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Original Article

Phytochemical Screening and FTIR Analysis of *Citrus maxima* Linn. Leaves

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ABSTRACT

Citrus maxima Linn. (Rutaece), commonly known as Shaddock or pomelo is indigenous to tropical parts of Asia. The present study was aimed to screen the phytochemicals and FTIR analysis of Citrus maxima ethanolic leaf extract. The results revealed that the alkaloids, steroids, flavonoids, phenolic compounds, proteins, carbohydrates, cardiac glycosides and saponins were present in ethanolic extract. The FTIR spectroscopic studies revealed different characteristics peak value with various functional compounds in the extracts. The FTIR analysis of ethanol leaf extracts of Citrus maxima confirmed the presence of amide, alkenes, alkyne, alkane, ether, alcohol, ketone, alkyl halides and aromatics groups in the leaf extracts. The study concluded that the ethanolic extract of Citrus maxima has potential bioactive compounds and it could be utilized in pharmaceutical industries.

Keywords: *Citrus maxima*, ethanolic extract, phytochemicals, FTIR.

1. INTRODUCTION

Medicinal plants are abundantly available all over the world and are more focused than ever because they have the ability to produce many benefits to human society, especially for the treatment of various types of human ailments. They serve as therapeutic agents as well as important raw materials for the manufacture of traditional and modern medicine [1]. They are the richest bio-resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs [2]. In most of the traditional systems of treatment, the use of medicinal plant include the fresh or dried parts, whole, chopped, powdered

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or an advanced form of the plant usually made through extraction with different solvents play a major role and constitute the backbone of the traditional medicine [3].

The therapeutic properties of medicinal plants are due to some chemical compounds they synthesize. These are regarded as secondary metabolites because the plants that synthesise them may have little need for them [4]. They are synthesized in all parts of the plant body; bark, leaves, stem, root, flower, fruits, and seeds [5]. Plant produces these chemicals to protect itself from herbivores but recent research demonstrates that many phytochemicals can protect humans against diseases. Different phytocontituents present in medicinal plants are flavonoids, carotenoids, alkaloids, anthocyanidins, phenolics and tannins, carboxylic acids, terpenes, amino acids, and inorganic acids etc [6].

Citrus maxima are a perennial shrub commonly known as Pamelo, distributed throughout India. Bark and root of *Citrus maxima* contain β-sitosterol, acridone alkaloid. Essential oil from the leaves and unripe fruits contain limonin, nerolol, nerolyl acetate and geraniol [7.] Like other citrus plant pommelos are rich in Vitamin C. They are generally used eaten as fruit. It has been used in indigenous system of medicine as sedative in nervous affections, convulsive cough and in the treatment of hemorrhagic diseases and epilepsy. It is said to poses appetizing, cardiac stimulant and antitoxic property [8] . *Citrus maxima* fruits also contains high amount of polyphenolic compound like hesperidin, naringin, caffeic acid, P-Coumaric acid, Ferulic acid and vanillic acid [7]. It shows various pharmacological activities which has been studied. The present study was carried out to screen the phytochemical constituents and FTIR analysis of *Citrus maxima* leaf.

2. MATERIALS AND METHODS

Collection of plant materials

Mature leaves were collected from the healthy plant in Mannargudi, Thiruvarur District. The collected plant materials were botanically authenticated by the Director S.John Britto, RAPINET Herbarium, St.Joseph's College, Tiruchirapalli.

Preparation of plant extract

The leaves were washed in tap water, shade dried for 10 days and made into a fine powder with 40 mesh. Following that, 100g of the powder was filled in the thimble and extracted using 500 ml of distilled ethanol in soxhlet apparatus for 8 to 10 hours. The extract was filtered through Whatman no.1 filter paper to remove all unextractable matter, including cellular materials and other constitutions that are insoluble in the extraction solvent. The entire extract was concentrated to dryness using water bath and stored for further use.

Qualitative phytochemical analysis

Qualitative phytochemical analysis were done using the procedures of Kokate, (1994) [9] . Alkaloids, carbohydrates, tannins and phenols, flavonoids, gums and mucilage, fixed oils and fats and saponins were qualitatively analyzed.

Fourier Transform Infrared Spectrophotometer (FTIR)

Fourier Transform Infrared spectrophotometer (FTIR) is perhaps the most powerful tool for identifying the types of chemical bonds (functional groups) present in compounds. The wavelength of light absorbed is characteristic of the chemical bond as can be seen in the annotated spectrum. By interpreting the infrared absorption spectrum, the chemical bonds in a molecule can be determined.

Dried powder of ethanol solvent extract of plant material was used for FTIR analysis. 10 mg of the dried extract powder was encapsulated in 100 mg of KBr pellet, in order to prepare translucent sample discs. The powdered sample of each plant specimen was loaded in FTIR spectroscope (Shimadzu, IR Affinity 1, Japan), with a Scan range from 400 to 4000 cm^{-1} with a resolution of 4 cm^{-1}.

