Research Article

Phytochemical Analysis of *Benincasa hispida*(Thunb.)Cogn. Fruit Using LC-MS Technique.

Sheemole M.S*, V.T. Antony, Kala K, Asha Saji
Research and Post Graduate Department of Botany, St. Berchmans College, Changanacherry, Kerala, India.
***Corresponding author's E-mail:** sheemolems@gmail.com

Accepted on: 08-12-2015; **Finalized on:** 31-12-2015.

ABSTRACT

Benincasa hispida(Thunb.)Cogn. a member of family Cucurbitaceae, is one of the nutritionally and medicinally important plant . A number of medicinal properties such as anti-diarrheal, anti-inflammatory, hypoglycemic, anti-obesity, anti- ulcer, anti-oxidant, anti-angiogenic and diuretic have been ascribed to this fruit of high economic value. It provides a good source of Primary metabolites such as carbohydrates, amino acids, fatty acids, organic acids, and also secondary metabolites like flavanoids, phenolic acids, alkaloids, coumarins, sterols, terpenoids etc. The present study aimed to screen the phytochemicals present in the fruit. Liquid Chromatography Mass Spectrophotometric Analysis (LC-MS) in *Benincasa hispida* (Thunb.)Cogn. fruits were done for the determination of the major component in it. The activities of the some selected compounds were predicted using Prediction of Activity Spectra for Substances (PASS) a computer based program.

Keywords: *Benincasa hispida* (Thunb.)Cogn., LC-MS, PASS, Cucurbitaceae, Phenolic compounds.

INTRODUCTION

Vegetables form the most important component of a balanced diet. Some vegetables have medicinal qualities and are used in ethnobotanical tradition of the folk medicine on the basis of traditional knowledge. Cucurbitaceae family is of considerable economic importance as a source of diverse commodities like vegetables, fruits, edible seeds rich in oils and protein, edible and industrial seed oils, domestic utensils and drugs.

Benincasa hispida(Thunb.)Cogn. belongs to the family Cucurbitaceae. It is also known as ash gourd, white gourd, wax gourd, fuzzy melon etc. It is an important vegetable mainly valued for its long storage life and having a good scope for value addition. Young fruit is fleshy, sacculent and hairy when it becomes mature the hairs change in to easily removable waxy bloom. The fruits are consumed as baked, fried, boiled, pickled or candied/preserved.[1] The fruits of this plant are traditionally used as a laxative, diuretic, tonic, aphrodisiac, cardiotonic, urinary calculi, blood diseases, insanity, and also in cases of jaundice, dyspepsia, fever, and menstrual disorders.[2] It is the main ingredient in Kusumanda rasayanam as rejuvenating agent against the treatment of nervous disorders. All most all parts of (leaves, flower, fruit and seeds) it have been used, either as food or as medicine. The young shoots, leaves and flowers can be used as vegetable. The immature as well as mature, large size fruits are often cooked as vegetable.

Phytochemicals are defined as the substances found in edible fruits and vegetables that exhibit a potential for modulating human metabolism in a manner beneficial for the prevention of chronic and degenerative diseases.[3] They protect the plants from various diseases and contribute to the plant's colour, aroma and flavour. Wide-ranging dietary Phytochemicals are found in fruits, vegetables, legumes, whole grains, nuts, seeds, fungi, herbs, spices.[4] *Benincasa hispida*(Thunb.)Cogn. has been valued as a high quality vegetable based on the index of Nutritional Quality (INQ) data.[5] Now a days many researchers are paying attention to the fruit of *Benincasa hispida*(Thunb.) Cogn. due to its potential health benefits. The objective of the study is to identify the different compounds present in the fruit pulp of *Benincasa hispida* (Thunb.)Cogn. using LC-MS technique and also list out the various phytochemically important compounds.

