Jurnal Jamu Indonesia (2023) 8(3): 84–89
ISSN 2407-7178
eISSN 2407-7763

Research Article
DOI: https://doi.org/10.29244/jji.v8i3.343

Effect of Starfruit Extract (*Averrhoa bilimbi* Linn) Using a Combination of Water, Acetone, and Methanol Solvents on Phenolic Content and Antioxidant Capacity

Pengaruh Ekstrak Belimbing Wuluh (*Averrhoa bilimbi* Linn) Menggunakan Kombinasi Pelarut Air, Aseton, dan Metanol Terhadap Kadar Fenolik dan Kapasitas Antioksidan

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Received May 31, 2023; **Accepted** October 19, 2023; **Available online** December 18, 2023

ABSTRACT

Starfruit leaves, which belong to the Averrhoa family and contain tannin, saponin, flavonoid, and terpenoid compounds, have been widely used in traditional medicine due to their pharmacological benefits. This study aims to investigate the effect of solvent maceration on the extraction of total phenolics from starfruit leaves and to evaluate their antioxidant activity capacity. The study utilized Wuluh starf leaves obtained from Biofarmaka IPB University, Bogor, Indonesia, in February 023. The total phenolic content (TPC) and antioxidant tests, such as ABTS and FRAP, were influenced by the maceration stage using solvents, such as a combination of water, ethanol, and methanol. The Folin-Ciocalteu reagent and sodium carbonate were used to determine the total phenolic content, and antioxidant tests, such as the reaction with radical cations (ABTS) and the antioxidant reaction with reduction of ferri-trpyridytriazine (FRAP), were used to evaluate the antioxidant activity capacity. The results indicate that extraction with 100% acetone solvent has the FRAP method's highest phenolic content and antioxidant capacity, with 10.6325 mg GAE/g DW and 34.26641 ± 1.82 µmol TE/g DW, respectively. On the other hand, the 50:50 ethanol:acetone solvent had the highest antioxidant activity using the ABTS method, with 100.31 ± 4.80 µmol TE/g DW. The TPC-FRAP standard curve shows a positive correlation with R2 = 0.9388, while the TPC-ABTS and FRAP-ABTS standard curves do not significant correlation with the R2 values of -0.09045 and 0.08038, respectively.

Keywords: Starfruit, Acetone, Antioxidants, Ethanol, Total Phenolics, ABTS, FRAP

ABSTRAK

Daun belimbing wuluh (Averrhoa bilimbi Linn) adalah bagian tanaman yang berasal dari klasifikasi famili Averrhoa yang mengandung senyawa tanin, saponin, flavonoid, terpenoid. lalu memiliki manfaat dalam dunia farmakologis dan banyak digunakan sebagai pengobatan tradisional. Oleh karena itu, penelitian ini bertujuan untuk menunjukkan pengaruh maserasi pelarut pada ekstraksi total fenolik dari daun belimbing wuluh, serta mengevaluasi kapasitas aktivitas antioksidannya. Daun-daun belimbing wuluh diperoleh dari Biofarmaka IPB University, Bogor, Indonesia, di bulan Februari 2023. Kemudian, perbedaan rendemen pengujian total fenolik (TPC) antioksidan seperti, ABTS dan FRAP dipengaruhi oleh tahapan maserasi yang menggunakan pelarut tertentu, seperti ekstraksi dengan menggunakan kombinasi pelarut air, etanol, dan metanol. Penentuan pengaruh ini, digunakan reagen Folin-Ciocalteu dan natrium karbonat untuk penentuan kadar total fenolik yang dibandingkan dengan uji antioksidan,seperti reaksi dengan kation radikal (ABTS) dan reaksi antioksidan dengan reduksi ferri-trpyridytriazine (FRAP). Berdasarkan penelitian ini, hasil menunjukkan bahwa ekstraksi dengan pelarut aseton 100% memiliki

