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Effect of extraction method on total phenolic content and antioxidant activity of *Terminalia catappa* (L.) leaves

Tubagus Akmal[1*], Andi Ika Julianti[1], Yenni Puspita Tanjung[1], Pipyt Mutiara[1], Sapitri Febriyanti[1]
[1]Akademi Farmasi Bumi Siliwangi,
Jl. Rancabolang no 104, Margahayu Raya, Sekejati, Kec. Buahbatu, Kota Bandung, Jawa Barat 40286 Indonesia

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✉ * E-mail Author: tubagus.akmal93@gmail.com

ABSTRACT

The potential for encountering free radicals poses a significant concern. Ketapang (Terminalia catappa L.) is a plant with antioxidant properties due to secondary metabolites, specifically phenolic compounds. The objective of this study was to determine the total phenolic content and antioxidant activity of ketapang leaves through the utilization of several extraction techniques. The extraction of Ketapang leaves is conducted using various methods, namely maceration, stirring-assisted extraction (SAE), ultrasound-assisted extraction (UAE), and microwave-assisted extraction (MAE) with 1:10 solid-solvent ratio. The total phenolic content of the Ketapang leaf extract was determined using the Folin-Ciocalteu technique and quantified using a UV-Visible spectrophotometer set at a wavelength of 782nm. Meanwhile, antioxidant activity measurements were carried out using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method and measured at a wavelength of 516nm. The study investigated the total phenolic content and antioxidant activity of Ketapang leaf extract using the maceration method of stirring-assisted extraction (SAE), ultrasound-assisted extraction (UAE), and microwave-assisted extraction (MAE), respectively, were 68.495%±2.891, 75.709%±0.106, 84.269%±0.159, and 65.065%±3.965 mgGAE/g and 20.891, 17.569, 15.427, and 21.353 µg/mL. The findings of this study indicate that the ultrasound-assisted extraction technique exhibits the greatest overall phenolic content and antioxidant activity.

Keywords: antioxidant, Terminalia catappa, total phenolic content, ultrasound-assisted extraction

ABSTRAK

Perkembangan ilmu dan teknologi tidak hanya memberikan manfaat terhadap kehidupan manusia. Salah satu dampak negatifnya adalah terjadinya kondisi stres oksidatif yang disebabkan oleh proses oksidasi radikal bebas. Stres oksidatif dapat dicegah dengan senyawa antioksidan. Ketapang (Terminalia catappa L.) merupakan tanaman yang mempunyai sifat antioksidan karena adanya metabolit sekunder khususnya senyawa fenolik. Tujuan penelitian ini adalah untuk mengetahui kandungan fenolik total dan aktivitas antioksidan daun ketapang melalui pemanfaatan beberapa teknik ekstraksi. Ekstraksi daun ketapang dilakukan dengan berbagai metode, yaitu maserasi, stirring-assisted extraction (SAE), ultrasound-assisted extraction (UAE), and microwave-assisted extraction (MAE) dengan perbandingan simplisia-pelarut 1:10. Kandungan fenolik total ekstrak daun ketapang ditentukan dengan menggunakan teknik Folin-Ciocalteu dan diukur menggunakan spektrofotometer UV-Visible pada panjang gelombang 782nm. Pengukuran aktivitas antioksidan dilakukan dengan metode 2,2-diphenyl-1-picrylhydrazyl (DPPH) dan diukur pada panjang gelombang 516nm. Hasil penelitian menunjukkan kandungan fenolik total ekstrak daun ketapang dengan metode maserasi stirring-assisted extraction (SAE), ultrasound-assisted extraction (UAE), and microwave-assisted extraction (MAE) masing-masing sebesar 68,495%±2,891, 75,709%±0,106, 84,269%±0,159, dan 65,065%±3,965 mgGAE/g dan aktivitas antioksidan masing-masing sebesar 20.891, 17.569, 15.427, dan 21.353 µg/mL. Temuan penelitian ini menunjukkan bahwa teknik ultrasound-assisted extraction (UAE) menunjukkan kandungan fenolik dan aktivitas antioksidan terbesar secara keseluruhan.

***Kata Kunci**: antioksidan, kadar fenolik total, Terminalia catappa, ultrasound-assisted extraction*

1. INTRODUCTION

The impact of technological advancements on human beings is not always favorable. The poor management of rapidly advancing technological breakthroughs gives rise to various issues. The advent of several types of diseases, including chronic diseases and degenerative diseases such as cancer, respiratory disorders, and cardiovascular disorders, is perceived by a significant number of individuals as having a substantial influence. One of the precipitating factors arises from the encounter with free radicals, which can be acquired by exposure to unfavorable surroundings, atmospheric contamination, UV radiation, and hazardous chemical pollutants.[1]

