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PHARMACOGNISTICAL AND PHYTOCHEMICAL EVALUATION OF *AMALAKI*

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Abstract: *Since ages, plants have remained important sources of medicines in our country, which is evidenced through their uses in traditional system of medicine. The Ayurvedic system of medicine is prevalent in India since the Vedic period and as early as the down of human civilization. The pharmacognosy and phytochemistry are necessary for authentification of crude drug and to prove therapeutic action as well. The present work deals with the pharmacognostical and preliminary phytochemical studies on the fruit of Amalaki (Emblica officinalis Gaertn). Amalaki is well known plant in Ayurveda. It has been mentioned in Vedas, Brihattrai and later it has been described in Nighantu. Pharmacognostical parameters for the fruit of Amalaki was studied with the aim of drawing the pharmacopoeial standards. The macroscopical and microscopical characters, physicochemical constants, quantitative microscopy parameters, extractive values with different solvents. The Preliminary phytochemical screening on the fruit of Amalaki was studied. The determination of these characters will help future researchers in their phytochemical as well as pharmacological analysis of this species. TLC profile was established for successive extracts of the root powder using TLC system.*
Keywords: *Ayurveda, Amalaki, Emblica officinalis, Pharmacognosy, Phytochemical.*

Introduction: Ayurveda is a science of life and one of oldest recognized systems of medicine gets origin from Vedas which are one of oldest repository of knowledge in the Indian history. Ayurvedic techniques of formulating compound mixtures developed gradually from the pre-Vedic period through the Vedic, the Samhita and the Samgraha periods and continue to develop. In the Samhita period, ancient indigenous science was at the peak of its glory and we find almost all the pharmaceutical modes [1].

Pharmacognosy is the study of identification of drugs derived from natural sources. The American Society of Pharmacognosy defines pharmacognosy as "the study of the physical, chemical, biochemical and biological properties of drugs, drug substances or potential drugs or drug substances of natural origin as well as the search for new drugs from natural sources." It is derived from the Greek word *pharmakon* meaning “a drug” and *gignosco* meaning “to acquire a knowledge”. Pharmacognosy is one of the five major divisions of the pharmaceutical curriculum [2]. The concept of standardization and quality control of drug can be found in ancient Ayurvedic texts. In those days, the physician himself identifies, checks the drugs based on habitat, morphology, taste, colour, texture and uses as medicine. But in modern times, these tests and tools are not sufficient to control the quality. Under these circumstances, pharmacognosy, pharmacology and phytochemistry are necessary for authentification of crude drug and to prove therapeutic action as well.

Amalaki (*Emblica officinalis* Gaertn.) is well known plant in Ayurveda. It has been mentioned in *Vedas*, *Brihattrai* and later it has been described in *Nighantu*. In present work preliminary phytochemical and pharmacognostical study on *Amalaki*, has been done as macroscopic study of powder, microscopic study of powder and phytochemical study of powder. Pharmacognostical parameters for the fruit of *Amalaki* (*Emblica officinalis* Gaertn.) was studied with the aim of drawing the

pharmacopoeial standards for this species. The macroscopical and microscopical Characters, physicochemical constants, quantitative microscopy parameters, extractive values with different solvents. Preliminary phytochemical screening for the fruit of Amalaki (*Emblica officinalis* Gaertn.) was studied. The determination of these characters will help future researchers in their phytochemical as well as pharmacological analysis of this species. TLC profile was established for successive extracts of the rhizome powder using TLC system.

Materials and Methods

Plant Material: *Amalaki* (*Emblica officinalis* Gaertn.) fruit was collected from vicinity of Varanasi (Mohanlal Rajnish shop, Goladeenanath, Varanasi) which authenticated by teachers of the department [3, 4].

I. Preliminary Pharmacognostic Characteristics

A. Macroscopic Characteristic of Drug: In present study, the *Amalaki* fruit was investigated for its macroscopic and microscopic characteristics.

Materials: Coarse powder of *Amalaki* fruit, Petri dish etc.

Method: 5 gm coarse powder of sample was taken in a Petri dish and examined with naked eye. (Observation and results as described in Table 1).

B. Microscopic Characteristic of Drug: The coarse powder of *Amalaki* fruit was pulverized in to fine powder. The powder was investigated for their microscopic characteristics.

