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PHARMACOGNOSTIC EVALUATION OF *PHYLLANTHUS EMBLICA* LINN (EUPHORBIACEAE)

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ABSTRACT

The present communication deals with the pharmacognostic investigations on *Phyllanthus emblica* Linn(Euphorbiaceae). It is a tree of small or moderate size with a greenish-grey bark and greenish-yellow flowers, formed in auxiliary clusters. The feathery leaves are linear-oblong, with a rounded base and obtuse or acute apex. The tender fruits are green, fleshy, globose and shining, and change to light yellow or brick-red when mature. It grows in tropical and subtropical parts of China, India, Indonesia, and on the Malay Peninsula. The fruits are known as Amalakam and Sriphalam in Sanskrit, Emblic myrobalam and Indian gooseberry in English. The paper aims to identify and differentiate various anatomical features such as root and stem vessels, anatomy of node, leaf anatomy, petiole anatomy and leaf epidermis includes quantitative microscopy *viz.*, like stomatal frequency (SF), stomatal index (SI), vein islet number (VIN) and vein termination (VTN), for evaluating the selected species. In addition, physicochemical analysis such as moisture contents, different ash values, different extractive values and effect of different chemicals on powder and extracts as well as quantitative estimation of various phytochemicals have been studied.

KEYWORDS : **Pharmacognosy, *Phyllanthus emblica,* Euphorbiaceae, gooseberry, phytochemicals.**

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INTRODUCTION

Phyllanthus emblica Linn. [Syn: *Emblica officinalis* Gaertn.] (Euphorbiaceae) is popularly known as '*Amla'* or '*Awala'* in Maharashtra. It is a tree of small or moderate size with a greenish-grey bark and greenish-yellow flowers, formed in axillary clusters. The feathery leaves are linear-oblong, with a rounded base and obtuse or acute apex. The tender fruits are green, fleshy, globose and shining, and change to light yellow or brick-red when mature. It grows in tropical and subtropical parts of China, India, Indonesia, and on the Malay Peninsula. The Malaysian variety has more scurfy branchlets and the immature fruit is top-shaped. The fruits are known as Amalakam and Sriphalam in Sanskrit, Emblic myrobalam and Indian gooseberry in English, and Phylontha emblic in French.

It is a well known remedy for the treatment of various types of disorders in the ayurvedic and homoeopathic systems of medicine in India. Its fruit contains a series of diterpenes referred to as the gibberellins, as well as the triterpene lupeol, flavonoids and polyphenols. It also shows the presence of phyllantine and zeatin alkaloid and a number of benzenoids including amalic acid, corilagin, ellagic acid, 3-6-diO-galloyl-glucose, ethyl gallate, 1,6-di-O-galloyl-ß-Dglucose, 1-di-O-galloyl-ß-Dglucose, putranjivain A, digallic acid, emblicol and alactaric acid. This fruit also contains significantly high amounts of ascorbic acid (vitamin C)[1-5].

P. emblica L. has been used for anti-inflammatory and antipyretic treatments by rural populations in its growing areas. Malays use a decoction of its leaves to treat fever[6]. In Indonesia, the pulp of the fruit is smeared on the head to dispel headache and dizziness caused by excessive heat[7]. The earlier chemical findings and biological activities have since been confirmed with more advanced techniques. Active principles or extracts of *P. emblica* L. have been shown to possess several pharmacological actions, *e.g.* analgesic, anti-inflammatory, antioxidant, chemoprotective, hypolipidaemic and anti-HIV-1 (Human immunodeficiency virus-1) activities[2,8-14].

Kapoor noted different contents from its fruit pulp. The fruit is rich in source of pectin and contains gallic acid, ellagic acid and glucose in its molecule which is naturally present in the fruit, prevents or retards the oxidation of vitamin-C and renders the fruit a valuable antiscorbutic, in the fresh as well as in the dry condition[15]. Tannin is found in different organs as fruit, twig bark, stem bark and leaf. In view of its varied medicinal importance and to ensure the quality of the drug, the present pharmacognostic investigation on *Phyllanthus emblica* has been undertaken.

