysed according to [11] Thenmozhi *et al*. method.

Determination of alkaloids

Take the 1mg of plant extracts (leaf and fruit) in test tube and Standard solutions from S1 to S5 in different test tubes and Blank solution in another test tube. Alkaloid content in the sample was precipitated using 0.5 ml of Dragendorff reagent and was allowed to stand for 10 minutes. This content was centrifuged and the precipitate was dissolved in 1ml of concentrated HNO_3. Yellow color was developed to above solution using 300 µl of 3% Thiourea solution. Now, absorbance was read at 435nm in spectrophotometer against Blank. The absorbance reading was noted and graph was plotted against Atropine standard [12].

Fourier transmittance infrared spectrometry

Identification of functional group

It is a valuable device for the identification and characterization of functional groups (chemical bonds) present in the compound. In addition, FT-IR spectra are unique that they are like a molecular "fingerprint". The drop forms a thin film between the cells. Solid samples can be milled with potassium bromide (KBr) and then compressed into a thin pellet using hydraulic press, which were then used for the analysis. The sample of the *Ziziphus oenoplia* Mill. hexane and ethanol extract were treated for FTIR spectroscopy IR-Affinity 1 (Shimadzu, Japan). The samples were run at infrared region between 400 nm and 4000 nm and standard DLATGS detector was used at 2.8 mm/sec mirror speed [13].

Results and Discussion

Preliminary qualitative analysis

Phytochemical analysis was conducted on the leaf and fruit samples of *Ziziphus oenoplia* Mill. that discovered the presence of phytochemical constituents, that renowned to exhibit medicinal still as physiological activities of this species. Analysis of this plant exposed the presence of alkaloid, phytosterols, phenol and protein (Table 1).

S.NO	Test compounds	A (Ethanol extract)		B (Hexane extract)	
		A1-Leaf	A2-Seed	B2-Leaf	B2-Seed
1	Alkaloids	+	+	+	-
2	Amino Acids	++	+	+	-
3	Carbohydrates	+	+	+	+
4	Fixed Oils and Fats	+	+	-	-
5	Glycosides	+	+	-	-
6	Phenolic Compounds and Tannins	++	+	+	-
7	Phytosterols	++	++	-	+
8	Proteins	++	+	+	-
9	Saponins	-	-	+	-
10	Gums and Mucilage's	+	+	-	+
11	Volatile Oils	-	-	-	-

Table 1: Qualitative screening of phytochemical constituents.

Note: (-): Negative (absence of constituents); (+): Weak positive (presence of constituents); (+ +): Positive test; (+ + +): Test strongly positive.

The ethanolic extract of leaf and fruit sample showed the presence of amino acid, phenolic compounds, tannins, phytosterols, protein in most concentration, that is relatively less concentration was ascertained within the dissolvent extract. Equally the phytoconstituents carbohydrate was found to be present in each extracts of leaf and seed sample respectively. The volatile oils are found to be absent in each extracts of leaf and seed sample. Whereas saponins content was found to be present solely in leaf hexane extracts. Phytoconstituents fixed oil, fats, glycosides, gums and mucilages were found to be present solely in ethanolic extracts of each the samples. The subsequent were the results obtained through the preliminary analysis of the plant extracts (Table 1).

The presences of phenolic compound are one in every of the most important and most ubiquitous team of plant metabolites [10]. They possess biological properties like anti programmed cell death, anti ageing, anti carcinogen, anti inflammation, anti atherosclerosis, cardiovascular protection and improvement of endothelial function, likewise as inhibition of angiogenesis and cell proliferation activities [14]. Several studies on medicative reportable to be flush in phenolic compound, that posses to own the inhibitory property [15]. Natural antioxidants mainly come from plants in the form of phenolic compounds such as flavonoid phenolic acids, tocopherols etc [16].

Quantification of alkaloid content in both the extract of leaf and fruit sample

Moreover, data regarding different phyto-constituents of plants may be a important and advantageous because it is away valuable within the production of advanced chemical compounds further as screening of their biological activities.

This study has been meted out for the quantification of the alkaloid content in ethanolic and hexane extracts of *Ziziphus oenoplia* Mill. leaf and fruit. The quantitative estimation of alkaloid content showed maximum amount in hexane extract compared to ethanol extract whereas high amount of alkaloid was ascertained in leaf extract. The contents of the alkaloid compounds within the crude extract, determined regression of Y on X of standardization curve (y = R^2) and expressed in atropine equivalent was 41.666 ± 4.784 mg/g of plant extract. The quality standardisation curve of Atropine was shown in figure respectively. The variation within the content was shown in figure 2.

Figure 2: Quantification of Alkaloid Content in *Ziziphus oenophia* Leaf and Fruit.

Fourier transform infrared Spectrometry

The FT-IR spectrum was used to determine the functional groups of the active components within the extract based on the peak values in the region of IR radiation. When the extracts were

passed into the FTIR, the functional groups of the components were separated based on its peak quantitative relation. The results of the FTIR peaks and its functional groups present in Ethanol and Hexane extracts are as follows.

