

ANTIOXIDANT ACTIVITIES OF SOME LESS UTILIZED EDIBLE FRUITS

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Received: 22 May 2017, Revised and Accepted: 22 Jul 2017

ABSTRACT

Objective: The objective of the present study was to evaluate the total phenolic (TPC) and flavonoid content (TFC) of five less utilized fruits such as *Aegle marmelos*, *Spondias pinnata*, *Limonia acidissima*, *Averrhoa carambola*, *Crescentia cujete* and was compared with *Phyllanthus emblica*, (Amla) well known for its antioxidant activities. Total phenolic and flavonoid contents of samples were correlated with antioxidant activities like 1,1-Diphenyl-2-Picryl-Hydrazyl (DPPH) free radical scavenging assay, Ferric reducing antioxidant potential (FRAP) assay and total antioxidant capacity (TAC).

Methods: The total phenolic of each fruit extract were determined by the Folin-Ciocalteu method with some modifications and the total flavonoids were estimated by Aluminum trichloride colourimetric method. The DPPH antioxidant assay, The FRAP assay and TAC were determined spectrophotometrically.

Results: The total phenolics were expressed as mg/100g Gallic acid equivalent (mg GAE/100 gm) and the total flavonoids were expressed as mg/100g Quercetin equivalent (mg QE/100 gm). TPC was found to be maximum in *Spondias pinnata* with 142.16 mg GAE/100 gm where as TFC was maximum in *Phyllanthus emblica* with 91.1 mgQE/100 gm. DPPH radical scavenging activity was expressed in percentage(%), FRAP values expressed as mg/100g Ascorbic equivalent (AAE) and the total antioxidant activity was expressed as mg/100g Ascorbic equivalent. Maximum DPPH radical scavenging activity was shown by *Spondias pinnata* (93.75%), FRAP values were maximum in *Phyllanthus emblica* with 72.6 mg AAE/100 gm and total antioxidant capacity was found to be highest in *Spondias pinnata* (50.1 mg AAE/100 gm).

Conclusion: *Spondias pinnata*, an underutilized fruit, was found to be promising with antioxidant activities comparable to *Phyllanthus emblica*.

Keywords: Less utilized fruits, Total phenolic, Total flavonoid, *Spondias pinnata*

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DOI: <http://dx.doi.org/10.22159/ijcpr.2017v9i5.22132>

INTRODUCTION

Studies have shown that fruits are the sources of diverse phytochemicals many of which display antioxidant properties [1]. The natural antioxidants present in fruits reduce the level of oxidative stress [2] and has been scientifically shown that by combating oxidative stress many diseases such as heart disease, cancer, diabetes, hypertension, stroke and Alzheimer's disease can be prevented [3, 4]. The antioxidative effect of the fruit is mainly due to phenolic components [5]. Presently much attention has been paid to the lesser known fruits with the principal purpose of utilizing them as a functional food and as ingredients in nutraceuticals [6, 7]. The food security becomes vulnerable when it is dependent on a few numbers of the crop [8]. Enriching the shrinking resource base of our food basket is urgently required [9] and this can be achieved by exploiting and utilizing the wild and less utilized sources. The present study was carried out with five less utilized fruits like *Aegle marmelos* (L.) Corr. Serr, *Spondias pinnata* (L. f.) Kurz, *Limonia acidissima* L., *Averrhoa carambola* L. and *Crescentia cujete* collected from different parts of South Gujarat. The Total Phenolic Content (TPC), Total Flavonoid Content (TFC), DPPH radical scavenging activity, Total Antioxidant Capacity (TAC), and Ferric Reducing Antioxidant Potential (FRAP) of the above-mentioned fruits were evaluated. total phenolic and Flavonoid contents of samples were correlated with the different antioxidant activities. *Phyllanthus emblica* L commonly known "Amla", well known for its rich polyphenol content and antioxidant properties was used as positive control [10, 11].

MATERIALS AND METHODS

Chemicals and reagents

Folin-Ciocalteu, sodium carbonate, aluminum trichloride (AlCl₃), methanol, DPPH, deionized distilled water, ethanol, hydrochloric acid (HCl), potassium ferricyanide sodium dodecyl sulfate (SDS), ferric chloride (FeCl₃), ammonium molybdate, sodium phosphate, Ascorbic Acid were purchased from Hi-meida, India. Gallic acid and Quercetin were purchased from Sigma.

