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Assessment of total phenolic content, anti-oxidant and anti-alpha-amylase activities in *B.flabellifer*, *S.pinnata*, *S.samrangense*, *C.carandus*, *A.chaplasha* and *A.carambolas*

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ABSTRACT

To find out a cheap source of dietary phenols and antioxidants along with anti-alpha-amylase activities, ethanol extracts of six Bangladeshi fruits were investigated. *C.carandus* had the highest total phenolic content (700.46±2.26 mg/100g), while *A.chapalisha* (76.27±7.02 mg/100mg) had the lowest phenolic content. All the sample extracts showed increasing reducing ability as the concentration of extracts increased. *S.pinnata* had the highest and, *B.flabellifer* and *A.Chaplisha* had lowest ability to reduce Fe (III), where as the remaining fruits had the intermediate values. All the tested fruits exhibited favorable concentration dependent radical scavenging and antioxidant activities and the IC₅₀ values. *S. pinnata* [2.11 mg/ml] have the highest IC₅₀ value whereas *C.caradus* has the lowest value[0.96 µg/ml] with no significant difference (P<0.05) among them. We examined the correlation between the reciprocal values of IC₅₀ values for DPPH and the amount of total phenolic content found in the extracts of the fruit and obtained a correlation of 0.85. In the present study, α-amylase was strongly inhibited by *B.flabellifer*, *C.carandus*. The highest inhibitory activity is found for *C. carandus* (94.28 ± 0.87%) where as the lowest activity is found for *S. samrangense* (9.00±1.37%). © 2011 Trade Science Inc. - INDIA

KEYWORDS

Total phenolic content;
Anti-oxidant;
Anti-alpha-amylase;
B.flabellifer;
S.pinnata;
S.samrangense;
C.carandus;
A.chaplasha;
A.carambolas.

INTRODUCTION

Phenolic phytochemicals are secondary metabolites, that occur naturally in plants, which play an important role in both humans and animals diet^[1,3]. These phenolic compounds exhibit protective functions against environmental and biological stress such as high energy radiation exposure, bacterial infection or fungal attacks^[4]. In addition phenolics are also essential for cell

structure, signaling and pigmentation. Phenolics are expressed in diverse cell and tissue types due to diversity of functions^[4,5]. Both artificial and naturally occurring antioxidants have been reported to play significant roles in inhibiting free radicals and xenobiotic-induced oxidative damage to membranes and tissues^[6,7]. Most living organisms possess enzymatic and non-enzymatic defence systems against excessive production of reactive oxygen species. However, different external fac-

tors (smoke, diet, alcohol, some drugs) and aging decrease the efficiency of such protecting systems, resulting in disturbances of the redox equilibrium established under healthy conditions. Thus, antioxidants compounds halt unexpected oxidation in the body, which involve the formation of free radicals and further deteriorate the condition of the body^[8]. Recently, more attention has been paid to the role of natural antioxidants, mainly phenolic compounds, which may have higher antioxidant activities than those of conventional vitamins C, E and β -carotene^[9]. The antioxidative effects of natural phenolic compounds, in pure forms or in their extracts from different plant sources (vegetables, fruits and medicinal plants), were studied in vitro using different model systems^[10,11]. Therefore, antioxidants, which can neutralize free radicals, may be of central importance in the prevention of carcinogenicity, cardiovascular, and neurodegenerative changes associated with aging^[12,13]. Epidemiological studies show that the consumption of vegetables and fruits could protect humans against oxidative damage by inhibiting or quenching free radicals and reactive oxygen species^[14].

Several studies have found phenolics from many common foods like capsicum, cinnamon and fenugreek have low α -amylase inhibition coupled with free radical scavenging-linked antioxidant activity, for potential diabetes management^[13-16]. This offers the potential for good postprandial blood glucose management via a α -glucosidase inhibition without the common side-effects associated with high α -amylase inhibition^[14,15]. In addition, these same foods have free radical scavenging-linked antioxidant activity which can help maintain the redox balance in susceptible cells^[14,15]. Phenolic, antioxidant and α -amylase inhibition of many fruits, vegetables and medicinal plants have been studied^[16-19]. The results of the studies showed that some of them could be rich sources of these compounds. Therefore, the investigation for plants source containing potent natural phenolics, antioxidants and α -amylase inhibitor attract the attention of researcher.

