International Food Research Journal 21(1): 119-124 (2014)
Journal homepage: *http://www.ifrj.upm.edu.my*

Total phenolic content, reducing power, antioxidative and anti-amylase activities of five Bangladeshi fruits

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Article history

Received: 8 August 2013
Received in revised form: 8 October 2013
Accepted: 13 October 2013

Keywords

Phenols
Antioxidant capacity
Reducing power
Anti-amylase activity
Fruits

Abstract

Phenolic content, antioxidant and anti-amylase activities were studied in the ethanolic extract of five available Bangladeshi fruits. The fruits were *Averrhoa bilimbi* (Bilimbi), *Artocarpus lacucha* (Monkey jack), *Cucumis melo* (Mask melon), *Phoenix sylvestris* (Wild date palm) and *Flacourita jangomas* (Indian plum). *P. sylvestris* had the highest total phenolic content (37.40 ± 1.72 mg GAE/10 g of extract), whereas *C. melo* had the lowest (6.02 ± 0.89 mg GAE/10 g of extract). All the fruits showed DPPH free radical scavenging activity with the IC_{50} values for *P. sylvestris*, *A. lacucha*, *F. jangomas*, *C. melo* and *A. bilimbi* were 1.90 µg/ml, 0.798 mg/ml, 1.144 mg/ml, 1.695 mg/ml and 3.683 mg/ml respectively. Highest level of reducing activity was found in *P. sylvestris* (O.D. 0.933 ± 0.02) and reducing power activity was lowest in *A. bilimbi* (O.D. 0.249 ± 0.01) at a concentration of 0.4 mg/ml. But all the fruits showed a dose-dependent increase in reducing power. The fruit extracts showed very weak inhibition of α-amylase activity. But highest activity found in case of *P. sylvestris* was 11.88 ± 3.69% and the lowest activity found for *F. jangomas* was 3.33 ± 0.64%. Considering the data, it can be concluded that among the five fruits *P. sylvestris* is the most health beneficiary.

Introduction

Free radicals are the cause of many pathological and physiological abnormalities such as aging, cardiovascular disorders, cancers and neurodegenerative diseases (Astley, 2003). Antioxidants which scavenge these free radicals can however reduce the risk of these diseases. Fruits have many health beneficiary functions. Recent research has confirmed that consumption of fruits and vegetables can reduce the risk of stroke and cancer (Beecher, 1999; Bae *et al*., 2008; Kawasaki *et al*., 2008; Wright *et al.*, 2008) as well as inflammation and problems caused by aging (Ames *et al.*, 1993). This risk reduction is related to the presence of antioxidative agents in fruits. Besides the antioxidative capacity, the phenolic compounds of plants also have other physiological roles: flavone and flavonoids inhibit α-amylase and α-glucosidase activities (Havsteen, 1983; Kim *et al*., 2000); polyphenols have anti-hyperglycemic effects (Hossain *et al.*, 2002; Hanamura *et al.*, 2006) and inhibit the development of diabetes (Zunino *et al.*, 2007). Besides the health beneficiary effect of fruits, the antioxidative agents of fruits may serve as natural source of antioxidants which may be used for increasing the self life of food items. At present most of the antioxidative agents are synthetic which have many side effects when consumed *in vivo* (Chen *et al*., 1992).

The determination of phenolic compounds in fruits, vegetables and other foods has been of increasing interest in recent years (Palma *et al.*, 2002). Although the phenolic content and antioxidative properties of many fruits of Bangladesh have been investigated, reports comparing the antioxidative and other physiological activities of Bangladeshi fruits are few (Hossain *et al*., 2008). Moreover, it is known that, amongst other factors, such as maturity stage or light exposure, phenolic composition varies with the cultivar (Ferreres *et al.*, 2009). So the secondary metabolites of fruits grown in Bangladesh may be different from fruits grown in other countries. The purpose of this study is to determine and compare the phenolic content, antioxidative and anti-amylase activities in the ethanolic extracts of five fruits, cheap and normally well grown in rural areas of Bangladesh, to find out fruits with good physiological activities and potentiality for industrial use as food supplement and preservative.

