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Antioxidant activities (DPPH and ABTS method) from extract of Bangle rhizome (*Zingiber cassumunar*) using different method of extraction

Aktivitas Antioksidan (Metode DPPH dan ABTS) dari ekstrak Rimpang Bangle (Zingiber cassumunar) menggunakan metode ekstraksi yang berbeda

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Abstract

Antioxidants are important for the prevention of oxidative stress, which can cause various degenerative diseases. Bangles contain bioactive components with antioxidant potential. Processing affects the active compounds in food, including angle. This study aimed to determine the effects of the blanching and extraction methods on the antioxidant activity of Bangle. This study focused on three factors: blanching treatment extraction technique, time, and type of extraction solvent. Analyses of antioxidants using the DPPH and ABTS methods were conducted in triplicate for each sample. Data were analyzed using ANOVA. The results of this study show that the method, time, and type of solvent significantly affect the antioxidant activity of bangle rhizome extracts. The best treatment for antioxidant activity of Bangle rhizome was blanching treatment by adding 0,05% citric acid solution in the sonication extraction method for 30 min with ethanol as the solvent. The IC_{50} value on the DPPH method was 20,61±0,76 mg/g, and the percent free radical scavenging value was 87,11±3,04%. The IC_{50} value on the ABTS method is 20,79±0,29 mg/g and a percent free radical scavenging activity value of 92,75±0,13%. This study provides key insights for choosing effective extraction methods to increase antioxidant activity in natural materials, such as Bangle rhizomes.
Keywords: Antioxidant, Bangle rhizome, blanching, extraction method

Abstrak

Antioksidan penting dalam mencegah oksidatif yang dapat menyebabkan berbagai penyakit degeneratif. Bangle mengandung komponen bioaktif dengan potensi antioksidan. Pengolahan mempengaruhi senyawa aktif dalam makanan, termasuk pada Bangle. Tujuan penelitian untuk mengetahui pengaruh metode *blanching* dan ekstraksi terhadap aktivitas antioksidan rimpang Bangle. Penelitian terdiri dari tiga faktor yaitu perlakuan *blanching*, teknik dan waktu ekstraksi dan jenis pelarut. Analisis antioksidan menggunakan metode DPPH dan ABTS dilakukan rangkap tiga untuk setiap sampel. Data dianalisis menggunakan ANOVA. Hasil penelitian menunjukkan bahwa metode, waktu, dan jenis pelarut berpengaruh nyata terhadap aktivitas antioksidan ekstraksi rimpang bangle. Perlakuan terbaik terhadap aktivitas antioksidan rimpang Bangle adalah perlakuan blanching dengan penambahan larutan asam sitrat 0,05% dengan metode ekstraksi sonikasi dan waktu 30 menit dengan pelarut etanol. Nilai IC_{50} pada metode DPPH sebesar 20,61±0,76 mg/g, dan nilai persen penangkal radikal bebas sebesar 87,11±3,04%. Nilai IC_{50} pada metode ABTS sebesar 20,79±0,29 mg/g dan nilai persen aktivitas penangkal radikal bebas sebesar 92,75±0,13%. Penelitian ini dapat

memberikan wawasan penting dalam memilih metode ekstraksi yang efektif untuk meningkatkan aktivitas antioksidan dalam bahan alami seperti rimpang Bangle.
Kata Kunci: Antioksidan, *blanching*, rimpang Bangle, metode ekstraksi

Introduction

Oxidative stress constitutes a significant risk factor contributing to the development of various chronic ailments. Oxidative stress arises from internal factors, such as reactive oxygen species (ROS), including hydroxyl, superoxide anion, hydrogen peroxide, singlet oxygen, nitric oxide, and hypochlorite radicals. External factors, such as smoking, ionizing radiation, pollution, organic solvents, pesticides, and pesticides, also contribute. External factors, such as smoking, ionizing radiation, pollution, organic solvents, pesticides, and pesticides, also contribute. These factors can assault nucleic acids, proteins, enzymes, and other minute molecules, leading to structural and functional deteriorations (González-Palma et al., 2016). These factors can assault nucleic acids, proteins, enzymes, and other minute molecules, leading to deterioration of structure and function. Free radicals and other reactive oxygen species contribute to the origin of conditions such as diabetes, Parkinson's disease, Alzheimer's disease, cancer, and aging in humans (Asmat et al., 2016; Gašparović, 2020; Percário et al., 2020; Viña et al., 2013).