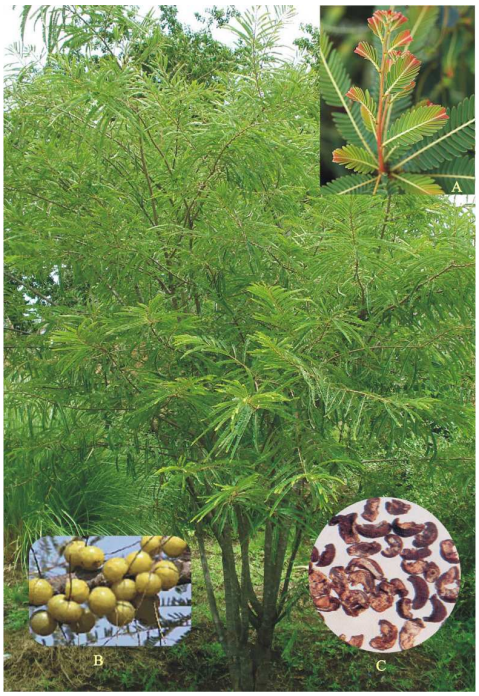

Figure1
Phyllanthus emblica Linn. a Plant showing Habit, A-Leaves of Plant, B- Fresh Fruits, C-Dried Fruits

MATERIALS AND METHODS

Parts of the fresh plant (leaves, stem, root and fruits) of *Phyllanthus emblica* Linn were collected from the Botanical Garden, Govt. Institute of Science, Caves Road, Aurangabad. The materials were washed and shade dried. The dried material was finely powdered sieved through muslin cloth and stored for chemical analysis. Some plant material was also preserved in 70% alcohol. Leaf epidermal studies were carried out on fresh specimens. Peels were removed mechanically using some chemicals. They were stained in 1% safranin mounted in glycerine and made semi-permanent by ringing with DPX solution. Stomatal index (SI) was calculated as defined by Salisbury[16, 17] *viz.*,

$$SI = \frac{S}{E+S} X\ 100$$

Where 'S' = number of stomata per unit area and 'E' = number of epidermal cells in the same area. Stomatal frequency and stomatal index have been calculated out of an average of 10 readings. Palisade ratios (PR) was calculated as the average of palisade cells (P) beneath each epidermal cell (E). Vein islet number is defined as the number of vein islets per sq. mm of the leaf surface midway between the midrib and the margins. The line and cellular sketches of the figures were drawn using a Camera Lucida. Transections of leaf and petiole were taken by free hand. Fresh and preserved materials were used. Sections were stained in safranin (1 %), light green (1 %) and mounted in DPX after the customary dehydration. Some hand sections were also examined in glycerine. Microphotographs of stem and root sections were taken by using Jenaval and Mirax Laborec Cameras affixed to microscope. For the observation of leaf architecture, leaves were first cleaned by in 10 to 20% aqueous sodium hydroxide solution followed by

trichloroacetic acid and phenol solution (2:1 by weight) and then stained with kores stamp pad purple ink[18].

The moisture content was determined by the standard methods[19]. It is be determined by heating the drug at 105 ^{o}C to constant weight and calculated the loss of weight. The extracts were prepared using various solvents by standard methods. Total ash, acid insoluble ash, and acid soluble ash value were obtained by heating the sample at 600 ^{o}C for 2 h in a muffle furnace.

Nitrogen (N) content in dry plant material was estimated by micro Kjeldal's method[20]. The crude protein percentage was calculated by percentage of total nitrogen multiplied with 6.25 as specified by AOAC[21]. Calcium (Ca) content was determined by titrating the sample against 0.01 N KMnO4 solution using methyl red as indicator. Phosphorus (P) content was analyzed by reacting the sample with ammonium molybdate solution at 660 nm by the colorimetric method[22]. Potassium (K) content was determined on a flame photometer (model Mediflame- 127) as suggested by Jackson[23]. The ethanolic extractable phenolic compounds were estimated by folin- phenol method. Amino acids and Reducing Sugar (RS) were calculating by standard methods[24].

RESULTS AND DISCUSSION

Microscopic characters

Epidermal features: The epidermal cells are polygonal, isodimetric or elongated in various directions. The adaxial epidermal cells are longer with thick walls and abaxial epidermal cells are smaller. A well-developed cuticle is always present on both the surfaces of the leaf. The cuticle on the epidermis of leaf may be smooth or may show papillae structure or a pattern of striation.

The leaves having paracytic stoma (Figure 2 A, B). The numbers of stomata per unit area is always higher on the lower surface than on the upper surface (Table 1). The shape of stomata is oval or elliptical in outline.

The trichomes are not present on leaf but they are present on stem. The trichomes are uniseriate multicelluar type and upper body consists of three to four cells with curved terminal cell (Figure 2D).

Leaf (Figure 2E)**:** The cells of upper epidermis are moderately larger with outer thick walls. The cuticle is thick. The cells of the lower epidermis are smaller. Stomata occur on both the upper and lower epidermis. Trichomes are missing on both surfaces. Mesophyll consists of palisade and spongy tissue. Palisade consists of large cells and spongy tissues of small loosely arranged cells. In midrib region, epidermis is followed by one layer of collenchyma on adaxial surfaces and one to two layered collenchyma on lower surface, follow by parenchymatous cortex. An arc shaped centrally placed Vascular bundle is present with primary xylem facing upwards.

