Study on Phytochemical and Antioxidant Properties of Water Apple (*Syzygium aqueum)* and Chappathikalli (*Opuntia ficus-indica)*

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ABSTRACT

The aim of this study is to determine the phytochemical and antioxidants properties, moisture, protein, fat, ash, crude fibre, acidity, TSS, pH and calcium content of wild fruits namely water apple (*Syzygium aqueum)* and chappathikalli (*Opuntia ficus-indica)* and it was collected from Kodaikanal hills, Tamilnadu, India. The total phenolic contents from water apple and chappathikalli fruits extract, determined following the Folin-ciocalteu assay were found in the range of 28.80 –30.70 mg/100g and 55.40 to 55.10 mg/100g. The total flavonoids, antioxidant activity and vitamin C contents from water apple (*Syzygium aqueum)* and chappathikalli (*Opuntia ficus-indica)* fruit extract were found in the range of 62.03 to 62.07µg/g and 69.50 to 69.51 144.50 µg/g, 144.50 to 138.40 to 144.50 mg AAeq/100g and 39.00 39.02 mg AAeq/100g and 13.06 to 13.08 mg/100g) and 30.00 to 30.03 mg/100g respectively. Whereas, the other chemical constituent's moisture in the range was 91.70 g%, protein 0.31g%, fat 0.29 g%, Ash 1.24g%, Crude fibre 1.37g%, Acidity 0.07 g%, TSS 2.3 bx, pH 4.14 and calcium 0.64mg in water apple. The moisture content in chappathikalli ranged was 83.25 g%, protein 0.52g%, fat 0.38g%, ash 1.98g%, crude fibre 0.89g%, acidity 0.05g%, TSS 6.4bx, pH 4.28 and calcium 18.02mg respectively.

Key words: Wild fruits, phytochemical, antioxidant, total phenols, vitamin C.

1. INTRODUCTION

Water Apple (*Syzygium samarangense*) is a species in the Myrtaceae, native to Philippines, Indonesia and Malaysia. Common names include wax apple, love apple, java apple, Chomphu (in Thai), Bellfruit (In Taiwan), jambu air (in Indonesian), water apple, mountain apple, jambu air ("water guava" in Malay), wax jambu, Rose apple, bell fruit, makopa, tambis (Philippines), and chambekka in Malayalam and jumbu (Sri Lanka). There are many varieties of water apple in the world. The most popular varieties of water apple in Kodaikanal are Rose water apple (*Syzygium samarangense).* Its pulp is crisp and watery, hence the name watery apple. *Opuntia ficus-indica* is a species of cactus that has long been a domesticated crop plant important in agricultural economies throughout arid and semiarid parts of the world. It is thought to possibly be native to Mexico. Some of the common English names for the plant and its fruit are Indian fig opuntia, barbary fig, cactus pear, spineless cactus, and prickly pear, although this last name has also been applied to other less common *Opuntia* species. Prickly pear is widely cultivated and commercially used in juices, jellies, candies, teas, and alcoholic drinks. American Indians used prickly pear juice to treat burns, and prickly pear has a long history in traditional Mexican folk medicine for treating diabetes. Its use in treating diabetes, lipid disorders, inflammation, and ulcers, as well as its other pharmacologic effects, have been documented. However, there is limited clinical information to support these uses. Having a diet rich in fruits will be able to provide some protection against the common diseases such as cardiovascular diseases, cancers and other age-related degenerative diseases

(Scalzo *et al*., 2005). Evidence shows that free radicals are responsible for the damage of lipids, proteins, and nucleic acid in cells could lead to these common diseases (Alothman *et al*., 2009a). Recent studies showed that frequent consumption of fruits and vegetables can reduce the risk of stroke and cancer which is related to the antioxidant micro constituents contained on the plant parts. Different fruits will exhibit different capacities due to the presence of different dietary antioxidants, such as vitamin C and E, carotenoids, flavanoids, and other phenolic compounds (Saura-Calixto and Goni, 2006).

2. MATERIALS AND METHODS

2.1. Materials

2.1.1. Study Area

The study was carried out in different areas of Tamil Nadu such as Kodaikanal, Ooty, Sirumalai hills, Kutralam, Thadiyankudisai, and Oothu were selected for this study. Based on the fruits availability the above mentioned areas were selected for this project (Fig.1).

