



## FESEM and FTIR spectroscopic characterization of *Aegle marmelos* (L.) unripe fruit

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### Abstract

*Aegle marmelos* (L.) is very important fruit plant in all over India which has great importance due to their economic value, nutritive value and major source of medicines. *Aegle marmelos* (L.) unripe fruit is considered as most natural medicinal fruit. In fruit plant the knowledge of elements are very important because it may influence the production of their bioactive constituents and their pharmacological action. Active constituents of plant are metabolic products of plant cells and a number of trace elements play an important role in metabolism. In present study characterization of *Aegle marmelos* (L.) unripe fruit powder was carried by Field Emission Scanning Electron Microscopy and Fourier Transform Infrared Spectroscopy. The *Aegle marmelos* (L.) unripe fruit powder showed various elements like C, O, N, K, Ca, Mg, S, Se and Pt. FTIR spectra showed various functional groups to elucidate its structure and composition. The FESEM technique with EDAX helps to characterize the biomaterial in *Aegle marmelos* (L.) at its elemental and morphological level. The present study concluded that, determination of functional groups by FTIR and characterization of elemental and morphological properties by FESEM techniques helps to identify the potential bioactive constituents of *Aegle marmelos* (L.) unripe fruit.

**Keywords:** *Aegle marmelos* (L.), FTIR, FESEM, EDAX, Bioactive constituents.

### Introduction

In the course of many centuries based on different medicinal systems such as Ayurveda, Unani and Siddha the knowledge of medicinal plants has been accumulated. For treatment of various diseases as well as a source for livelihood human population depends on medicinal plants in a large number of countries<sup>1</sup>. *Aegle marmelos* (L.) commonly known as Bael, belongs to family Rutaceae<sup>2</sup>. It is cosmopolitan distributed in the deciduous forests of India<sup>3</sup>. *Aegle marmelos* (L.) is very important fruit plant shows economic value, nutritive value and major source of medicines<sup>4</sup>.

Fruits are major sources of minerals, fibers and vitamins provides nutrients for the human. *Aegle marmelos* (L.) unripe fruit is considered as most natural medicinal fruit shows various nutritional and therapeutic properties and it contains many functional and bioactive compounds such as phenolics, alkaloids, flavonoids, coumarins, carotenoids, terpenoids and other antioxidants which may protect us against chronic diseases like cancer, cardiovascular diseases and other aging-related pathologies<sup>5-9</sup>. Many researchers reported that *Aegle marmelos* (L.) fruit contain vitamin C, vitamin A, thiamine, riboflavin and many trace elements in the body play an important role in metabolism<sup>10,11</sup>.

Metallic and nonmetallic elements are required for growth and health of human beings within certain permissible limits<sup>12</sup>. Metallic products of plant cells are the active constituents of plants and in various metabolism trace elements play an

important role<sup>13</sup>. Very less literature is reported about trace elemental composition in fruit plants, the literature survey on these fruit plants is mostly concern with their bioactive components, organic compositions and their pharmacological effects<sup>14</sup>. Identification of secondary metabolic compounds has been investigated by various scientists using different methods<sup>15-19</sup>. Scanning electron microscopy (SEM) and energy dispersive X-ray microanalysis (EDX) is a prominent method can provide information about the surface morphology and the elemental compositions of the sample. The Fourier-transform infrared is a rapid, reputable and valuable method for the structural determination of bioactive compounds. Hence, the aim of the present study was to determine the functional groups and characterization of elemental composition and morphological properties of *Aegle marmelos* (L.) unripe fruit by FESEM and FTIR spectroscopic method.

### Materials and methods

**Plant material:** *Aegle marmelos* (L.) unripe fruits were collected by taking all the precautions and by avoiding damage to the plant life. The *Aegle marmelos* (L.) plant was authenticated by Scientist D. Botanical Survey of India, Pune, and Maharashtra. The Specimen No. (TGD-1) was kept to herbarium department in Botanical Survey of India, Pune. The unripe fruits were washed and manually their hard rind was broken. Then the pulp separated from fruit and dried. The dried pulp powder was prepared and preserved in airtight container for further experimentation.

