IJPSR (2011), Vol. 2, Issue 5 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 01 February, 2011; received in revised form 24 March, 2011; accepted 28 April, 2011

PHARMACOGNOSTICAL AND PRELIMINARY PHYTOCHEMICAL PROPERTIES OF *AEGLE MARMELOS L. CORREA FRUIT* PULP

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Keywords:

Aegle marmelos, Phytochemical Characters, Pharmacognostic Characters

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ABSTRACT

The present study deals with the Pharmacognostical and preliminary phytochemical studies of fruit pulp of *Aegle marmelos*. Macroscopy, organoleptic characters, microscopic features, physiochemical standardization parameters such as total ash, acid insoluble ash, water soluble ash, alcohol, water and other organic solvent extractives, preliminary phytochemical assay, fluorescence analysis and microbial limit assay were performed using standard textual methods. The Pharmacognostical results showed the following observations that are total ash (3.8%), acid insoluble ash (2.05%), water soluble ash (2.9%), water extractives (12%), alcoholic extractives (10.4%), foaming index (333.3U), and swelling index (3.3%). Fruit pulp reveals the presence of steroids, terpenoids, flavonoids, Saponins, phenolic compounds, lignin, fat and oil, proteins, carbohydrates, amino acids and reducing sugars. These observations would be of immense value in the botanical identification and standardization of drugs in a crude form.

INTRODUCTION: India has a rich heritage of traditional knowledge and is a birth place to several important time-honored systems of health care like Ayurveda, Siddha and Unani. It has been estimated that the proportion of medicinal plants in India (7, 500 of the 17, 000 higher plant species are medicinal plants) is higher than any other country of the world [1, 2]. *Aegle marmelos* L. Correa commonly known as *Bael* in English and Vilvam in Tamil belonging to the family Rutaceae has been widely used in Indian medicine due to its various medicinal properties.

Although this plant is native to northern India it is also widely found throughout the Indian peninsula and in Ceylon, Burma, Thailand and Indo-China. All parts of this tree such as root, leaf, trunk, fruit and seed are useful in several ailments. The unripe fruit is said to be an excellent remedy for diarrhoea and is especially useful in chronic diarrhoea [3, 4, 5].

The effectiveness of *Aegle marmelos* fruit in diarrhoea and dysentery has resulted in its recognition into the British Pharmacopoeia. The present work therefore attempts to report various necessary phytochemical and pharmacognostical standards of *Aegle marmelos* fruit pulp.

MATERIALS AND METHODS:

Collection and Authentication of Plant Material: The fruit pulp of *Aegle marmelos* were collected from the herbal garden of Thiuvanai koil temple, Tiruchirappalli and authenticated by Professor Dr. John Britto, Taxonomist, Department of Botany, St. Joseph's College, Thiruchirapalli, India. After authentication, the fruit pulp were collected by scooping the pulp using sterile scoop and blade, shade dried and then milled into coarse powder by a mechanical grinder.

Organoleptic Evaluation: Organoleptic evaluation refers to evaluation of the formulation by color, odor, taste, texture, etc. The organoleptic characters of the samples were evaluated based on the textual methods [6].

Microscopy: Free hand section of the materials were taken using a sharp blade, suitably stained and subjected to microscopic observations. Photomicrographs were taken using Nikon compound microscope attached with digital camera. Photomicrographs were taken at different magnifications depending upon the anatomical details [7].

Physicochemical Parameters: The determination of various physicochemical parameters such as total ash, acid insoluble ash, water soluble ash, water soluble extractive value, alcohol soluble extractive value, swelling index, foaming index, foreign matter were analyzed by the methods given in the ayurvedic Pharmacopoeia of India [8, 9].

Fluorescence Analysis: Fruit pulp powder were subjected to analyze fluorescence features under ultra violet light and day light after giving treatment for 48 hours with various chemical and organic solvents like ethanol, 50% sulphuric acid, 10% sodium hydroxide and dilute hydrochloric acid [10,11].

Qualitative Phytochemical Screening: Freshly prepared *Aegle marmelos* fruit pulp extracts were tested for the presence of phytochemical constituents using standard methods [12, 13].