Free radicals are chemical species that possess one or more unpaired electrons, resulting in their characteristic high reactivity.[2] Free radicals are composed of three components: oxygen, nitrogen, and sulfur. Reactive oxygen species (ROS) encompass a group of oxygen-centered free radicals, namely superoxide, hydroxyl, peroxyl, alkoxyl, and nitrogen monoxide. Hydroxyl and alkoxyl groups exhibit high reactivity, facilitating rapid interaction with adjacent molecules.[3]

Free radicals can originate from external and internal sources in the human body. In essence, human beings generate free radicals as a byproduct of metabolic processes involved in energy production. Energy is acquired by oxidative phosphorylation, which occurs within mitochondria through the reaction between hydrogen and oxygen.[4] The overproduction of free radicals has the potential to cause harm to macromolecules, including nucleic acids, proteins, and lipids. This phenomenon induces tissue injury and triggers oxidative stress.[5]

Antioxidant molecules can safeguard metabolic processes against the detrimental impacts of reactive oxygen species (ROS) and oxidative stresses. Antioxidants are chemical compounds that possess the ability to prevent the process of oxidation and the generation of free radicals.[6] The antioxidant activity is also influenced by the quantity and composition of hydroxyl groups attached to the aromatic ring. Antioxidants possess the ability to function as hydrogen donors in a broad sense. The addition of hydroxyl groups to the phenol ring has been observed to have an inhibitory effect on the oxidation process.[3] Antioxidants, owing to their tight association with phenolic chemicals, are inherently present in several plant components, encompassing fruits, vegetables, nuts, seeds, leaves, roots, and skin. There is a positive correlation between the concentration of phenolic chemicals in a plant and its antioxidant capacity.[5]

Several plants containing phenolic compounds, including the ketapang plant (*Terminalia catappa* L.), have been shown to possess potent antioxidant properties.[7] The majority of ketapang plants possess utility, with ketapang leaves being one notable example. Flavonoid chemicals found in Ketapang leaves have been identified as a phenolic content with potential therapeutic applications in the treatment of several degenerative diseases, including diabetes, anti-cholesterol, anti-hypertension, and other associated advantages. Numerous investigations have substantiated the presence of diverse substances, including alkaloids, phenols, flavonoids, saponins,

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tannins, and carotenoids, inside ketapang. These compounds have been observed to have significant antioxidant activity. The utilization of this substance in traditional medicine has been observed to possess antibacterial, anti-inflammatory, antidiabetic, hepatoprotective, and anticancer properties.[8,9]

The extraction method can affect the amount of bioactive compounds extracted from a plant including the amount of phenolic compounds. Multiple extraction procedures can be employed, including maceration, reflux, Soxhlet, ultrasonic, and microwave methods.[10] Nonetheless, the utilization of unsuitable extraction techniques may have an impact on the extent of phenolic component retrieval from botanical specimens. Hence, the selection of the extraction technique holds significant importance in achieving phenolic compounds that align with the specified criteria.[11]

In light of this, it is essential to conduct a number of antioxidant assays to evaluate the antioxidant potential of plant extracts. This assessment serves as a crucial criterion for determining the usefulness of these extracts. The objective is to identify the optimal extraction process for obtaining ketapang extract with the highest antioxidant value.

2. METHODOLOGY

Materials and Tools

The materials used in this research include *Terminalia catappa* leaves (Nano brothers herbal), distilled water (Rofa laboratory), gallic acid (Merck), 2,2-Diphenyl-1-picrylhydrazyl (Sigma Aldrich), methanol p.a (Merck), folin-ciocalteu's phenol (Merck), sodium carbonate anhydrous (Merck). The tools employed in this study include digital overhead stirrer (DLAB, Beijing, China), magnetic hotplate stirrer (DLAB, Beijing, China), sonicator bath (EECOO, China), microwave (SHARP, Karawang, Indonesia), UV-Visible spectrophotometer (Shimadzu, Kyoto, Japan), micropippete (Socorex, Switzerland).

Methods

a. Extraction

For all extraction methods, 50 grams of ketapang leaf powder added with distilled water with a solid-solvent ratio of 1:10 (w/v) was put into a glass beaker. Maceration is carried out by soaking ketapang leaf powder for 72 hours. Every 24 hours, the solution was stirred for two minutes with stirring rod.[12] Stirring-assisted extraction (SAE) process commences by positioning a beaker onto a hotplate set at a temperature of 50°C. Subsequently, stirring is initiated using an overhead stirrer operating at a velocity of 200 revolutions per minute. The extraction process was conducted for 60 minutes.[13]

The extraction method using ultrasound-assisted extraction (UAE) is according to that used by Kunarto et al., (2019), with slight adjustments. The extraction process was conducted using a bath sonicator operating at 40 kHz and a temperature of 50°C for 30 minutes. During the extraction, the extract was periodically stirred every 5 minutes for 1 minute using a stirring rod.[14] The microwave-assisted extraction (MAE) method was employed to perform extraction using a microwave oven. Place the beaker in the microwave and adjust the power setting to 540 watts, allowing it to operate for 10 minutes. The findings obtained from each extraction method are then subjected to

filtration and concentration processes to create a concentrated extract.[15] The extraction yield of *Terminalia catappa* leaves was calculated by the following equation:

$$Extraction\ Yield\ (\%) = \frac{W_e}{W_s}\ x\ 100$$

Where W_e is the mass of *Terminalia catappa* extract and W2 is the mass of the sample.

