Antioxidant Activities of some Local Bangladeshi Fruits (*Artocarpus heterophyllus*, *Annona squamosa*, *Terminalia bellirica*, *Syzygium samarangense*, *Averrhoa carambola and Olea europa*)**

一些孟加拉当地产水果的抗氧化活性**

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Abstract In the present study, antioxidant activities of the fruits of *A. heterophyllus*, *A. squamosa*, *T. bellirica*, *S. samarangense*, *A. carambola and O. europa* were investigated. For this, at first matured fruits of them were sliced into small pieces and dried in the sun and finally crushed in a grinder to make powder. Ethanolic extracts of fruit powder were prepared using 99.99% ethanol. The antioxidative activities of these extracts were determined according to their abilities of scavenging 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical . It was demonstrated that all the ethanolic extracts of *A. heterophyllus*, *A. squamosa*, *T. bellirica*, *S. samarangense*, *A. carambola and O. europa* showed antioxidant activities. The IC50 of the ethanolic extracts of *A. heterophyllus*, *A. squamosa*, *T. bellirica*, *S. samarangense*, *A. carambola and O. europa* were 410, 250, 34, 200, 30 and 76μg/mL, respectively. Among them, *A. carambola* showed the highest antioxidant activities followed by *T. bellirica*, *O. europa*, *S. samarangense*, *A. squamosa and A. heterophyllus* indicating that fruits of *A. carambola*, *T. bellirica and O. europa* are very beneficial to human health.

Key words antioxidant, ascorbic acid, ethanolic extracts, free radicals, UV spectrophotometer

摘 要 研究了 *A. heterophyllus*, *A. squamosa*, *T. bellirica*, *S. samarangense*, *A. carambola and O. europ* 水果的抗氧化活性。首先将这些成熟的水果切成小片,然后用日光晒干,最后用研磨器磨成粉末。这些水果粉末用 99.99% 的乙醇进行乙醇提取。这些提取物的活性通过其清除稳定的 1, 1-diphenyl-2-picrylhydrazyl (DPPH)自由基的能力进行测定。实验结果表明,所有 *A. heterophyllus*, *A. squamosa*, *T. bellirica*, *S. samarangense*, *A. carambola and O. europa* 这些水果的乙醇提取物都具有抗氧化活性,这些水果的乙醇提取物的的 IC_{50} 值分别是 410, 250, 34, 200, 30 和 76μg/mL。其中 *A. carambola* 具有最高的抗氧化活性,其次是 *T. bellirica*, *O. europa*, *S. samarangense*, *A. squamosa* 和 *A. heterophyllus* 。结果表明 *A. carambola*, *T. bellirica* 和 *O. europa* 对人体健康是非常有益的。

关键词 抗氧化剂,抗坏血酸,乙醇提取物,自由基,紫外分光光度计
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An antioxidant is a chemical that prevents the oxidation of other chemicals. In biological systems, the normal processes of oxidation (plus a minor contribution from ionizing radiation) produce highly reactive free radicals, which can

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readily react with and damage other molecules. In some cases the body uses this to fight infection; in other cases, the damage may harm their own cells[1]. In recent years, a great deal of attention has been directed to the possible therapeutic potential of antioxidants in controlling degenerative diseases associated with marked oxidative damage. Several plant extracts and different classes of phytochemicals have been found to have quite prominent antioxidant activity[2]. The present study was conducted to determine the antioxidant activities of some local varieties of Bangladeshi fruits namely *Artocarpus heterophyllus* (Jackfruit), *Annona squamosa* (Custard apple), *Terminalia bellirica* (Beleric myrobalan), *Syzygium samarangense* (Rose apple), *Averrhoa carambola* (Carambola/Star fruit) and *Olea europa* (Olive tree).