There is an abundance of fruits in Bangladesh because of fertile landforms and climate. Phenol content, anti-oxidative, anti- α -amylase and anti- α -glucosidase activities of Bangladeshi few fruits are studied by Hossain et al.^[20,21]. Scientific data of addressing antioxidant activity, phenolic content and anti- α -amylase inhibitor of

Borassus flabellifer L. (immature kernel), *Spondias pinnata* Kurz, *Syzygium samrangense*, *Carissa carandus* L., *Artocarpus chaplasha* Roxb. and *Averrhoa carambolas* L are not available. The present research is aimed at investigating and recording the phenol content, anti-oxidative and anti- α -amylase activities of these fruits.

MATERIALS AND METHODS

Collection of fruits and sample extraction

Fresh fruits (*Borassus flabellifer* L., *Spondias pinnata* Kurz, *Syzygium samrangense*, *Carissa carandus* L., *Artocarpus chaplasha* Roxb. and *Averrhoa carambolas* L.) were collected from Chittagong district of Bangladesh. The collected fruits were chopped into pieces and sun-dried. The dried samples were then grained to powder. The powdered samples are stored in air tight containers. To conduct experiments, 20 gm powder of each was macerated in 540 ml pure ethanol for 12 days at room temperature with occasional stirring. After that, ethanol extract was filtered with whatman no.1 filter paper. The extract was concentrated under reduced pressure below 500C through vacuum evaporator. The concentrated extracts were collected in Petri dish and allow to air dry for complete evaporation of ethanol. Obtained extract was kept in a refrigerator at 40C.

Determination of total phenolic compounds (TPH)

The total phenolic TPH in the extracts was determined according to the Folin-Ciocalteu method^[22] with gallic acid (GA) as the standard and expressed (mg) as gallic acid equivalents (GAE)/g of extract^[20]. 20 l of the sample extract was diluted with 2.08ml distilled water and mixed with 100 l Folin-Ciocalteu reagents. After 1 minute interval 300 l of 20% sodium carbonate was added. The mixture was then allowed to 2h incubation at room temperature. Absorbance of the supernatant was then measured at 765 nm. All samples were analysed in triplicates. Total phenolic content was determined from standard curve of gallic acid.

Anti-oxidant activity

Reducing power activity

Reducing power was determined by the method of

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Oyaiza^[23]. Phosphate buffer (2.5ml, 0.2M, pH 6.6) containing different concentrations of the extract of the samples were prepared. Then 2.5ml of 1% potassium ferricyanide was added to the mixture. The mixture was incubated at 50 °C for 20 min. An aliquot (2.5 ml) of 10% trichloroacetic acid was added to the mixture, followed by centrifugation at 3000 rpm for 10 min. The upper layer of solution (2.5 ml) was mixed with 2.5 ml of distilled water and 2.5 ml of 0.1% ferric chloride (FeCl₃). Then absorbance of the solution was read at 700 nm. One mM potassium ferrocyanide in one buffer solution, which is produced from potassium ferricyanide by reduction, produced the absorbance at 700nm. Ascorbic acid 40 g/ml phosphate buffer served as positive control.

2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

DPPH radical-scavenging activity was measured, using the method of Yen and Wu^[11]. The hydrogen atom or electron donation ability of ethanol extract was measured from the bleaching of the purple coloured ethanol solution of DPPH. This spectrophotometric assay used stable radical DPPH as a reagent. 1.5 ml various concentrations of ethanol extracts were added to 1ml of 20mg/ml ethanol solution of DPPH. After 30 min of incubation period at room temperature, the absorbance was measured against a blank at 517nm. DPPH radical scavenging activity was calculated according to the following equation^[11]:

$$\text{Antioxidant activity} = \left(1 - \frac{A_s}{A_c}\right) \times 100\%$$

Where, AS is the absorbance of sample, AC is the absorbance of control. Extract concentration providing 50% inhibition (IC₅₀) was calculated using the graph by plotting inhibition percentage by extract concentration. The entire tests were carried in triplicates.