Materials: Fine powder of fruit of *Amalaki*, Chloral hydrate, Plain water, Microscope, Slide & Cover slip, Watch glass.

Method: 5 gm powder of *Amalaki* was boiled separately with chloral hydrate solution in small quantity respectively. Cleaved powder was removed in three separate watch glasses respectively and stained with one drop each of phloroglucinol and conc. HCL. A little of the treated powder of *Amalaki* was mounted in Dil. Sulphuric Acid, Alcoholic picric acid and saffarin; the slide was observed under microscope at low power respectively. (Observation and results as described in Table 2 and 3).

II. Standardization of *Amalaki*: The parameters which were used for evaluation are nature, odour, colour, taste and texture, Determination of water soluble extractive value, ethanol soluble extractive value, determination of total ash, acid insoluble ash, water soluble ash of the drug and determination of foreign matter etc.

Procedure for Different Parameters

(A) Extractive Values: Since drug was to be used in the form of powder of *Amalaki*. The extractive values of these drugs are determined.

i. Determination of Aqueous Soluble Extractive Value: 5 gm of the air dried fruit of *Amalaki* was coarsely powdered and macerated with 100 ml of Aqueous (70:30) of the specified strength in a closed flask for twenty-four hours shaking frequently during six hours and allowed to stand for 18 hrs. It was filtered rapidly taking precautions against loss of aqueous 25 ml of the filtrate was evaporated to dryness in a tarred flat bottomed shallow dish (Temp at 105^0C) and weighed. After that the percentage of aqueous soluble extract was calculated with reference to the air dried drug. (Observation and results as described in Table 4).

ii. Determination of Hydro-alcoholic (70:30) Soluble Extractive Value: 5 gm of the air dried seeds coarsely powdered and macerated with 100 ml of ethanol of the specified strength in a closed flask for twenty-four hours shaking frequently during six hours and allowed to stand for fourty-two hours. It was filtered rapidly taking precautions against loss of ethanol. 25 ml of the filtrate was evaporated to dryness in a tarred flat bottomed shallow dish (Temp at 105^0C) and weighed. After that the percentage of ethanol-soluble extract was calculated with reference to the air dried drug. (Observation and results as described in Table 4).

B. Determination of Total Ash: 5 gm of air dried parts of fruit of *Amalaki* was accurately weighed separately in a tarred platinum or silica dish and incinerated at a temperature not exceeding 450^0C until free from carbon, cooled and then weighed again each. The percentage of ash was calculated with reference to the air dried drug.

$$\text{Total Ash value of the sample} = \frac{100\,(Z-X)}{Y}\ \%$$

Z= weight of the dish + ash (after complete incineration)

X= weight of the empty dish; Y= weight of drug taken.

C. Determination of Acid-insoluble Ash: The ash was boiled for 5-10 minutes with 25 ml of 2M HCl, and then the insoluble matter was collected in a Gooch crucible or on an ash less

filter paper, then washed with hot water, ignited and weighed. The percentage of acid insoluble ash was then calculated with reference to the air dried drug.

Acid-insoluble Ash value of the sample = $\frac{100\times a}{y}\%$

a = weight of the residue; y = weight of the drug taken.

D. Determination of Water-soluble Ash: The ash was boiled for 5-10 minutes with 25 ml of water, and then the insoluble matter was collected in a Gooch crucible or on an ash less filter paper; and then washed with hot water, ignited to constant weight at a low temperature. The weight of insoluble matter was subtracted from the weight of the total ash and the difference in weight represents the water soluble ash. The percentage of water soluble ash was then calculated with reference to the air dried drug.

Water-soluble ash value of the sample = $\frac{100\times a}{y}\%$

a = weight of the residue; y = weight of the drug taken.

E. Determination of Foreign Matter: 50 gm of plant material was accurately weighed and spread it as a thin layer and sorted the foreign matter into groups either by visual inspection using a magnifying lens (6X or 10X), or with the help of a suitable sieve, according to the requirements for the specific plant material. The remainder of the sample was shifted through a no 250 sieve; dust was regarded as mineral admixture. The portions of this sorted foreign matter weighed within 0.05gm. The content of each group was then calculated in gm/ 100gm of air dried sample.