An antioxidant is a stable molecule capable of providing an electron to a highly reactive free radical, effectively neutralizing it and diminishing its potential to cause harm. These antioxidants mitigate cellular damage by efficiently scavenging free radicals (Zulaikhah 2017). Endogenous antioxidants are also produced in humans makes endogenous antioxidants. Certain antioxidants, such as glutathione, ubiquinol, and uric acid, are synthesized as part of regular metabolic processes within the body (Shi et al., 1999). Superoxide dismutase (SOD) is an antioxidant enzyme produced in the human body that catalyzes the dismutation of superoxide radicals into hydrogen peroxide (H_2O_2) and molecular oxygen (O_2) and serves as a crucial defence mechanism(Sari et al., 2020). However, it remains important to enhance the diet with antioxidants present in food to reduce oxidative harm. An antioxidant is a stable molecule capable of providing an electron to a highly reactive free radical, effectively neutralizing it and diminishing its potential to cause harm. These antioxidants primarily mitigate or hinder cellular damage by scavenging free radicals efficiently. These antioxidants, characterized by their low molecular weights, can interact safely with free radicals, interrupting the chain reaction before the essential molecules incur damage.

Indonesia is endowed with rich and valuable biodiversity, which plays a vital role in advancing the chemical industry. Bangle (*Zingiber cassumunar*) is a well-known plant in Indonesia commonly used as a culinary spice and medicinal plant. Scientists have identified various categories of compounds that exhibit potential antioxidant properties, including phenylbutenoids, curcuminoids, sesquiterpenoids, benzaldehydes, quinones, and essential oils containing monoterpenoids. Many research studies have been conducted to investigate the phytochemical composition and biological effects of *Z. cassumunar,* aiming to establish a solid scientific basis for its traditional medicinal applications. A collective body of biological research on *Z. cassumunar* has delineated its various medicinal attributes, including antioxidative, anti-inflammatory, anticancer, neuroprotective, neurotrophic, cosmeceutical, antifungal, and antimicrobial properties (Han et al. 2021).

The processing of food ingredients, including bangles, can affect the activity of active compounds. Several studies have shown that the blanching heat treatment can lead to an increase in these functional components. For instance, Pujimulyani et al. (2010) found that white turmeric rhizomes blanched in 0,05% citric acid medium at 100°C for 5 min showed significantly enhanced antioxidant activity, total phenol levels, total flavonoids, and condensed tannin levels compared to white turmeric without blanching. Pujimulyani et al. (2022) also report, black saffron powder with the blanching process has shown better

antioxidant activity than that without the blanching process. Blanching using citric acid media 0,05% for five minutes resulted in the best antioxidant activities, as indicated by the high contents of total phenolics, total flavonoids, and tannins. Similar findings have been observed in studies involving materials beyond rhizomes, such as pomegranate peel extracts. Antioxidant assays, including DPPH, ABTS, and FRAP assays, revealed that blanching enhanced the antioxidant potential of pomegranate peel extracts, as evidenced by higher levels of polyphenols, catechin, and epicatechin (Magangana et al., 2021).

Moreover, the extraction method affected the bioactive components obtained. It is crucial to select an appropriate extraction process to maximize the yield of the material. In a study by Momchev et al. (2020), the extraction of Echinacea purpurea using a mixed solvent of water-ethanol and water-glycerol demonstrated anti-radical properties. Additionally, ultrasonic wave extraction can achieve significantly higher yields of phenolic acids in a shorter extraction time. This finding was also supported by Oroian et al. (2020), who highlighted that ultrasonic extraction yielded better results than microwave extraction and maceration when extracting propolis.

