



E-ISSN: 2321-2187  
P-ISSN: 2394-0514  
IJHM 2017; 5(4): 17-24  
Received: 06-05-2017  
Accepted: 07-06-2017

**BM Meghashree**

Ph.D. Research Scholar,  
Drug Standardization Research  
Unit, Regional Ayurveda  
Research Institute for Metabolic  
Disorders, (CCRAS, Ministry of  
AYUSH, Govt. of India),  
Bangalore, India.

**TR Shantha**

Research Officer (Botany),  
Drug Standardization Research  
Unit, Regional Ayurveda  
Research Institute for Metabolic  
Disorders, (CCRAS, Ministry of  
AYUSH, Govt. of India),  
Bangalore, India.

**G Venkateshwarlu**

Research Officer (S-3,  
Ayurveda), Advanced Centre for  
Ayurveda and Metal Health &  
Neuro Sciences, NIMHANS  
campus, Bangalore, India

**Sulochana Bhat**

Research Officer (S-3,  
Ayurveda), Regional Ayurveda  
Research Institute for Metabolic  
Disorders, (CCRAS, Ministry of  
AYUSH, Govt. of India),  
Bangalore, India.

**Correspondence****BM Meghashree**

Ph.D. Research Scholar,  
Drug Standardization Research  
Unit, Regional Ayurveda  
Research Institute for Metabolic  
Disorders, (CCRAS, Ministry of  
AYUSH, Govt. of India),  
Bangalore, India.

## Comparative pharmacognostical and histochemical studies on *Benincasa Hispida* (Thunb.) Cogn.– Fruit and Seed

**BM Meghashree, TR Shantha, G Venkateshwarlu and Sulochana Bhat**

**Abstract**

According to Ayurvedic classics fruits of different stages of growth have different properties which possess different effects on consumption; therefore choosing a right stage of fruit for consumption or formulation preparations is very important. This study aims to establish the pharmacognostic standards and evaluate the difference between 3 growing stages of fruit and its seeds along with histochemical analysis on an important medicinal & dietetic fruit *Benincasa hispida* (Thunb.) Cogn. Known as kushmanda in Sanskrit. The present study revealed important characters like presence of discontinuous patches of stone cells in tender stage, powder study revealed more parenchyma cells in tender stage and more xylem vessels and starch grains in mature stage. Difference in percentage of histochemicals in fruit and seed showed more of phytochemical in mature stage.

**Keywords:** rasayana, pharmacognosy, histochemical studies, *benincasa hispida*, fruit.

**1. Introduction**

The Rasayana drugs and formulations provides longevity to users, with age stabilization, retaining youth for longer with maintaining strength of all organs optimally, enhanced intelligence, improved complexion, voice and allied positive health attributes, memory, freedom from disorders, youthful age, excellence of luster, respectability and brilliance<sup>[1]</sup>. There are several standardization procedures available for the proper identification and authentication of the medicinal plants, among these identification parameters the pharmacognostic study is accepted method for the quality control analysis of the crude drugs<sup>[2,3]</sup>. Among the rasayana drugs kushmanda is a dietary drug which is distributed and cultivated in tropical and sub-tropical parts of India. The plant is an annual creeper, large leaves, thickly covered with bristly hairs. Fruits are pepo, large and oval shaped, not ribbed. Seeds are many. *B. hispida* is commonly known as Ash gourd which is beneficial for diabetic and obese persons. It eliminates constipation and soothes the gastrointestinal tract, stabilize nerve cells. The juice induces relief in acidity and peptic ulcers. Seeds are anabolic and hence promote tissue growth<sup>[4]</sup>.

**2. Regional names in India**<sup>[5]</sup>

Hindi: Petha

Kannada: Boodagumbalakayi

Sanskrit: Kushmanda

Tamil: Pusanikayi

Telugu: Budidegumnudu.

**3. Materials and Methods****3.1. Plant material collection**

Fruit of *B. hispida* was collected from Gandsi, Arsikere, Karnataka; identified by Taxonomist from Survey of Medicinal Plant Unit, Regional Ayurveda Research Institute for Metabolic Disorders, Bangalore. The plant material cut into small pieces and was shade dried, powdered and preserved in airtight container.

