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RESEARCH ARTICLE

Phytochemical Screening and FTIR Analysis of two Important Medicinal Plant Species of Madurai District

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ABSTRACT

The present study deals with the phytochemical screening and FTIR analysis of available parts (mainly leaves and seeds) of two traditional medicinal plants of two different families found in selected region of Madurai District. Test plants were extracted with methanolic solvent for the presence of flavonoids, glycosides, saponins, tannins, steroids, terpenoids, resins, phenolic compounds, proteins and aminoacids and acidic compounds. We found that the selected plants are good source of various phytochemicals. This study revealed the presence of various biologically active secondary metabolites which could be helpful in the prevention of chronic diseases.

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Keywords: Screening, Alcoholic extracts, secondary metabolites, FTIR analysis

1. Introduction

Plants produce various bioactive phytochemicals which can be grouped under two catagories; primary and secondary metabolites. Primary metabolites include proteins, carbohydrates, amino acids and chlorophyll while polyphenols, alkaloids, terpenoids are some examples of secondary metabolites. Secondary metabolites are the chemicals that are not required for the immediate survival of the plant but synthesized to increase the survival of the plant by allowing it to interact with pathogens, herbivores insects and environment. The plant kingdom is a treasure house of potential drugs and in the recent years there has been an increasing awareness about the importance of medicinal plants. Plants are the richest resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs (1). The use of plants and plant products as medicines could be traced as far back as the beginning of human civilization. The earliest mention of medicinal use of plants is founds in "Rigveda", which is said to have been written between 4500 - 1600 B.C. and is supposed to be the oldest repository of human knowledge.

Ayurveda is the foundation of medicinal science, in its eight division deals with specific properties of drugs and various aspects of science of life and the art of healing (2). The world health Organization (WHO) estimated that 80% of the population of developing countries still relies on traditional medicines, mostly plant drugs, for their primary health care needs (3). Medicinal plants are a source of great economic value all over the world. Plant products have been part of phytomedicines since time immemorial. Knowledge of the chemical constituents of plants is desirable because such information will be valuable for synthesis of complex chemical substances. There is widespread interest in evaluating drugs derived from plant sources. This interest mainly arises from the belief that green medicine is safe and dependable, compared to costly synthetic drugs which are invariably associated with adverse effects (4). The adverse effects of the drugs available today, necessitate the discovery of new harmless pharmacotherapeutic agents from medicinal plants (5, 6). A knowledge of the chemical constituents of plants is desirable, not only for the discovery of therapeutic agents, but also because such information may be of value in disclosing new sources of such economic materials such as flavoniods, tannins, essential oils, gums, precursors for the synthesis of complex chemical substances, etc. The present work is aimed to screen different phytoconstituents found in two important traditional medicinal plants of Madurai District.

2. MATERIALS AND METHODS

2.1. Collection and Identification of Plant Material

Fresh plant/plant parts were collected from Goripalayam in and around region of Madurai District, Tamilnadu. The plants and the parts were screened, together with their family and vernacular names. The taxonomic identities of these plants were confirmed by Dr. D. Stephen, The American College, Madurai, Tamilnadu, and

the voucher specimen numbers of the plants were preserved. Fresh plant material was washed under running tap water, air dried and then homogenized to fine powder and stored in tight air bottles.

2.2. Preparation of plant extracts

To know the presence of major phytochemicals, the healthy leaf and seed samples were collected and dried in the shade for 2-3 weeks. Then the shade dried leaf and seed samples of *Ziziphus jujuba* and *Myristica fragrans* respectively were made into a fine powder. Following that, 30 g of the powder was filled in the filter paper and successively extracted using 250 ml solvent *viz.*, Methanol using the soxhlet extractor for 8 – 10 hours (7). The extract was filtered through Whatman No.1 filter paper to remove all undissolved matter, including cellular materials and other constitutions that are insoluble in the extraction solvents.

2.3. Preliminary phytochemical studies

The extract was tested for the presence of bioactivecompounds by using following standard methods (8, 9 & 10). The extracts were subjected to preliminary phytochemical tests to determine the groups of secondary metabolites present in the plant material as follows:

2.3.1. Test for flavonoids

The stock solution (1 mL) was taken in a test tube and added few drop of dilute NaOH solution. An intense yellow colour was appeared in the test tube. It became colourless when on addition of a few drop of dilute acid that indicated the presence of flavonoids.

2.3.2. Test for glycosides

Salkowski's test: To the 2 ml of extract, add 2 ml of concentrated sulphuric acid. The appearance of reddish brown colour indicates the presence of glycosides.