Anti alpha-amylase activity

The α -amylase inhibition assay was carried out using the starch-iodine method. The starch solution (1% w/v) was obtained by boiling and stirring 1gm of starch in 100ml of deionized water for 15 min. The enzyme solution was prepared by mixing 10 l of α -amylase solution was mixed 390 l of phosphate buffer (0.02M containing 0.006M NaCl, pH7.0) containing different

concentrations of extract. After incubation at 37°C for 10min, 100 l of the starch solution (1 or 5%) was added and the mixture was re-incubated for 1h. Next 0.1 ml of 1% iodine solution was added and then add 5ml distilled water. The absorbance was taken at 565nm. Samples, substrate and α -amylase blank determinations were undertaken under the identical conditions. Inhibition of enzyme activity was calculated as (%)

$$I_{\text{anti-}\alpha\text{-amylase}} \% = \left[\frac{A-C}{B-C}\right] \times 100$$

where A is the absorbance of the sample, B is the absorbance of the blank (no α -amylase) and C is the absorbance of the control (no extract).

Statistical analysis

Experimental results were mean \pm SD of 3 parallel measurements. One-way analysis of variance (ANOVA) followed by Turkey's post test was used to assess the presence of significant difference ($P < 0.05$) between the extract. Results were processed by Origin 7 and Microsoft Excel 2007 softwares.

RESULTS AND DISCUSSIONS

Total phenolic contents of the selected fruits

The total phenolic contents of *Borassus fabellifer* L., *Spondias pinnata* Kurz, *Syzygium samrangense*, *Carissa carandus* L., *Artocarpus chaplasha* Roxb. and *Averrhoa carambola* L were estimated in gallic acid equivalent using the Folin-Ciocalteu method, which relies on the transfer of electrons from phenolic compounds to the Folin-Ciocalteu reagents in alkaline medium. Total phenolic content was expressed as gallic acid equivalent (mg/100g dry extract) from the calibration curve using the equation:

$$Y = 0.0846X \quad R^2 = 0.9968$$

where X was the gallic acid equivalent and Y was the absorbance.

The yield of the sample extracts and concentration of total phenolic content (mg/100 g dry weight) is shown in Figure 1. Phenolics present in fruits and vegetables have received considerable attention because of their potential antioxidant activity. Phenolic compounds undergo a complex redox reaction with the phosphotungstic and phosphomolybdic acids present in the Folin-Ciocalteu reagent^[24]. *C. carandus* had the highest total

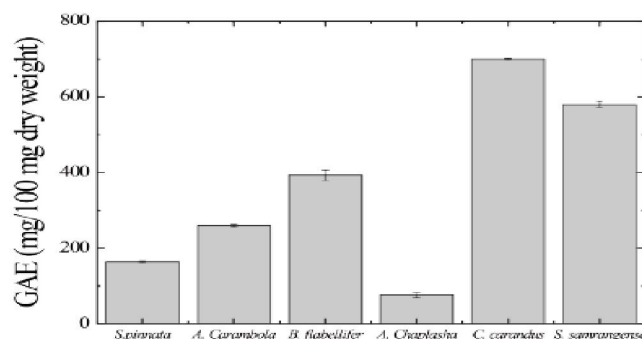


Figure 1. Phenolic content of the studied six fruits using Folin-Ciocalteu method. The values of total phenolic content are means \pm SDs (bars) from three experiments.

