WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 11, Issue 2, 1647-1656. Research Article ISSN 2277– 7105

DETAILED PHARMACOGNOSTICAL AND PHYTOCHEMICAL SCREENING OF THE FRUIT OF *AMLAKI* (*EMBELICA OFFICINALIS* GAERTN.)

Dr. Parul Anand*[1] and Dr. A. R. Murthy[2]

[1]Ph.D Scholar, Department of *Dravyaguna Vigyana*, ITRA Jamnagar.

[2]Professor, Department of *Dravyaguna Vigyana*, NIA Jaipur.

Article Received on
06 December 2021,

Revised on 26 Dec. 2021,
Accepted on 16 Jan. 2022,

DOI: 10.20959/wjpr20222-22963

***Corresponding Author**
Dr. Parul Anand
Ph.D Scholar, Department of *Dravyaguna Vigyana*, ITRA Jamnagar.

ABSTRACT

Amlaki (*Embelica officinalis* Gaertn.) is one of the most important herbs in the Indian traditional medicine system, especially Ayurveda and also known as the king of all medicinal plants. It is famous Ayurvedic herb is likely one of the most useful drug treatments within the Indian pharmacopoeia, and is considered to be one of best tonics (*Rasayana*), particularly for the blood, bones, liver, and heart. If the plant drugs are adulterated, then the quality of preparation cannot give the desirable results. So before using a drug it is very much essential to carry out its detailed Pharmacognostical study as it is not only helpful for correct identification but also to get a clue for its phytochemical, pharmacological and medicinal properties. The present study includes macroscopy, microscopy, physico-chemical and preliminary phytochemical evaluation of market sample of dried *Amlaki*. The findings of the study may be helpful to identify, standardize and for quality assessment of fruit of *Embelica officinalis*.

KEYWORDS: Pharmacognostical, Phytochemical, Yashtimadhu, Powder microscopy.

INTRODUCTION

Physicochemical analysis provides the objective parameters to fix up the standards for quality of raw drugs as well as finished products. Analytical study of a drug also helps to interpret the pharmacokinetics and pharmacodynamics of the same.[1] Standardization starts right from the collection of raw materials upto their clinical application. In case of *Ayurvediya* medicines, the therapeutic efficacy is also related to its chemical constituents. The quality and purity refers to the total profile of the drug rather than any of its character. The detailed

Pharmacognostical study of plant help us to differentiate between closely related species of the same genus or related genera of the same family. It is also the first step to standardize a drug which is the need of the day. Every drug has got its own physical and chemical characteristics which help to separate it from other closely related drug. Therefore, a multidimensional approach is essential for standardizing an *Ayurvedic* drug.

Amlaki is one of the oldest oriental medicines mentioned in Ayurveda as potential remedy for various ailments. It is widely used in the Ayurvedic medicines and refered to increase defence or immune power against diseases. Several parts of the plant are used to treat a variety of diseases, but the most important is the "fruit." The fruit is rich in quercetin, phyllemblic compounds, gallic acid, tannins, flavonoids, pectin, and vitamin C and also contains various polyphenolic compounds. A wide range of phytochemical components including terpenoids, alkaloids, flavonoids, carbohydrates, and tannins have been shown to possess useful biological activities. Many pharmacological studies have demonstrated the ability of *Amlaki* as antioxidant, anticarcinogenic, antitumor, antigenotoxic, anti-inflammatory activities, anticancer, antidiabetic, antidepressant, hair growth tonic, wound healing activities and many more. The purpose of this article is to evaluate pharmacognostical, physico-chemical parameters and preliminary phytochemical constituents including TLC profile of the fruit of *Embelica officinalis* Gaertn.

MATERIALS AND METHODS

Amlaki fruit was used as material for the Pharmacognostical study. The study was conducted as per the guidelines of Ayurvedic Pharmacopeia of India.[2]

1. Collection and Authentication of samples

Amlaki fruit (dried) was taken from Jaipur raw drug market after proper identification. The plant material was identified and authenticated for their botanical identity from Raw Material Herbarium and Museum, Delhi (RHMD) of National Institute of Science Communication And Information Resources (NISCAIR) Delhi. *Emblica officinalis* Gaertn. was authentified under the reference number NISCAIR/RHMD/Consult/2017/3131-80-2.

2. Preservation and processing of samples

Samples were shade dried, powdered with mechanical grinder, sieved through 80 mesh and stored in an air-tight glass vessel. This powder was utilized for powder microscopy.

