PHARMACOGNOSTIC AND PHYTOCHEMICAL INVESTIGATION OF 
BENINCASA HISPIDA (THUNB.) COGN. FRUIT

J. K. Patil¹*, M. R. Patel²

¹Research Scholar, Shri Jagdishprasad Jhabarmal Tibrewala University, Dist. Jhunjhunu, Rajasthan – 333 001, India.
²Shree B. M. Shah College of Pharmaceutical Education & Research, Modasa, Gujarat, India.

ABSTRACT
The present study was aimed at pharmacognostic and preliminary phytochemical evaluations of Benincasa hispida Thunb. fruit belonging to family Cucurbitaceae. It is a perennial, large trailing gourd climbing by means of tendrils, cultivated in India, Burma and Ceylon. The fruits are sweet, cooling, styptic, laxative, diuretic, tonic, aphrodisiac and antiperiodic. They are useful in asthma, diabetes, haemorrhages from internal organs, epilepsy, fever and vitiated conditions of pitta. The pharmacognostic investigations were carried out in terms of organoleptic, microscopic and physical parameters. The extracts, obtained after successive Soxhlet extraction of dried fruit powder using benzene, chloroform, alcohol and water, were subjected to a preliminary phytochemical screening which revealed the presence of carbohydrates, amino acids, steroids, triterpenoids.

Keywords: Benincasa hispida, Fruit, Microscopic and physical parameters, Phytochemistry.

INTRODUCTION

Benincasa hispida (Cucurbitaceae) (Thunb.) Cogn.

Benincasa hispida also known as White gourd melon (English), Petha (Hindi)¹ is plant probably a native of Malaysia, now found throughout the tropics. It is cultivated in India, Burma and Ceylon and on the hills up to 4,000’. A perennial, large trailing gourd climbing by means of tendrils; leaves large, 10-15 cm in diameter, heart-shaped, covered with rather rough bristly, hispid beneath; flowers yellow, unisexual, male peduncle 7.5-10 cm long, female peduncle shorter; fruits broadly cylindric, 30-45 cm long, hairy throughout, ultimately covered with a waxy bloom.¹,²

The fruits are sweet, cooling, styptic, laxative, diuretic, tonic, aphrodisiac and antiperiodic. They are useful in asthma, cough, diabetes, haemoptysis, haemorrhages from internal organs, epilepsy, fever and vitiated conditions of pitta. The seeds are sweet, cooling and anthelmintic, and are useful in dry cough, fever, urethrorrhea, syphilis, and
hyperdipsia. Seeds have the principal functions of diuresis. Used in the treatments of different edema of liver and beriberi. Seed extract facilitates mucus secretion and expectorant effect, prevents gastric ulcer, histamine inhibitory effects and anti-tumor effects.

*Benincasa hispida* has shown the presence of four triterpenes and two sterols together with a flavonoid C-glycoside, an acylated glucose, and a benzyl glycoside. Seeds are mainly composed of saponins, urea, citrulline, linoleic acid, oleic acid and fatty acids and triterpenoids known as isomultiflorenol, proteins such as trigonelline, fooffeeain, and osmotin, steroids such as beta-sitosterol and stigmast-5-ene-3-beta-ol, alkaloids such as 5-methylcytosine, and triterpenoids such as cucurbitacin B. The fruit also contains mucins, mineral salts, vitamins B and C, cucurbitine, acid resin, myosin. Phytochemical studies indicate two triterpenes, alunsenol and mutiflorenol, with mast cell stabilising effects in rats. Fruit extract of *Benincasa hispida* has gastroprotective effect, and has showed protection against acetylcholine and histamine induced bronchospasm in guinea pigs. The fruit is an important source of water-soluble and hemicellulosic polysaccharides. From sarcocarp a protease has been purified by two steps of chromatography and identified that protease in a cucumisin like serine protease. Different extracts of *Benincasa hispida* has shown anti-angiogenic effect, anti-ulcerogenic effect, hypoglycemic effect, anxiolytic-like effect, antidepressant activity, nootropic activity, prevent morphine withdrawal in mice, fruit rind is anti-inflammatory.

**MATERIALS AND METHODS**

**Collection of Plant Material**

The fruit was collected from local area of Nandurbar, Maharashtra and authentificated by Dr. S. K. Tayde, Dept. of Botany, Art’s, Science and Commerce College, Shahada, Dist-Nandurbar, Maharashtra, India. The voucher specimen has been preserved in the Dept. of Pharmacognosy and Phytochemistry, College of Pharmacy, Shahada for future reference. The collected plant material was air dried under shade and used for the study of macroscopic and microscopical characters. Finally dried pieces of fruit were subjected to size reduction to get coarse powder and then passed through sieve no. 40 to get uniform
powder. Then uniform powder was subjected for the determination of ash values, extractive values, loss on drying, fluorescence analysis and phytochemical constituents.