2.1.2. Design of the study

The design of the study is collection of wild fruits from the wild areas and assesses the phytochemical and antioxidant properties of wild fruits (Fig. 2).

2.1.3. Collection of wild fruits

Two wild fruits were surveyed and collected for the analysis of phytochemical and antioxidant properties and also other chemical constituents such as moisture, protein, fat, ash, crude fibre, Total Soluble Solids (TSS), pH, Acidity, Ascorbic acid, etc.

The locally available wild fruits such as water apple (*Syzygium aqueum)* and Chappathikalli (*Opuntia ficus-indica),* were collected from the Kodaikanal, Oothu, Thadiyankudisai and Sirumalai etc.

2.1.4. Assessment of Chemical constituents

The chemical constituents such as Phytochemical, antioxidant, moisture, protein, fat, ash, crude fibre, calcium, phosphorus,pH and TSS etc., were assessed using standard methods.

2.1.5. Chemicals

The chemicals and reagents used for the study were both Analytical Reagent, Laboratory Reagent and Guaranteed Reagent (GR) grade. Chemicals such as acetone, methanol, hydrochloric acid, ferric chloride, ferrous sulphate, sodium acetate and acetic acid were obtained from Madurai chemical laboratory.

2.1.6. Proximate analysis

Two fruits were sliced into small portions, and were arranged on an aluminum foil, then; the samples were placed inside the oven at 63°C for 48 hours. After 48 hours, the fruits were dried. The dried fruits were then blended into a powder form using a blender (Multipro, Kenwood, Japan). The powder was used for ash, fat, protein and fiber analysis. The moisture content was measured using an oven method according to Association of Official Analytical Chemists (AOAC International) standard. The Kjeldahl method was used for protein determination and the Soxhlet method was used for the fat content. The determination of fiber was based on the method by Lees (1968). For ash content, the sample was weigh and transfer to a muffle furnace at 550°C until a white or light grey ash is obtained. Three replications of all of these measurements were carried out.

2.2. Methods

2.2.1. Estimation of moisture

The moisture content of the sample was estimated by hot air oven method as per the procedure given by AOAC (1995). The sample was dried at 110^{o} and the drying was continued till a constant reading was obtained. The moisture content was expressed as percentage.

$$\% \text{ of moisture content} = \frac{W2 - W3}{W2 - W1} X100$$

2.2.2. Estimation of Protein

Protein was analyzed by the amount of nitrogen available in the sample by Micro Kjedhal Method. Hundred gram of sample was transferred into 250ml of digestion flash along with three grams of catalyst mixer and 10 ml of concentrated sulpuric acid. The catalyst mixer consists of sodium or potassium sulphate and copper sulphate (5:1 ratio). The sample was digested until the solution become colourless.

The digested sample was placed in the distillation unit for ammonia recovery. The solution was distilled and the ammonia was collected in the receiver solution. The solution was titrated against the 0.1N hydrocholoic acid for the end point, until the colour changes. The same procedure was repeated to get the blank titre value and the nitrogen content of the sample can be calculated. The nitrogen value multiplied by factor 6.25 gives the crude protein content of the sample in per cent.

2.2.3. Estimation of Fat

The lipid in the sample was extracted with petroleum ether (60-80^o) in soxplus apparatus for two hours. Then the solvent was evaporated and the remaining residue was weighed. The fat content was expressed as percentage.

2.2.4. Estimation of crude fibre

The dried sample was taken in a beaker and 200 ml of 1.25 percentage sulphuric acid was added and boiled for 30 minutes. The content were filtered through musclin cloth and washed with distilled water until the washings were no longer acidic. The residue was transferred into the same beaker and boiled with 1.25 per cent NaOH for 30 minutes and filtered through a musclin cloth, washed with 50 ml distilled water and 25 ml of alcohol. The residue was transferred into pre weight slica crucible, dried for two to four hours at 130^o, cooled and weighted. It was ignited and asked for 30 minutes at 600^o cooled and weighed. The loss in weight on ignition was expressed as percentage.

2.2.5. Estimation of ash content

The ash of the sample was determined by the method described by Hart and Fisher (1971). A sample of five gram was ashed in an electronic muffle furnace at 500^o to 600^{o}C. The ash content was expressed as percentage.

