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Pharmacognostical and Histochemical analysis of *Phyllanthus emblica* Linn. Fruit – A dietary rasayana drug

BM Meghashree, TR Shantha and Sulochana Bhat

Abstract

Standardization of crude drugs is still a challenge in the research and development of herbal formulation preparations. This study aims to establish the pharmacognostic standards and understand the difference between 3 growing stages of fruits along with histochemical analysis on an important medicinal & dietary fruit *Phyllanthus emblica* Lin., belonging to the family euphorbiaceae, and known as Amalaki in Sanskrit texts. *P. emblica* is the only fruit which consists of all 5 Rasa's. Hence it is the best Aahara Rasayana. Present study macro, micro, powder microscopy along with histochemical analysis revealed different and important characters like absence and presence of stone cells in tender and mature stage respectively. Difference in percentage of histochemicals representing is also highlighted along with the figures respectively.

Keywords: Amalaki, Rasayana, Pharmacognosy, Histochemical studies

1. Introduction

Rasayana drugs play a vital role in Ayurvedic system of medicine. ‘Rasayanas’ are “rejuvenators”, nutritional supplements and possess strong antioxidant activity. They also have antagonistic actions on the oxidative stressors, which give rise to the formation of different free radicals. Their ant stress/ adapt genic actions have made them therapeutically more important. Ramayana’s a measure which may delay aging and prevent disease. Longevity and improvement in mental faculties have been claimed as the classical Ramayana effects. Ramayana drugs are administered to person according to constitution or Prakriti. Depending upon the status of different Dhatus and Dhatusara one has to select specific Ramayana ingredients for the benefit of different specific Dhatus. This has to be determined in consideration of Pharmacodynamics properties and Panchmahabautika [1, 2]. A persons undergoing rejuvenation attains longevity, memory, intellect, freedom from disease, youth, excellence of lusture, complexion, voice, excellent potentialities of the body and sense organs, the means by which one gets the excellence of Rasa is known as Rasayana or a Rejuvenation therapy [3]. Among all rasayana drugs, amalaki is called as king of rejuvenation and it is the best ‘aahararasayana’. Amalaki is commonly known as Indian gooseberry/aml. It is a medium sized deciduous tree distributed through tropical India, found cultivated or growing wild and ascending to 1300m on hills. Fruits fleshly roundish, marked with six lobes or ridges; with seeds six angular. Amla in diet acts as a cardiac tonic and helps in anemia, clears respiratory system in infection as it reduces kapha, enhances memory power and strengthens the nervous system. It sharpens the sensitivity of sense organs. Normalizes digestion, reduces acidity and rejuvenates liver, reduces burning sensation of skin. It strengthens the hair follicles and reduces the inflammation of scalp skin. Amla boosts immunity of skin and helps to prevent acne and pimples. It relieves constipation when taken in more quantity. It alleviate ‘tridoshas’ and specially normalizes pitta. Hence it is very useful in diseases which arise due to aggravation of pitta [4, 5]. Literature review revealed that this type of studies has not been carried out so far, hence present study has taken up which provides knowledge on its rasayana properties.

2. Regional Names in India [6]

Hindi	:	Amla
Kannada	:	Nelli
Sanskrit	:	Amalaki
Tamil	:	Nillikal
Telugu	:	Usharinki.

3. Materials and Methods

3.1. Plant material collection

Fruits of *P. emblica* was collected on 18th November 2016, from Raamtek, Nagpur, Maharashtra; identified by Taxonomist from Survey of Medicinal Plant Unit, Regional Ayurveda Research Institute for Metabolic Disorders, Bangalore. The plant material was cut into small pieces, shade dried, powdered and preserved in airtight container.

3.2. Pharmacognostic Study [7-9]

3.2.1. Macroscopy: The morphological as well as taxonomical characters in Fresh samples and macroscopical characters like odour, taste, colour, texture, (organoleptic characters) was documented by direct observation methods (Naked eye observation) in the dried samples.

3.2.2. Microscopy: The fruit was fixed in Formalin-Acetic acid-Alcohol for histological studies, to detect the location site of various constituents. The microscopical characters like anatomical/ histology, (arrangements of different tissues in definite manner) cell contents in different tissues, arrangements of cells in length and breadth carried out by using standard protocols to observe the characteristic fragments of different tissues and images was captured using Catcam camera and processed using computer software.

Finally Diagnostic characters was listed out, (for each growing stage of fruit), in turn which was useful for the identification of genuine drug.