Block *et al* (1992)[3] stated that the crushed fruits of jackfruit had diuretic action and could expel bladder and kidney stones. A fluid extract of it was employed as an antioxidant and an antibacterial agent against cariogenic bacteria. Morton (1987)[4] reported that *Annona squamosa* of family Annonaceae had varied medicinal effects including insecticide, anti-ovulatory and abortifacient. The fruits of Annona could be used as haematinic, cooling, sedative, stimulant, expectorant, maturant and tonic agents. Its seeds had abortifacient and insecticidal effects and were useful in destroying lice in the hair. Barthakur and Arnold (1991)[5] found the edible tissue of *Terminalia chebula* contained 10.3 times more vitamin C and 14.5 times more protein in comparison with apple. *T. chebula* contains 5% lysine, which increases the biological value of its protein. It was used to treat digestive diseases, urinary diseases, diabetes, skin diseases, parasitic infections, heart diseases, irregular fevers, flatulence, constipation, ulcers, vomiting, colic pain and hemorrhoids[6]. Larson (1988)[2] reported that the fresh ripe fruit of *Syzygium samarangense* was eaten by people though it was considered insipid. However, its taste could be improved by stewing with cloves or other flavoring and serving with cream as a dessert. Babu and Madhavi (2001)[7] tated that the potential medicinal use of carambola fruits was due to its antibacterial activity against cariogenic bacteria, ability to increase plasma vitamin C and antioxidant activity. Zalom *et al* (2003)[8] reported that olive fruits were generally eaten fresh, but occasionally used in desserts and juices. The active components in olive oil are thought to be monounsaturated fats (primarily oleic acid), squalene and phenolic compounds that function as antioxidants in the body.

The people of Bangladesh suffered from deficiency of nutrition and don't know nutrition content of foods. The average age of Bangladeshi people is less than that of the people of other countries in the world, because Bangladeshi people don't consume highly antioxidant bearing fruits or vegetables. The antioxidant activities of international fruits such as apple, grape, banana, orange, mango are known, but these fruits are very costly. Bangladeshi poor people cannot buy these fruits. On the contrary, many fruits of Bangladesh are very cheap. Therefore, to fulfill the needs of cheaper sources of antioxidants from Kathal* (Jackfruit), Ata* (Custard apple), Bohera* (Beleric myrobalan), Jamrul* (Rose apple), Camranga* (Carambola/Star fruit), Jalpai* (Olive tree) for the poor people, this research was conducted. The experimental design was focused to the following objectives:

- To know the antioxidant activities of some local fruits of Bangladesh.
- Quantitation of antioxidant of these fruits.

1 Materials and Methods

1.1 Preparation of ethanolic extract

The collected green-matured fruits were separated from undesirable materials. They were then cut into small parts and then sun-dried for thirty five days. The dried fruits were ground into a coarse powder with the help of a suitable grinder. The powder was stored in an air tight container and kept in a cool, dark and dry place until analysis. 25 ~ 120g of each powered material was taken into clean, flat-bottomed glass containers and soaked in 99.99% ethanol. The containers with its contents were sealed and kept for a period of 12 days accompanying regular shaking and stirring. Each mixture was then underwent coarse filtration by a piece of clean, white cotton material. The filtrates (ethanolic extracts) thus obtained were evaporated under ceiling fan and in a water bath until dried.

1.2 Determination of antioxidant activity

The antioxidant activity of the ethanolic extracts were determined on the basis of their activities to scavenge the stable 1,1-diphenyl-2-picryl hydrazyl (DPPH) free radical. DPPH is a stable free radical containing an odd electron in its structure and usually utilized for detection of the radical scavenging activity in chemical analysis. 1mL of each solution

* indicates the names are in native Bengali language.

of different concentrations (1 ~ 500μg/mL) of the extracts was added to 3mL of 0.004% ethanolic DPPH free radical solution. After 30min the absorbance of the preparations were taken at 517 nm by a UV spectrophotometer and compared with the corresponding absorbances of standard ascorbic acid concentrations (1 ~ 500μg/mL). The method described by Hatano *et al* (1988)[9] was used to measure the absorbance with some modifications. Then the % inhibition was calculated by the following equation：% radical scavenging activity = (absorbance of blank − absorbance of sample)/(absorbance of blank) × 100.

