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Determination of elemental and morphological properties of *Aegle marmelos* (L.) by FTIR and FESEM technique

Jadhav M.G.*, Pawar S.S. and Deokar T.G.
Department of Zoology, Yashwantrao Mohite College of Arts, Science and Commerce, Bharati Vidyapeeth Deemed University Erandwane, Pune-411038, India
mgjvvk11@yahoo.com

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Abstract

Aegle marmelos (L.) is a supreme indigenous medicinal plant. It's all parts have been used in ethnomedicine to make use of its medicinal properties including antidiarrhoel, antidysentric, antioxidant, anti-inflammatory etc. Different parts of Aegle marmelos (L.) contains alkaloids, flavonoids, tannins, steroids, polyphenols etc. The objective of this study was to identify the functional groups present in Aegle marmelos (L.) leaves by Fourier Transform Infrared Spectroscopy and to determine its elemental and morphological properties by Field Emission Scanning Electron Microscopy. Result of the FTIR spectra of Aegle marmelos (L.) leaves revealed the presence of different functional groups indicating the presence of aldehydes, amines, alcohols, phenols, ethers, esters, carbohydrates etc. These results confirm the presence of secondary metabolites like alkaloids, saponins, tannins, flavonoids, steroids, polyphenols etc. The FESEM technique with EDAX showed the presence of various elements like C, O, N, K, Ca, Mg, Al, Cl, Se, Pt, Na, S, P, Be, Ba, Cu, Ag, Cr, Ni, Co, Mn, Zn and Fe. The present study on Aegle marmelos (L.) leaves will provide useful information about qualitative, quantitative and pattern of composition of these biocomponents responsible for its medicinal properties.

Keywords: *Aegle marmelos* (L.), FTIR, FESEM, EDAX, Secondary metabolites.

Introduction

From ancient times plants have been used in traditional medicine. Medicinal plants are considered as wide source of active pharmacological constituents which are used in home for multiple ailments in India[1]. Secondary metabolites of plants possess different biological activities[2-5]. *Aegle marmelos* (L.) is an indigenous plant belongs to the family Rutaceae. Lots of work has been carried by several workers on various parts of *Aegle marmelos* (L.) and some important phytoconstituents such as triterpenes, coumarins, flavonoids, alkaloids, sterols and essential oils was observed in their investigation[6-10]. *Aegle marmelos* (L.) is of very high value in treating cardiac disorders, dysentery, diarrhea, diabetes, fever, inflammation, and pain[11]. The antioxidant, anticancer, antihyperglycemic, anti-inflammatory, antipyretic, analgesic and chemo protective activities of the leaves of this plant have also been studied[12]. The leaves of *Aegle marmelos* (L.) are used as laxative, febrifuge and expectorant, also in deafness, ophthalmic defects, catarrh, antifungal and asthmatic complaints[13]. Natural plants are very healthier and safer rather than synthetic drugs. For diagnosis, treatments and in pharmaceutics phenomenal progress has been made in the field of medicine with the advancement in science and technology. Recent natural drug discovery techniques based on structure activity relationships, computer modeling, combinatorial chemistry, high throughput screening and spectroscopy have triggered and spearheaded the discoveries of many natural and synthetic drugs[14]. Various advanced techniques like HPTLC, NMR, GC-MS have been employed to characterize the medicinal plants for the identification, isolation and quantitative determination of biocomponents, key metabolites and their molecular structure[15-19]. The FTIR is a valuable tool for the characterization and identification of compounds or functional groups present in plant. In addition FTIR spectra of pure compounds are usually so unique that they are like a molecular fingerprint. Elements play crucial roles in various activities as a chemical constituent used by body in many ways[20,21]. Plants are the rich source of minerals and for normal life processes every form of living matter requires these elements[22,23]. Mineral elements play significant role in health and disease states of humans. Metal content must be properly balanced for the proper functioning of the human body[24]. The reliable measurement of elements in herbal medicinal plant is becoming more important. Therefore the aim of the present study was to identify the functional groups present in *Aegle marmelos* (L.) leaves by FTIR and to find out its elemental and morphological properties by FESEM technique.

Materials and methods

Plant material: Fresh leaves of *Aegle marmelos* (L.) were collected by taking all the precautions and by avoiding damage to plant life. The herbarium of *Aegle marmelos* (L.) was prepared and authentication has been obtained (MGJ - 2) from Scientist D and HOD, Botanical survey of India, Pune, Maharashtra. The shade dried leaves of *Aegle marmelos* (L.) were powdered and used for further analysis.