This study aimed to assess the antioxidant activity of *Z. cassumunar* using the DPPH and ABTS methods. This study evaluated the effects of various processing and extraction methods on the rhizomes of the plant, specifically 1) blanching techniques (non-blanching, blanching, and blanching with citric acid addition); 2) extraction technique and time (24 h maceration, 48 h maceration, 15 min sonication, and 30 min sonication); and 3) type of extraction solvent (water and ethanol).

Methods

The aim of this study was to assess the radical scavenging activity of Bangle rhizome extracts using the DPPH and ABTS methods. This research was conducted from January to August 2023 at the Post-Harvest and Packaging Laboratory, University of Mulawarman.

Material and Tools

The Raw materials used in this study were sourced from Bangle Rhizome, which was procured from the experimental garden of the Faculty of Agriculture at Teluk Dalam, Samarinda, East Kalimantan, Indonesia. Other ingredients included distilled water (One Med), Technical Ethanol (Medical Alcohol 96%), Absolute Ethanol (Fulltime), Citric Acid (Cap Gajah), potassium iodide, DPPH (2,2 dipenyl-l-picrylhydrazyl) (Smart Lab), and ABTS (2,2 azinobis (3-ethylbenzothiazoline)-6-sulfonic acid (ABTS) (Roche).

The tools used in this research were a Drying Oven, a UV spectrophotometer (Eppendorf BioSpektrometer basic), Analytical Scales (FS-AR210), Digital Scales (AD-600i; ACIS), Rotary Evaporator (RV 10 Digital Diagonal IKA), and Ultrasonic Cleaner (DELTA D68H).

Preparation of Bangle Rhizome

The rhizome of the bangled plants was used as the primary material for this study. The bangled rhizomes were cleaned to remove the roots, stems, and any attached soil. The specimens were then sliced to a thickness of 2 mm using a slicing tool.

Preparing Bangle Rhizome Powder Samples

The procedure used to create samples of bangle rhizome Simplisia involved blanching 2 mm-thick sliced rhizomes at 100°C for 10 min. Citric acid (0,05%) and 1.700 ml of water were used during this process. Subsequently, the steamed products were dried in an oven at 50°C for 18 h. Following drying, the samples were processed using a flour blender until a smooth consistency was achieved. The resulting mixture was then sifted through an 80 mesh sieve.

Process of Extracting Simplicia Bangle Powder

Bangle powder was extracted by dissolving it in a mixture of distilled water and ethanol (at a ratio of 1:10). Maceration was performed at intervals of 24 and 48 h, and sonication was

conducted at intervals of 15 and 30 min. Extraction by sonication was facilitated using a sonicator operating at a frequency of 20 kHz. Following extraction, the obtained mixture was subjected to filtration, employing filter paper to remove the ethanol solvent, and vacuum filtration of distilled water, leading to the separation of the filtrate and powder. The filtrate was then subjected to rotary evaporation (RV 10 Digital Diagonal IKA) at 50°C for 36 h until a dry extract was obtained, which was subsequently placed in a glass bottle.

Antioxidant Activity using DPPH Method

The antioxidant activity test was performed using the spectrophotometric method by calculating the inhibition of 2,2-diphenyl-1-picrylhy-drazyl (DPPH) (Sigma-Aldrich, Germany) reduction. A total of 1 mL of diluted extract in ethanol was added to 1 mL of DPPH (0,15 mM in ethanol), and at the same time, a control consisting of 1 mL of DPPH with 1 mL of ethanol was prepared. The solution was thoroughly mixed and incubated in the dark at room temperature for 30 min. The absorbance was measured at 517 nm (Rahmadi et al., 2017).

Antioxidant Activity using ABTS Method

Antioxidant activity using the ABTS method (2,2-Azinobis (3-ethyl benzothiazoline) -6-sulfonic acid) following the approach outlined by Sami and Rahimah (2015) with some modifications. First, two stock solutions were prepared: (A) containing 7.1015 mg ABTS and (B) containing 3.500 mg K2S2O8.

