A PHARMACOGNOSTICAL & PHYSIOCHEMICAL STUDY OF BENINCASA HISPIDA WITH
AYURVEDIC REVIEW
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ABSTRACT
Benincasa hispida (Thunb.) of Cucurbitaceae family commonly known as Kushmanda, Winter melon, Wax gourd, is used in Ayurvedic system of medicine. It is cultivated throughout the plains of India and on the hills up to 1200 m altitude, as a vegetable. The Fruits of Kushmanda are considered as Diuretic (mutral), Aphrodisiac (vrisyha), Appetizer (dipana); used in Acid reflux syndrome(Amlapitta), Purpura (Raktapitta), Emaciation (Kshaya), Mental disorder (Chetovikara), etc. In the present study fruit of Benincasa hispida was authenticated Pharmacognostically in the department of Pharmacognosy, I.P.G.T. & R.A., Gujarat Ayurveda University of Pharmacy, Jamnagar, Gujarat, India. Each and every drug has its own physical and chemical characteristics that help for separating it from other closely related drugs. Hence physicochemical studies of a particular drug by making use of various parameters help in standardizing the drug and validate it. Chromatographic techniques were adopted for the separation of active principles present in the fruit powder. Therefore, an attempt has been made to standardize Kushmanda powder, an Ayurvedic medicine based on the TLC and HPTLC fingerprint profile.

KEY WORDS: Benincasa hispida, Cucurbitaceae, Kushmanda, Phytochemical Analysis, Chromatography, Ayurveda.

INTRODUCTION
Benincasa hispida is an extensive trailing or climbing annual herb cultivated throughout the plains of India and on the hills up to 1200 m altitude, as a vegetable. The genus name was given to it by famous Italian botanist, Gaetano Savi, in 1818 to honor Giuseppe Benincasa, an Italian patron of botany. He called the plant as Benincasa cerifera " wax bearing", Hispida means " rough hairy" as whole parts of plant contains rough hair1. By maturity, the fruit loses its hairs and develops a waxy coating, giving rise to the name wax gourd, and providing a long shelf life. The melon may grow as large as 80 cm in length. Although the fruit is referred to as a "melon," the fully grown crop is not sweet. Originally cultivated in South-East Asia, the winter melon is now widely grown in East Asia and South Asia as well. Winter melon is also a common name for members of the Inodorus cultivar group of the muskmelon (Cucumis melo L), more commonly known as casaba or honeydew melons. It is also called as Ash gourd, Green pumpkin, White gourd2. As per Ayurveda classics the herb is known as Kushmanda3-6 (Ku nasti ushma andesu bijesu yasya sa), Pushpaphala7,8, Brihatphala5, Pitapushpaha6. In the description of Shodasakala Chikitsa (Sixteen Pre-requisite Qualities), Ayurvedic classics have given special importance to the physician's ability9 to understand the rationale behind the utility of natural compounds in modifying the disease conditions and in re-establishing the equilibrium of Doshas (Humour), Dhatu (Tissues) and Malas (Waste Products) in other words, maintaining the Health.

In the description of Chikitsa Chatuspada (Four Limbs of Treatment), Drugs have the second most importance in treating the diseases and also the drug should possess the good qualities in it10. So, proper identification and standardization of the drug is essential. Each and every drug has its own physical and chemical characteristics that help for separating it from other closely related drugs. Hence physicochemical studies of a particular drug by making use of various parameters help in standardizing the drug and validate it. Chromatographic techniques were adopted for the separation of active moieties present in the fruit powder. Therefore, an attempt has been made to standardize Kushmanda powder, an Ayurvedic medicine based on the TLC and HPTLC fingerprint profile.

Scientific Classification
Kingdom : Plantae (unranked): Angiosperms (unranked): Eudicots (unranked): Rosids
Order : Cucurbitales
Family : Cucurbitaceae
Subfamily : Cucurbitoideae
Tribe : Benincasae
Subtribe : Benincasinae
Genus : Benincasa
Species : B. hispida
Binomial name2: Benincasa hispida (Thunb.) Cogn.

