Market samples of *Terminalia chebula* (Harde) fruit: an in-depth study of their phytochemical and pharmacognostic characters

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ABSTRACT

Of the two types of *Terminalia chebula* fruits available in the market, Haritaki (mature fruit rinds) and Himaj (unripe tender fruits), only the former one is studied, that too very cursorily and all these studies are found incomplete as many of the cellular features are missing. During an in-depth study involving anatomy, it is found that the samples contained a good percentage of stones which are the hard endocarp enclosing seeds. The new distinguishing characters of the mature fruits (Harde) unearthed in the present study are more than a dozen. The aggregation, arrangement and orientation of various types of macrosclereids and brachysclereids to produce an endoskeleton in mesocarp of fruit are well documented. The components of endocarp such as tightly packed macrosclereids, resins and resin chambers as well as the cell architecture of testa and tegmen provide absolute new data. It is for the first time tender fruits (Himaj) are subjected to a detailed study on their phytochemical and pharmacognostic characters. Chemically they are having the same constituents as of mature fruits, albeit in differing amounts. The anatomical/pharmacognostic characters, which here also, amount to a dozen or more, consist of a wide variety of macro- and brachysclereids of different levels of maturity, their shapes and sizes, arrangement, resin chambers and cells constituents. All these can be effectively used in quality control measures

Keywords : *Haritaki, Himaj, Macrosclereids, Pharmacognosy, Phytochemistry, Terminalia chebula*

INTRODUCTION

Terminalia chebula (Retz.) is a very important medicinal plant of India, the fruits of which, are widely used as part of "Triphala". It is a preferred laxative and has tonic, restorative and also used as an antiageing drug. Two types of fruit samples are met with in trade, the larger one named "Harde", is the mature fruits devoid of the hard endocarp and seed enclosed within and the younger small ones, called "Himaj", consisting of tender immature seedless fruits (Fig.1A). The small fruits are preferred for constipation, purgation and piles [1]. All the available studies on the drug Haritaki are based on the mature fruit samples. This drug is proved to be having excellent antioxidant, antibacterial, antiviral, anti-inflammatory, hypoglycaemic, cardioprotective and anticancer properties[2]. Though very widely used in almost all parts of India, the quality control studies including pharmacognostic characters are very poorly conducted because almost all the workers studied the powder only and not the fruits. In such a method many characters are found missing. A correct study involves the anatomical and micro morphological studies of the intact fruits to locate the shape and characters of all possible types of cells, cell modifications and cell inclusions and one has to search for all of them in powder and record the pharmacognostic characters. The absence of clear pharmacognostic characters prompted as many as four authors [3-6] to publish their observations on these characters in last 5 years. As these reports are from non-Botany scholars, there appeared many conceptual errors in identifying different parts of fruit, tissues etc. Actual Harde sample should consist of the outer shell composed of epicarp and mesocarp and no endocarp or seed. But in a market sample procured by the Senior author for manufacturing purposes, it is observed to contain about 10-15% of fruits with seeds. Over and above these glaring gaps, there is no study on Himaj, the immature fruit, with regards to its phytochemistry and pharmacognosy. Therefore the objective of the present study is to analyse both mature fruit sample and immature fruits for their chemical constituents and pharmacognostic characters so that the two samples could be properly identified in sample and extracts. This study should also give better quality and efficacy standards for the two drugs, mature and immature fruits.

MATERIALS AND METHODS

Samples of mature and immature fruits were procured from "Paras Herbal Pharma", Vadodara and their identity was confirmed by comparing with samples collected from Botanical Garden, The M.S. University of Baroda, Vadodara, Gujaat, India. Plant materials were extracted with methanol in Soxhlet's extractor for flavonoids and later with water for mucilage. The extracts were concentrated, water soluble extract separated, hydrolysed and aglycones separated in solvent ether and analysed for flavonoids and phenolic acids by Paper chromatography, Thin Layer chromatography and co –chromatography with standards by methods recommended by Harborne[7] and Daniel and Denni[8]. Total flavonoids were estimated gravimetrically by lead acetate method[7]. Total tannins were extracted by indigocarmine method[7]. The plant residue after methanol extract was extracted with boiling water and the mucilage was precipitated by adding methanol to the concentrated aqueous extract. The mucilage precipitated was filtered off and quantified gravimetrically.