**Characterization: Field emission scanning electron spectroscopy:** The unripe fruit powder of *Aegle marmelos* L. was subjected to elemental analysis by using Field Emission Scanning Electron Microscopy (FESEM) technique with energy dispersive x-ray spectroscopy EDAX (FESEM, Hitachi-S3000H). Secondary electron modes were employed under x3000 and x5000 magnification to examine the nature, homogeneity and microstructure of the sample. The micrograph of sample was taken after coating with Platinum to avoid charging. The elemental composition of the sample was done using energy dispersive x-ray spectrometer with accelerating voltage at 15 KV and high vacuum mode (HV).

**Fourier transform infrared spectroscopy:** *Aegle marmelos* (L.) unripe fruit powder was analyzed by Fourier Transform Infrared Spectroscopy (Shimadzu, Japan 8000 Series). 10 mg of

the dried fruit powder was encapsulated in 100mg 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  $\text{cm}^{-1}$  with a resolution of 4  $\text{cm}^{-1}$  and FTIR spectrum was recorded.

## Results and discussion

**Field Emission Scanning Electron Microscopy (FESEM) and EDAX:** The elemental composition of the sample was confirmed by Energy Dispersive X-ray spectroscopy (EDX) and Scanning Electron Microscopy (SEM) provides information about the shape and surface morphology. In the present study, various elements like C, O, N, K, Ca, Mg, S, Se, Pt were observed in *Aegle marmelos* (L.) unripe fruit powder and depicted in Table-1, Figure-1 and 2.

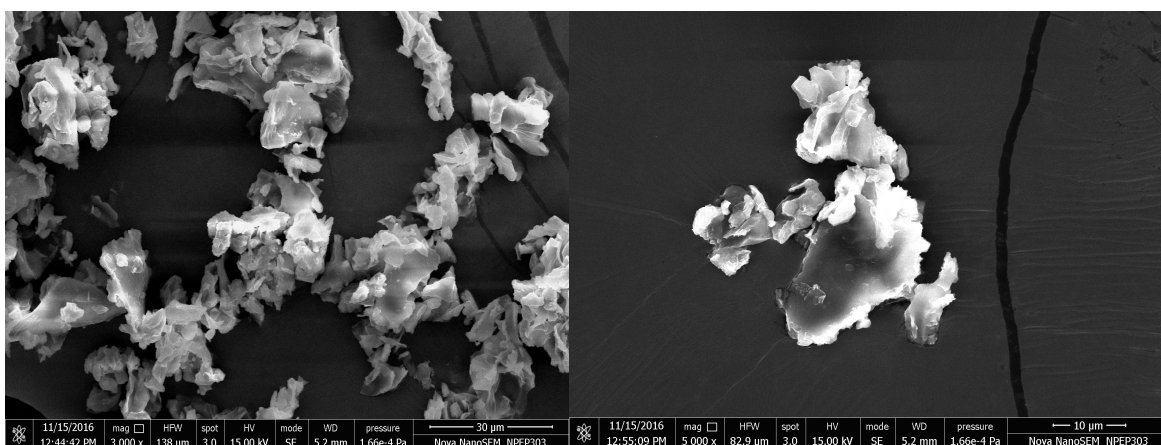


Figure-1: Field Emission Scanning Electron (FESEM) Microscopy Images of *Aegle marmelos* L. Unripe Fruit.