kandungan fenolik dan kapasitas antioksidan metode FRAP tertinggi, yakni 10.6325 mg GAE/g DW dan 34,26641 ± 1,82 µmol TE/gr DW. Di sisi lain, pengujian kapasitas antioksidan melalui metode ABTS menunjukkan pelarut etanol:aseton 50:50 memiliki aktivitas tertinggi, yakni 100,31 ± 4,80 µmol TE/gr DW. Kurva standar TPC-FRAP menunjukkan korelasi positif dengan R2 = 0.9388****, sedangkan kurva standar TPC-ABTS dan FRAP-ABTS tidak memiliki korelasi yang signifikan dengan nilai R2 secara berturut-turut sebesar -0.09045 dan 0.08038.

Kata Kunci: Belimbing Wuluh, Aseton, Antioksidan, Etanol, Total Fenolik, ABTS, FRAP

INTRODUCTION

The starfruit (Averrhoa bilimbi Linn.) is a plant that belongs to the Averrhoa family. It is a tree that thrives at an altitude of 5-500 meters above sea level. The stem of this plant is rough with bumps and few branches. The young branches resemble fine brown hair, while the leaves are pinnately compound and odd-numbered with 21-45 pairs of children. The fruit has a sour taste and flat seeds. Originating in Indonesia and mainland Malaya, the Starfruit plant is commonly found in home gardens and is easy to cultivate. In addition to its traditional medicinal uses, starfruit is often used as a food flavoring, cooking spice, preservative, and to make food taste fresh. It can also remove stains from clothes, clean dirty bodies, and rust from metals and ceramics. The leaves of the starfruit plant can treat stomach aches, rheumatism, mumps, and fever. The fruit can be used to treat whooping cough, acne, high blood pressure, bleeding gums, canker sores, cavities, disorders, and inflammation of the digestive function (Aseptianova & Yuliany, 2020).

Starfruit comprises various components, including sugars, phenolic compounds, calcium ions, amino acids, citric acid, vitamins, and cyanidin 3-o-h-D-glucoside. Additionally, starfruit contains flavonoids and triterpenoids that exhibit antibacterial properties. Muchtadi et al., (2010) reported that starfruit also contains organic acids, which could be antibiotics to eliminate Salmonella sp. bacteria and stabilize the microflora of the digestive system. Starfruit contains the highest amount of citric acid (92.6-133.8 mg/100 g), which is an organic acid. According to Silalahi & Sinaga (2012), incorporating 0.5% star fruit juice into the ratio can enhance body weight.

The goal of using ethanol solvents to extract starfruit leaf metabolites is to obtain the bioactive compounds present in starfruit leaves. Ethanol solutions proved to be more effective than water solutions based on physicochemical analysis due to the ethanol solvent's different chemical compositions and properties. The use of ethanol as a solvent in the extraction process can affect several aspects, such as the structure and physical shape of the leaves, the active compounds, and the components present in the leaves. A higher ethanol concentration can reduce the polarity of the solvent, making it easier to degrade low-polarity cell walls and extract active compounds from starfruit leaves. An alcohol solvent can also be used to test the color of the leaves, indicating the presence of certain contents in the leaves. Starfruit leaves contain flavonoids, tannins, and alkaloids, which are indicated by orange or red, blue, and orange-brown, respectively. Wuluh starfruit leaves contain tannins that are extracted more effectively using a 70% acetone solvent than an alcohol solvent, as acetone can inhibit the interaction of tannins with proteins.

METHODS

1. Sample preparation and solvents

The starfruit leaves were ground using a blender until they became a fine powder and then sifted using a sieve. The solvents were made into seven compositions. The first solvent contained 100% water/distilled water. The second solvent consisted of water and ethanol in a 1:1 ratio. The third solvent consisted of water and acetone in a 1:1 ratio. The fourth solvent contained ethanol only. The fifth solvent contained ethanol and acetone at a 1:1 ratio. The sixth solvent was acetone. The seventh or final solvent contains water, ethanol, and acetone in a ratio of 1:1:1.

