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Phytochemical screening and comparative study of antioxidative properties of the fruits and leaves of *Spondias mombin* in Bangladesh

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Abstract
Objective: To explore the phenolic contents and antioxidant activity of the fruits and leaves of *Spondias mombin* (*S. Mombin*) grown in Bangladesh.
Method: Different solvents (Water, methanol, ethanol, acetone and n-hexene) were used for the extraction of fruits and leaves of *S. Mombin*. Polyphenolic contents and antioxidant activities were estimated by this crude extracts. Different in vitro methods were used for the investigation of antioxidant properties including phosphomolybdenum assay, DPPH free radical scavenging assay and reducing power assay.
Results: The study showed that the leaves and fruits contained saponin, steroid, tannin, alkaloid and flavonoid. Among the other extracts the methanolic extract showed the highest phenolic content, flaovonoid content and antioxidant activity. Fruits extracted with methanol showed the total phenolic content 719.25±0.94mg GAE/100gm of fresh weight and leaves extract showed the total phenolic content 161.36 ± 0.67 mg GAE/100gm of fresh weight.
Conclusion: The study recommended that *Spondias Mombin* exhibits significant polyphenolic activity and antioxidant activity and there is a strong correlation between phenolic content and antioxidant activity.

Keywords: *Spondias Mombin*, antioxidant activity, phenolic content, phytochemical screening

Introduction
In recent years different types of degenerative diseases are increasing day by day. Many chronic degenerative diseases are appeared by oxidative stress. Oxidative stress means the imbalance between the formation and neutralization of free radicals in our body. Through electron pairing with biological macromolecules (proteins, lipids, and DNA) these free radicals intend to neutralize themselves. As a result healthy human cells leading to protein and DNA damage along with lipid peroxidation [1]. These changes provide to introduce of cancer, atherosclerosis, cardiovascular diseases, aging, inflammatory diseases, and other degenerative syndrome.

Naturally damage of the body cells prevented themselves against free radicals through antioxidant compounds such as vitamin E, vitamin C and glutathione and via the enzymes such as superoxide dismutase and catalase [2]. But sometimes due to several physiological and pathological abnormalities free radicals are continuously generated. So the antioxidant compound of human cells cannot neutralize the free radicals leading to different chronic diseases [3].

Plants are the valuable source of diverse chemical compounds and medicinal value that play an important role in excavating new arsenals against different degenerative diseases. In addition to various nutritional values of plants protect the human cells from oxidative stress [4]. In present time cancer is a proverbial disease has become the major cause of human mortality in the world and it is reported that approximately half of incidence and mortality occurs in Asia [5]. Chemotherapeutic drugs are still regarded as the most effective treatments for cancer but it has numerous side effects as a result scientists have currently engaged in finding natural medicine resources as an alternative to current chemotherapeutic drugs. Also antioxidant containing phytochemicals exhibited capacity to inhibit carcinogenesis [6].

Among the 17 species of genus spondias (Anacardiaceae), 10 species are grown in tropical Asia and 7 species are grown in neotropics [4]. The plants are used in traditional medicine. Pharmacological investigation of different Spondias species demonstrated that due to the wide range of phytoconstituents, these plants possess cytotoxic, antioxidant, ulcer protective, hepatoprotective, photoprotective, anti-inflammatory, antiarthritic, antidementia, antipyretic,

analgesic, thrombolytic, hypoglycemic, antifertility, antihypertensive, antimicrobial, and anthelmintic activities [2]. The fruits are commonly known as amra in Bangladesh. Along with the fruits all section of the plants are used as herbal medicine. Fruits are used as antipyretic and diuretic. Fruits are also used as medicine for ulcer, inflammation, and sore throat. The barks are used for the treatment of diarrhea, dysentery, gonorrhoea and leucorrhoea [7]. The leaves of *Spondias Mombin* are extensively used to induce labour and diminish the pain and bleeding during and after the childbirth [8]. It is also reported that the leaves of *Spondias Mombin* have anti-viral [9, 10], antimicrobial [11-13], anti-malarial [14] and wound healing properties [15].

Therefore, the main objectives of the study were to evaluate phenolic content and details antioxidant activities of the fruit and leaf of *Spondias Mombin* grown in Bangladesh.

