Evaluation of Total Phenolic, Flavonoid Content and Antioxidant Capacity of Different Parts of *Averrhoa carambola* L.

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ABSTRACT: *Averrhoa carambola* L. belonging to the Oxalidaceae family has fruits with attractive star shape. Total phenolic and flavonoid content and antioxidant capacity of various parts of *A. carambola* have been studied to explore active extracts for novel antioxidant compounds. Methanol extract of bark contained the highest amount of phenolic constituent (125 mg GAE/g of dry extract). However, hexane, dichloromethane and methanol extracts contained the maximum number of flavonoids and were 45, 92 and 72 mg QE/g of dry extract, respectively. Since the bark possessed the highest antioxidant capacity, it contained more phenolic compounds than other extracts of fruits and leaves. Total antioxidant capacity of hexane, dichloromethane and methanol extracts of bark were 62, 117 and 151 mg AAE/g of dry extract, respectively. The results recommend that the bark part can be a good source of phenolic compounds as it showed the highest antioxidant capacity. The fruit and leave parts are also rich sources of antioxidant compounds.

Key words: Antioxidant capacity, polyphenols, star fruit, total flavonoid content, total phenolic content.

INTRODUCTION

Medicinal plants have important role for the development of new drugs. Reactive oxygen species (ROS) such as superoxide anion, hydroxyl radical, hydrogen peroxide, singlet oxygen, peroxynitrite etc. are continually generated in living organism during usual physiological activities. However, their excess generation can affect necessary biomolecules like lipids, proteins, nucleic acids and carbohydrates.[1] Over 100 ailments including cancer, diabetes, aging and hypertension have been connected to ROS.[2,3] Antioxidants are helpful to protect cells from such oxidative damage. Synthetic antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxyanisol (BHA) are used in foods as additives but these are associated with cancer and liver damage.[4-7] The ample antioxidants in the diet are polyphenols, mostly carried out from natural sources like fruits, vegetables and cereals. These polyphenols as dietary antioxidants are considered to be present in the diet.[8] There is a rising eagerness for polyphenols in the study of flavonoids from natural dietary sources because of having their health benefits.[9]

Averrhoa carambola L. locally known as kamranga belongs to the oxalidaceae family. It is also known as star fruit because of its diacritic shape. It is a small tree grows slowly and cultivated in tropical and subtropical regions for its fruits and medicinal uses. Though it is native in Indonesia and Malaysia, it is distributed in Australia, Brazil, China, Colombia, India, Laos, Myanmar, Philippines, South America, Thailand and Vietnam as a cash crop for its edible fruits.[10] It is slow growing, bushy, broad, rounded crown and reaches up to 6-9 m in height. This fruit mixing with mustard is seasonal and very popular food in Bangladesh. It has low calories but possesses good amount of vitamin A and C, potassium, calcium and secondary phenolic antioxidants.[11] This plant is generally used as a traditional herbal medicine for the treatment of several ailments and the pharmaco-

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logical activities of various parts of the plant, such as fruits, leaves and stems have been reported.[12] It was shown that the plant possesses antioxidant, antimicrobial, anti-inflammatory and anti-ulcer activities.[13] It is also used in ayurvedic medicine as antimalarial, anti-helminth, antiscorbutic, antidote, febrifuge and digestive tonic for poison.[14] We report here, the total phenolic & flavonoid content and antioxidant capacity of different parts of locally grown *A. carambola*.

MATERIAL AND METHODS

Chemicals and reagents. Solvents and reagents were collected from E. Merck, Germany. Rotary vacuum evaporator (Heidolph, Germany) was used to dry extracts removing organic solvents. For freeze drying a freeze-drier (LABCONCO, USA) was used. Absorbance was measured by a double beam UV-Visible spectrophotometer (SHIMADZU UV–1800, Japan).