MATERIALS AND METHODS

Plant Material

Taxonomical description of Benincasa hispida (Thunb.)Cogn

Benincasa hispida(Thunb.)Cogn. is a climber, stem branched, hairy, diffuse. Leaves alternate; Petioles hirsute, 4-15 cm long; lamina 10-20 cm long and as broad, reniform-rotund, deeply cordate, upper surface scabrous, lower shortly hispid, 5-7 lobes, margin sinuate, dentate; nerves hirsute at lower surfaces. Tendrils slender; 2 fid. Flowers large yellow; axillary, unisexual; Peduncles hirsute, male 6-15 cm long, Female 2-4 cm long. Probracts foliaceous; opposite the tendril, ovate, 0.7-12 X 5-10 mm. Calyx tube (male) densely villose , 12-15 mm broad; lobes lanceolate, 8-12 X 3-5 mm. Corolla 5, nearly separate, obtuse, mucronate, 4-6 x 2.5-4 cm. Stamens 3, inserted near the mouth of the tube; filaments hispid. Ovary hairy; inferior style thick with three flexuous stigma; ovules numerous. Fruits spherical, large, fleshy, hairy when young, waxy bloom when mature; Seeds are many

compressed ovoid, smooth, yellowish white, distinctly marginate, 10-12 X 5-7 mm.

Collection and Authentication

The fruits of *Benincasa hispida*(Thunb.)Cogn were collected from different localities and are authenticated from the Regional Herbarium of S.B. College (RHK), Changanacherry, Kerala. The voucher specimens of the plant sample were deposited in the Herbarium of S.B. College Changanacherry.

Extraction

The fruits were sliced in to two halves and the peel was carefully removed with a manual peeler and the seeds were selectively collected and removed manually then the pulp obtained was homogenized in a warring commercial blender (Dynamics Corporation, CT, USA). Samples were ground in a Coffee grinder and dried under hot-air oven then stored in airtight container. 2g each of the fruit sample were separately extracted using petroleum ether (60-80) and methanol sequentially using Soxhlet's apparatus for about 3 hours each using 30 ml of the corresponding solvent. The extracts were then dried and dissolved in 10 ml petroleum ether/methanol (HPLC Grade, Merck). It was then filtered through 0.20 mm membrane filter. The extract was used for this analysis.

LC-MS Analysis

10µl of the filtered sample was then injected to the manual injector using a micro syringe (1-20µl Shimadzu). The mobile phase used was water: methanol(50:50) in an isocratic mode. The column used was RP-C-18(phenomenex). The separated compounds were then ionized using APC method using the split mode (50:50). The flow rate was maintained to 2 ml/mn with temperature 25±2° C. The class VP integration software were used for the data analysis. The Library used for the analysis was Metwin–LS. The version of the library was version 1.0-52.09.

PASS

Any biologically active compounds has a wide spectrum of effects. Computer Program PASS(Prediction of Activity Spectra for Substance) provides the activity spectra of these compounds.[6]

RESULTS AND DISCUSSION

Plant synthesize a vast range of organic compounds that are majorly classified in to Primary and secondary metabolites. Importance of plant lies in their biological active compounds. Primary metabolites such as sugars, amino acids, proteins, fats, chlorophylls etc are involved in the growth and development, respiration and photosynthesis, hormone and protein synthesis. Plant produce diverse compounds that have no direct participation in the role of growth and development are secondary metabolites. Secondary metabolites such as flavanoids, carotenoids, sterols, phenolic acids, alkaloids and glucosinolates determine the color of vegetables, protect plants against herbivores and microorganisms, attract pollinators and seed-dispersing animals and act as signal molecules under stress conditions.[7,8] *Benincasa hispida* (Thunb.)Cogn.fruit is a good source of valuable nutrients including organic acids, natural sugars, amino acids, vitamins and mineral elements.[9,5,10]

Phytochemical analysis of methanol and petroleum ether extract revealed the presence of carbohydrate, fatty acids, organic acids, aminoacids, carotenoids, Flavanoids, phenolic acids, alkaloids, terpenoids etc.