3.2.3. Powder Microscopy: Powder microscopy was carried out by using standard protocols, sieved ingredient powder in 40-50mesh and treated with chloral hydrate solution and water, to observe the characteristics of fragments of different tissues by observing the powder in 8-10 slides to know the each characteristics of powdered ingredient in fragmented form.

3.3. Histochemical study [10]

The fruit was fixed in Formalin-Acetic acid-Alcohol for histological studies, to detect the location site of various constituents. Histochemical tests provides an initial knowledge about the presence of various group of phytochemical in the cellular and histological structures of the fruit. The tests for minerals like calcium, calcium oxalate and magnesium; carbohydrates like cellulose and calcium pectate; lignin; polyphenols and tannins were carried out with fresh samples using different reagents.

4. Results

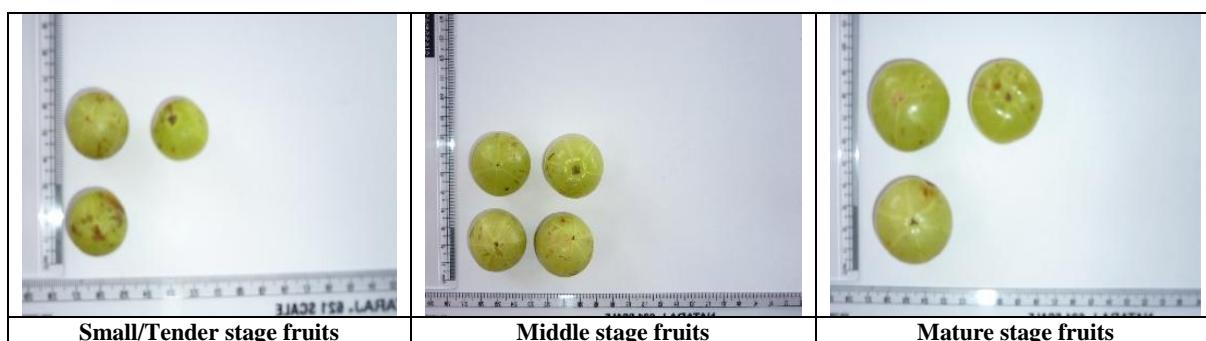
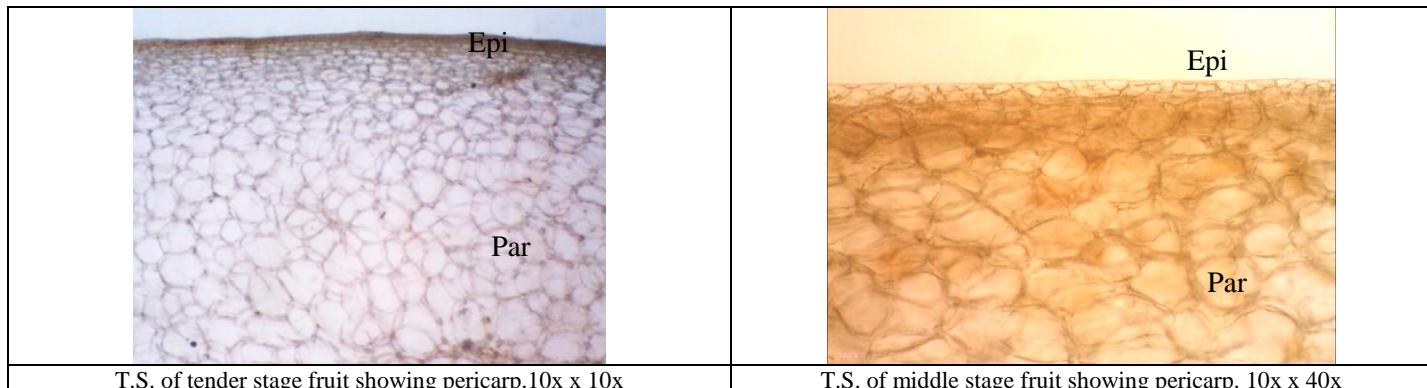


