Journal of Phytology 2010, 2(2): 36–43
© Journal of Phytology, 2010
ISSN: 2075-6240
Available Online: www.journal-phytology.com

REGULAR ARTICLE

DEVELOPMENT OF QUALITY STANDARDS OF *AEGLE MARMELOS* L. LEAVES

N.A. Siddique[1], M. Mujeeb[1*], S.H. Ansari[1], S. Ahmad[1], B. Ali[1], Junaid Aslam[2]

[1]*Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, Jamia Hamdard (Hamdard University), New Delhi-110062, India*
[2]*Cellular Differentiation and Molecular Genetics Laboratory, Department of Botany, Faculty of Science, Hamdard University, New Delhi – 110062, India*

SUMMARY

Aegle marmelos L (Rutaceae) is a moderately size deciduous tree, growing wild throughout the deciduous forest of India. It is commonly used in day to day life. In present investigation an attempt has been made for the pharmacognostical standardization and evaluation of *Aegle marmelos* leaves. The pharmacognostical evaluation comprises of detailed macroscopy, powdered microscopy, fluorescence analysis, quantitative microscopy and physical constants such as ash and extractive values. The leaves extracts were subjected to preliminary phytochemical screening. The data obtained in present study will serve as valuable tool for identification, authentication and detection of adulterants, standardization and quality control of the drug. The developed technique will also be useful for the standardization of formulations containing *A. marmelos*.

Keywords: *Aegle marmelos*, Extractive values, Ash values.

N.A. Siddique et al. Development of Quality Standards of *Aegle marmelos* L. Leaves. J Phytol 2 (2010) 36-43
*Corresponding Author, Email: mujeeb_zaid@yahoo.co.in

1. Introduction

Aegle marmelos (L.) (Rutaceae) commonly known as bael or koovalam (Malyalam, India) growing wildly throughout deciduous forest of India, ascending to an altitude of 1,200 m in western Himalayas and also occurring in Andaman Islands. The fruits and leaves are valued in indigenous medicine [1]**.** The plant has been employed for long time in folk therapy. Poultice made of leaves is used for ophthalmia and ulcers. The leaves are used to reduce blood glucose level [2]. Other actions like antifungal [3], antibacterial [4], antifungal [5], antioxidant [6], antidiarrhoetic [7], pesticidal, antidote, anti-inflammatory properties [8], antispermatogenic [9] has been reported. Certain biochemical constituents namely alkaloids, aegelinol, coumarin, steroid [7], terpenoid [5] and tannin [10], D-glucoside, marmesinine [11], lupeol [12], tannins, phlobatannins, flavonoids, umbelliferone, quercetin and volatile oils (Eugenol and methyl eugenol) are reported in different parts of the tree. It has been reported that leaves possess cardiotonic, antiasthmatic, antifungal, analgesic and antioxidant activities [13]. The drug is collected from the wild sources and varies in constituents and efficacy due to the geographical diversity. Improper collection and storage condition lead to the deterioration of the raw material. Keeping in view the above mentions problems, it was essentials to

standardize the leave of *A. marmelos* for the establishment of quality and identity profile of the drug for the purpose of safety monitoring and overall quality assurance of the industrially as well as commercially important drug i.e *A. marmelos.* Since there is no report in literature regarding the standardization of *A. marmelos* leaves. Therefore, in the present investigation an attempt has been made to standardize *A. marmelos* leaves by using macroscopy and microscopic characters, powder microscopy, fluorescence analysis, quantitative microscopy and physico-chemical values.

2. Material and Methods

Chemicals and reagents

All the chemicals and reagents used were of analytical grade, purchased from Sigma chemical co. (St Louis, MQ, USA) and Merck (Darmstadt, Germany). Leaves of *A. marmelos* were collected from campus of Hamdard University, New Delhi, India, (July -2007), which was identified by Taxonomist (Professor M.P. Sharma), Department of Botany, Hamdard university New Delhi. The voucher specimen was deposited in Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, Jamia Hamdard (JHFP-2023).

Morphological studies

The morphological studies were carried out for shape, size, colour, odour, taste and fracture of the *A. marmelos* leaves.

Microscopic studies and powder analysis

The transverse section of leaf and stem were prepared by standard method. Slides of powdered leaf material were also prepared and studied. Microphotography on different magnifications was carried out with motic microscopic unit. Polarized light was used for the study of crystals, starch granules and lignified cell.