Fresh plant materials and fruits were collected from Botanical garden of M.S. University of Baroda and compared with the Herbarium (BARO) in Department of Botany. Plant material was collected and dried at 60°C. They were then extracted with methanol and analysed for flavonoids, tannins, phenolic acids and mucilage using standard procedures[7, 8] . The plant residue after methanol extract was extracted with boiling

water and the mucilage was precipitated by adding methanol to the concentrated aqueous extract. The mucilage precipitated was filtered off and quantified gravimetrically.

Dry fruits were kept in water and warmed in a Microwave oven to soften and used for initial studies using Stereozoom microscope model ST6 which involved hand sections. Later they were fixed in FAA[9.] . Trimmed parts were embedded in paraffin and transverse sections were taken in a rotary microtome (Senior precision microtome model RMT-30). Sections were selected and stained with safranin and after dehydration mounted in DPX. The sections are then observed and photographs were taken in trinocular compound microscope with camera under 400X magnification. The size (dimensions) of various cells and crystals were measured using stage and ocular micrometers. The quantitative data are based on the average of 20 readings.

RESULTS AND DISCUSSION

Phytochemistry

a. Mature fruit (Harde)

Mature fruits contained tannins, flavonoids like quercetin, kaempferol, phenolic acids like vanillic, gallic, ellagic acids, waxes and mucilage. Waxes amount to 1.3% and mucilage 4%. Tannins amounted to 40% and flavonoids about 10%.

b. Immature fruits (Himaj)

Himaj also contained same phytochemicals but the amount varies. Tannin content was much higher, about 68%. Total flavonoids amounted to 16% and consist of quercetin, kaempferol and their derivatives. Phenolic acids located were vanillic, gallic and ellagic acids. Waxes were about 1% and mucilage 3.9%.

Pharmacognostic studies

Of the two types of fruits available in market, the larger "Haritaki" or Harde, are mature broken shells of fruits, brownish yellow in colour and 3-5 cm long, 1.5 – 2 cm broad and weigh 4-5g. These samples consists of a major amount of open fruit walls having epicarp and a porous mesocarp and up to 20-30% of fruits containing a large single stone (seed enclosed in hard endocarp) in the centre (Fig.1A). The stone also is spindle shaped having a height of 2.7 cm and a breadth of 1cm (Fig.1B). Seed within also is spindle shaped having a height of 2.3 cm and a width of 4mm (Fig.1C). The stone weighs about 1.5g and seed approx. 0.15g The smaller fruits, known as "Himaj" locally, consists of small and comparatively larger immature fruits. They are small black in color, consist of immature entire fruits, unopened and contain seed-less hard stones in centre. The fairly larger ones here are spindle shaped or oval with 5 vertical ribs having a height of 2-3 cm and a breadth (diameter) of 1- 1.4 cm having a smooth yellow surface and weighing about 1 to 1.2g. The smaller ones are very young fruits having a weight varying from 0.5 – 0.7g (Fig 1A).

Fig.1, *T.chebula* fruit morphology: A. Mature fruits (Haritaki) left and immature fruits (Himaj) right. B. Stone enclosing seed, C. Seed, D. T.S of fruit showing endocarp and mesocarp fused and yellow endocarp enclosing seed; E. L.S. of stone showing outer endocarp containing small brown resin chambers as dots, more on the inner side and seed in centre. Seed coat is partially peeled off at the top.