Instruments

Hot air oven grinder, digital balance water bath, hot plate, magnetic stirrer, centrifuge, thermometer, microwave oven, spectrophotometer, refrigerator, glassware (conical flasks, beakers, test tubes, petri plates, glass rod), micro pipettes.

Fruit sample collection and extract preparation

Fresh fruit samples of *Aeglemarmelos*, *Spondias pinnata*, *Limonia acidissima*, *Averrhoa carambola*, *Crescentia cujete* and *Phyllanthus emblica* were collected from Dharampur and nearby areas of South Gujarat in the month of December-January. Fruits were botanically identified with the help of local flora and authenticated by experts. All the fruits were washed with running water and then finally with distilled water. The edible portion of fruits (1 gm) was homogenized with 10 ml of an aqueous methanol solution (70% methanol). The homogenate was stirred with a magnetic stirrer at 900 rpm at room temperature for 30 min. The extract was centrifuged at 3000 rpm for 20 min. The supernatant was removed and filtered with Whatman filter paper (No.1). The process under vacuum and dissolved in methanol: water (4:1) ratio [7].

Determination of total phenolic

The total phenolic of each fruit extract was determined by the Folin-Ciocalteu method with some modifications [7]. The diluted aqueous solution of each extract (0.5 ml) was mixed with Folin Ciocalteu reagent (0.2 N, 2.5 ml). This mixture was allowed to stand at room temperature for 5 min and then sodium carbonate solution (75 g/l in water, 2 ml) was added. After 2 h of incubation, the absorbance was measured at 760 nm against a water blank. A standard calibration curve was plotted using Gallic acid.

Determination of total flavonoid

The total flavonoids were estimated by aluminium trichloride colorimetric method [12]. A diluted methanolic solution (2 ml) of each

fruit extract was mixed with a solution (2 ml) of $AlCl_3$ in methanol (2 %). The absorbance was read at 415 nm after 10 min against a blank sample consisting of a methanol (2 ml) and with $AlCl_3$. Quercetin was used as reference compound to produce the standard curve.

DPPH free radical scavenging assay

The DPPH antioxidant assay was determined as described by [13]. Briefly, 0.1 mmol DPPH (1 ml) dissolved in ethanol was added to an ethanol solution (3 ml) of the tested compound at different concentrations (0, 40, 60, 80, 100 μ g/ml). An equal volume of ethanol was added in the control test. The mixture was shaken vigorously and allowed to stand at room temperature for 30 min. Then the absorbance at 517 nm was measured with a UV spectrophotometer. The percentage of scavenging of DPPH was then calculated in the following way:

$$\text{DPPH scavenging effect (\%)} = [1 - (\text{Test sample absorbance} / \text{blank sample absorbance})] \times 100(\%)$$

Modified ferric reducing antioxidant potential (FRAP) assay

The FRAP assay was done according to Oyaizu method with some modifications [14]. To 0.1 ml of each fruit extract, 0.9 ml 96% ethanol, 5 ml of distilled water, 1.5 ml of 1M Hydrochloric acid (HCl), 1.5 ml of 1% potassium ferricyanide $K_3[Fe(CN)_6]$, 0.5 ml of 1% Sodium dodecyl sulfate (SDS) and 0.2% 0.5 ml ferric chloride ($FeCl_2$) were added to each extract. The mixture was boiled in a water bath at 50 °C for 20 min after boiling the mixture was rapidly cooled and mixed well. The increase in the absorbance at 750 nm was used to measure the reducing power of the fruit extract. Ascorbic acid was used as a positive control.

Total antioxidant capacity (TAC) by phospho- molybdenum method

The total antioxidant capacity of the methanol extract was evaluated by the phosphomolybdenum method according to the procedure described by [15]. A 0.2 ml extract was combined with 2 ml of reagent solution (0.6M sulfuric acid, 28 mmol sodium phosphate and 4 mmol ammonium molybdate). The absorbance of the reaction mixture was measured at 695 nm using a spectrophotometer against a reagent blank after incubation it at 95 °C for 90 min and cooling to room temperature. Reagent (2 ml) in the place of the extract was used as the blank. The antioxidant activity is expressed as the number of mg/gm equivalent of Ascorbic Acid. (AAE).