2.3.3. Test for saponins

One ml of extract was taken in a test tube and 5 ml of distilled water was added and vigorously shaken. A persistent froth that lasted for at least 15 minutes indicated the presence of saponins.

2.3.4. Test for tannins

Two ml of the extracts were diluted with distilled water in separate test tubes and 2-3 drops of 5 % ferric chloride ($FeCl_3$) solution was added. A green-black or blue-black colouration indicated the presence of tannins.

2.3.5. Test for steroids

2 ml of chloroform and 1ml of concentrated sulphuric acid were added with the 5 ml aqueous plant extract. In the lower, if chloroform layer shows red color appearance that indicates the presence of steroids.

2.3.6. Test for terpenoids

5ml of extract were mixed with 2ml of chloroform and 1ml of concentrated sulphuric acid to form a layer. A reddishbrown coloration of the interface shows the presence of terpenoids.

2.3.7. Test for Resins

1ml of extract was dissolved in acetone and then 1 ml of distilled water is added. Turbidity indicates the presence of resin.

2.3.8. Test for phenolic compounds

Tothe 3 ml of extract, 2 ml of lead acetate solution is added and observed for formation of precipitate.

2.3.9. Test for Proteins and Amino acids

To 2 ml of extract, few drops of nitric acid is added by the sides of the test tube and observed for formation of yellow colour.

2.3.10. Test for Acidic compounds

To 2 ml of the extract, 3 ml of sodium bicarbonate solution is added and observed for the production of effervescences.

2.4. FTIR analysis

The FTIR analysis was conducted on non-extracted leaf and seed powder of *Ziziphus jujuba* and *Myristica fragrans* sample respectively. The 50ºC oven-dried leaves (*Ziziphus jujuba)* and seeds (*Myristica fragrans*) were blended into a fine powder. As much as 1 mg of the sample was mixed with 50 mg KBr (FTIR-grade); then, some of the mixture was placed into the sample holder. All investigations were performed with an IRPrestige-21 (Shimadzu). The scanning absorption range was 400 to 4000 cm^{-1}.

3. RESULTS AND DISCUSSION

The present study subjected to screen the phytochemical constituents and identification of functional group of two medicinal plants namely *Ziziphus jujuba* (Rhamnaceae) and *Myristica fragrans* (Myristicaceae) generally inhabiting at Goripalayam and in and around region of Madurai district, Tamil Nadu, India were selected. The qualitative phytochemical analysis of *Ziziphus jujuba* and *Myristica fragrans* leaf and seed samples respectively with alcoholic solvent, viz., methanol extracts were presented in Tables 1 and 2.

Preliminary phytochemical screening of methanolic leaf extract of *Ziziphus jujuba* was carried out using different methods in order to identify either the presence or absence of secondary metabolites such as tannins, saponins, flavonoids, glycosides, resins, terpenoids, steroids, protein, phenolic compounds and acetic compounds are presented in Table 1.The leaf extract showed positive result for saponins, flavanoids, glycosides andsteroids and the

phytochemicals like tannins, resins, terpenoids, protein, phenolic compounds and acetic compounds were absent.

Another plant species *Myristica fragrans*, study also revealed that the methanolic seed extract was carried out using different methods in order to identify either presence or absence of bioactive compounds such as tannins, saponins, flavonoids, glycosides, resins, terpenoids, steroids, protein, phenolic compounds and acetic compounds are presented in Table 2. The seed extract showed positive result for tannins, saponins, flavanoids, resins, terpenoids, steroids and phenolic compounds and the phytochemicals like glycosides, protein and acetic compounds were absent.

FTIR analysis

The chemical bonds or functional groups present in the dried leaf and seed powder of *Ziziphus jujuba* and *Myristica fragrans* respectively were predicted using FTIR (KBr method). The bonds were determined by interpreting the infrared absorption spectra. Figure I shows the FTIR spectrum of the dried leaf powder, while Table 3 shows the interpretation of the chemical bonds in the non-extracted leaf powder of *Z. jujuba*. Seven major peaks are 3420.14, 2919.7, 1637.27, 1542.77, 1455.08, 1245.79 and 1056.8cm-1in the region between 400-4000 cm-1 (Figure I). Functional groups like amine group, carboxylic acid, conjugation of allergies with two aromatic ring, amide, aromatic ring, aliphatic nitro, amine group, alcohol, ether, carboxylic acid, alcohol and ether were identified. The corresponding functional groups are amine group (3420.14), carboxylic acid (2919.7), conjugation of allergies with two aromatic ring, amide (1637.27), aromatic ring, aliphatic nitro (1542.77), amine group (1455.08), alcohol, ether, carboxylic acid (1245.79), and alcohol and ether (1056.8) respectively (Table 3).