Sample collection. The fruits, leaves and barks of *A. carambola* were collected from Savar, Dhaka. The fresh fruits, leaves and barks of the plants were washed with clean running water to remove all the soils and dirt and then air dried followed by grinding into powder.

Extraction. The powder of fruits (40.0 g), leaves (40.0 g) and barks (40.0) were extracted individually with n-hexane, dichloromethane (DCM) and MeOH successively at room temperature. The extracts were dried under reduced pressure using rotary vacuum evaporator at 40º C and thus n-hexane, DCM and MeOH extracts from various parts of *A. carambola* were obtained (Table 1).

Table 1. Amount of different extracts obtained from fruits, leaves and bark of *A. carambola*.

Parts of *A. carambola* extracts	Hexane ext. (g)	DCM ext. (g)	Methanol ext. (g)
Fruits	1.4	1.2	1.6
Leaves	1.5	1.0	1.4
Barks	1.0	1.0	0.5

Total phenolic content (TPC). The total phenolic contents were determined by modified Folin-Ciocalteu method.[15,16] Briefly, 0.5 mL (1 mg/ml) of n-hexane, dichloromethane and methanol extracts of fruits, leaves and bark of *A. carambola* were taken separately in different test-tube. Then 5 mL of Folin-Ciocalteu's reagent and 4 mL sodium carbonate solution were added in each test tube. The solutions were vortexed for 15 seconds for proper mixing and allowed to stand for 30 min at 40° C for color development. After 30 minutes of reaction absorbance was measured against the blank in a double beam UV/Visible spectrophotometer (UV-1800) at absorption maximum 765 nm. Three readings were taken per each experimental sample to get reproducible results. The total phenolic content was determined and expressed as mg gallic acid equivalents (GAE) per gram of dry extract using the equation obtained from a standard gallic acid calibration curve, $y = 0.0137x + 0.0611$; $r^2 = 0.999$.

Total flavonoid content (TFC). Aluminium chloride colorimetric method was used for the determination of total flavonoid content of the *A. carambola* extracts.[17,18] 5 mL (1mg/mL) of each of the extracts was individually mixed with 2.5 mL equal mixture of aluminium chloride ($AlCl_3$) and 1M sodium acetate solution. They were allowed to stand for 30 min at room temperature and the absorbance of the reaction mixture was measured at 415 nm with a double beam spectrophotometer. The total flavonoid content was determined as mg quercetin equivalent (QE) per gram of dry extract using the equation obtained from a standard quercetin calibration curve, $y = 0.0048x + 0.0204$; $r^2 = 0.994$.

Total antioxidant capacity (TAC). The total antioxidant capacity of the *A. carambola* sample extracts was evaluated by the phosphomolybdenum assay method[15,17,19] which is based on the reduction of (VI) to Mo (V) and the subsequent formation of a green phosphate-Mo (V) complex in acidic condition. The 0.3 mL of each extract was allowed to mix with 3.0 mL of the reagent solution (0.6 M H_2SO_4, 28 mM Na_3PO_4, 4 mM ammonium molybdate). This reaction mixture was incubated at 95 °C for 90 min. After

letting the solution cool back to room temperature, the absorbance was measured at 695 nm using a spectrophotometer against a blank solution. The total antioxidant capacity was determined and expressed as mg ascorbic acid equivalents per gram of dry extract using the equation obtained from a standard ascorbic acid calibration curve, y = 0.0041x - 0.0092; $r^2 = 0.998$.

RESULTS AND DISCUSSION

Total phenolic content (TPC). Gallic acid, a standard phenolic compound, was used for preparing various concentrations to make a calibration curve. At first stock solution 1000 µg/mL was prepared in methanol and it was further diluted to make 10- 250 µg/mL and six points calibration curve was obtained where $r^2 = 0.999$ was found (Figure 1).