The results of the all these study were presented as the mean of three determinations along with standard deviation. Statistical analysis was done using MS Excel software (CORREL Statistical function).

RESULTS

Total phenolic and flavonoids content

The total phenolic was expressed as mg/100g Gallic acid equivalent (mg GAE/100 gm) using the standard curve equation: $y = 0.0087x$

$+0.018$, $R^2 = 0.9994$, Where y is an absorbance at 760 nm and x is the total phenolic content of the fruits (fig. 1).

In the present study, all the fruits were found to be quite rich in phenolic. The total content of phenolic ranges from 101.20 ± 2.39 in *Crescentia cujete* to 142.16 ± 0.7 as found in *Spondias pinnata* (table 1). The total flavonoids were expressed as mg/100g Quercetin equivalent (mg QE/100 gm) using the standard curve equation: $y = 0.005250x + 0.123$, $R^2 = 0.998$, Where y is an absorbance at 415 nm and x is total flavonoids content (fig. 2). Total flavonoids varied from 42.4 ± 0.41 mgQE/100 gm in *Averrhoa carambola* to 91.1 ± 0.55 mgQE/100 gm in *Phyllanthus emblica* (table 1). Variation in total phenolic and flavonoids was observed among the studied fruits. TPC was higher in *Spondias pinnata* where as TFC was maximum in *Phyllanthus emblica* (table 1).

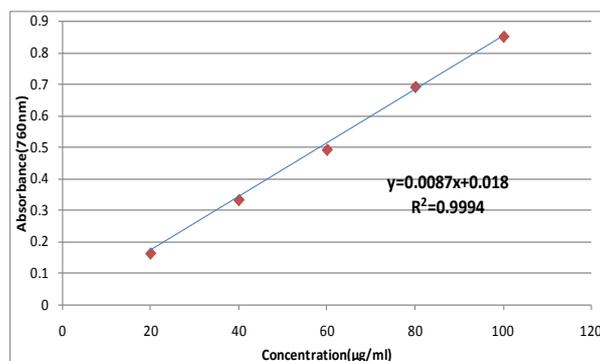


Fig. 1: Standard curve of gallic acid

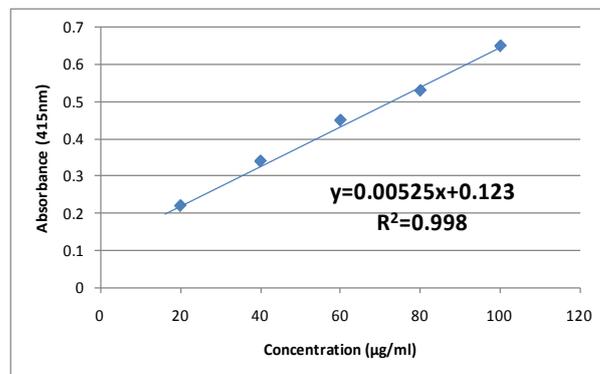


Fig. 2: Standard curve of quercetin acid

Table 1: Total phenolic and total flavonoid of the studied fruits

Fruit species	Total phenolics (mgGAE/100 gm)	Total flavonoids (mgQE/100 gm)
<i>Phyllanthus emblica</i>	130.33 \pm 1.8	91.1 \pm 0.55
<i>Spondias pinnata</i>	142.16 \pm 0.7	77.10 \pm 0.43
<i>Aegle marmelos</i>	117.33 \pm 1.3	52.2 \pm 0.30
<i>Averrhoa carambola</i>	104.32 \pm 2.42	42.4 \pm 0.41
<i>Limonia acidissima</i>	103.66 \pm 3.01	47.7 \pm 0.35
<i>Crescentia cujete</i>	101.20 \pm 2.39	49.1 \pm 0.7

Each value represents the mean of three replicated+Standard Deviation.

