

Phytochemical screening of *Averrhoa* species: *Averrhoa carambola* L. and *Averrhoa bilimbi* L.

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Abstract

The genus *Averrhoa* belongs to the family Oxalidaceae. It consists of two species *Averrhoa carambola* L. and *Averrhoa bilimbi* L. The main purpose of this study was to evaluate the phytochemical screening of the fruit extract of *Averrhoa carambola* L. and *Averrhoa bilimbi* L. The fruits have great nutritional value, traditional medicine, and have numerous health benefits. The fruits are a good source of antioxidants and are used traditionally in mouth ulcers, toothache, nausea, diarrhea, ascites, and asthma. In preliminary phytochemical investigation, *Averrhoa carambola* leaves and fruit showed the presence of alkaloids, α -amino acid, carbohydrate, reducing sugars, glycosides, phenolic compounds, tannins, flavonoids, saponins, alkaloids, glycosides, phenols, terpenoids, emodols, coumarins, and starch. The ethyl acetate fraction BE underwent phytochemical screening, analyzed using reverse-phase high-performance liquid chromatography (RP-HPLC), 2,2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS. +) radical scavenging activity, and hydroxyl radical scavenging activity. star fruit leaves contain flavonoids apigenin and quercetin as potential anti-inflammatory and anticancer agents. The raw material for the drug in Indonesia is mostly obtained through imports from other countries. To support the independence of traditional medicine raw materials, it is important to standardize the quality of conventional medicine raw materials, in this case is star fruit leaves, by the High-Performance Liquid Chromatography (HPLC) method.

Keywords: *Averrhoa carambola* L. and *Averrhoa bilimbi* L., Qualitative and Quantitative phytochemical analysis, RP-HPLC, HPLC.

Introduction

The genus *Averrhoa* belongs to the family Oxalidaceae. It has two species, *Averrhoa carambola* L. and *Averrhoa bilimbi* L. *Averrhoa carambola* L. of Oxalidaceae is native to South-East Asia and cultivated in some parts of India,

and is commonly known in various places as ‘star-fruit’ or ‘kamrakh’. The plant is distributed throughout Myanmar and the warmer regions of the world. The fruits are five-lobbed, fleshy, and yellow-greenish. This fruit is used as traditional medicine for a variety of diseases. It has drooping branches and white wood, which turns reddish. It takes a bushy shape, having many branches producing a broad, rounded crown. The soft leaves are compound in nature, medium-green, and are spirally alternately arranged around the branches. The flowers are lilac or purple-streaked and come up in the axils of leaves at the end of twigs. The attractive fruits are oblong-shaped and in measurement they are longitudinally 5 to 6 angled and 6.35-15 cm long and up to 9 cm wide. The fruits are orange-yellow, having a thin, waxy skin. The fruits are juicy and yellow inside when ripe and have a crisp texture and look like a star when cut in cross-section. Usually, the fruits contain oxalic acid in Odor, which varies from strong to mild depending on the plants. Each fruit may contain up to twelve 6-12.5 mm long seeds, and they are flat, thin shaped and brown. Some cultivated forms produce seedless fruit. (Chakraborty et al.,2012). Quantitative analysis as the determination of the active compounds using high-performance liquid chromatography (HPLC). Star fruit leaves contain major flavonoid compounds such as apigenin and quercetin. Other compounds identified in star fruit leaves are Amarin, rutin, saponin, and tannin. According to the Cabrini report, apigenin compounds in star fruit leaf extracts and fractions can inhibit the formation of edema (redness) on the ear skin of mice and effectively inhibit the migration of leukocytes to the inflammatory. (Araho et al.,2005 and Cabrini et al.,2011). Quercetin-3-O-rhamnoside compound isolated from star fruit extract has high antioxidant activity (5.19 µg/mL) and potential as an anticancer. (Artanti et al., 2006). *A. bilimbi* (common name: Bilimbi) is a medicinal plant belonging to the family Oxalidaceae. The genus Averrhoa was named after an Arab Philosopher, physician, and Islamic Jurist, Ibn Rushd, often known as Averroes (1126-98). *A. bilimbi* is a small tree that grows up to 15 m high with sparsely arranged branches. It has compound leaves with twenty–forty leaflets each and is 5–10 cm long (De Lima et al., 2001). The leaves are hairy with pinnate shapes and form clusters at the end of branches. (Orwa et al., 2009 and Lim, 2012). The tree is cauliflorous with 18–68 flowers in panicles that form on the trunk and other branches. The flowers are heterotrichous with petals 10–30 mm long, yellowish green to reddish purple (Orwa et al., 2009 and Ganders, 1979). The fruits are produced on the bare stem and trunk. The fruits are greenish with a firm and juicy flesh, which becomes soft on ripening (De Lima et al., 2001). The fruit juice is sour and extremely acidic. *A. bilimbi* holds great value in complementary medicine as evidenced by the substantial amount of research on it. Therefore, we aimed to compile an up-to-date and comprehensive review of *A. bilimbi* that covers its traditional and folk medicine uses, phytochemistry, and pharmacology.