2. Extraction with MAE

The starfruit extract was obtained using the Microwave-assisted Extraction (MAE) method, with modifications. We weighed 4 g of starfruit powder and put it in a 250 mL vial. Then, 20 mL of the solvent was added. The sample was then homogenized and heated in a microwave at a low temperature for 3 min. The resulting extract was filtered using Whatman No.1 filter paper, and the volume was adjusted to 20 mL.

3. Total Phenolic Content (TPC) Test (Nisar et al. 2015 with Modifications)

Determination of TPC was carried out using a microplate reader using Folin-Ciocalteu (FC) and Na_2CO_3 reagents. Next, 20 µL of the sample was prepared, and then 120 µL of Folin-Ciocalteu 10% was added and shaken until homogeneous. The mixture was incubated for 5 min in a dark room. Next, 80 µL of 10% Na_2CO_3 was added and the mixture was left for 30 min in the dark. The absorbance of the sample solution was measured using a microplate reader at 750 nm. Gallic acid standards vary between 20-300 ppm. The total phenolic content was expressed as milligram gallic acid equivalents per gram of dry weight (mg GAE/g DW).

4. ABTS Antioxidant Test

The ABTS method was used to determine the antioxidant activity. The ABTS stock solution was prepared by adding 90 mg of ABTS with distilled water to 25 ml in a measuring flask, which was then stored in a dark bottle and wrapped in aluminum foil. The solution was then refrigerated. Calcium persulfate was prepared by adding 66,289 mg/K.

5. FRAP Antioxidant Test

Antioxidant activity was tested using the FRAP method. The FRAP solution was prepared by mixing acetate buffer (pH 3.6), 10 M tripyridyl-s-triazine (in 40 mM HCI), and 20 mM FeCI at a ratio of 10:1:1 (v/v/v), and then stored in a dark bottle. A total of 10 µL of sample extract was placed into a 96-well-microplate and then 300 µL of FRAP solution was added. Subsequently, it was incubated for 30 min in a dark room. Absorbance was measured at 593 mm using a microplate reader (Spectrostar Nano, BMG LABTECH). Standard curves were generated using Trolox (0-600 M). The antioxidant activity was expressed as micromoles of Trolox equivalent per gram of extract (µmol TE/g).

6. Statistic analysis

All data were analyzed using the t-test and interpreted using IBM SPSS statistics version 26. Data were visualized using GraphPad Prism 8. The significance level was determined at $p < 0.05$ for all analyses.

RESULTS & DISCUSSION

Phenolic compounds are the most common secondary metabolites found in the greatest abundance in plants. This compound is easily soluble in polar solvents because it contains hydroxyl groups. In addition, it has redox properties because it can be easily oxidized by strong bases or enzymes (Julianto, 2019). One of the tests that can be used to determine the phenolic content is the total phenolic content or the Folin–Ciocalteu method. This test used Folin-Ciocalteu reagent and sodium carbonate, which was incubated for 30 minutes and then measured using absorbance at a wavelength of 750 nm. The color change to blue was due to the electron transfer process of the sample, and the Folin-Ciocalteu reagent was measured. The samples used in this test are expressed in mg gallic acid equivalent (GAE)/g dry weight (Calvindi et al., 2020).

Several factors must be considered in the extraction rate process, such as the type of solvent, solvent ratio, time, temperature, particle size, and amount of solvent used. The active ingredients are then dissolved in a solvent that matches its polarity during extraction. One of them is polar phenolic compounds because they have hydroxyl groups that neutralize free radical compounds. Therefore, a polar solvent was required. The active ingredients were dissolved in a solvent that matched its polarity during the extraction process. Extraction solvents, such as ethanol, methanol, and acetone, are solvents often used to extract phenolic compounds from plants and herbal plants. During the extraction process, the yield increased as the amount of solvent increased because a higher amount of solvent was used. Thus, the target compound obtained is optimal, and solvent saturation can also be avoided. However, increasing the amount of solvent by a certain amount can slightly increase the yield and tend to be constant. Based on the results obtained, the highest total phenolic content was produced in the water: ethanol solvent ($\pm$ 0.2217 mg GAE/g DW), while the lowest phenolic activity was produced in the ethanol solvent ($\pm$ 0.1340 mg GAE/g DW), as shown in **Figure 1c**.