Whereas, Table 4 shows that the interpretation of the chemical bonds in the non-extracted seed powder of *M. fragrans*. Thirteen major peaks are 3391.21, 2922.59, 2852.2, 1707.6, 1635.34, 1514.81, 1460.81, 1375, 1239.04, 1128.15, 1047.16, 862.989 and 720cm-1in the region between 400-4000 cm-1 (Figure II). Functional groups like alcohol, carboxylic acid, carboxylic acid, aldehyde hydrogen, carboxylic acid, carboxylic acid, conjugation of aldehyde with two aromatic ring, amide, aromatic nitro, aromatic ring, amino related, aliphatic nitro, ether, alcohol, carboxylic acid, alcohol, ether, alcohol, ether, aromatic ring and alcohol, ether, aromatic ring were identified. The corresponding functional groups are alcohol, carboxylic acid (3391.21), carboxylic acid (2922.59), aldehyde hydrogen, carboxylic acid (2852.2), carboxylic acid (1707.6), conjugation of aldehyde with two aromatic ring, amide(1635.34), aromatic nitro, aromatic ring (1514.81), amino related (1460.81), aliphatic nitro (1375), ether, alcohol, carboxylic acid (1239.04), alcohol ether (1128.15), alcohol, ether (1047.16), aromatic ring (862.989) and alcohol, ether, aromatic ring (720) respectively (Table 4).

Qualitative phytochemical analysis of *Ziziphus jujuba* and *Myristica fragrans* leaf and seed extracts showed possible presence of chemical principles respectively. The methanolic solvent used to leaf extract from *Ziziphus jujuba* and seed extract from *Myristica fragrans* determined the bioactive compounds. Among the two extract methanolic solvents, the seed methanolic extracts of plant species showed the positive results for seven bioactive compounds like tannins, saponins, flavanoids, resins, terpenoids, steroids and phenolic compounds. Whereas, the methanol leaf extracts showed the positive results for only four bioactive compounds like saponins, flavanoids, glycosides and steroids.

Based on the results, the methanolic seed extracts of the *M. fragrans* plants showed positive results for many bioactive compounds and followed by methanol leaf extracts showed moderate positive results of active compounds. It is explained that the polarity level and species nature are playing major role in extracting the secondary metabolites. Many researchers also informed that the components arranged plants are largely polar. There are different factors that will affect the quantity and composition of the phytocompounds present in an extract. Among these are the types of extraction, time of extraction, temperature, nature of the solvent, solvent concentration and lastly polarity of the solvent (11). These differences may be attributed to the microclimate, processing method as well as the type of solvent employed (12, 13) and genetic variation (14). It also reported that the plants components are more soluble in high polar solvents. It can therefore, be deduced that the amount of extracts recovery is polarity dependent (15). Aqueous could dissolve alkaloid and glycoside compounds, but ethanol was effective to extract sterol, flavonoid, phenolic, and alkaloid (16). FTIR spectrum is generally used tool in plant biological studies (17).

Various chemicals have been used to extract bioactive compounds from plants. In this extraction, the high polar solvent like methanol showed differential extraction i.e., more compounds from seed and some of the compounds from leaf organs of the two studies plant species. The differential extractions may be due to degrading enzymes that may be active or denatured in the alcoholic extractants (18). These secondary metabolites are reported to have many biological and therapeutic properties (19 & 20). The presence of phenolic compounds in the plants indicates that these plants may be an antimicrobial agent (21). Tannins are known to be inhibiting pathogenic fungi (22). Saponin has the property of

precipitating and coagulating red blood cells (23 & 24). Glycosides also have vast therapeutic efficacy as they are found in almost every medicinal plant. Proteins are the building blocks of life. The body needs protein to repair and maintain itself (25).

Therefore, the data obtained from the experiments have provided the chemical basis for the wide use of these plants as therapeutic agents for treating different ailments. The traditional medicine practice is recommended strongly for these plants as well as it is suggested that further work should be carried out to isolate, purify, and characterize the active constituents responsible for the activity of these plants. Also additional work is encouraged to elucidate the possible mechanism of action of these extracts.

4. CONCLUSION

In conclusion, the study findings support these plants can also be used to discover bioactive natural products that may serve as leads for the development of new pharmaceuticals. Hence, the above plants extract could be explored for its highest therapeutic efficacy due to the presence of bioactive components by pharmaceutical companies in order to develop safe drugs for different ailments in future.