Primary metabolites present in the fruit of *Benincasa hispida* (Thunb.)Cogn are the following

Carbohydrates

Carbohydrates in vegetables occur as sugar monosaccharides, disaccharides, sugar alcohol, oligosaccharides and polysaccharides. Sugars like galactose, maltose, fructose etc are present in the fruit.

Fatty acids

Fatty acids are involved in the absorption of certain vitamins, blood clotting, and the immune response.[11,12] Fatty acids like Oleic acid, Ricinoleic acid, Linoleic acid are present.

Linoleic acid is one of the important Essential Fatty Acid play a key role in preventing many diseases and abnormal differentiation problems.[13] Oleic acid is one of the sources of good cholesterol. It is rich in antioxidants. It improves the functioning of heart.

Essential Fatty Acid mainly contribute towards the production of prostaglandins which regulate body functions such as heart rate, blood pressure, blood clotting, fertility, conception, and also play positive role in immune system by regulating inflammation and encouraging the body to fight against infections.[14,15]

Organic acid

Organic acids give the vegetables tartness, and affect flavour by acting on the perception of sweetness.[16]Organic acids influence the colour of vegetables since many plant pigments are natural pH indicators.[17] Organic acids like pipecolic acid, Cucurbic acid, Malonic acid are present in the fruit.

Aminoacid

Aminoacids play a major role in plant and animal metabolism. Amino acids like Methyl Amino L Analine is present.

Sl.No	Carbohydrates	Fatty acids	Organic acids	Aminoacids
1	Maltose	Oleic acid	Pipecolic acid	Methyl Amino L Alanine
2	Fractose	Linoleic acid	Cucurbic acid	
3	Galactose	Ricinoleic acid	Malonic acid	

Primary metabolites present in the fruit of *Benincasa hispida*(Thunb.)Cogn

Secondary metabolites

The secondary metabolites present in the fruit of *Benincasa hispida*(Thunb.)Cogn are as follows.

Plant name	Flavonoids	Phenolic acids	Terpenoids	Alkaloids	Coumarin	Sterols
Benincasa hispida(Thunb.)Cogn.	Isovitexin Dihydroxyflavan Hydroxyflavan Myricetin	Ferulic acid Syringic acid Benzoic acid	Marasmic acid Hirsuitic acid B vetivone	Palmatine	Umbelliferone	Sitosterol

Phenols

High consumption of vegetables containing phenolic antioxidants which inhibit the oxidation of LDL are said to slow down the process of atherosclerosis and may also reduce the risk of cancer and other diseases.[18,19] Plant phenolics have multiple biological functions such as anti-oxidant, anti-inflammatory, and anti-microbial activities.[20]

Phenolic compounds are major anti-oxidants of our diet.[21] In addition, there have been reported to have other biological effects, including anti-microbial, anti-inflammatory, anti-mutagenic, anti-carcinogenic, anti-allergic, anti-platelet and vasodilatory actions.[22,21]

Flavonoids

Flavonoids are the most numerous of the Phenolics and are found throughout the plant kingdom.[23] They are present in high concentrations in the epidermis of leaves and the skin of fruits and have important and varied roles as secondary metabolites. Flavonoids have been reported to exert a wide range of biological activities. These includes: anti-inflammatory, anti-oxidant, anti-bacterial, anti-viral, anti-allergic.[24-26] Flavonoids like Myricetin, Hydroxyflavan, Dihydroxiflavan, Isovitexin etc, are present in the fruit.

Phenolic acid

Phenolic acids are one such group of aromatic secondary plant metabolites widely spread throughout the plant kingdom.[27] Syringic acid, Benzoic acid, Ferulic acids etc are present in *Benincasa hispida* (Thunb.)Cogn fruit. Syringic acid may contribute to the bitter & astringent taste of Vegetables.[28] Benzoic acid have anti-microbial and antifungal action, probably due to the enzyme inhibition by the oxidised compounds.[29]

Terpenoids

Terpenoids like Beta Vetivone, Hirsuitic acid, Marasmic acid ect are present in fruit. Terpenoids exhibit various important pharmacological activities i.e., anti-inflammatory, anticancer, anti-malarial, inhibition of cholesterol synthesis, anti-viral and anti-bacterial activities.[30] Terpenoids of different sizes and composition are found all classes of living things and are the largest group of naturally occurring chemicals.[31]