PHARMACOGNOSTIC EVALUATION

Organoleptic Evaluation: \cite{15, 16}

In organoleptic evaluation, various sensory parameters of the plant material, such as color, odour, taste, and nature of the fruit were recorded and shown in table no. 1.

Microscopical Investigation: \cite{15, 16}

Transverse Section of *Benincasa hispida* Thunb. fruit

The transverse sections of the fruit and seed were taken to observe microscopic characteristics like epicarp, mesocarp, endocarp of fruit and testa & endosperm of seed. The characteristic are shown in microscopic pictures as fig. no. 1, 2A & 2B.

Powder Analysis

The pulverized powder of fruit was boiled with chloral hydrate solution in small quantity. To a little quantity of powder taken onto a microscopic slide, 1–2 drops of 0.1% phloroglucinol solution and a drop of concentrated hydrochloric acid were added, mounted in dilute glycerine, covered with a cover slip and observed under microscope with 10 × 10 magnification. The characteristic structures observed for the powdered fruit of *Benincasa hispida* were shown in fig. no. 3a, 3b, 3c & 3d.

PHYSICAL EVALUATION

In physical evaluation, ash values viz., total ash, acid insoluble ash and water soluble ash, and extractive values viz., alcohol soluble extractive value, water soluble extractive and ether soluble extractive values were determined.

1. **DETERMINATION OF ASH VALUES** \cite{17}

A) Determination Total Ash Value:

Accurately weighed (2 gm) of air-dried fruit powder of *Benincasa hispida* was taken in a silica dish and incinerated at a temperature not exceeding 450°C until free from carbon. The resultant ash was cooled and weighed. The percentage of ash was calculated with reference to the air-dried drug.

B) Determination of Acid Insoluble Ash:

The total ash obtained from 2 gm of fruit powder was boiled for 5 minutes with 25 ml of dilute hydrochloric acid and the insoluble matter was collected on an ashless
filter paper. It was washed with hot water, ignited and weighed. The percentage of acid insoluble ash was calculated with reference to the air-dried drug.

C) Determination of Water Soluble Ash:

The total ash obtained from 2 gm of fruit powder was boiled for 5 minutes with 25 ml of water; the insoluble matter was collected on an ashless filter paper, washed with hot water, and ignited for 15 min at a temperature not exceeding 450ºC. The weight of insoluble matter was subtracted from the weight of the ash, the difference in weight represent the water-soluble ash. The percentage of water soluble ash was calculated with reference to the air-dried drug.

The results were given in table 2.

2. EXTRACTIVE VALUES

A) Water-soluble extractive value:

Accurately weighed (5 gm) of fruit powder of *Benincasa hispida* was added to 50ml of boiled water at 80ºC in a stoppered conical flask. It was then shaken well and allowed to stand for 10 minutes so as to cool it and filtered. 5ml of filtrate was transferred to an evaporating dish, which was 7.5 cm in diameter, the solvent was evaporated on water bath, allowed to dry for 30 minutes, finally dried in an oven for 2 hours at 100ºC and residue was weighed. Percentage of water-soluble extractive was calculated with reference to the air-dried drug.

B) Alcohol soluble extractive value:

Accurately weighed (5 gm) of fruit powder of *B. hispida* was macerated with 100 ml of Alcohol in a closed conical flask, shaking frequently during the first 6 hours and allowed to stand for 18 hours. Thereafter, it was filtered rapidly taking precaution against loss of Alcohol. Evaporated 25ml of filtrate to dryness in a tared flat bottom shallow dish dried at 105ºc and weighed. Percentage Alcohol soluble extractive was calculated with reference to the air-dried drug.

C) Chloroform soluble extractive value:

Accurately weighed (5 gm) of fruit powder of *B. hispida* was macerated with 100 ml of chloroform in a closed conical flask, shaking frequently during the first 6 hours and allowed to stand for 18 hours. Thereafter, it was filtered rapidly taking precaution against loss of Chloroform. Evaporated 25ml of filtrate to dryness in a tared flat bottom shallow
dish dried at 105°C and weighed. Percentage chloroform soluble extractive was
calculated with reference to the air-dried drug.

D) Benzene soluble extractive value:

Accurately weighed (5 gm) of fruit powder of *B. hispida* was macerated with 100
ml of benzene in a closed conical flask, shaking frequently during the first 6 hours and
allowed to stand for 18 hours. Thereafter, it was filtered rapidly taking precaution against
loss of benzene. Evaporated 25ml of filtrate to dryness in a tarred flat bottom shallow
dish dried at 105°C and weighed. Percentage benzene soluble extractive was calculated
with reference to the fruit.