It is reported that the extraction efficiency of phenolic compounds from plant materials is influenced by various factors including the nature of solvent used for extraction (Pinelo *et al*., 2005). Although there are some published articles on the phenolic content and antioxidative activities of these fruits separately from their methanolic extract

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(Ikram *et al*., 2009; Hasan *et al*., 2009; Ismail *et al.*, 2010; Rahman *et al*., 2012; Prakash *et al*., 2013; Hassanuzzaman *et al.*, 2013) but no study was found on the phenolic content and antioxidative activities of these five fruits independently or in a single study from their ethanolic extract except *A. bilimbi* (Kolar *et al*., 2011). So it is important to investigate the phenolic content and antioxidative activities of these fruits from their ethanolic extracts. Again this is the first study on the phenolic content, antioxidative and anti-amylase activities of *P. sylvestries* and *C. melo* grown in Bangladesh.

Methods and Materials

Fruit samples

The experimental fruits were collected from different markets and areas of Chittagong, Bangladesh and were identified as *Averrhoa bilimbi* L. (Oxalidacae), *Artocarpus lacucha* Buch.-Ham (Moraceae), *Cucumis melo* L. (Cucurbitacae), *Phoenix sylvestris* (L.) Roxb. (Arecaceae) and *Flacourtia jangomas* (Lour.) Rausch (Flacourtieae).

Chemicals

Potassium ferricyanide [$K_3Fe(CN)_6$], 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH•), trichloroacetic acid, $AlCl_3$, ascorbic acid and $FeCl_3$ were purchased from Sigma-Aldrich (St. Louis, Mo., U.S.A.). Folin-Ciocalteus's phenol reagent, ethanol, and sodium carbonate were from Merck Chemical Supplies (Merck KGaA, Darmstadt, Germany). Gallic acid was purchased from Nacalai Tesque, Kyoto, Japan and bacterial α-amylase was purchased from Wako Pure Chemical Industry Ltd., Osaka, Japan. All the other chemicals used including solvents were of analytical grade.

Preparation of samples taken for analysis

The freshly collected green-matured fruits were initially washed with tap water thoroughly until the attached dust particles, unicellular algae etc. were removed. Finally they were washed with distilled water. Green-matured fruits were cut into small pieces and sun-dried. The dried fruits were ground into powder with a grinder. The powders were stored separately in air-tight containers and kept in a cool, dark and dry place.

Preparation of fruit extracts

The powder of the each dried fruit was socked in 99.5% ethanol in separate conical flask for 12 days at room temperature with occasional shaking and stirring. The conical flasks were sealed to avoid evaporation. After that the contents were filtered and the filtrate were evaporated and solidified with rotary evaporator at 45°C. The weight of the final crude extracts was expressed as a percentage of the dry weight (% d.w.) of the powder, and it was 47.7, 24.1, 32.5, 25.9 and 45.8% for *A. bilimbi*, *A. lacucha*, *C. melo*, *P. sylvestris* and *F. jangomas* respectively. Twenty milligrams of each extract was dissolved in 1 ml of ethanol to prepare a stock-solution for experiments.

Determination of total phenolic content

The total concentration of phenol (TPH) in the extracts was determined according to the Folin-Ciocalteu method (Ough and Amerine, 1998) with Gallic acid (GA) as the standard and expressed (mg) as Gallic acid equivalents (GAE)/10 g of extract (Aoshima and Ayabe, 2007). 20 µl of sample extract was added to 1.58 ml distilled water. Then 100 µl of Folin-Ciocalteu reagent was added. After 1 min interval 300 µl of 20% sodium carbonate solution was added. After 2 hour incubation at room temperature resulting blue color was read at absorbance of 765 nm. Samples were analyzed in triplicates.