Alkaloids

Alkaloids have many pharmacological activities including antihypertensive effects, antiarrhythmic effect, antimalarial activity and anticancerous actions. Plant alkaloids Palmatine have antimicrobial and cytotoxic activities.[32]

Coumarin

Coumarin comprise a very large class of compounds in the plant kingdom. They have great interest due to its various biological activities. Umbelliferone is one of the important coumarin present in the fruit. Umbelliferone have antioxidant property and also have the property to prevent the complications of Type 2 diabetes.[33,34]

Sterols

Plant sterols may possess anti-cancer, anti-atherosclerosis, anti-inflammation, and anti-oxidant activities.[35] Sitosterol is present in the fruits of *Benincasa hispida* (Thunb.) Cogn.

Compound like Quercitol, Hydroquinone, Luteolin and its glucosides etc are also present in the fruit. Luteolin and its glucosides are widely distributed in the plant kingdom. It shows wide range of biological activities such as antimicrobial, anti-inflammatory, antioxidant activities.[36,37] Secondary metabolites have various type of biological activities. In which antioxidants give more important. Various types of diseases especially diabetes, atherosclerosis, cancer, aging, and inflammations are caused due to oxidative damage that reduced by the action of antioxidants. In this scenario the role of vegetable containing these anti-oxidants are very important. Majorly the phenolics provide the anti-oxidant capacity.

Activity of compounds

Ferulic acid- Cytoprotectant, Carminative, Lipid metabolism regulator, Pulmonary hypertension treatment, Hypercholesterolemic (Pass).

Oleic acid- Hematoxic, Anti-seborrheic, Antivirus, Anti-mutagenic, Antithrombotic, Mucomembranous protector, Sickle cell Anemia treatment, Anti-toxic, Hypercholesterolemic, Skin disease treatment(Pass).

Pipecolic acid- Convulsant, Neoroprotector, Fibrinolytic, Urologic disorders treatment, Dopamine release stimulant.(Pass)

Myricetin - Myocardial ischemia treatment, Cardioprotectant, Antioxidant, Antineurotoxic, Cytoprotectant, Free radical scavanger, Emetic, Antiseborrheic, Histamine release protectant, Chemoprotective, Mucomembraneous protector(Pass).

Quercitol - Hematotoxic, Anti-seborrheic, Insulin sectretagoues, Hypercholesterolemic, Antineurotoxic, Emetic, Cardiotoxic, Sickle cell anaemia treatment.(Pass)

Syringic acid - Fibrinolytic, Hematotoxic, Hypercholesterolemic, Antiseborrheic, Sickle cell anemia treatment, Myocardial ischemia treatment.(Pass).

Umbelliferone - Cardiovascular analeptic, Hematotoxic, Myocardial Ischemia treatment, Anti-seborrheic, Pulmonary hypertension treatment, Spasmolytic, Anti-carcenogenic, Emetic, Cardioprotectant (Pass).

CONCLUSION

Through Pharmacognosy, Phytochemistry and pharmacology of the plant products all over the world tremendous progress has been made in research on herbal medicine. Cucurbitaceae family vegetables are well recognized for their medicinal value. They are widely used in the Indian traditional medicine. *Benincasa hispida*(Thunb.)Cogn. fruit is one of the nutraceutically important fruit vegetable currently gaining popularity among researchers due to its various health benefits. A number of medicinal properties such as anti-diarrheal, anti-obesity, anti-diabetic, anti-inflammatory, anti-microbial, anti-oxidant, diuretic and nervous disorders have been ascribed to the fruit. It may due to the presence of functionally important compounds such as phenolics, terpenoids, alkaloids, sterols and glycosides. Phytochemically active compound like myricetin, hydroxyflavan, dihydroxyflavan, synaptic acid, ferulic acid, β Vetivone, hirsuitic acid, marasmic acid, palmatine, umbelliferone, sitosterol etc are obtained from the fruit.