III. Preliminary Screening of Phytochemicals: The preliminary phytochemical studies were performed for testing the different chemical groups present in the drug. 10% (w/v) solution of extract was taken unless otherwise mentioned in the respective individual test. General screening of various extracts of the plant material was carried out for qualitative determination of the groups of organic compounds present in them (Observation and results as described in Table 5).

1. Alkaloids

Dragendorff's Test: Dissolve a few mg of test solution until an acid reaction occurs, and then add 1 ml of Dragendorff's reagent, an orange or orange-red precipitate is produced immediately.

2. Carbohydrates

Anthrone Test: To 2 ml of Anthrone test solution, adding 0.5 ml of aqueous extract of the drug. A green or blue colour indicates the presence of carbohydrates.

3. Flavonoids

Shinoda's Test: In a test tube containing 0.5 ml of alcoholic extract of the drug, adding 5-10 drops of dil. hydrochloric acid followed by a small piece of magnesium. In the presence of flavonoids a pink, reddish pink or brown colour is produced.

4. Proteins

Biuret's Test: To 1 ml of hot aq. extract of the drug adding 5-8 drops of 10% w/v sodium hydroxide solution followed by 1 or 2 drops of 3% w/v copper sulphate solution. A red or violet colour is obtained.

5. Saponins

In a test tube containing about 5 ml of an aqueous extract of the drug, adding a drop of sodium bicarbonate solution, shaking the mixture vigorously and leave for 3 minutes. Honeycomb like froth is formed.

6. Steroids

Liebermann-Burchard's Test: Adding 2 ml of acetic anhydride solution to 1 ml of test solution extract of the drug in chloroform followed by 1 ml of conc. sulphuric acid. A greenish colour is developed which turns to blue.

7. Tannins: To 1–2 ml of plant test solution, adding a few drops of 5% FeCl3 solution was added. A green colour indicates the presence of gallotannins while brown colour tannins.

8. Glycosides: Detection of glycoside on paper spray solution No. 1 (0.5 % aqueous sol. of Sodium metaperiodate) & waiting for 10 minutes after then spraying solution No. 2 [0.5% Benzidine (w/v) in solution of Ethanol–acetic Acid (4:1)], white spot with blue back ground shows presence of *glycoside.*

IV. Thin Layer Chromatography (TLC) of Extracts of *Amalaki*: Thin Layer Chromatography (TLC) is a type of planar chromatography. TLC is routinely used by researcher in the field of phytochemicals, biochemistry etc. to identify the components in a compound mixture like alkaloids, phospholipids, amino acids etc. It is a semi quantitative method of analysis and its sophisticated version or highly

precise quantitative version is High performance thin layer chromatography (HPTLC). Similar to other chromatographic methods TLC is also based on the principle of separation. The separation depends on the relative affinity of compounds towards stationary and mobile phase. The compounds that under the influence of mobile phase (driven by capillary action) travel over the surface of stationary phase. During this movement the compounds with higher affinity to stationary phase travel slowly while the others travel faster. Thus separation of components in the mixture is achieved. Once separation occurs individual components are visualized as spots at respective level of travel on the plate. Their nature or characters are identified by means of suitable detection techniques. TLC System consists of a TLC plates preferably readymade with stationary phase: These are stable and chemically inert plates on to whose surface a thin layer of stationary phase is applied. The stationary phase on the plates is of uniform thickness and consists of fine particle size. TLC system consists of:

1. TLC plate preferably readymade with stationary phase: These are stable and chemically inert plates on to whose surface a thin layer of stationary phase is applied. The stationary phase on the plates is of uniform thickness and consists of fine particle size.
2. TLC chamber: This is used for the development of TLC plate. The chamber maintains uniform environment inside for proper development of spots. It also prevents the evaporation of solvents and kept the process dust free) Mobile phase: This comprises of a solvent or solvent mixture recommended for the purpose. The mobile phase used should be particulate free and of highest purity for proper development of TLC spots. The solvents recommended are chemically inert with the sample, stationary phase.) A filter paper moistened in the mobile phase, to be placed inside the chamber. This helps uniform rise in mobile phase over the length stationary phase.

