IJPSR (2020), Volume 11, Issue 7 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 01 March 2020; received in revised form, 21 May 2020; accepted, 25 May 2020; published 01 July 2020

PRELIMINARY QUALITATIVE PHYTOCHEMICAL, PHYSICO-CHEMICAL AND PHYTO-PHARMACOGNOSTICAL STANDARDISATION EVALUATION OF THE PULP (WITHOUT SEED), PERICARP, LEAVES AND SEED OF *AEGLE MARMELOS* L. (BAEL)

Sneh Lata Shakyawar [*1], Md. Akhlaqur Rahman [2], Shanthy Sundaram [1] and Shashi Alok [3]

Centre of Biotechnology (CBT) [1], Science Faculty, University of Allahabad, Prayagraj (Allahabad) - 210001, Uttar Pradesh, India.
Department of Biotechnology [2], S.S. Khanna Girls' Degree College, Prayagraj - 211003, Uttar Pradesh, India.
Institute of Pharmacy [3], Bundelkhand University, Jhansi - 284128, Uttar Pradesh, India

Keywords:

Aegle marmelos (L.), Phytochemical screening, Pharmacognostical evaluation, HPTLC, R_f value, Microbial limit test, Fluorescence analysis, Microscopic, Tannin, Flavanoids

Correspondence to Author:
Sneh Lata Shakyawar

Centre of Biotechnology (CBT), Science Faculty, University of Allahabad, Prayagraj (Allahabad) - 210001, Uttar Pradesh, India.

E-mail: snehlata.biotech88@gmail.com

ABSTRACT: *Aegle marmelos* L. (Bael), is a commonly occurring herb which has biomedical potency to treat many types of diseases such as diarrhea, hepatoprotective, epilepsy, stomachalgia, digestion related problems *etc*. The different parts of *Aegle marmelos* (L.) were collected, dried and coarsely powdered. The present study deals with the physio-chemical constants standardization parameters such as pH, solubility including water and methanolic extraction, total ash, acid insoluble ash, water soluble ash, extractive values, dry and wet particle size, foaming index, swelling index, foreign matters, loss of drying (LOD) and acidity were observed. Fluorescence analysis was performed for study of behavioral characters of the pulp without seed, pericarp, leaf and seed powder treated with chemical reagents. Extractive values of powder with solvents such as water and methanol were determined. Macroscopical characters help in identifying the fruit, pulp, pericarp, leaf and seed. Anatomical observations showed the presence of palisade cell, trichomes, mesophyll cell, collenchymas, xylem, phloem, pith, upper epidermal cells and lower epidermal cells with stomata. Preliminary qualitative phytochemical screening reported the presence of carbohydrates, protein, alkaloids, tannins, phenolic compounds, starch, steroids, terpenoids, glycosides, amino acids, reducing sugars, saponins, lignin, fat and flavanoids. The reported phytoconstitutents of the plant *Aegle marmelos* (L.) may be responsible for the pharmacological and pharmaceutical activities. Microbial limit assay were performed using standard textual methods. The qualitative screenings along with primary HPTLC fingerprinting were performed was showed the R_f values at 254 nm with their respective UV-visible spectrum wavelengths scanned in between 200 to 400 nm. The results obtained about traditional information which will further increase the usage of this plant and evoke towards the scanning of the plants. These studies will provide immense value in the botanical referential information for the correct identification and standardization of the drug in a crude form.

INTRODUCTION: Most of the plants and their parts have been used to cure and treatment of many diseases since ancient time.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.11(7).3489-06

The article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.11(7).3489-06

In current scenario, synthetic drugs are easy available and more effective in treatment of various diseases but caused some side effect in human body. Most of the person still prefers using traditional phytomedicines because of their minute side effects for health. Phytomedicinal plants are precious items for getting novel drugs that forms the constituent in conventional systems of medicine, nutraceuticals, modern medicines, folk medicines, food supplements, bioactive principles, pharmaceutical intermediates, and lead compounds

in synthetic drugs [1]. There is a wide diversity of compounds, especially secondary metabolites like an alkaloid, phenols and flavonoid found and isolated by plants. Studies have shown that these compounds have analgesic, anticancer, anti-bacterial, antitumor, anti-inflammatory, antiviral and many other activities to a greater or lesser extent [2, 3].

Eminent examples of these phyto-chemical compounds include phenols, flavonoids, and saponins, phenolic glycosides, cyanogenic glycosides, stilbenes, nitrogen compounds (alkaloids, amines, betalains), terpenoids, tannins and some other endogenous metabolites [3, 4]. This drives the need to screen medicinal plants for novel bioactive compounds as they are safe, less side effects and have biodegradable [5].

Aegle marmelos (L.) Corr., golden apple belongs to the family Rutaceae and also known as Bael tree [6, 7]. It is native to India and is used in traditional medicines. *A. marmelos* is a slow growing tree of a medium size (up to 12-15 m tall) throughout the deciduas forest of India of height up to 1200 meters with flaking, soft, short trunk, thick bark and spreading spiny branches. It occurs all over India, especially in dry forest on hilly slopes and plains of Eastern Central, sub- Himalayan forest, Bengal and south India.

From Homoeopathic point of view, the unripe fruit and ripe fruit are being used as digestive, astringent and stomachic problems and it is prescribed for diarrhoea and dysentery treatment [8]. The fresh juice of the leaves is taken with honey as a febrifuge and laxative; it is also used in asthmatic complaints. The ripe fruit is used as a remedy for diarrhoea. Beverages prepared with ripe fruit pulp are used to relive body heat. Cologne is also obtained by distillation from flowers [9]. All parts of *Aegle marmelos* (L.) tree such as pulp, bark, flower, root, leaf, fruit, trunk and seed are useful in many ailments. The unripe fruit is also said to be an excellent remedy for diarrhoea and is mainly useful in case of chronic diarrhoea [10, 11, 12].

In the present study, an attempt for morphology, physicochemical standards, fluorescence analysis, preliminary phytochemical screening and high performance thin layer chromatography were performed.

MATERIALS AND METHODS:

Collection of Plant Samples: The different parts (fruit pulp without seed, pericarp, leaf and seed) *of Aegle marmelos* (L.) were collected from Deendayal Research Institute, Chitrakoot (M.P.) INDIA in April 2016 and identification was done in Department of Botany, University of Allahabad, India and also herbarium of *Aegle marmelos* (L.) plant was deposited in the Department of Botany, University of Allahabad. Uttar Pradesh, India.

Preparation of Powder: The different parts (fruit pulp without seed, pericarp, leaf and seed) were collect. They were rinsed with tap water followed by distilled water to remove the dirt on the surface individually. Pulp, pericarp and seed were removed from whole fruit and pericarp cut into small pieces. They were shade air-dried for 3 days. Dried samples were ground into fine powder with help grinder mixer and kept in desiccators then store in air tight container. The ingredients of powder were observed under microscope. The powder was treated with different chemical reagents and observations were made. A little amount of drug mounted in different reagents was observed under UV light and fluorescence was recorded.

Preparation of Aqueous and Methanolic Extracts for Phytochemical Test: The extraction was carried out in solvent. 10 gm dried powdered of plant parts was dissolve in 100 ml distilled water and methanol (1:10) individually for 72 hours in a round bottom flask at room temperature by placing on shaking water bath. After 3 days, all samples were filtered through Whatman filter paper no. 1. The filtrate extract was store in durable glass bottle and stored at 4ºC for further Phytochemical experimental use [13].

Instruments and Chemicals: UV chamber (Dolphin, Mumbai), Afcoset digital balance (Afcoset Balances, Mumbai, E-R-180A, Leica photographic microscope (Leica Microsystems, Mumbai, DM-3000), TLC silica gel 60 F254 5 × 10 cm plate (Marck), The HPTLC system used consisted of a TLC Scanner 4 supported by win CATS software version 1.4.7 equipped with Linomat 5 sample spotter and camag Reprostar 3 system for photo-documentation (all from CAMAG, Muttenz, Switzerland), analytical grade toluene, formic acid (98-100%), ethyl acetate,

acetone (Merck, Mumbai), n-butanol, chloroform , petroleum ether (40-60°C) (Sd Fine-Chem Ltd, Mumbai), methanol etc. were used for the study and experiments.

Macroscopy Morphogical Evaluation: Macroscopic morphological evaluation features of fresh and dried *Aegle marmelos* (L.) fruit, pulp, pericarp, leaf and seed were studied directly in the field and were photographed [14].

Organoleptic Characters: The macroscopical study includes the evaluation of organoleptic characters colour, odour, shape, size, fraction and external features of the various parts of selected plant material were evaluated based on the textual methods [14].

Microscopic Study (Transverse Section) of Leaf: Microscopic evaluation of fresh leaf was performed according to the procedure mentioned in the Ayurvedic Pharmacopoeia of India [15]. The vascular bundle, stomata types, calcium oxalates, xylem, mesophyll and epidermal cells, trichomes, starch grains, *etc*. were studied. For leaf surface preparation to make leaf transparent, a small piece of leaf were cut in to two portion, turns one piece upside down, the add a small drops of chloral hydrate S and heat to boil directly on a slide. As soon as bubbles start, remove the slide from the flame. Heat until the bubbles are disappeared and fragments are transparent. Add few drops of chloral hydrate S then apply cover glass and observe under microscope.

Microscopic Powder: The dried powder of pulp without seed, pericarp, leaf and seed of *Aegle marmelos* (L.) was cleared with sodium hydroxide and mounted in glycerin medium after staining. 1gm of sample taken in small petriplate / beaker then adds few ml of chloral hydrate solution mixed properly. Add 10 ml distilled water and boil on hot plate. All the solvent was evaporated and obtained semi-solid solution were cooled. After cooling it was washed with distilled water seven times until glycerin is properly removed. Now the clear particles are seen. Few small size particles of sample were put on the glass slide with the help of brush and one drop of various staining reagents such as safranin, toluidine blue, fast green and iodine were used to study calcium oxalates crystals, starch grains, stomata, trichomes, epidermal cells, xylem parenchyma, parenchyma and lignified xylem fibers. After that add three drops of glycerin on every slide with the help of dropper and put coverslip on the slide, and then dry at room temperature. The photographs at different magnifications were taken by Leica photographic microscope [16].