Antioxidant activity can be measured using several methods, such as 2,2-diphenyl-1-picrylhydrazyl (DPPH) and Ferric Reducing Antioxidant Power (FRAP). Other methods that can be used include ABTS and CUPRAC. The research process that was carried out used the ABTS and FRAP methods to measure the antioxidant activity of starfruit extract (Setiawan et al., 2018). Trolox or

gallic acid is used as a comparison or standard substance in antioxidant assays. Troloks also have the same or equivalent per gram of dry weight (µmol TE/g DW), which can be a benchmark for measuring antioxidant activity in a sample. Thus, the higher the total standard substance, the higher the antioxidant activity if the extraction is carried out with this solvent (Rumpf et al., 2023).

The ABTS method can be used to measure antioxidant activity based on the working principle of a reaction with organic radical cations using a mixed-method reaction mechanism (HAT and SET). The final product was determined using a calorimeter (color change) at a length of 734 nm with a working pH of approximately 3-6. The antioxidant polarity of this method was hydrophobic and lipophilic (Rumpf et al., 2023). Furthermore, analysis of antioxidant capacity using the ABTS method on a scatter plot showed that the extraction of starfruit samples with a solvent ratio of water: acetone: ethanol (33:33:33) showed the highest antioxidant activity, namely 32,789 ± 2,294 µmol TE/g DW, as shown in **Figure 1a**. Meanwhile, low activity was observed when using ethanol solvent with a value of 11.76 ± 0.421 µmol TE/g DW.

Then the FRAP method for measuring antioxidant activity has its working principle in the form of the antioxidant's ability to reduce ferric-tripyridyltriazine (Fe(III)TPTZ), which is orange in color into a ferro-tripyridyl triazine (Fe(II)TPTZ) complex which is dark blue in acidic conditions (Benzie & Devaki, 2017). The final product was determined using a calorimeter (color formation) at a wavelength of 593 nm, a working pH of approximately 3.6, and the antioxidant polarity was hydrophilic (only in solution) (Rumpf et al., 2023).

The results of the capability analysis using the FRAP method showed that the solvent water:acetone:ethanol (33:33:33) had the highest capacity of 27,479 ± 1,094 µmol TE/g DW. Meanwhile, the antioxidant activity decreased when extracted using ethanol and ethanol:acetone solvents, namely 8,428 ± 0.214 µmol TE/g DW and 8,287 ± 1,308 µmol TE/g DW, respectively, as shown in **Figure 1b**.