3. Macroscopic study

Macroscopic observations were made with necked eyes and with the help of dissecting Quasmo binocular compound microscope. Evaluation of the raw sample as well as powder were carried out for various characters like, colour, texture, odour, taste.[3]

4. Powder microscopy

Powder microscopic inspection of medicinal plant materials is indispensable for the identification of broken or powdered materials; the specimen has to be treated with chemical reagents.[4] An examination by microscopy alone cannot always provide complete identification, though when used in association with other analytical methods it can frequently supply invaluable supporting evidence.

Procedure

For examining the characters of the powder take sufficient amount of powder in different chemical reagents on a slide and warm over a low flame for a short time. Put drop of glycerin on the slide, cover it with the cover slip and observe under the microscope.

Chemical reagents used for staining of the powder samples were as follows

- ✓ Safranin
- ✓ Dilute Ferric chloride
- ✓ Eosine
- ✓ Methylene blue

5. Physicochemical analysis

Coarse powder of *Amlaki* fruit was used to carry out different parameters as mentioned in Ayurvedic Pharmacopoeia of India. Loss on drying, foreign matter, ash value, acid insoluble ash, water soluble extractive, alcohol soluble extractive, petroleum ether soluble extractive value and pH, and were determined following standard procedure in API,[5] and other standard texts.[6]

6. Preliminary phytochemical screening

For qualitative analysis, the presence of various secondary metabolites dissolved in water and alcohol extract was carried out following standard procedures.[7] Freshly prepared extracts were tested for the presence of various active phyto compounds like phenols, tannins, flavonoids, proteins, reducing sugar, carbohydrates, lipids, saponins, alkaloids.

7. **Chromatography study**

Thin layer Chromatography (TLC) study was carried out following standard procedures.[8]

Chromatography plates

T.L.C. plate coated with 0.25 mm layer of silica gel 60 F_{254} with fluorescent indicator was used. (Each plate dimension is 10 cm long and 2 cm width)

Activation of pre-coated Silica gel 60 F_{254}

Plates were dried in hot oven at 105^0 C for one and half hour.

Preparation of mobile solution

Toluene: Ethyl Acetate: Formic Acid (7:2:1)

Preparation of test solution

4 gm test drug was extracted with 100 ml of ethanol (90 percent) in a Soxhlet apparatus consecutively three times. Extract was filtered and concentrated to 10 ml. The extract was filtered and concentrated under vacuum to 10 ml with ethanol.

Sample application

Sample was applied with the help of capillary 1cm above the base of T.L.C. plate. Then it was dipped in mobile solution. T.L.C. plate was removed from the mobile solution immediately after the spot reached the 1cm below the top of the T.L.C. plate.

Visualization

1. U.V
2. Iodine Vapour
3. The plate was sprayed with p-Anashaldehyde sulphuric acid reagent and heated at 105˚ for 5 to 10 min. Rf value and colour of the resolved bands are recorded.

Calculation of Rf Value

$$Rf = \frac{\text{Distance travelled by solute from origin line}}{\text{Distance travelled by solvent from origin line}}$$

RESULTS AND DISCUSSION

Macroscopic study

Drug consists of curled pieces of pericarp of dried fruit occuring either as separated single segment; 1-2 cm long or united as 3 or 4 segments; bulk colour grey to black. Pieces show, a broad, highly shrivelled and wrinkled external convex surface to somewhat concave, transversely wrinkled lateral surface, external surface shows a few whitish specks, occasionally some pieces show a portion of stony testa (Fig.1). Detailed results of organoleptic study are given in table 1.

Table 1: Organoleptic examination of *Embelica officinalis* fruit.

S. No.	Organoleptic Characters	In fresh Fruit	In dry Powder of fruit
1	Colour	Green, Yellow	Yellow-Brown
2	Odour	Citrus	Citrus
3	Taste	Citrus-Sweet	sour and astringent
4	Texture	Globose, 2.5-3.5 cm in diameter, fleshy, smooth with six prominent lines	Rough

Fresh fruit **Fruit powder**

Fig. 1: Macroscopy of Fresh and dry powder of *Embelica officinalis.*

Microscopic study

Transverse section

Transverse section of fruit shows epicarp consisting of a single layered epidermis, cell appearing tabular and polygonal in surface view; cuticle present. Mesocarp cells are tangentially elongated parenchymatous and crushed, differentiated roughly into peripheral 8 or 9 layers of tangentially elongated smaller cells, rest consisting of mostly isodiametric larger cells with walls showing irregular thickenings. Ramified vascular elements occasionally present; stone cells present either isolated or in small groups towards endocarp. Pitted vascular fibres, walls appearing serrated due to the pit canals, leading into lumen. (Fig. 2 & 3).