Characterization: Fourier Transform Infrared Spectroscopy (FTIR): The dried leaf powder of *Aegle marmelos* (L.) was analyzed using Fourier Transform Infrared Spectroscopy (Shimadzu, Japan 8000 Series). 10 mg of the dried leaf powder was encapsulated in 100 mg of KBr pellet, in order to prepare translucent sample discs. The powdered sample was loaded in FTIR spectrophotometer with scan range from 400 to 4000 cm^{-1} with a resolution of 4 cm^{-1} and FTIR spectrum was recorded.

Field Emission Scanning Electron Microscopy (FESEM): The dried leaf powder of *Aegle marmelos* (L.) was subjected to elemental analysis using Field Emission Scanning Electron Microscopy equipped with Energy Dispersive X Ray Spectroscopy (FESEM, Hitachi- S-3000H). Secondary electron modes were employed under 1500x and 5000x magnification to examine the homogeneity and microstructure of the sample. The micrograph of sample was taken after coating with Platinum to avoid charging. The elemental analysis of major and minor elements present in the sample was done using Energy Dispersive X-Ray Spectrometer with accelerating voltage 15kV and high vacuum mode (HV).

Results and discussion

Fourier Transform Infrared spectroscopy (FTIR): Results of FTIR spectroscopic study revealed structural information of various chemical constituents in the leaf powder of *Aegle marmelos* (L.) which showed variations in their peaks. By interpreting the infrared absorption spectrum various absorption bands and wave numbers of prominent peaks were obtained from FTIR spectra (Figure-1 and Table-1). The peaks observed at 3391.64 cm^{-1} and 3194.04 cm^{-1} corresponding to O-H broad and N-H stretch representing phenols, amines and amides. The peak intensities at 2918.67, 2849.58 and 1731.40 cm^{-1} in the IR spectrum display the characteristic absorption of methylene, alkyl, methoxy, methyl ether and aldehyde groups respectively. The strongest peak at 1600.79 cm^{-1} assigned to the C=C bending which indicates the presence of aromatic compounds. The band at 1557.55 cm^{-1} represents carboxylic acid and aliphatic nitro compounds. The peak at 1515.87 cm^{-1} showed C-C stretching that is mainly generated by aromatic compounds. Cyclic ethers and aliphatic fluoro compounds were detected at 1137.90 and 1081.19 cm^{-1}. More intense absorption bands were observed at 1416.74, 1242.32, 1041.73 and 1011.08 cm^{-1} corresponding to vinyl, carboxylic acid, aromatic ethers, primary amines, cyclohexanes and alkynes.

Ramamurthy and Kannan used FTIR spectroscopy to reveal qualitative characters regarding the organic molecules in *Calotropis gigantea* collected from industrial area – SIPCOT[25]. The more intense bands occurring at 3419, 2924, 2854, 1635, 1406, 1242, 1070, and 617 cm^{-1} corresponding to O-H/N-H, C-H, C-O and C-CI/C-CS stretching/bending vibrations respectively suggest the presence of amino acids, alkenes, nitrates, ethers, organic halogen compounds and carbohydrates in plants[26]. Presence of alkyl, methyl groups, alcohols, ethers, esters, carboxylic acid, anhydrides and deoxyribose indicates the appearance of C=O, C-H, C=C, C-O, C-C bonding structures[27,28]. The findings of the present study confirms the presence of secondary metabolites like alkaloids, saponins, tannins, flavonoids, steroids, polyphenols etc. responsible for the medicinal property of *Aegle marmelos* (L.).