DPPH free radical scavenging assay, modified ferric ion reducing assay and total antioxidant capacity

In the present study, all the extracts showed free radical scavenging properties at different levels. Maximum DPPH radical scavenging activity was shown by *Spondias pinnata* (93.75 ± 1.06) followed by *Phyllanthus emblica* (93.5 ± 1.76). *Averrhoa carambola* showed the least activity of 77.5 ± 3.53 (table 2).

FRAP values were obtained by comparing the absorbance change at 750 nm in test reaction mixtures with those containing ferrous ions in a known concentration of Ascorbic acid and were expressed as mg/100g Ascorbic equivalent (AAE) using the standard curve equation: $y = 0.0291x + 0.2348$, $R^2 = 0.966$ (fig. 3). The value ranges from 30.6 ± 0.11 mg AAE/100 gm in *Limonia acidissima* to 72.6 ± 0.4 mg AAE/100 gm in *Phyllanthus emblica* (table 2). *Spondias pinnata* also showed very high activity (70.0 ± 0.2 mg AAE/100 gm).

The total antioxidant activity is expressed as the number of equivalents of ascorbic acid. The value was expressed as mg/g Ascorbic equivalent using the standard curve equation: $y = 0.00415x + 0.075$, $R^2 = 0.9800$ (fig. 4).

Among the extracts of the different fruits undertaken for the study, total antioxidant capacity was found to be highest in *Spondias pinnata* (50.1±0.5 mg AAE/100 gm) followed by *Phyllanthus emblica* (46.0±1.2 mg AAE/100 gm). The least value of 10.2±0.5 mg AAE/gm was found in *Averrhoa carambola* (table 2).

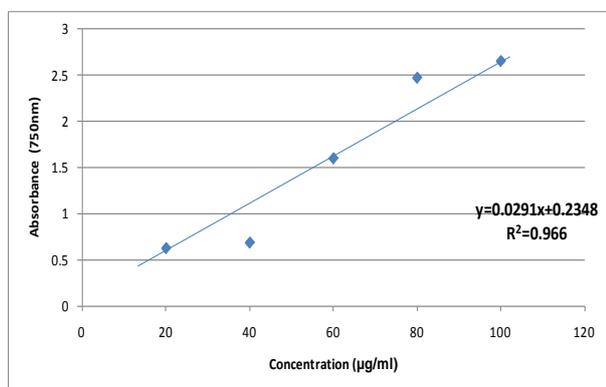


Fig. 3: Standard curve of ascorbic acid for for FRAP assay

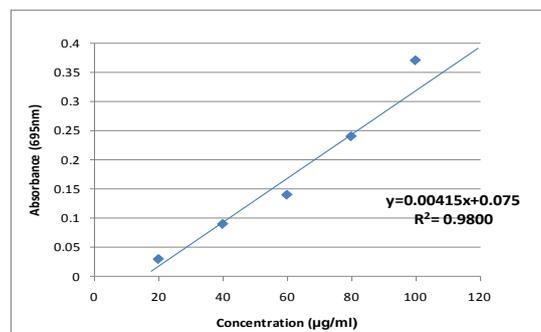


Fig. 4: Standard curve of ascorbic acid for TAC

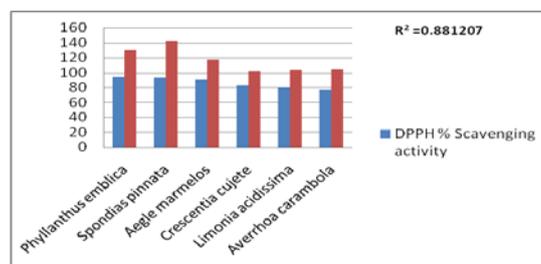


Fig. 5: Linear correlation between DPPH scavenging activity and total phenolics

Table 2: DPPH Free radical scavenging assay, FRAP assay and TAC of the studied fruits