Vernacular name
Sanskrit : Kusmanda, Brihatphala, Pushpaphala, Pitapushpa, etc.
Hindi : Petha, Pethakaddu

Global Distribution
Plant probably a native of Malaysia, but is now found throughout tropics. It is cultivated for its fruits throughout the plain of India, Burma and Ceylan, and on hills up to 4000ft. It is very well known vegetable in India, China, Philippines, Thailand, Vietnam and elsewhere in Asia14. In plains seeds are sown during February-March and on hills in March-May.

Significance List
Nationally endangered (seriously), formerly it was used as container for oil10.

Propagation & Cultivation
Crop can be grown in plains in late winter by adopting poly house technology. It involves raising the seedlings during Dec-Jan in small polythene bags. Seedlings having 3-4 true leaves should be transplanted in the pits in 1st week of Feb when danger of frost is over. The optimum temperature when growing is 73 -82°F and moderate rainfall but no hot, humid tropics. It requires long growing season of 5 months to develop to maturity, though they can be harvested at different immature stages. Wax gourds are drought tolerant. Where large fruits are wanted they are grown on flat grounds otherwise they are allowed to climb on fences. Cultivated largely in Uttar Pradesh, West Bengal, Punjab, Rajasthan, and Bihar11.

Parts Used - Fruit, Seed & Seed oil12.
Aims & Objectives
2. Physico-chemical analysis of powdered Kushmanda.

MATERIALS & METHODS
The fresh fruit of Benincasa hispida was collected in the month of August, 2011 from the local vegetable market of Jamnagar, Gujarat. The authentication & macroscopic study of the plant were done in the department of Pharmacognosy, I.P.G.T. & R.A., Gujarat Ayurveda University of Pharmacy, Jamnagar, Gujarat, India. The fruits were dried, seeds were separated, powdered and sieved through # 60 and fine powder was collected. The powder was subjected to powder microscopy.

Pharmacognostical Study
Macroscopic - Stem - Stem is much branched, 5-sided, thick, furrowed and covered with sharp bristles. Tendrils - split at 3cm into 2-3, each to 15cm, are slender, rarely simple. It sprawl over ground or climb like pumpkin. Leaves – large, roundish, kidney-shaped, base is deeply heart-shaped. Upper surface is rough, lower surface shortly bristly, blade 10-25 cm long and as much broad, 5-7-lobed, lobes ovate-triangular, margin sinuate or toothed alternate; edge scalloped into teeth; stalk is 20cm long with sharp bristles. Flowers - solitary, sexes separate, yellow, flat-faced. Male flowers are 5-15 cm long with 3 stamens of 1cm length; female flowers are 2-4 cm long and style dividing into 3. Calyx tube is 10-15 mm long, densely hairy, lobes are lanceolate, acute, 6-12 mm long. Petals are spreading, blunt but ending in a short point, 3-5 x 2-4 cm, 5 in number and oval. Filaments of the stamens are inflated and hairy at the base, anthers tri lobbed (Figure 1). Flowering period – July to September. Fruit - spherical, hairy & fuzzy when young, ripens dark blue depending on the variety. The flesh is white and the fruit is spherical, to round with flattened ends or just like ‘Chinese pillow shape’. Broadly cylindrical, 30-45 cm long, hairy throughout 10 weighing around 11 kg and 30 – 38 cm long (Figure 2).

Microscopic - Mature fruit shows cuticularised epicarp consisting of single layered, square or slightly tangentially elongated cells of epidermis, outer tangential walls of epidermis thickened and cuticularised; a few epidermal cells divide peripherally and become 2 or 3 layered; mesocarp has a heterogeneous structure consisting of multilayered hypodermis composed of tangentially elongated, thin-walled, parenchymatous cells; immediately within this is a zone of thick-walled, multilayered, lignified sclerides with the outer one to three layers thicker than the inner 2 to 6 or more layers; beneath this zone, thin walled tangentially elongated, parenchymatous cells present, their size gradually increasing from those at periphery to those inside of mesocarp, the latter becoming circular having conspicuous intercellular spaces; vascular bundles poorly developed, bi-collateral, found scattered throughout mesocarp.

Powder microscopy – The fruit was dried, seeds were separated and powdered and passed to 60 mesh. The dried powder was mounted in the distilled water to detect the numerous fragments of thin-walled, tangentially elongated and circular loosely arranged parenchymatous cells (Figure 3). Parenchyma with starch grains (Figure 4). Prismatic crystal (Figure 5) and numerous sclerides in groups and singles and a few fragments of xylem vessels having spiral thickenings. After staining with Phloroglucinol & conc. HCl, powder microscopy shows Lignified parenchyma cells (Figure 6).