Physiochemical Parameters Analysis:

1. Determination of pH Value: The pH value of a liquid can be determined potentiometrically by means of the glass electrode, a reference electrode and a pH meter either of the digital or analogue type.

Procedure: Firstly standardize the pH meter. Take standard tablet/capsue of different pH and dissolve in 100 ml of distilled water to prepare solutions of different pH 4, 7 and 9 (buffer solutions) or as directed in the label. Switch ON the instrument. Leave it for some time unless or on the board requirement of different pH solution appears. Take the buffer solution in the beaker and dip the electrode in it. Carry out the same exercise for another buffer solution also, after washing the electrode thoroughly with distilled water. Take the test sample (for solid preparation 10% aqueous solution) and dip the electrode in it and note the value of pH [17].

2. Solubility Test:

Water Soluble-Extractive (WSE): Extractive values gives the idea about nature of active constituents present in the given plant material. Higher solubility indicates the possibility of considerable amount of polar compounds. Weighed accurate 2 gm powder sample and transfer in 250 ml iodine flask and add 100 ml water solvent. Provide continuous shaking for 6 h and leave for 18 hours (maceration). After this extract was filtered using Whatman filter paper no. 1 and weighted in thin porcelain dish, solvent was evaporated on water bath and residue was weighed. Percentage of extractive value (w/w) was determined [18].

Average = Differences of sample / Total no. of sample

Percentage of WSE = Average × 500

Alcohol Soluble Extractive (ASE) Value: Alcohol soluble extractive value was determined using the procedure described for water soluble

extractive, except that alcohol used for maceration or extraction procedure [18].

3. Loss on Drying: LOD value was used to determine the amount of water and volatile matters present in a sample when the sample is dried under specified conditions. 2gm of powder sample was transferred to weighed thin porcelain dish and kept in hot air oven at 105 °C for 5 h. Dish was cooled in desiccators and weighed. The dish again kept in oven for 30 min. Dish again cooled in desiccators and weight taken. The loss in weight is calculated as percentage [18.]

Average = Differences of sample / Total no. of sample

Percentage of LOD = Average × 100 / Weight of Sample Taken

4. Acidity: Take 10gm of the sample in a suitable titration flask and dissolve in 7 5ml of carbon dioxide free water (dd/w). Mix it properly and titrate against standard sodium hydroxide solution (0.05N) using 4-6 drops of phenolphthalein indicator (Dissolve 0.5gm phenolphthalein in 100ml of 50% ethyl alcohol (v/v)) till pink color persists for 10 sec. Determine blank on water and indicator and correct the volume of sodium hydroxide solution used [19]. The Acidity was calculated by applying the formula as given below:

Acidity as formic acid (%) by weight = 0.23 × V / M

Where, V = Corrected volume of 0.05 N sodium hydroxide used, M = Weight in a gram of the sample taken for the test (10 gm).

Physicochemical standards are generally used for determining the identity, purity, and strength of the plant powder. These characters also used to check the genuine nature of the sample.

5. Foreign Matters: The sample shall be free from visible signs of mold growth, sliminess, stones, rodent excreta, insects or any other noxious matter when examined as given below:-

Procedure: Take appropriate quantity of sample to be tested. Spread the sample in a thin layer on the dish or trey. Examine in daylight with unaided eye. Transfer suspected particles (if any) to a Petridish. Examine with 10x lens in daylight and weight. Record the value in lab data register and calculate the percentage with respect of sample taken [17].

6. Total Ash: The ash value is useful to determine the quality and purity of the drug. Ash contains inorganic radicals like phosphate, carbonates and silicates of sodium, potassium, magnesium, calcium, *etc*. Different ash values such as total ash value, acid insoluble ash value and water soluble ash was determined. 2 gm of leaf powder were taken in silica crucible (previously weighed). Material was incinerated with the help of the muffle furnace at 450 ºC, until vapors almost cease to evolve. Dish was heated until all carbon was burnt off. Dish was cooled, and percentage of total ash was calculated [18].

Average = Differences of sample / Total no. of sample

Percentage of total ash = Average × 100 / Weight of sample taken

7. Acid Insoluble Ash: Ash was boiled with 25 ml 5% hydrochloric acid for 5 min. Insoluble matter was collected in the ash less filter paper no. 42. It was washed with the hot water until neutral, ignited, cooled in desiccators and weighted. Percentage of acid insoluble ash was calculated [18].

Average = Differences of sample / Total no. of sample

Percent of acid-insoluble ash = Average × 100 / Weight of sample taken

8. Water Soluble Ash: Boil the ash for 5 min with 25 ml of water; collect insoluble matter in a Gooch crucible or on an ash less filter paper, wash with hot water, and inflame for 15 min at a temperature not exceeding 450 ºC. Subtract the weight of the insoluble matter from the weight of the ash; the difference in weight represents the water–soluble ash. Calculate the percentage of water-soluble ash with reference to the air-dried drug [18].

Average = Differences of sample / Total no. of sample

Percentage of water-soluble ash = Average × 100 / Weight of sample taken

9. Dry Powder Particle Size: In Ayurveda, it is mentioned that, drug are proper cleaned, dried and grind to fine particle size powder. The powder is fine of at least 80 mesh sieves. It should be free from moisture content. The fine powder has better therapeutic value and should be kept in air tight container [16].

10. Wet Powder Particle Size: In Ayurveda, it is mentioned that, drug are proper cleaned and dry and grind to fine particle size powder. The powder is fine of at least 80 mesh sieves. It should be free from moisture content. The fine powder has better therapeutic value and should be kept in air tight container [16].

11. Foaming Index: 1gm of the plant samples were taken (coarse powder) and transferred to a 500ml of conical flask containing 100 ml of boiling distilled water. It was moderate boiled for 30 minutes, cooled and then filtered into a 100 ml volumetric flask through Whatman no.1 filter paper and added sufficient water through the filter to dilute to volume. Now poured the decoction into 10 stoppered test tubes of height-16cm, diameter-16mm in successive portions of 1ml, 2ml, 3ml, etc. up to 10ml and adjusted the volume of the liquid in each tube with water up to 10ml. All the tubes are stoppered and shaken in a length wise motion for 15 sec, at the rate of two shakes per second. Allowed to stand for 15 min and measured the height of the foam [20].

The results were assessed as follows.

1. If the height of the foam in every tube is less than1cm, the foaming index is less than 100.

2. If a height of foam of 1cm is measured in any tube; the volume of the plant material decoction in this tube (a) is used to determine the index. If this tube is the first or second tube in a series, prepare an intermediate dilution in a similar manner to obtain a more precise result.

3. If the height of the foam is more than 1cm in every tube; the foaming index is over 1000. In this case repeat the determination using a new series of dilutions of the decoction in order to obtain a result. The foaming index was calculated by using the following:

$$\text{Foaming Index} = 1000 / A$$

Where, A = the volume in ml of the decoction used for preparing the dilution in the tube where foaming to a height of 1 cm is observed.

12. Swelling Index: The swelling index of many types of herbal materials are of specific therapeutic or pharmaceutical quality and utility because of their swelling properties - especially gums and those containing an appreciable amount of mucilage, pectin or hemicelluloses**.**

All samples were cleaned by passing through 20 or 40 meshes. Unwanted materials like grains, husk, etc. were removed from it. 1 gm of pulp without seed, pericarp, leaf and seed was transferred to the measuring cylinders as 25, 50, 100, 250 ml and required volume of solvent like distilled water, chloroform water, 90% alcohol, 0.1 N HCl, 0.1 N NaOH, ethyl acetate, solvent ether and alcohol moistened samples + distilled water was added and stoppered. The measuring cylinders were agitated at every 10 min. For first 1 hour and then every 3 hours and the volume occupied by seeds along with mucilage was noted. This process was continued for 24 h. Final results were taken at the end of 24 h. The cylinders were kept for observation for next 24 h to note any change in swelling factor [21, 22].

Extraction of Different Parts of *Aegle marmelos* (L.): Freshly collected *Aegle marmelos* (L.) pulp without seed, pericarp, leaves and seed were dried in shade. Course powder of dried different parts (300gm) was extracted successively in methanol and distilled water solvents by maceration process of extraction for the period of three days with occasional stirring. The macerate mixtures were filtered by Whatman paper no. 1 separately and thereafter extract fractions obtained were concentrated and dried. The yields percentage and consistencies of extract fractions were recorded [23].

Primary Qualitative Phytochemical Screening: Primary phytochemical screening of extract fractions of *Aegle marmelos* (L.) pulp without seed, pericarp, leaves and seed were performed to identify the presence of class of phytoconstituents like, alkaloids, amino acids, carbohydrates, flavo-noids, glycosides, proteins, saponin, steroids, tannins and phenols [24, 25].

Fluorescence Analysis: Dried powder of *Aegle marmelos* (L.) pulp without seed, pericarp, leaves and seed was studied for fluorescence study as such and after treating independently with water, 1 N of HCl, HNO_3, H_2SO_4, NaOH, $KMnO_4$, KOH, alcoholic NaOH and KOH and ammonia using normal and ultra-violet light (254 nm). The physical and chemical parameters when felt

inadequate, as it often happens with the powdered drugs, the plant material may be identified from their adulterants on the basis of fluorescence study [26, 27].

Chemical Qualitative Profiling of *Aegle marmelos* (L.) using HPTLC fingerprinting Technique: The mobile phase used in qualitative identification of different parts of *Aegle marmelos* (L.) by HPTLC method, was prepared by mixing toluene: ethyl acetate (7:3, v/v).

Preparation of Stock Solution: For HPTLC, the stock solutions of different parts of *Aegle marmelos* L. (1mg/ml) were prepared in methanol.

Sample Preparation: *Aegle marmelos* (L.) parts were dried and extracted with distilled water to obtain various aqueous extracts. For HPTLC each extracts were weighted accurately, 1 gm powder was dissolve in 20 ml methanol and sonicated for 30 min and then extracted with methanol under reflux on water bath at 60 ºC and finally made up to 5ml. Each extracts were then filtered through Whatman no. 1 filter paper. 20µl sample were applied to the plates in duplicates.