Materials and Methods

Plant collection

Fresh fruits and leaves samples with no apparent physical or microbial damage were collected separately in July 2017 from Chittagong, Bangladesh. All the fruits were edible quality and were identically selected in terms of shape, size, color, and matured stage. The freshly collected matured fruits were washed with water thoroughly until the attached dust particles were removed. The fruits were chopped and seeds were removed from fruits. The resulted chopped fruits and leaves were desiccated in Economy Incubator (Size 2) at 50°C. Subsequently, dried fruits and leaves were ground into powder and stored separately in air-tight containers at -40^{0}C.

Extraction

10-gram powder of plant samples were kept separately in a conical flask and soaked in 100 ml of a different solvent system. Each flask was sealed and left for a period of 6 days with occasional shaking. On the 7th day, the extracts were then filtered using sterilized muslin cloth followed by Whatman No.1 filter paper and stored them at 4°C refrigerator. After this, the extracts were evaporated in a rotary evaporator at 50°C to get crude extract. The obtained crude extracts were kept in a refrigerator at 4^{0}C for further analysis. Five different pure solvents (methanol, ethanol, acetone, n-hexane, water) systems were used for extraction. All extracts were dissolved in dimethyl sulfoxide (DMSO) for experimental analysis.

Chemicals and reagents

Methanol, ethanol, acetone, haxene, sulphuric acid, HCl, glacial acetic acid, Disodium hydrogen phosphate, ascorbic acid were purchased from MERCK, Darmstadt, Germany. Folin-ciocalteu reagent, Sodium carbonate, Sodium nitrate, Ferric chloride, Sodium acetate was from BDH Chemicals Ltd. Poole, England. Gallic acid was purchased from Ashland, boonton, USA. $K_3Fe(CN)_6$was procured from UNI-CHEM, China. Ammonium molybdate, Aluminium chloride, Sodium hydroxide, Sodium dihydrogen phosphate were procured from MERCK, Mumbai, India.

Phytochemical Screening

Distinct solvents extracts were used for identifying the presence of saponin, steroid, tannin, alkaloid and flavonoid according to the methods of Trease and Evans [16] and Harborne [17] with slide modification.

Total phenolic content

Total phenolic content of five solvents extracts were determined by Folin-Ciocalteu method with slide modification (18). 20 µL of sample extract was added with 2.58 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 and mixed by vortex. After 2-hours incubation at room temperature, the resulting blue color supernatant was read at an absorbance of 765 nm (UV-1601 Shimadzu, Kyoto, Japan). All samples were analyzed in triplicates. A standard calibration curve of gallic acid (0.002-0.01mg/ml) was plotted. The total phenolic content was expressed as mg GAE/100g fresh weight**.**

Total flavonoid content

Total flavonoid content of each extract of S. mombin was measured by aluminum chloride ($AlCl_3$) colorimetric method (19). 200µl of fruit extract was mixed with 4.8 ml of distilled water. At zero time, 0.3 ml. of (5% w/v) $NaNO_2$ was added. After 5 min, 0.3 ml of (10% w/v) $AlCl_3$ was added. At 6 min, 2 ml of 1 M solution of NaOH were added. Immediately adding 2.4 ml of distilled water the volume was made up to 10 ml. The mixture was shaken vigorously and the absorbance of the mixture was read at 510 nm. A calibration curve was prepared using a standard solution of catechin (0.002-0.02 mg/ml, r2 = 0.987). The results were also expressed on a fresh weight basis as mg catechin equivalents (CEQ) / 100 g of sample.

DPPH radical scavenging activity assay

DPPH radical scavenging activity assay was measured by Hossain *et al*. 2008 (20) with slight modification. Total reaction mixture was made by 2.0 ml each extract at different concentration (2, 4, 6, 8, 10µg/ml) were mixed with 3.0 ml of DPPH ethanol solution (20 µg/ml). After an incubation period of 30 min, the absorbance was measured at 517 nm. The radical scavenging activity (%) was calculated based on the following formula:

% inhibition of DPPH= (Abs control -Abs sample)/ Abs control X 100

Where Abs control is the absorbance of DPPH solution without extracts.