The results of extractive values are given in table 2.

3. LOSS ON DRYING [19]

Accurately weighed (2 gm) quantity of fruit powder was taken in a tarred glass
bottle and initial weight was taken. The sample was heated at 105°C in an oven and
weighed. This procedure was repeated until a constant weight was obtained. The moisture
content of the sample was calculated with reference to air-dried drug and the results are
in table 2.

4. FLUORESCENCE ANALYSIS [20]

The fruit of *B. hispida* (entire and powder) are examined in short and long
ultraviolet radiation to detect the fluorescent compounds and report its authenticity. The
results are given in table 2.

PRELIMINARY PHYTOCHEMICAL SCREENING [21]

The fruit powder was subjected to successive extraction in a Soxhlet apparatus
using petroleum ether (40-60°C), chloroform, alcohol and water; and the extracts were
evaporated to dryness. The dried extracts were weighed, and percentage yields were
calculated. The results are given in table 3.

The extracts were used for preliminary phytochemical screening with a battery of
chemical tests viz., Molisch’s, Fehling’s and Benedict’s tests for carbohydrates; Biuret
and Millon’s tests for proteins; Ninhydrin’s test for amino acids; Salkowski and
Liebermann-Burchard’s reactions for steroids; Borntrager’s test for anthraquinone
glycosides; foam test for saponin glycosides; Shinoda and alkaline tests for flavonoid
glycosides; Dragendorff’s, Mayer’s, Hager’s and Wagner’s tests for alkaloids; and ferric
chloride, lead acetate, potassium dichromate and dilute iodine tests for tannins and phenolics. The results are given in table 4.

RESULTS:
In the present study the fruit of *Benincasa hispida* was evaluated for its pharmacognostic, and phytochemical aspects which revealed the following results.

A. Pharmacognostic Evaluation

1. Organoleptic Evaluation: The results of organoleptic evaluations are given in table no. 1.

**TABLE 1: ORGANOLEPTIC / MACROSCOPIC CHARACTERISTICS OF FRUIT OF BENINCASA HISPIDA THUNB.**

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Parameters</th>
<th>Observation of Fruit</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Colour</td>
<td>Yellowish brown</td>
</tr>
<tr>
<td>2.</td>
<td>Odor</td>
<td>Characteristic</td>
</tr>
<tr>
<td>3.</td>
<td>Taste</td>
<td>Sweet</td>
</tr>
<tr>
<td>5.</td>
<td>Nature</td>
<td>Coarse powder</td>
</tr>
</tbody>
</table>

2. Microscopical Investigation:

(i) Transverse Sections:

![Figure 1](image_url)

**Figure 1**
Transverse section of *Benincasa hispida* fruit (pericarp)
Pericarp showing three regions,

1. Epicarp: Outermost thick coating, the outer epidermis is having cuticle & many hairs, few hypodermal layers of parenchyma & several layers lignified tissue.

2. Mesocarp: Middle layer composed of spongy parenchyma tissue & vascular strands ramify in tissue of mucilaginous mesocarp. Mesocarp constitutes maximum bulk of pericarp.

3. Endocarp: Innermost thin layer forming casing of numerous seeds.

**FIGURE 2: TRANSVERSE SECTIONS OF BENINCASA HISPIDA SEED**

![Figure 2A](image1.png)  
T. S. passing through micropyle of seed,  

![Figure 2B](image2.png)  
T. S. passing through seed wall

1. Testa: a) Multilayered epidermis: layer of lignified sclereids, layer containing scattered sclereids & mucilaginous layer, b) Sclerenchyma: one to more layers having strongly thickened walls, c) Nutrient tissue: Several layers of thin walled parenchyma.

2. Endosperm: Composed of cellulose walled parenchyma.

(ii) Powder Analysis:

**FIGURE 3: MICROSCOPIC CHARACTERISTIC OF BENINCASA HISPIDA FRUIT POWDER**
3. Physical Evaluation:

TABLE 2: PHYSICAL CONSTANTS FOR FRUIT OF BENINCASA HISPIDA THUNB.