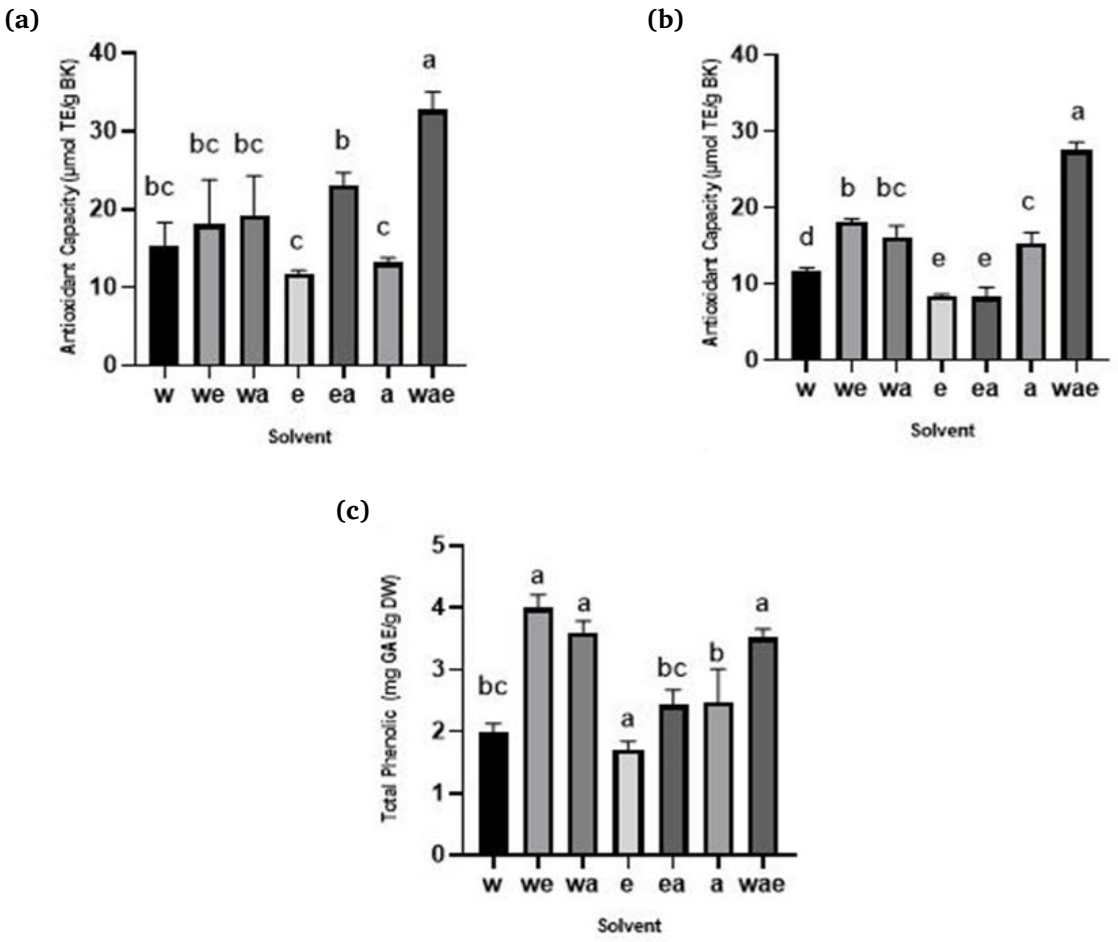

Figure 1. (a) ABTS test, (b) FRAP test, (c) TPC. Each value is shown to be the average of three replicates ± standard deviation (SD), respectively.

Determination of antioxidant activity via the FRAP and ABTS methods using a scatter plot showed a correlation between the two tests. This can be observed in the R2 value of 0.6277** (**Figure 2**). According to Rumpf et al. (2023), results related to FRAP and ABTS have a strong positive correlation with an R2 value of 0.763.

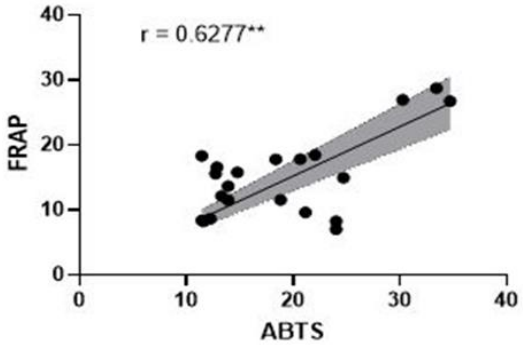

Figure 2. FRAP and ABTS standard curves. The correlation value obtained was R2 = 0.6277**, which explains the positive correlation between FRAP and ABTS, which is indicated by the narrower curve area, meaning that the wider the area on the curve, the closer the R2 value is to one.

In addition, the Scatter Plot on TPC and FRAP showed a positive correlation of R2 = 0.7193***, with the area of the curve getting narrower, indicating that the R2 value approached one (**Figure 3**). According to research by Dudonné et al. (2009), phenolic compounds in plant extracts have an important role that significantly influences the ability of antioxidant activity. Thus, phenolic compounds impact the formation of phenoxy radicals from one or more hydroxyl groups to stop free radicals (Bors & Michel, 2002).