2, 2-Diphenyl picryhydrazyl (DPPH) radical scavenging activity

DPPH radical scavenging activity and the reducing power of the fruit extracts were determined according to the standard methods (Yen and Wu, 1999). Ethanol aqueous solution with different concentrations of the extract was prepared. To 4.0 ml of sample solutions, 1.0 ml of 0.2 mM DPPH was added and mixed vigorously. After incubation at room temperature for 30 min, the absorbance of the resulting solutions was measured at 517 nm using a spectrophotometer (UV-1601 Shimadzu, Kyoto, Japan).The control was conducted in the same manner, except that distilled water was added instead of sample. DPPH radical scavenging activity was calculated according to the following equation:

$$\text{DPPH radical scavenging (\%)} = [1 - (As / Ac) \times 100]$$

Here, Ac = absorbance of control, As = absorbance of sample solution.

Then % of inhibition was plotted against respective concentrations used and from graph IC_{50} was calculated.

Reducing power activity

The reducing power of the fruit extracts was determined according to the method of Oyaizu, (1986). 2.5 ml of 0.2M phosphate buffer, pH 6.6 containing different concentrations of the extract were prepared. Then it was added to 2.5 ml of 1% potassium

ferricyanide, and mixed. After incubation at 50°C for 20 minutes, the mixtures were mixed with 2.5 ml of 10% trichloro-acetic acid and then centrifugation at 3000 rpm for 10 min. The supernatant (2.5 ml) was mixed with 2.5 ml of distilled water and 0.5 ml of 0.1% ferric chloride. Absorbance of this resulting solution was measured at 700 nm. Increased absorbance of the reaction mixture indicated increasing reducing power.

α-amylase assay

α-Amylase activity was carried out using the starch-iodine method. Briefly, 10 µl of α-amylase solution (0.025 mg/ml) was mixed with 390 µl of phosphate buffer (0.02 M containing 0.006 M Nacl, pH 7.0) containing different concentrations of extract. After incubation at 37°C for 10 min, 100 µl of the 1% starch solution was added, and the mixture was re-incubated for 1 h. Next, 0.1 ml of 1% iodine solution was added, and after adding 5 ml distilled water, the absorbance was taken at 565 nm. Sample, substrate and α-amylase blank determinations were undertaken under the same conditions. Inhibition of enzyme activity was calculated as (%) = (A-C) × 100/ (B-C), Where, A = absorbance of the sample,

B = absorbance of blank (no α-amylase), and

C = absorbance of control (no starch).

Results and Discussion

In this research work, five different locally available fruits were analyzed for their total phenolic content, reducing power activity, antioxidant activity and anti-amylase activity in their ethanolic extracts. These fruits are very cheap and grown well in Bangladesh.

Total phenolic (TPH) content

The total phenolic contents of five fruits determined by Folin-Ciocalteu method are shown in Table 1. Out of the five fruits, *P. sylvestris* showed highest phenolic contents (37.40 ± 1.72 mg GAE/10 g extract). The other four fruits showed poor and very close phenolic contents compare to *P. sylvestris.*

Antioxidative activity

Fruits contain large variety of antioxidants. Many methods are available to measure the antioxidative capacity of plant materials. Owing to the complexity of the oxidation-antioxidation process, no single testing method is capable of providing a comprehensive view of the antioxidative profile of a sample (Parejo *et al.*, 2002). Therefore, a multi-method approach is necessary to assess antioxidative activity. In this study we used two different methods: DPPH free radical scavenging assay and ferric reducing power assay.

Table 1. Total phenolic content, reducing power activity, anti oxidant activity and anti-amylase activity of the five fruit extracts

Name of fruits	Total phenolic content g GAE/10 g extract	Reducing power activity (O.D.) at 0.4 mg/ml	2, 2-Diphenyl-picryhydrazyl (DPPH) radical scavenging activity (%) at 1.25 mg/ml, *P. sylvestris* at 10 µg/ml	α-Amylase activity (%) at 0.4 mg/ml
A. bilimbi	6.08±0.9	0.249±0.01	28.37±1.63	4.94±1.03
A. lacucha	9.9±0.46	0.548±0.03	72.84±0.41	11.82±0.57
P. sylvestris	37.40±1.72	0.933±0.02	90.96±1.50	11.88±3.69
F. jangomas	6.81±0.18	0.786±0.01	54.45±0.75	3.33±0.64
C. melo	6.02±0.89	0.698±0.01	37.29±2.53	10.64±0.61

Values are given as mean ± SD of 3 replicates.

