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Research Article

PHARMACOGNOSTIC AND PRELIMINARY PHYTOCHEMICAL ANALYSIS ON *Benincasa hispida* FRUIT

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ABSTRACT

Aim: To study the plant drug formulation that has medicinal property due to the presents of secondary metabolites with fewer side effects and less expensive.

Objective: The present study was designed to investigate the Pharmacognostic, preliminary phytochemical studies and fluorescence analysis of powder sample of *Benincasa hispida* fruit.

Method: The preliminary phytochemical analysis of *Benincasa hispida* was done using extracts like Ethyl acetate, Ethanol, Hydro alcohol and Aqueous. Microscopic analysis of *Benincasa hispida* fruit was done using Toluidine Blue staining reagent. Ash value, extractive value and loss on drying, powder analysis with chemical agents and fluorescence analysis of powder sample were performed.

Result: Pharmacognostic study with Toluidine blue staining reagent shows Parenchyma, Vascular strands, Xylem, Phloem, ground tissue and crystals in transverse section of fruit. The preliminary phytochemicals studies revealed the presence of carbohydrates, Phenols, alkaloids, glycosides, flavonoids, tannins, terpenoids and saponins. Total ash is 6.89%, Acid insoluble ash 2.35%, Water soluble ash 3.12%, sulphated ash 5.42% and water soluble extract value is 45.4%, Hydro alcohol soluble extract value is 7.14, Ethanol soluble extract value is 12.58 and Ethyl acetate extract value is 6.6.

Conclusion:

Study reveals that phytochemicals present in the extract may have beneficial effects on many pathological conditions.

Keywords: *Benincasa hispida*, Macroscopic, Microscopic, Physiochemical and Phytochemical Parameters.

INTRODUCTION

The traditional Indian system mentions herbal remedy for the several of the diseases. Herbal medicines are promising choice over modern synthetic drugs. They show minimum side effects [1]. Plants contain important phytochemicals like alkaloids, flavonoids, tannins, glycosides, phenolic compounds and saponins [2]. Polyphenols have high free radical scavenging activity, which helps to reduce the side effects of diseases like neurodegenerative diseases, cardiovascular, cancer, liver cirrhosis and hepatitis [3]. Tannins exhibit significant inhibitory effect on pancreatic lipase activity and fat absorption from the intestine [4].

Benincasa hispida belong to cucurbitaceae family. *Benincasa hispida* is a tendril climber which is cultivated throughout India. *Benincasa hispida* is commonly known as kolu or safedkolu (Gujarati), Petha (Hindi), white pumpkin or wax gourd or ash gourd (English) and Kushmanda(Sanskrit). The fruit is a large fleshy pepo. It consists of a thin skin of epidermis fleshy and juicy mesocarp and swollen, thick placenta. The fruit is tricarpellary syncarpous with peripheral placentation. Fruit of this plant are traditionally used to treat a renal diseases, jaundice, dyspepsia, fever and menstrual disorders. The methanolic extract of the fruit is reported to possess antiulcer [5], anti-inflammatory [6], antihistaminic, and antidepressant activities [7]. Fruits of *Benincasa hispida* are traditionally used for treatment of epilepsy and other nervous diseases [8]. The present study was designed to investigate the Pharmacognostic, preliminary phytochemical studies and fluorescence analysis of *Benincasa hispida* fruit which is an important medicinal plant in the Indian system of medicine.

MATERIALS AND METHODS

Collection of plant materials

Plant specimen for the proposed study was collected from medicinal plant vendor. Care was taken to select healthy fruits. The fruit was authenticated by Dr. P. Jayaraman, Director of National Institute of Herbal science, Plant Anatomy Research Center, Chennai. The fruit specimen was used for the studies.

Macroscopic analysis

The characters of the fruit such as color, odour, taste, nature, texture were studied for morphological investigation.

