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PHYTOCHEMICAL ANALYSIS AND ANTIOXIDANT ACTIVITY OF SELECTED FRUITS

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ABSTRACT
Consumption of fruits has been associated with reduced risk of chronic diseases such as cardiovascular disease and cancer. Phyto chemicals, especially phenolics, in fruits are suggested to be the major bioactive compounds for the health benefits. Fruits such as amla, guava, pomegranate and sweet orange were analyzed comparatively in order to determine the total polyphenols, total flavonoids, tannin content and measuring the antioxidant activity using four different assays, 2,2-diphenylpicrylhydrazil radical scavenging capacity (DPPH), ferric reducing antioxidant power assay (FRAP), Nitric oxide scavenging activity (NOSA) and Superoxide anion scavenging activity (SOSA). Ascorbic acid content ranged from 24.21 - 526.80 mg/100g, total poly phenols content ranged from 128.47 - 2904 mg gallic acid equivalent (GAE)/100g, total flavonoids ranged from 64.20 - 369.46 mg quercetin equivalent (QE)/100g and tannin content ranged from 386.76 - 2140.54 mg tannic acid equivalent (TAE) 100g. Amla fruit showed the highest antioxidant activity based on DPPH, FRAP and NOSA assay (2763.95, 1723.29 and 2562.50 mg AAEAA/100g). The lowest level was obtained for Sweet orange (71.39, 69.63 and 70.05 mg AAEAA/100g). The ranking order for antioxidant ac-tivity of fruits was: Amla > Guava > Pomegranate > Sweet orange in all antioxidant assays expect in SOSA assay.

INTRODUCTION

Antioxidants may be defined as compounds that inhibit or delay the oxidation of other molecules by inhibiting the initiation or propagation of oxidizing chain reactions. The main characteristic of an antioxidant is its ability to trap free radicals (Gulcin *et al.,* 2010). A large number of antioxidants, both nutritive and non-nutritive occur in fruits. Besides the nutrients such as â carotene, vitamin C and vitamin E, number of carotenoids, flavonoids and phenols also occur naturally in fruits and act as antioxidants which serve free radical scavenging activities and play a significant role in the prevention of many diseases (Prakash and Kumar, 2011).

The three major groups of natural antioxidants in fruits are vitamin C, phenolics and carotenoids; especially â-carotene is responsible for the defensive effect of antioxidants in fruits. Vitamin C and phenolics are known as hydrophilic antioxidants and carotenoids are known as lipophilic antioxidants (Thaipong *et al.,* 2006). Phenolic compounds are secondary metabolites in fruits and vegetables. They have been reported to exhibit antioxidant activity which allows them to scavenge both active oxygen species and electrophiles, to inhibit nitrosation and to chelate metal ions, to have the potential for autoxidation and the capability to modulate certain cellular enzyme activities (Huda-Faujan *et al.,* 2009). Carotenes have been proved to possess antioxidant activity due to their ability to quench singlet oxygen and inhibit lipid peroxidation. Beta-carotene is the most common carotenoid with lipophilic antioxidant activity and pro-vitamin A activity (Alquezar *et al.,* 2008).

Since many degenerative human diseases have been recognized as being a consequence of free radical damage, there have been many studies undertaken on how to delay or prevent the onset of these diseases. The most likely and practical way to fight against degenerative diseases is to improve body antioxidant status, which could be achieved by higher consumption of vegetables and fruits. Foods from plant origin usually contain natural antioxidants that can scavenge free radical. Epidemiological evidence has clearly shown that diets based on fruits and vegetables, with high content of natural antioxidants, contribute to reduced mortality from cardiovascular and cerebrovascular disease (Alia *et al.,* 2003).

In view of huge importance of fruits as antioxidant sources, in the present intends to analyse and compare the total antioxidant activity and photochemical properties of commonly consumed fruits and four different *in vitro* antioxidant methods have been used to compare the antioxidant activity of fruits.

MATERIALS AND METHODS

Samples

The fruits selected for the study include amla (*Phyllanthus emblica* syn. *Emblica officinalis)*, guava (*Psidium guajava),* pomegranate (*Punica granatum*) and sweet orange (*Citrus sinensis*). Fresh and fully mature, good quality fruits purchased from the local market of Madurai city. The procured fruits were inspected to discard the spoiled, bruised and imma-ture ones.