Microbial Limit Assay: Dissolved 1gm of powdered plant material in 10mL of distilled water. It was serially diluted using phosphate buffer as diluent. The sample was inoculated in Nutrient agar by pour plate, Rose Bengal agar and SS agar by spread plate techniques for Bacteria, Fungi and *Salmonella* respectively. For bacteria, the plates were incubated at 37°C for 48 hrs and for fungi; the plates were incubated 25°C for 96 hrs [13].

RESULT AND DISCUSSION: Medicinal plants are the backbone of Traditional system of medicines like Ayurveda and Siddha. Good raw material provides better therapeutic values. Pharmacognostical study will contribute in determining characteristic features of

standard and raw materials. Standardization of herbal drug is a topic of great concern recently. They are subjected to variability due to its origin from heterogeneous sources. Hence, in the present investigation efforts were made to provide scientific data to determine standards for the plant materials. The present study on microscopical features and other physicochemical, phytochemical and fluorescence parameters on the *Aegle marmelos* fruit pulp will help in identifying the correct species of the plant.

Microscopy: Presence of characteristic stone cells, compact parenchyma cells and oil globules. Phloem cells were characteristically dark pinkish color, radially flattened cork cells. Presence of brownish granules, laticiferous ducts were unique. Medullary ray were mostly uniseriate (**Figure 1-10**).

