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Study of Antioxidant Activity by Various Methods in *Averrhoa carambola* L.: A Review

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Abstract

The fruit of *Averrhoa carambola* L. belongs to the family Oxalidaceae, is probably native to Ceylon and Moluccas, and is commonly known in various places as 'star-fruit' or 'kamrakh'. It is used as traditional medicine in various diseases. Fruits having many phenolic compounds that are beneficial in health. Now a day's there is extensive research on the phenolic compounds and the antioxidant activity of the fruit of *A. carambola* L. In this study antioxidant activity of these fruits content of polyphenols, vitamin C and β-carotene are reviewed. The antioxidant activities were determined by various methods of total polyphenol content y(TP), total flavonoids content (TFC), total antioxidant activity method and 1,1-diphenyl-2-picrylhydrazyl method (DPPH), and 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS). Whereas HPLC analysis was performed to analyse the content of phenolic acids, flavonoids, vitamin C and β-carotene.

Keywords: *A. carambola* L.; Antioxidant Methods, Total Polyphenol Content, Total Flavonoid Content; HPLC.

Introduction

There are many species in the genus *Averrhoa*. One of these is *A. carambola* L., also referred to as kamrakh or star-fruit. It belongs to the family Oxalidaceae, a perennial tree native to tropical and subtropical regions. It is native to Ceylon and Moluccas (Kurup & Mini, 2017; Manda et al., 2012; Payal et al., 2012). Southeast Asia, Malaysia, southern China, Taiwan, India, the Philippines, Queensland, Australia, and

several Pacific regions are among the places where it is cultivated. The fruit is available from March to August (Dembitsky et al., 2011; Payal et al., 2012). Plant secondary metabolites known as phenolic compounds serve as defences against both biotic and abiotic stress factors (Li et al., 2018). They are classified as phenolic acids, simple phenols, flavonoids, lignans, and tannins based on their chemical structure, which consists of one or more hydroxyl components joined to an aromatic ring (Valduga et al., 2019). In India cultivated the edible fruit of *Averrhoa carambola* L., which is also used to treat a number of illnesses (Thomas et al., 2008). The purpose of the current study was to screening of phytochemicals and examine the antioxidant activity and brine shrimp lethality of the methanolic extract of *Averrhoa carambola* L. Typically fruit is oblong has a tart-sweet taste. The fruit gets its name because it looks like a star in cross section. It is used in traditionally, it has been used to cure stomach, kidney and bladder disorders, fever, and eye difficulties. This fruit has been the subject of numerous scientific investigations to ascertain its hypoglycemic potential, antioxidant activity, anti-inflammatory, antibacterial, and anti-ulcer properties (Manda et al., 2012; Muthu et al., 2016; Payal et al., 2012). The fruit is used to treat piles and hepatic problems and has laxative, antidysenteric, antiphlogistic, febrifuge, anti-inflammatory, and antispasmodic properties (Khare et al., 2007). In addition to their nutritional value, *Averrhoa carambola* L. fruits include phytochemicals that antioxidant activities, such as polyphenols, carotenoids, or vitamin C. Numerous research are currently being conducted on natural bioactive chemicals that may be useful in preventing a number of illnesses. Plant polyphenols in human diet are beneficial to health as their consumption decreases the incidence of cardiovascular diseases, diabetes, and cancers and produce antimicrobial effects (Kranz et al.,2020).

Antioxidant Activity: An antioxidant is a substance that can delay or prevent the oxidation of cell contents such as DNA, proteins, carbohydrates, and lipid in plants.

Methodology

1. DPPH Method

The antioxidant capacity of the fruit juices was determined by a modified Yen and Chen method, using 0.1 mmol/L methanol solution of 1,1-diphenyl-2-picrylhydrazyl (DPPH, Sigma-Aldrich, St. Louis, MO, USA). An amount of 0.1 mL of a sample was added to 2.9 mL of DPPH solution and mixed. The absorbance was measured on a Rayleigh UV-1800 V/VIS spectrophotometer at 517 nm after 30 min of incubation at room temperature in the dark. For each juice, samples were analyzed in three replicates and the results were used to calculate an average value. The percentage of DPPH scavenging was calculated using the equation:

$$\% \text{ scavenging} = [(ADPPH - A \text{ juice})/ADPPH] \times 100$$

where ADPPH is the absorbance of the DPPH blank solution and A juice is the absorbance of the sample solution.