Leaf microscopic characters are summarized in Table 1.

Table 1

Quantitative microscopy of P. emblica.

Parameter	Mean
Stomatal frequency	
a) Adaxial Epidermis	154.0 *
b)Abaxial Epidermis	218.0 *
Stomatal index	
a) Adaxial Epidermis	31.5*
b)Abaxial Epidermis	21.81*
Vein islet number	17.0 /mm^2
Vein termination	31.5 / mm^2
Palisade ratio	2.96 Palisade/Epidermal cell

Note : * - microscopic field.

Petiole (Figure 2F): The shape of petiole is somewhat triangular. The epidermis consists of small thick walled cells with cuticle. Stomata are few in number. Epidermis is followed by several-layered parenchymatous cortex. The petiole vasculature consists of centrally placed an arc shaped median vascular bundle. Xylem elements are in linear rows and facing upwards. Papillae are absent.

Node (Figure 2 G): A solitary arc shaped vascular strand is emerged out from the stele at the nodal region. A single gap is formed. The node is thus unilacunar one-traced. Phylotaxy is alternate.

Stem (Figure 2H)**:** A transverse section shows a circular outline, presence of non-glandular trichomes; the former being present in small depressions of the epidermis. Following the epidermis is a band of collenchyma of 1-2 layers of cells and a wide zone of parenchyma. The stem possesses a siphonostele. Starch grains, calcium oxalate crystals of acicular are found abundantly in the region of the stem.

Root (Figure 2I)**:** The transverse section of the root shows an irregular outline. Epidermis has small cells which are compactly arranged. The cork consists of 4 to 5 rows of nearly cubical to rectangular cells of which, the cells of the peripheral rows are thick walled, but not lignified while the innermost one or two rows are thin walled. The cortex is narrow, consists of 4 to 5 rows of tangentially elongated cells and small group of stone cell. Starch grains of simple type are present in the cortex. Each phloem group is composed of narrow tangential strips of phloem fibres alternating with wide zones of regular thin-walled phloem-elements transversed by medullary rays. Xylem developed in large amount. Medullary rays are straight, uni-tri-seriate.

Vessel Elements (Figure 2 J)**:** In stem of *P. emblica* vessels length are from 71.45 μm to 342.96 μm, diameter ranges from 22.2 μm to 42.87 μm (Table 2). They are Tubular, column like, and cylindrical. Lateral wall thickening – Simple pitted; Pits arrangements– Alternate; Perforation plate– Simple; Shape of perforation in plate– Circular, oval; Position of plate– Transverse, oblique (Figure 2 J-II,III).

In root length of vessels ranges from 79.92 μm to 385.83 μm, diameter ranges from 5.5 μm to 53.28 μm (Table 2). They are long, cylindrical with a tail- like structure at the end; Length, width, perforation plate, end walls and lateral wall thickening were recorded; Shape– Tubular, column like, cylindrical; Lateral wall thickening– Reticulate, simple pitted; Pits arrangements– Simple; Perforation plat– Simple, with scalariform thickening (i.e. transverse bands present in perforation plate.) (Figure 2 J-II, III).

Table 2
Vessel elements in Stem and Root of P. emblica

Name of Plant Part	Length of vessel members (μm)			Diameter of vessel members (μm)		
	Minimum length	Maximum length	Average length	Minimum diameter	Maximum diameter	Average diameter
Stem	71.45	342.96	207.2	22.2	42.87	32.53
Root	79.92	385.83	232.88	5.55	53.28	29.41

Physio-chemical characters

The physio-chemical characters in drug samples are play an important role in the drug evaluation. In present studies records are summarized in Table 3. The details of effect of different chemicals on powder drug are shown in Table 4.

Table 3
Physico-chemical evaluation of P. emblica

Parameter	Leaf	Stem	Fruit
Moisture content (%)	5.95	7.8	9.35
Extractive values (%)			
a) Petroleum Ether	1.5	1.5	3.5
b) Alcohol	14.5	24	43
c) Water	18	14	52.5
Ash values (%)			
a) Total ash	4.5	5.0	2.8
b) A.I.A.	3.5	2.5	0.5
c) A.S.A.	1.0	2.5	2.3