PHYSICOCHEMICAL STUDY
Organoleptic characters – The Organoleptic characters are tabulated in Table 1

Physicochemical parameters: The powders were evaluated for physicochemical parameters like Foreign matter, Total Ash Value, Acid soluble and water-soluble extractive values and Water-soluble extractive. The results were placed at Table 2

Thin Layer Chromatography - Preliminary thin layer chromatography (TLC) study was carried out for checking the presence of phytoconstituents as beta-sitosterol, lupeol, iso-vitexin, isomultifloreinol, cucurbitacin-B, different amino acids, etc. which have been reported to be present in the fruit of Benincasa hispida.

Extraction procedure
The powder of fruit (500 g) was first defatted with petroleum ether (500 ml × 6; yield was 1% w/w). The defatted powder was then dried and extracted with methanol (500 ml × 6; yield was 20% w/w) and thereafter with ethyl acetate (500 ml × 4; yield was 3% w/w) successively. The remaining powder was dried and extracted with distilled water to give aqueous extract (500 ml × 6; yield was 5% w/w).

TLC is mentioned as a primary tool for identification as part of monographs on all medicinal plants. Alkaloid fraction was used for the spotting of the TLC plate (Silica gel G Pre-coated plates). Then the spotted TLC was run with the solvent systems [Benzone (9 ml) & Ethyl acetate (1 ml)] separately. And the resulting TLC pattern was viewed under long wave ultra violet light at 366 nm or Short wave ultra violet light at 254 nm. Then after spraying with the Vanillin-Sulphuric acid reagents and drying in a hot air oven and the number of spots viewed under daylights. TLC of alcoholic extract of drug on silica gel G Plate using Benzene (9 ml): Ethyl acetate (1 ml): shows two fluorescent zones Under 366 nm U.V. at Rf.0.71 and 0.79 (both violet). On exposure to Iodine vapor eight spots appear at Rf. 0.07, 0.18, 0.28, 0.40, 0.50, 0.59, 0.71 and 0.79 (all yellow). On spraying with Vanillin-Sulphuric acid reagent and heating the plate at 105°C for ten minute six spots appear at Rf. 0.07, 0.18, 0.40, 0.50, 071 and 0.79 (all violet). The results were placed at Table no. 3 & 4 respectively.

High Pressure Thin Layer Chromatography (HPTLC): Further, quantitative estimation of phytoconstituents using high pressure thin layer chromatography (HPTLC) fingerprinting was also carried out for both the extracts using RP-18 silica, bried as follows:
1. Betasitosterol in petroleum ether extract (PEBHI) - Petroleum ether: Acetonitrile: Methanol (1:2:2)
2. Lupeol in PEBHI - Benzene: Ethyl acetate (9.5: 0.5)
3. Iso-vitexin in methanol extract (MEBHI) - Ethyl acetate: n-butanol: Water (2:1:3)

Phytochemistry
Major compound identified are1; (E) – 2- hexenal, n – hexenal, n-hexyl formate is powerful green fruity, pungent odour n-hexanal is powerful, penetrating fatty green, grassy odour and n- hexyl formate is strongly penetrating, ethered fruity odor. It contains 0.5gm glucose & 0.5 gm fructose/ 100gm but no sucrose. However, 2,5-dimethylpyrazine, 2,6-dimethylpyrazine, 2,3,5-trimethylpyrazine, 2- methylpyrazine, and 2-ethyl-5-methylpyrazine were the major compounds in the wax gourd beverage. Roots – Pentacyclic triterpene, Bynonic acid. Fruits - Lupeol, β- sitosterol & their acetates, Cucurbitin, Rhamnose, Mannitol, Triaccontal, alkakali, fat, vitamin, glucose, adenine, trigonelline, histidine. The waxy layer of fruit contains Pentacyclic triterpene isomultifloreol acetate. The aroma of fruit & beverage differs because former contains hexanal compound & latter Pyrazine compound. Seeds – 24z – ethylidene cholesterol- 7 enol (Avenasterol) & 24β- ethyl cholesterol – 7,25
PHARMACOLOGICAL / BIOLOGICAL ACTIVITIES