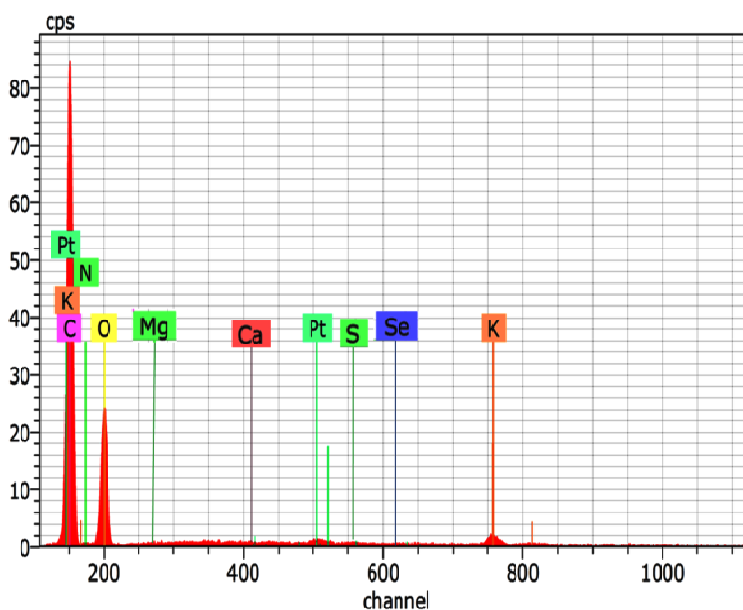


Figure-2: Energy Dispersive X-Ray Analysis (EDAX) Spectra of *Aegle marmelos* L. Unripe Fruit

**Table-1:** Energy Dispersive X-Ray Analysis (EDAX) Elemental Composition of *Aegle marmelos* L. Unripe Fruit

Element	Atomic Number	Atomic (%)
C	6	57.70
O	8	37.85
N	7	2.55
K	19	0.66
Ca	20	0.73
Mg	12	0.15
S	16	0.12
Se	34	0.11
Pt	78	0.09
Total		100

From detected elements, C (57.70 %) and O (37.85%) were observed in higher amount than that of other elements. While N (2.55%) and Ca (0.73%) observed at moderate amount in unripe fruit powder of *Aegle marmelos* (L.). For building and maintaining strong bones and teeth, functioning of blood coagulation and in extra cellular fluid calcium is necessary while nitrogen is useful element of the nucleic acid DNA and RNA, other molecules derived from the nitrogen bases<sup>20</sup>. The concentration of potassium is 0.66%. The most abundant cations and the primary electrolyte, potassium ions are located inside the body cells and store in muscle fibers. It carries transport of glucose into the muscle cell<sup>21</sup>.

In unripe fruit of *Aegle marmelos* (L) the concentration of magnesium was found to be 0.15%. Magnesium helps bone growth, maintain a stable metabolism, keeps blood vessels flexible, prevents cardiovascular diseases and repair injured cerebral cells<sup>22</sup>. Important element sulphur required in trace amounts to construct all body parts of human<sup>21</sup>. The concentration of sulphur was found to be 0.12% in *Aegle marmelos* (L.) plant. Selenium (0.11%) and platinum (0.09%) was found in trace amount. Selenium is important trace element that can function as both as an essential nutrient and an environmental toxicant<sup>23</sup>. The essential mineral elements in human, animal and plant nutrition has been well distinguished<sup>24-25</sup>.

Because of maximum percentage of important minerals the fruit plants has high nutritive value can be used for health care and also in preparation of nutritive products<sup>16</sup>. In present work the C, O, N, K, Ca, Mg, S, Se and Pt elements was observed in varying concentrations. Al, As, Pb, Cd and Hg like non essential

toxic elements were not observed in unripe fruit of *Aegle marmelos* (L.). The FESEM technique with EDAX helps to characterize the biomaterial in *Aegle marmelos* (L.) at its elemental and morphological level.

**Fourier Transform Infrared Spectroscopy (FTIR):** The interpretation of the infrared spectrum involves the correlation of the absorption bands with the chemical constituents in the sample. The observations of FTIR spectroscopic studies revealed the presence of various functional groups in crude powder of *Aegle marmelos* L. unripe fruit which showed variations in their peaks depicted in Figure no.3 and FTIR spectra analysis was given in Table-2.