The obtained value was then substituted into the equation of a previously

prepared 6- hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox-Sigma-Aldrich) calibration curve. The antioxidant capacity of the samples was expressed as milligrams of Trolox equivalents (Sigma-Aldrich) per litre of sample (mg Tx L−1).

2. ABTS Method

The antioxidant capacity was determined using the (Re et al., 1999) method with small modifications. In the ABTS method, 2,20 -azinobis-(3-ethyl-benzothiazoline-6-sulfonic acid) diammonium salt (ABTS, Sigma-Aldrich, St. Louis, MO, USA) and potassium persul-fate solutions were mixed and stored overnight at room temperature in the dark for 12–16 h. ABTS solution was diluted with methanol to an absorbance of 0.70 ± 0.02 at 734 nm. After addition of 1.0 mL of diluted ABTS solution (A734 nm = 0.700 ± 0.020) to 0.01 mL of antioxidant compounds or Trolox standards in methanol, the absorbance was measured on a Rayleigh UV-1800 V/VIS spectrophotometer at 734 nm against methanol after 1 min. Quantification was performed using a Trolox standard curve. The antioxidant capacity of the samples was expressed as milligrams of Trolox equivalents (Sigma-Aldrich, St. Louis, MO, USA) per litre of sample (mg Tx L−1).

Total Flavonoid Content

The total flavonoid content was measured using the colorimetric assay developed by Kapci et al. Briefly, 0.3 mL of 5% sodium nitrite was added to 1 mL of the sample at zero time. After 5 min, 0.3 mL of 10% aluminium chloride was added. At the 6th min, 2 mL of 1M sodium hydroxide was added. The mixture was diluted by addition of 2.4 mL distilled water and mixed. The absorbance was measured on a Rayleigh UV-1800 V/VIS spectrophotometer at 510 nm. The total flavonoids content was determined using a (+)-catechin (Sigma-Aldrich, St. Louis, MO, USA) standard curve and was expressed as milligrams of catechin equivalents per litre of sample (mg CAE L−1).

Total Polyphenol Content

The total polyphenol content (TP) of the samples was determined in the Folin–Ciocalteu method (Sigma-Aldrich). First, 0.3 mL of a sample was placed in a 10-mL capacity tube; next, 0.05 mL 2 mol/L Folin–Ciocalteu reagent (Sigma-Aldrich, St. Louis, MO, USA) and 0.5 mL 20% sodium carbonate solution were added. The mixture was diluted by addition of 4.15 mL distilled water and mixed. The absorbance was measured on a Rayleigh UV-1800 V/VIS spectrophotometer at 765 nm after 30 min incubation in the dark at room temperature. A calibration curve was performed with gallic acid. The results were expressed as milligrams of gallic acid equivalents per litre of sample (mg GAE L−1).

HPLC Analysis of Ascorbic Acid and β-Carotene

Ascorbic acid was determined using HPLC methods of AOAC with slight modification. The separation was performed on a Thermo Electron Co., column BetaBasic-18 (150 mm × 4.6 mm, 5 µm). The isocratic mobile phase consisted of 0.1% o-phosphoric acid in water.

β-carotene was determined using HPLC normalised methods of EN-12823-2:2000 with slight modification. The separation was performed on a Kinetex

C18 (Phenomenex) column (150 mm × 4.6 mm, 2.6 µm).

Results

Antioxidant capacity of the Averrhoa carambola L. fruit were analyzed by DPPH method (1268+ 80b) and ABTS method (1906+146b) for their antioxidant capacity. Total polyphenol content was determined by Folin-ciocalteu method (Ruiz-Torralba et al.,2018). Results investigated that total polyphenol content (2464+153b) and high level of vitamin C (1345+17a). The total flavonoid content was measured using the colorimetric assay, results stated that juice of Averrhoa carambola had high content of total flavonoids (1345mgCAEL-1) and the composition of these compounds was dominated by flavanols Epicatechin (Vasco et al.,2008).

Conclusions

In this review we concluded that highest antioxidant activity which had a very high vitamin C content as well as total phenolic content, regardless of the analytical method used. Polyphenols were the key compounds in the composition of juices from *Averrhoa carambola* L. affecting their antioxidant activity. Furthermore, the flavonoids determined in the *Averrhoa carambola* L. juice contained mostly flavanols (mainly epicatechin).

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