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Antioxidant Activity and Nutrient Analysis of Benincasa Hispida (Ash Gourd) Leaves, Pulp and Seeds: A Comparative Study

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ABSTRACT
Ash gourd, scientifically termed *Benincasa hispida* and commonly known as Kushmanda is a green vegetable. It has been well-regarded for its significant medicinal values since ancient times and is widely documented in Ayurvedic texts. This study investigates the antioxidant, nutritional, and mineral properties of different parts of the Ash gourd (Benincasa hispida), including leaves, pulp, and seeds, to evaluate their potential health benefits. the Ash gourd components were subjected to various analytical methods including DPPH, FRAP, and ABTS assays for antioxidant activity, and biochemical techniques for vitamin C, fiber, protein, carbohydrate, fat, ash, moisture content, and mineral estimation. Results demonstrated that Ash gourd seeds exhibit the highest antioxidant activity and nutrient content, including carbohydrates, proteins, and fats, compared to the leaves and pulp. The leaves were found to have the highest calcium content, while the seeds showed significant potassium and iron levels. Comparative analysis with pumpkin samples revealed that Ash gourd has higher carbohydrate and fiber content, whereas pumpkin seeds are richer in proteins, fats, and iron. The findings revealed the nutritional and medicinal value of Ash gourd, supporting its use in dietary and therapeutic applications. Biochemical activity of the fruit and other parts of the plant includes anti-oxidative, anti-inflammatory, anti-angiogenic, de-toxicant, and curative effects in treating various ailments.

Keywords: Ash Gourd, *Benincasa hispida*, antioxidant activity, nutrient analysis, mineral analysis

INTRODUCTION

Ash gourd, scientifically termed Benincasa hispida, goes by several vernacular names such as “Petha” in Hindi, “Boodida Gummadi” in Telugu, “Neer Pooshnikkai” in Tamil and “Kumbalam” in Malayalam (Bimakar et al., 2012).. Ash gourd is a green vegetable has been well-regarded for its significant medicinal values since ancient times and is widely documented in Ayurvedic texts. Today, it continues to be heralded for its immense health benefits and is widely incorporated, in popular local cuisine across India, as well as in alleviating illnesses of the stomach, liver and skin, to name a few. Ash gourd naturally grows in the wilderness in South-East Asian countries such as India, Sri Lanka, China, Nepal and Indonesia, as well as in the warmer southern regions in Australia. Ash Gourd It is a creeper which produces big yellow flowers. The leaves are long around 10 to 20 centimeters and have a hairy long

stem. The fruits are usually oval in shape and grow up to 30 centimeters in diameter. Plant propagation is done by seeds. This vegetable when immature has white thick flesh which is sweet. By the time it reaches maturity, it loses the hairs and a waxy coating is developed which provides a long shelf life.

Ash gourd is commonly considered a vegetable, which is used in cooking a variety of staple Indian dishes such as kootu, curry, sabzi and dal, besides sweets and candies called petha. The therapeutic and remedial traits offered by the vegetable, as well as ash gourd seeds and leaves are extensive. Moreover, the roots and juice of ash gourd also have applications in skin and hair care. Also Ash gourd (Benincasa hispida) commonly known as Kushmanda, has been used as a vegetable in various countries and has significant medicinal properties. In Indian traditional medicine, it has been used for treating diverse diseases such as diabetes melitus, diuresis diseases, urinary infections, chronic inflammatory disorders, epilepsy, cough, fever, heart conditions and liver disorders. Ash gourd plant, including fruit peel, flower, seed and leaves are used. biochemical activity of the fruit includes anti-oxidative, anti-inflammatory, anti-angiogenic, de-toxicant, and curative effects in treating various ailments. The essential minerals Ca, Mg, Fe, Cu, Zn and Se are present. Hence of ash gourd may be examined as food and for medicinal uses. *Benincasa hispida* (Prerna Gupta etal., 2019) is an annual growing as a trailing vine reaching 80cm in length. Its solitary yellow flowers are 08-10 cm wide and unisexual. Hairy leaves are heart shaped at the base and typically plamately lobed (Pandey et al., 2007). . Round, or oblong, fruit can reach up to 40cm in length and are often covered by a white, chalky, wax which deters microorganisms and helps impart longevity to the gourd. Mature fruit is green containing flat white seeds about 1 cm long. The taste is bland. Warm, humid conditions with an optimum temperature of 24-30 ºC and a soil pH of 6.0-7.5 is required for growth (Zaman, 2006). The fruit forms different shapes, color and sizes. Average weight of fruit varies from 0.5 - 03 kgs, length of 18-35cm, width 15-33cm, and circumference 30-37cm. Fruit an ovoid-oblong, ellipsoid or globose berry 20–60(–200) cm × 10– 25 cm, dark green to speckled pale green or glaucous, hispid when immature, thinly hispid or sub glabrous when ripe, covered with a chalk-white, easily removable wax layer; flesh greenish white, juicy, slightly fragrant, spongy in the middle, containing many seeds. Seeds ovate elliptical, flattened, 1–1.5 cm long, yellow- brown, sometimes prominently ridged (Grubben, 2004.). Harvesting generally starts 90-100 days after sowing and is complete in about 140-160 days. mature fruit is harvested when the ashy/waxy bloom on the surface disappears. Average yield is 25-30 tonnes/ha (Dewan et al., 2014).