These solutions were dissolved in 5 mL distilled water and incubated for 12 h. Following the incubation period, solutions A and B were combined in the dark and supplemented with absolute ethanol until the total volume reached 25 ml. The ABTS blank solution was prepared by mixing 1 mL of the ABTS stock solution with absolute ethanol, resulting in a 5 mL solution. Subsequently, 500 µL of 1.000 µg/mL sample solution was added to 1 mL of ABTS solution and absolute ethanol, resulting in a final volume of 5 mL at a concentration of 100 µg/mL. The absorbance values of the samples and blank were then measured within the wavelength range of 745-755 nm and computed.

Data Presentation

Data are presented as % scavenging activity and IC_{50} using the following equation: the ability of the extract to inhibit DPPH and ABTS reduction was calculated by dividing the absorbance of the reduced control sample by the absorbance of the control.

$$Scavenging\ Activity\ [\%] = \frac{[A - B\ ]}{A}\ x\ 100\ \%.$$

Note:
A : Blank Absorbation
B : Sample Absorbation

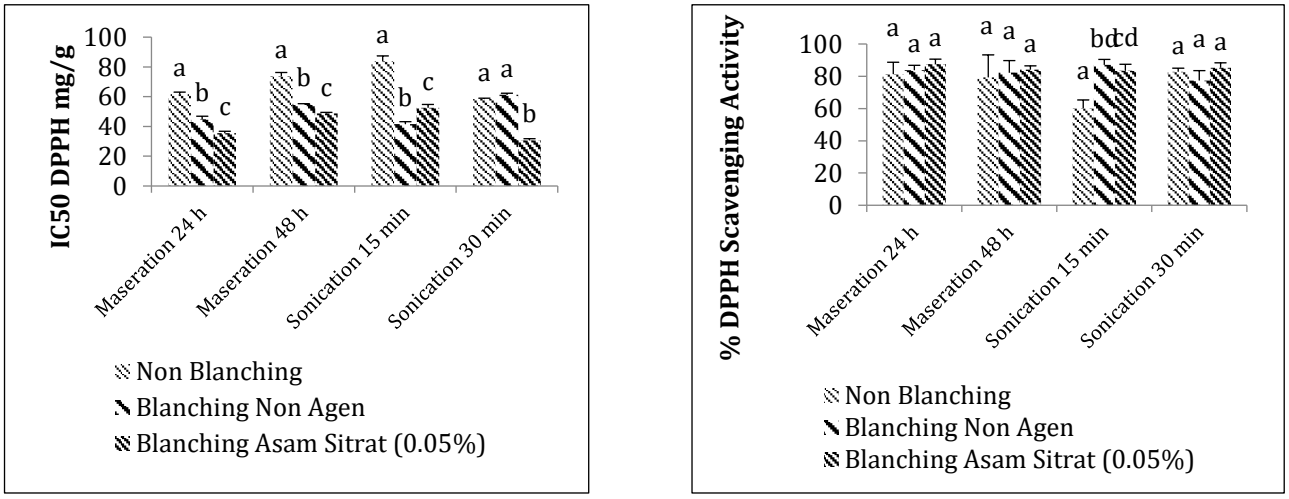
The inhibition percentage was plotted on a curve as variable y, and the sample concentration was plotted as variable x. The capacity value is shown as IC_{50}. IC_{50} is the concentration of the sample solution that reduces the DPPH radical activity by up to 50%. The smaller the IC_{50} value, the greater the antioxidant activity. The total antioxidant activity was plotted using a linear regression equation to obtain the IC_{50} Value.

[Antioxidant potential] = [ingredients in ppm] + b

The results are presented as the mean ± standard deviation of three replicates. ANOVA was used to analyze the antioxidant activity.