Fruit species	DPPH % scavenging activity	Ferric reducing power assay (mg AAE/100 gm)	Total antioxidant capacity (mg AAE/100 gm)
<i>Phyllanthus emblica</i>	93.5±1.76	72.6±0.4	46±1.2
<i>Spondias pinnata</i>	93.75±1.06	70.0±0.2	50.1±0.5
<i>Aegle marmelos</i>	90.62±1.94	67.6±0.1	18.5±0.7
<i>Crescentia cujete</i>	83.25±1.06	60.6±0.5	23.1±0.1
<i>Limonia acidissima</i>	80.00±0.71	50.0±0.15	30.0±0.4
<i>Averrhoa carambola</i>	77.5±0.53	30.6±0.11	10.2±0.5

Each value represents the mean of three replicates+Standard deviation

DISCUSSION

Phenolics are a class of antioxidant agents with very strong redox properties, acting as hydrogen donors, singlet oxygen quenchers, metal chelators and scavengers of free radicals [16, 17]. In the present study, the methanolic extract of *Spondias pinnata* was found to possess maximum total phenolic. The high content of total phenolic of *S. pinnata* was also reported in a previous work conducted in Nepal [18]. Flavonoids also possess high antioxidant activity which is due to their ability to reduce free radical formation and to scavenge free radicals [19]. The free radical scavenging activity of methanolic extracts of different fruits was studied by their ability to reduce the DPPH. DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule [20]. A freshly prepared DPPH solution is of deep purple colour with an absorption maximum at 517 nm and in the presence of antioxidant this colour disappears due to quenching of DPPH free radicals and converting them into a colourless product i.e. 2, 2-diphenyl-1-hydrazine. Antioxidant mechanism performed by providing hydrogen atoms or [21]. In the FRAP assay, the antioxidants reduce Fe^{3+} to Fe^{2+} . This ion then conjugates with Ferricyanide ion to form Prussian blue coloured product which was spectrophotometrically measured at 750 nm wavelength. [22]. Total antioxidant capacity by phospho- molybdenum method assay is based on the reduction of Mo (VI) to Mo (V) by the sample analyte and the subsequent formation of green phosphate/Mo (V) complex at acidic pH. In the present study, the strong and positive correlation was observed between total phenolics and total flavonoids of the undertaken fruits with antioxidant assays like DPPH radical

scavenging activity, FRAP assay and with total antioxidant capacity (TAC). Maximum correlation ($R^2=0.881$) was observed between total phenolics and DPPH radical scavenging activity (fig. 5).

This suggests that 88% of the antioxidant potential with respect to the ability to scavenge DPPH radical results from the TPC of the fruits undertaken for the study. It was also observed that *Spondias pinnata* which has the highest phenolic content possess maximum DPPH radical scavenging activity as compared to other fruits undertaken for the study (table 2). In a previous work with different leaf extracts of blackberry species, similar types of the result of high DPPH radical scavenging activity correlating with high phenol content was observed [23].

The results in the present study indicate that though phenol and flavonoids are the major contributors of antioxidant activities of the fruits are not limited to the phenolic only, other secondary compounds such as ascorbic acid, β -carotene, α -carotene and different xanthophylls might be playing a significant role for the antioxidant activity [24].

CONCLUSION

The present study showed that all the less known edible fruits which were never compared together for their antioxidant activities have immense potential as a source of antioxidant. Of these, *Spondias pinnata*, a wild edible fruit [25] was found to possess higher TPC value than that of Amla, with equally significant TFC value and antioxidant capacity.

However, more detailed study with respect to phytochemical content and other *in vivo* assays are required to establish these less known edible fruits as powerful antioxidants.

Sustainable use of wild fruits needs to be promoted. Wider and sustained acceptance of wild fruits as important dietary components must be stimulated to solve the problems of food scarcity [26].

CONFLICT OF INTERESTS

None of the authors declared any conflict of interest.

ACKNOWLEDGEMENT

We are indebted to Dr. P. V. Desai, Principal, and Dr. S. Desai, Vice Principal, Bhagwan Mahavir College of Science and technology for supporting us and providing us the necessary facilities.

AUTHOR CONTRIBUTION

The above study was carried out by research students Ms. Maitry Pandya and Ms. Nirali Patel under the supervision of Dr. Sumita Dasgupta.

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How to cite this article

- Sumita Dasgupta, Maitry Pandya, Nirali Patel. Antioxidant activities of some less utilized edible fruits. *Int J Curr Pharm Res* 2017;9(5):28-31.