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Physical Constants</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Ash Values</td>
<td></td>
</tr>
<tr>
<td></td>
<td>A) Total Ash</td>
<td>5.76 %w/w</td>
</tr>
<tr>
<td></td>
<td>B) Acid Insoluble Ash</td>
<td>2.88 %w/w</td>
</tr>
<tr>
<td></td>
<td>C) Water Soluble Ash</td>
<td>3.60 %w/w</td>
</tr>
<tr>
<td>2.</td>
<td>Extractive Values</td>
<td></td>
</tr>
<tr>
<td></td>
<td>A) Water soluble extractive</td>
<td>40.0 %w/w</td>
</tr>
<tr>
<td></td>
<td>B) Alcohol soluble extractive</td>
<td>14.0 %w/w</td>
</tr>
<tr>
<td></td>
<td>C) Chloroform soluble extractive</td>
<td>2.00 %w/w</td>
</tr>
<tr>
<td></td>
<td>D) Benzene soluble extractive</td>
<td>2.54 %w/w</td>
</tr>
<tr>
<td>3.</td>
<td>Loss on Drying</td>
<td>25 %w/w</td>
</tr>
<tr>
<td>4.</td>
<td>Fluorescence Analysis</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• At 254 nm (Short wave length)</td>
<td>Dark brown</td>
</tr>
<tr>
<td></td>
<td>• At 366 nm (Long wave length)</td>
<td>Bluish brown</td>
</tr>
</tbody>
</table>

4. Preliminary Phytochemical Screening:
TABLE 3: PERCENTAGE YIELD OF FRUIT OF BENINCASA HISPIDA THUNB. AFTER SOXHLET EXTRACTION

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Extract</th>
<th>% Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Benzene extract</td>
<td>1.68</td>
</tr>
<tr>
<td>2.</td>
<td>Chloroform extract</td>
<td>2.16</td>
</tr>
<tr>
<td>3.</td>
<td>Alcohol extract</td>
<td>14.40</td>
</tr>
<tr>
<td>4.</td>
<td>Aqueous extract</td>
<td>47.20</td>
</tr>
</tbody>
</table>

The preliminary phytochemical investigation revealed the presence of various phytoconstituents in each extracts of fruit; they shown the presence of carbohydrates, amino acids, steroids, and triterpenoids as shown in table 4.

TABLE 4: QUALITATIVE CHEMICAL EXAMINATION OF VARIOUS EXTRACTS OF BENINCASA HISPIDA THUNB. FRUIT

<table>
<thead>
<tr>
<th>Sl.No.</th>
<th>Name of the Test</th>
<th>Aqueous Extract</th>
<th>Alcoholic Extract</th>
<th>Chloroform Extract</th>
<th>Benzene Extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>For Carbohydrates</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a)</td>
<td>Molisch’s test</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>b)</td>
<td>Fehling’s test</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>c)</td>
<td>Benedict’s test</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2.</td>
<td>For Proteins</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a)</td>
<td>Biuret test</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>b)</td>
<td>Millons test</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3.</td>
<td>For Amino acids</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a)</td>
<td>Ninhydrin test</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4.</td>
<td>For Alkaloids</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>b)</td>
<td>Dragendroff’s test</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>c)</td>
<td>Mayer’s test</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>d)</td>
<td>Hager’s test</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>e)</td>
<td>Wagner’s test</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5.</td>
<td>For Sterols</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a)</td>
<td>Salkowsky test</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>b)</td>
<td>Libermann reaction</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6.</td>
<td>For Phenolics &amp; Tannins</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a)</td>
<td>Ferric chloride test</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>b)</td>
<td>Lead acetate test</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>c)</td>
<td>Dil HNO₃ test</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>7.</td>
<td>For Triterpenoids</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a)</td>
<td>Libermann Burchard’s Test</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>b)</td>
<td>Salkowskasi test</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>8.</td>
<td>For Flavonoid glycosides</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
9. For Saponins
   a) Haemolytic test - - - -
   b) Foam test - - - -

   Shinoda test
   Alkaline test

‘+’ – Positive, ‘-’ - Negative

DISCUSSION:

Benincasa hispida is extensively used in the traditional system of medicine for the
treatment of number of ailments. The results of these investigations could serve as a basis
for proper identification, collection and investigation of the plant. The parameters
determined in quantitative microscopy can be useful to differentiate closely related
species. The physical constants are important parameters in detecting and/or improper
handling of drugs. Presence of various phytoconstituents can serve to treat diseases by
using various pharmacological activities. Physical standards may be used to determine
the quality this plant material in future investigation. It will also be of immense use in
carrying out further research and revalidation of its use in traditional system of medicine.

ACKNOWLEDGEMENT

Authors are thankful to P. S. G. V. P. Mandal’s College of Pharmacy, Shahada (M.S.) for
providing all necessary facilities to carry out this research work and to Dr. S. K. Tayde
for authentification of plant.

REFERENCES:

1. Arya Vaidya Sala. Indian Medicinal Plants a Compendium of 500 species. Orient
2. The Wealth of India. A dictionary of Indian Raw Materials and Industrial
3. Keyon HL, Hye RC and Chang HK: Anti-angiogenic effect of the seed extract of
   prevents development of experimental ulcers. Journal of Ethnopharmacology
   2001; 78(2-3): 159-164.
7. http://www.medind.nic.in


For Correspondence:
J. K. Patil
Email: javesh4u@gmail.com