2.2.6. Estimation of Acidity

Acidity of the sample was estimated by the method described by Sain *et al.*(2001). About 5 gram of the sample was weighed and discolored in a known quantity of water and made up to 50 ml and filtered. From the filtrate an aliquot of sample was taken and titrated against 0.01 N NaOH, using phenolphthalein as indicator till the appearance of pale pink colour. The titration was repeated to obtain concordant values. The result was expressed as percentage.

2.2.7. Estimation of Ph

The pH of the sample was estimated by the method of described by Hart and Fisher (1971). Ten grams of the sample was mixed well by stirring with 50 ml of distilled water using glass rod and the pH of the suspension was determined in the pH Meter.

2.2.8. Estimation of Ascorbic acid

Pipette out 5 ml of the working standard solution into a 100ml conical flask. Add 10ml of 4% oxalic acid and titrate against the dye (V1M1). End point is the appearance of pink colour which persists for a few minutes. The amount of dye consumed is equivalent to the amount of ascorbic acid. Extract the sample (0.50 – 5g depending on the sample) in 4% oxalic acid and make up to known volume (100ml) and centrifuge. Pipette out 5 ml of this supernatant and 10 ml of 4% oxalic acid and titrate against the dye (V2Ml).Amount of ascorbic acid mg/100g of sample

2.2.9. Estimation of total carotenoids and lycopene

Total carotenoids and lycopene can be extracted in the sample using petroleum ether and estimated at 450nm and 503nm respectively. The sample (0.5g) was homogenized and saponified with 2.05ml of 12% alcoholic potassium hydroxide in a water bath at 60^o for 30 minutes. The saponified oxlate was transferred to separating funnel containing 10-15ml of petroleum ether and mixed well. The lower aqueous layer was transferred to another separating funnel and the upper petroleum ether layer containing the carotenoids was collected. The extraction was repeated until the aqueous layer become colourless. A small amount of anhydrous sulphate was added to the petroleum extract to remove excess moisture. The final volume of the petroleum ether extract was noted. The absorbance of the yellow colour was read in a spectrophotometer at 450 nm and 503 m using petroleum ether as blank. The amount of total carotenoids and lycopene was calculated using the formulae

2.2.10.Estimation of total phenols

The amount of total phenols in the plant tissues was estimated by the method proposed by Mallick and Singh (1980). The sample (0.5g) was homogenized in 10% volume of 80% ethanol. The homogenate was centrifuged at 10000 rpm for 20 minutes. The extraction was repeated with 80% ethanol. The supernatant were pooled and evaporated to dryness. The residue was dissolved in a known volume of distilled water. Different aliquot were pipette out and the volume in each tube was made upto 3.0ml with distilled water. Folin – reagent (0.5 ml) was added and the tubes were placed in a boiling water bath for exactly one minute. The tubes were cooled and the absorbance was read at 650 nm in a spectrophotometer against a reagent blank. Standard catechol solution 0.2 – 1ml) corresponding to 2.0 to 10µg concentrations were also treated as above. The concentration of phenols is expressed as mg/g of sample.

2.2.11.Estimation of Total Flavonoids

The flavonoids were measured using aluminum chloride calorimetric assay, as described by maximum *et al.*(2005).

2.2.12. Estimation of Flavonoids

The samples (0.5g) were first extracted with methanol: water mixture (2:1) and secondly with the same mixture in the ratio 1:1. The extracts were shaken well and they were allowed to stand overnight. The supernatants were pooled and the volume was measured. This supernatant was concentrated and then used for the assay.

A known volume of the extract was pipette out and evaporated to dryness. Vanillian reagent (4.0ml) was added and the tubes were heated in a boiling water bath for 15 minutes. Varying concentrations of the standard were also treated in the same manner. Optimal density was read in a spectrophotometer at 340nm. A standard curve was constructed and the concentration of flavonoids in each sample was calculated. The values of flavonoids were expressed as mg/g sample.

2.2.13. Estimation of Flavonoids

Total antioxidant activity is measured by ferric reducing antioxidant power (FRAP) Assay of Benzie and strain (1799). FRAP assay user antioxidants as reductants in a redox – linked colorimetric method employing an easily reduced oxidant system present stoichiometric excess.