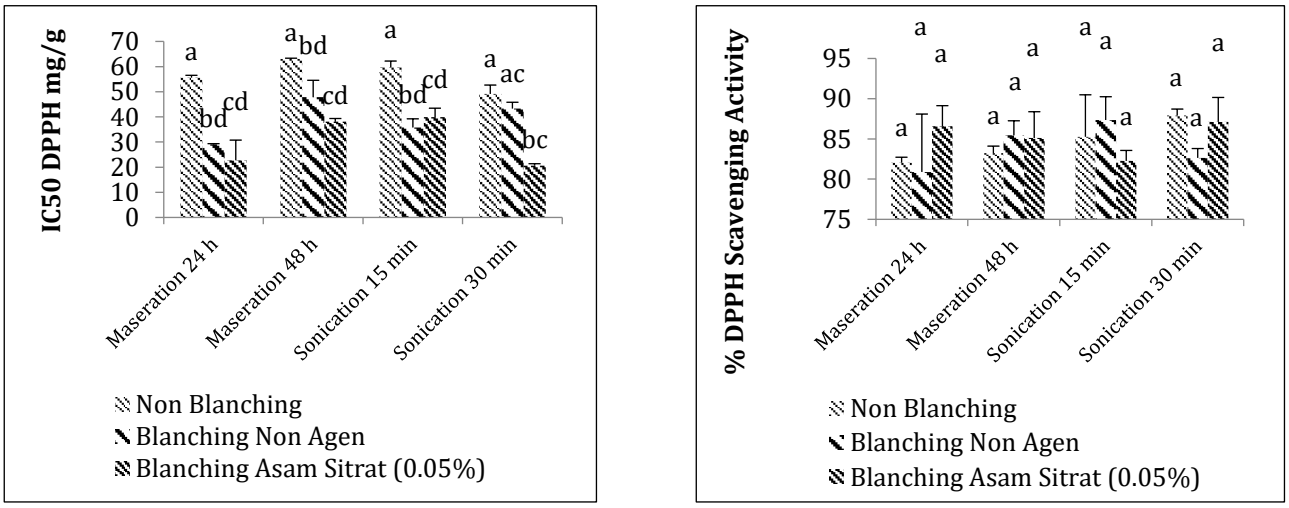
Result and Discussion

The antioxidant activities of the Bangle rhizome extracts with different blanching and extraction treatments using the DPPH method are presented in Figure 1 and 2. In extraction using distilled water, the best antioxidant activity was found in the 0,05% citric acid blanching treatment after 30 min of sonication, with an IC_{50} value of 30,67 ± 1,09 mg/g, and the percent scavenging activity value was 85,27 ± 3,05%. Meanwhile, the use of bangle rhizome ethanol, which had the highest antioxidant activity, was obtained by blanching treatment with 0,05% citric acid at 30 minutes of sonication with an IC_{50} value of 20,61 ± 0,76 mg/g with a percent scavenging activity value of 87,11 ± 3,04%. BHT and catechin as positive controls had IC_{50} values of 14,36±3,00 mg/g and 8,54±0,88 mg/g, respectively, while the percent Scavenging activity values were 83,35±0,26% and 86,69±0,32% respectively.



(a) (b)