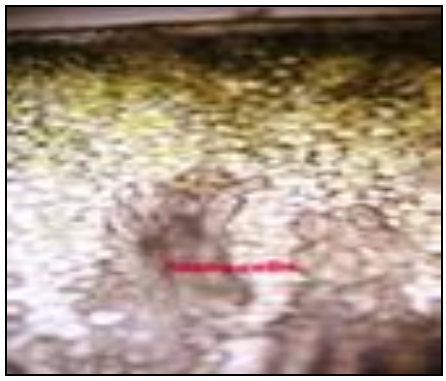

FIG. 1: FRUIT PORTION ENLARGED

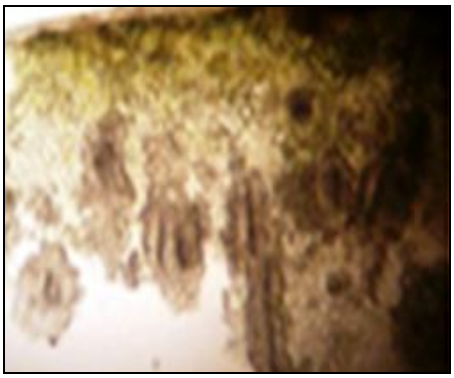

FIG. 2: FOCCUSSED VIEW OF ENDODERMAL

FIG. 3: ENDODERMAL CELLS ENLARGED

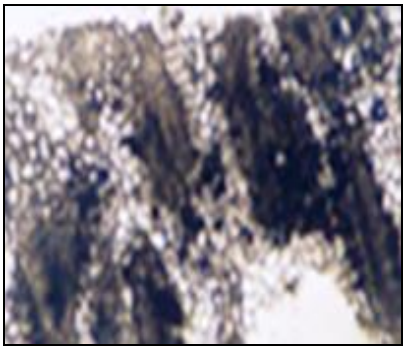

FIG. 4: STONE CELLS ENLARGED

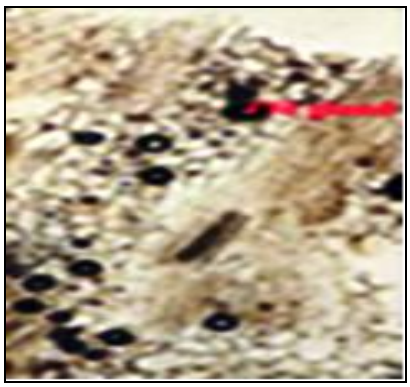

FIG. 5: VASCULAR TRACES

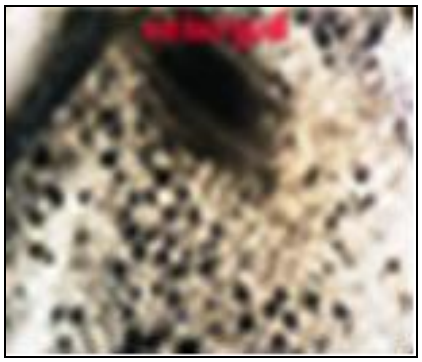

FIG. 6: VASCULAR TRACES ENLARGED

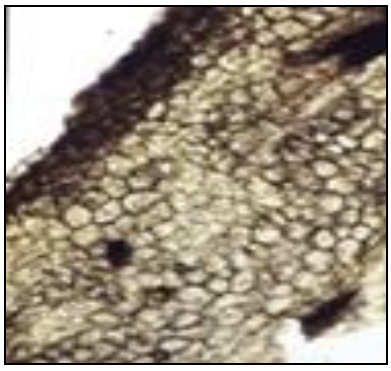

FIG. 7: PARENCHYMA CELLS

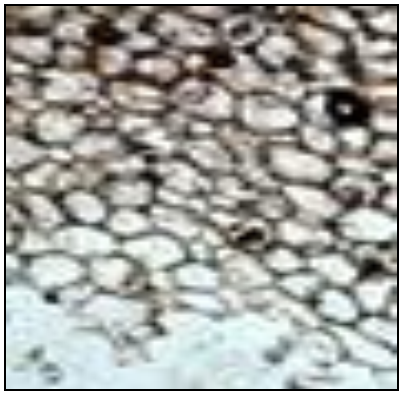

FIG. 8: ENDOCARPIC CELLS

FIG. 9: VASCULAR ELEMENTS

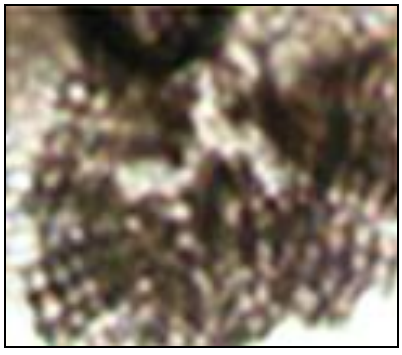

FIG. 10: ENLARGED VESICULAR ELEMENTS

Macroscopy: Fruits occur as piece about up to 3.95±0.43 cm long and up to 0.21±0.6cm width, cylindrical thick and hard. The fruit of *Aegle marmelos* were directly plugged from the tree and the fruit was cut into two halves and transverse sections were prepared (**Figure 11-14**).

FIG. 11: UNRIPE FRUIT; FIG. 12: FRUIT EXPOSED; FIG. 13: FRUIT PULP; FIG. 14: FRUIT PULP POWDER

Organoleptic characters: The following organoleptic characters were indicated when observing powdered fruit pulp. Good powder of *A. marmelos* fruit pulp was yellowish orange in color, bitter taste and latex cum aromatic odor and smooth texture (**Table 1**).

TABLE 1: ORGANOLEPTIC CHARACTERS OF *AEGLE MARMELOS* FRUIT

CHARACTER	OBSERVATION
Color	Yellowish orange
Odor	Latex odor and mild aromatic odor
Taste	Bitter taste
Size	Length-9.6 cm; Width- 22.6 cm
Texture	Smooth

Physicochemical characters: The fruit pulp was dried, powdered and analyzed for its physicochemical characters, which were presented in **table 2**. Total ash content of the powdered fruit pulp was found to be 3.8%, acid insoluble ash 2.05% and water soluble ash 2.9%. Water extractive value was higher (12%) than alcohol extractive value (10.4%).