Anatomical and pharmacognostic details of mature fruit (Haritaki)

In T.S of fruit, 3 regions are recognised; outer epicarp, middle mesocarp and an inner endocarp enclosing a single seed in the centre (Fig.1D-E). The hard endocarp containing the seed is designated as"stone". Mesocarp and epicarp together form the commercially available drug which amounts to about 66% of the fruit. Endocarp when present forms 29% of the fruit and the seeds the smallest part, a mere 5-6% (Fig. 1C, D, E).

Epicarp is small in size having a thickness of about 350-380 mμ consisting of epidermis and a single layered hypodermis and a large cortical region (Fig.2A). Epidermis consists of rectangular (35 mμ in breath and 18mμ in height) containing abundant chromoplasts. There is a moderately thick waxy cuticle over it. Hypodermis is of slightly broader colorless parenchyma cells (40-45 mμ) without any cellular inclusions (Fig.2 B). Below this is the cortical region composed of large elliptical/rectangular parenchyma (50 x 30 mμ) filled with small spherical starch grains having a diameter of 3 mμ. A few cells are found to contain oil globules (Fig. 2A)

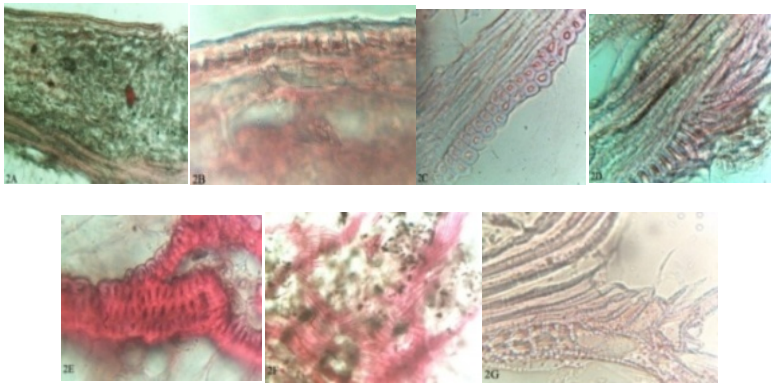


Fig.2 . *T.chebula* fruit anatomy: A. Epicarp showing epidermis, cuticle, one layered hypodermis and 5-6 layered cortex. Red region below is the outer region of mesocarp; B.Epidermis, cuticle and hypodermis in magnified view. C. T.S of fruit mesocarp showing outer 3-4 rows of parallely arranged macrosclereids (in X axis) with cross section of 1-2 rows of macrosclereids at right angles(Y axis) appearing like "stone cells". D. Same structure as C, but another group of macrosclereids proceeding inwards attached at right angles (Z axis) to outer ring, E. TLS of fruit showing longitudinal sections of transversely running macrosclereids appearing as sclerenchyma and the branching chains, F Anastomosing network of macrosclereids with ground parenchyma trapped in between., G. Macrosclereids oriented in all three directions.

Mesocarp is the broadest region, lined at outside by vertical sheets of various types of macrosclereids (fibre-sclereids) and an anastomising network of long chains or sheets of brachysclereids and macrosclereids. The outermost layer, running parallel to the periphery of fruit, consists of sheets of transversely running fibre like macrosclereids (in "x" axis) and inner to this is a single layer of sclereids appressed positioned at right angles running upwards parallel to the periphery and thus appear as sclerenchyma in TLS (Fig 2 C,D). The outer layer, mostly 2-3 sclereids thick, is found to frequently branch and rejoin afterwards (Fig.2E). Each macrosclereid in these two layers has a diameter of 16 mμ and a lumen of 3 - 11 mμ. In TLS the sections of these fibers appear rectangular with a length 16 mμ and breadth of 12 mμ enclosing a rectangular lumen of 11 mμ long and 3 mμ wide. These two layers appressed together form the outer solid sheet of mesocarp. Then there are thick chains of brachysclereids and macrosclereids attached at right angles to this sheet running towards the centre of fruit (Z axis) which branch into sheets of one sclereid thick or uniseriate filamentous chains which anastomose to form a network of macrosclereids enclosing patches of parenchyma (Fig. 2 F.). The macrosclereids are very heterogeneous, showing a wide variety in shapes ranging rectangular to elongated filaments (Fig.2 G.). Short trapezoidal sclereids are seen at places of joining with outer sheet. The size varies but, on an average, the rectangular/trapezoidal sclereids have a width of 18-24 mμ and a length of 150 mμ and a broad lumen of 13-15 mμ. The long fibre like macrosclereids have a length of 260 mμ or more and a breadth, 20 mμ breadth and a broad lumen. They are distinct from fibres in having angular projections on sides and are branched at the ends. The parenchyma located oval or rectangular, having a diameter of 155 mμ and a length of 180 mμ. Almost all parenchyma are filled with spheroidal starch grains (Fig 3H) of 3 mμ diameter or contain a ring of grains in periphery and or scarcely a sphaeraphide of 30 mμ diameter.