Table – 1 Phytochemical screening of Methanol leaf extract of *Ziziphus jujuba*

S.No	Tests	Leaves of *Ocimum tenuiflorum*
1.	Tannins	-ve
2.	Saponins	+ve
3.	Flavanoids	+ve
4.	Glycosides	+ve
5.	Resins	-ve
6.	Terpenoids	-ve
7.	Steroids	+ve
8.	Protein	-ve
9.	Phenolic compounds	-ve
10.	Acetic compounds	-ve

Table – 2 Phytochemical screening of Methanol seed extract of *Myristica fragrans*

S.No	Tests	Leaves of *Ocimum tenuiflorum*
1.	Tannins	+ve
2.	Saponins	+ve
3.	Flavanoids	+ve
4.	Glycosides	-ve
5.	Resins	+ve
6.	Terpenoids	+ve
7.	Steroids	+ve
8.	Protein	-ve
9.	Phenolic compounds	+ve
10.	Acetic compounds	-ve

Table – 3: FTIR spectral peak values and functional groups of dried leaf powder of *Ziziphus jujuba*

Spectrum No.	Wave Number cm-1	Functional group	Predicted compound
1	3420.14	N-H :stretching mode	Amine group
2	2919.7	O-H: Stretching mode	Carboxylic acid
3	1637.27	C=C,C=O :stretching mode	Conjugation of allergies with two aromatic ring, amide

4	1542.77	C=C: stretching mode, N-H:Bending in secondary amine, -NO2:Asymetric stretching mode	Aromatic ring, Aliphatic nitro
5	1455.08	N-H : Bending in secondary amine	Amine group
6	1245.79	C-O, C-F Stretching mode, C=O, C-O-H:Bending mode	Alcohol, Ether, Carboxylic acid
7	1056.8	C-O : Stretching mode C-O : Stretching mode	Alcohol Ether

Table – 4:FTIR spectral peak values and functional groups of dried seed powder of *Myristica fragrans*

Spectrum No.	Wave Number cm-1	Functional group	Predicted compound
1	3391.21	Trible Bound C-H, N-H, O-H:stretchingmode,Hydrogen bonded O-H band	Alcohol, Carboxylic acid
2	2922.59	O-H :stretching mode	Carboxylic acid
3	2852.2	C-H, O-H: stretching mode	Aldehyde hydrogen, Carboxylic acid
4	1707.6	C=O: stretching mode	Carboxylic acid
5	1635.34	C=C,C=O:stretching mode, N-H Bending mode	Conjugation of Aldehyde with two aromatic ring,Amide
6	1514.81	C=C:Stretching mode, N-H: Bending in secondary amine, -NO2:Asymmetric stretching mode	Aromatic nitro, aromatic ring
7	1460.81	N-H: Bending in secondary amine,	Amino related
8	1375	C-O-H bending mode, -NO2:symmetric stretching mode	Aliphatic nitro
9	1239.04	C-O-H, C=O bending mode, C-O, C-F: stretching mode	Ether, Alcohol, Carboxylic acid
10	1128.15	C-O : Stretching mode C-O : Stretching mode	Alcohol Ether
11	1047.16	C-O : Stretching mode C-O : Stretching mode	Alcohol Ether
12	862.989	=C –H : out of- plane bending mode	Aromatic ring
13	720	=C-H: out of plane bending mode, C-CI: stretching mode	Alcohol, Ether, Aromatic ring