Preparation of sample extract

Methanol was used to extract antioxidant phyto chemicals from samples. Fifty grams of sample was ground in a domestic blender and 1 g of the ground sample was crushed using a pestle and mortar with 30 ml of methanol. The homogenate was centrifuged at 4,500 rpm for 10 min to obtain a clear supernatant liquid. The residue was re-extracted with methanol (15 ml) and centrifuged. The supernatants were pooled together and filtered using What man No.1 filter paper and made up to a known volume (Gupta and Prakash, 2009). The extract was then kept in freeze for future use.

Phytochemical analysis and antioxidant activity

Ascorbic acid content was estimated by titration method and total polyphenols were determined by the spectro photometric method, as described by Sadasivam and Manickam (2008). Total flavonoids were measured using aluminium chloride colorimetric assay, as described by Marinova *et al*. (2005). The analysis of antioxidant activity was carried out using Diphenyl- picryl- hydrazyl (DPPH) assay (Goupy *et al*., 1999), Nitric oxide scavenging activity (NOSA) (Garrat, 1964), Superoxide anion scavenging activity (SOSA) (Winter bourn *et al*., 1975) and Ferric reducing antioxidant power (FRAP) assay (Benzie and Strain, 1996) with some modifications.

Statistical analysis

Data from all experiments were performed in triplicate for each sample. The results of the three replicates were pooled and expressed as mean ± standard deviation. Data were analyzed using Data Entry Module for AGRES Statistical Software. The 50% inhibitory concentration (IC_{50}) was calculated according to Concentration-Effect regression line.

RESULTS AND DISCUSSION

Phytochemical analysis of fruits

The phytochemical content of the fruits was determined by different assays and the results are shown in Table 1. The ascorbic content ranged from 24.21 mg/100g in pomegranate to 526.80 mg/100g in amla fruit. Among the fruits studied amla contains highest amount of ascorbic acid followed by guava, sweet orange and pomegranate. The total polyphenols content in the fruits analyzed were in the range of 128.47 - 2904 mg GAE/100g. Among all the fruits analyzed, the amla fruit revealed the highest total polyphenols at 2904 mg GAE/ 100g followed by guava (204.53 mg GAE/100g), pomegranate (148.61 mg GAE/100g) and sweet orange (128.47 mg GAE/ 100g) . The total flavonoids content ranged from 64.20 mg QE/100g in sweet orange to 369.46 mg QE/100g in amla fruit. For the other fruits the total flavonoids content were as follows: guava (287.44 mg QE/100g and pomegranate (209.83 mg QE/100g). The highest tannin content was found in amla (2140.54 mg TAE/100g), followed by pomegranate (1620.45 mg TAE /100g), guava (403.86 mg TAE /100g) and the lowest was found in sweet orange (386.76 mg TAE /100g). There were highly significant differences was noted in phytochemical content among fruits ($p < 0.05$).

Among the nine tropical fruits studied by Lim *et al*. (2007), guava (both seeded and seedless) have the largest ascorbic acid content values, which are higher than that of orange. The ranking order observed in total polyphenols content is similar to the observation made by Ali *et al*. (2010) although the values of total polyphenols observed in the present study are higher. The most probable reason for observed higher values may be the quality of the fruits and difference in the variety of fruits. Liu *et al*. (2008) reported that total phenolic content of amla fruit was ranged from 81.5 to 120.9 mg GAE/g and the flavonoid content was varied from 20.3 to 38.7 mg QE/g. Ardekani *et al*. (2011) stated that the flavonoid content in the pulp extracts of Persian pomegranate cultivars ranged from 0.84 to 2.14 mg catechin equivalents/g and the total phenolic contents varied from 11.62 to 21.03 mg GAE/g of extract. Khomdram and Shantibaladevi, (2010) reported that the vitamin C content of *Emblica officinalis* was 379.7 mg/100g and lowest in *Punica granatum* (14.4 mg/100g).

Antioxidant activity of fruits

Different antioxidant components may act *in vivo* through different mechanisms, no single method can fully evaluate the total antioxidant activity of foods. So, more than one type of measurement for antioxidant activity needs to be performed to take into account the various modes of action of antioxidants. In this study four different assays which have been widely used to determine the antioxidant activities of fruits were applied to obtain data on antioxidant activity of the selected fruits. Table 2 includes the mean values for antioxidant activity mea-sured. Amla fruit showed the highest antioxidant activity based on DPPH, FRAP and Nitric oxide scavenging activity assay (2763.95, 1723.29 and 2562.50 mg AAEAA/100g). The lowest level was obtained for Sweet orange (71.39, 69.63 and 70.05 mg AAEAA/100g). The ranking order for antioxidant ac-tivity of fruits was: Amla > Guava > Pomegranate > Sweet orange expect in superoxide anion scavenging activity assay. In superoxide anion scavenging activity assay, amla fruit showed the highest an-tioxidant activity (2236.19 mg AAEAA/ 100g) followed by pomegranate (181.13 mg AAEAA/100g), guava (165.89 mg AAEAA/100g) and the least activity was measured in sweet orange (65.81mg AAEAA/100g). There were significant differences found between fruits ($p < 0.05$).