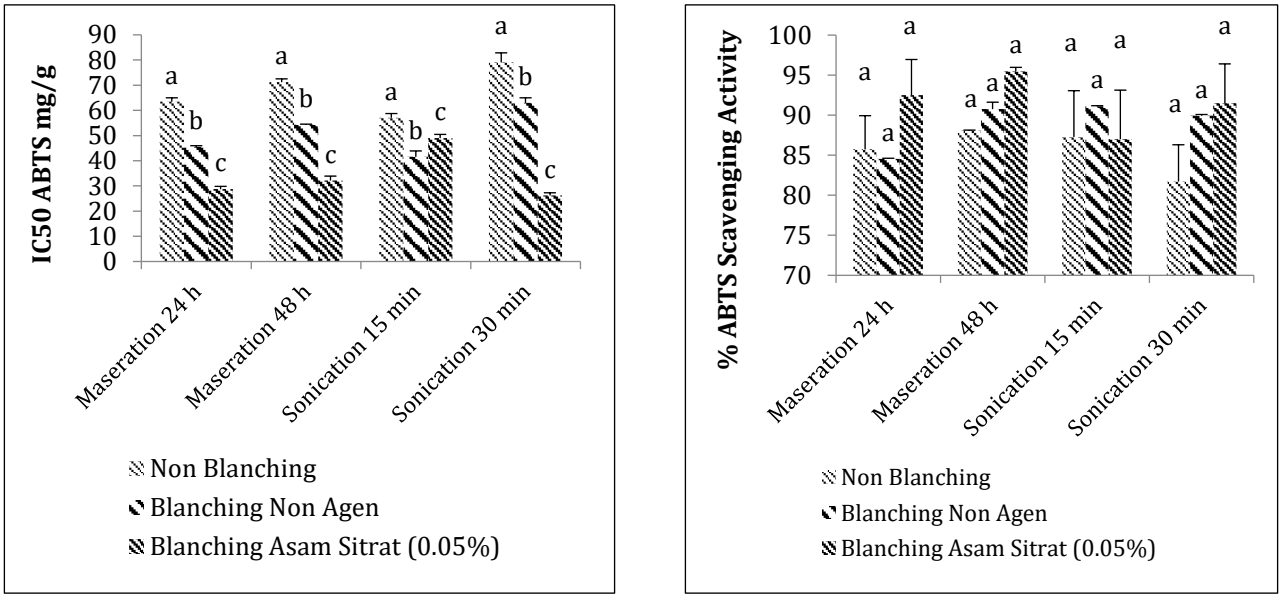
Figure 1. Effect of method and time using distilled water solvent on antioxidant activity IC_{50} (a) and % of free radical scavenging activity *100 ppm (b) Extraction of bangle rhizomes using the DPPH method. Note: numbers quoted with the same letters are not significantly different for each extraction method



(a) (b)

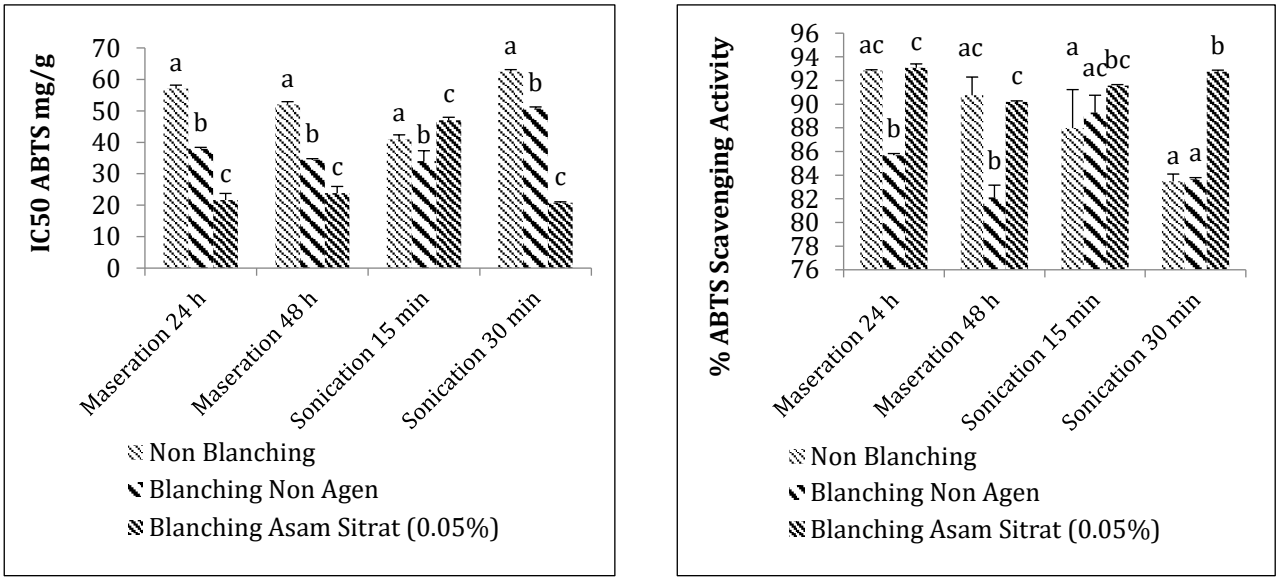
Figure 2. Effect of method and time using ethanol solvent on antioxidant activity IC_{50} (A) and % inhibition of free radical scavenging activity *100ppm (B) extraction of bangle rhizomes using the DPPH method Note: The numbers quoted with the same letters indicate that they are not significantly different for each extraction method.