Sample (100ml) is mixed with 3 ml of working FRAP reagent and absorbance (593nm) is measured at 0 minute after vortexing. Thereafter samples are placed at 37^{o} in water bath and absorption is again measured after 4 minutes. Ascorbic acid standards (100µm - 1000 µm) were presented in the same way.

2.2.14. Estimation of Tannins

The Tannin content was estimated as per the method described by Sadasivam and Manikkam (2008). An aliquot of 0.1 ml of the methanolic extract on standard solution of tannic acid prepared using distilled water (10µ to 100µg per ml) was added with 7. 5 per cent of distilled water and add 0.5 ml of folin denis reagent, 1ml of 35 % sodium carbonate solution and diluted 10 ml with distilled water. The mixture was shaken well, kept at room temperature for 30 minutes and absorption was measured against reagent blank at 700 nm in a double beam UV – Vis Spectorphotometer 2201. Tannin content was expressed as mg of tannic acid equivalent (TAE) per 100g of sample on FWB. The scavenging ability of the matured antioxidants towards the stable free radical DPPH was measured by the method of Mensor *et al.* (2001).

2.2.15. Estimation of Antioxidant Activity

The extracts (20ml) were added to 0.5ml of methonolic solution of DPPH and 0.48ml of methanol. The mixture was allowed to react room temperature for 30 minutes. Methanol served as the blank and DPPH in control, without the extracts, served as the positive control. After 30 minutes of incubation, the discoloration of the purple colour was measured at 518 nm in a spectrophotometer.

2.2.16. Total soluble solid (TSS)

The total soluble solid (TSS) of fruit juice (^{o}Brix) was determined using a Digital refractometer (AR 2008, Kruss, Germany) at 25^{o}C. All experiments were conducted at room temperature and the average values of three replications were reported.

3. RESULTS AND DISCUSSION

3.1. Collection of Information

The wild fruits such as water apple (*Syzygium aqueum)* and Chappathikalli (*Opuntia ficus-indica)* were collected from the wild areas of Kodaikanal, Oothu, Thadiyankudisai, Ooty and

Sirumalai and analysed chemical constituents such as phytochemicals, antioxidants, moisture, protein, fat, TSS, pH, acidity, ascorbic acid and crude fibre etc.

3.2. Phytochemical And Antioxidant Properties

The phytochemical and antioxidant properties of water apple (*Syzygium aqueum),* and Chappathikalli (*Opuntia ficus-indica)* were presented in Table1.

Table. 1. Phytochemical and Antioxidant Properties of Fresh Wild Fruits

Particulars	Water apple			Chappathikali		
	R_1	R_2	R_3	R_1	R_2	R_3
Ascorbic acid (mg/100g)	13.08	13.6	13.8	30.0	30.01	30.03
Total phenols(mg GAE/100g)	28.8	29.6	30.7	55.4	55.3	55.1
Total flavonoids (µg/g)	62.03	62.05	62.07	69.5	69.51	69.51
Antioxidant activity (mg AA eq /100g)	144.5	138.4	140.3	39.0	39.02	39.05
Beta carotene(µg/g)	37.21	37.28	37.25	2.90	2.91	2.92