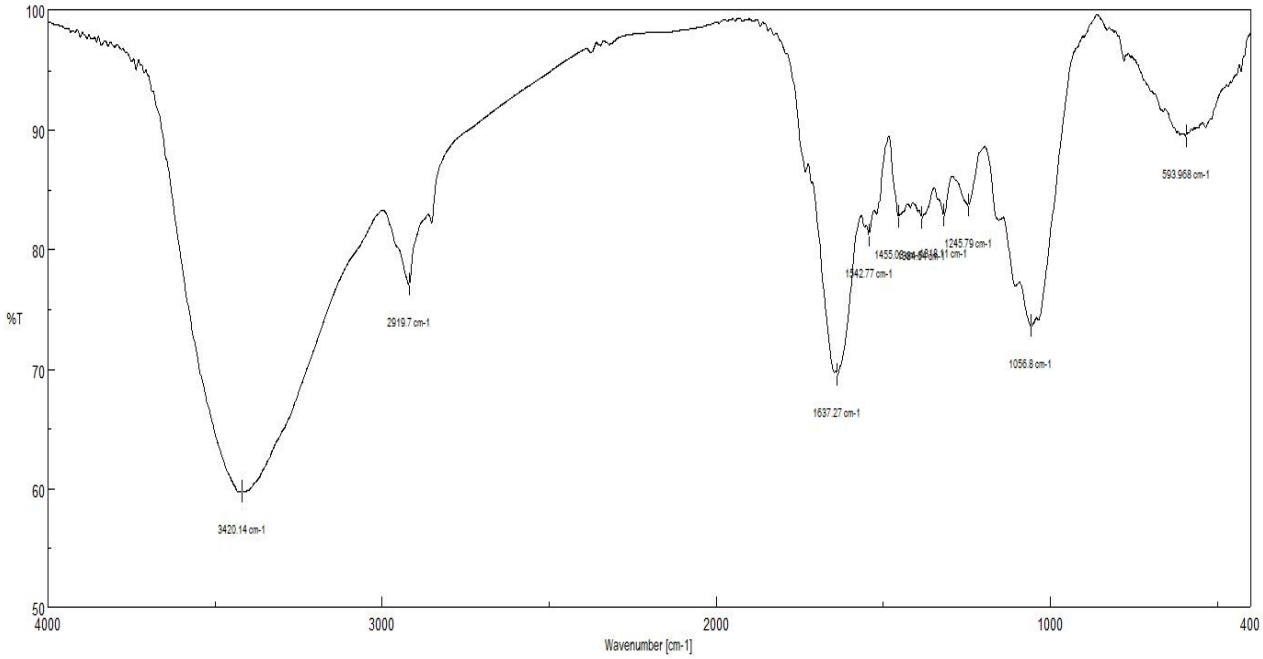

Fig.I: FTIR spectrum of dried leaf powder of *Ziziphus jujube*

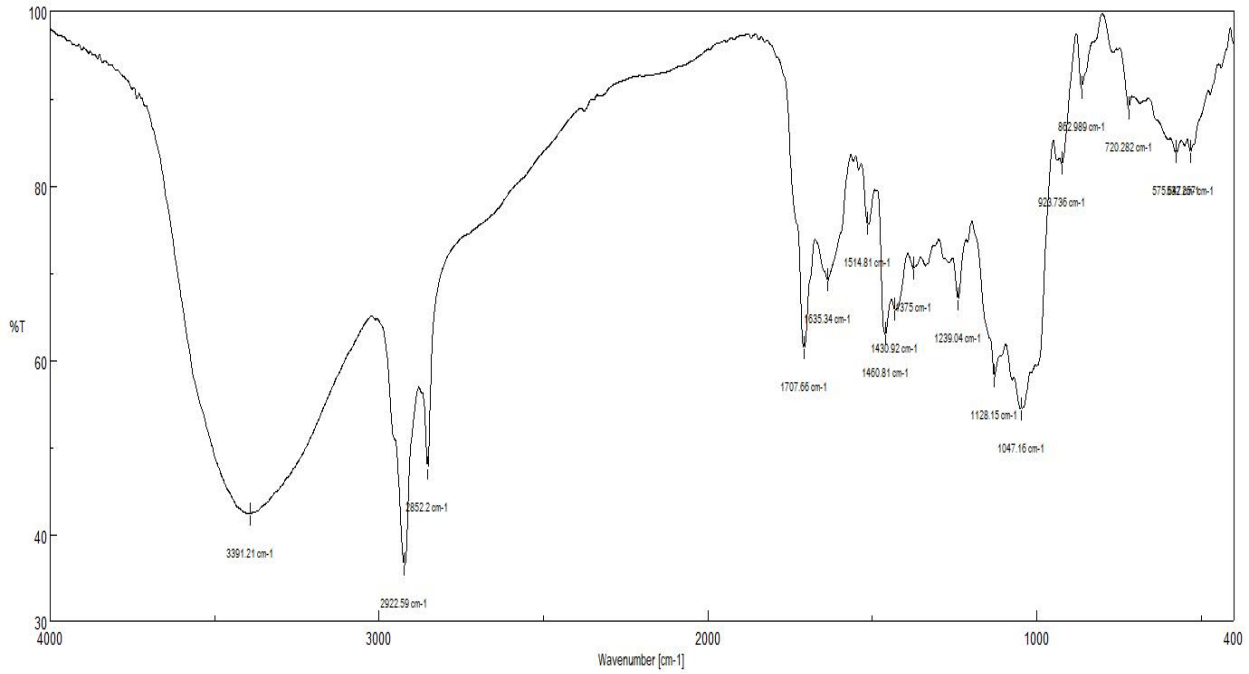

Fig.II: FTIR spectrum of dried seed powder of *Myristica fragrans*

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