In Indian traditional medicine, it has been used for treating diverse diseases such as diabetes mellitus (Majumdar et al. (2010)), diuresis related diseases, urinary infections (Bhalodia et al., 2009)., chronic inflammatory disorders (Park et al., 2009). epilepsy, cough, fever, heart conditions and liver disorders. Ash gourd plant, including fruit peel, flower, seed, and leaves are used. Biochemical activity of the fruit and other parts of the plant includes anti-oxidative (Rao et al., 2007), anti-inflammatory, anti-angiogenic, detoxificant, and curative effects in treating various ailments (Dhiman et al., 2012).

MATERIALS AND METHODS

Collection of Ash Gourd leaves, fruit and seeds.

The leaves and fruits of Ash gourd were collected in the month of June 2024 from the agriculture fields around Jagtial , Telangana. The seeds were extracted from fruit before consumption for studies.

Solvent Extraction (Methanol Extraction)

10 grams of Ash gourd leaves were weighed and added to 100ml of organic solvent (methanol) in a conical flask. After 24 hours, it was filtered using muslin cloth and centrifuged at 5000 rpm for 15

minutes. The supernatant was then gathered in a round base jar and the dissolvable was dissipated to make the last volume of one-fourth of the first volume, giving a convergence of 40 (**µg** /0.1ml. It was put away at 40 in water/air proof jugs for additional studies.

Determination of Vitamin C

About 100 g of Ash gourd leaves were completely uprooted with ethanol. The excerpt was concentrated to a residue. The crude excerpt was stored in a castrated bottle and kept in a refrigerator for further use. 10 ml of each filtrate was mixed with 20ml glacial acetic acid in a 100 ml standard beaker which was made up to 100 ml of distilled water.

Color medication: The standard color result was prepared by dissolving 50 mg of blue color in 50 ml of distilled water. The admixture was adulterated to 200 ml, filtered, and kept.

Preparation of standard ascorbic acid: This was prepared by dissolving 100 mg liquid ascorbic acid in 50 ml of 20 glacial acetic acid and lacing it to 100 ml with distilled water. (Biochemical methods, 2nd Edn. 1996).

Titration procedures:

10 ml of the ascorbic acid result was titrated with the color result. Each drop of the color in contact with the result turns pink. The endpoint was reached when the pink color lasts for 10 seconds. also, 10 ml of each sample prepared was in turn titrated with the due, and the titre values were noted (Pankaj. Tyagi1, and Shruti Tyagi, 2018).

Determination of Fiber

Determine independently the sample humidity by hot in a roaster at 105 °C to constant weight. Cool in a desiccator. Weight directly about 01 gm of grinded sample (1 mm about) roughly with 1 mg. => W1. Add 1.25 ml sulfuric acid up to the 150 ml notch, after preheating by the hot plate in order to reduce the time needed for boiling. Add 3- 5 drops of n- octanol as antifoam agent. Boil 30 twinkles exactly from the onset of boiling. Connect to vacuum for draining sulfuric acid. Wash three times with 30 ml (gauntlet filled up to the top) of hot de-ionized water, connecting each time to compressed air for stirring the content of gauntlet. After draining the last marshland, add 150 ml of preheated potassium hydroxide (KOH)1.25 and 3- 5 drops of antifoam. Boil 30 twinkles. Perform a last washing with cold deionized water aimed to cool the trials and also wash three times the gauntlet content with 25 ml of acetone, stirring each time by compressed air. Remove the trials and determine the dry weight after drying in an roaster at 105 °C for an hour or over to constant weight. Let cool in a desiccator. This weight(W2) represents the crude fiber plus ash content in comparison to original weight (Fiber plus system).