**Table-2:** Fourier Transform Infrared Spectroscopy (FTIR) Spectra Analysis of *Aegle Marmelos* (L.) Unripe Fruit

Functional groups	Component(peaks)	Wave number (cm-1)
Alcohols	O-H Stretch	3206.16
Alkanes	C-H Stretch	2924.72
Silica	Si -H-Silane	2359.44
Amines and amides	N-H Stretch	1598.42
Alkenes	C=C Stretch	1516.65
Phenol or tertiary alcohol	OH Bend	1417.02
Ethers	C-O Stretch	1229.16
Carboxylic acid	C-O Stretch	1138.83
Aromatic cyclic ethers	C-O-C Stretch	1071.66
Primary amines	C-N Stretch	1039.41
1 <sup>o</sup> and 2 <sup>o</sup> amines	N-H Stretch	833.21
Aliphatic chloro-compounds	-C-Cl	766.50

The broad peak obtained at the 3206.16 cm<sup>-1</sup> is result from the stretching of the -OH bond of alcohol groups and it indicates bonded hydroxyl (-OH) group. The more intense absorption peak at 1598.42 cm-1 representing amines and amide functional groups. It gives strong peak between 3000-2800 cm<sup>-1</sup> represent C-H stretching vibration generated by lipids. The peak obtained at 2359.44 cm<sup>-1</sup> indicated the presence of silica. The peak observed at 1120.64 cm-1 -C-O stretch representing carboxylic acids, esters, ethers, group of proteins and metabolites present in the fruit extract of *Aegle marmelos* L.<sup>26</sup>. Same functional groups were observed in our study in *Aegle marmelos* L. unripe fruit powder at 1138.83 cm-1. Antraquinone were present as aromatic cyclic ethers with C-O stretch at the 1071.66 cm<sup>-1</sup> and. The strongest absorption band at 1039.41cm<sup>-1</sup> could be due to C=N

stretching vibrations of aliphatic amines. The peak obtained at the  $833.21\text{ cm}^{-1}$  which indicates the presence of  $1^0$  and  $2^0$  amines.

FTIR spectroscopic study of *Aegle marmelos* L. unripe fruit showed the hydroxyl (-OH), carboxyl (-C=O) and amine (-NH) groups of coumarins, alkaloids or tannins. Krupa carried identification of functional groups in *Aegle marmelos* L. fruit extract and its characterization through Fourier transform infrared spectroscopy (FTIR), X-ray diffractometer (XRD), UV-Vis spectrophotometer and atomic force microscopy (AFM)<sup>27</sup>. In FTIR spectra of synthesized silver nanoparticles of *Aegle marmelos* L. fruit extract bands of absorption observed at around 740, 999, 1246 and  $3153\text{ cm}^{-1}$  and these bands are matching to *Aegle marmelos* L. leaf extract FTIR spectrum<sup>18</sup>.

In FTIR spectroscopic study Moses predicted the presence of O-H, N-H, C-H, C=O, C-N, C=N, C=C stretching of the detected functional groups<sup>28</sup>. Kayani reported amines, amides, aldehyde, alkenes, alcohol, phenols, aromatic, carboxylic acids, ethers, quinines and esters belong to secondary plant metabolites<sup>29</sup>. In the present study amines, amides, aldehyde, alkenes, alcohol, phenols, aromatic, carboxylic acids, ethers, quinines and esters were detected in *Aegle marmelos* L. unripe fruit powder.

## Conclusion

For the interpretation of the pharmacological actions of the medicinal plants the data on trace, minor, major and toxic

elements in plants is of great importance. The result obtained in the FESEM with EDAX analysis of *Aegle marmelos* (L.) unripe fruit powder showed sufficient amount of essential elements. In present investigation no potentially toxic elements were observed.