Objective

1. To separate the constituents using thin layer chromatography (TLC) method.
2. To analyze and detect their spots using UV and spraying agents.
3. To develop skills including use of solvent system for TLC separation method.

Material Required: Extracts from lab, Toluene, chloroform, methanol, ethanol, hydrochloric acid, diethyl ether, distilled water and glacial acetic acid, Aluminum TLC plate, Developing tanks, spraying agent: 10% Sulfuric acids, heating oven, UV Lamp Detector.

Procedure

1. The stationary phase is applied onto the plate uniformly and then allowed to dry and stabilize.
2. A thin mark is made at the bottom of the plate with a pencil to apply the sample spots.
3. Then samples solutions are applied on the spots marked on the line at equal distances.
4. The mobile phase is poured into the TLC tanks to a level few centimeters above the tanks bottom.
5. Then the plate prepared with sample spotting is placed in TLC tanks such that the side of the plate with sample line is towards the mobile phase. Then the chamber is closed with a lid.
6. The plate is immersed such that sample spots are well above the level of mobile phase but not immersed in the solvent as shown in the picture for development. Sufficient time is allowed for development of spots. Then the plates are removed and allowed to dry. The sample spots are visualized in suitable UV light chamber. (Observation and results as described in Table 6 and 7).

Results and Discussion

Table 1: Macroscopic characteristic of powder of *Amalaki* (Fruit)

S. No.	Parameters	Observation of seeds
1	Nature	Coarse powder
2	Colour	Dark brown
3	Odour	Characteristic
4	Taste	Astringent and Sour
5	Texture	Rough & fibrous
6	Size	Seive with mesh aperture of 1.70 mm

Table 2: Powder Microscopy of Fruit of *Amalaki*:

S.No.	Reagents	Observations	Characterstics
1.	Phloroglucinol + Conc. Hcl	Pink	Lignified Vessels
2.	Dil. Sulphuric Acid	White	Calcium oxalate crystals
3.	Alcoholic Picric Acid	Yellow	Starch grains
4.	Phloroglucinol + Conc. Hcl	Pink	Sclereids

Figure 1: Powder microscopy of fruit of *Amalaki*


Starch grains


Prismatic crystals


Lignified Tissues


Sclereid

Table 3: Certificate of analysis of *Amalaki*

S.No.	Parameters	Observation
I	Physical tests	
	Nature	Coarse powder
	Colour	Yellowish
	Odour	Aromatic
	Taste	Bitter
II	Foreign matter	Nil
III	Ash value (% w/w)	
	Total ash	7.20
	Acid insoluble ash	8.30
	Water soluble ash	2.50

Table 4: Percentage yield of Extracts of *Amalaki*

S.No.	Extracts	Nature of Extract	Weight (gm)	% Yield w/w
I	**Aqueous**	Viscous	12.436	10.277
II	**Hydro-alcohol**	Viscous	110.75	55.375

Table 5: Genuine sample of *Amalaki* gave the presence of following phytochemicals

S.No.	Test Sample	Aqueous Extract	Hydroalcoholic extract
1.	***Test for alkaloids***		
	Dragendorff's test	+	+
	Hager's test	+	+
	Wagner's test	+	+
	Mayer's test	+	+
2.	***Test for Carbohydrates***		
	Anthrone test	_	+
	Benedict's test	_	+
	Fehling's test	_	+
	Molisch's test	_	+
3.	***Test for Flavonoids***		
	Shinoda's test	_	+
4.	***Test for Proteins***		
	Biuret's test:	+	+
	Millon's test:	+	+
5.	**Test for Cardiac Glycosides**	_	+
6.	**Test for Saponin Glycosides**	+	+
7.	**Test for Coumarin Glycosides**	_	_
8.	**Test for Anthraquinone Glycosides**	_	_
9.	**Test for steroids**	_	_
10.	**Test for Tannins & phenolics**	+	+
11.	**Test for Amino acid**	+	+
12.	**Test for Non-reducing sugar**	+	_
13.	**Test for volatile oils**	_	_