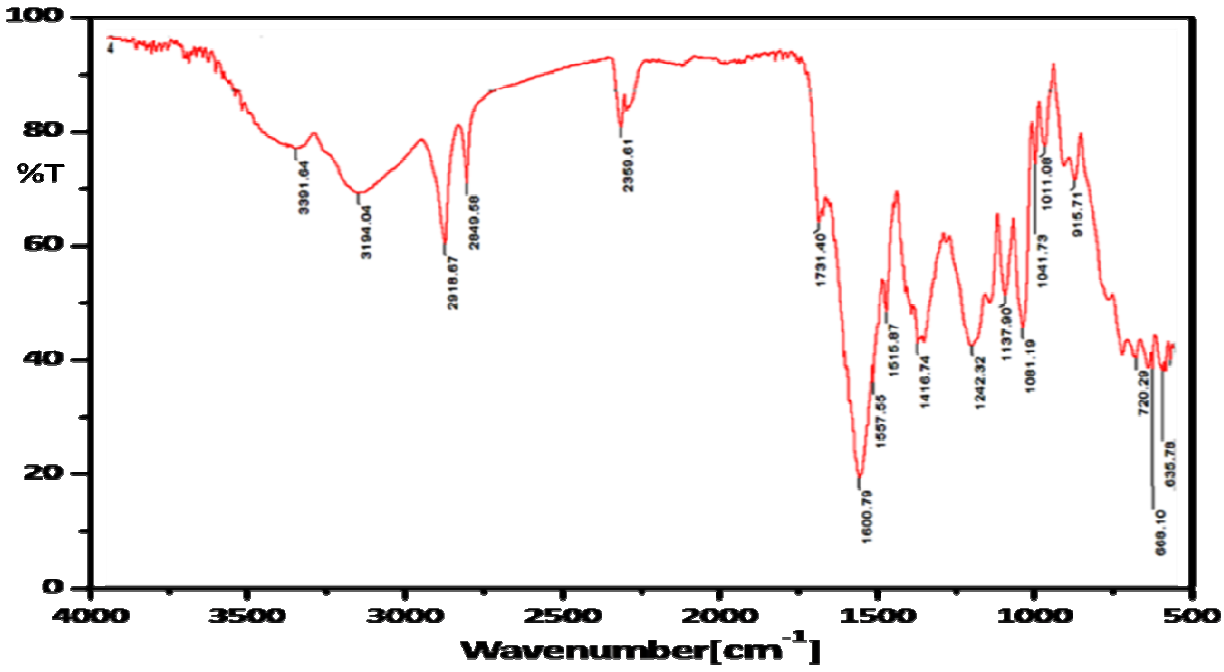

Figure- 1: Fourier Transform Infrared Spectroscopy (FTIR) spectrum of leaf powder of *Aegle marmelos* (L.).

Table-1: Fourier Transform Infrared Spectroscopy (FTIR) spectrum analysis of leaf powder of *Aegle marmelos* (L.).

Functional groups	Components (Peaks)	Wavenumber (cm^{-1})
Alcohols, Phenols	O-H Broad	3391.64
Amines and Amides	N-H Stretch	3194.04
Methylene	C-H Stretch	2918.67
Alkyl	C-H Stretch	2849.58
Silica	Si-H- Silane	2359.61
Aldehyde	C=O Stretch	1731.40
Aromatic	C=C Bend	1600.79
Carboxylic acid and Aliphatic nitro compound	C-H Stretch	1557.55
Aromatic ring	C-C Stretch	1515.87
Venyl	C-H Bend	1416.74
Aromatic ethers	Aryl-O Stretch	1242.32
Cyclic ethers	-C-O Stretch	1137.90
Aliphatic fluoro compounds	C-F Stretch	1081.19
Aliphatic fluoro compounds Primary amines	C-F Stretch C-N Stretch	1041.73
Aliphatic fluoro compounds	Cyclohexane ring vibrations C-F Stretch	1011.08
Venyl	C-H Out of plane bend	915.71
Methylene	-(CH_2)n- Rocking	720.29
Alcohol	O-H Out of plane bend	668.10
Alkylene	C-H Bend	635.78

Field Emission Scanning Electron Microscopy (FESEM): The data obtained from FESEM with EDAX analysis showed the surface morphology and elemental composition of *Aegle marmelos* (L.). The microstructures obtained by FESEM and elemental compositions screened by EDAX were depicted in Figure-2, 3 and Table-2 respectively. FESEM images were obtained under high and low magnification. In the present study various elements of biological importance in human metabolism were found. Total twenty three elements were detected in dried leaf powder of *Aegle marmelos* (L.). The observations revealed that the concentration of C, O, N, Ca, Mg, S and Na is 49.37%, 35.22%, 1.96%, 3.57%, 3.52%, 1.33%, 0.92% and 0.69% respectively. For normal functioning of cardiac muscles, blood coagulation, milk clotting and regulation of cell permeability Calcium is necessary while Magnesium carries various biochemical and physiological functions[29].

Sodium is the principal cation in extracellular fluids[30]. The concentration of iron is 0.06%. Iron is involved in cellular respiration[20]. The concentration of Nickel is 0.19%. Manganese concentration is 0.13%. Manganese is the vital micro nutrient for animals and plants, its insufficiency in humans has been associated with several diseases[31].

The concentration of copper is 0.27%. Copper is involved in protein metabolism and provides metabolic control over auxin synthesis. Lack of Copper in body shows demonetization of bones, anemia, diarrhea, neutropenia and amyelination of the central nervous system in the newborn[32]. In the present investigation the concentration of zinc is 0.17%. For the normal functioning of cells including carbohydrates metabolism, cell growth and cell division Zinc is an essential element [33]. Cobalt is a useful constituent of vitamin B12. Its concentration in *Aegle marmelos* (L.) is 0.08%. Selenium is essential trace element. It is a constituent of antioxidant enzyme which is a part of defense system that protect against harmful effect of free radicals[34].

The concentration of selenium in the studied plant is 0.13%. Other essential elements such as Al (6.54%), Cl(0.62%), P(0.54%), Be(0.12%), Ba(0.08%), Ag (0.21%), Cr(0.09%) and Pt(0.19%) are found present in sufficient concentration which are necessary for plant and animal life. If any of these metals get lacks from nutrition one can suffer from health problems. In our study no any toxic element was detected in *Aegle marmelos* (L.) From the present investigation of elemental analysis it was observed that *Aegle marmelos* (L.) can be used as a potential source of herbal medicine for various disorders.