Determination of Antioxidants by DPPH Method

The HCl buffer(pH7.4) in a testing tube. And also 200µl of testing sample result was added and mixed snappily. The result was kept at room temperature for 30 min. The absorbance of the result at 517nm was recorded. A mixed result with 1,200 µl of ethanol and 800µl of Tris HCl buffer (pH7.4) was used as the blank. The inhibition rate was attained from the following equation Inhibition rate()(A1 − A2) × 100 ⁄ A1, where A 1ist the absorbance of the addition of ethanol rather of testing sample and A2 is the absorbance of testing sample result (Debasis nayak, 2012.) %DPPH radical scavenging effect = A0 – A1/A0 x 100

Determination of Total Antioxidant Activity by FRAP Method

FRAP (3.6 mL) add to distilled water (0.4 mL) and incubated at 37℃ for 5 min. also this result mixed with certain attention of the factory excerpt (80 mL) and incubated at 37℃ for 10 min. The absorbance of the response admixture was measured at 593 nm. For construction of the estimation wind, five

attention of FeSO4, 7H2O (0.1, 0.4, 0.8, 1, 1.12, 1.5 mM) were used and the absorbance values were measured as for sample results (Monika skowyra, 2014).

Determination of Total antioxidant Activityby ABTS method

Experiments were performed according to (Pellegrini, et al., 1997) with small modifications. ABTS and potassium persulfate were dissolved in distilled water to a final concentration of 7 mM and 2.45 mM respectively. These two solutions were mixed and the mixture allowed to stand in the dark at room temperature for 16 h before use in order to produce ABTS radical (ABTS +). For the study of phenolic compounds, the ABTS radical solution was diluted with distilled water to an absorbance of 1.00 at 734 nm. Phenols (final concentrations 0.0001-0.01 mg/ml) or Trolox standards (final concentration 0-20 mM) were added to diluted ABTS + solution and the absorbance reading was taken 6 min after mixing using the spectrophotometer. Results are presented as the ability of phenols to scavenge 50% of free radical ABTS + (IC50) and TEAC (Trolox equivalent antioxidant capacity). Parameters IC50 (μM) and TEAC (μM) were determined with a relative uncertainty of less than five percent.

Determination of Moisture Content

Weigh a small vessel. Weigh 10 g of the material into the vessel. Sot the sample for 24 hours at 105- 110 ºC roaster. Reweigh the sample, abate the weight of the vessel, and determine the humidity content using the following equation Mn = ((Ww- Wd)/ Ww) x 100. (Mayur- Chandrasekhar- Patil 2019, JAOAC 17, 215(1934); 18, 80(1935)).

Determination of Ash

Find out the weight of a clean dry gauntlet. Place about 2 g of sample and weigh this to find out accurate weight of the sample taken. Precisely place the counted gauntlet over electric burner. The gauntlet should be incompletely opened. The sample will get scorched with original expatriation of bank. Place the gauntlet in a muffle furnace and heat to 600 ºC. Keep it for 2 hours. At this temperature all organic matter will be burnt leaving behind minerals. Remove the gauntlet from the furnace precisely and cool it in a desiccator to room temperature and weight again (AOAC Official Method 942.05).

Determination of total phenolic content

The total phenolic content of the gourd vegetable extract was determined by using Folin-Ciocalteu reagent following a slightly modified method (Ainsworth et al., 2007). Gallic acid was used as a reference standard for plotting calibration curve. A volume of 0.5 mL of the plant extract (100 μg/mL) was mixed with 2 mL of the Folin-Ciocalteu reagent (diluted 1:10 with de-ionized water) and were neutralized with 4 mL of sodium carbonate solution (7.5%, w/v).

The reaction mixture was incubated at room temperature for 30 min with intermittent shaking for colour development. The absorbance of the resulting blue color was measured at 765 nm using UV-VIS Double Beam Spectrophotometer. The total phenolic contents were determined from the linear equation of a standard curve prepared with Gallic acid. The content of total phenolic (TP) compounds was expressed as μg/g Gallic acid equivalent (GAE) of fresh weight. (Biochemical methods, 2nd Edn. 1996).

Estimation of Calcium

Initially, calcium oxalate is precipitated out using ammonium oxalate. Dissolve this precipitate in concentrate sulphuric acid and calcium is calculated by Permanganometric titration (AOAC Official Method 927.02)

Estimation of Iron

Dichromatometric titration was used to estimate Iron in food samples. From the titrate value the strength as well as the amount of Iron present in the whole of the food sample was calculated. (Mrinal Kanti si, et

al, 2021.)