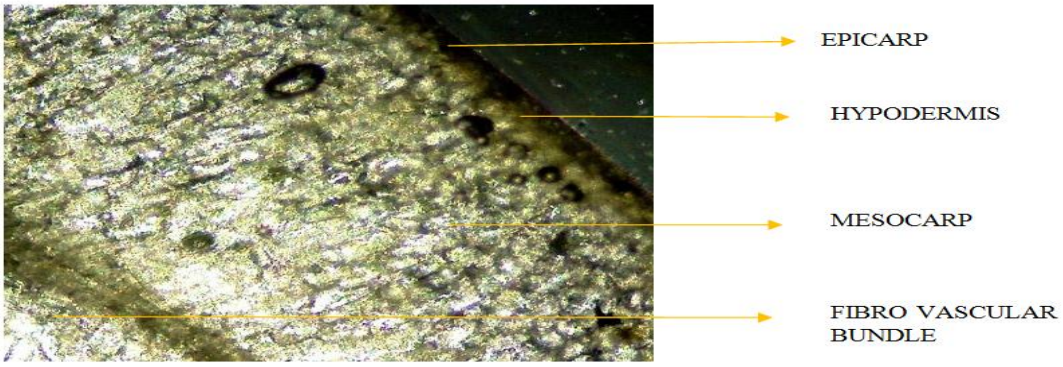


Fig. 2: Transverse section of fruit of *Embelica officinalis* Gaertn.

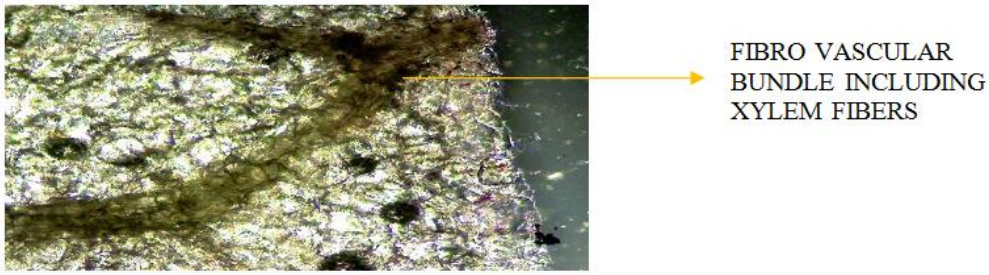
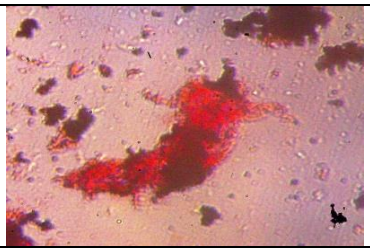
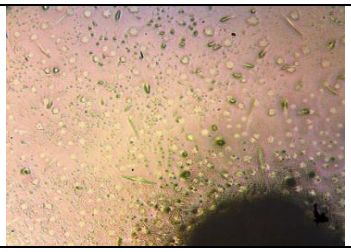
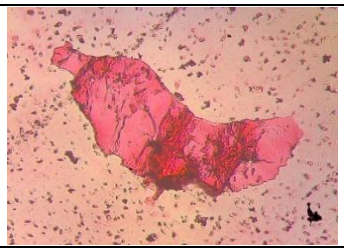
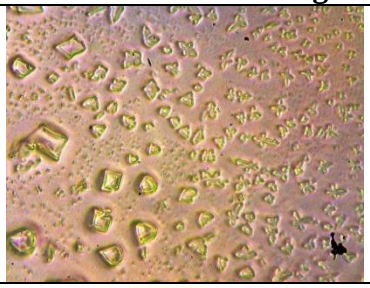
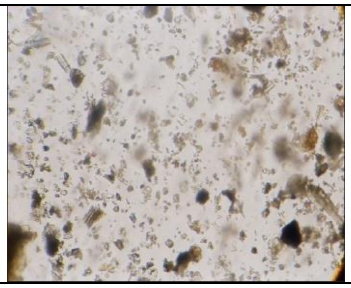
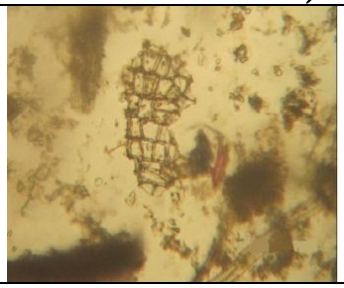
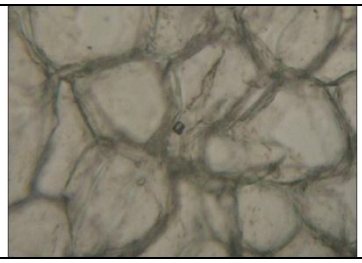