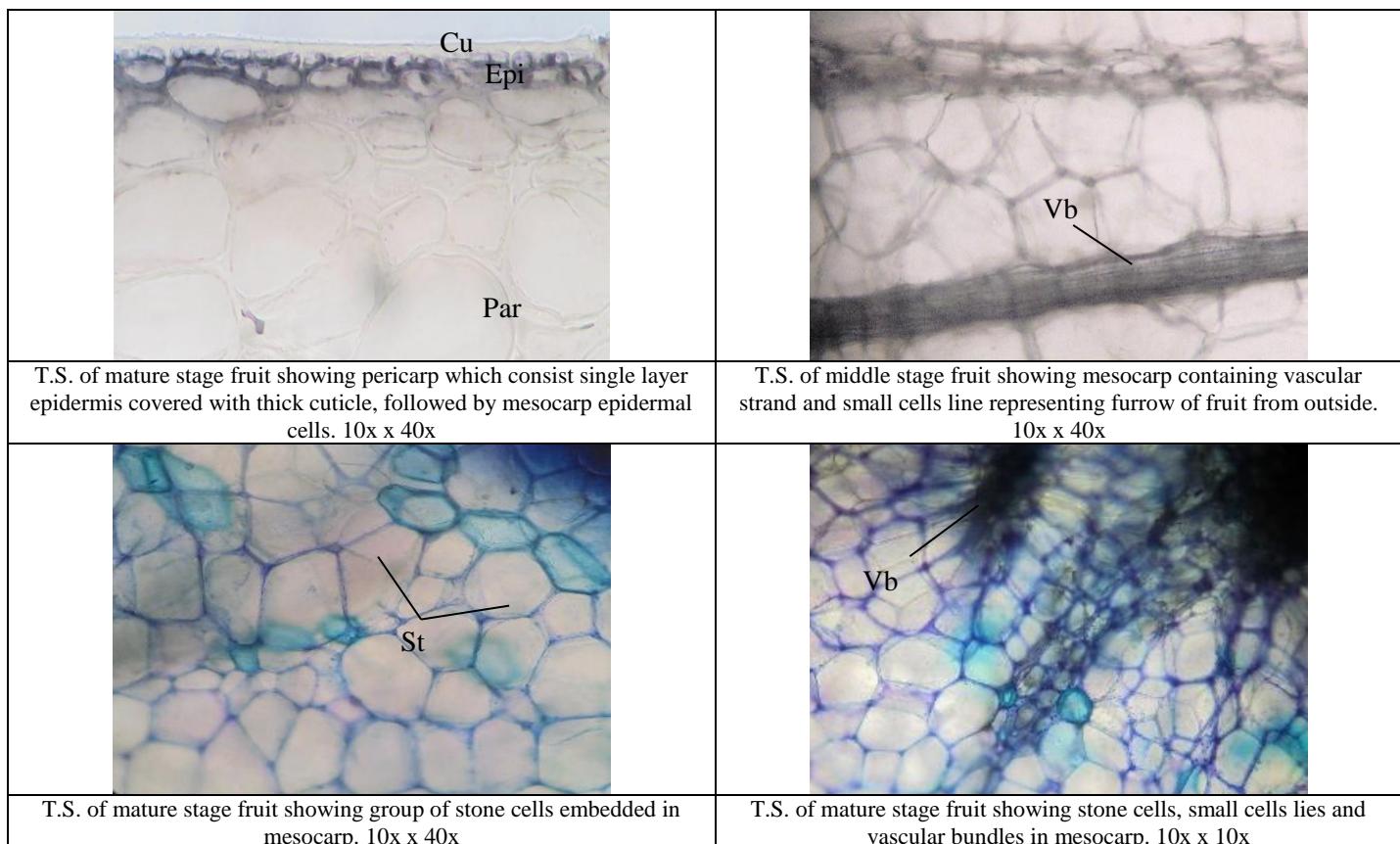
Plate 1: Macroscopic Characters

4.1. Macroscopical characters (Plate 1)

The Indian gooseberries are compact, fleshy, six seeded drupes. The fruits are globular in shape, varies from green to yellow colour, the tender fruits shows green colour whereas after maturity they shows greenish to yellowish ting colouration. The mature and middle stage fruit has smooth and shiny surface, but the tender fruits shows less-smooth (skinny) and dull surface. There are six longitudinally furrows running from top to base in all 3 stages. In mature stage two depressions were found, one at base which indicates the scar of pedicel and one at top which indicate the scar/ of style; both depressions are found in middle stage also but the % of

depression will be less compare to mature fruits. But scars of pedicel and style are found in tender stage without depressions. On drying the fruits they splits longitudinally along the running furrows, when compared with tender fruit, and the mature fruits splits first. Endocarp will be attached with fibrous vascular strand of mesocarp which can be seen when mesocarp is cut and endocarp is exposed. Odour characteristic, taste of tender fruit is very bitter, middle is bitter, sour and astringent and the mature fruit will be slightly bitter, sour, and astringent, followed by a sweetish feeling.