**3.2. Pharmacognostic Study**<sup>[6, 7, 8]</sup>

**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:** Freehand sections of each stage were taken, cleared with chloral hydrate solution and water, stained with safranin according to standard prescribed methods, and 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 were observed and images was captured. 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<sup>[9]</sup>:**

The fruit was fixed in Formalin-Acetic acid-Alcohol for histological studies, to detect the location site of various constituents. Histochemical tests provide 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, magnesium and silica; carbohydrates like starch and calcium pectate; lignin; polyphenols; and tannins were carried out with fresh samples using different reagents.

**4. Results**

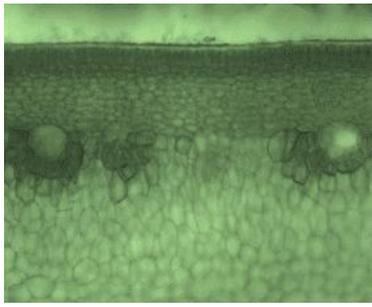
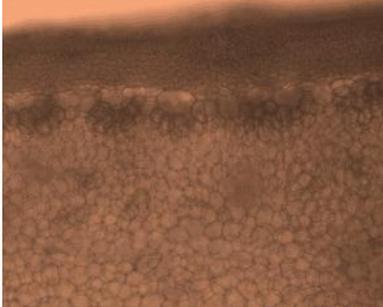
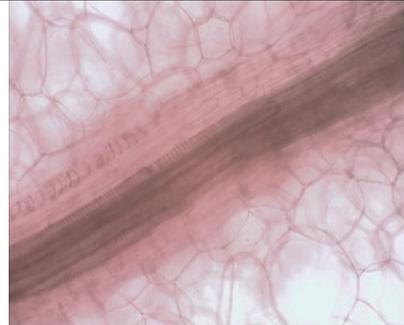


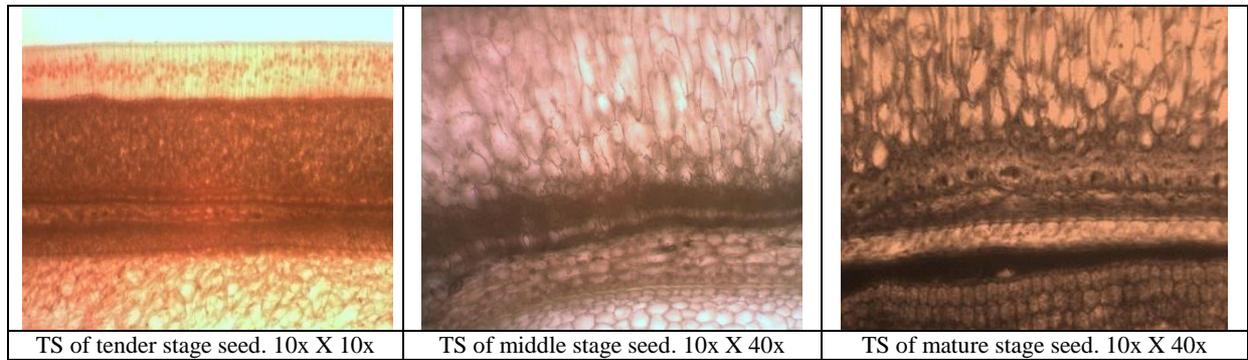
**Plate 1: Macroscopic Characters**

**4.1. Macroscopical characters (Plate 1):**

It is a pepo type of fruit, which are large, green in colour, oval shaped, not ribbed, the epicarp (skin) bears white bristly hairs in tenders stage of fruit growth; the waxy ash deposition on fruit skin starts from stock region when the fruit is in middle stage and waxy ash covers whole fruit when the fruit attains

mature stage of growth. Seeds are many, slimy, flat and compressed marginally; seeds in tender stage of fruit are small, white and easy to cut in hands; in middle stage the seed coat starts hardening; and in the mature stage of fruit the seed is brownish, big, hard to break up by hand and surface rough. Fruits and seeds are odourless and taste slightly sweetish.