The antioxidant activities of Bangle rhizome extracts with different blanching and extraction treatments using the ABTS method are presented in Figure 3 and 4. In extraction using distilled water, the best antioxidant activity was found in the 0,05% citric acid blanching treatment after 30 min of sonication, with an IC_{50} value of 30,67±1,09 mg/g, and the percent scavenging activity value was 85,27±3,05%. Meanwhile, the use of bangle rhizome ethanol, which had the highest antioxidant activity, was obtained by blanching treatment with 0,05% citric acid at 30 minutes of sonication with an IC_{50} value of 20.61±0,76 mg/g with a percent scavenging activity value of 87,11±3,04%. BHT and catechin as positive controls had IC_{50} values of 14,36±3,00 mg/g and 8,54±0,88 mg/g, respectively, while the percent scavenging activity values were 83,35±0,26% and 86,69±0,32% respectively.



(a) (b)

Figure 3. Effect of method and time using distilled water on antioxidant activity IC_{50} (A) and % Scavenging activity *125ppm (B) Bangle rhizome extraction method Note: the numbers quoted with the same letters indicate that they are not significantly different for each extraction method.



(a) (b)

Figure 4. Effect of method and time using ethanol solvent on antioxidant activity IC_{50} (A) and % Scavenging activity *125ppm (B) Bangle rhizome extraction ABTS method Note: the numbers quoted with the same letters indicate that they are not significantly different for each extraction method

Based on the analysis of variance, the results of testing the antioxidant activity using the DPPH and ABTS methods on bangle rhizome extraction showed that the interaction between method and time with different treatments of bangle rhizome extraction had a significant effect. Bangle rhizome antioxidant activity was measured using IC_{50} and inhibition percentage. In this study, BHT (synthetic antioxidants) and catechins (pure compounds) were used as the positive controls.

The ABTS and DPPH methods have their own advantages. The ABTS method can be used in both aqueous and organic systems, with faster reaction times and the ability to operate over a wide range of pH levels. However, this method is highly sensitive to light and requires a relatively long incubation time, specifically 12-16 hours under dark conditions (Hidayati et al., 2017). In contrast, the DPPH method can be applied to both solid and liquid samples but does not work specifically for particular antioxidant components. This method measures the overall antioxidant capacity of a sample by determining the hydrogen-capturing reaction of DPPH from the antioxidant substances. The DPPH method relies on the reduction of the unpaired electron of the nitrogen atom by a hydrogen atom, forming a yellow hydrazine group. The ABTS test relies on the generation of a blue/green ABTS+ compound that can be removed by antioxidants (Baliyan et al., 2022). Although these two methods have the same principle, namely, prevention of free radicals, our research determined that the 2,2 Diphenyl-1-picrylhydrazyl (DPPH) method offers a fast, easy, and cost-effective way to evaluate antioxidant characteristics.

The average value of the antioxidant activity, both the IC_{50} value and the percent inhibition of free radical scavenging increased with the method, time, and type of solvent for extracting bangle rhizomes after blanching treatment. Adding 0,05% citric acid to the blanching also significantly increased antioxidant capacity. Blanching can potentially enhance the concentration of bioactive compounds because it can help break down the cell walls and membranes of plant materials, making these compounds more accessible and extractable during subsequent processing steps. In addition, Xiao et al. (2017) stated that thermal blanching can cause structural changes in plant tissues, such as disruption of cell membranes; loosening of hemicellulose, cellulose, and pectin networks; and alternating cell wall porosity. This would improve the extraction of bioactive compounds. This finding aligns with Pujimulyani et al. (2010) and Pujimulyani et al. (2022), who reported that rhizomes of white turmeric and black saffron, when subjected to a blanching process using 0.05% citric acid for five minutes, exhibited better antioxidant activity than non-blanching.