Fig. 3: Transverse section of fruit of *Embelica officinalis.*

Powder Microscopy

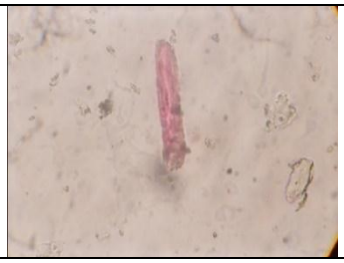
Fine powder shows epidermis with uniformly thickened straight walled, isodiametric parenchyma cells with irregular thickened walls, occasionally short fibres and tracheid. Detailed powder microscopy with different reagents has been depicted in Fig 4.

		
Safranin (Red Colour Indicates Presence of Lignin)	**Crystal of Calcium Oxalates**	**Eosin (Red Colour Indicates Presence of Protiens)**
		
Iodine (Crystal Shape Particles of Starch)	**Prismatic crystal of calcium oxalate**	**Cork Cells**



Prismatic crystal

Bordered pitted vessels



Group of lignified fibres

Fig. 4: Powder microscopy of fruit powder of *Embelica officinalis.*

Preliminary physico-chemical analysis

Foreign matter was found to be as per limit in fruit powder sample which may be due to the good harvesting practice followed during the collection of the drug. Loss on drying was to be found 3.568% w/w. The loss on drying of any sample is directly related to its moisture content. An excess of water in medicinal plant materials will encourage microbial growth, the presence of fungi or insects and may affect its preservation.[9] It is found that aqueous soluble extractive value (63.5 % w/w) was more than that of alcohol soluble extractive (43.94% w/w). Water soluble and alcohol soluble extractive values are indicative of the bioavailability of the plant. Hence, proposed that fruit powder was more soluble in water. Ash value was found to be 2.19 % w/w. The ash value shows the presence of inorganic and salt materials in the sample. Acid-insoluble ash value was 1.21 % w/w. Acid-insoluble ash indicates the presence of more siliceous matter in the drug. pH value of water extract of fruit was 2.5 which indicates its strong acidic nature. Detailed results of physicochemical analysis are given in table 2.

Table 2: Physio-chemical Analysis of fruit powder of *Embelica officinalis* Gaertn.

S. No.	Physico-chemical parameters	Results	API Ref Value
1.	**Foreign Matter**	0.45%	NMT 3%
2.	**Loss on Drying**	3.568%	---
3.	**Aqueous Extractive Value**	63.5%	NLT 50%
4.	**Alcoholic Extractive Value**	43.94%	NLT 40 %
5.	**Total Ash**	2.19%	NMT 7%
6.	**Acid Insoluble Ash**	1.21%	NMT 2%
7.	**Water Soluble Ash**	1.09%	--
8.	**pH**	2.8	--

Preliminary phytochemical analysis

Preliminary phytochemical test revealed presence of carbohydrates, amino acids, proteins, saponins, tannins and phenolic compounds in aqueous and alcoholic extract of fruit powder. The results of tests performed are portrayed in table 3.

Table 3: Qualitative phytochemical tests of extracts of fruit powder of *Embelica officinalis* Carbohydrates.