Abbreviations: cu: cuticle; epi: epidermis; par: parenchyma; st: stone cells; vb: vascular bundles.

Plate 2: Microscopical Characters

4.2. Microscopical characters (Plate 2)

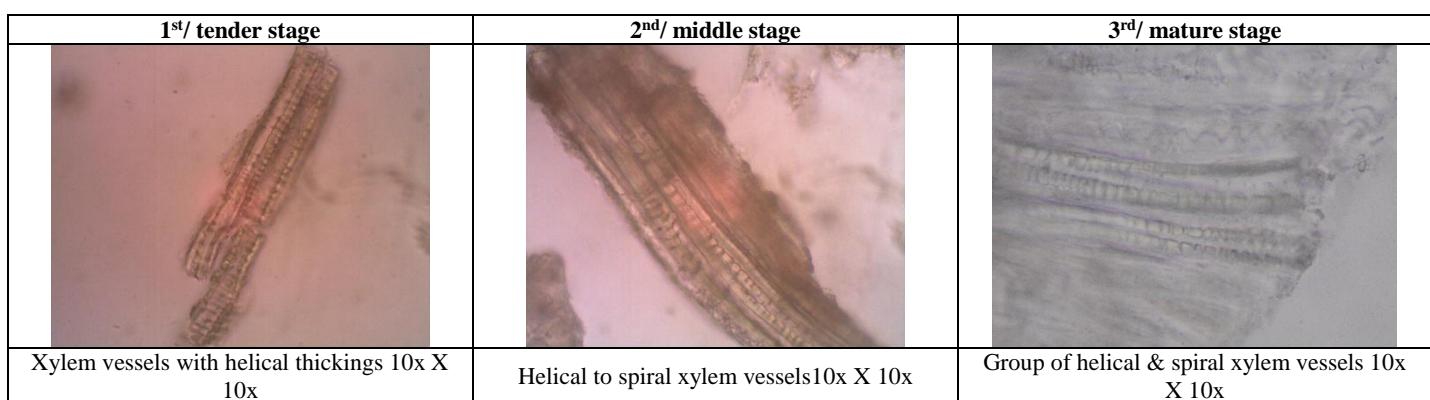
T.S. of fruit fleshypulp shows pericarp which consists of 2 parts, outer epicarp, followed by mesocarp tissue which forms the bulk of the fleshy portion. Epicarp to its outer side is covered with thick cuticle in mature stage but less cuticle in tender; and a single layer of epidermal cells which are narrow, tubular cells and thick walled in mature but not much in tender; underneath this lies few rows of small cells called hypodermis. The remaining cells of mesocarp slowly increases in size from epicarp to endocarp region in all three growing stages; (the sizes of epidermal cell, hypodermis and increasing size of mesocarp cells varies according to its growth, they all are not clearly distinguished in tender stage but well identified in mature stage). Fibro – vascular bundles

are seen embedded in mesocarp which are interconnected in various directions and found more towards inner region. Stone cells are not found in tender stage but found in mature stage embedded throughout the mesocarp along with parenchyma especially on surroundings of small cells of six longitudinal furrows. Fibers are located mostly towards mesocarp's inner region. Fibers are very less or not present in tender stage.

4.3. Powder Microscopy (Plate 3)

Powder light brown in colour, smooth to touch, smell characteristic and taste is bitter to astringent. When powder treated with Chloral hydrate, water and safranin, following different fragments of tissues was observed under microscope.

Plate 3: Powder microscopy study of 3 growing stages of *P. emblica* Fruit



Epidermis with Parenchyma cells 10x X 40x	Group of elongated stone cells 10x X 10x	Group of stone cells with highly lignified thickening and broad lumen 10x X 40x
Tracheids 10x X 40x	Elongated stone cells 10x X 10x	Single stone cell with highly lignified thickening 10x X 40x
Parenchyma cells 10x X 40x	Pitted xylem vessels 10x X 10x	Stone cells with broad lumen 10x X 40x
Group of elongated stone cells 10x X 40x	Single stone cell with broad lumen 10x X 40x	Group of elongated stone cells 10x X 40x
Elongated stone cells 10x X 40x	Elongated stone cells 10x X 40x	Fibers 10x X 10x