Chromatographic Conditions: The HPTLC densitometry scanning was performed on CAMAG TLC scanner III connected to PC running WINCATS software under MS Windows, connected with 100µl syringe and linked to a nitrogen tank. The samples were spotted on pre-coated silica gel aluminium plate 60 F254 (20 cm × 20 cm with 0.2 mm thickness) 5 × 10 cm plate using a CAMAG Linomat V (Switzerland) sample applicator for spotting. The plates were prewashed by methanol and activated at 105 ºC for 20 min, prior to chromatography. The mobile phase consisted of toluene: ethyl acetate (7:3 v/v) used for chromatography. Linear ascending development was carried out in 20 cm × 10 cm twin trough glass chamber (CAMAG, Switzerland) saturated for 30 min with the development solvent system allowed to start at the position 10 mm and migrate up to a height of 80 mm from the lower edge at room temperature (25±2 °C) and relative humidity of 60±5%. The chromatogram was developed for 30 min to a distance of 80 mm and dried in a current of hot air using an air dryer. Deuterium and tungsten lamps, in the absorbance mode at 190 and 400 nm emitting a continuous UV spectrum was used as the source of radiation. A constant application rate of 150 mL/sec was used. The scanner was set for maximum light optimization and with the settings of slit dimension, scanning speed 20 mm/sec and data resolution speed 100 m/step. The scan started at position 10 mm and ended at position 80 mm [28].

Procedure: 5µl sample loaded in 100µl syringe and run up to 15 min in solvent system. It is dried in hot air. After dry, plates were evaluated in UV spectrum chamber. Then 10 ml spray (5% methanolic - sulphuric acid reagent) on a TLC plate, heated at 105 ºC for 5 -10 min. After derivatization (visibilities) TLC plates was evaluated in visible or UV- 365 nm in UV spectrum chamber [29].

Microbial Limit Assay: Dissolve 1gm of powdered plant material in 10 mL 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 and Fungi respectively. For bacteria, the plates were incubated at 37 °C for 48 h and for fungi; the plates were incubated 25 °C for 96 h [30].

RESULTS AND DISCUSSION:

Macroscopic Morphological Characters of Different Parts of *Aegle marmelos* (L.): The Bael tree attains a size about 12 to 15 meter in tall in the harsh and dry climates in india. Fruit of *Aegle marmelos* (L.) are use as dietary purpose. It has either oval, pyriform, sub-globous or oblong in shape but it size yield depend on the geoclimatic condition and variety of Bael. The fruit having length 10.4 cm and width 23.6 m was observed. The fruit was cut into two halves and transverse sections were prepared. The pulp of *Aegle marmelos* (L.) are found in hard central core of Bael. It has 8 to 22 diameters of triangular segments that are dark orange in color. The pulp is basically pale orange, sweet, resinous, pasty, pleasantly more or less astringent but highly aromatic characteristic are present in Bael pulp. They contain 10 to 20 seeds. The seed are flat, oblong in shape and about 1 cm long with white woody cotton like hair on their outer layer. They having length 0.5 cm and width 0.2 cm was observed. The Bael fruit has pericarp (ringed) about

10.4 cm length and 1.5-3 mm thick, hard and woody shell with a hammer or machete. It has smooth or slightly granular circular scars at the point of attachment with peduncle. The leaf of *Aegle marmelos* (L.) was deciduous, alternate and 3-folilate leaflet arrangement. The shape of leaf is oval, pointed, shallowly toothed leaflet 4.5 cm long; 2.4 cm wide, the terminal one with 2.5 cm a long petiole are observed **Fig. 1**.

FIG. 1: MACROSCOPIC PHOTOGRAPHS OF *AEGLE MARMELOS* (L.) – (A): Bael fruit, (B): Fruit cut part, (C): Pulp with seed, (D): Pulp without seed, (E): Bael pericarp, (F): Bael seed, (G): Bael leaf

Organoleptic Characters: The organoleptic characters were observed in powdered fruit pulp (without seed), pericarp, leaf and seed. Fine powder of *Aegle marmelos* (L.) fruit pulp (without seed) was bright yellowish orange in color, bitter taste and latex cum mild aromatic odor and smooth texture. The pericarp powder was having light yellowish orange in color, latex odor, less bitter taste and rough texture. The leaf powder was having dark green in color, aromatic odor, bitter taste and smooth texture. The seed powder was a off white in color, mild aromatic odor, bitter taste and oily with smooth texture observed **Table 1**.

TABLE 1: ORGANOLEPTIC CHARACTERS OF DIFFERENT PARTS OF BAEL (*AEGLE MARMELOS* L.) FRUIT

S. no.	Name of Characters	Results			
		Pulp	Pericarp	Leaf	Seed
1	Color	Bright yellowish-orange	Light yellowish-orange	Green	Off white
2	Odor	Latex odor and mild aromatic odor	Latex odor	Aromatic odor	Mild aromatic odor
3	Taste	Bitter taste	Less bitter taste	Bitter taste	Bitter taste
4	Texture	Smooth	Rough	Smooth	Smooth

Microscopic Study of Leaf (Transverse Section): The Bael **(***Aegle marmelos* L.) Leaf is 3 leaflets in nature. Transverse section (T.S.) of the leaf showed the upper arched epidermal cells with stomata. The fibers were extent beneath the epidermis. Mesophyll cells include sclerenchyma and collenchymas fibers that proliferate from the sheath around the vein. Small acicular crystals, xylem, phloem, pith and trichom were formed in idioblasts of mesophyll and lower epidermis was showed **Fig. 2**.

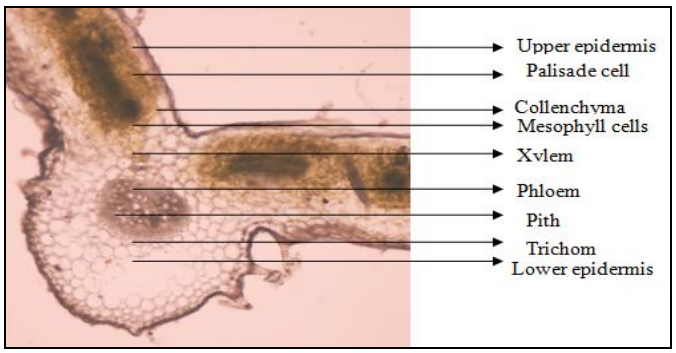

FIG. 2: MICROSCOPY TRANSVERSE SECTION (T.S.) OF *AEGLE MARMELOS* (L.) GREEN LEAF

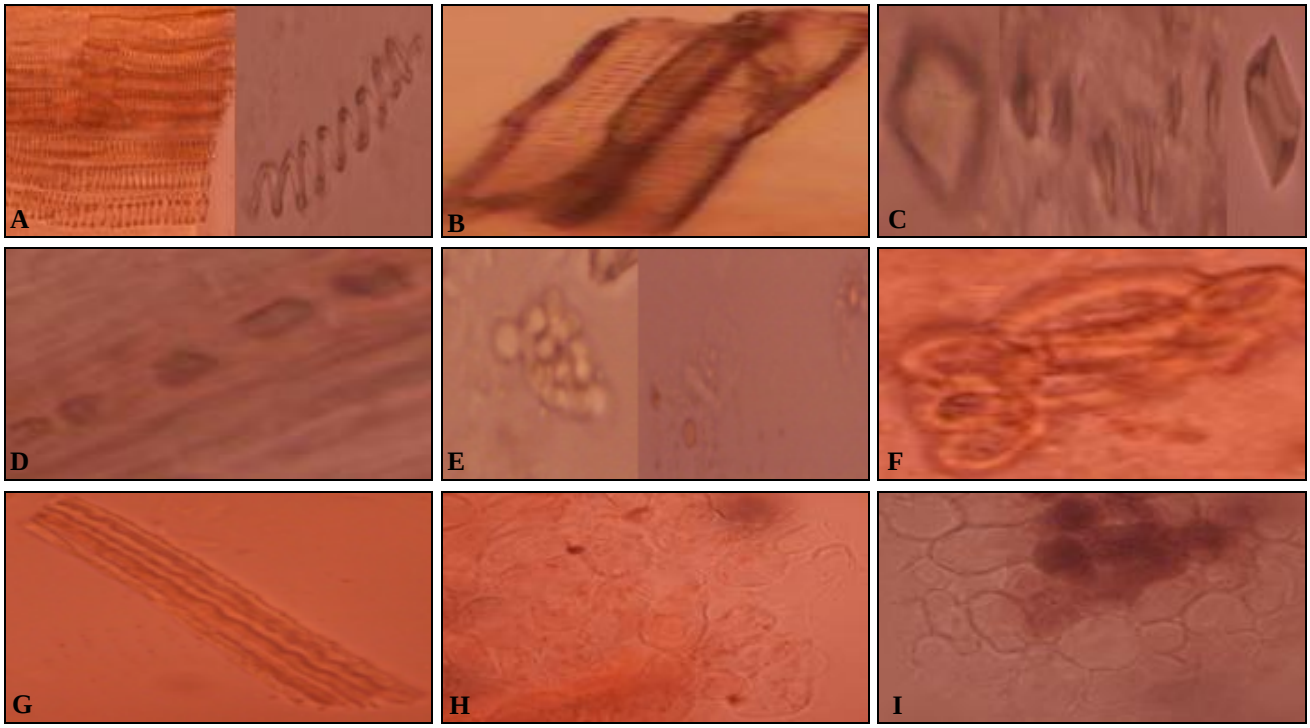

FIG. 3: MICROSCOPIC PHOTOGRAPHS OF POWDER OF *AEGLE MARMELOS* (L.) PULP (WITHOUT SEED) – (A): Fragments of isolated, (B): Tracheids, (C): Prismatic crystals of calcium oxalate, (D): Crystal fibers, (E): Single and compound starch grains, (F): Groups of stone cells, (G): Fibres, (H): Parenchymatous cell containing oil globules and calcium oxalate in surface view, (I): Parenchymatous cells containing starch grains in surface view

Microscopy Study of Power: The different parts of Beal (*Aegle marmelos* L.) like pulp (without seed), pericarp, leaf and seed powder were observed under microscope. The pulp powder (without seed) showed the presence of characteristic prismatic crystal of calcium oxalate, simple and compound starch grains, oil globules. Fragments of isolated vessels of scalariform thickenings containing starch grains and calcium oxalate crystals in surface view. Group of parenchymatous cell containing oil globules and starch grains, isolated stone cells, group of stone cells, fragments of isolated vessels of spiral thickening and fragment of pitted tracheid element **Fig. 3**.