Microscopic analysis

The fruit was fixed in FAA (Formalin-5ml+ Acetic acid-5ml + 70% Ethyl alcohol-90ml). After 24 hrs of fixing, the specimens were dehydrated with graded series of Tertiary –Butyl alcohol (TBA) as per the schedule given by [9]. For anatomical studies customary technique of microtome was followed [10]. Infiltration of the specimens was carried by gradual addition of paraffin wax (melting point 58-60 C) until TBA solution attained super saturation. The specimens were cast into paraffin blocks and sliced (Transverse section). Paraffin sections of 10-12µm thick were stained with Toluidine blue as per the method published by [11]. The dye rendered pink colour to the cellulose walls, blue to the lignified cells, dark green to suberin, violet to the mucilage and blue to the protein bodies. Different cell components were observed under microscope.

Photomicrographs

Microphotographs of section at different magnifications were taken with Nikon labphoto 2 microscopic Unit. For the study of crystals, starch grains and lignified cells, polarized light was employed. Descriptive terms of the anatomical features are as given in the standard Anatomy books [12].

Powder microscopy

Shade dried fruits were powdered with the help of an electric grinder till a fine powder was obtained. This fine powder of fruit was subjected to powder microscopy as per standard procedure [13].

Physical Evaluation

The ash values, extractive values and loss on drying were performed according to the methods prescribed in Indian pharmacopeia [14].

Preparation of extract for phytochemical study

The fruit was peeled off and seeds were removed. The pulp were taken and cut into pieces, dried and then ground to powder form. The different extracts from the dried powder was extracted with solvents like ethyl acetate, ethanol, hydroalcohol, and aqueous using soxhlet apparatus. All the extracts were stored in a glass bottle and refrigerated throughout the period of experiment.

Phytochemical analysis (qualitative)

Phytochemical analysis was carried out with Ethylacetate, ethanol, hydroalcohol and aqueous extract to identify the constituents using standard procedures as described by Sofowara, Trease and Harborne [15,16,17].

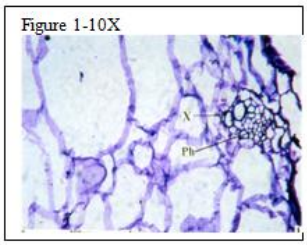
RESULTS

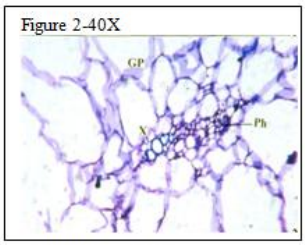
In the present study the fruit of *Benincasa hispida* was evaluated for pharmacognostic and phytochemical analysis which revealed the following results.

Microscopic investigation

Anatomy of the fruit

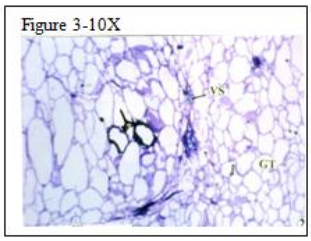
The fruit is a large fleshy pepo. It consists of a thin skin of epidermis, fleshly and juicy mesocarp and swollen, thick placenta. The fruit is tricarpellary syncarpous and has peripheral placentation.

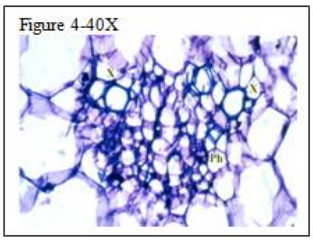




Legends: GP-Ground parenchyma; Ph-Phloem; X-Xylem

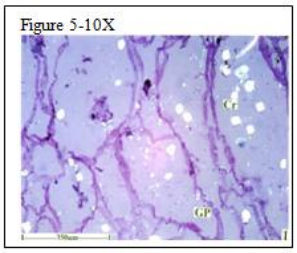
Fig 1 & 2: Transverse section of fruit showing ground parenchyma and vascular strand.