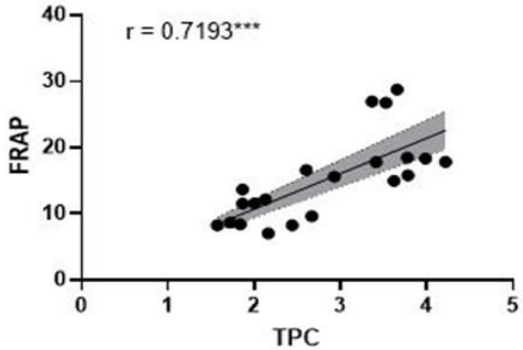

Figure 3. TPC and FRAP standard curves. The correlation value obtained was R2 = 0.7193***, which explains the positive correlation between TPC and FRAP, which is indicated by the narrower curve area, meaning that the smaller the area on the curve, the closer the R2 value is to one.

Then related to the results of the correlation between TPC and ABTS which shows an insignificant correlation of R2 = 0.4632* (**Figure 4**). These results accordance with the research by Kim & Lee (2020), which explains that in the research conducted, there was a strong correlation between TPC and ABTS, giving a value of R2 = 0.817 or a positive correlation.

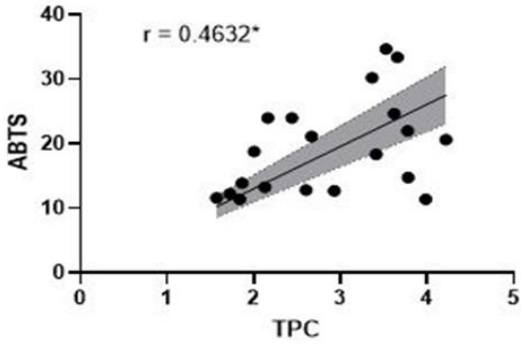

Figure 4. TPC and ABTS standard curves. The correlation value obtained was R2 = 0.4632*, which explains the positive correlation between TPC and ABTS, which is indicated by the curve area not being too narrow, indicating that the sample will not detect a small amount of secondary metabolites when using these two tests.

CONCLUSION

The combination of water, acetone, and ethanol solutions in the process of extracting the phenolic and antioxidant contents of starfruit was used to show the effect of solvent maceration on the extraction of total phenolics from starfruit leaves and to evaluate its antioxidant activity capacity using the FRAP and ABTS methods. The results showed that the highest total phenolic content, with a quadratic curve shape and an R-squared value of 0.6277, was found in the water:ethanol solvent extract with a concentration of ± 0.2217 mg GAE/g DW. The highest antioxidant activity of FRAP, with a quadratic curve and an R-squared value of 0.7193, was found in the solvent water:acetone:ethanol (33:33:33), which had the highest capacity, namely 27,479 ± 1,094 µmol TE/g DW. The highest antioxidant activity of ABTS with a linear curve and an R-squared value of 0.4632 was found in the solvent water:acetone:ethanol with a ratio of 33:33:33, showing the highest antioxidant activity of 32,789 ± 2,294 µmol TE/g DW.

ACKNOWLEDGMENT

The author would like to thank the Department of Biochemistry, Faculty of Mathematics and Natural Sciences, IPB University, and the Center for Tropical Biopharmaceutical Studies.

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Citation Format: Putri, D. A., Gultom, M. F., Binukajati, F. A., Maulana, A. S. T., Putra, R. P., Liwanda, N., & Nurcholis, W. (2023). Effect of Starfruit Extract (*Averrhoa bilimbi* Linn) Using a Combination of Water, Acetone, and Methanol Solvents on Phenolic Content and Antioxidant Capacity. *Jurnal Jamu Indonesia, 8*(3), 84–89. https://doi.org/10.29244/jji.v8i3.343