Note:* '-' = *Absent*; '+' = *Present

Table 6: Rf values for Aqueous extract of *Amalaki*

S. No.	Spots	Rf Value
1.	Spot 1 (Ethyl acetate:Methanol:Water; 15:1.5:1)	0.20
2.	Spot 2 (Ethyl acetate:Methanol:Water; 15:1.5:1)	0.33
3.	Spot 3 (Ethyl acetate:Methanol:Water; 15:1.5:1)	0.63

Table 7: Rf values for Hydro alcoholic extract of *Amalaki*

S. No.	Spots	Rf Value
1.	Spot 1 (Toluene:Ethyl acetate: Acetic acid; 5:10:5)	0.27
2.	Spot 2 (Toluene:Ethyl acetate: Acetic acid; 5:10:5)	0.38
3.	Spot 3 (Toluene:Ethyl acetate: Acetic acid; 5:10:5)	0.55
4.	Spot 4 (Toluene:Ethyl acetate: Acetic acid; 5:10:5)	0.76

The aqueous extract of fruit of *Amalaki* was also prepared. A large number of solvent systems were tried to achieve a good resolution. Finally the solvent system Ethyl acetate: Methanol: Water (15:1.5:1) ratio was selected for aqueous extract. The three bands are appeared at Rf 0.20, 0.33 and 0.63 by kept TLC plate in Iodine chamber**.**

$$\text{Rf value} = \frac{\text{Distance travelled by the substance}}{\text{Distance travelled by the solvent}}$$

Solvent system (Ethyl acetate : Methanol : Water; 15:1.5:1)

For Spot 1 Rf Value - 1.2/6.0 = 0.20
For Spot 2 Rf Value -2.0/6.0 = 0.33
For Spot 3 Rf Value -3.8/6.0 = 0.63

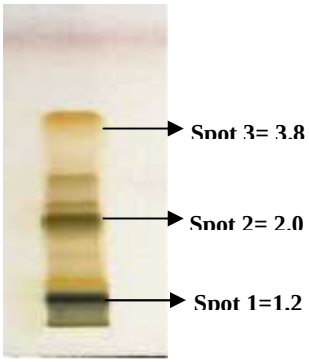

Figure 2: TLC of aqueous extract of *Amalaki*

2. The Hydro-alcoholic extract of fruit of *Amalaki* were prepared. A large number of solvent systems were tried to achieve a good resolution. Finally the solvent system Toluene: Ethyl acetate: Acetic acid (5:5:0.5) ratio was selected for Hydro alcoholic extract. The four bands are appeared for solvent system at Rf 0.27, 0.38, 0.55 and 0.76 by kept TLC plate in Iodine chamber**.**

For Spot 1 Rf Value - 1.8/6.5 = 0.27
For Spot 2 Rf Value - 2.5/6.5 = 0.38
For Spot 3 Rf Value - 3.0/6.5 = 0.55
For Spot 4 Rf Value - 5.0/6.5 = 0.76

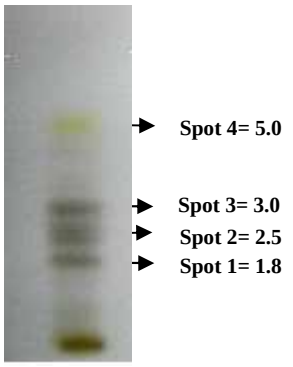

Figure 3: TLC of Hydroalcoholic extract of *Amalaki*

Conclusion: The pharmacognosy is define as "the study of the physical, chemical, biochemical and biological properties of drugs, drug substances or potential drugs or drug substances of natural origin as well as the search for new drugs from natural sources." In present study pharmacognostical standards have been established with regards to fruit of *Amalaki* (*Emblica officinalis* Gaertn). Powder microscopy of fruit showed the presence of lignified cells, calcium oxalate crystals, starch grains and sclereids. The physical evaluation furnished different ash values, extractive values in different solvents. Total ash, acid insoluble ash and water soluble ash values were also determined. The phytochemical investigation shows the presence of alkaloid, carbohydrate, flavenoids, cardiac glycosides, saponin glycosides, tannins & phenolics, amino acid and non reducing sugar compounds in the fruit of *Amalaki*. Study was carried out in order to assess the quality of fruit of *Amalaki* and also to detect the foreign matter, adulteration and substitution etc., which may be helpful to researchers in future.

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