The application of computerized system PASS result shows several pharmacological activities such as antivirus, anti-carcenogenic, anti-seborrheic, free radical scavenger, hypercholesterolemic, cardioprotectant, sickle cell anemia treatment, chemoprotective, Lipid metabolism regulator etc. Based on the findings from this study we concluded that it is a potential source of high value components for pharmaceutical and nutraceutical industry.

REFERENCES

1. Robinson, R.W, & Decker-Walters, D.S. Crop production science in horticulture. Cucurbit (pp.6). New York: CAB International. 1999.
2. Kirtikar KR, Basu BD, *Benincasa hispida*, In: Blatter E, Claius J F, Mhaskar KS, editors, Indian Medicinal Plants Vol 2.2 nd ed. Dehrradun: M/s Bishen Singh Mahendra Palsingh; 1975, 1126-8.
3. Tripoli E, Guardia ML, Giammanco S, Majo DD, Giammanco M. Citrus flavanoids: Molecular structure, biological activity and nutritional properties : A review. Food Chemistry, 104, 2007, 466-479.
4. Mathai K, Nutrition in the Adult Years, In Krause's Food, Nutrition, and Diet Therapy, 10 th ed., L.K. Mahan and S. Escott Stump, 271, 2000, 274-275.
5. Mingyu D., Migzhang L., Qinghong Y., Weiming F., Jianiang X., & Weiming X.(1995). A study on Benincasa hispida contents effective for protection of kidney. Jiangsu Journal of Agricultural Sciences, 11, 1995, 46-52.
6. Filimonov D.A, Poroikov V.V, Karaicheva E.I, Karayan, R.K, Boudunova A.P, Mikhailovsky E.M, Rudnitskih A.V, Goncharenko LV, Burov, Yu V, Computer aided prediction of biological activity of spectra of chemical substance on the basis of their structural formulae; computerized system PASS, Experimental and Clinical Phramacology(Rus), 8(2), 1995, 56-62.
7. Seiger DS. 1998. Plant Secondary metabolism. Dordrecht, The Netherlands: Kluwer Academic Publishers. 529 p.
8. Crozier A, Clifford M, Ashihara H. 2006. Plant secondary metabolites: occurrence structure and role in the human diet. Oxford,U.K: Blackwell Publishing Ltd, 2006, 384.
9. Wills, R.B.H., A.W.K. Wong, F.M. Scriven and H. Greenfield. 1984. Nutrient composition of Chinese vegetables. J.Agric. Food Chem., 32, 1984, 413-416.
10. Zaini, N.A.M., Anwar, F., Hamid, A.A. AND Saari, N. Kundur (Benincasa hispida(THUNB.) Cogn.): A Potential source for valuable nutrients and functional foods. Food Research International, 44(7), 2010, 2368-2376.
11. Nettleton JA. Omega-3 fatty acids and health. New York: Chapman Hall. 1995, 384 p.
12. Shahidi F, Miraliakbari H. Omega -3 fatty acids in health and disease: part 2- health effects of omega -3 fatty acids in autoimmune diseases, mental health and gene expression. J Med Food, 8(2), 2005, 133-48.
13. Kirmizigui S., Boke N., Sumbul H., Gokturk R.S. and Arda N. Essential fatty acid components and antioxidant activities of eight Cephalaria Species from southwestern Anatolia. Pure and Applied Chemistry, 79, 2007, 2297-2304.
14. Yehuda S.S. Rabinovitz & D.I. Mostofsky. Essential fatty acids and the brain: From infancy to aging. *Neurobiol. Aging*., 26, 2005, 98-102.
15. Youdim K.A., A. Martin, J.A. Joseph. Essential fatty acids and the brain: Possible health implications. *Int.J.Dev.Neurosci*., 18, 2000, 383-399.
16. Fisher C, Scott TR. Food flavours: biology and chemistry. Cambridge, U.K: The Royal Society of Chemistry. 1997, 176 p.
17. Davies K. Plant pigments and their manipulation. Annual plant reviews V 14. Oxford, U.K: Blackwell Publishing Ltd. 2004, 368 p.
18. Beteridge D. Diabetes, lipoprotein metabolism and atherosclerosis, *Br.Med.Bul*, 45, 1989, 285-311.
19. Baynes J.W. Perspectives in diabetes: role of oxidative stress in development of complications in diabetes, *Diabetes*, 37, 1991, 550-557.