Tender stage	Middle stage	Mature stage
		
TS of tender stage fruit showing pericarp and bundles of stone cells. 10x X 10x	TS of middle stage fruit showing pericarp and discontinuous layer of stone cells. 10x X 10x	TS of mature stage fruit showing epidermis, starch grains in hypodermis and continuous layer of stone cells. 10x X 40x
		
TS of tender stage fruit showing mesocarp and vascular bundle embedded in it. 10x X 10x	TS showing annular and spiral xylem vessels in middle stage fruit. 10x X 40x	TS showing group of xylem vessels in mature stage fruit. 10x X 40x



**Plate 2:** Microscopical characters

**4.2. Microscopical characters (Plate 2)**

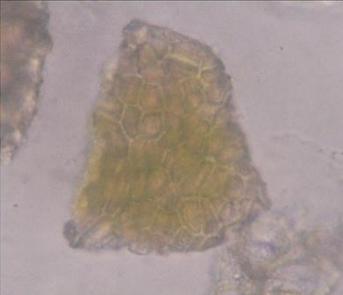
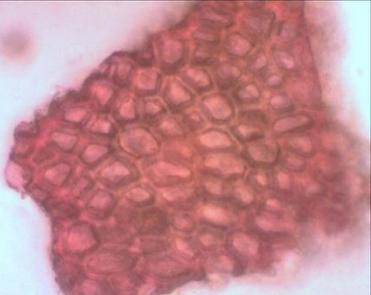
T.S. of fruit shows a single layer of epidermis, made up of rectangular shaped parenchyma cells which are covered by a waxy ash on outer surface, followed by the epidermis multiple layers of hypodermis composed of tangentially elongated, thin walled, parenchymatous cells and starch grains are seen embedded in these cells. Next to hypodermis, 4 to 6 layers of stone cells runs continuously in mature stage fruits and found in patches in tender stage fruits. This is followed by mesocarpparenchyma cells which are of different sizes, the size of the cells increases from periphery region to inside mesocarp. The cells are thin walled and oval shaped and the mesocarp is very much watery. Interior to this seed are present which are arranged in parietal placentation. T.S. of

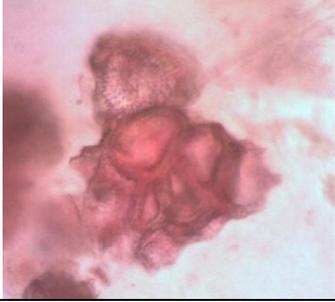
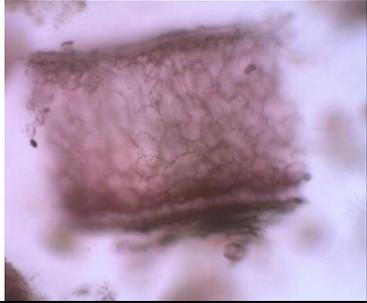
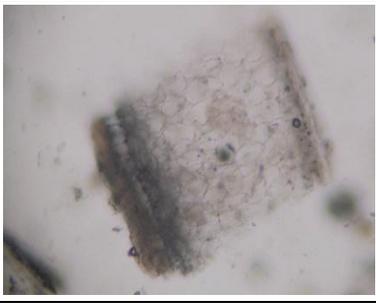
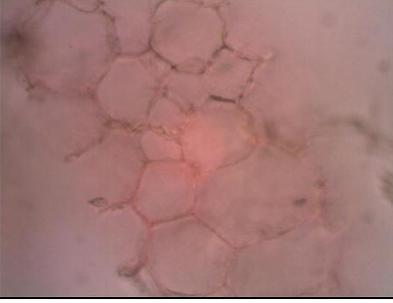
seed shows outer epidermis which is slimy in nature, next to this is outer sclereids which are thin walled in tender seeds. It is followed by stone cells, perisperm, endosperm and collapsed cells layer and in the centre cotyledon portion which contains loosely arranged parenchyma cells in tender seeds and compactly arranged palisade parenchyma is present in mature stage seeds and these cells contains starch grains.