Quantitative microscopy

Leaf constants such as stomatal index, stomata number, vein islet, vein termination and palisade ratio of the drug were determined according to the method described [14].

Physicochemical Standardization

The various physico-chemical values of leaves such as ash values, extractive values, loss on drying, were determined according to the Pharmacopoeial method [11].

Phytochemical screening

The phytochemical evaluation of drug was carried out as per the method described [12]. Previously dried powdered leaves (5 gm) were extracted in a Soxlet apparatus with petroleum ether, chloroform, methanol and water successively. The extracts were evaporated to dryness under vacuum. These extract were used for the analysis of different phyto-constituents *viz*. alkaloids, carbohydrates, phenolics, flavonoids, proteins, amino acids, saponins, mucilage and resins etc.

Fluorescence Analysis

The fluorescence nature of powder drug was analyzed [15] and the observations with different chemicals were also carried out and recorded.

3. Results and Discussion

Macroscopical evaluation

The leaves of *A. marmelos* were subjected to macroscopical examination and observations were recorded. The proper examination of the leaves was carried out under sun light and artificial source similar to day light. The leaves are attenuate, trifoliate, occasionally digitally five foliate with crenate margin, acuminate apex and long petiolate. Surface smooth and shiny, taste bitter, and green in colours. The results of macroscopical evaluation are presented in the Table 1; Plate 1.

Microscopical evaluation

The slides of T.S of different parts of plant were prepared and subjected to microscopical examination. The histology of different parts of plant was examined and the observations were recorded. The T.S of *A. marmelos* leaves (Plate

2C, D) showed groups of fibres with calcium oxalate crystals and also exhibit outer and inner epidermis with round to oval cells, covered with striated cuticle. A multilayered strip of collenchymas (3-4 layered) appear above the lower epidermis and below the upper epidermis, midrib compose of xylem and phloem arranged in an arc. The leaves show paracytic stomata, more in number on upper epidermis and lesser in lower epidermis. Calcium oxalate crystals were numerous and mainly of cluster crystal type. It contains numerous covering trichomes scattered in the powder. Some xylem vessels (pitted vessels) were also visible which were lignified. Cells of palisade and spongy parenchyma were also visible.

Table 1. Macroscopical characters of leaf of *Aegle marmelos.*

Description of the macroscopic structure	Observation
External Colour	Green
Size	7- 8 cm
Shape	Lanceolate
Apex	Acute
Surface	Smooth and shiny
Margin	Entire
Odour	Characteristic
Taste	Bitter
Others	Compound leaves,alternate, petiolate and paripinnate.

Table 2. Quantitative Microscopy of leaf of *Aegle marmelos*

Plant	Vein termination	Vein islet	Stomatal number	Stomatal index	Palisade ratio
A. marmelos	8-9	7-8	6-11	16.0	7-10

Table 3 Showing the effect of different chemical reagents on the fluorescence behavior of crude drug powder.

Treatment	Day light	UV light 254 nm	UV light 366 nm
Powder as such	Green	Dark green	Green
Powder treated with distilled water	Light green	Dark green	Black
Powder treated with 1N NaOH in water	Greenish brown	Greenish black	Brown
Powder treated with HNO_3	Light brown	Dark green	Dark violet
Powder treated with H_2SO_4	Green	Black	Blue
Powder treated with iodine	Green	Blue	Dark brown
Powder treated with conc. HCl	Dark green	Radish brown	Greenish black
Powder treated with ammonia	Light green	Dark green	Greenish brown
Powder treated with ferric chloride	Green	Radish black	Greenish brown
Powder treated with Iodine	Dark brown	Brown	Brown
Powder treated with Glacial acetic acid	Yellow	Dark yellow	Yellow
Powder treated with Picric acid	Dark yellow	Yellow	Dark yellow
Powder treated with Petroleum ether	Dark green	Green	Dark green
Powder treated with Chloroform	Dark green	Dark green	Dark green

Table 4. Showing the Phytochemical screening of different extract

Extract → / H Constituents	Petroleum ether	Chloroform	Alcoholic	Aqueous
Alkaloids	-	+	+	+
Carbohydrates	-	-	+	+
Phenolic compounds	-	+	+	+
Flavonoid	-	+	+	+
Proteins and amino- acids	-	-	+	+
Saponins	-	-	+	+
Mucilage	-	-	+	+
Resins	+	+	+	-
Lipids / Fats	+	-	-	-

(-: Absent, + : Present)