From calibration curves, obtained from different amounts of extract, the IC_{50} (Inhibitory concentration 50%) was determined. IC_{50} value denotes the concentration of sample required to scavenge 50% of the DPPH free radicals[10].

2 Results and Discussion

The work presented here deals with the fruits of *Artocarpus heterophyllus* (Jackfruit), *Annona squamosa* (Custard apple), *Tetminalia bellirica* (Beleric myrobalan), *Syzygium samarangense* (Rose apple), *Averrhoa carambola* (Carambola/Star fruit), and *Olea europa* (Olive tree) of Bangladesh.

The dried edible parts of the fruits were used to prepare the ethanolic extracts. Considerable amounts of extracts were collected from small amounts of these fruit powder (Table 1).

Table 1 Amounts of extracts collected from small amounts of fruit powders of the tested plants

Serial No.	Name of the plant	Amount of fruit powder/g	Amount of extract/g
1	*Artocarpus heterophyllus*	100	18.50
2	*Annona squamosa*	100	18.20
3	*Tetminalia bellirica*	100	14.30
4	*Syzygium samarangense*	100	17.80
5	*Averrhoa carambola*	100	15.40
6	*Olea europa*	100	22.20

DPPH is one of the free radicals widely used for testing preliminary radical scavenging activity of a compound or a plant extract. In the present study, ethanolic extracts of the above mentioned fruits showed free-radical scavenging activity (Tables 2 ~ 8).

Table 2 Absorbance and evaluation of % inhibition of standard ascorbic acid

Concentration of ascorbic acid/(μg/mL)	Absorbance/nm			Absorbance of blank solution/nm			% inhibition
	1	2	Average	1	2	Average	
1	0.340	0.342	0.3410				21.25
5	0.269	0.267	0.2680				38.11
10	0.192	0.193	0.1925	0.428	0.438	0.433	55.54
50	0.092	0.091	0.0915				78.87
100	0.019	0.026	0.0225				94.80
500	0.009	0.013	0.0110				97.46

Table 3 Absorbance and evaluation of % inhibition of the fruit extract of *Artocarpus heterophyllus* (Jackfruit)

Concentration of *Artocarpus heterophyllus*(μg/mL)	Absorbance/nm			Absorbance of blank solution/nm			% inhibition
	1	2	Average	1	2	Average	
1	0.424	0.425	0.4245				01.96
5	0.410	0.408	0.4090				05.54
10	0.397	0.396	0.3965	0.428	0.438	0.433	08.43
50	0.373	0.375	0.3740				13.63
100	0.335	0.340	0.3375				22.06
500	0.166	0.163	0.1645				62.01

Table 4 Absorbance and evaluation of % inhibition of the fruit extract of *Annona squamosa* (Custard apple)

Concentration of *Annona squamosa* /(μg/mL)	Absorbance/nm			Absorbance of blank solution/nm			% inhibition
	1	2	Average	1	2	Average	
1	0.378	0.384	0.3810				12.01
5	0.328	0.334	0.3310				23.56
10	0.313	0.311	0.3120	0.428	0.438	0.433	27.94
50	0.300	0.296	0.2980				31.18
100	0.267	0.266	0.2665				40.76
500	0.060	0.059	0.0595				86.26

Table 5 Absorbance and evaluation of % inhibition of the fruit extract of *Terminalia bellirica* (Beleric myrobalan)

Concentration of *Terminalia bellirica*/(μg/mL)	Absorbance/nm			Absorbance of blank solution/nm			% inhibition
	1	2	Average	1	2	Average	
1	0.425	0.431	0.4280				01.15
5	0.385	0.389	0.3870				10.62
10	0.354	0.360	0.3570	0.428	0.438	0.433	17.55
50	0.090	0.090	0.0900				79.21
100	0.060	0.061	0.0605				86.03
500	0.040	0.039	0.0395				90.88