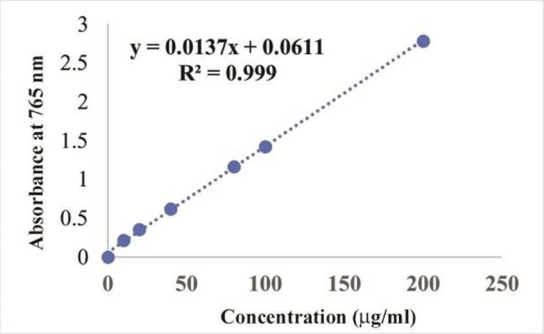

Figure 1. Calibration curve of gallic acid.

Folin-Ciocalteu reagent used in this method reacts with OH group of any phenolic type compound and it is an oxidation process. Hexane, dichloromethane and methanol extracts of three main parts (fruits, leaves and bark) of *A. carambola* were individually used for determining total phenolic content. Among three extracts methanol extract contained the highest amount of phenolic compound with approximately 42.57 ± 4.25, 70.65 ± 2.16 and 125.34 ± 4.25 mg GAE/g for dry fruits, leaves and bark, respectively and it was followed by dichloromethane extract. The least phenolic compounds were present in hexane extracts. Though hexane extract of bark contained about 60 mg GAE/g, it was lower compared to dichloromethane and methanol extracts (Table 2). From our previous research, TPC of hexane, DCM and methanol extracts of *Syzygium samarangense* were found to be 46.32 ± 0.2, 87.77 ± 0.09 and 49.59 ± 0.16 mg GAE/g, respectively for red variety.[17] In *Syzygium jambos*, the TPC were 15.39 ± 0.24, 31.32 ± 0.25 and 42.19 ± 0.16 mg GAE/g for hexane, DCM and methanol extracts, respectively.[20] It was reported that methanol extract of sour and sweet star fruit plant's leaves grown in Indonesia showed phenolic content 2830.99/100 mg GAE and 1959.77/100 mg GAE, respectively.[21] Khanam reported that the total phenolic content of aqueous and ethanolic extracts of *A. carambola* fruits exhibited 77.00 ± 2.89 and 97.16 ± 4.29 mg GAE/g of extract (dw), respectively using Folin–Ciocalteu colorimetric method and based on the decrease of phosphomolybdic acid by phenols in the presence of alkali.[22]

Table 2. Total phenolic content (mg GAE/ g of dry ext.) in different solvent extracts of *A. carambola* fruits, leaves and bark.

Extracts	fruits	leaves	bark
n-Hexane	24.75 ± 4.28	45.61 ± 4.3	60.70 ± 4.3
DCM	35.76 ± 4.22	47.95 ± 4.31	82.62 ± 4.28
MeOH	42.57 ± 4.25	70.65 ± 2.16	125.34 ± 4.25

Values are expressed as mean ± SD (n = 3).

Total flavonoid content. Quercetin dissolving in water, a standard flavonoid compound, was used for preparing various concentrations to make a calibration curve. At first stock solution 1000 µg/mL was prepared and it was further diluted to make 2.5-100 µg/mL and six points calibration curve was obtained where $r^2 = 0.994$ was found (Figure 2).

Aluminium chloride reacts with flavonoids at alkaline medium and gives red chelate products.[23] Among three extracts, DCM extract exhibited the highest flavonoid content in fruits, leaves and bark of *A. carambola*. Leaves contained more flavonoid content than fruits and bark. The flavonoid contents of hexane, DCM and MeOH extracts were found about 31, 60 and 41 mg QE/g in fruits; 45, 92 and 72

mg QE/g in leaves and 41, 75 & 55 mg QE/g in bark extracts (Table 3). Noticeably, the hexane extract showed the presence of higher flavonoids in fruits, leaves and bark. It may be possible when the hexane is the first solvent during the successive extraction and small molecules with phenolic nature are possible to extract out in hexane fraction. The method used in this research is not specific to phenolic substances and reagent can react with any compound having OH group and suitable structural features. Khanom reported that TFC of ethanol and aqueous fruit extracts of *A. carambola* were found approximately 42 and 18 mg QE/g of dry extract.[22] In this study, the TFC of methanol extract in fruits was found approximately 41 mg QE/g which corelates with previous study.