The pericarp powder is showing diagnostic characters various shapes and sizes of stone cells, thick-walled cork cells in surface view, parenchymatous cells, thick-walled fibers, and epicorp cells **Fig. 4**.

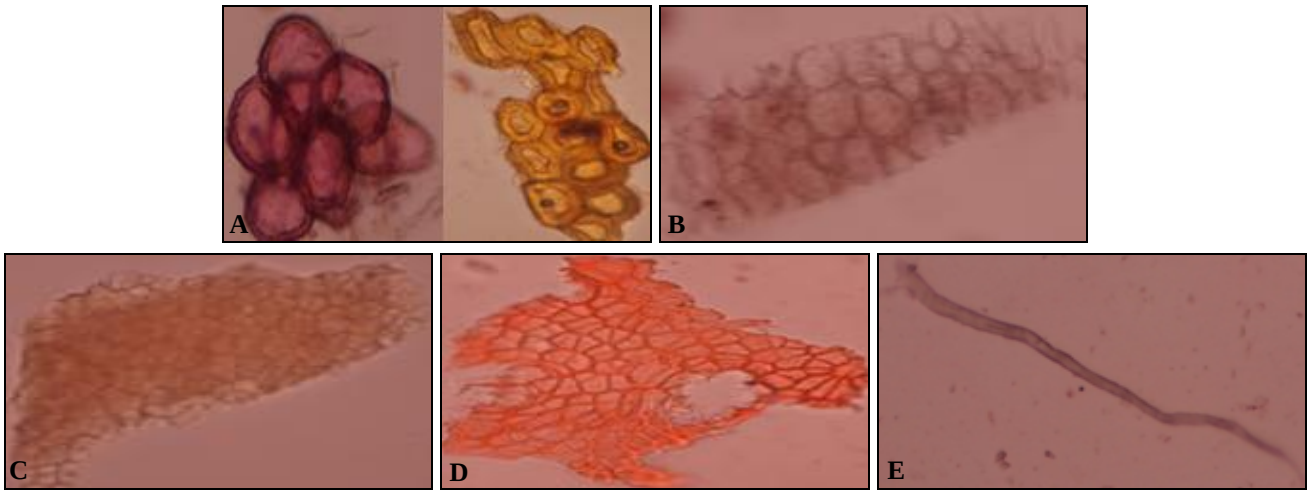

FIG. 4: MICROSCOPIC PHOTOGRAPHS OF POWDER OF *AEGLE MARMELOS* (L.) PERICARP – (A): Groups f stone cells in various shapes and sizes, (B): Parenchymatous cells, (C): Epicorp cells, (D): Cork cells in surface view, (E): Fibres

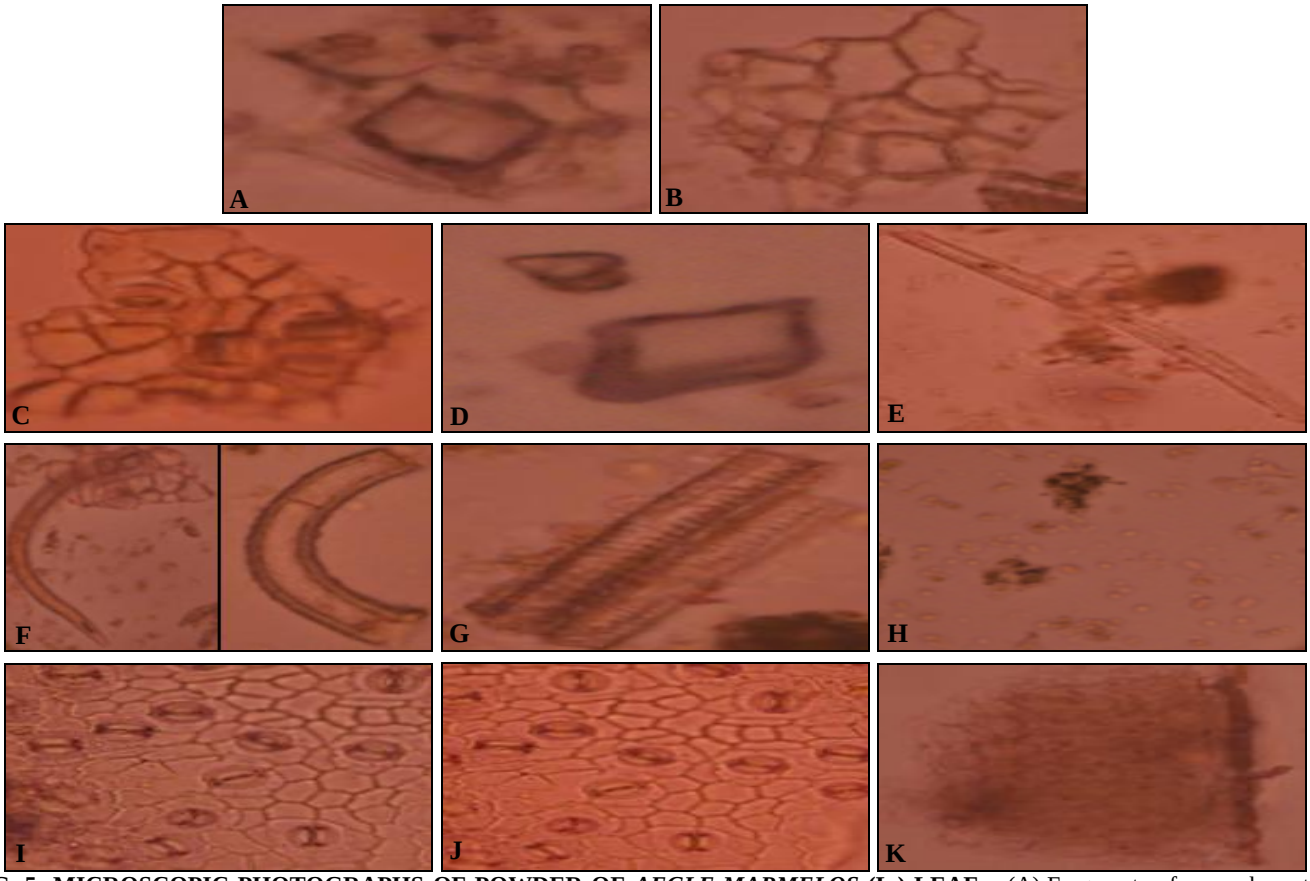

FIG. 5: MICROSCOPIC PHOTOGRAPHS OF POWDER OF *AEGLE MARMELOS* (L.) LEAF – (A):Fragments of parenchymatous cells filled with calcium oxalate crystals, (B): Parenchyma filled with calcium oxalate crystals & starch grains, (C): Parenchymatous cells filled with calcium oxalate crystals, (D): Prismatic crystals of calcium oxalate, (E): Thick-walled fibres, (F): Various types of Trichomes, (G): Reticulate thickenings, (H): Single and compound starch grains, (I): Upper epidermis with stomata, (J): Lower epidermis with stomata, (K): Palisade cells

The leaf powder Shows prismatic crystals of calcium oxalate, single & compound starch grains, Reticulate thickenings, Simple pitted vessel, Spiral thickening, Stone cells, fragments of parenchymatous cells filled with calcium oxalate crystals, parenchymatous cells filled with calcium oxalate crystals, Thick walled fibers, Various types of trichomes, parenchyma filled with calcium oxalate crystals & starch grains, Lower epidermis with stomata, Upper epidermis with stomata, thick walled parenchymatous cells from rachis **Fig. 5**. The seeds powder diagnostic characters shows oil globules, aleurone grains, simple starch grains, fragments of cotyledone in surface view, fragments of endosperm showing epidermis and collapsed cells, thick walled long warty trichomes **Fig. 6**.

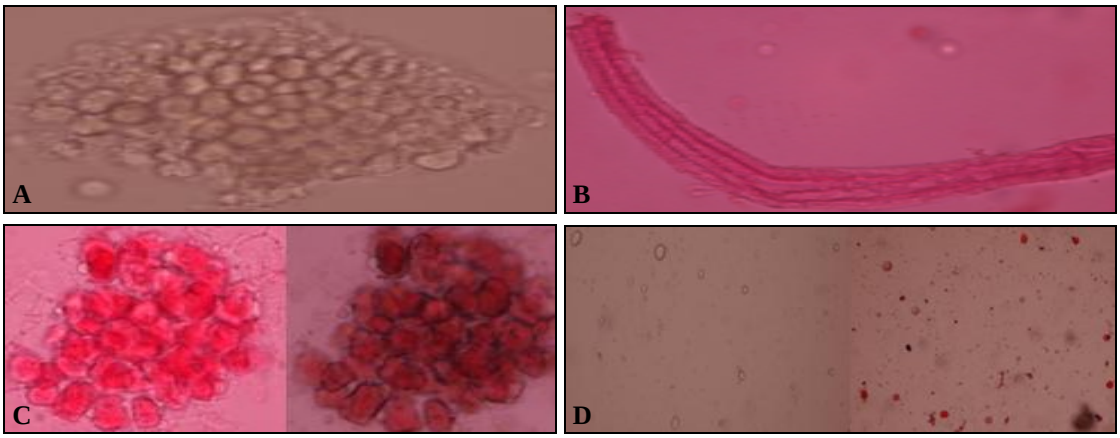

FIG. 6: MICROSCOPIC PHOTOGRAPHS OF POWDER OF *AEGLE MARMELOS* (L.)SEED – (A): Cotyledone in surface view, (B): Long, thick-walled warty trichomes, (C): Fragments of endosperm showing epidermis and collapsed cells, (D): Oil granules, Aleurone gains & Starch grain

Physico-chemical Parameters: The physico-chemical parameters are the most important tool for identifying adulterants, impurities, quality, purity and improper handling of powder drugs. The result of physicochemical parameters of powdered drug, carried out by following standard procedures. The ash values used to identifying quality and purity of drug including matter, organic, inorganic composition and other impurities present in the powder drugs. It was found that ash values of the powdered leaf reveal a high percentage in compare to total Ash value, acid insoluble ash and water soluble ash. The solubility test (extractive values) given an idea about the many chemical constituents found in the powder drug as well as it is more useful in the determination of exhausted impurities and adulterated drug. They are most helpful in estimation of particular chemical constituents soluble in main solvent chemical. The loss on drying (LOD) reveals the percentage of moisture, Acidity, Foreign Matter, Dry Powder particle size, Wet Powder particle size, Dry Powder particle size, Foaming Index and Swelling Index was present in the powder drug **Table 2**.