Sr. no.	Name of test	Aqueous extract	Alcohol extract
A.	Molisch test	-ve	-ve
B.	Benedict test	+ve	+ve
C.	Barfoed's tests	-ve	+ve
D.	Fehling test	-ve	+ve

Alkaloids

A.	Dragondroff test	-ve	-ve
B.	Wagner's test	-ve	-ve
C.	Hager's test	-ve	-ve

Amino acids

A.	Ninhydrine test	+ve	+ve

Proteins

A.	Biuret test	+ve	-ve
B.	Xanthoprotic test	-ve	+ve
C.	Millon's test	+ve	-ve

Saponins

A.	Foam test	+ve	-ve

Glycosides

A.	Borntragor's test	-ve	-ve

Phenolic compound

A.	Phenolic test	+ve	+ve

Steroids

A.	Salkowaski reaction	-ve	-ve

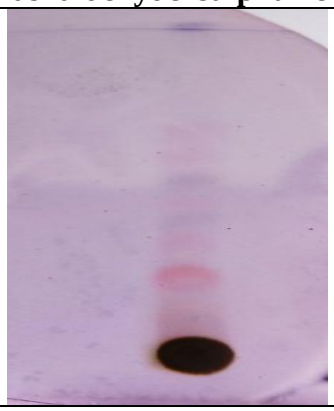
Tannins

A.	FeCl3 test	+ve	+ve
B.	Lead acetate test	+ve	+ve
C.	Potassium dichromate test	+ve	+ve

Thin layer Chromatography (TLC)

Detail result of TLC of fruit powder are given in table 4.

Table 4: TLC of ethanol extract of fruit powder (before and after derivatization).

Iodine Vapours	p-Anashaldehyde sulphuric acid
	
Rf values: 0.14, 0.45, 0.75, 0.82, 0.87, 0.90, 0.98	Rf values: 0.25, 0.32, 0.39, 0.46, 0.71, 0.73, 0.97

Thin layer Chromatography is a tool for separation and identification of chemical constituent. Thin-layer chromatography is a technique in which a solute undergoes distribution between two phases, a stationary phase acting through adsorption and a mobile phase in the form of a liquid. The adsorbent is a relatively thin, uniform layer of dry finely powdered material applied to a glass, plastic or metal sheet or plate. Glass plates are most commonly used. Separation may also be achieved on the basis of partition or a combination of partition and adsorption, depending on the particular type of support, its preparation and its use with different solvent.[10]

Identification can be effected by observation of spots of identical Rf value and about equal magnitude obtained, respectively, with an unknown and a reference sample chromatographed on the same plate. A visual comparison of the size and intensity of the spots usually serves for semi-quantitative estimation.

CONCLUSION

The fruit powder depicts the presence of Crystal shape particles of starch, Prismatic crystal of calcium oxalate, Bordered pitted vessels and group of lignified fibres are the key identification characters of the fruit. Fruit powder portrays the presence of different types of functional groups like carbohydrates, amino acids, proteins, saponins, tannins and phenolic compounds. Physicochemical and phytochemical parameters, TLC results will help in further standardization and act as standards for quality assurance.

REFERENCES

1. Pravin H. Nikam, Joseph Kareparamban, ArunaJadhav and VilasraoKadam, Future Trends in Standardization of Herbal Drugs, Journal of Applied Pharmaceutical Science, 2012; 02(06): 38-44.
2. Anonymous, The Ayurvedic Pharmacopoeia of India, 1st ed, Govt. of India. Ministry of Health and Family welfare, Department of I.S.M. & H., New Delhi, 1999; I: 2.
3. Khandelwal K. R.., Practical pharmacognosy, Nirali Prakashan, Pune: Edition 19th, 2008; 137-82.
4. Dr. K. R. khandelwal. Practical pharmacognosy, 20th edition, 3-5.
5. Anonymous, The Ayurvedic Pharmacopoeia of India, Ed. 1st, Govt. of India, Ministry of Health and Family Welfare, Department of I.S.M. & H., New Delhi, 1999; 1(1).
6. Laboratory guide for the analysis of *Ayurveda* and *siddha* formulations, CCRAS, Dept. Of Ayush, ministry of health and family welfare, govt. of India New Delhi, 27.
7. Shukla V.J. and Bhatt U.B. Methods of Qualitative Testing of some Ayurvedic Formulations. Gujarat Ayurvedic University, Jamnagar, 2001; 5-10.
8. Laboratory guide for the analysis of *Ayurveda* and *siddha* formulations, CCRAS, Dept. Of Ayush, ministry of health and family welfare, govt. of India New Delhi, 89-92.
9. E book. Quality control methods for medicinal plant materials. Geneva: World Health Organization, 31.
10. Laboratory guide for the analysis of *Ayurveda* and *siddha* formulations, CCRAS, Dept. Of Ayush, ministry of health and family welfare, govt. of India New Delhi, 89-92.