Endocarp forms the protective covering of the single seed and it is highly sclerified. It is uniform in having only macrosclereids closely packed. Benches of sclereids are packed parallel or at right angles or at different angles to each other (Fig 3 A). Many of them are elongated dumb-bell shaped (osteosclereids) with a very thick wall traversed by simple pits and narrow lumen (Fig.3B). Larger ones have a length of 520 mμ, breadth of 30 mμ and a lumen of 2 mμ or less. Towards the inner half of endocarp are large number of schizogeneous chambers containing yellow resin (Fig.3C). Each chamber is oval or elliptical having a breadth of 310-370 mμ and a height of 450- 610 mμ . Each chamber contains yellow colored globular resinous material.

Seed is single, spindle shaped, 18 mm long and 3-4 mm broad in the middle. Testa and tegmen are seen fused. Testa is thick and three layered with cells decreasing in size towards inner layers. Outer layer is made of square thin walled, pitted sclereids of 50 mμ on one side (Fig.3D). These cells appear rectangular on surface view (L.S.) having a length of 130 mμ and a breadth of 60 mμ. Middle layers are smaller , but having a thicker pitted wall with dimensions of 40 mμ length and 25-30 mμ wide. The innermost layer is still smaller cells having an average length of 20 mμ and breadth of 15 mμ (Fig.3E). Tegmen is single layered, composed of tubular thick walled cells (appearing rectangular in T.S.) having a spiral or double spiral thickening (Fig 3F). Cotyledons are seen as a spirally twisted against each other and covered by an epidermis of rectangular cells (17 x 12 mμ) enclosing an undifferentiated parenchymatous tissue(Fig.3G). Parenchyma cells are isodiametric

(diameter 35 mμ), heavily packed by starch grains and oil globules (Fig 3H). A few sphaeraphides are seen scattered.

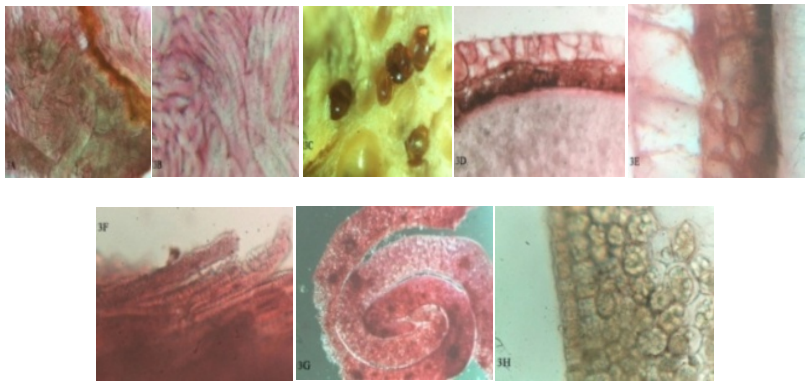


Fig.3. *T.chebula* fruit Endocarp (A-C) and seed.; A. Benches of parallely arranged heavily thickened macrosclereids and osteosclereids arranged criss-cross one above the other; B. Heavily thickened and pitted walls of macrosclereids/osteosclereids with little or no lumen, C. Brownish yellow Resin and empty resin chambers, D. Outer testa with square pitted cells and inner tegmen of spirally thickened thick walled cells, appearing rectangular in cross section; E. Enlarged view of Testa and Tegmen, F. Spirally thickened cells of tegmen appearing cylindrical, G. Two cotyledons spirally arranged in seed; H. Cells of cotyledon packed with starch grains and oil globules.