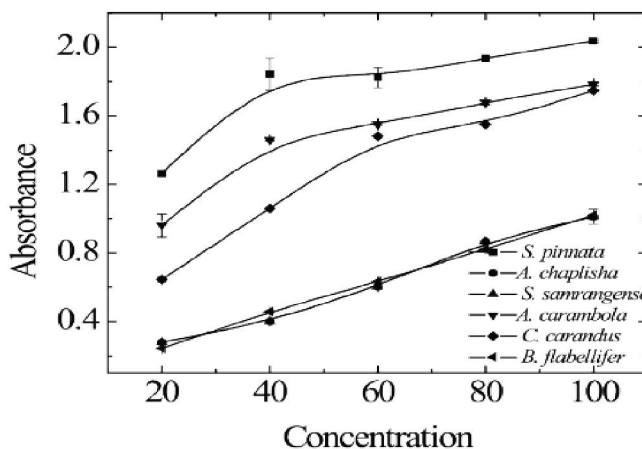


Figure 2. Reducing power ability of the sample extracts of the selected fruits. The value of absorbance means \pm SDs (vertical bars) from three experiments.

phenolic content (700.46 ± 2.26), while *A. chapalisha* (76.27 ± 7.02) had the lowest phenolic content. Among six fruits *B. flabellifer*, *C. carandus* and *S. samrangense* have significantly higher phenolic content than others. There was no significant difference in phenolic content values ($P < 0.05$). The total phenolic content of these fruits were very high when compared with other vegetables and fruits

reported in literatures^[16, 18, 19]. However, it should be also noted that some chemical groups of ascorbic acid, organic acids, sugars, aromatic amines can also react with Folin-Ciocalteu reagent^[25]. Thus, due to these compound the determination of total phenolic contents by the Folin-Ciocalteu method, which leads to an over-valuation of the phenolic contents. Furthermore, different phenolics might present different response with Folin-Ciocalteu reagent such as gallic acid, which leads to an under estimation of various compounds.

Among six fruits *B. flabellifer*, *C. carandus* and *S.*

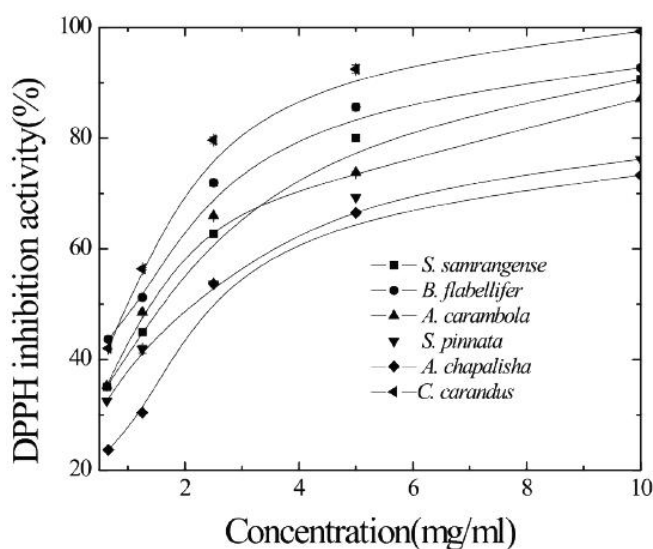


Figure 3. Antioxidant activities of the studied fruits extracts using DPPH. The values of DPPH activity are means \pm SDs (vertical bars) from three experiments.

samrangense have significantly higher phenolic content than other. *C. carandus* had the highest total phenolic content (700.46 ± 2.26), while *A. chapalisha* had the lowest phenolic content.

Antioxidant activity of the selected fruits

Reducing power ability

Reducing antioxidant power is a measure of the reductive ability, and it is evaluated by the transformation of Fe (III) to Fe (II) in the presence of sample extracts^[26]. The reducing powers of the six collected samples are summarized in Figure 4.3. From the figure, reducing power increased with an increase in extracts concentration. This result is similar to that reported by G, İçin et al^[26]. The ability to reduce Fe (III) may be attributed to hydrogen donation from phenolic compound^[27] which is also to the presence of reductant agents^[28].

From Figure 2, all the sample extracts showed increasing reducing ability as the concentration of extracts increased. *S. pinnata* had the highest and, *B. flabellifer* and *A. Chapalisha* had lowest ability to reduce Fe(III), where as the remaining fruits had the intermediate values. This results similar to that reported by other studies^[27, 28]. No correlation is observed between phenolic compounds and reducing power.