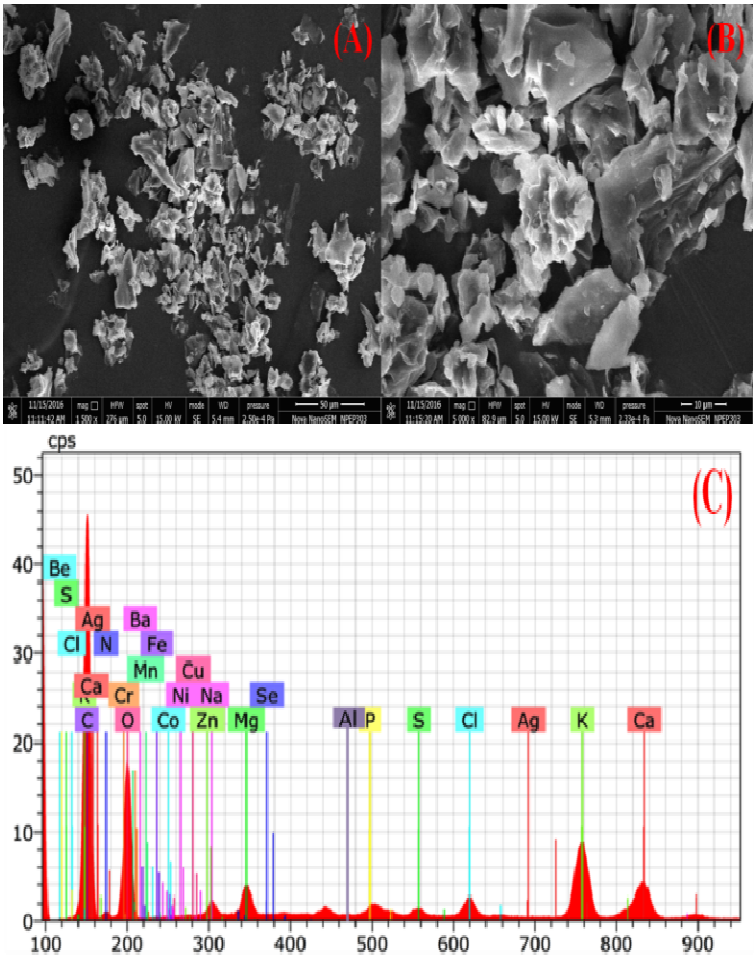

Figure-2: (A) and (B) Field Emission Scanning Electron Microscopy (FESEM) Images, (C) EDAX analysis of leaf powder of *Aegle marmelos* (L.).

Table-2: EDAX elemental analysis of leaf powder of *Aegle marmelos* (L.).

Element	Atomic No.	Atomic%	Element	Atomic No.	Atomic%
C	6	49.37	P	15	0.54
O	8	35.22	Be	4	0.12
N	7	1.96	Ba	56	0.08
K	19	3.57	Cu	29	0.27
Ca	20	3.52	Ag	47	0.21
Mg	12	1.33	Cr	24	0.09
Al	13	0.54	Ni	28	0.19
Cl	17	0.62	Co	27	0.08
Se	34	0.13	Mn	25	0.13
Pt	78	0.19	Zn	30	0.17
Na	11	0.69	Fe	26	0.06
S	16	0.92	Total		100

Conclusion

From the results it can be concluded that FTIR spectroscopy is valuable technique to fingerprint and to analyze the different biomolecules in plants. This method also offers a rapid and nondestructive investigation to identify bioconstituents. The functional groups present in *Aegle marmelos* (L.) leaf powder are aldehydes, amines, amides, alcohols, phenols, aromatic compounds, carbolic acids, esters, ethers, mythelene, aliphatic fluoro compounds and alkynes. All these compounds belong to secondary metabolites and could be responsible for medicinal properties of *Aegle marmelos* (L.). From FTIR analysis, more characteristic peaks of flavonoids, alkaloids and tannins were observed. The FESEM technique with EDAX can help to confirm morphological ultra structure and elemental compositions in *Aegle marmelos* (L).

The image obtained by FESEM technique will be useful in future to investigate the nanostructure of *Aegle marmelos* (L). EDAX analysis revealed that, *Aegle marmelos* (L.) contains sufficient amount of essential elements which can be considered as a possible source of elements other than diet. Based on FTIR and FESEM analysis in *Aegle marmelos* (L.) no toxic compound was observed. Therefore, the present study on *Aegle marmelos* (L.) leaves will be useful for quality control, to determine the dosage, for ensuring purity, safety and efficacy of natural products.

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