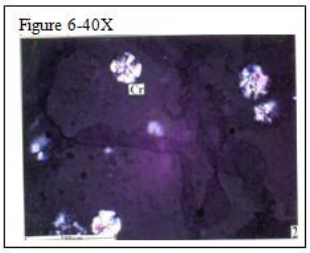




Legends: GT-Ground Tissue; Ph-Phloem; VS- Vascular strand; X-Xylem

Fig 3 & 4: Vascular strand enlarged





Legends: Cr-Crystals; GP-Ground parenchyma

Fig 5 &6: Distribution of calcium oxalate crystals.

The fruit consists of homogeneous parenchymatous tissue which was large, thin walled compact and the cells were variable shape and size (Figure 1) Scattered in the ground tissue are vascular strand which consists of a few xylem elements and fairly more number of phloem elements. The xylem and phloem are arranged in collateral position as shown in (Figure 2).

The xylem elements are thick walled and wide (Figure 3). These are so large, thick walled secretary cells, sparely seen in the ground tissue (Figure 4). Calcium oxalate crystals were observed in the ground tissue. They are diffuse in distribution and it was 30µm in diameter. (Figure 5 & 6).

Macroscopic characteristics

Macroscopic characteristics of *Benincasa hispida* fruit is shown in Table 1.

Table1: Macroscopic characteristics of *Benincasa hispida* fruit.

S. No.	Parameters	Observation of fruit
1.	Color	Yellowish white
2.	Odour	Characteristic
3.	Taste	Sweet
4.	Nature	Coarse powder

Powder Microscopy with chemical reagents

Characteristics colour changes of the fruit power are mainly used to identify the drugs present in it. The colour changes due to chemical reaction are shown in Table 2 & 3.

Table 2: Powder analysis with chemical reagents

S. No.	Reagents	Color observed
1	Powder as such	Pale yellow
2	Powder +Concentrated HNO_3	Brown
3	Powder +Concentrated H_2So_4	Brownish black
4	Powder +Concentrated Hcl	Brown
5	Powder +Glacial acetic acid	Brown
6	Powder +5% NaOH solution	Yellowish brown
7	Powder +5% KOH solution	Yellowish brown
8	Powder +5% Ferric chloride solution	Yellowish brown
9	Powder +Picric acid	Yellowish green
10	Powder+Ammonia	Yellow

Table 3: Fluorescence analysis of powdered sample

S. No.	Reagents	Color observed
1	Powder as such	Pale yellow
2	Powder +1N NaOH in methanol	Yellow
3	Powder +1N NaOH in water	Yellow
4	Powder +50% Hcl	Light brown
5	Powder +50% H_2So_4	Blackish brown
6	Powder +50% HNO_3	Yellowish brown
7	Powder +Petroleum ether	Brown
8	Powder + Chloroform	Brownish yellow
9	Powder +Picric acid	Yellowish green
10	Powder+5% Ferric chloride solution	Yellowish green
11	Powder+ 5% Iodine solution	Light green
12	Powder+ Methanol	Brown
13	Powder+(HNo_3+NH_3)	Brown

Physical evaluation

The loss on drying, ash values and extractive values of various solvents is shown in Table 4.

Table 4: Physical evaluation parameters

S.No	Parameter	Value (%)(W/W)
1.	Loss on Drying	25%
2.	Ash value	
	a. Total ash	6.89%
	b. Acid insoluble ash	2.35%
	c. Water soluble ash	3.12%
	d. Sulphated ash	5.42%
3.	Extractive values	
	a. Water soluble extract	45.4%
	b. Hydro alcohol soluble extract	7.14%
	c. Ethanol soluble extract	12.58%
	d. Ethyl acetate soluble extract	6.6%

Phytochemical Screening

The different extracts of *Benincasa hispida* were subjected to phytochemical screening. The extracts were found to contain carbohydrates, Phenols, alkaloids, glycosides, terpenoids, flavonoids, tannins and saponins as shown in Table 5.