The highest antioxidant activity observed for amla in the present

Table 1: Phytochemical analysis of fruits

Fruits	Ascorbic acid(mg/100g)	Total Polyphenols(mg/100g)	Total Flavonoids(mg/100g)	Tannins(mg/100g)
Amla	526.80 ± 3.42	2904.00 ± 7.29	369.46 ± 4.26	2140.54 ± 3.19
Guava	236.00 ± 8.44	204.53 ± 3.68	287.44 ± 12.43	403.86 ± 5.09
Pomegranate	24.21 ± 0.40	148.61 ± 3.75	209.83 ± 4.72	1620.45 ± 7.30
Sweet orange	102.20 ± 0.18	128.47 ± 2.43	64.20 ± 2.48	386.76 ± 2.44
S.ED	7.4374	52.3814	6.4828	13.3382
CD(0.05)	17.1508**	120.7932**	14.9496**	30.7582**

Table 2: Antioxidant activity of fruits

Fruits	AAEAA mg/100g DPPH free radical scavenging activity	Ferric reducing antioxidant power	Nitric oxide scavenging activity	Superoxide anion scavenging activity
Amla	2763.95 ± 59.59	1723.29 ± 18.78	2562.50 ± 80.08	2236.19 ± 64.50
Guava	218.75 ± 7.09	151.31 ± 1.77	198.75 ± 1.07	165.89 ± 2.24
Pomegranate	198.31 ± 5.36	143.24 ± 5.55	192.25 ± 6.23	181.13 ± 7.02
Sweet orange	71.39 ± 3.02	69.63 ± 0.87	70.05 ± 0.75	65.81± 1.72
S.ED	9.9916	19.9023	13.6882	30.6591
CD(0.05)	23.0409**	45.8953**	31.5655**	70.7008**

AAEAA – Ascorbic Acid Equivalent Antioxidant Activity

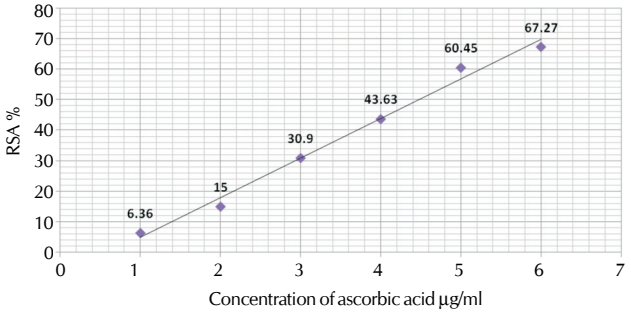

Figure 1: IC_{50} value of free radical scavenging activity of ascorbic acid by DPPH method

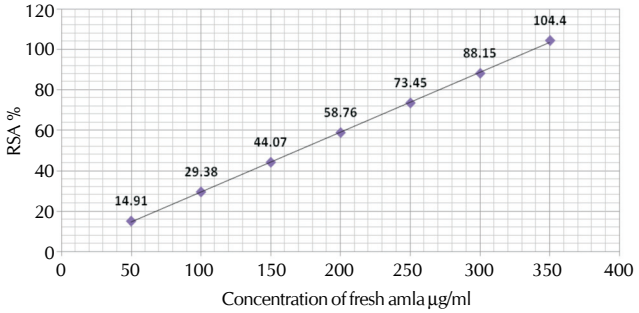

Figure 2: IC_{50} value of free radical scavenging activity of fresh amla by DPPH method

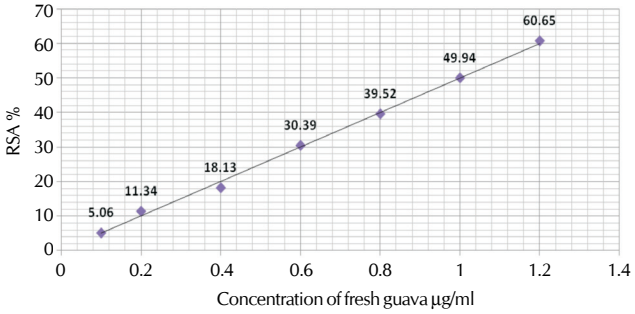

Figure 3: IC_{50} value of free radical scavenging activity of fresh guava by DPPH method