Table 6 Absorbance and evaluation of % inhibition of the fruit extract of *Syzygium samarangense* (Rose apple)

Concentration of *Syzygium samarangense*/(μg/mL)	Absorbance/nm			Absorbance of blank solution/nm			% inhibition
	1	2	Average	1	2	Average	
1	0.368	0.370	0.3690				14.78
5	0.350	0.357	0.3535				18.36
10	0.342	0.341	0.3415	0.428	0.438	0.433	21.13
50	0.312	0.314	0.3130				27.71
100	0.299	0.306	0.3025				30.14
500	0.123	0.113	0.1180				72.75

Table 7 Absorbance and evaluation of % inhibition of the fruit extract of *Averrhoa carambola* (Carambola/Star fruit)

Concentration of *Averrhoa carambola*/(μg/mL)	Absorbance/nm			Absorbance of blank solution/nm			% inhibition
	1	2	Average	1	2	Average	
1	0.421	0.419	0.4200				03.00
5	0.375	0.383	0.3790				12.47
10	0.302	0.309	0.3055	0.428	0.438	0.433	29.45
50	0.128	0.121	0.1245				71.25
100	0.075	0.087	0.0810				81.29
500	0.052	0.045	0.0485				88.80

Table 8 Absorbance and evaluation of % inhibition of the fruit extract of *Olea europa* (Olive tree)

Concentration of *Olea europa*/(μg/mL)	Absorbance/nm			Absorbance of blank solution/nm			% inhibition
	1	2	Average	1	2	Average	
1	0.408	0.413	0.4105				05.20
5	0.394	0.387	0.3905				09.82
10	0.321	0.334	0.3275	0.428	0.438	0.433	24.36
50	0.268	0.259	0.2635				39.15
100	0.178	0.181	0.1795				58.55
500	0.093	0.087	0.0900				79.21

The IC_{50} values of these extracts were compared with that of ascorbic acid and summarized in Table 9.

Table 9 Comparison between achieved result and result of standard ascorbic acid

Serial No.	Name of the plant	IC_{50} of sample /(μg/mL)	IC_{50} of ascorbic acid (μg/mL)
1	*Artocarpus heterophyllus*	410	8
2	*Annona squamosa*	200	8
3	*Terminalia bellirica*	034	8
4	*Syzygium samarangense*	250	8
5	*Averrhoa carambola*	030	8
6	*Olea europa*	076	8

From Table 9, the antioxidant strength of the tested fruits can be arranged as follows: *A. carambola* > *T. bellirica* > *O. europa* > *A. squamosa* > *S. samarangense* > *A. heterophyllus*.

The water extract of carob pods reduced 13% DPPH free radicals at a concentration of 53μg/mL whereas the ethanolic extract of the pods of *A. marmelos* showed the same effect at 49.4μg/mL. In the case of grape pomace, its methanolic extract exhibits 73.65% hydroxyl radical scavenging activity at 200μg/mL[11]. On the other hand, 0.2 mg methanolic extract of oil seeds showed 50% inhibition of DPPH free radicals[12]. In the present study, the 50% DPPH free radicals was reduced by 410, 200, 34, 250, 30 and 76μg/mL of the fruit extracts of *A. heterophyllus*, *A. squamosa*, *T. bellirica*, *S. samarangense*, pods of *A. carambola and O. europa* respectively. Apparently, these results extend our understanding of the resources of plants rich antioxidants.

It is accepted that the DPPH free radical scavenging ability of antioxidants is due to their hydrogen donating ability[13,14]. The antioxidant activities of the individual compounds present in the extracts may depend on structure factors such as the number of phenolic hydroxyl or methoxyl groups, flavone hydroxyl, keto groups and other structural features. This work demonstrates the presence of antioxidative compounds in the tested fruits, but no specific antioxidant has been identified. Therefore, much work should be done to characterize individual antioxidant compounds in the extracts of these fruits in order to assign certain fruit(s) as the cheapest source for the poor people in Bangladesh as well as in the world.

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