Table 5
Effect of Chemicals on Powder of P. emblica

Sr. No.	**Reagent**	**Leaf**	**Stem**	**Fruit**
1	Powder	Olive green	Light brown	Light brown
2	Powder + Iodine	Yellowish orange	Dark orange	Redish orange
3	Pd + 5% ferric chloride	Greenish brown	Greenish brown	Dark brown
4	Pd + NaOH	Dark brown	Dark brown	Yellowish green
5	Pd + acetic acid	Yellowish green	faint yellow	Transparent
6	Extract + acetic acid + 50% H_2SO_4	Yellow Perple	White Turbid	Yellowish turbid
7	Pd + 50% Conc. HCL	Dark brown	Orange brown	Brown
8	Pd + ammonia	Light Yellow	Yellowish green	Greenish black
9	Pd + Ammonia + pot. Ferrocyanide	Light brown	Lemon brown	Faint yellow
10	Extracts + 4% NaOH + 1% CuSO4	Light brown	Yellowish purple	Greenish turbid
11	Extracts + 40% NaOH+ 1% Lead acetate	Yellowish purple	Yellowish purple	Greenish Turbid
12	Pd + 50% Nitric acid + picric acid	Yellowish	Orange yellow	light brown
13	Pd + Sat. Picric acid	Faint brown	Lemon yellow	Light brown

Pd= Powder

Phytochemical evaluation

The phytochemical quantitative percentage showed great variations of various phytochemicals estimated. The values obtained in drug sample are presented in Table 5.

Table 5

Quantitative estimation of Different Chemical of P. emblica

Sr. No.	Phytochemicals	Leaf (%)	Stem (%)	Fruit (%)
1	Calcium	1.07	1.1	0.42
2	Phosphorus	0.10	0.13	0.04
3	Potassium	0.27	0.69	0.69
4	Phenols	2.79	1.62	2.94
5	Reducing sugar	4.47	3.12	8.60
6	Nitrogen	2.14	1.00	0.58
7	Crude protein	15.06	6.25	3.63
8	Amino acid	8.52	7.32	4.14

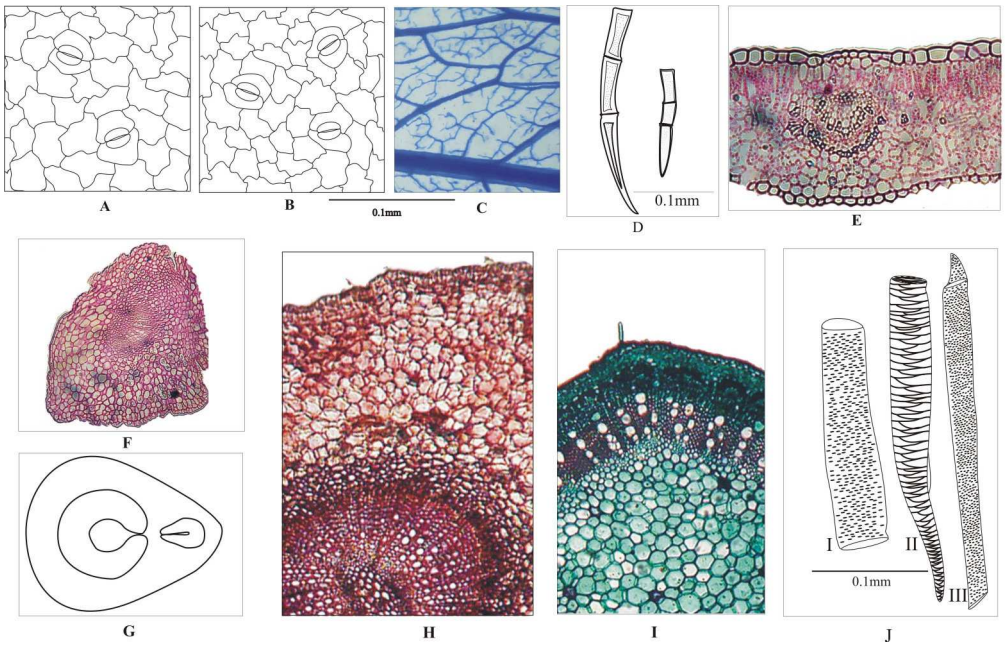

Figure 2

Anatomical Features A- Adaxial epidermis with Stomata, B- Abaxial epidermis with Stomata, C- Cleared Leaf showing Venation Pattern, D- Trichomes Observed on Stem, E- T.S.of Leaf, F- T.S.of Petiole, G- T.S.of Stem Node, H- T.S.of Stem, I- T.S. of Root, J.I- Stem Vessels, J.II,III- Root Vessels.

CONCLUSION

Different plant parts (leaves, stem, root and fruits) of *Phyllanthus emblica* Linn have been studied to give reports on pharmacognostical and preliminary phytochemical features. These results will help the fellow scientists, Ayurvedic-pharmaceuticals companies and others who will use and research in *Phyllanthus emblica* Linn to identify, authentication and further research regarding to its chemistry.

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