**4.3. Powder Microscopy (Plate 3)**

Powder pale green in colour, smooth to touch, odourless and tasteless. 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 *B. hispida*- whole fruit

Tender stage	Middle stage	Mature stage
		
Single spiral xylem vessel 10x X 40x	Single spiral xylem vessel 10x X 40x	Group of spiral vessels 10x X 40x
		
Spiral xylem vessel 10x X 10x	Group of helical & spiral vessels 10x X 40x	Group of Spiral xylem vessels 10x X 40x
		
Epidermal cells in surface view with stomata 10x X 40x	Epidermal cells in surface view 10x X 40x	Epidermal cells in surface view 10x X 40x

		
Stone cell 10x X 40x	Group of stone cells from epicarp 10x X 40x	Group of stone cells from epicarp 10x X 40x
		
Parenchyma cells from seed 10x X 40x	Parenchyma cells from seed 10x X 40x	Parenchyma cells from seed 10x X 40x
		
Group of parenchyma cells 10x X 40x	Elongated parenchyma cells 10x X 40x	Starch grains embedded in parenchyma cells 10x X 40x

**4.3.1. Diagnostic characters of tender stage fruit:**

- Abundant parenchyma cells present
- Xylem vessels lignification is wider
- Abundant epidermis present with stomata
- Abundant spiral xylem vessels present

**4.3.2. Diagnostic characters of middle stage fruit:**

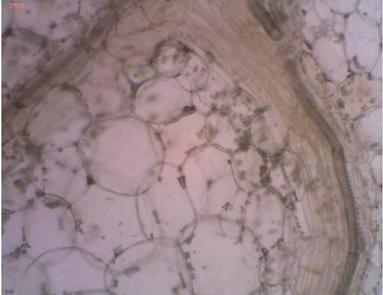
- Abundant spiral and helical xylem vessels present
- Xylem vessels lignification is narrow
- Abundant stone cells present
- Parenchyma cells of seed present
- Elongated parenchyma cells present

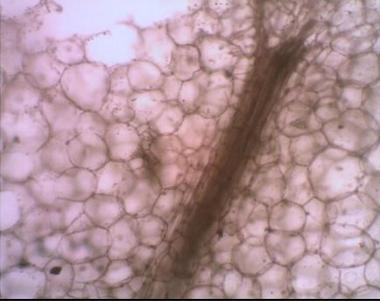
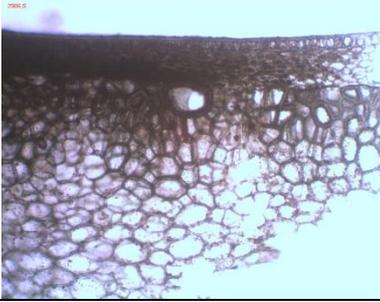
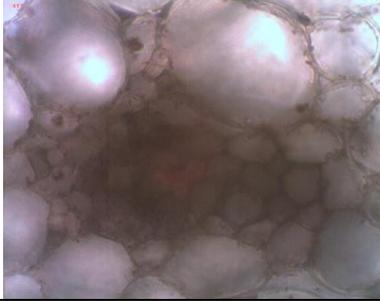
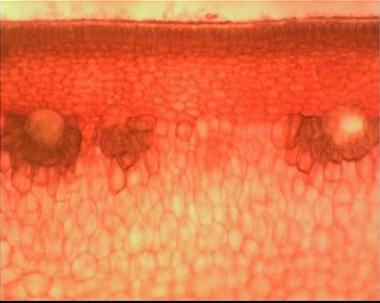
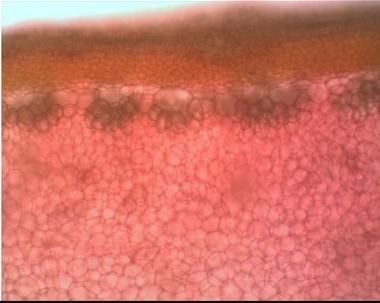
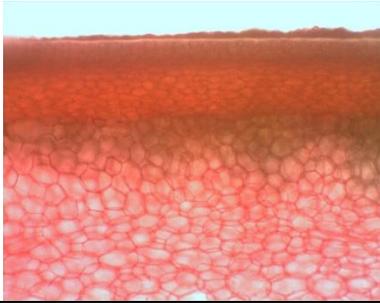
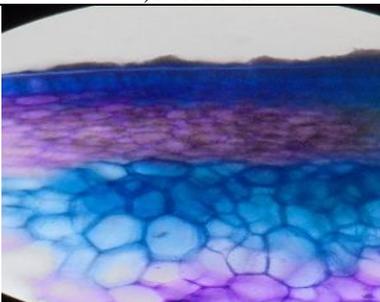
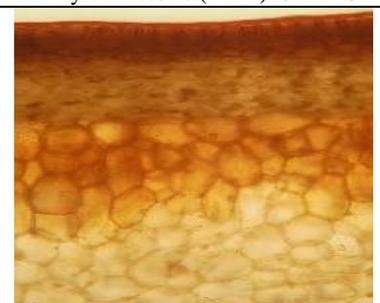
**4.3.3. Diagnostic characters of mature stage fruit:**