Evaluation of total antioxidant capacity by phosphomolybdate assay

The total antioxidant capacities of extracts were evaluated by phosphomolybdenum method according to Prieto *et al*. 1999 [21]. 400µl of the sample solution (in water, methanol, ethanol, acetone, n-hexane) was combined in a screw-cap tube with 4ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). The tubes were capped and incubated in a thermal block at 95°C for 90 min and cooled to room temperature. The absorbance was measured at 695 nm (UV-1601, Shimadzu, Kyoto, Japan) against a blank. All assays were run in triplicate.

Reducing power activity

The reducing power of the extracts was determined according to the method of Oyaizu [22]. Phosphate buffer (2.5 mL, 0.2 M, pH 6.6) containing different concentrations of the extract were prepared. Then it was added to 2.5 mL of 1% (w/w) potassium ferricyanide, and mixed. After incubation at 50°C

for 20 minutes, the mixtures were mixed with 2.5 mL of 10% (w/w) trichloroacetic acid followed by centrifugation at 650g 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. Then the absorbance of this solution was measured at 700 nm. A calibration curve was prepared using a standard solution of ascorbic acid (0.02, 0.04, 0.08, 0.12 and 0.16 mg/ml, r2 = 0.989).The antioxidant capacity of extracts was expressed as mg equivalent of ascorbic acid/100g of fresh weight (mg EAA/100g).

Result and Discussion

Phytochemical Screening- Phytochemical screening of the crude extract revealed the presence of saponin, steroid, tannin, alkaloid and flavonoid in fruits and leaves. The phytochemical screening of the crude extracts is described in Table 1. All the secondary metabolites play a crucial role in our body's defense mechanism. Phytochemicals may reduce the risk of coronary heart disease by preventing the oxidation of low density lipoprotein (LDL) cholesterol and they also reduce the synthesis or absorption of cholesterol, normalizing blood pressure and clotting, and improving arterial elasticity [23]. Phytochemicals have also been used for the prevention and treatment of diabetes and high blood pressure [24]. Phytochemicals play a vital role in preventing or treating cancer and heart disease [23].

Table 1: Phytochemical screening of the crude extract of fruit and leaves of *S. mombin.*

Sample	Alkaloid	Tannin	Steroid	Saponin	Flavonoid
Fruit	+	+	+	+	+
Leaf	+	+	+	+	+

Table 2 provides the findings of the total phenolic and flavonoid content in the ten extracts of fruits and leaves considered in this study. The highest phenolic and flavonid content were found in the methanolic extracts of fruit and leaf. The phenolic content of methanolic extract of fruit was (719.25±0.94) mg GAE/100gm of fresh weight and flavonoid content was (581.60±0.88) mg quercetin equivalents/100 gm of fresh weight. The Phenolic content of methanolic extract of leaf was 161.36± 0.67 mg GAE/100gm of fresh weight and flavonoid content was (112.23±0.93) mg quercetin equivalents/100 gm of fresh weight. Among all extracts n-haxane exhibited lowest phenolic content and flavonoid content for both leaf and fruit. Previous study showed that methanolic extract of fruit and leaf of *spondias dulcis* exhibited highest phenolic and flavonoid content [4]. Ehimwenma *et al* also used methanol as solvent and demonstrated that methanolic leaf extract of *spondias mombin* appeared highest phenolic content [25].

Table 2: Total phenolics and flavonoids content of the different extracts of *S. mombin*

Extract	Phenolic content mg/100 gm fresh weight		Flavonoid content mg/100 gm fresh weight	
	Fruit	Leaf	Fruit	Leaf
Methanol	719.69±0.94	161.41±0.67	581.92±0.88	112.63±0.93
Ethanol	639.25±0.54	124.36±0.86	394.60±0.56	101.23±0.67
Acetone	444.87±0.76	134.70±0.24	300.08±0.01	107.21±0.25
n-Haxane	295.81±0.24	115.64±0.29	217.86±1.65	98.56±0.51
Water	314.56±0.78	127.43±0.76	272.76±0.89	103.43±0.23

Values are means (n = 3) ± SD. Values with the same superscript letter are not statistically signifcant at the 5% level.

Enormous varieties of antioxidant compounds are present in fruits and leaves. So it is very difficult to measure the antioxidant compounds by single method. So, in this study different methods are used to determine the antoxidant capacity of the fruit and leaf. Table 3 demonstrates the findings of Antioxidant capacity of fruit and leaf extracts obtained from different solvents. Antioxidant capacity was measured by DPPH assay, total antioxidant capacity by phosphomolybdate assay and reducing power assay. In this study methanolic extracts of fruit and leaf showed the highest antioxidant capacity.