TABLE 2: PHYSICOCHEMICAL PARAMETERS OF *AEGLE MARMELOS* FRUIT PULP POWDER

PARAMETERS	RESULTS
Foreign matter	<0.1%
Dry Powder Particle size	0.344 µm
Wet powder Particle size	0.348 µm
Foaming index	333.3U
Swelling index	3.3 %
Acid insoluble ash value	2.05%
Water soluble ash value	2.9 %
Total ash	3.8%
Water extractive	12%
Alcoholic extractive	10.4%

Phytochemical Analysis: The results of preliminary quantitative phytochemical screening of aqueous and alcoholic extracts of *Aegle marmelos* fruit pulp revealed the presence of multiple polar and non-polar chemical constituents (**Table 3**). Steroids, terpenoids, flavonoids, phenolic compounds, lignin, fat, inulin, proteins, carbohydrates were present in both extracts. Alkaloids and tannins were present only in alcoholic extracts whereas saponins and cardiac glycosides were present only in aqueous extract. Terpenoids therapeutically exert wide spectrum of biological activities such as antiseptic and antihelmentic [14]. Phenolic compounds are used in the treatment of burns as they precipitate the proteins of exposed tissue to form a protective covering [15]. They are also used as healing agents in inflammation, leucorrhoea, gonorrhoea, burns, piles and as antidote [16].

TABLE 3: QUALITATIVE PHYTOCHEMICAL ANALYSIS *AEGLE MARMELOS* FRUIT PULP EXTRACTS

Test	Aqueous extract	Alcoholic extract
Alkaloids	Negative	Positive
Steroids	Positive	Positive
Terpenoids	Positive	Positive
Flavonoids	Positive	Positive
Saponins	Positive	Negative
Phenolic compounds	Positive	Positive
Tannins	Negative	Positive
Lignin	Positive	Positive
Phlabo tannins	Negative	Negative
Fat and Oil	Positive	Positive
Inulin	Positive	Positive
Cardiac glycosides	Positive	Negative
Proteins	Positive	Positive
Carbohydrates	Positive	Positive
Amino acids	Positive	Positive
Reducing sugars	Positive	Positive

Fluorescence analysis: Magenta to red colored chromophore was produced when the drug powder is treated with Aqueous and alcoholic NaOH, HCl, Benzene. Black coloration was noted after 48 hrs of H_2SO_4 treatment under visible light. These chromatophoric colors under UV and visible light illustrated the nature of raw materials (**Table 4**). Fruit pulp exhibited characteristic colors when treated with different chemicals that may be due to chromatophores present in the powder. This may help to assess the purity of the drug [17].

TABLE 4: FLUORESCENCE ANALYSIS OF *AEGLE MARMELOS* FRUIT PULP AT 24 HOURS

TEST PLANT POWDER +	DAY LIGHT	UV LIGHT
Chloroform	Orange	Greenish yellow
Hexane	Light Brown	Greenish yellow
Benzene	Red	greenish yellow
Aqueous NaOH	Dark red	Dark Brown
Alcoholic NaOH	Dark red	Greenish yellow
1NHcl	Red	Yellowish red
Ethanol	Sandal	greenish yellow
Ethyl acetate	Pale yellow	Greenish yellow
Acetone	Dark brown	greenish yellow
50% H_2SO_4	Magenta	Dark pink

Microbial Limit assay: Total bacterial load available in the fruit powder was within the limits of ayurvedic pharmacopeia of India. Only $7X10^2$ CFU of bacteria and $3X10^1$ funguses were isolated per gram of plant powder. Plant powder was free from enteric bacteria like *Escherichia coli*, *Salmonella sp.,* and *Shigella sp*.

CONCLUSION: The following Pharmacognostical standards were determined in the present work. Fruits occur as piece about up to 3.95±0.43 cm long and up to 0.21±0.6 cm width, cylindrical thick and hard, presence of characteristic stone cells, compact parenchyma cells and oil globules. Phloem cells were characteristically dark pinkish color, radially flattened cork cells, presence of brownish granules. Laticiferous ducts were unique. Medullary ray were mostly uniseriate. Color of the fruit pulp power was found to be yellowish orange, mildly aromatic odor and bitter taste. The Pharmacognostical results showed the

following observations that are total ash (3.8%), acid insoluble ash (2.05%), water soluble ash (2.9%), water extractives (12%), alcoholic extractives (10.4%), foaming index (333.3U), and swelling index (3.3%). Foreign matter was found to be <0.1%. Flavonoids, phenolic compounds and tannins were the major secondary metabolites present in both the extracts. Microorganisms were present within the limits of ayurvedic pharmacopoeia of India. These observations would be of immense value in the botanical identification and standardization of drugs in a crude form.

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