- Abundant spiral and helical xylem vessels present
- Xylem vessels lignification is narrow
- Abundant stone cells present
- Abundant epidermal cells present with thick wall
- Abundant Starch grains present

**4.4. Histochemical studies (Table 1, Plate 4 & 5):**

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

Tender stage	Middle stage	Mature stage
		
Calcium: present in xylem parenchyma of vascular bundle. (+) 10x X 10x	Calcium: present in xylem parenchyma of vascular bundle. (+ +) 10x X 10x	Calcium: present in xylem parenchyma of vascular bundle and parenchyma cells. (+ +) 10x X 10x

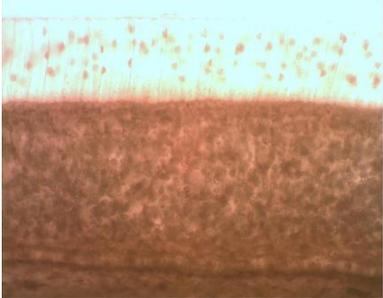
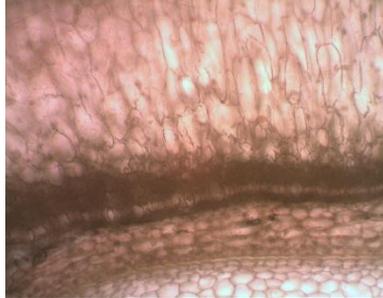
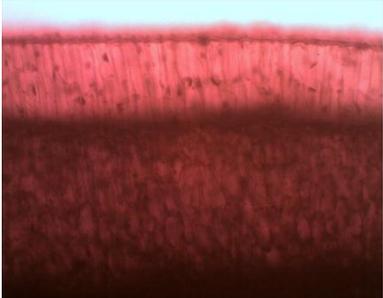
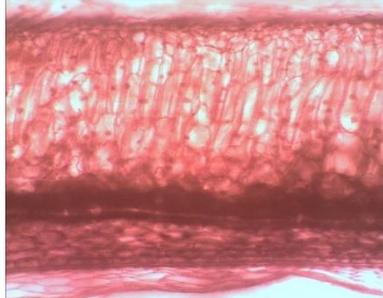
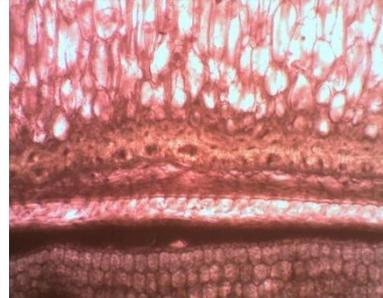
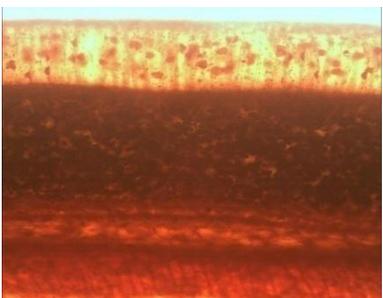
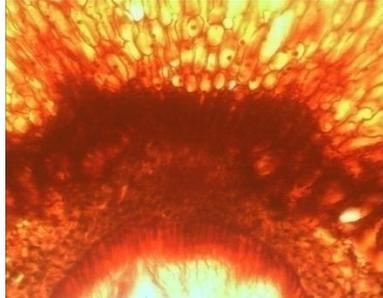
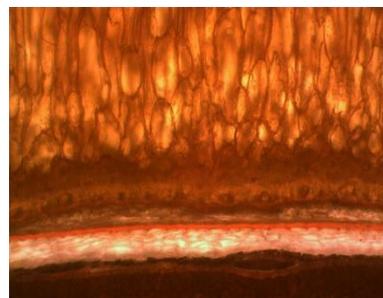
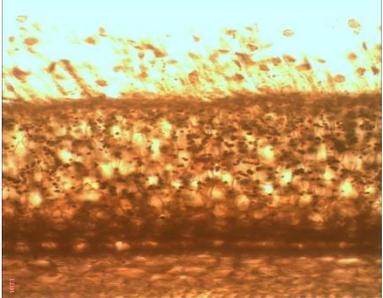
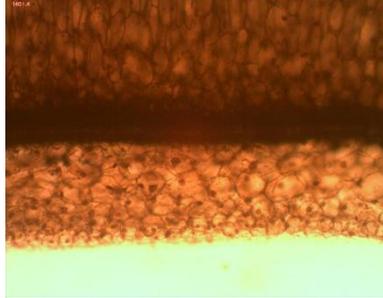
		