20. John A., and Grohmann K. Phenols in citrus peel byproducts, concentration of hydroxycinnamates and polymethoxylated flavones in citrus peel molasses. J. Agricul. Food Chem. 49, 2001, 3268-3273.
21. Scalbert A., C. Manach, C. Morand and C. Remesy. Dietary Polyphenols and prevention of diseases. Critical Reviews in Food Science and Nutrition, 45, 2005, 287-306.
22. Boyer J., and R.H. Liu Apple phytochemicals and their health benefits. Nutrition Journal, 3, 2004, 5.
23. Harborne J.B. The Flavanoids: Advances in Research Since 1986. Chapman & Hall, London, 1993.
24. Murray, MT. Quercetin: Nature's antihistamine. Better Nutrition, 1998.
25. Cushnie TPT, Lamb AJ. Antimicrobial activity of flavanoids, International Journal of Antimicrobial Agents, 26, 2005, 343-356.
26. Cook NC, Samman S. Flavanoids; Chemistry, metabolism, cardio protective effects and dietary sources. Nutritional Biochemistry 1996, 7, 66-76.
27. Herrmann K. Occurrence and content of hydroxycinnamic and hydroxybenzoic acid compounds in foods. *Crit. ReV. in Food Sci. Nutr.* 28, 1989, 315-347.
28. Drewanowski A, Gomez-Carneros C. Bitter taste phytonutrients and the consumer: a review. Am J Clin Nutr, 72, 2000, 1424-35.
29. Cowan, M.M. Plant products as antimicrobial agents. Clin Microbial Rev, 12(4), 1999, 564-82.
30. Mahato SB, Sen S. Advances in triterpenoid research, 1990-1994. Phytochemistry, 44, 1997, 1185-1236.
31. De las Heras B, Rodríguez B, Boscá L, Villar AM. Terpenoids: sources, structure elucidation and therapeutic potential in inflammation. Curr. Top. Med. Chem. 3, 2003, 171-185.
32. Facchini P.J. Alkaloids biosynthesis in plants: biochemistry cell biology molecular regulation and metabolic engineering applications. Ann Rev Plant Physiol Plant Mol Biol, 52, 2001, 29-66.
33. Okada Y, Miyauchi N, Suzuki K, Kobayashi T, Tsutsui C, Mayuzumi K, Nishibe S, Okuyama T. Search for naturally occurring substances to prevent the complications of diabetes. II. Inhibitory effect of coumarin and flavanoid derivatives on bovine lens aldose reductase and rabbit platelet aggregation. Chemical & Pharmaceutical Bulletin; 43, 1995, 1385-1387.
34. Dhalwal K, Shinde VM, Namdeo AG, Mahadik KR, Antioxidant Profile and HPTLC-Densitometric Analysis of Umbelliferone and Psoralen in *Aegle marmelos*. Phytotherapy; 46(2), 2008, 266-272.
35. Awad AB, Fink CS. Phytosterols as anticancer dietary components: evidence and mechanism of action. J Nutr, 130, 2000, 2127-30.
36. Ko F.N, Chu C.C, Lin C.N, Teng C.M. Isoorietin a water soluble antioxidant isolated from Gentiana arisanensis. Biochim, Biophys, Acta, 1389, 1998, 81-90.
37. Zian L; Yongmei Z; Nan Z, Niag T, Baolin L. Evaluation of the anti-inflammatory activity of Luteoline in experimental animal models Planta Med; 73, 2007, 221-6.

Source of Support: Nil, **Conflict of Interest:** None.