3. RESULTS AND DISCUSSION

Phytochemical Screening

The present study has shown that *Citrus maxima* leaf contains medicinally active constituents. Preliminary phytochemical screening tests for ethanol extract of *Citrus maxima* showed the active phytochemical classes as alkaloids , amino acids, carbohydrates , carotenoids , coumarins , flavonoids, monoterpenes , sesquiterpenes , steroids as presented in **Table 1.**

Table1: Phytochemical Analysis of *Citrus maxima* leaves

S.No	Phytochemicals	Results
1	Alkaloids	+
2	Aminoacid	+
3	Carotinoids	+
5	Triterphenoids	-
6	Carbohydrate	+
7	Coumarins	+
8	Flavonoids	+
11	Protein	-
12	Tannin	-
13	Saponin	-
14	Sesquiterpene	+
15	Phenol	-
16	Anthroquinones	-
17	Steroids	+

(+) indicate presence whereas (-) indicates absence

Medicinal plant extracts are reported to have health beneficial properties that are due to secondary metabolites such as alkaloids, amino acids, carbohydrates, carotenoids, coumarins, flavonoids monoterpenes, sesquiterpenes, steroids etc., present in them. These bio-components are known for their versatile biological effects and are implicated in treatment of variety of diseases. Tannins are considered as superior antioxidants as they prevent cellular damages by shielding the proteins from oxidation and glycation reactions, besides their copper scavenging action [10]. The relaxing effect of tannins on vascular segments indicates their protective effect in cardiovascular complications [11]. Saponins are reported to enhance glucose

utilization by regulating glucagon [12] and insulin secretion thus implicating its role in hypoglycemic action in medicinal plants [13]. Alkaloids have been shown to exhibit cytotoxic effect on tumour cell lines emphasizing its role in prevention of cancer, neurodegenerative diseases, chronic inflammation etc.,[14]. Potential free radical scavenging and termination of chain reaction in lipid peroxidation reactions merits flavonoids as one of the famous class of polyphenols having beneficial effect in stress related complications [15]. The bioactivity of polyphenolics as free radical scavenger is due to their ability to chelate metal ions, redox properties and inhibition on lipooxygenase enzymes [16]. Incidentally polyphenols are effective secondary antioxidants as they can stabilize the oxidised form of the metal ions which otherwise are instrumental in formation of most toxic reactive hydroxyl radicals from reaction between superoxide anion and hydrogen peroxide [17]. Bio-functionalities of these secondary metabolites present in the extracts influence the biological activities of the plants. In addition, protective ability of plant extracts against the pathological diseases is related to total phenolics and flavonoids in the plant samples as they have been recognized to exhibit various biological activities [18,19].

Fourier Transform Infrared Spectroscopic Analysis (FT-IR)

The FTIR spectrum of ethanol extract of *Citrus maxima is* presented in **Table 2 and Figure 1.** The data on the peak values and the probable functional groups (obtained by FTIR analysis) present in the ethanol extracts of *Citrus maxima* are represented. The region of IR radiation helps to identify the functional groups of the active components present in extract based on the peaks values of the FTIR spectrum. When the extract was passed into the FTIR, the functional groups of the components were separated based on its peaks ratio. The results of FTIR analysis confirmed the presence of alcohol, aldehyde, alkyne, alkene, amines and ester. The absorbance bands analyses in bioreduction process are observed in the region between 400–4000 cm^{-1} are 1015.14, 1105.48, 1644.52, 2141.50, 2838.40 and 3354.08. Major peaks were observed at 3354.08 cm^{-1} that could be assigned to the 0-H stretching vibrations of O-H Alcohol.

Table 2: FTIR analysis of *Citrus maxima* Leaves

S.No	Peak value	Functional Group
1	3422.13	-N-H Stretch
2	2923.24	-C-H aldehyde
3	2834.33	-C-H Stretch
4	2117.63	C≡C Stertch
5	1627.01	C=C alkene
6	1421.75	C=C aromatic
7	1384.38	CH3 bend
8	1818.74	C=O anhydrite
9	1238.30	C-O-C Stretch
10	1101.25	C-OH Stretch
11	1065.42	C-OH Stretch
12	894.45	C-F
13	780.58	C-Cl
14	666.92	C-Br
15	833.88	C-F

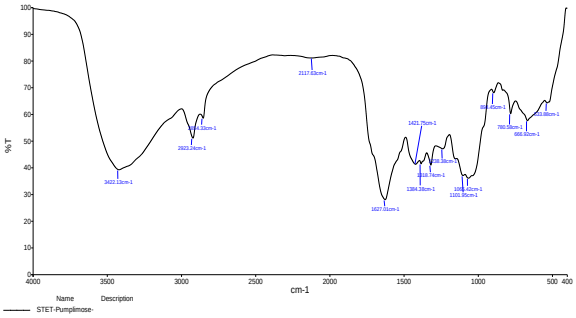

Fig 1: FT-IR Spectra of *Citrus maxima* L. leaves

The FTIR analysis of methanol and aqueous leaf extracts of *Bauhinia racemosa* revealed the presence of protein, oil, fats, phenolic compounds, flavonoids, saponins, tannins and carbohydrate as major functional groups analyzed [20, 21] the ethanol extracts of *Ichnocarpus frutescens* using FTIR analysis that revealed functional group components of amino acids, amides, amines, carboxylic acid, carbonyl compounds, organic hydrocarbons and halogens [22] also worked in the methanol leaf extracts of *Solanum torvumto* confirm the presence of alcohol, alkanes, aromatic carboxylic acid, halogen compound, alkyl halide through the FTIR analysis. The characterization and antibacterial effect of plant-mediated silver nanoparticles using *Ceropegia thwaitesii* was carried out [23] and the presence of triterpenoids and methoxy groups played an important reduction role in the synthesis process was also authorized by them using FTIR. The absorbance bands analysis in bioreduction is observed in the region of 400–4000 cm^{-1} are 1024.02, 1383.68, 1629.55, 2921.63 and 3449.30 cm^{-1} , Major peaks were observed at 2921 cm1 that could be assigned to the C-H stretching vibrations of methyl, methylene and methoxy groups. But major peaks observed in the crude methanol extract of *Citrus maxima* are 3354.08 and 2838.40 that are indicating the presence of O-H alcohol and =C-H aldehyde groups. The result of the FTIR analysis is contradictory to the results [24, 25]. The results of the present study confirmed that *Citrus maxima* may be wealthy resource of phytoconstituents which can be isolated and examined for further pharmacological activities.

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