From present study it can be concluded that, determination of functional groups by FTIR and characterization of elemental and morphological properties by FESEM techniques with EDAX helps to identify the potential bioactive constituents of *Aegle marmelos* (L.) unripe fruit. The presence of characteristics of functional groups alcohols, phenols, alkane, amines, ethers, aromatic compounds could be responsible for the various medicinal properties of *Aegle marmelos* (L.) unripe fruit powder were observed. FTIR analysis is useful for the identification and to elucidate the structure of bioactive compounds. Therefore the presence of the important or potential bioactive constituents in unripe fruit of *Aegle marmelos* (L.) may attribute health benefits by providing the protective effects during the progressive stages of chronic disorders.

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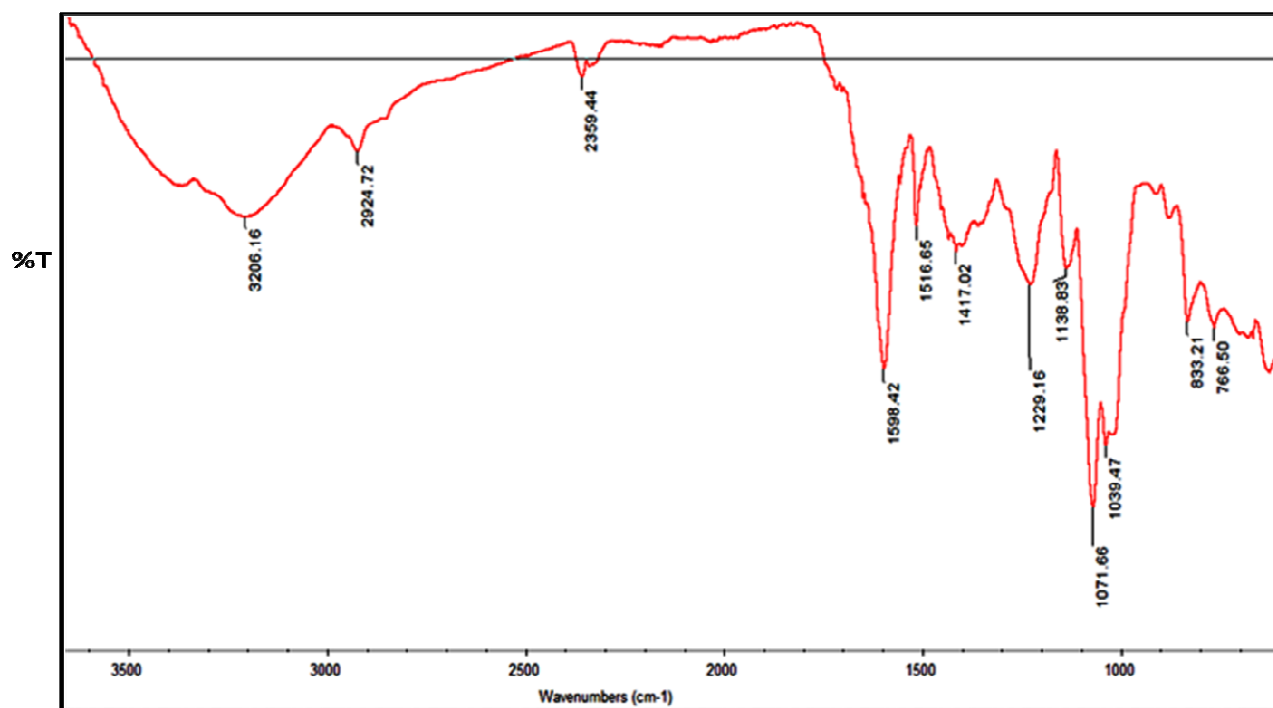


Figure-3: Fourier Transform Infrared Spectroscopy (FTIR) Spectrum Unripe Fruit Crude Powder Of *Aegle Marmelos* (L.).

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