2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

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The antioxidant activity of each extract was monitored, using the DPPH radical assay. DPPH is a commercial oxidizing radical which can be reduced by antioxidants. In this assay, the violet colour of DPPH was reduced to a pale yellow color due to the abstraction of hydrogen atom from antioxidant compound. The more antioxidants present in the extract, the more the DPPH decreases. High reduction of DPPH is related to the high scavenging activity performed by particular sample^[29]. IC₅₀ was calculated as amount of antioxidant present in the sample necessary to decrease the initial DPPH concentration by 50%. The lower the IC₅₀ value, the higher is the antioxidant activity. Figure 3 describes DPPH activity of the fruits extracts. Among the fruit *B. fabellifer* has the highest free radical scavenging activity where as *A. chaplasha* has the lowest free radical scavenging activity. The lower the IC₅₀ value, the higher is antioxidant activity. All the tested fruits exhibited favorable concentration dependent radical scavenging and antioxidant activities and the IC₅₀ values. *B. fabellifer* [1.11mg/ml], *S. pinnata* [1.06mg/ml], *S. samrangense* [1.04µg/ml] and *A. carambola* [1.01µg/ml] showed the highest antioxidant capacities, with no significant difference ($P < 0.05$) among them. In the measured range, 50% inhibition was not observed for *C. carandus* and *A. chaplasha*.

The correlation between antioxidant activity and total phenolic content

Generally, extracts with a higher phenolic content show more DPPH radical scavenging activity and vice-versa. The correlation between antioxidant activity and total phenolic content of experimental six fruits is shown in Figure 4. We examined the correlation between the reciprocal values of 50% inhibition for DPPH and the amount of total phenolic content found in the extracts of the fruit. Phenolic content present in the fruit extracts showed a correlation of 0.85, with a p value 0.025 being considered significant. It is evident from the Figure 4 that lower the IC₅₀ value higher the total phenolic content, which demonstrates that higher is antioxidant activity. These results indicated that the phenolic compound could be the main contributor of the antioxidant activity of these fruits. This result was in agreement with many previous studies^[30, 31]. Antioxidants are believed to intercept the free radical chain

oxidation and to denote hydrogen from their phenol hydroxyl groups, thereby forming a stable end-product, which does not initiate or propagate further oxidation of lipids^[32]. Our results, however, were unable to show a good correlation between total phenolic content and deducing power activity of the extracts ($r = -0.16$).

Anti-α-amylase activities of the selected fruits

α-Amylase is one of the main enzymes in human

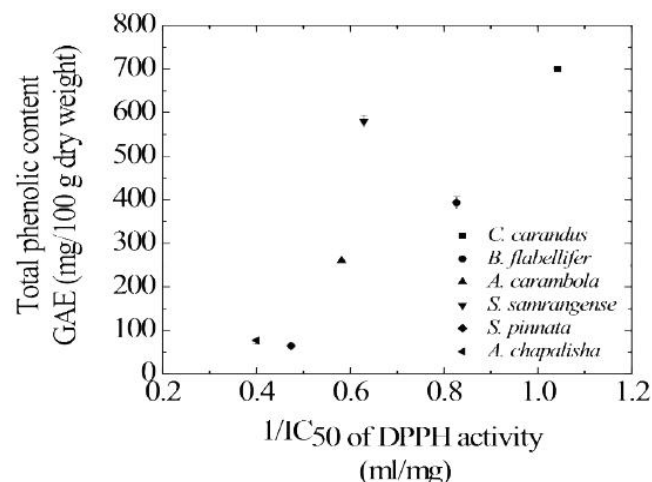


Figure 4. Relationship between total phenolic content and the reciprocal of IC₅₀ values for DPPH radical scavenging activities of different extracts of fruits. The values of total phenolic content are means ±SDs (bars) from three experiments.