Table 5. Percentage of loss on drying, ash values and resin contents of *A. marmelos*

Parameters	*A. marmelos* %
Loss on drying	0.7433
Total ash	6.3027
Water soluble ash	1.2796
Acid insoluble ash	2.5525
Resin content	0.2100

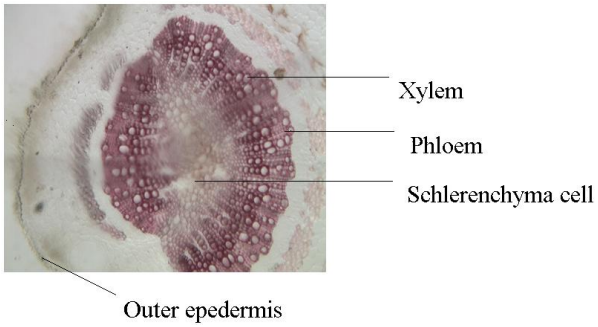
Powder microscopy

The microscopic examination of powdered leaf material was performed to detect and established various identifying microscopic characters which will be help full in differentiation of the substitute of the drug supplied in the form of dried powder. The photomicrographs of the identifying features of the plant material are shown in (Plate 1; Plate 2A-E). The covering trichomes and stomata were present in the sample. The covering trichomes were multicelluler, uniseriate and the stomata were paracytic type. It was found that the powdered leaf showed groups of fibres with calcium oxalate crystals. Calcium oxalate crystals were numerous and mainly of cluster crystal type. Some xylem vessels (pitted vessels) were also visible which were lignified and cells of palisade and spongy parenchyma were also observed.

Quantitative Microscopy

The slides of surface preparation of leaf were prepared and subjected to quantitative microscopic examination. The parameters such as vein termination, vein islet and stomatal numbers, stomatal index and palisade ratio of the leaf of *A. marmelos* were observed and recorded [14]. The observations and results are summarized in the Table 2; Plate 2E.

Plate 1. T.S of leaf of *A. marmelos*



Physicochemical standardization of leaves

The air dried, powdered plant materials were subjected for determination of various physicochemical standardization parameter as per the method described in WHO guide lines.

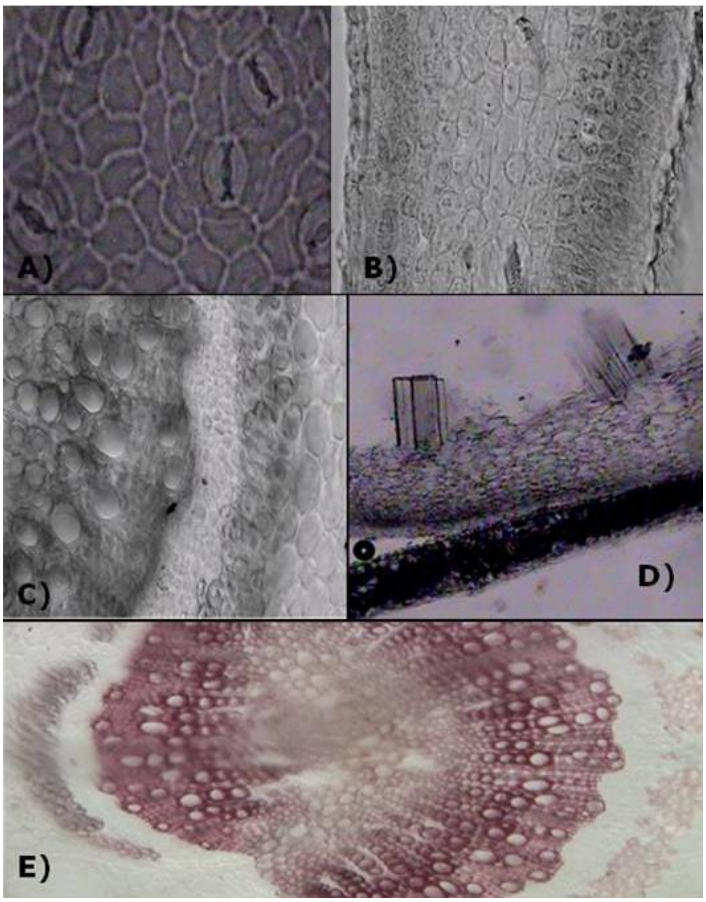


Plate 2. T.S.of of *A marmelos* Leaf section showing different tissues

A) Epidermis showing paracytic stomata
B) Lamina portion in sectional view
C) Vascular tissue of leaf
D) Calcium oxalate crystal
E) Magnified view of vessel of leaf