Epidermal cells in surface view 10x X 10x	Tracheids 10x X 10x	Group of elongated stone cells with highly lignified thickenings 10x X 40x
Group of xylem vessels with parenchyma 10x X 10x	Group of elongated stone cells with narrow lumen 10x X 10x	Group of elongated stone cells 10x X 10x
Group of spiral xylem vessels along with parenchyma cells 10x X 40x	Helical and spiral xylem vessels 10x X 40x	Epidermal cells in surface view 10x X 10x
Spiral xylem vessels 10x X 10x	Group of xylem vessels along with parenchyma cells 10x X 10x	Group of spiral xylem vessels 10x X 10x
Group of Parenchyma cells. 10x X 40x	Epidermal cells in surface view along with stomata. 10x X 10x	Group of spiral xylem vessels with fibres. 10x X 10x

4.3.1. Diagnostic characters of tender stage fruit:

- Abundant epidermal cells in surface view
- Abundant parenchyma cells present
- Minute crystals and starch grains present
- Abundant spherical and annular xylem strands present
- Regular stone cells absent but elongated type of stone cells present in few numbers

4.3.2. Diagnostic characters of middle stage fruit:

- Minute crystals and starch grains present
- Both elongated type and regular type of stone cells present
- Average of 9-11 stone cells found per slide
- Most of regular type stone cells shows less lignification border and mostly without pits in lumen

4.3.3. Diagnostic characters of mature stage fruit:

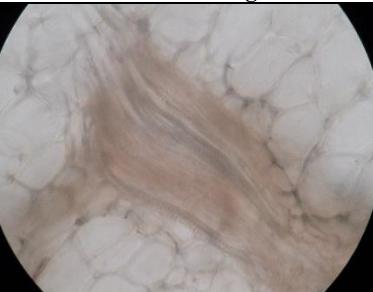
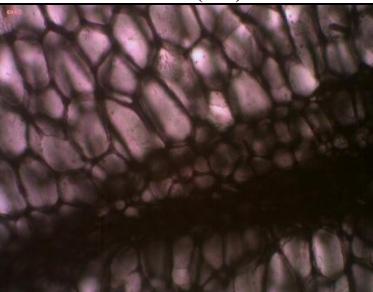
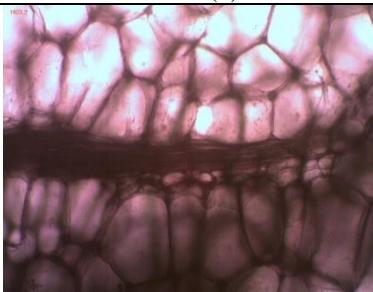
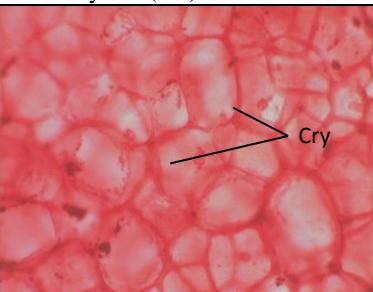
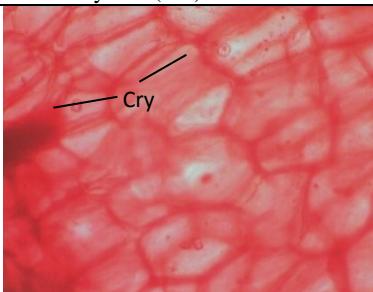
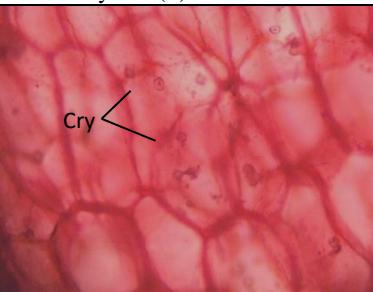
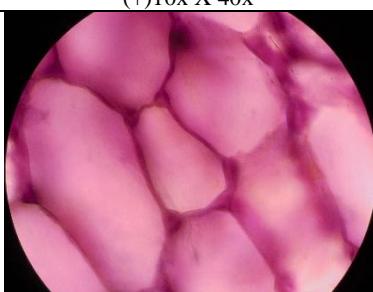
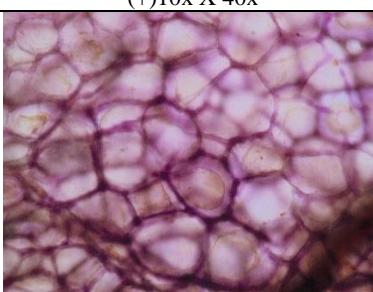
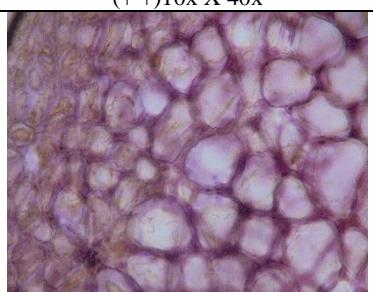
- Both elongated type and regular type of stone cells present
- Average of 3-6 stone cells found per microscopic view
- Abundant spiral and pitted xylem vessels present
- Most of regular type stone cells shows thick lignification border and pits in lumen