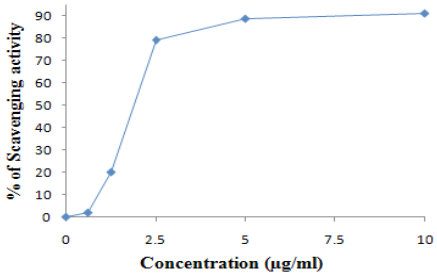

Figure 1. Dose dependency of DPPH free radical scavenging of *P. sylvestris* at different concentration of fruit extract

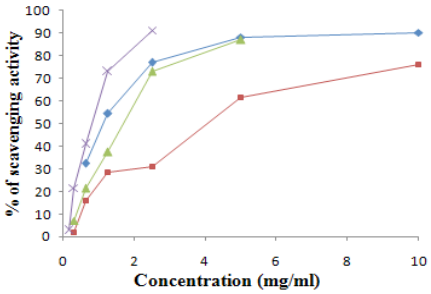

Figure 2. Dose dependency of DPPH free radical scavenging activity of *A. bilimbi* (—■—), *C. melo* (—▲—), *F. jangomas* (—◆—) & *A. lacucha* (—×—) fruit extracts at different concentrations.

The DPPH free radical scavenging activity of five fruits' extracts are shown in Table 1. Extract of fruit *P. sylvestris* showed very high antioxidative activity. It showed 90.96 ± 1.50% free radical scavenging activity at a concentration of only 10 µg/ml. Other fruits showed very poor antioxidative activity compare to *P. sylvestris.* The second highest activity was found for fruit *A. lacucha*. This fruit inhibited DPPH free radical 72.84 ± 0.41% at a concentration of 1.25 mg/ml. Thus *P. sylvestris* showed at least 100 times higher antioxidative activity than *A. lacucha.* The antioxidative power was lowest in fruit *A. bilimbi* (28.37 ± 1.63% activity at concentration 1.25 mg/ml).

All the fruit extracts showed dose dependency in scavenging DPPH free radical. The IC_{50} value was determined from the dose dependency curve. The IC_{50}

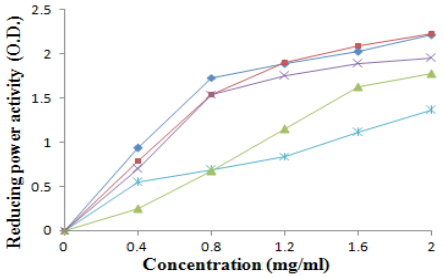

Figure 3. Reducing power activity of *P. sylvestris* (✦), *F. jangomas* (■), *A. bilimbi* (▲), *C. melo* (✕) & *A. lacucha* (✳) fruit extracts at different concentrations.

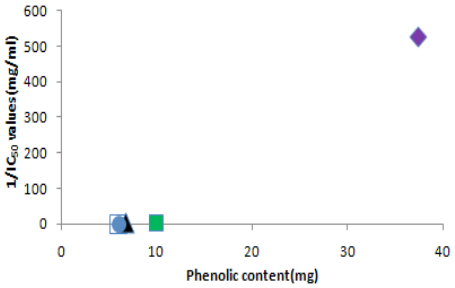

Figure 4. Relationship between total polyphenol content and the reciprocal of IC_{50} values for DPPH free radical scavenging activities of different fruit extracts. *F. jangomas* (▲), *A. bilimbi* (□), *C. melo* (●), *A. lacucha* (■) & *P. sylvestris* (◆).