Powder study of Harde.

The powder of Harde contained 1). Pieces of epidermis with lamellate collenchymas (Fig 4A), 2) thin walled long pitted macrosclereids of outer mesocarp(Fig 4B), 3)short broad macrosclereids(Fig 4C), 4) Anastomosing macrosclereids(Fig 4D), 5) Short oval or spherical sclereids (Fig 4E),. 6) Large Parenchyma enclosing starch grains (Fig 4F), 7). Small spheroidal starch grains abundant everywhere(Fig 4G), 8) heavily thick walled long macrosclereids of endocarp(Fig 4H), 9) resin masses(Fig 4I), 10) outer layers of cotyledons containing rows of small sphaeraphides(Fig 4J), 11) cells of tegmen with spiral or double spiral thickenings (Fig 4K),and 12) large sphaeraphides of mesocarp (Fig 4L).

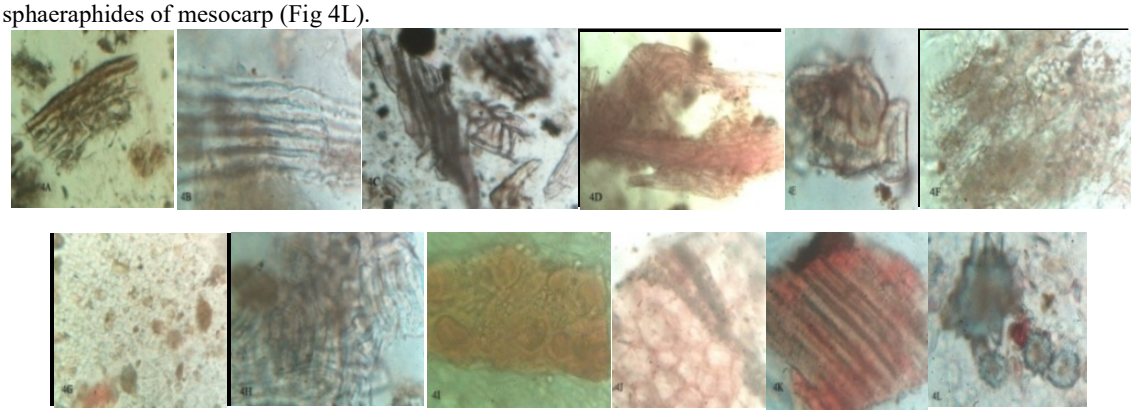


Fig 4. *T.chebula* (Harde) Powder characteristics: A. Pieces of epidermis with lamellate collenchyma, B. Thin walled pitted macrosclereids of outer mesocarp, C. Short broad macrosclereids, D. Anstomosing macrosclereids, E. Short oval or spherical sclereids. F. Large Parenchyma enclosing starch grains, G. Small spheroidal starch grains abundant everywhere, H. Heavily thick walled long macrosclereids of endocarp, I. Resin masses, J. Outer layers of cotyledons containing rows of small sphaeraphides, K. Cells of tegmen with spiral or double spiral thickenings and L. large and small sphaeroraphides of mesocarp.

Anatomical and pharmacognostic details of unripe fruit (Himaj)

Compared to mature fruits (Harde), younger fruits are soft consisting of tender cells getting differentiated. The epicarp, almost indistinguishable from mesocarp, amounts to about 10%. Mesocarp is the largest portion consisting of 50 to 55%. Endocarp appears clear enclosing an seedless empty chamber. (Fig.5A). In some very small fruits the central chamber is absent. Endocarp amounts to about 20% of the fruit and the chamber, when present, accounts for 5-10%.

