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[1]. 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[2,3]. Among the rasayana drugs kushmanda is a dietary drug which is distributed and cultivated in 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[4].

2. Regional names in India[5]
Hindi: Petha
Kannada: Boodagumbalakayi
Sanskrit: Kushmanda
Tamil: Pusanikayi
Telugu: Budidegummu.

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[6,7,8]

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:\cite{9}:
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](image)

<table>
<thead>
<tr>
<th>Tender stage</th>
<th>Middle stage</th>
<th>Mature stage</th>
</tr>
</thead>
<tbody>
<tr>
<td>TS of tender stage fruit showing pericarp and bundles of stone cells. 10x X 10x</td>
<td>TS of middle stage fruit showing pericarp and discontinuous layer of stone cells. 10x X 10x</td>
<td>TS of mature stage fruit showing epidermis, starch grains in hypodermis and continuous layer of stone cells. 10x X 40x</td>
</tr>
<tr>
<td>TS showing annular and spiral xylem vessels in middle stage fruit. 10x X 40x</td>
<td>TS showing group of xylem vessels in mature stage fruit. 10x X 40x</td>
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</tr>
</tbody>
</table>

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.
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 mesocarp parenchyma 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 2: Microscopical characters

<table>
<thead>
<tr>
<th>Plate 3: Powder microscopy study of 3 growing stages of B. hispida- whole fruit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tender stage</td>
</tr>
<tr>
<td><img src="image1" alt="Image" /></td>
</tr>
<tr>
<td>Single spiral xylem vessel 10x X 40x</td>
</tr>
<tr>
<td>Spiral xylem vessel 10x X 10x</td>
</tr>
<tr>
<td>Epidermal cells in surface view with stomata 10x X 40x</td>
</tr>
</tbody>
</table>
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.
Calcium pectate: cell walls and xylem. (+ +)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

Starch: present in hypodermis and mesocarp parenchyma cells. (+ +) 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

<table>
<thead>
<tr>
<th>Histochemical Analysis</th>
<th>Tender stage</th>
<th>Middle stage</th>
<th>Mature stage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium</td>
<td>Calcium: present in outer sclereides, collapsed cells layer. 10x X 40x</td>
<td>Calcium: present in outer sclereides, collapsed cells layer. 10x X 40x</td>
<td>Calcium: present in outer sclereides, collapsed cells layer and cotyledon. 10x X 10x</td>
</tr>
<tr>
<td>Calcium pectate</td>
<td>Calcium pectate: present in cell walls and stone cells. 10x X 40x</td>
<td>Calcium pectate: present in cell walls and stone cells. 10x X 40x</td>
<td>Calcium pectate: present in cell walls and stone cells. 10x X 40x</td>
</tr>
<tr>
<td>Magnesium</td>
<td>Lignin: present in outer sclerids and stone cells. 10x X 40x</td>
<td>Lignin: present in outer sclerids and stone cells. 10x X 40x</td>
<td>Lignin: present in outer sclerids and stone cells. 10x X 40x</td>
</tr>
<tr>
<td>Polyphenols</td>
<td>Starch: present in outer sclerids. 10x X 40x</td>
<td>Starch: present in collapsed cells layer. 10x X 40x</td>
<td>Starch: present in paliside of cotyledon. 10x X 40x</td>
</tr>
<tr>
<td>Starch</td>
<td>Present more in mature stage</td>
<td>Moderate in all 3 stages</td>
<td>Moderate in all 3 stages</td>
</tr>
<tr>
<td>Tannin</td>
<td>Present moderate in middle and mature stage</td>
<td>Present moderate in middle and mature stage</td>
<td>Present moderate in middle and mature stage</td>
</tr>
</tbody>
</table>
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, Vastamayantaka Gritha and Dhatryadi 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. Venkateshwarlu for giving me the opportunity to carry out studies; at RARIMD, Bangalore.

8. References

7. Evans WC, Trease D. Pharmacognosy, Edinburgh