TABLE 2: PHYSIO-CHEMICAL PARAMETERS OF BAEL (*AEGLE MARMELOS* L.) FRUIT

S. no.	Name of Experiments	Results (percentage)			
		Pulp (without seed)	Pericarp	Leaf	Seed
1	PH %	5.43	5.21	6.11	6.22
2	Solubility test (%)				
3	Aqueous extract	54.56	25.98	39.43	8.64
4	Methanol extract	61.32	25.74	15.74	22.03
5	Total Ash value	3.84 ± 0.19	2.51 ± 0.02	19.18 ± 0.01	5.75 ± 0.02
6	(a) Acid Insoluble Ash	0.13 ± 0.05	0.08 ± 0.04	3.43 ± 0.13	0.07 ± 0.02
7	(b)Water Soluble Ash	0.43 ± 0.03	0.62 ± 0.02	16.29 ± 0.06	5.23 ± 0.01
8	LOD (Loss of Drying) %	6.80	6.20	6.10	4.18
9	Acidity as Citric Acid%	0.94	0.74	137.36	119.11
10	Foreign Matter	< 0.1%	< 0.01%	< 0.2%	< 0.05%
11	Dry Powder particle size	0.346 µm	0.446 µm	0.159 µm	0.601µm
12	Wet Powder particle size	0.350 µm	0.446 µm	0.163 µm	0.609 µm
13	Foaming index	331.2 U	223.1 U	337.5 U	198.1 U
14	Swelling Index	4.5 %	2.7 %	3.5 %	2.9 %

Extraction Analysis: The extraction of the different parts of pulp (without seed), pericarp, leaf and seeds of *Aegle marmelos* (L.) plant were performed. The percentage (%) yields and consistencies of aqueous and methanolic extract fractions are shown in **Table 3**.

The extract was subjected to qualitative phyto-chemical investigation to detect different phyto-constituents physiological test, antioxidant, antibacterial, drug toxicity, nutritional analysis, TLC, HPLC and different *in-vitro* methods.

TABLE 3: % YIELD OF EXTRACTS OF DIFFERENT PARTS OF *AEGLE MARMELOS* (L.) PLANTS

S. no.	Name of solvents	Results				
		Parts of plant	Color of extraction	Consistencies	Yield (in gms)	Percentage yield (w/w)
1	Aqueous	Pulp (without seed)	Yellowish Brown	Viscous	82.3	11.14
2	100% methanol	Pulp (without seed)	Yellowish Brown	Viscous	92.3	13.14
3	Aqueous	Pericarp	Yellowish Brown	Viscous	56.8	9.90
4	100% methanol	Pericarp	Yellowish Brown	Viscous	65.3	8.76
5	Aqueous	Leaf	Greenish	Crystals	62.3	10.05
6	100% methanol	Leaf	Dark Greenish	Viscous	72.3	11.12
7	Aqueous	Seed	Light white	Crystals	77	10.09
8	100% methanol	Seed	White	Viscous oily	80	12

Qualitative Phytochemical Screening: The qualitative preliminary phytochemical constituents screening is helpful in a forecast of the nature of drugs and also useful for detection of many types of chemical constituents in different polarity solvent. The results for primary phytochemical screening of aqueous and methanolic extract of *Aegle marmelos* (L.) of diffentent parts such as pulp (without seed), pericarp, leaf and seed revealed the presence of different polar and non-polar chemical constituents **Table 4**.

TABLE 4: PHYTOCHEMICAL SCREENING OF AQUEOUS EXTRACT AND METHANOL EXTRACT

S. no.	Name of experiments	Pericarp Extracts		Pupl Extracts		Leaf Extracts		Seed Extracts	
		A	M	A	M	A	M	A	M
1	Alkaloids	+	+	+	+	+	+	+	+
2	Carbohydrates	+	+	+	+	+	+	+	+
3	Protein	+	+	+	+	+	+	+	+
4	Starch	+	+	+	+	+	+	+	+
5	Resin	+	+	+	+	+	+	+	+
6	Coumarins	+	+	+	+	+	+	+	+
7	Phenols	+	+	+	+	+	+	+	+
8	Tannin	+	+	+	+	+	+	+	+
9	Phloba- Tannin	+	+	+	+	+	+	-	-
10	Flavonoids	+	+	+	+	+	+	+	+
11	Terpenoids	+	+	+	+	+	+	+	+
12	Triterpenoid	+	+	+	+	+	+	+	+
13	Glycosides	+	+	+	+	-	-	-	+
14	Vitamin C	+	+	+	+	+	+	+	+
15	Anthra-quinones test	+	+	+	+	+	+	+	+
16	Polyphenols	+	+	+	+	+	+	+	+
17	Steroids	+	+	+	+	+	+	+	+
18	Saponins	+	-	-	-	+	-	-	-
19	Quiones	-	-	-	-	-	-	-	-
20	Carboxylic Acid	-	-	-	-	-	-	-	-

Fluorescence Analysis of Powder Pulp, Pericarp, Leaf and Seed of *Aegle marmelos* (L.) Plant: The fluorescence analysis of the pulp, pericarp, leaf and seed parts of *Aegle marmelos* (L.) plant as well as powder was studied in UV short light, UV long light and day light. The powder showed different fluorescence color in UV light when with treated different chemical solvents which indicates the presence of chromophore in the powder as well as drugs. These different types of chromatophoric colors under UV and visible light illustrated the nature of raw materials. It may be help to assess the purity and quality of the drug **Table 5-8**.

TABLE 5: FLUORESCENCE ANALYSIS OF PULP (WITHOUT SEED) POWDER OF *AEGLE MARMELOS* (L.)

S. no.	Treated with Chemical reagents	Day light	Fluorescence Results (colour base)	
			UV light	
			UV 254 nm	UV 366 nm
1	Pulp powder	Yellowish orange	White	Yellow
2	1N HCL	Yellow	Brown	Rock
3	Aqueous 1N NaOH	Pale Yellow	Red	Yellowish red
4	Methanol 1N NaOH	Pale Yellow	Dark red	Dark Brown
5	50% KOH	Pale Yellow	Dark red	Greenish yellow
6	50% H_2SO_4	Dark orange	Brown	Dark pink
7	conc. H_2SO_4	Pale orange	Light brown	Pale yellow
8	50% HNO_3	Yellow orange	Brown	Light Black
9	Acetic acid	Pale Yellow	Light Brown	Yellow
10	Iodine water	Brown	Light black	Yellow

TABLE 6: FLUORESCENCE ANALYSIS OF PERICARP POWDER OF *AEGLE MARMELOS* (L.)

S. no.	Treated with Chemical reagents	Daylight	Fluorescence Resuts (colour base)	
			UV light	
			UV 254 nm	UV 366 nm
1	Pericarp powder	Yellow	White	Yellow
2	1N HCL	Pale Yellow	Light yellow	Brown
3	Aqueous 1N NaOH	Yellow	Dark Red	Dark brown
4	Methanol 1N NaOH	Pale Yellow	Dark red	Brown
5	50% KOH	Dark Yellow	Light Brown	Black
6	50% H_2SO_4	Yellowish orange	Brown	Dark pink
7	conc. H_2SO_4	Dark brown	Light brown	Pale yellow
8	50% HNO_3	Pale Yellow	Brown	Black
9	Acetic acid	Pale Yellow	Light Brown	Light Black
10	Iodine water	Yellowish orange	Light Brown	Light Black

TABLE 7: FLUORESCENCE ANALYSIS OF LEAF POWDER OF *AEGLE MARMELOS* (L.)

S. no.	Treated with Chemical reagents	Day light	Fluorescence Results (colour base)	
			UV light	
			UV 254 nm	UV 366 nm
1	Leaf powder	Green	Greenish brown	Rock white
2	1N HCL	Dark green	Light green	Grayish Brown
3	Aqueous 1N NaOH	Brown	Dark Brown	Pale green
4	Methanol 1N NaOH	Pale green	Yellowish green	Grayish Brown
5	50% KOH	Dark green	Light Brown	Dark brown
6	50% H_2SO_4	Light Brown	Pale green	Brown
7	conc. H_2SO_4	Dark orange	Pale golden	Brown
8	50% HNO_3	Pale green	Reddish Brown	Brown
9	Acetic acid	Pale green	Yellowish green	Dark green
10	Iodine water	Brown	Reddish Brown	Light brown

TABLE 8: FLUORESCENCE ANALYSIS OF SEED POWDER OF *AEGLE MARMELOS* (L.)

S. no.	Treated with Chemical reagents	Day light	Fluorescence Results (colour base)	
			UV light	
			UV 254 nm	UV 366 nm
1	Seed powder	Dusky white	Yellow	White
2	1N HCL	Pale Yellow	Pale yellow	golden
3	Aqueous 1N NaOH	Dull white	Pale golden	Faint yellow
4	Methanol 1N NaOH	Dull white	Brown	Whitish yellow
5	50% KOH	Pale Yellow	Dull white	Brown
6	50% H_2SO_4	Light brown	Greenish brown	Light brown
7	conc. H_2SO_4	Brown	Pale brown	Brown
8	50% HNO_3	Light Brown	Brown	Dark brown
9	Acetic acid	Pale Yellow	Pale golden	Pale brown
10	Iodine water	Light brown	Pale golden	Pale yellow

Qualitative Primary HPTLC Fingerprinting Analysis of Methanolic Extract Pulp, Paricarp, Leaf and Seed of *Aegle marmelos* (L.) Plant: The HPTLC fingerprinting of methanolic extract fraction of pulp was showed the R_f values at 254 nm, 366 nm and day light with their respective UV-visible spectrum wavelengths scanned.