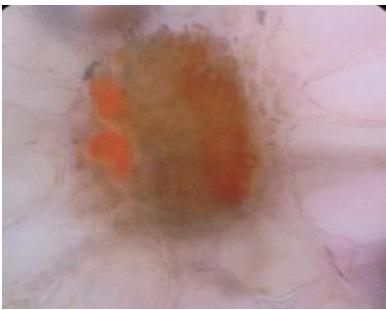
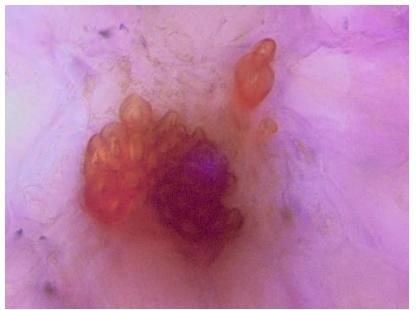
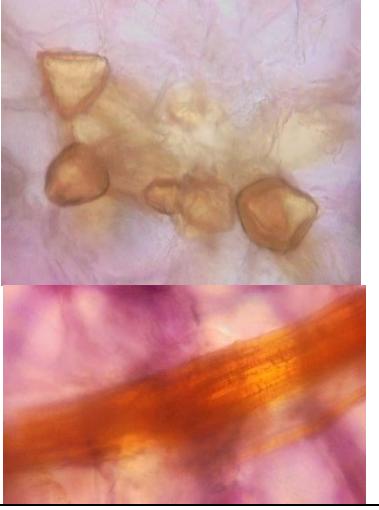
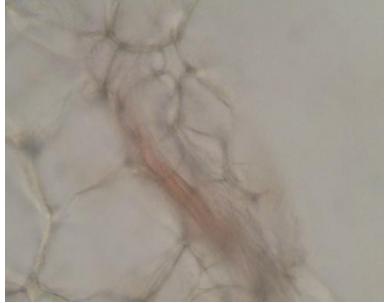
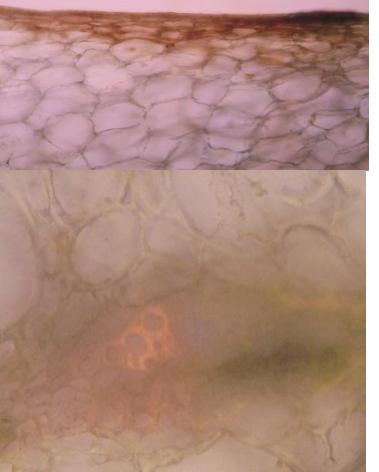
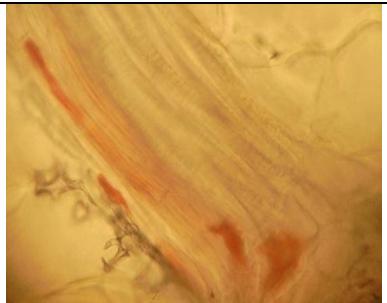
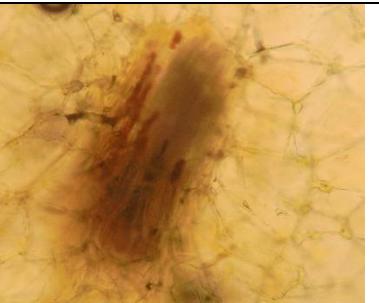
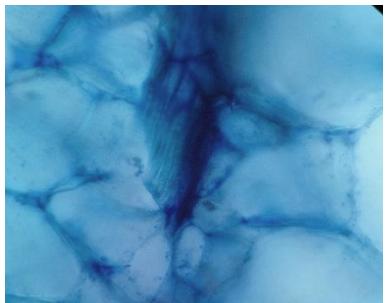
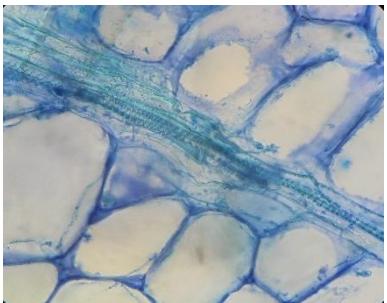
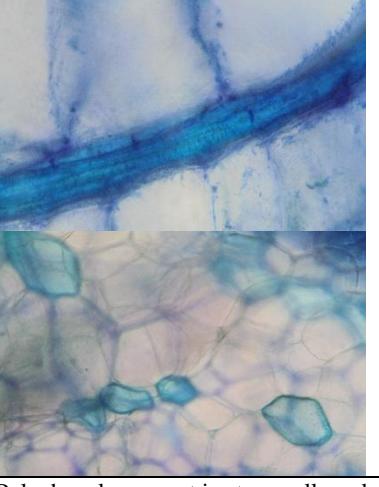
4.4. Histochemical studies (Table 1 & 2, Plate 4):