Ethanol extract of *Ziziphus oenoplia* leaf

C-I (Halo compounds), C-Br (Halo compounds), C-Br (Halo compounds), =C-H (Alkene), C=C (Alkene, Aromatic), C-Cl (Halo compounds), C-O (Secondary alcohol, Aliphatic ether, Tertiary alcohol, Ester, Vinyl ether, Alkyl aryl ether), C-N (Amine, Aromatic amine), S=O (Sulfone, sulfonamide, sulfonate), O-H (Phenol, Alcohol, Carboxylic acid), N=O (Nitro compound), C=O (Carbonyl, Conjugated ketone, Conjugated acid, Conjugated acid halide, Anhydride), C=N (Imine/Oxime), C-H (Aromatic compound, Aldehyde, Alkane), N-H (Amine salt) (Figure 3a).

Figure 3a: Ethanol leaf FT-IR Spectrum Profile of *Ziziphus oenoplia*.

Hexane extract of *Ziziphus oenoplia* leaf

C-I (Halo compounds), C-F (Halo compounds), C-Br (Halo compounds), C-Cl (Halo compounds), C=C (Alkene), =C-H (Alkene), C-O (Alcohol, Primary alcohol, Vinyl ether, Alkyl aryl ether), C-N (Amine, Aromatic ester, Aromatic amine), C-H (Alkane), N-O (Nitro compound), O-H (Alcohol intermolecular bond, Carboxylic acid), C=C (Aromatic), C=O (Carbonyl, Carboxylic acid, Aliphatic ketone- cyclohexanone or cyclopentanone, α,β unsaturated esters-formates), N-H (Amine salt, Aliphatic primary amine) (Figure 3b).

Ethanol extract of *Ziziphus oenoplia* fruit

C-I (Halo compounds), C-F (Halo compounds), C-Br (Halo compounds), C-Cl (Halo compounds), =C-H (Alkene), S=O (Sulfoxide, Sulfate), C-O (Primary alcohol, Vinyl ether, Alkyl aryl ether, Aromatic ester), C-H (Alkane), C-H (Alkyne), O-H (Alcohol, Carboxylic acid), C=O (Aromatic, Conjugated alkene), N-H (Amine, Amine salt, Aliphatic primary amine), C=C (Aromatic, Alkene, Cyclic alkene, Conjugated alkene), S-C ≡ N (Thiocyanine) (Figure 3c).

Figure 3b: Hexane leaf FT-IR Spectrum Profile of *Ziziphus oenoplia*.

Figure 3c: Ethanol fruit FT-IR Spectrum Profile of *Ziziphus oenoplia*.

Hexane extract of *Ziziphus oenoplia* fruit

C-Cl (Halo compound), C-F (Halo compounds) =C-H (Alkene), C=C (Alkene, Aromatic), S=O (Sulfonamide, Sulfonic acid, Sulfoxide, Sulfonate), C-N (Amine, Aromatic amine), C-O (Tertiary alcohol, Alkyl aryl ether, Aromatic ester), C-H (Alkane, Aldehyde, Aromatic compound), O-H (Phenol, Alcohol, Carboxylic acid), N-H (Amine, Amine salt), C=O (Carbonyl, Aldehyde, δ-Lactone, Esters, Conjugated anhydrides), N=C=S (Isothiocyanate), O=C=O (Carbon dioxide) (Figure 3d).

Figure 3d: Hexane fruit FT-IR Spectrum Profile of *Ziziphus oenoplia*.

The FTIR spectrum profile was illustrated in figure 3a and 3c. The FTIR of Ethanol extract of Leaf sample gave broad peaks at 1517.98 cm^{-1} which indicates the presence of aromatic nitro compounds. Similarly, in FTIR of Hexane extract of Leaf sample gave prominent peaks at 1624.06 cm^{-1} and 1056.06 cm^{-1} which indicate the presence of alkene, primary alcohol, and vinyl ether. In case of Ethanol extract of Fruit sample show broad peaks at 3379.29 cm^{-1} which indicate the presence of alcohol and aliphatic primary amine. The Hexane extract of Fruit sample showed prominent peak at 1739.79 cm^{-1} which indicate the presence of carbonyl, aldehyde, esters, conjugated anhydrides, and δ-Lactone.

These results show that *Ziziphus oenoplia* Mill. does not contain any toxic substances. The FTIR spectrum confirmed the presence of alcohol, alkanes, aromatic compounds and nitro compounds in the powdered extract.

FTIR is a high resolution analytical technique to identify chemical constituents and elucidate the structural compounds [17]. FTIR offers a rapid and non-destructive investigation to fingerprint plant extracts or powders [18].

Conclusion

Plants are a veritable supply source of medications. However, man tends to ignore the portance of seasoner drugs. Medicative plants have formed the basis of health care throughout the planet since the earliest days of humanity and are still wide used and have respectable importance in international trade. The plant was shade dried and pulverised. The pulverised plants samples were extracted in different solvents (Hexane and Ethanol). The various solvents extract were investigated for phytochemicals, alkaloid estimation and FTIR analysis.

Phytochemical screening of the hexane and ethanol extracts of *Ziziphus oenoplia* Mill" discovered the presence alkaloids, amino acids, carbohydrates, proteins, phenols, phytosterols and gums and mucilage. Alkaloids were present in higher proportion in hexane extract when compare to ethanol extract. The IR analysis has revealed the presence of many active groups of phytochemical compounds in leaves and seed. The findings of the recent study reveal that the plant samples are good sources for the ethnomedicinal use.

Conflict of Interest

No financial interest or any conflict of interest exists.

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