Rf value was 0.28 at 254 nm and 0.21, 0.29, 0.37, 0.43, 0.69 and 0.83 at 366 nm (1mg/ml concentration) of sample. After derivatization using 5% methanolic – sulphuric acid reagent spray Rf value were 0.14, 0.20, 0.27, 0.36, 0.41, 0.53, 0.61, 0.69, 0.85, 0.91 and 0.59, 0.71, 0.81 (day light) were found at 366 nm. HPTLC plate were scanned at 254 nm, 366 nm and day light after derivatization are shown in **Fig. 7** and the Rf value and color of the resolved band at 254 nm, 366 nm and day light after derivatization are shown in **Table 9**.

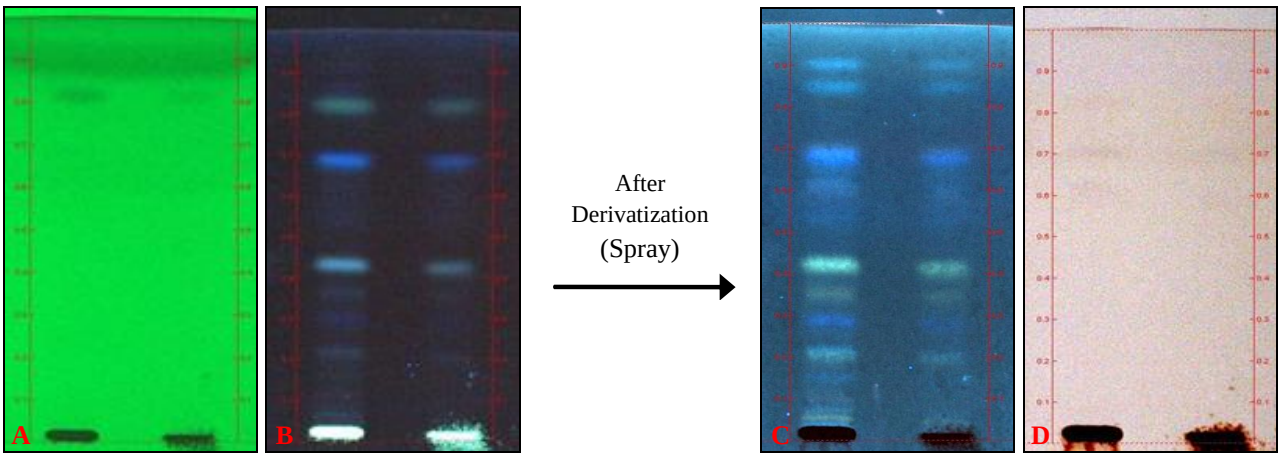

FIG. 7: SCANNED HPTLC DEVELOPED PLATES OF *AEGLE MARMELOS* (L.) PULP (WITHOUT SEED) EXTRACT. A: Scanned HPTLC plate at 254 nm; B: Scanned HPTLC plate at 366 nm; C: Scanned HPTLC plate after derivatization at 366 nm; D: Scanned HPTLC plate after derivatization at Day light

TABLE 9: R_F VALUE SCAN METHANOLIC EXTRACT OF PULP BY USING HPTLC FINGERPRINTING

S. no.	Rf value of Pulp (Retention Factors)			
			After Derivatization (after spray) 5% Methanolic – Sulphuric acid reagent	
	254 nm	366 nm	366 nm	Day Light
1	0.28	0.21 (light blue)	0.14 (light blue)	0.59
2		0.29 (light blue)	0.20 (light brown)	0.71
3		0.37 (grey)	0.27 (blue)	0.81
4		0.43 (sky blue)	0.36 (grey)	
5		0.69 (blue)	0.41 (light green)	
6		0.83 (light green)	0.53 (light blue)	
7			0.61 (grey)	
8			0.69 (blue)	
9			0.85 (blue)	
10			0.91 (blue)	

The HPTLC fingerprinting of methanolic extract fraction of pericarp was showed the Rf values at 254 nm, 366 nm and day light with their respective UV-visible spectrum wavelengths scanned. Rf value was 0.30 at 254 nm and 0.03, 0.05, 0.07, 0.10, 0.17, 0.24, 0.31, 0.38, 0.44, 0.54, 0.81, 0.85 at 366 nm (1mg/ml conc.) of sample. After derivatization using 5% methanolic - sulphuric acid reagent spray R_f value were 0.09, 0.19, 0.37, 0.44, 0.55, 0.64, 0.71, 0.85 and (day light) 0.63, 0.70 found at 366 nm. HPTLC Plate were scanned at 254 nm, 366 nm and day light with after derivatization are shown in **Fig. 8** and the R_f value and color of the resolved band at 254 nm, 366 nm and day light with after derivatization are shown in **Table 10**.

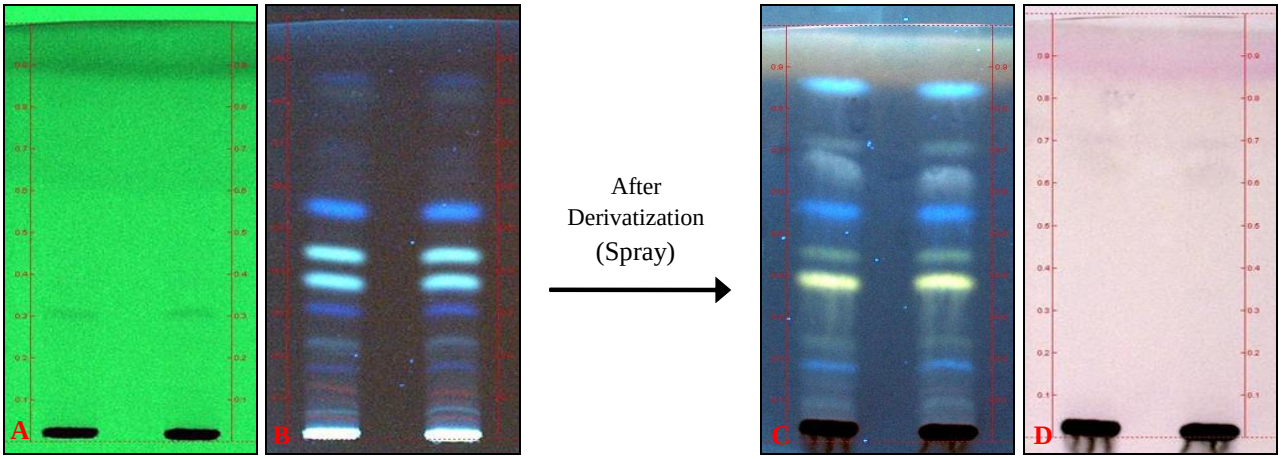

FIG. 8: SCANNED HPTLC DEVELOPED PLATES OF *AEGLE MARMELOS* (L.) PERICARP EXTRACT. A: Scanned HPTLC plate at 254 nm; B: Scanned HPTLC plate at 366 nm; C: Scanned HPTLC plate after derivatization at 366 nm; D: Scanned HPTLC plate after derivatization at Daylight

TABLE 10: R_F VALUE SCAN OF METHANOLIC EXTRACT PERICARP BY USING HPTLC FINGERPRINTING

S. no.	Rf value of Pericarp (Retention Factors)			
			After Derivatization (after spray) 5% Methanolic – Sulphuric acid reagent	
	254 nm	366 nm	366 nm	Day Light
1	0.30	0.03 (sky blue)	0.09 (grey)	0.63
2		0.05 (red)	0.19 (blue)	0.70
3		0.07 (light green)	0.37 (yellow)	
4		0.10 (red)	0.44 (light green)	
5		0.17 (light blue)	0.55 (blue)	
6		0.24 (grey)	0.64 (brown)	
7		0.31 (blue)	0.71 (grey)	
8		0.38 (sky blue)	0.85 (sky blue)	
9		0.44 (sky blue)		
10		0.54 (blue)		
11		0.81 (grey)		
12		0.85 (blue)		

The HPTLC fingerprinting of methanolic extract fraction of **leaf** was showed the Rf values at 254 nm, 366 nm and day light with their respective UV-visible spectrum wavelengths scanned. Rf value were 0.11, 0.28 (major spots) 0.36, 0.49, 0.61, 0.69, 0.80 at 254 nm and 0.08, 0.12, 0.16 0.22, 0.27, 0.36, 0.43, 0.50, 0.55, 0.62, 0.67, 0.76, 0.80, 0.82, 0.85, 0.91, 0.97 at 366 nm (1mg/ml conc.) of sample. After derivatization using 5% methanolic – sulphuric acid reagent spray Rf value were 0.05, 0.11, 0.16, 0.22, 0.27, 0.36, 0.43, 0.50, 0.55, 0.61, 0.76, 0.80, 0.86, 0.91, 0.96 and (day light) 0.05, 0.11, 0.15, 0.26, 0.36, 0.48, 0.61, 0.70 found at 366 nm. The HPTLC plate were scanned at 254 nm, 366 nm and day light with after derivatization are shown in **Fig. 9** and the Rf value and color of the resolved band at 254 nm, 366 nm and day light with after derivatization are shown in **Table 11**.