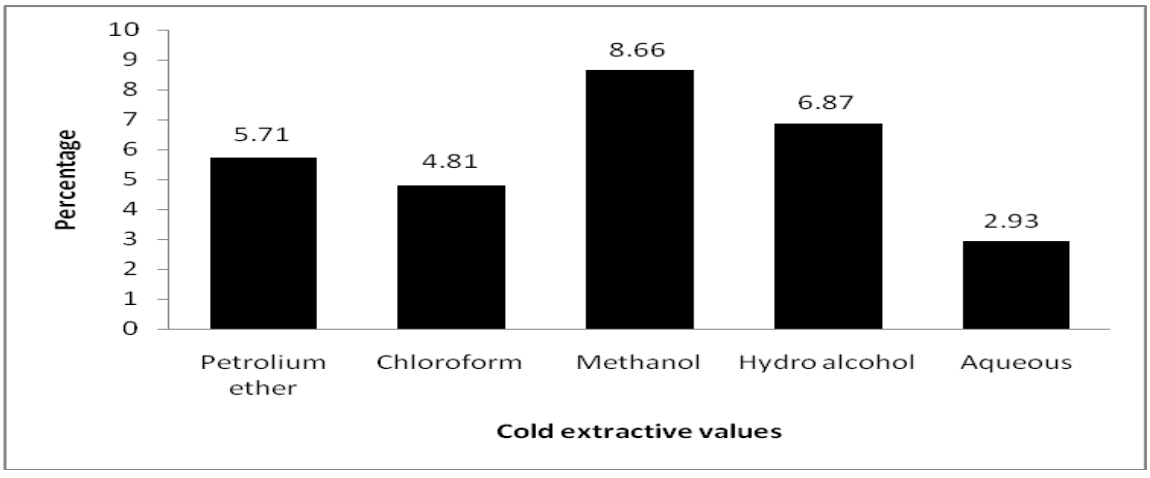


Fig. 1. Showing the percentage of cold extractive values

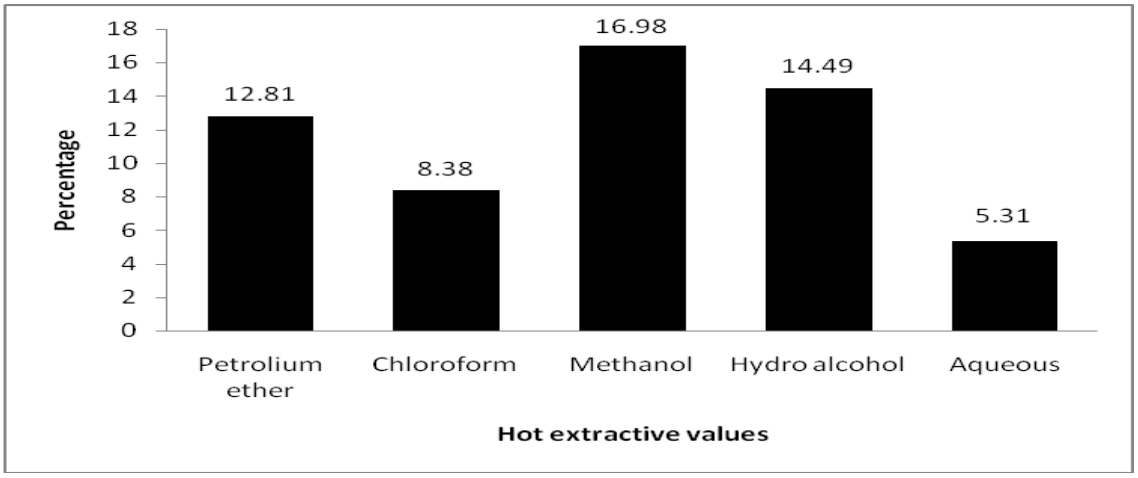


Fig. 2. showing the percentage of hot extractive values

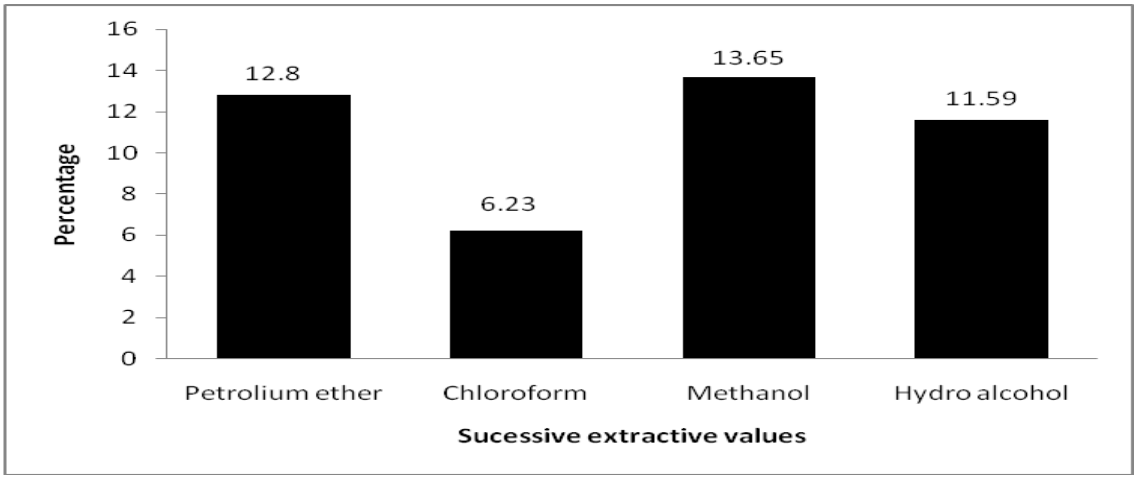


Fig. 3. Showing the percentage of successive extractive values

Extractive value

Estimation of extractive values determines the amount of the active constituents in a given amount of plant material when extracted with solvent. It is employed for that material for which no chemical and biological assay method exit. The extractions of any crude drug with a particular solvent yield a solution containing different phytoconstituents. The compositions of these phytoconstetuents depend upon the nature of the drug and solvent use. The use of a single solvent can be the means of providing preliminary information on the quality of particular drug. Extractive value also give the information regarding the quality of the drug (whether drug is exhausted or not).

Determination of individual extractive values (Cold extraction)

The air dried, powdered plant materials were extracted with petroleum ether, chloroform, alcohol, hydroalcohol and water separately in a conical flask at a room temperature. Methanol followed by hydroalcohol proved to be highly effective for high cold extractive values. Comparative accounts of extractive values are presented in Fig. 1.

Determination of individual extractive values (Hot extraction)

The air dried powdered plant materials were extracted with Petroleum ether, chloroform, alcohol, hydroalcohol and water separately in a Soxhlet apparatus. The observations are presented in Fig. 2. The maximum hot extractive values noticed in methanol extract.

Determination of Successive extractive values

The dried and coarsely powdered material (10g) is subjected to successive extraction in a Soxhlet apparatus with different solvents like petroleum ether, chloroform and methanol. The extracts are evaporated to dryness and their constant extractive values are recorded. The maximum successive extractive values recorded in methanol (Fig. 3).

Fluorescence Analysis

The air dried plant materials were subjected to different chemicals and lights. Table 3 showed a detail fluorescence behavior of crude drug powder.