Determination of Fat Content

Weigh 10 g of Ash gourd sample in a thimble, later close the thimble with cotton plug hen add glass beads in fat flask and note the initial weight of the flask then add 150 ml of petroleum ether in fat flask along with condenser, heat it on distillator at 60 ^{0}C for 4 hours and take petroleum ether after 4 hours and soxhlet beaker is kept in hot air oven for 15 min until the hexane evaporates and cool it in desiccator, the final weight of the sample is the fat content in the given sample. (Katherine M. Phillips, et al, 1997, JAOCS)

Determination of protein content

Weigh 1g of ash gourd sample in a butter paper. Take 6g of digestion mixture [Copper Sulphate & Sodium sulphate 1:10] in a digestion tube. Now add 15ml of concentrated Sulfuric acid. Now put the digestion tube in the digestive system for the digestion tube. The addition of Na OH and water, is automatically done by the protein analyzer. The process of distillation is programmed until the pink color turns to yellow color in conical flask. Now tit rated against 0.1 NH2SO4 and calculate as per the given formula. Digestion Distillation (According to AOAC 984.13)

Determination of carbohydrate content

The dried pulverized sample was extracted with petroleum ether (boiling point 40-60 0C) using a soxhlet apparatus to obtain the crude lipid content while crude fibre content was estimated by consecutive acid and alkali digestion of sample followed by washing, drying, ashing at 600 ºC and calculating the weight of ash free fibre and carbohydrate was calculated by difference (AOAC)1990 15th edition)

RESULTS AND DISCUSSION

The preliminary phytochemical investigation of the methanolic extracts of Ash gourd leaves, pulp and seeds showed the presence of Antioxidants and vitamin c (Table 1). Quantitative estimation of Anti oxidants and Vitamin C are represented below

TABLE-1: ANTI- OXIDANT PROPERTY OF ASH GOURD LEAVES, PULP AND SEEDS

Parameters	Method of Analysis	Ash Gourd leaves	Ash Gourd pulp	Ash Gourd seeds	Units
anti-oxidant activity	DPPH Method	45	64	78	IC 50 value in ppm
	FRAP method	2.4	1.6	0.5	µ mol
	ABTS assay	40.45	30.25	110.15	GAE/g
Vitamin C	Biochemical methods, 2nd Edn. 1996	30.55	24.15	12.40	mg/100 g
Total phenols content	Biochemical methods, 2nd Edn. 1996	75.25	50.52	30.42	GAE/g

The anti-oxidant property of Ash Gourd Seeds are comparatively higher than that of the leaves and pulp. While the total phenolic content remains almost same in all the tested plant parts. Moreover, the nutrient and calorific values of seeds are much higher due to presence of minerals like potassium, calcium, iron and macro nutrients in large composition than that of pulp and leaves. Interestingly the leaves and pulp showed similar anti-oxidant and nutrient values.

TABLE-2: NUTRIENT PROPERTIES OF ASH GOURD LEAVES, PULP AND SEEDS

Parameters	Ash Gourd leaves	Ash Gourd pulp	Ash Gourd seeds	Units
Carbohydrates	4.45	6.2	37.0	g/100 g
Proteins	4.5	0.65	21.4	g/100 g
Fat	1.25	0.15	14.8	g/100 g
Fiber	3.8	2.0	7.2	g/100 g
Ash content	3.0	1.0	4.5	g/100 g
Moisture content	83	90	15	g/100 g

Carbohydrates per 100 grams of Ash Gourd Leaves contains 4.45 grams, pulp contains 6.2 grams and seeds contains 37.0 grams of carbohydrates. The seeds have significantly higher carbohydrate content compared to the leaves and pulp, indicates that they are richer in sugars and starches.

Proteins per 100 grams of Ash gourd leaves contains 4.5 grams, Pulp Contains 0.65 grams and seeds contains 21.4 grams of proteins. All parts of the Ash Gourd are relatively low in protein content, with the seeds having slightly higher protein compared to the leaves and pulp.

Fat per 100grams of Ash gourd leaves contains 1.25 grams, pulp has 0.15 grams and seeds contains 14.8 grams of fat. Similar to proteins, all parts of the Ash Gourd have low fat content, with the seeds having the highest fat content among the leaves, pulp and seeds of Ash gourd.

Fiber content per 100 grams of ash gourd leaves is 3.8 grams and pulp is 2.0 grams whereas seeds contain 7.2 grams.

Ash content in ash gourd leaves is 3.0 grams, pulp is 1.0 grams and seeds is 4.5 grams per 100grams. Seeds have higher amount and pulp has lower amount of ash content.

Moisture content per 100 grams of ash gourd leaves is 83 grams, pulp is 90 grams and seeds is 15 grams. Pulp has the higher and seeds has the lower amount of moisture content.