• Anti-Ulcer: Extracts of Kushmanda prevent development of experimental ulcers. In Ayurveda a study showed extracts of Kushmanda may be a natural drug with anti-ulcer activity.11

• Anti-angiogenic Effect: Study showed the seed extract of Kushmanda decreased bFGF-induced endothelial cell proliferation and tube formation in a dose-dependent manner. It showed no cytotoxicity and showed potent inhibitory effect on bFGF-induced angiogenesis in vivo. Seed extract of Kushmanda supports its anti-angiogenic property through inhibition of endothelial cell proliferation.11

• Gastroprotective / Anti-Ulcer / Antioxidant: (1) Study results were comparable with the omeprazole treated group. Study suggested Kushmanda possesses significant antiulcer as well as antioxidant property. (2) Study showed decrease in ulcer index in animals treated with fruit extract of Kushmanda, showed the antioxidant property of oxidation treatment of morphine withdrawal. Results suggest a potential for Kushmanda activity histamine in guinea pigs: The ME of BH showed excellent protection against histamine-induced bronchospasam probably through an antihistamine activity (H1 receptor-antagonism).11

• Opioid Withdrawal Benefit: Study showed the juice of Kushmanda showed significant activity against symptoms of morphine withdrawal. Results suggest a potential for Kushmanda in preventing the development of morphine addiction and suppression of Opioid withdrawal in animals.15

• Antipyretic: Study results indicate that the ethanol extract of Kushmanda possesses potent antipyretic effects and pharmacologically justifies its folkloric use for fever and pain conditions.11

• Anti-diarrheal: Study showed the methanol extract of fruit of Kushmanda showed significant inhibitory activity against castor oil-induced diarrhea and inhibited PGE2 induced enteric pooling in rats. Results establish its efficacy as an anti-diarrheal agent.14

• Antioxidant / Alzheimer's disease: Results revealed chronic treatment of Kushmanda pulp extract markedly decreased lipid per oxidation level, significantly increased superoxide dismutase, CAT and reduced glutathione level in different parts of the brain. Study showed the antioxidant property of Kushmanda may be beneficial in the management of colchicines-induced rat model of Alzheimer's disease.11

• Anorectic / Potential Anti-Obesity Benefit: Study investigated the anorectic effect of the methanol extract of Kushmanda in Swiss albino mice. Results reveal, for the first time, a possible anorectic activity of Kushmanda probably through CNS mediation, with no effect on gastric emptying. Further studies are suggested for its anti-obesity potential.11

CONCEPTUAL STUDY

Description of Kushmanda as per Brihatrayee - Bagbhat described that Kushmanda is the best fruit among all valliphasa.14 Acharya Charaka and Sushruta have described Kushmanda in Shakhavarga12,5,7. Rasa, Gun, Virya, Vipaka, Prabhava, Doshaghnata and Action are tabulated in Table 5 & Table 6 respectively. Acharya Charaka has also mentioned another species of Kushmanda named Karkaruka.18 Sushruta has described Kushmanda under madhura varga also.19

Description of Kushmanda as per Bhavaprakasha nighantu: Rasa, Gun, Virya, Vipaka, Doshaghnata and Action of Kushmanda are tabulated in Table 7.

Preparatory Classical medicine of Kushmanda with indication

1. Kushmanda avaleha: Raktapitta(purpa), Kshaya(consumption), Jvara(Fever), Shosa(emaciation), Trishna(thirst), Bhirama(vertigo), Shvasa(respiratory disease), Kasa (cough), Kshata(ulcer in lungs), Urahsandhanakrid, Vishrya(aphrodisiac), Brimhana(nutrient), Balakrit(Tonic).

2. Vasakhandha Kushmandaka avaleha: Shvasa(respiratory disease), Kasa(cough), Kshaya(consumption), Hikka(hiccup), Raktapitta(purpa), Amlapitta(Acid reflex syndrome), Pinasa(Rhinitis), Halmaka.

3. Kushmanda rasayana: Jvara(Fever), Shvasa(respiratory disease), Kasa(cough), Kshaya(consumption), Kshata(ulcer in lungs), Urahsandhanajana, Medha-Smriti-Balakrit(memory booster & tonic).