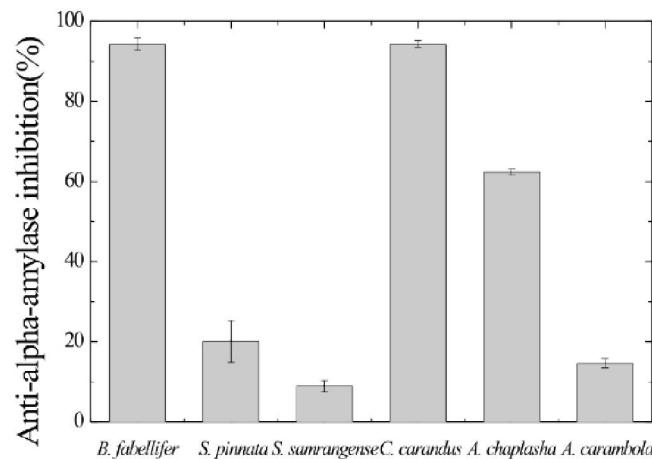


Figure 5. The α-Amylase inhibition of the studied six fruits extract. The values of α-Amylase inhibition are means ±SDs (bars) from three experiments.

that is responsible for the breakdown of starch to more simple sugars thus, the inhibitors of this enzyme can delay the carbohydrate digestion and reduce the rate of glu-

TABLE: 1 Yield of ethanolic extract (%) of dry fruits and phenolic content

Species	% Yield of ethanolic extract	GAE mg/100g dry weight	±SD
<i>S. pinnata</i>	25.35	164.26	2.53
<i>A. Carambola</i>	32.05	260.03	3.16
<i>B. flabellifer</i>	13.35	393.17	13.94
<i>A. Chaplasha</i>	34.05	76.27	7.02
<i>C. carandus</i>	23.05	700.46	2.26
<i>S.samrangense</i>	20.30	579.73	7.96

cose absorption. Consequently, postprandial rise in blood glucose is decreased. Hence, they have long been thought to improve glucose tolerance in diabetic patients^[15,17]. On the other hand, Natural polyphenols have been reported to inhibit the activity of carbohydrate hydrolyzing enzymes^[33]. Besides, phenolic compounds are currently regarded natural antioxidants and considered to be important ingredients for human health^[34]. Therefore, in this study, α -amylase inhibitory activities of the ethanol extracts obtained from six Bangladeshi fruits (*B. fabellifer*, *S. pinnata*, *S. samrangense*, *C. carandus*, *A. chaplasha* and *A. carambolas*) were determined and compared to each other.

The inhibition of α -amylase varied widely among the tested fruits. As can be observed in Figure 5, Therefore, the α -amylase inhibitory activities of the extracts in decreasing order was *C. carandus* > *B. fabellifer* > *A. chapalisha* > *S. pinnata* > *A. carambola* > *S. Samrangense*. The highest inhibitory activity is found for *C. carandus* ($94.28 \pm 0.87\%$) where as the lowest activity is found for *S. samrangense* ($9.00 \pm 1.37\%$), with no significant difference among them ($P < 0.05$). The lower inhibitory effect is probably due to the fact that at high extract concentrations of the sample, there may be a conformational change derived from binding of compounds to the enzyme^[36].

CONCLUSION

Maximum sample extracts of six fruits showed potent free radical scavenging activity against DPPH. Among all the local herbs extract analysed, *Carissa carandus L.* showed the highest total phenolic content (700.46 mg GAE/ 100 g dry weight) and the greatest antioxidant activity. Antioxidant activity of sample extracts varied with increasing sample concentration. In

our study, study traditional fruits are investigated for their α -amylase inhibitory activity. The highest inhibition of α -amylase is found for *Carissa carandus L.* ($94.28 \pm 0.87\%$) and *Borassus fabellifer L.* ($94.25 \pm 1.59\%$) where as the lowest activity is found for *Syzygium samrangense* ($9.00 \pm 1.37\%$). It is concluded that *Carissa carandus L.* and *Borassus fabellifer L.* is a rich source of anti- α -amylase compounds. Results of this study showed significant anti- α -amylase activity at very low concentration (10 μ ml) of fruits extracts. In the quest for antidiabetic compound from plants, the focus has been on medicinal fruits used traditionally and this study reported the inhibition of α -amylase at very low concentration (less than 1mg). Although this study reports α -amylase inhibition at low concentration, these fruits offer a safe method to control or supplement treatment strategy through its α -amylase inhibitory effect in addition to beneficial nutritional effects.

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