Phytochemical screening

The extracts were subjected to preliminary chemical tests to detect the presence and absence of various phytoconstituents. Alkaloids, carbohydrates, phenolic compounds, flavonoid, proteins and amino acids, saponins and mucilage were absent in petroleum ether, however, resins and lipids were present. Chloroform extract showed the presence of alkaloids, phenolic compounds, flavonoid, resin and lipids. Morever, in aqueous extract only resins and lipids were absent, while; only lipids were not detected in alcoholic extract. Table 4. showed the presence and absence of various phytoconstitutents in different extracts. Phytochemical evaluation of the plant extracts may provide the information regarding various types of phytoconstituents present. Presence or absence of particular types of phytoconstituents in the plant of the interest may be helpful, partly in the development of analytical profile and in the differentiation of contravention plants.

Determination of ash values

The percentage of loss on drying, total ash values, water soluble ash, acid insoluble ash and resin content were determined. The results noticed were; loss on drying (0.7433%), total ash (6.3027%), water soluble ash (1.2796%), acid insoluble ash (2.5525%) and resin (0.2100%) respectively. The ash value of any organic material is composed of their non volatile inorganic components. Control incineration of crud drugs result in ash residue consisting of an inorganic material (metallic salt and silica). This value varies within fairly wide limits and is there for an important parameter for the purpose for evaluation of crude drugs. In certain drug, the percentage variation of ash from sample to sample is very small and any marked difference indicates the change in quality. Unwanted parts of drug, some time posses a character that will raise the ash value. Ashing involves an oxidation of the components of the product. A high value is indicative of contamination, substitution, adulterations or carelessness in preparing the crude drug for marketing. The total ash value, acid insoluble ash value, water-soluble ash values were determined as per WHO guide lines. The results and observation are presented in Table 5.