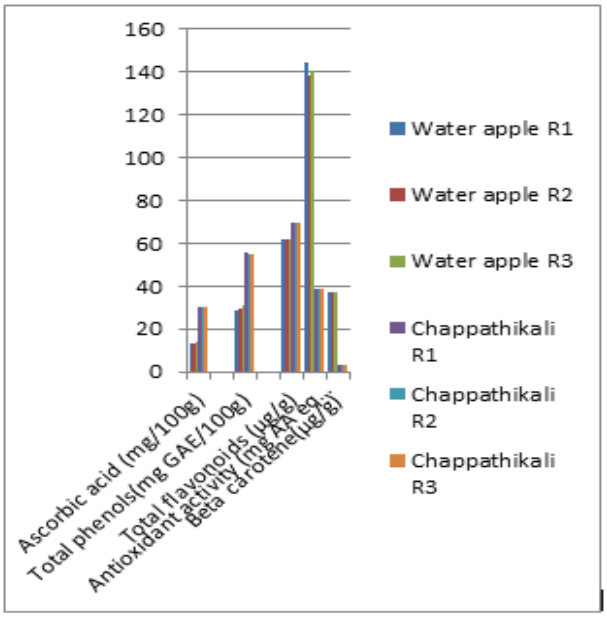

Fig. 1. Phytochemical and antioxidant properties of fresh wild fruits

The ascorbic acid content of water apple was ranged from 13.08 to 13.8mg and chappathikalli ranged from 30.00 to 30.03mg/100g. The phenol content of water apple and chappathikalli were ranged from 28.8 to 30.07 and 55.1 to 55.4 mg GAE/100g. The total flavonoids, antioxidant activity and beta carotene content of water apple and chappathikalli were 62.03 to 62.07 µg/g and 69.50 to 69.51 µg/g, 138.4 to 144.5 mg AA eq /100g and 39.00 to 39.05 mg AA eq /100g, and

37.21 to 37.28 and 2.90 to 2.92 µg/g respectively. The percentage (%) inhibition of DPPH obtained within the assay time in general reflects the antioxidant capacity of the samples. The assay time would usually range from 10-20 min but could be up to 6 hr (Alothman *et al*., 2009b).

Table 2. Proximate Composition of Fresh Wild Fruits

Particulars	Water apple			Chappathikalli		
	R_1	R_2	R_3	R_1	R_2	R_3
Moisture (g%)	91.12	91.70	91.70	83.17	83.35	83.25
Protein (g%)	0.30	0.31	0.30	0.54	0.54	0.52
Fat (g%)	0.19	0.29	0.35	0.38	0.31	0.41
Ash (g%)	1.02	1.37	1.24	1.73	2.03	1.98
Crude fibre (g%)	1.02	1.37	1.24	0.84	0.89	0.79
Acidity (g%)	0.07	0.05	0.07	0.05	0.05	0.05
TSS	5.2	5.4	5.3	6.5	6.3	6.4
pH	4.24	4.08	4.14	4.28	4.28	2.20
Calcium (mg)	0.63	0.62	0.64	18.0	18.02	18.04

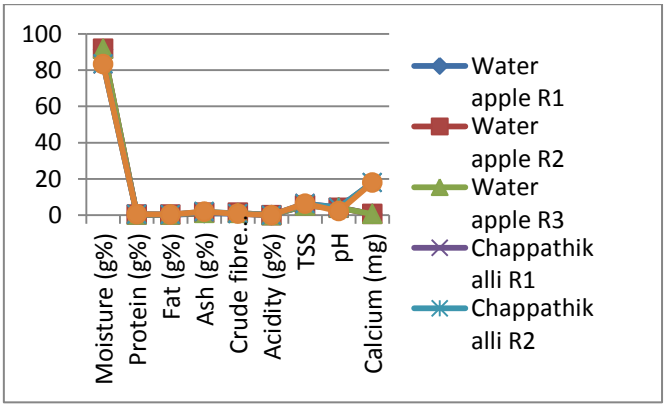

Fig. 2. Proximate composition of fresh wild fruits

The moisture content of water apple was 91.70% and Chappathikalli 83.35%. The high content of moisture in the samples suggested that they have high perishability (Adeleke and Abiodun, 2010). The protein content of water apple and chappathikalli were 0.31, and 0.54. The fat content of the fresh samples were 0.29 and 0.31. They were lower compared to the fat content in Dragon Fruit (Hylecereus polyhizus) reported by Ruzainah et al. (2009), which was 4.5% for freeze-dried sample and 5.5% for oven dried sample. Ash content 1.37, and 2.03, crude fibre 1.37, and 0.89, acidity 0.05, and 0.05, TSS 5.4, and 6.3, pH 4.08, and 4.28, and Calcium 0.62, and 18.02 were found in water apple and chappathikalli fruits respectively.

4. CONCLUSIONS

Based on the result, the highest amount of ascorbic acid, total phenols and total flavonoids were found in chappathikalli fruits when compared to water apple, whereas the total antioxidant activity and beta carotene content were highest in water apple fruit only. The other chemical constituents such as protein, fat, pH, TSS and calcium were highest percentage in chappathikalli compared to water apple. Hence, it can be concluded that chappathikalli has more amount of phytochemical and antioxidant properties, which is very much essential for human health for preventing cancer, hypertension, blood cancer and skin diseases etc. Thus eat fruits for healthy life.

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