Preliminary Phytochemical Investigation of the fruits of *Averrhoa carambola* L. and of *Averrhoa bilimbi* L.

For preliminary phytochemical investigation, the air-dried powders of the fruits were used. Tests for alkaloids, α -amino acids, carbohydrates, starch, reducing sugars, glycosides, phenolic compounds, saponins, tannins and flavonoids were done by using various solvents. These results were carried out according to the (British Pharmacopoeia, 1968; Marrini Bettalo et al., 1981) (Central Council for Research in Unani Medicine, 1987 and Trease and Evans, 2002.)

Test for Alkaloids

The powdered sample (2 g) was boiled with 1% HCL (50 ml) for 20 minutes and filtered off. The filtrate was divided into two portions and tested with Dragendroff's reagent and Wagner's reagent. The precipitates treated with the above-mentioned reagents showed the presence of alkaloid (Central Council for Research in Unani Medicine, 1987).

Test for α -amino acids

The powdered sample (2 g) was boiled with distilled water (25 ml) for 20 minutes and then filtered. And then a few drops of each filtrate were spotted on a filter paper by using a capillary tube. This paper with filtrate was allowed to dry and sprayed with ninhydrin reagent. It was dried at room temperature and then kept in oven at 110°C for a few minutes. The colour of spots changes to the pink due to the presence of α -amino acids (Marrini Bettolo et al., 1981).

Test for Carbohydrates

The powdered sample (2 g) was boiled with distilled water (25 ml) for 20 minutes and then filtered. The filtrate was then placed into a test tube and a few drops of 10% α -naphthol was added and shaken. The test tube was kept inclined at an angle of 45° and about 1 ml of concentrated sulphuric acid was slowly introduced along the inner side of the test tube. A red ring was formed between the two layers (Trease and Evans, 2002). 2nd Myanmar Korea Conference Research Journal 465

Test for Starch

The powdered sample (2 g) was boiled with distilled water (25 ml) for 20 minutes and then filtered. Two drops of iodine solution were added to this filtrate. Bluish black precipitates indicate the presence of starch (Central Council for Research in Unani Medicine, 1987).

Test for Reducing Sugars

The powdered sample (2 g) was boiled with distilled water (25 ml) for 20 minutes and then filtered. A mixture of Benedict's solution was added to this filtrate and boiled on a water bath for a few minutes. Brick red precipitates are

due to the presence of reducing sugars (Trease and Evans, 2002).

Test for Glycosides

The powdered sample (2 g) was boiled with distilled water (25 ml) for 20 minutes and then filtered. To this filtrate, 10% lead acetate solution was added. Yellow precipitates showed the presence of glycosides (Marrini Bettolo et al., 1981).

Test for Phenolic compounds

The powdered sample (2 g) was boiled with 1% hydrochloric acid (25 ml) for about 20 minutes and filtered. The filtrate was treated with 3% ferric chloride solution. The yellow precipitates appeared due to the presence of phenolic compounds (Marrini Bettolo et al., 1981).

Test for Saponins

The powdered sample (2 g) was put into a test tube and some distilled water was added into a test tube. The mixture was vigorously shaken for a few minutes. The frothing appeared due to the presence of saponins (Marrini Bettolo et al., 1981).

Test for Tannins

The powdered sample (2 g) was boiled with distilled water (25 ml) for 20 minutes and then filtered. To this filtrate, 3% ferric chloride solution was added. The observation indicated the greenish brown precipitates because of the presence of tannins (Central Council for Research in Unani Medicine, 1987).

Test for Flavonoids

The powdered sample (2 g) was boiled with 95% ethanol (25 ml) for 20 minutes and filtered. A few drops of concentrated hydrochloric acid and 0.5 g of Mg were added to this filtrate. Pink color appeared due to the presence of flavonoids (Central Council for Research in Unani Medicine, 1987).

Test of protein

4-5 ml of plant extract was taken and added few drops of Melons reagent were added, mixed properly, and heated. We found which precipitate was formed, and the precipitate turns brick red after boiling. (Manda et al., 2012)

Test of coumarins:

A few drops of ammonia were added to a filter paper. A drop of extract was added to it. The fluorescence indicates the presence of coumarins. (Manda et al., 2012)

Test of terpenoids:

To 1 mL of the solvent extract, 2 mL of chloroform and 3 mL of concentrated H₂SO₄ were added carefully to form a layer. A reddish-brown coloration of the interface indicates the presence of terpenoids. (Manda et al.,2012)

Antioxidant capacity:

1] ABTS. + radical scavenging activity

2,2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS. +) radical scavenging activity of the BEA extract was conducted using Trolox as a standard [1], and TEAC of the extract was calculated using the standard curve produced by percentage inhibition of Trolox from the following formula:

$$\% \text{ Inhibition} = [1 - A (\text{Test}) / A (\text{Control})] \times 100$$

2] Hydroxyl radical scavenging activity

The measured scavenging ability of hydroxyl radicals by the method of Kunchandy and Rao [13] is expressed as an IC₅₀ value using the following formula:

$$\% \text{ Inhibition} = [1 - A (\text{Test}) / A (\text{Control})] \times 100$$

Quantitative analysis of apigenin in extract and fraction using high-performance liquid chromatography (HPLC)

The apigenin standard calibration curve was done by plotting six concentrations, i.e., 10, 20, 40, 60, 80, and 100 ppm. Standards solution and samples (extract and fraction) were analyzed using HPLC (Waters), with a 4.6 × 150 mm Sun Fire C18 column size, 1.0 mL/min flow rate, 20 µL injection volume, and Photodiode Array (PDA) detector at 340 nm. The mobile phase used isocratic system, comprising acetonitrile and distilled water (45:55, v/v). 9

Quantitative analysis of quercetin in extract and fraction using high-performance liquid chromatography (HPLC)

The quercetin standard calibration curve was done by plotting six concentrations, i.e., 10, 20, 40, 60, 80, and 100 ppm. Standards solution and samples (extract and fraction) were analyzed using HPLC (Waters), with a 4.6 × 150 mm Sun Fire C18 column size, 1.0 mL/min flow rate, 20 µL injection volume, and Photodiode Array (PDA) detector at 273 nm. The mobile phase used isocratic system, comprising acetonitrile: water: acetic acid (10:90:0.2, v/v). 10

High-performance liquid chromatography Averrhoa bilimbi L. (HPLC)

A BDS HYPERSIL C-18 column (150×4.6 mm, 5 µm particle size) equipped with PDA/ultraviolet detector with 310 nm as the detecting wavelength in room temperature (27°C) enabled chromatic separations under the following conditions: 1 ml min⁻¹; solvent A, 10% acetic acid in water; and solvent B, 15% methanol in water starting from 0 to 20 min (40–52% A), 20–40 min (52–80%

A), and 40–60 min (80% A).

Result:

The fruits of *Averrhoa carambola* have been investigated from the point of view of medicinal aspects in this research. In preliminary phytochemical investigation, the presence of fruits showed that alkaloids, α -amino acid, carbohydrate, reducing sugars, glycoside, phenolic compound, tannin, flavonoids, saponin, and starch. The phytochemical analysis of the *Averrhoa bilimbi* fruit extract showed the presence of carbohydrates, flavonoids, phenols, glycosides, protein, and amino acids. ABTS. + Radical scavenging activity, the percentage inhibition of BE was dose dependent, wherein as the concentration of extract increased, the percentage inhibition increased. The ABTS. + Radical scavenging activity of BE was found to be 11.5 μ M TEAC. Hydroxyl radical scavenging activity was measured by the inhibition of degradation of deoxyribose by the free radicals generated by the Fenton reaction. The BE inhibited to production of hydroxyl radicals, wherein the IC₅₀ value of the extract and Trolox was 69.42 μ g/ml and 45.58 μ g/ml, respectively. HPLC After running the extract in HPLC, chromatographic separations showed prominent peaks at 23, 27, and 37 min under 310 nm HPLC spectrum of apigenin compounds in the extract, ethyl acetate fraction and water fraction has a retention time 4.055 minutes with a mobile phase acetonitrile water (45:55, v/v) is similar with retention time of apigenin standard. The hexane fraction did not show any apigenin spectrum. HPLC spectrum of quercetin only appears in the extract and ethyl acetate fraction, has a retention time of 6.116 minutes with a mobile phase of acetonitrile, water, acetic acid (10:90:0.2, v/v) is similar to the retention time of quercetin standard. While in water and hexane fractions, HPLC spectra did not show any quercetin.

Conclusion

Preliminary phytochemical analysis of *Averrhoa carambola* L. and *Averrhoa bilimbi* L. revealed the presence of alkaloids, α -amino acid, carbohydrate, reducing sugars, glycoside, phenolic compound, tannin, flavonoids, saponin, starch, carbohydrates, flavonoids, phenols, protein, and glycosides. *Averrhoa carambola* L. and *Averrhoa bilimbi* L. both have the highest antioxidant properties, with a very high vitamin C content. The results of apigenin and quercetin assays the highest content of apigenin was found in the ethyl acetate fraction. While the hexane fraction did not contain apigenin compound, which is proven by zero apigenin levels, the highest quercetin level was found in the ethyl acetate fraction. While water and hexane fractions did not contain any quercetin compound, it is proven by zero quercetin level, in HPLC spectra.

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