Calcium pectate: cell walls and xylem. (+) +10x X 10x	Calcium pectate: cell walls and xylem. (+) +10x X 10x	Calcium pectate: cell walls and xylem. (+) +10x X 10x
		
Magnesium: present in xylem vessels, hypodermis and parenchyma cell walls. (+)10x X 10x	Magnesium: present in xylem vessels, hypodermis and parenchyma cell walls. (+) +10x X 10x	Magnesium: present in xylem vessels, hypodermis and parenchyma cell walls. (+) +10x X 10x
		
Polyphenols: present in epidermis, stone cells and xylem vessels. (+)10x X 40x	Polyphenols: present in epidermis, stone cells and xylem vessels. (+ +)10x X 40x	Polyphenols: present in epidermis, stone cells and xylem vessels. (+ + +)10x X 40x
		
Starch: present in hypodermis and mesocarp parenchyma cells. (+ +) 10x X 40x	Starch: present in hypodermis and mesocarp parenchyma cells. (+ +) 10x X 40x	Starch: present in hypodermis and mesocarp parenchyma cells. (+ +) 10x X 10x
		
Tannin: present in stone cells and vascular bundle. (+)10x X 10x	Tannin: present in stone cells and vascular bundle. (+ +)10x X 40x	Tannin: present in stone cells and vascular bundle. (+ +)10x X 10x

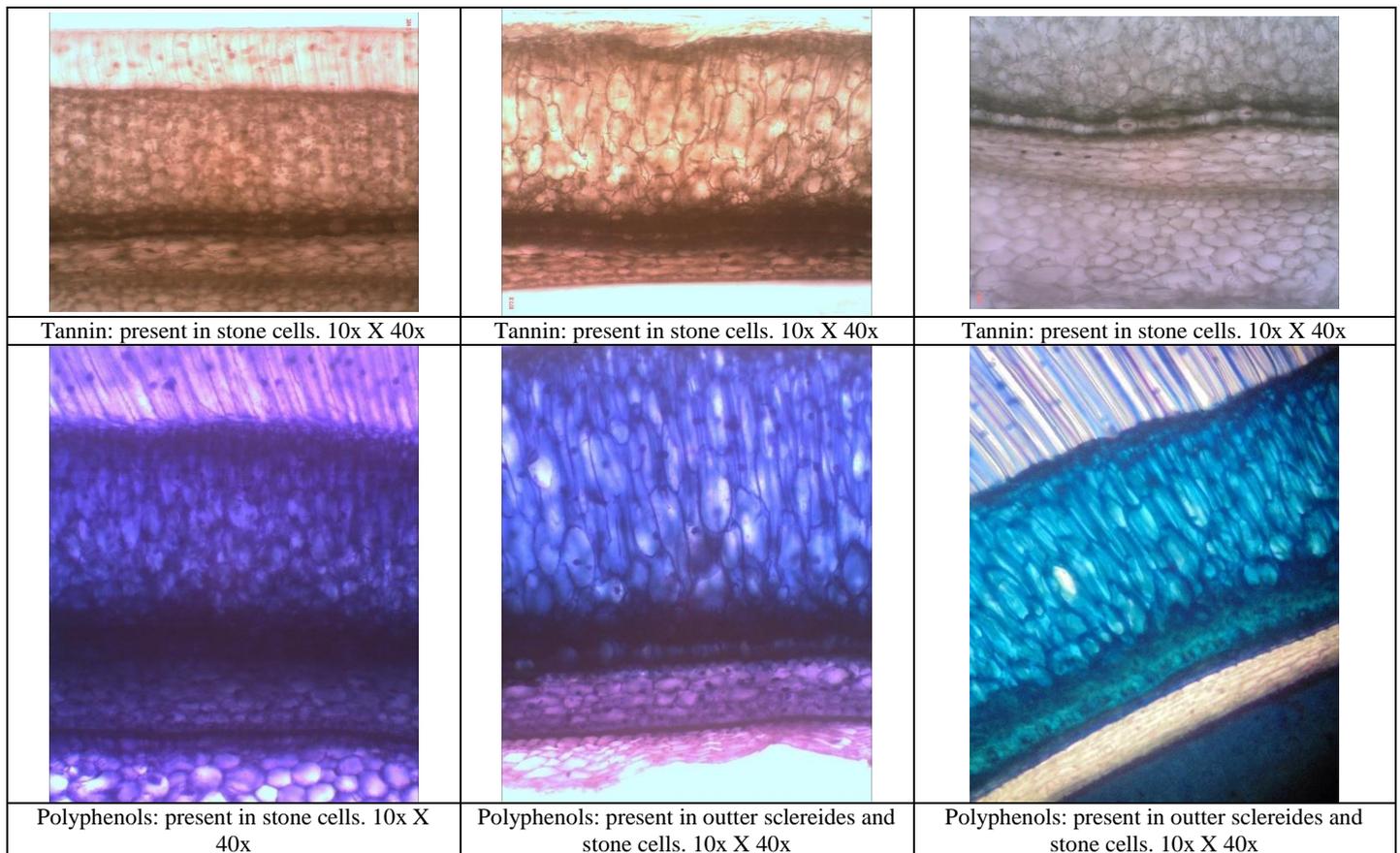
(+) = less; (+ +) = moderate; (+ + +) = more/abundant