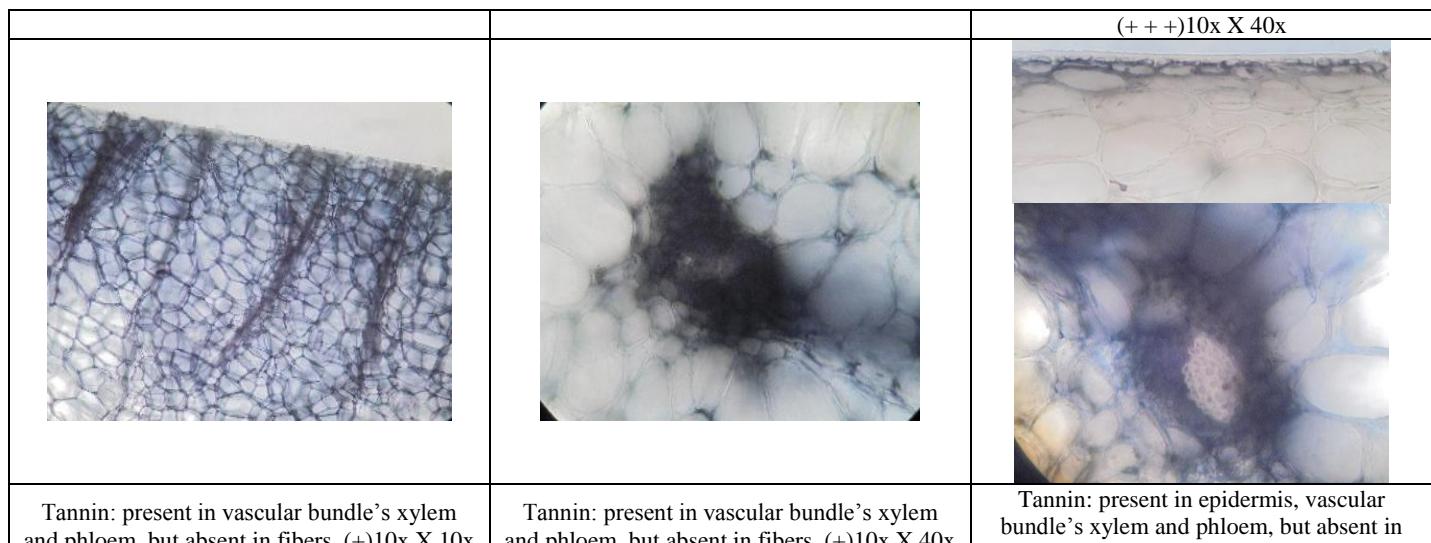
Revealed the presence of different phytochemicals in various tissues of fruit, the colour intensity taken up by the tissues represents the percentage of phytochemical present in different stages of fruit.

Table 1: Histochemical studies details

Stain/Reagent	Histochemical	Indication
Toluidine Blue O	Polyphenols	Blue green
Urea, Acetic acid, Sodium Nitrite and Sodium Hydroxide	Phenol	Cherry red
Alkaline Pyrogallol reagent	Calcium	Yellow brown
Silver hydrogen peroxide	Calcium oxalate	Crystals stain black, with red background
Titian yellow and Sodium Hydroxide	Magnesium	Brick red
Potassium iodide iodine and H_2SO_4	Cellulose	Bright blue
Tannic acid and Ferric chloride	Calcium pectate	Blue black
Phloroglucinol	Lignin	Yellow to Red
Ferric chloride in formalin	Tannin	Navy blue

1 st / tender stage	2 nd / middle stage	3 rd / mature stage
		
Calcium: present in xylem parenchyma of vascular bundle. (+)10x X 10x	Calcium: present in xylem parenchyma of vascular bundle. (+)10x X 40x	Calcium: present in xylem parenchyma of vascular bundle. (+)10x X 10x
		