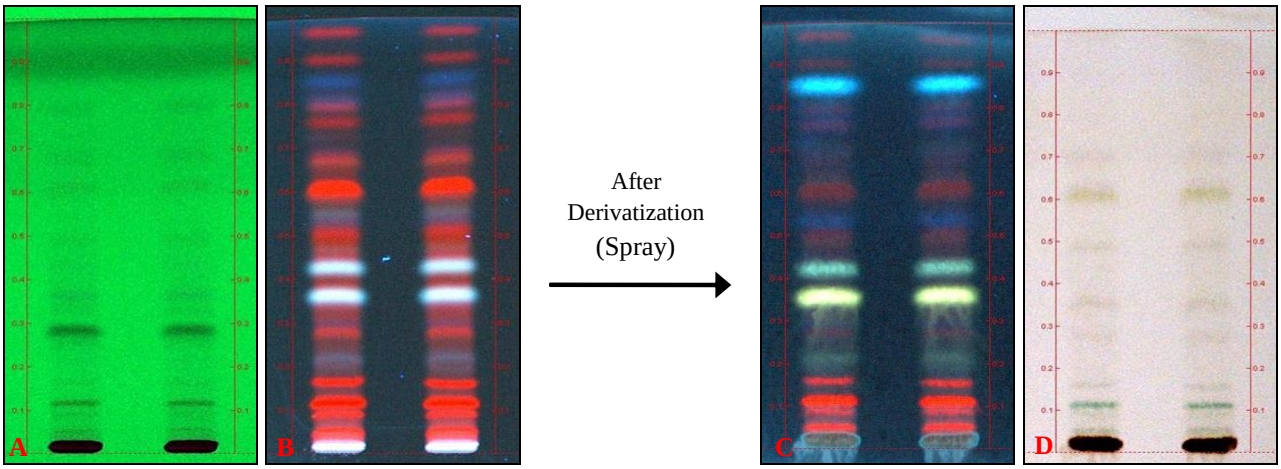

FIG. 9: SCANNED HPTLC DEVELOPED PLATES OF *AEGLE MARMELOS* (L.) LEAF EXTRACT. A: Scanned HPTLC plate at 254 nm; B: Scanned HPTLC plate at 366 nm; C: Scanned HPTLC plate after derivatization at 366 nm; D: Scanned HPTLC plate after derivatization at Day light

TABLE 11: R_F VALUE SCAN OF METHANOLIC EXTRACT LEAF BY USING HPTLC FINGERPRINTING

S. no.	R_f value of Leaf (Retention Factors)			
			After Derivatization (after spray) 5% Methanolic – Sulphuric acid reagent	
	254 nm	366 nm	366 nm	Day Light
1	0.11	0.08 (red)	0.05 (red)	0.05 (light green)
2	0.28	0.12 (red)	0.11 (red)	0.11 dark green)
3	0.36	0.16 (red)	0.16 (red)	0.15
4	0.49	0.22 (blue)	0.22 (greenish brown)	0.26
5	0.61	0.27 (red)	0.27 (red)	0.36
6	0.69	0.36 (brown white)	0.36 (yellowish brown)	0.48
7	0.80	0.43 (brown)	0.43 (brown)	0.61 (yellowish green)
8		0.50 (light blue)	0.50 (dark red)	0.70
9		0.55 (light blue)	0.55 (blue)	
10		0.62 (red)	0.61 (mahroon)	
11		0.67 (red)	0.76 (purple)	
12		0.76 (red)	0.80 (purple)	
13		0.80 (red)	0.86 (sky blue)	
14		0.82 (purple)	0.91 (light red)	
15		0.85 (blue)	0.96 (light red)	
16		0.91 (red)		
17		0.97 (red)		

The HPTLC fingerprinting of methanolic extract fraction of seed was showed the R_f values at 254 nm, 366 nm and day light with their respective UV-visible spectrum wavelengths scanned. R_f value was 0.83 at 254 nm and 0.05, 0.16, 0.21, 0.29, 0.34, 0.41, 0.47, 0.54, 0.69, 0.73, 0.81 at 366 nm (1mg/ml conc.) of sample. After derivatization using 5% methanolic – sulphuric acid reagent spray R_f value were 0.16, 0.21, 0.35, 0.41, 0.46, 0.54, 0.61, 0.73, 0.79, 0.85, 0.87 and (day light) 0.59, 0.71, 0.80, 0.87 found at 366 nm. HPTLC plate were scanned at 254 nm, 366 nm and day light with after derivatization are shown in **Fig. 10** and the R_f value and color of the resolved band at 254 nm, 366 nm and day light after derivatization are shown in **Table 12**.

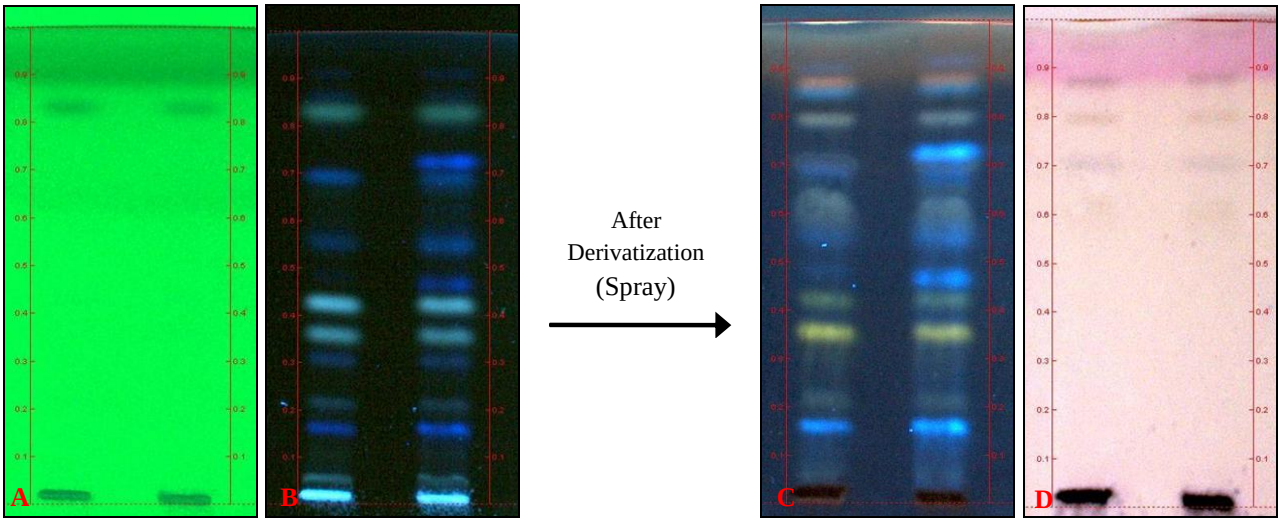

FIG. 10: SCANNED HPTLC DEVELOPED PLATES OF *AEGLE MARMELOS* (L.) SEED EXTRACT. A: Scanned HPTLC plate at 254 nm; B: Scanned HPTLC plate at 366 nm; C: Scanned HPTLC plate after derivatization at 366 nm; D: Scanned HPTLC plate after derivatization at Day light

TABLE 12: R_F VALUE SCAN OF METHANOLIC EXTRACT SEED BY USING HPTLC FINGERPRINTING

S. no.	R_f value of Seed (Rentention Factors)			
			After Derivatization (after spray) 5% Methanolic – Sulphuric acid reagent	
	254 nm	366 nm	366 nm	Day Light
1	0.83	0.05 (grey)	0.16 (sky blue)	0.59
2		0.16 (blue)	0.21 (grey)	0.71
3		0.21 (grey)	0.35(yellow)	0.80
4		0.29 (light blue)	0.41 (light yellow)	0.87
5		0.34 (light sky blue)	0.46 (blue)	
6		0.41 (light blue)	0.54 (light blue)	
7		0.47 (light blue)	0.61 (grey)	
8		0.54 (light blue)	0.73 (sky blue)	
9		0.69 (light blue)	0.79 (grey)	
10		0.73 (blue)	0.85 (blue)	
		0.81 (light green)	0.87 (red)	

Microbial Limit Assay: The total bacterial load present in the fruit (without seed) powder, pericarp, leaf and seed was within the limits as reported by Ayurvedic pharmacopeia of India. Only 200 CFU /g of bacteria were present in pulp (without seed) powder, 40 CFU/g of bacteria were present in pericarp powder, 30 CFU/g of bacteria were present in leaf powder and 400 CFU/g of bacteria were present in seed powder as well as pharmacopeia limits 10^5CFU/g. Only 30 CFU/g funguses were isolated per gram of pulp (without seed) powder, 180 CFU/g of fungus in pericarp powder, 200 CFU/g of fungus in leaf powder and 200 CFU/g of fungus in seed powder of *Aegle marmelos* (L.) plant compare as well as pharmacopeia limits of 10^3CFU/g. Finally, it was shown that every parts of plant powder was free from enteric bacteria like *Escherichia coli*, *Salmonella* sp., *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The results of microbial limits test plate are shown in **Fig. 11** and **Table 13**.

TABLE 13: MICROBIOLOGICAL LIMIT TEST IN DIFFERENT PARTS OF *AEGLE MARMELOS* (L.) CORR.

S. no.	Parameters	*Aegle marmelos* (L.) Corr.				Permissible Limits API part II Appendix 2.4
		Pulp	Pericarp	Leaf	Seed	
1	*Staphylococcus aureus /g*	Absent	Absent	Absent	Absent	Absent
2	*Salmonella sp. /g*	Absent	Absent	Absent	Absent	Absent
3	*Staphylococcus aureus /g*	Absent	Absent	Absent	Absent	Absent
4	*E.coli*	Absent	Absent	Absent	Absent	Absent
5	Total microbial plate count. (TPC)	200 cfu/g	40 cfu/g	30 cfu/g	400 cfu/g	10^5 cfu/g
6	Total Yeast and mould.	300 cfu/g	180 cfu/g	200 cfu/g	200 cfu/g	10^3 cfu/g