Table 5: Qualitative Phytochemical examination of various extracts of *Benincasa hispida* fruit

S. No	Compounds	Ethyl acetate extract	Ethanolic extract	Hydro alcohol extract	Aqueous extract
1.	Flavonoids	+	+++	+++	+++
2.	Terpenoids	+	+++	+++	+++
3.	Tannins	-	++	++	++
4.	Anthocyanin & Betacyanin	-	+++	+++	+++
5.	Phenols	++	+++	+++	++ +
6.	Cardiac glycosides	-	+	+	+
7.	Amino acids	++	+++	++	+
8.	Reducing sugar	+	+++	+	++
9.	Sterols	++	+++	+++	+++
10.	Phytosterols	+	+++	+++	+++
11.	Saponin	++	+++	+++	+++
12.	Carbohydrate	+++	+++	+++	+++
13.	Coumarin	-	++	+++	+++
14	Alkaloids	+	++	+	++

Legends: +++ -Highly present; ++ -Moderately present; + - Present; --Absent

DISCUSSION

A variety of herbs and herbal extracts contain different phytochemicals with biological activity that can be of therapeutic value. Different phytochemicals have been found to possess a wide range of activities, which may help in protection against chronic diseases. Phytochemicals screening of ethyl acetate, ethanol, hydroalcohol and aqueous extracts of *Benincasa hispida* fruit revealed that the crude extracts contain alkaloids, aminoacids, flavonoids, glycosides, phytosterols, saponins, steroids, tannins and triterpenoids. Flavonoids are polyphenols naturally present in all plant materials [18]. Flavonoids have high antioxidant potential which attributes to scavenge harmful reactive oxygen species (ROS) [19]. Phenolic compounds are effective hydrogen donors, and this makes them good antioxidants [20]. Secondary metabolites like tannins, flavonoids and steroids showed antibacterial activities [21][22]. Saponins in diet of rats have shown to decrease the plasma cholesterol and increase the bile acid production. Cardiac glycosides influence the sodium and potassium ion movement of cardiac membrane. It also inhibits ATP-ase activity which regulates the sodium potassium pump [23]. Tannins also exhibit strong antibacterial, antiulcer, anti-inflammatory, anti-leishmanial, antimutagenic, enzyme regulating, blocking signal transduction pathways and apoptotic activities. [24]. Alkaloids are one of the diverse groups of secondary metabolites which are found to have antimicrobial activity by inhibiting DNA topoisomerase in the micro organisms [25]. Phytochemical analysis of *Benincasa hispida* shows the presence of flavonoids, Saponins, terpenoids, phenolic compounds and alkaloids which may act as better antioxidants and have beneficial effects on many pathological conditions.

CONCLUSION

Medicinal plants are the local heritage with the global importance. World is endowed with a rich wealth of medicinal plants. The transverse section of the fruit of *Benincasa hispida* shows the presence of phloem, xylem, ground tissue, ground parenchyma, vascular strand and calcium crystals. Phytochemical analysis of *Benincasa hispida* shows the presence of tannins, saponins, terpenoids, phenolic compounds and alkaloids in all the extracts. Compared to other extracts ethanolic extract showed more number of phytochemical constituents. The medicinal properties of *Benincasa hispida* extracts may be due to the presence of above mentioned phytochemicals. Further studies are in progress to identify the active components in *Benincasa hispida* and to elucidate the antioxidant potential.

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No conflict of interest.

REFERENCES

1. Hetal J. Gorasiya, Archana Paranjape, Krishna Murti. Pharmacognostic and Pharmacological profile of *Lagenaria siceraria* (molina) Standley: a review. Pharmacologyonline. 2011; 3: 317-324.
2. Iqbal Hussain, Riaz ULLah, Rooh Ullah, Muhammad Khurram, Naseem Ullah, Abdul Baseer et al.,. Phytochemical analysis of selected medicinal plants. African journal of Biotechnology. 2011; 10(38): 7487-7492.