Calcium pectate: parenchyma cells wall and xylem. (+)10x X 10x	Calcium pectate: parenchyma cells wall and xylem. (+)10x X 10x	Calcium pectate: parenchyma cells, wall and xylem. (+)10x X 10x
		
Calcium oxalate: minute crystals found embedded in monocarp's parenchyma cells. (+)10x X 40x	Calcium oxalate: minute crystals found embedded in monocarp's parenchyma cells. (+)10x X 40x	Calcium oxalate: minute crystals found embedded in monocarp's parenchyma cells. (+)10x X 40x
		
Cellulose: present in walls of all parenchyma cells (+)10x X 40x	Cellulose: present in walls of all parenchyma cells. (+)10x X 10x	Cellulose: present in walls of all parenchyma cells. (+)10x X 10x

		
Lignin: present in xylem lignification. (+)10x X 40x	Lignin: present in xylem lignification and fibers. (++)10x X 40x	Lignin: present in stone cells, xylem lignification and fibers. (+++)10x X 40x
		
Magnesium: present in xylem vessels consists it. (+)10x X 40x	Magnesium: present in xylem vessels consists it. (++)10x X 40x	Magnesium: present in epidermis and xylem vessels consists it. (+)10x X 40x
		
Phenols: present in xylem parenchyma cells. (+)10x X 40x	Phenols: present in xylem parenchyma cells. (++)10x X 40x	Phenols: present in xylem parenchyma cells. (+++)10x X 40x
		
Polyphenols: present in xylem vessels. (+)10x X 40x	Polyphenols: present in stone cells and xylem vessels. (++)10x X 40x	Polyphenols: present in stone cells and xylem vessels.



Tannin: present in vascular bundle's xylem and phloem, but absent in fibers. (+)10x X 10x

Tannin: present in vascular bundle's xylem and phloem, but absent in fibers. (+)10x X 40x

Tannin: present in epidermis, vascular bundle's xylem and phloem, but absent in fibers. (++)10x X 40x

(+) = less; (++) = moderate; (++) = more/abundant

Abbreviations: cry: crystals.

Plate 4: Histochemical studies of 3 growing stages of *P. emblica* Fruit

Table 2: Histochemical analysis results

Histochemical	Analysis
Polyphenols	Present more in mature stage
Phenol	Amount of phenol in tender stage is less and in mature stage is more. It gradually increases with the growth of fruit.
Calcium	% of calcium content is more in tender stage when compare to middle and mature stage.
Calcium oxalate	More in mature stage compare to tender
Magnesium	Less in tender stage and moderate in middle and mature stage
Cellulose	Present more in mature stage since it contains completely developed parenchyma cells
Calcium pectate	More in tender stage and gradually decrease to middle and to mature stage
Lignin	Present more in mature stage
Tannin	Present more in mature stage

5. Discussion

Previous pharmacognostic studies have not mentioned any growth stage and may be carried out in mature stage fruits [12-14]. Present study with three different growth stages revealed the presence of abundant xylem vessels & stone cells with highly lignified boarder in mature *P. emblica* fruits; presence of parenchyma and epidermal cells is observed abundantly in tender stage. Histochemical studies based on intensity of colouration revealed more percentage of polyphenols, phenols, calcium oxalate crystals, lignin and tannins; likewise tender stage consist more percentage of calcium and calcium pectate; and middle stage fruits consist moderate percentage of magnesium, cellulose, lignin, tannin and more of calcium pectate were observed.

6. Conclusion

Ancient Indian systems of medicines has given information on many formulations and treatments for several diseases and they have also provided the information about selection of crude drug for those preparations according to their 'rasa panchaka'. Hence studies like this should be carried out to prove literally the ancient texts, and such studies are important to overcome the adulterations. Tender one or unseasonal grown one will have difference in chemical constituents and with the presence of different types of stone cells, fibers, crystals and other tissues. Thus using those mixed with important stage like mature stage (drug) may not give the expected effect of health for consumers and may not serve as Rasayana drug. This type of study will be helpful to detect the adulterations and to certify the genuineness with different stages of the fruit, which in turn will be more useful

for Ayurvedic physicians, research scholars and students to know the efficacy of rasayana effect in different stages of fruits, which will substantiate the usage of Amalaki as "Rasayana" drug. Further studies like HPTLC can be carried out for revalidation of the drugs in 3 different stages.

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