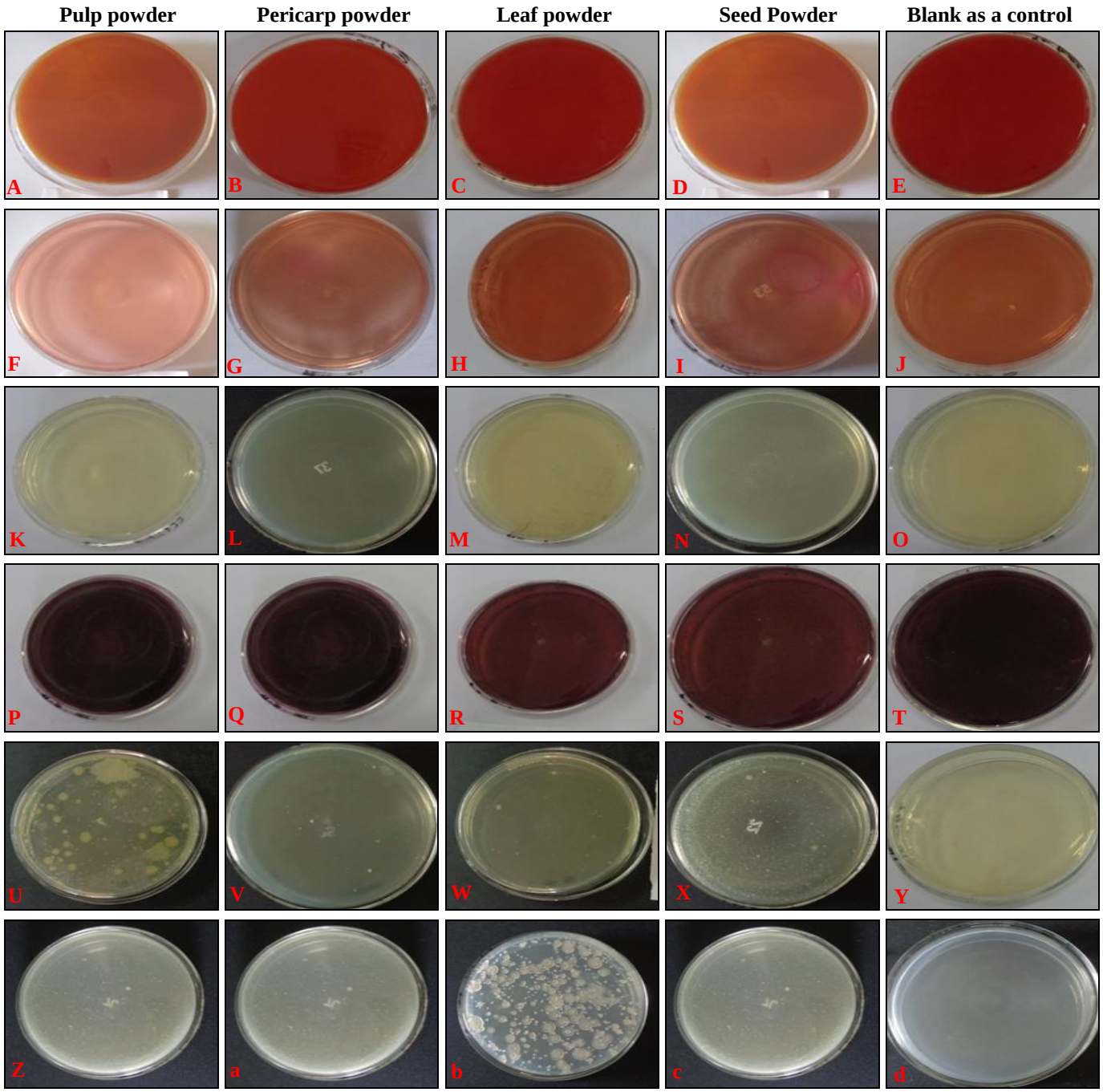

FIG. 11: MICROBIAL LIMIT TEST OF *AEGLE MARMELOS* (L.) PULP (WITHOUT SEED), PERICARP, LEAF, SEED AND CONTROL: Showing negative results for Salmonella (A – E), Showing negative result for *Staphylococcus aureus* (F – J), Showing negative result for *Pseudomonas aeruginosa* (K – O), Showing negative result for *E. coli* (P – T), Showing Total Bacterial Count (U – Y), Showing Yeast & Moulds (Z – d)

CONCLUSION: *Aegle marmelos* L. (Bael) pulp without seed, pericarp, leaf and seed powder was subjected to pharmacognostical evaluation including the organoleptic, powder microscopy, transverse section of leaf, fluorescence analysis of powder, anatomical studies, microbial limit test, HPTLC for qualitative analysis, physiochemical constituent and preliminary phytochemical screening. The organoleptic analysis which provided simple and fastest means to establish the identifying of a particular sample. The organoleptic studies was confirm shape, size, colour, odour, tecture and taste of different parts of *Aegle marmelos* (L.). Microscopic examination of powder shows the presence of cork cells, starch, vessels and fibers and histochemical studies of transverse section of leaf with different reagents showed calcium oxalate crystals, stone cell, phloem cells, cork cells, presence of brownish granules cells, etc. which is responsible for the medicinal importance of the

plant and many types of secondary plant metabolites. The standard behavior has been shown by different powders under day light, short UV and long UV light. Fluorescence analysis of particular powder drug gives the much information about the presence of chromophore.

Many details obtained by preliminary phytochemical screening will be useful in locating out ingenuousness of powder drug. The Ash values, forming index, extractive values can be used as reliable help for detection many types of adulteration. Microorganisms were found within the limits test as reported by Ayurvedic Pharmacopoeia of India.

These observations and results would be of great value in the standardization and botanical identification of drugs in a crude form. The observation and results of the present study adds more praise to existing phytomedicinal information helps in alignment development and to draw Pharmacopoeia standards which will be fruit-bearing for the future researchers towards the study of new herbal phytochemical medicine of indigenous resources entities.

ACKNOWLEDGEMENT: Author is thankful to University Grants Commission, New Delhi, India for financial assistance under Rajiv Gandhi National Senior Research Fellowship to carry out this work.

CONFLICTS OF INTEREST: The authors declare that there is no conflict of interests regarding the publication of this paper.

REFERENCES:

1. Ncube NS, Afolayan AJ and Okoh A: Assessment techniques of antimicrobial properties of natural compounds of plant origin: current methods and future trends. African Journal of Biotechnology 2008; 7(12): 1797-06.
2. Cai YZ, Luo Q, Sun M and Corke H: Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. Life Sci 2004; 74: 2157-84.
3. Miliauskas G, Venskutonis PR and Beek TA: Screening of radical scavenging activity of some medicinal and aromatic plant extracts. Food Chem 2004; 85: 231-37.
4. Abdelwahab SI, Abdul AB, Elhassan MM, Mohan S and Mariod AA: Phenolic Content and antioxidant activities of *Goniothalamus umbrosus* extracts. Int J Nat Prod Pharm Sci 2010; 1: 1-6.
5. Prusti A, Mishra SR, Sahoo S and Mishra SK: Anti-bacterial Activity of Some Indian Medicinal Plants. Ethnobotanical Leaflets 2008; 2: 227-30.
6. Gamble JS Flora of the Presidency of Madras. Adlard and Son's Ltd, London, UK 1935.
7. Matthew KM: The Flora of Tamil Nadu Carnatic. In The Rapinat Herbarium. St Joseph's College, Tiruchirapalli, India 1983.
8. Bakhru HK: Foods that Heal. The Natural Way to Good Health Orient Paperbacks. ND, India 1997.
9. Bakhru HK and Sastry ARK: Threatened Plants in India. Botanical Survey of India. Calcutta, WB, India 1979.
10. Chopra R: Indigenous drugs of India. Calcutta; Academic Publishers 1982.
11. Nadkarni AK: Indian Materia Medica. Volume 1. 3rd edition. Mumbai; Popular Prakashan 1954.
12. Satyavati GV, Gupta AK and Tandon N: Medicinal Plants of India. New Delhi: Indian Council of Medical Research 1976; 1.
13. Pandey A and Mishra R: Antibacterial properties of *Aegle marmelos* leaves, fruits and peels against various pathogens. JPBMS 2011; 13(13): ISSN NO- 2230 – 7885.
14. Brain KR and Turner TD: The Practical evaluation of phytopharmaceuticals. Bristol: Wright Scientechnica 1975; 4-9.
15. The Ayurvedic Pharmacopoeia of India. Part-I, Vol. I, Ed., 1st Controller of Publications. Ministry of Health and Family Welfare, Department of Indian Systems of Medicine and Homoeopathy, Government of India. New Delhi: National Institute of Science Communication (CSIR) 2001.
16. Quality standard of Indian medicinal plants, Indian council of medical research, Appendix I, The Ayurvedic Formulary of India, part- II, First English edition, government of India, ministry of health and family welfare, Department of India system of medicine & homoeopathy, New Delhi, 109. 2008; 5: 323-24.
17. Laboratory guide for the analysis of Ayurveda and Suddha Formulations "publisher by Central Council for Research in Ayurveda and Siddha, New Delhi. Department of AYUSH, Ministry of health and family Welfare, Government of India 2010; 32-33.
18. The Ayurvedic Pharmacopoeia of India, part 2 (Formalations), first edition, published by the controller of publications civil lines delhi ministry of health and family welfare, Department of Ayurveda, Yoga& Naturopathy, Unani, Siddha and Homoeopathy, New Delhi. page no. 2008; 2: 160-61.
19. The Ayurvedic Pharmacopoeia of India, part 1, 1st edition. published by the controller of publications civil lines delhi ministry of health and family welfare, Department of Ayurveda, Yoga & Naturopathy, Unani, Siddha and Homoeopathy, New Delhi. 2008; 6: 242.
20. Kokate CK: Practical Pharmacognosy. Vallabh Prakashan, New Delhi 1986; 112-13.
21. Indian Herbal Pharmacopoeia 1999; 2: 106.
22. Shah CS and Quadry JS: Textbook of Pharmacognosy. B. S. Shah Prakashan 1995.
23. Ng AS: Diterpenes from *Euphorbia neriifolia*. Phytochemistry 1990; 29(2): 662-4.
24. Khandelwal KR: Practical Pharmacognosy: Techniques and experiments. 19th ed., Pune: Nirali Prakashan 2008.
25. Kokate CK. Practical Pharmacognosy. 4th ed., New Delhi: Vallabh Prakashan 2007.
26. Chase CR and Pratt R: Fluorescence of powdered vegetable drugs with particular reference to development

of a system of identification. J Pharm Sci 1949; 38(6): 324-31.
27. Kokoski CJ, Kokoski RJ and Slama FJ: Fluorescence of powdered vegetable drugs under ultraviolet radiation. J of the American Pharma Association 1958; 47(10): 715-7.
28. Samir ME, Laila AR, Maher AM, Amal MS, Allia MA and Asmaa SM: Chemical investigation and antioxidant activity of phenolic acids from the leaves of *Terminaliaarjuna*. GJP 2013; 7(4): 448-456.
29. Thin layer chromatographic allas of Ayurvedic Pharmacopoeial drugs, part-1, 1st edition, published by the controller of publications civil lines, Delhi- 110054, 1: 35.
30. Anonymous: Indian Pharmacopoeia, Vol. I & II. Government of India, Ministry of Health and Family Welfare, the Controller of Publications, Civil Lines, Delhi - 110054, 1996.

How to cite this article:
Shakyawar SL, Rahman MA, Sundaram S and Alok S: Preliminary qualitative phytochemical, physico-chemical and phyto-pharmacognostical standardisation evaluation of the pulp (without seed), pericarp, leaves and seed of *Aegle marmelos* L. (Bael). Int J Pharm Sci & Res 2020; 11(7): 3489-06. doi: 10.13040/IJPSR.0975-8232.11(7).3489-06.