Conclusion

The results obtained in the present investigation are encouraging and will be used as reference data for the standardization of *A. marmelos* and the formulations containing *A. marmelos* as a main ingredient. The plant is collected from wild sources and varies in constituents and efficacy due to geographical diversity. Improper collection and storage conditions lead to contamination of microorganism and heavy metals. Standardization is the prime need of time because standardization establishes quality and identify profile that can be used for the purpose of safety monitoring and overall quality assurance of herbal medicines. An Indian medicinal plant are used frequently in many traditional systems throughout the globe,

there acceptably in modern medicine and in developed world is remarkably low, largely due to the lack of standardization. Moreover, Indian herbal products are exported and marketed in various developed countries of the world under the name of food supplement not the drugs due to quality and safety point of view. There is an urgent need for evaluation and analysis of herbal drugs using sophisticated modern techniques of standardization.

Reference

1. Charakbraty, B., C. Malik and S. Bhatthacharya, 1960. Studies on the effect of green leaves of *Aegle marmelos* and *Piper nigrum* on the glucose and cholesterol levels of blood in diabetes mellitus. Indaian. Med Forum. 9: 285-28
2. Anonymous, 1988. The Ayurvedic Pharmacopoeia of India. Vol- IV, Part- I, Ist-Edition, Govt. of India, Ministry of health and family welfare, Deptt. Of AYUSH, New Delhi.
3. Renu A. 1983. Fungitoxicity of leaf extracts of some higher plants against *Rhizoctonia solani* Kuehn. Natl. Acd. Sci. Lett. 6: 245-246
4. Chattopadhyay, R.R., S.K. Bhattacharyya and C. Medda, 2008. Antibacterial activity of some medicinal plants commonly used in Indian systems of medicine. Biomed. 2(4): 367-374
5. Rana, B.K., U.P. Singh and V. Taneja, 1997. Antifungal activity and kinetics of inhibition by essential oil isolated from leaves of *Aegle marmelos.* J. Ethnopharmacol. 57:29-34
6. Dhalwal, K., V.M. Shinde, A.G. Namdeo and Mahadik, K.R. 2008. Antioxidant Profile and HPTLCDensitometric Analysis of Umbelliferone and Psoralen in *Aegle marmelos*. J. Pharmaceutical Biology. 46(4): 266 – 272
7. Mazumder, R., S. Bhattacharya, A. Mazumder, A.K. Pattnaik, P.M. Tiwary and S. Chaudhary, 2006. Antidiarrhoeal evaluation of *Aegle marmelos* (Correa) Linn. root extract. Phytother Res. 20 (1): 82-84
8. Misra, K.K. 1999. New Crop Fact Sheet. India. Chatterjee, A and Bose, S, J. Indian Chem Soc. 29:425-429
9. Sur, T.K., S. Pandit and T. Pramanik, 1990. Antispermatogenic activity of leaves of *Aegle marmelos,* corr. In albino rats: a preliminary report. Biomed. 19: 199-202
10. Paricha, S. 2004. Bael (*Aegle Marmelos*) Nature's Most Natural Medicinal Fruit. *Orissa Review*.
11. Sharma, B.R. and Sharma, P. 1980. Constituents of *Aegle marmelos* II. Alkaloids and coumarin from fruits. Planta Med 43, 102-103.
12. Patra, A., A.K. Mukhopadhyay, A. Ghosh and A.K. Mitra, 1979. Constituents of *Aegle marmelos* : Carbon-13 NMR spectra of aurapten and marmin. Indian J Chem. 17B:85-387
13. Rai, M.K. 1996. *In vitro* evaluation of medicinal plant extract against *Pestalotiopsis mangiferae.* Hindustan Antibiot. Bull. 38 (1-4): 53-56
14. Anonymous, 1985, Trease, G.E., Evans, W.C., 1985. Pharmacognosy. Bailliere Tindall, London, 12th Edition.
15. Woltering, E.J. and Van Doon W.G. (1988). *Journal of Experimental Botany* 39: 1605-1616.