The epicarp is about 7-8 cells thick. Epidermis consists of heavily cutinised square cells filled with tannin contents. Cutin layer is about 6 mμ thick and the square epidermal cells have one side about 17 mμ. Hypodermis cannot be distinguished. (Fig 5B). The cortical collenchymas are lamellate and are square (20 x 20 mμ) in outer layers, rectangular (24 mμ long, 20 mμ wide) in layers below and oval (35 mμ long and 22 mμ) towards Mesocarp and are filled with tannins and chloroplasts/chromoplasts (Fig.5C).
Below epicarp is the broad mesocarp, essentially similar to that of mature fruits, which begins with 2-3 layers of very long rectangular(180 mμ long and 20 mμ wide) undifferentiated macrosclereids

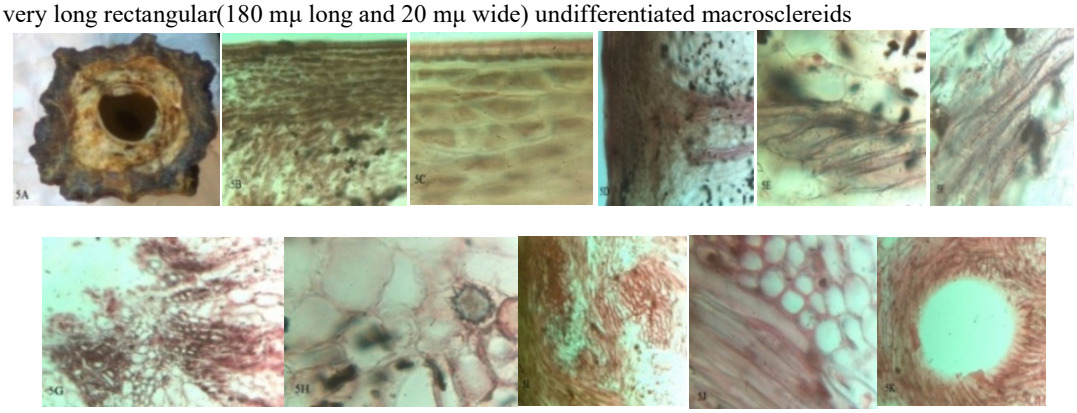


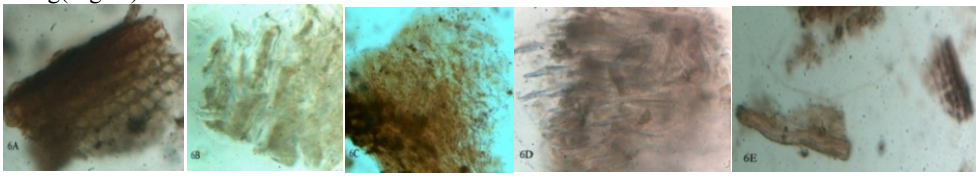
Fig 5. *T.chebula* (Himaj) Anatomy. A T.S of fruit with central seedless chamber. B. Epicarp almost undifferentiated from mesocarp. C. Epicarp with lamellate collenchyma containing chromoplasts, D. Outer mesocarp with bundles of macrosclereids running parallel to periphery and bench of macrosclereids attached at right angles running to centre of fruit. E. Trapezoidal macrosclereids, F. Very long rectangular macrosclereids, G. Vascular bundles, H. Mesocarp parenchyma with sphaeraphides, I. Macrosclereids arranged criss- cross in endocarp, J. Thin walled bunches of macrosclereids arranged at right angles to each other, K. Empty resin chamber of endocarp.