**Plate 4:** Histochemical study of 3 growing stages of *B. hispida* Fruit

**Table 1:** Histochemical analysis results

Histochemical	Analysis
Calcium	Amount of calcium content is less in tender stage when compare to middle and mature stage.
Calcium pectate	Moderate in all 3 stages
Magnesium	Less in tender stage and moderate in middle and mature stage
Polyphenols	Present more in mature stage
Starch	Moderate in all 3 stages
Tannin	Present moderate in middle and mature stage

Tender stage	Middle stage	Mature stage
		
Calcium: present in outer sclereides, collapsed cells layer. 10x X 40x	Calcium: present in outer sclereides, collapsed cells layer. 10x X 40x	Calcium: present in outer sclereides, collapsed cells layer and cotyledon. 10x X 10x
		
Calcium pectate: present in cell walls and stone cells. 10x X 40x	Calcium pectate: present in cell walls and stone cells. 10x X 40x	Calcium pectate: present in cell walls and stone cells. 10x X 40x
		
Lignin: present in outter sclerieds and stone cells. 10x X 40x	Lignin: present in outter sclerieds and stone cells. 10x X 40x	Lignin: present in outter sclerieds and stone cells. 10x X 40x
		
Starch: present in outter sclerieds. 10x X 40x	Starch: present in collapsed cells layer. 10x X 40x	Starch: present in paliside of cotyledon. 10x X 40x



**Plate 5:** Histochemical study of 3 growing stages of *B. hispida* seed

#### 4.4.1. Histochemical study of seed

Histochemical studies of 3 growing stages of *B. hispida* seed revealed the presence of calcium, calcium pectate, lignin, tannin, starch and polyphenols and the percentage of histochemical colour change of respective phytochemical is more in mature stage when compared with middle and tender stage.

#### 5. Discussion

The present study revealed the presence of abundant xylem vessels in all 3 stages; presence of parenchyma and epidermal cells is observed abundantly in tender stage; and abundant spiral and helical xylem vessels, stone cells, thick wall epidermal cells and Starch grains are present in mature stage fruit powder microscopy. Histochemical studies based on intensity of colouration revealed percentage of calcium, calcium pectate, lignin, tannin, starch and polyphenols more in mature stage seed; likewise moderate percentage of calcium and calcium pectate, magnesium, polyphenols, Starch and Tannins observed in mature stage fruit.