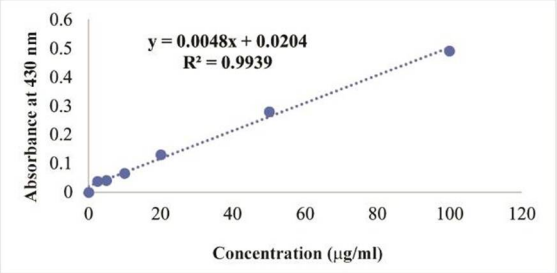

Figure 2. Calibration curve of quercetin.

Table 3. Total flavonoid content (mg QE/ g of dry ext.) in various solvent extracts of *A. carambola* fruits, leaves and barks.

Different solvent extracts of *A. carambola*	fruits	leaves	bark
n-hexane ext.	30.90 ± 1.95	45.1 ± 3.5	40.65 ± 3.55
DCM ext.	60.10 ± 2.45	92.4 ± 2.65	75.65 ± 3.57
MeOH ext.	40.95 ± 2.65	72.5 ± 4	55.4 ± 3.75

Values are expressed as mean ± SD (n = 3)

Total antioxidant capacity. At first stock solution of standard ascorbic acid 1000 µg/mL was prepared and it was further diluted to make 2.5-100 µg/mL and six points calibration curve was obtained where $r^2 = 0.998$ was found (Figure 3).

The study revealed that methanol extract in fruits, leaves and bark contained the highest antioxidant capacity with about 113, 82 and 251 mg ascorbic acid equivalent (AAE)/g of dry extract, respectively. It is also observable that barks showed more antioxidant capacity than fruits and leaves. In bark, TAC of hexane, DCM and methanol extracts were found to be about 62, 117 and 151 mg AAE/g of dry extract. DCM extract showed approximately 49, 78 and 117 mg AAE/g in fruits, leaves and bark respectively (Table 4).

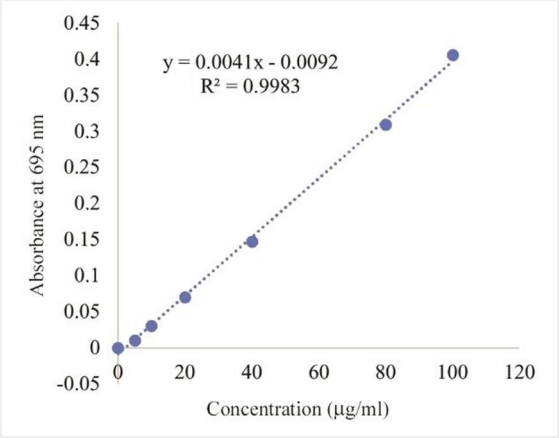

Figure 3. Calibration curve of ascorbic acid.

Table 4. Total antioxidant capacity (mg AAE/ g of dry ext.) in various solvent extracts of *A. carambola* fruits, leaves and barks).

Different solvent extracts of *A. carambola*	fruits	leaves	bark
n-Hexane	42.49 ± 2.85	13.85 ± 2.65	62.5 ± 2.65
DCM	49.32 ± 2.95	78.27 ± 2.95	116.8 ± 2.85
MeOH	113.22 ± 2.83	82.39 ± 2.61	151.2 ± 2.85

Values are expressed as mean ± SD (n=3)

CONCLUSION

In this study, the assessment of total phenolic & flavonoid content and antioxidant capacity of fruits, leaves and bark of *A. carambola* indicates that this plant is a potential source of natural antioxidants. The determination of antioxidant capacity can be helpful as a guide to use this plant for diseases related to ROS. Further investigations about the isolation and identification of individual antioxidant compound and their mechanism of action are vital issue to know their ability to control diseases which have significant impact on human life.

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