having thinly lignified walls with small pores and long walls parallel to epidermis (Fig 5D). Adjoining this is a layer of similar macrosclereidal cells attached at right angles appearing small rectangular 20 mμ long and 10 mμ) with lightly sclerified walls. These three layers form the outer solid sheet of mesocarp which later get lignified to become thick walled mature macrosclereids. Attached at right angles towards centre are filaments of long rectangular/ trapezoidal cells (Fig. 5E, F) similar to those present in outermost layers of mesocarp. These filaments may be 2-3 cells thick and branch at times or anastomoses as in mature fruit. Arranged between these filaments are isodiametric (about 60 mμ in dia) or oval (70 mμ long and 60 mμ) parenchyma cells and vascular bundles (Fig 5 G, H)

Endocarp contains various types of macro- and osteosclereids as in mature fruits, but with very thin walls (2-3 mμ thick) which are getting gradually lignified (Fig. 5. I). Long rectangular sclereids have a length of 170-190 mμ and a breadth of 20 mμ. Then there are oval and round sclereids also, all with poorly thickened walls containing many unthickened areas which correspond to pits in mature sclereids (Fig. 5J). Towards the inner side are a number of oval or round schizogenous resin ducts which may come to 400 mμ in diameter(Fig. 5K). Starch grains are not seen and very few sphaeraphides were present (Fig. 5.H).

Powder study of Himaj

The powder of tender fruits contains square cortical cells of epicarp (Fig 6A), cortical lamellar collenchyma rich in chloroplasts (Fig 6B), mesocarp parenchyma with chloroplasts and tannins (Fig 6C), macrosclereids with large lumen and poorly thickened walls (Fig 6D), rectangular macrosclereids (Fig 6E), macrosclereids and surrounding parenchyma of mesocarp (Fig 6F), parenchyma of mesocarp without intercellular spaces and rich in chloroplasts and other contents(Fig 6G), vascular bundles(Fig 6H), macrosclereids of endocarp arranged in criss-cross pattern(Fig 6 I) and pieces of mesocarp from which flavonoids (yellow in color) are seen oozing out and spreading(Fig 6J).



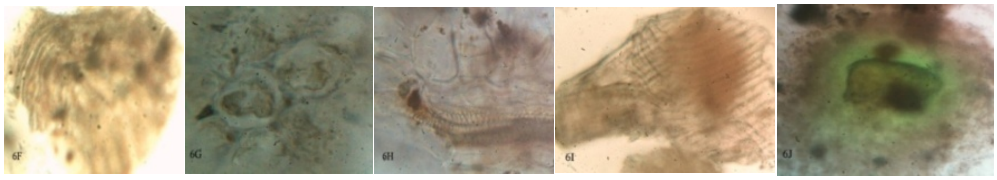


Fig 6. *T.chebula* (Himaj) Powder characteristics: A. Square epidermal cells and square cortical cells of epicarp, B. Cortical lamellar collenchyma rich in chloroplast, C.Mesocarp parenchyma with chloroplasts and tannins, D. Macrosclereids with large lumen and poorly thickened walls, E. Single rectangular macrosclereid and part of epicarp, F. Macrosclereids and surrounding parenchyma of mesocarp. G. Parenchyma without intercellular spaces and rich in chloroplasts and other contents , H.vascular bundles, I. Criss -cross pattern of macrosclereids of endocarp and J. Pieces of mesocarp from which flavonoids (yellow in color) are seen oozing out and spreading.

Phytochemically there appeared only quantitative differences between Harde (mature fruits) and Himaj (immature fruits). The tannin and flavonoid contents are about 50% higher in Himaj samples. They share the same flavonoids, phenolic acids and tannins. The amount of tannins and flavonoids in Himaj would have been the reason of them used more in constipation, purgation and piles.