4. Kushmanda Khandha: Amlapitta(Acid reflux syndrome)

Previous Research Works On Kushmanda In Ayurveda


2. Dev Dhanya R - Comparative experimental study of two varieties of Kushmanda (Benindasa hispida (Thumb) with special reference to its Asmareeghna (Lithotripric) property, Kerala University, Thrivunanathapuram, 2005.

3. Arasi Senth - A Randomized controlled trial on the effect of Kushmanda avaleha in Malnutrition in preschool children, Kerala University, Thrivunanathapuram, 2005.


CONCLUSION

The plant Benincasa hispida used from the ancient time for its medicinal values and many of the Ayurvedic formulations prescribed for various diseases have Kushmanda as one of the ingredients. The plant Benincasa hispida was identified and authenticated phamacognostically and was used as a unique ingredient. The formulation namely, Kushmanda powder was subjected to phytochemical, physicochemical, TLC and HPTLC studies. It is inferred that the formulation meets the minimum qualitative standards as reported in the API at a preliminary level. Through Ayurvedic review it is revealed that Kushmanda is effective in Chetovikara (mental diseases), Panatayya (alcoholism), Amlapitta (Acid reflux syndrome, etc) and also acts as Dipana (appetizer), Bhedana (purgative), Mutrala (diuretic), Vridhva19 (aphrodisiac) & Vastishodhana (bladder purifier). All these activities have been proved scientifically through pharmacological studies. This study may be used as reference standard in the further quality control researches. Further studies may be carried out based on identification and separation of active ingredients with the help of Biomarkers.

REFERENCES

Table 2: shows Physico-chemical Parameters of Kushmanda powder

<table>
<thead>
<tr>
<th>SL. No.</th>
<th>Parameters</th>
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<tbody>
<tr>
<td>1</td>
<td>Rasa</td>
<td>Madhura</td>
</tr>
<tr>
<td>2</td>
<td>Guru</td>
<td>Madhura</td>
</tr>
<tr>
<td>3</td>
<td>Vriddha</td>
<td>Madhura</td>
</tr>
<tr>
<td>4</td>
<td>Vipaka</td>
<td>Madhura</td>
</tr>
<tr>
<td>5</td>
<td>Doshaghanta</td>
<td>Madhura</td>
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Table 3: shows TLC of Methanol Extract of Kushmanda powder

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<tr>
<th>Extract</th>
<th>Solvent System</th>
<th>Wavelength</th>
<th>No. of spots</th>
<th>RF value</th>
<th>Observation under UV light</th>
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<td>2</td>
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Table 4: shows TLC - After spraying with Vanillin-Sulphuric acid

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<th>Solvent</th>
<th>No. of spots</th>
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Table 5: shows Rasa, Gunā, Virya, Vipaka, Doshaghanta of Kushmanda

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<tr>
<th>Property</th>
<th>Charaka Samhita⁴</th>
<th>Sushruta Samhita⁶</th>
<th>Ashtanga Hridaya¹⁷</th>
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<td>Rasa</td>
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<td>Gunā</td>
<td>Pakva – Laghu, Kshara yukta</td>
<td>Pakva – Laghu, Udsina,</td>
<td>Guru</td>
</tr>
<tr>
<td>Virya</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Vipaka</td>
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<td>Madhura</td>
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Table 6: shows Action of Kushmanda

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<th>Sushruta Samhita⁶</th>
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Table 7: shows Rasa, Gunā, Virya, Vipaka, Doshaghanta & Action of Kushmanda

<table>
<thead>
<tr>
<th>Property</th>
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<tr>
<td>Rasa</td>
<td>Vridhā - Svadu</td>
</tr>
<tr>
<td>Gunā</td>
<td>Bala (innature) - Shita</td>
</tr>
<tr>
<td>Virya</td>
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<tr>
<td>Vipaka</td>
<td>-</td>
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<tr>
<td>Doshaghanta</td>
<td>Bala - Pittapaha</td>
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Fig 1: Male & female flower of the plant *Benincasa hispida*

Fig 2: Fruit of *Benincasa hispida*

Fig 3: Loosely arranged parenchyma cells

Fig 4: Parenchyma with starch grains

Fig 5: Prismatic crystal

Fig 6: Lignified parenchyma cells

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