Table 3: Antioxidant capacity of fruit and leaf extracts obtained from obtained from different solvent extraction system.

Extract	DPPH free radical scavenging activityIC_{50}(µg/ml)		Total antioxidant capacity(mg AAE/g fresh weight		Reducing power activity (mg AAE/g fresh weight	
	Fruit	Leaf	Fruit	Leaf	Fruit	Leaf
Methanol	3.92	5.31	36.32±1.91	21.56±0.93	88.55±2.98	34.08±1.90
Ethanol	4.61	6.91	32.62±0.89	21.05±0.26	80.54±1.07	32.40±0.98
Acetone	5,87	7.21	24.62±1.20	16.95±0.81	69.26±0.98	27.29±0.76
n-Haxane	10.02	13.10	8.98±0.99	7.02±0.47	40.54±0.79	19.67±0.54
Water	7.43	9.29	14.36±0.86	12.23±0.67	52.05±1.07	24.86±0.29
Ascorbic acid	3.21	3.21				

Values are means (n = 3) ± SD. Values with the same superscript letter are not statistically signifcant at the 5% level.

DPPH free radical-scavenging activity

1,1-Diphenyl-2-picrylhydrazyl (DPPH) is a stable organic nitrogen radical. Through two different processes DPPH react with phenol. One is by direct separation of H atom from phenol and other is electron transfer. Both of these processes depend on either the nature of solvents or solvents or the redox potential of the involved species. DPPH react with antioxidants and neutralize the free radical. The color of the reaction mixture turns from purple to yellow. The appearance of changing color measures the capability of antioxidant scavenging activity [26].

Table 3 shows both methanolic fruit and leaf extract produced maximum free radical scavenging activity with IC_{50} value 3.92µg/ml and 5.31µg/ml respectively. The IC_{50} value of methanolic fruit extract was slightly higher than reference antioxidant in this study. Furthermore the total phenolic content and DPPH scavenging activity exhibited strong correlation (0.931) between them. On the other hand fruit and leaf extract of n-hexane showed minimum free radical scavenging activity among the five solvents.

Total antioxidant capacity

Total antioxidant capacities of different solvent extracts of *S. Mombin* was measured by phosphomolybdenum method. From table 3 it was found that methanolic fruit and leaf had highest total antioxidant capacity (36.32±1.91 and 21.56±0.93 mg AAE/g of fresh weight respectively).The potency of different solvent extracts was decreased in the following

order: methanol > ethanol > acetone> water> n-hexane. The potency of the extracts are same as other methods of the study. When compared the total phenolic content showed strong correlation with total antioxidant activity (0.917).

Reducing power assay

The reducing potential of plants acts as a remarkable indicator of its potential antioxidant activity. The formation of liver peroxidation and liver injury is inhibited by the reducing potential. Many studies have mentioned that the reducing potential of bioactive compounds depends on the capacity of electron donation that is associated with antioxidant activity. Reducing potential properties are generally coupled with the presence of reductones and these exhibit antioxidant actions by breaking the free radical chain. The electron donating ability of antioxidants is measured by reducing power assay by using the potassium ferricyanide reduction method. Antioxidants reduce the Fe3+/ferricyanide complex and convert it to the ferrous form.

The reducing power of different solvent extracts is presented in Table 3. Result showed that best reducing power of fruit and leaf was obtained from methanol (88.55±2.98 and 34.08±1.90 mg AAE/g of fresh weight). Methanol extracts showed the highest reducing power activity in which highest phenolic compound was present. Similarly n-hexane expressed lowest reducing power activity and n-hexane contained lowest phenolic compound. And the correlation between TPC and reducing power was 0.895.

Conclusion

In conclusion, this study represents that fruit and leaf of *S. mombin* may serve as valuable source for human health as an antioxidant agent. A robust correlation between the total phenolic content and antioxidant activities proved that phenolic compounds contribute to the antioxidant activity. Further studies are covetable to distinguish and isolate the unknown bioactive compound in order to establish their pharmacological properties which could provide valuable lead components in the respective therapeutic area.

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