The results of the DPPH and ABTS method analyses indicated that employing a blanching treatment with the addition of 0,05% citric acid significantly enhanced antioxidant activity compared to both the non-blanching and blanching treatments without the added agent. This improvement can be attributed to the preservation of bioactive compounds such as alkaloids, tannins, and flavonoids during the blanching process with citric acid. Incorporating organic acids during blanching can safeguard the nutritional attributes. Organic acids are employed to enhance color preservation and maintain the overall quality of the end product. Chelating agents such as citric acid can offer stability and protection against oxidation in the Bangle rhizome. Citric acid is widely used because of its ability to reduce heat requirements by lowering pH. Additionally, citric acid exhibits antioxidant properties, particularly in mitigating lipid peroxidation, deactivating undesirable enzymes, and enhancing flavor(Martínez et al., 2013).

Two solvents were chosen to obtain the right target compounds as antioxidants for extracting bangle rhizomes. A compound dissolves in solvents of the same polarity. Ethanol and distilled water were used as polar solvents. Wakeel et al. (2019) also stated that solvents can dissolve extractions with the same polarity. The amount of ethanol used was higher than that of distilled water. Ethanol has a similar polarity level and is more effective in dissolving flavonoid compounds in Bangle rhizomes; therefore, Bangle rhizome extract using ethanol solvent produces high levels of flavonoid compounds. Among the Bangle rhizome extracts, distilled water had the lowest antioxidant activity. Distilled water is the most polar solvent compared to other solvents, so polar components, such as carbohydrates, are also extracted, causing the antioxidant activity per sample weight to be low. This is supported by Sopee et al. (2019), who found that the DPPH radical scavenging activity of Nephelium mutabile rind extract was higher when using 80% ethanol as a solvent than when using water and lower concentrations of ethanol. This is in line with a study by Suryanto and Taroreh (2020), who found that corn cob had a higher free radical scavenging activity from ethanol extract than from distilled water extract during sonication extraction.

Maceration and sonication were the extraction methods used in this study. These two extraction methods were selected because they were relatively simple and easy to perform. These two extraction methods were used to determine the optimal extraction method. The results showed that sonication was more effective than maceration in extracting bangle rhizomes. The sonication extraction method uses ultrasonic waves more significantly than 20 kHz, which can vibrate bangled rhizome powder during extraction. This accelerates the contact between the sample and the solvent to extract the resulting compounds more efficiently. However, the maceration method involves an extraction process in a stationary state, which can decrease the extraction efficiency of active compounds.

The antioxidant activity was optimal after 24 h of maceration. After 24 h of maceration, antioxidant activity decreased. This is because the extraction time is too long, which causes the extraction to be hydrolyzed; therefore, it is not appropriately extracted from the material. This research is in line with the study by Sayuti (2017), who stated that the highest increase in antioxidant activity at 24 h of maceration extraction time was 39,94%, and the lowest was at 48 h of maceration extraction time of 33,77%. The highest sonication extraction time was obtained at 30 min, and the lowest sonication extraction time was obtained at 15 min. This is because the longer the contact time between the solute and solvent during the extraction process, the greater the amount of chemical constituents extracted, thereby increasing the extract yield.

Conclusion

Based on this study, the method, time, and type of solvent significantly affected the antioxidant activity of bangle rhizome extract. The highest antioxidant activity was obtained from the DPPH method, namely, the blanching treatment with the addition of 0,05% citric acid solution in the 30 min sonication method using an ethanol solvent.

This research can offer crucial insights into the selection of effective extraction methods to preserve or enhance antioxidant activity in natural materials, such as Bangle rhizomes. Antioxidant testing of Bangle rhizome extract using alternative methods such as FRAP and CUPRAC still needs to be evaluated to determine their compatibility, and should be conducted with different measurement principles. This study is expected to serve as a foundation for further research on the testing of specific bioactive components that may have potential applications as antioxidants or for other purposes such as anti-inflammatory, antidiabetic, and antimicrobial properties.

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