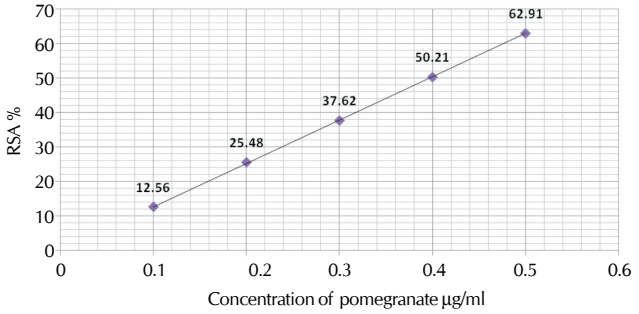

Figure 4: IC_{50} value of free radical scavenging activity of fresh pomegranate by DPPH method

investigation may be due to high content of vitamin C and other compounds which have antioxidant activity. It is nutritionally and medicinally important fruit due to high contents of vitamin C and used as a principle ingredient in the preparation of famous ayurvedic tonic chavyanpras. Recently, consumption of amla juice has also increased. Guava fruits has the richest content of ascorbic acid from other temperate fruit (Yan *et al*., 2006), that support our findings having amla the highest antioxidant activity as its vitamin C content was the highest from the rest of the fruits. From our findings among the fruits, pomegranate fruit that is found to be have considerable activity of antioxidant, though its vitamin C content was in reasonable level and at same time sweet orange having high amount of vitamin C content next to amla and guava. These may be attributed to presence of other antioxidant phytochemicals. It is known that majority of the antioxidant activity of the fruits are contributed by polyphenols, vitamin C, vitamin E, Maillard reaction products, â- carotene and lycopene. The differences in the antioxidant activities among the fruits could be attributed to their differences in phenolic contents and compositions and to other non-phenolic antioxidants present in the fruits (Hassanien, 2008). Jamuna *et al*. (2011) stated that *P. emblica* has exhibited highest antioxidant activity among the various fruits studied. The total antioxidant capacity of *Phyllanthus emblica* was 248.50 µg ascorbic acid/mg of extract, 57.30 µg ascorbic acid/mg of extract for *Psidium guajava* and 19 µg ascorbic acid/mg of extract for *Citrus sinensis* of ascorbic acid/mg of extract by DPPH assay. The ascorbic acid equivalents for reducing power were 595.00, 23.60 and 11.60 µg/mg of extract in amla, guava and sweet orange respectively.

Free radical scavenging activity (IC_{50}) of fruits by DPPH method

The IC_{50} value for each fruit extract defined as the concentration of extract causing 50 per cent inhibition of absorbance was

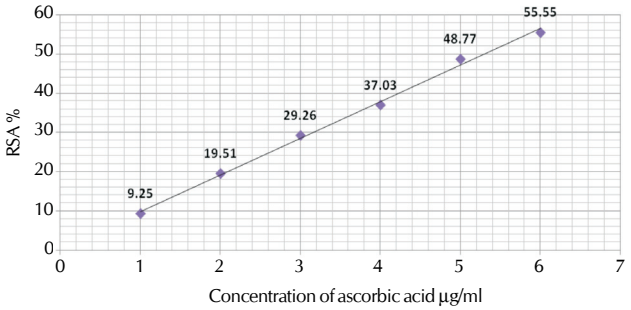

Figure 5: IC_{50} value of free radical scavenging activity of fresh sweet orange by DPPH method

calculated, since IC_{50} is a measure of inhibitory concentration, a lower IC_{50} value would reflect greater antioxidant activity of the sample. The methanolic extracts of fresh fruits were tested for their antioxidant activity by DPPH assay and its antioxidant activity is comparable to the standard ascorbic acid.

IC_{50} values of both standards and extracts in the DPPH assay are shown in Fig. 1 to 5. Compounds presenting the lowest IC_{50} values are considered the best antioxidant. IC_{50} value of standard ascorbic acid was 6.70 μg/ml. Among the fruits, amla was found to have lowest IC_{50} value (170 µg/ml) followed by pomegranate (0.4 mg/ml), guava fruit (1 mg/ml) and sweet orange (5.2 mg/ml). Khomdram and Shantibaladevi (2010) stated that the IC_{50} value of *Emblica officinalis* (amla) and *Punica garanatum* (pomegranate) was 181µg/ml and 398ìg/ml respectively. Qusti *et al*. (2010) found that the IC_{50} value of pomegranate was 0.53 mg/ml in fresh weight. Yan *et al*. (2006) found that the IC_{50} value of guava and orange was 1.71 mg/ml and 5.4 mg/ml respectively.

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