With regard to Pharmacognostic characters, this is the first time the immature fruits are recognised and studied. Basically they have the same design of cellular organisation as that of mature fruits, but almost all types of cells are young and in the initial stages of differentiation. Though the mature fruits were studied by many , that also recently, the present study unearths a large array of distinguishing diagnostic characters unseen by all previous workers. The chains of macrosclereids (fibre sclereids) in the outer layers and seen permeating the inner layers of mesocarp were called "xylem" by a few workers. But xylem is already present separately in vascular bundles in mesocarp with distinctive tracheids. In having pits the macrosclereids are similar to tracheids, but do not have any spiral or annular thickenings characteristic of primary xylem tracheids. The walls are not straight but bent out to form angles unlike the uniform wall of tracheids. Moreover there is no phloem tissue anywhere near these structures. In addition, the transverse orientation of these macrosclereids from outside to the centre indicate its role as a mechanical tissue. The great variety of shapes and sizes of sclereids is mind boggling. They are brachysclereids (rare) or macrosclereids having shapes varying from broadly oval to rectangular, trapezoidal, or tubular always with simple pits. The thickness of walls varies; heavily thickened in brachysclereids and thin walled in others. Another characteristic feature is the network of anastomising macrosclerieds. The ground tissue of parenchyma, arranged among the network of sclereids, are filled with spherical starch grains and or tannins.

This is the first time the endocarpic tissues and the seed structure of the plant are studied, of course in detail. As the commercial samples of Harde contain about 15-20% intact fruits with seed, these characters are bound to be present in all samples. Moreover the young seeds of Himaj invariably contain endocarp. These characters were observed by none of the earlier workers. The uniform nature of endocarp in consisting of heavily thickwalled macrosclereids, the layers of which are closely appressed in a criss-cross pattern is a unique character of Harde fruits. Some of these sclereids are dumbbell shaped- osteosclereids. Absolutely there are little or no lumen in these sclereids. In addition the multitude of large resin chambers filled with globules of yellow resin (also seen with naked eye) also is located for the first time. To sum up the new pharmacognostic characters unearthed in present study are more than a dozen in number. Similarly this is the first time, the immature fruits are studied for their phytochemical and pharmacognostic details. These immature fruits are the ones which falls off the tree due to various reasons and are normally collected from the base of the trees. The fact that they are specially used or preferred for constipation, purgation and piles (Pandey, 2018) indicate its market share. Being immature they posses almost all phytochemical and pharmacognostic features. The tannin and flavonoid contents are much higher (up to 50%) in these fruits. All the cells and tissue systems here are in nascent stage or at varying levels of differentiation. Thus we encounter with square hypodermal collenchyma, macrosclereids of thin walls (poorly lignified), brachysclereids with larger lumen, parenchyma with chromoplasts and tannin and endocarp with thin walled macrosclereids having empty resin chambers devoid of any resin. The starch grains abundant in mature fruits are virtually absent here. As the fruits are immature, seeds also are absent.

In contrast to the earlier studies, the powder of both Harde and Himaj provide a large amount of very characteristic features which were unreported so far. The new distinguishing characters of the former unearthed in the present study are more than a dozen like lamellate chlorenchyma, macrosclereids of varied shapes (rectangular, trapezoidal), anastomosing macrosclereids, endocarpic thick walled macro- and osteosclereids, their criss-cross arrangement, resin canals, yellow resin, square testa cells, double spirally thickened tegmen cells and parenchyma of cotyledons. The characters of tender fruits reported also are of an equal number. All these can be effectively used in quality control measures.

CONCLUSION

Of the two types of *Terminalia chebula* fruits available, Haritaki (mature fruit rinds) and Himaj (unripe tender fruits), only the former one was subjected to ambiguous and incomplete studies. During an in-depth study involving anatomy, a good percentage of stones consisting of endocarp and seeds were seen. The aggregation, arrangement and orientation of various types of macrosclereids and brachysclereids to produce an endoskeleton of fruit are well documented. The components of endocarp such as tightly packed macrosclereids, resins and resin chambers as well as the cell architecture of testa and tegmen provide absolute new data. The new distinguishing characters of the mature fruits (Harde) unearthed in the present study are more than a dozen. Similarly the tender fruits (Himaj) are having the same constituents as of mature fruits , albeit in differing amounts. The distinguishing anatomical/pharmacognostic characters found here also are more than a dozen similar to Harde but tissues are poorly differentiated.

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Conflict of Interest

The authors declares no competing or conflict of interest

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