3. Ames BM, Shigena MK & Hagen TM. Oxidants, antioxidant and the degenerative diseases of aging proceeding of the National Academy of sciences. 1993; 90: 7915-7922.
4. F Lei XN Zhang, W Wang, DM Xing, WD Xie, H Su and LJ Du. Evidence of antiobesity effects of pomegranate leaf extract in high fat diet induced obese mice. International journal of obesity. 2007; 31:1023-1029.
5. Grover JK, Adiga G, Vats V, Rathi SS. Extracts of Benincasa hispida prevent development of experimental ulcers. J Ethnopharmacol. 2001; 78: 159-64.
6. Chandrababu S, Umamaheshwari S. Studies on the anti-inflammatory activity of fruit rind extract of Benincasa hispida Cogn. Indian Drugs. 2002; 39: 651-3.
7. Anilkumar D, Ramu P. Effect of methanolic extract of Benincasa hispida against histamine and acetyl choline induced bronchospasm in guinea pigs. Indian J Pharmacol. 2002; 34: 365-6.
8. Wealth of India. Publication and information Directorate CSIR, New Delhi, 1998;2: 108.
9. Sass JE. Elements of Botanical Micro technique. McGraw Hill Book Co; New York; 1940.
10. Johansen DA. Plant Micro technique. McGraw Hill Book Co; New York; 1940.
11. O'Brien TP, Feder N and Mc Cull ME. Polychromatic Staining of Plant Cell walls by toluidine blue-O.Protoplasma. 1964; 59:364-373.
12. Easu K. Plant Anatomy. John Wiley and sons, New York, 1964.
13. Sandhiya S, Jaffery SAH, Vinod KR. Pharmagnostical studies on the leaf and root of physalis angulatalis. International Journal of Pharma Research and Development. Online- 2010;2:1-8.2010; 2: 1-8.
14. Indian Pharmacopoeia. Government of India, Ministry of Health and Family Welfare. Controller of Publication. 4th ed; New Delhi 1996.
15. Sofowara A. Medicinal plants and Tradional medicine in Africa spectrum Books Ltd. 1993.
16. Trease GE and Evans WC. Pharmacognosy 11th edition Brailliae Tiridal Can. Macmillan Publisher's; 1989.
17. Harborn JB. Phytochemical methods: A guide to modern techniques of plant analysis. 1973.
18. Bravo L. Polyohenols: Chemistry, dietary sources, metabolism & nutritional significances. Nutr Rev. 1998; 56: 343-355.
19. Bors W, Heller W, Michel C, Saran M. Flavonoids as antioxidants: Determination of radical-scavenging efficiencies. Methods enzymol. 1990; 186: 343-355.
20. Takuo Okuda and Hideyuki Ito. Tannins of constant structure in medicinal and food plants Hydrolyzable Tannins and Polyphenols related to Tannins. Molecules. 2011; 16:2191-2217.
21. Tona LK, Kumbu DN and Manga KC. Antimicrobial activity of tannins. Fitoterapia. 1999; 2: 279.
22. Rhoda MK. Antibacterial activity of Ajuge remota. Fitoterapia. 2001; 72: 177.
23. Schild HO. The colas of Nigeria & Cameroon. Cameroon Tropical Agric. Tnnida. 1995; 32: 210-240.
24. Prabavathy D and Valli nachiyar C. Cytotoxic potential and Phytochemical analysis of *Justicia beddomei* and its *Endophytic Aspergillus sp.* Asian J Pharm Clin Res.2013; 6 Suppl 5:159-161.
25. Praveena A and suriyavathana M. Phytochemical characterization of *Toddalia asiatica*.l var. Floribunda stem Asian J Pharm Clin Res. 2013; 6 Issue 4,148-151.