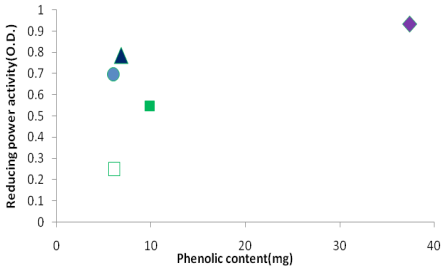

Figure 5. Relationship between total polyphenol content and reducing power activities of different fruit extracts. *F. jangomas* (▲), *A. bilimbi* (□), *C. melo* (●), *A. lacucha* (■) & *P. sylvestris* (◆).

value of fruits *P. sylvestris* and *A. lacucha* were 1.90 µg/ml and 0.798 mg/ml respectively. The highest IC_{50} value was 3.683 mg/ml for *A. bilimbi* (Figures 1 and 2). The IC_{50} value of reference ascorbic acid was 2.20 µg/ml. Reducing power assay method is based on the principle that substances, which have reduction potential, react with potassium ferricyanide (Fe^{3+}) to form potassium ferrocyanide (Fe^{2+}), which then reacts with ferric chloride to form ferric ferrous complex that has an absorption maximum at 700 nm. The reducing power of ethanolic extracts of five Bangladeshi fruits at a concentration of 0.4 mg/ml is shown in Table 1. Like DPPH free radical scavenging activity, *P. sylvestris* showed the highest and *A. bilimbi* showed the lowest reducing power activity. For all the fruits reducing power was increased concomitantly with increasing the concentration of fruit extract (Figure 3). Some studies report a strong correlation between phenolic content and antioxidant activity in fruits, vegetables and grains (Velioglu *et al.*, 1998) while other reports do not (Dasgupta and De, 2007). In this study, with the increase concentration of phenolic content the $1/IC_{50}$ value was also increased (Corelation coefficient 0.99) (Figure 4) suggested that with increase in polyphenol content, the antioxidative activities increase (Duh *et al.*, 1999). Correlation was also found between phenolic content and reducing power (correlation coefficient 0.62) (Figure 5). These correlations confirm that the phenolic compounds are the main microconstituents contributing to the antioxidant activities of these fruits.

Inhibition of α-amylase activity

In the present study, α-amylase activity was not strongly inhibited. But comparatively higher activity was found in case of *P. sylvestris* (11.88 ± 3.69%), *A. lacucha* (11.82 ± 0.57%) and *C. melo* (10.64 ± 0.61%) than the activity found for *A. bilimbi* (4.94 ± 1.03%) and *F. jangomas* (3.33 ± 0.64%) (Table 1). Recent studies have shown that phenolic phytochemicals exert anti-diabetic activity through inhibition of carbohydrate-hydrolyzing enzymes, such as alpha-amylase and alpha-glucosidase. Natural alpha-amylase inhibitors offer an attractive approach to the management of postprandial hyperglycemia by decreasing glucose release from starch (Kim *et al.*, 2005). Several findings (Kwon *et al.*, 2006; Apostolidis *et al.*, 2007) suggest that phenolic synergies may play a role in mediating amylase inhibition and therefore have the potential to contribute to the management of type 2 diabetes.

Conclusion

Out of the studied five Bangladeshi fruits, *P. sylvestris* showed very high phenolic content, antioxidative and anti-amylase activities compared to other four fruits. Correlation was also found between the phenolic contents and antioxidative activities of these fruits. This study demonstrates that these common fruits of Bangladesh have potential health beneficiary functions. Ethanolic extracts of these fruits can be used as easily accessible source of natural antioxidants and as a possible food supplement or in pharmaceutical industry. Further studies will be needed to evaluate its potential in various *in vitro* and *in vivo* systems. The components responsible for the antioxidant and anti-amylase activities of these fruits are currently unclear. Therefore, further works will have to perform on the isolation and identification of the antioxidant and anti-amylase components present

in the ethanolic extract of these fruits.

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