Ash Gourd seeds are significantly rich in carbohydrates, protein, fiber and fat compared to the leaves and pulp, while moisture content is lower than other parts.

TABLE 3: MINERAL CONTENT OF ASH GOURD LEAVES, PULP AND SEEDS

Parameters	Ash gourd leaves	Ash gourd pulp	Ash gourd seeds	Units
Potassium	428	370	445	mg/100 g
Calcium	285	20.45	214	mg/100 g
Iron	5.5	0.55	4.2	mg/100 g

There are some minerals present in ash gourd leaves, pulp and seeds with some content. Potassium content per 100 grams of ash gourd leaves is 428mg and pulp is 370mg and seeds contain 445mg of potassium. Potassium content is higher in the seeds and lower in the pulp among them.

Calcium content per 100 grams of leaves is 285mg and pulp is 20.45mg and seeds contain 214mg. Ash gourd pulp is low in calcium content while leaves have significantly more calcium among them. Iron content per 100 grams of ash gourd leaves is 5.5mg and pulp is 0.55mg and seeds have 4.2mg. Iron content is higher in the leaves and lower in pulp. Ash gourd seeds have higher potassium than other parts, while potassium and calcium is higher in leaves across all parts.

The study analyzed the antioxidant activity, nutrients of Ash gourd leaves, pulp and seeds. Ash Gourd leaves have a high carbohydrate content, with seeds having a higher carbohydrate content. Proteins are low, with seeds having slightly higher protein than leaves and pulp. Fat content is low, with seeds having the highest fat content. Fiber content is 3.8 grams, with pulp 2.0 grams and seeds 7.2 grams. Ash content is 3.0 grams, with seeds having a higher amount. Moisture content is 83 grams, with pulp having the highest and seeds having the lowest. Overall, Ash Gourd leaves, pulp, and seeds have low protein, fat, fiber, ash, and moisture content. Ash Gourd seeds are mostly rich in carbohydrates, protein, fiber and fat compared to the leaves and pulp, while moisture content is lower than other parts.

Ash gourd leaves, pulp, and seeds contain minerals with varying content. The leaves have higher potassium content (428mg per 100 grams), while the pulp has 370mg and 445mg. The leaves have higher calcium content (285mg per 100 grams), and the seeds have higher iron content (5.5mg per 100 grams). The leaves have higher iron content, while the leaves have higher potassium and calcium content. The T-test values for Anti-oxidant activity, nutrient analysis and minerals are significant that defines the evident differences between these samples.

We can compare the ash gourd samples with the Pumpkin samples of leaves, pulp and seeds. Carbohydrates in the 100grams of pumpkin leaves, pulp and seeds are 2.33g, 6.5g and 17.81g. Proteins present in pumpkin leaves, pulp and seeds per 100grams are 3.15g, 1g and 24.54g. The values of fats are 0.4g, 0.1g and 45.85g in the samples of pumpkin leaves, pulp and seeds. Fiber present in the 100grams of leaves, pulp and seeds are 0g, 0.1g and 3.9g. Potassium content in leaves, pulp and seeds per 100grams are 436mg, 340mg and 807mg. Calcium present in the 100grams of leaves, pulp and seeds are 39mg, 21mg and 43mg. Iron content per 100grams of pumpkin leaves, pulp and seeds are 2.22mg, 0.8mg and 14.97mg.

carbohydrates are rich in ash gourd leaves and seeds than the pumpkin samples. Protein is rich in the pumpkin samples than the ash gourd samples. Fiber is higher in the samples of ash gourd than the samples of pumpkin. Potassium is higher in the pumpkin seeds than any other samples. Calcium is rich in the Ash gourd leaves than other samples and Iron is higher in the pumpkin seeds than any other samples.

CONCLUSION

This study highlights the substantial nutritional and medicinal benefits of Ash gourd (*Benincasa hispida*), with distinct differences observed among its various parts—leaves, pulp, and seeds. The seeds exhibit the highest levels of carbohydrates, proteins, fats, and fiber, making them nutritionally richer compared to the leaves and pulp. While the leaves have the highest calcium content, the seeds are notably richer in potassium. The antioxidant activity, measured by various methods, reveals that Ash gourd seeds possess superior antioxidant properties compared to the leaves and pulp. Comparatively, Ash gourd seeds also show higher mineral content and lower moisture compared to the other parts. This comprehensive analysis underscores the potential of Ash gourd seeds as a valuable dietary component with considerable health benefits, while also highlighting the nutritional and medicinal value of its

leaves and pulp. The comparative analysis with pumpkin samples further illustrates the unique nutritional profile of Ash gourd, supporting its use in traditional medicine and dietary applications.

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