#### 6. Conclusion

Ayurveda is one of the Ancient Indian systems of medicines which is practised throughout India. 'Rasayana' is the seventh division in Ayurveda classification, which is further classified into many subclasses; out of it 'Aahara Rasayana' is one of the sub classification. Kushmanda is one of the drug which consists dried or fresh fruit, and it is an example for aahararasayana. It can be consumed as such in raw state, or by preparing recipes, or through formulations like Kushmanda Rasayana, Vastiyamayantaka Gritha and Dhatriyadi Gritha. This fruit can be consumed daily and directly as a single drug also and it act as best rejuvenator with no side effects. Apart from this, this type of studies help to know the medicinal uses and properties of kushmanda and also helps in substantiating

the genuine Rasayana properties in three different stages and also further studies like preliminary chemical analysis, HPTLC, nutrition analysis provides uses of Rasayana drug.

#### 7. Acknowledgement

I am thankful to my beloved parents Mr. S.S. Mohan and Mrs. M.A. Padmini Mohan, for supporting & helping me in every aspect of my studies. I am thankful to my Ph.D. supervisor Dr. T.R. Shantha for guiding me throughout this work and I am thankful to Dr. Sulochana Bhat and Dr. G. Venkateswarlu for giving me the opportunity to carry out studies; at RARIMD, Bangalore.

#### 8. References

- Mishra RN. Rasayan–The Ayurvedic Perspective, Research Journal of Pharmaceutical, Biological and Chemical Sciences. 2011; 2(4):269-282.
- Folashade KO, Omoregie EH, Ochogu PA. Standardization of herbal medicines - A review, International Journal of Biodiversity and Conservation. 2012; 4(3):101-112.
- Sumitra C. Importance of pharmacognostic study of medicinal plants: An overview, Journal of Pharmacognosy and Phytochemistry. 2014; 2(5):69-73.
- Anonymous. The Ayurvedic Pharmacopoeia of India Government of India Ministry of Health and Family Welfare Department of Ayush, India. 4, 62-63.
- Anonymous. The Wealth of India: A dictionary of Indian Raw material and Industrial Products, Raw materials volume-2: B, Publications and Information Directorate, Council of Scientific & Industrial Research, New Delhi, 1988, 348-349.
- Anonymous. Quality control methods for medicinal plant materials, World Health Organization, Geneva, 1998.
- Evans WC, Trease D. Pharmacognosy, Edinburgh

- Saunders Company. 2002, 519-20.
8. Wallis TE. Text book of Pharmacognosy, New Delhi CBS Publishers and Distributors. 1985, 572-575.
  9. Krishnamurthy KV. Methods in Plant Histochemistry, S. Viswanathan Printers and Publishers Pvt Ltd, 1988.
  10. Al-Snafi AE. The pharmacological importance of *Benincasa hispida*-A review, International Journal of Pharma Sciences and Research. 2013; 4(12):165-170.
  11. Ghosh K, Baghel MS. A pharmacognostical & physiochemical study of *Benincasa hispida* with ayurvedic review, International Journal of Research in Ayurveda & Pharmacy. 2011; 2(6):1664-1668.
  12. Nadhiya K, Haripriya D, Vijayalakshmi K. Pharmacognostic and preliminary phytochemical analysis on *Benincasa hispida* fruit, Asian Journal of Pharmaceutical and Clinical Research. 2014; 7(1):98-101.
  13. Nadhiya K, Vijayalakshmi K. Evaluation of total phenol, flavonoid contents and *invitro* antioxidant activity of *Benincasa hispida* fruit extracts, International Journal of Pharmaceutical, Chemical and Biological Sciences. 2014; 4(2):332-338.
  14. Patil JK, Patel MR. Pharmacognostic and phytochemical investigation of *Benincasa hispida* (Thunb.) Cogn. Fruit, An International Journal of Pharmaceutical Sciences. 2012; 3(1):146-156.
  15. Shiromani M, Sharma PP, Pandya TN, Ravi Shankar B. Pharmacognostical evaluation of kushmanda (*Benincasa hispida* (Thumb.) Cong. Beeja, Journal of Ayurveda & Holistic Medicine. 2014; 2(8):15-18.