b. Total Phenolic Content Determination

The total phenolic content (TPC) was measured using the Folin-Ciocalteu (FC) method. Before measuring the total phenolic content of Ketapang leaf extract, the maximum wavelength and incubation time were first determined. 0.5 ml of gallic acid (GA) (100 µg/ml) was mixed with 2.5 ml of 10% FC reagent (v/v) and then incubated for 5 minutes at room temperature. Then, 2 ml of 7.5% sodium carbonate (Na_2CO_3) was added and incubated at room temperature.[16] Every 5 minutes of incubation for 60 minutes, the solution was measured using UV-Visible spectrophotometry at a wavelength of 400–900 nm.

To determined TPC of sample, 2.5 ml of 10% FC reagent, 0.5 ml of known dilution of the Ketapang leaf extract (1000 µg/ml), incubated at room temperature for 5 minutes, and 2 ml of 7,5% Na_2CO_3 solution were added. After incubation time of incubation at room temperature in the dark, the absorbance at maximum wavelenght was measured. GA (10-50 µg/ml) was used as a standard for constructing the calibration curve. Total phenolic content was expressed as milligrams of gallic acid equivalents per gram dry weight (mg GAE/g DW).[17]

c. Antioxidant Activity Determination

The antioxidant activity of katapang leaf extract was determined based on the scavenging ability of DPPH free radicals expressed in the form of IC50 concentrations. Determination of the maximum wavelength of DPPH was carried out by measuring the absorbance of a DPPH solution with a concentration of 40 µg/ml at a wavelength of 400-600 nm. Incubation time was determined by measuring the absorbance of a 40 µg/ml DPPH solution measured at maximum wavelength every 5 minutes for 60 minutes and observed that the solution began to produce a stable absorbance.[18]

One milliliter of each sample with varying concentrations (6.25 – 100 µg/ml) were mixed with 2 mL of 40 µg/ml DPPH in a test tube. The mixture was homogenized and incubated at room temperature in the dark for incubation time. The absorbance of the solution was measured using a UV–Visible Spectrophotometer at maximum wavelength.[19] The percentage value of free radical scavenger can be calculated using the equation:

$$Scav.Act_{DPPH}\ (\%) = \frac{Abs_c - Abs_s}{Abs_c}\ x\ 100$$

Where Abs_c is absorbance of control solution and Abs_s is absorbance sample (extract)

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3. RESULT AND DISCUSSION

In this study, samples of ketapang leaves were obtained from the coastal area of Situbondo Regency. The leaves used are ketapang leaves, which are brown and have fallen from the tree. Ketapang leaves are processed into ketapang leaf powder by Nano Brothers Herbal. The authenticity of these specimens was confirmed by the Herbarium Jatinangor, located at Padjadjaran University in Indonesia.

The quality of the extract is greatly influenced by the extraction method used and the type of solvent. Phytochemical compounds from plants can be extracted utilizing extraction procedures that involve polar solvents such as ethanol and water.[20] Water is employed as the solvent during the extraction procedure due to its suitability for the extraction of phenolic compounds. The amount of phenolic compounds extracted will increase with increasing solvent polarity.[21]

Maceration, stirring-assisted extraction, ultrasound-assisted extraction, and microwave-assisted extraction were the comparison techniques. One characteristic shared by all extraction methods is the implementation of a soaking procedure. Maceration is a frigid process in which the extraction temperature is maintained at room temperature, whereas SAE, UAE, and MAE are heated. SAE can augment extraction yields by employing stirring from a head stirrer implement.[22] UAE employs sonicator vibrations to accelerate the extraction of compounds present in plants.[23] The MAE method has several benefits, including a relatively brief extraction duration, which results in comparatively less solvent and energy and a greater extract yield.[24]

Effect of Extraction Method on Yield Extract

According to the data presented in Table 1, it can be observed that the MAE produced the highest %yield and weight of extract in Terminalia catappa aqueous extract (32.04%; 16.02 g), followed by UAE (22.14%; 11.07g), SAE (21.67%; 10.83g), and maceration with the lowest %yield and weight of extract (17.05%; 8.52g). Variations in yield and extract weight arise due to disparities in temperature and duration of extraction. The extraction temperatures utilized in the maceration, SAE, UAE, and MAE are as follows: room temperature (25-30°C), 50°C, 50°C, and 80°C, respectively. In contrast, the duration of extraction is 72 hours, 60 minutes, 30 minutes, and 10 minutes, respectively.

Temperature and time are crucial extraction parameters that must be optimized even to reduce the energy cost of the process. Extraction is facilitated by temperature and time, which increase the solubility of the solute and the coefficient of diffusion.[25,26] An increase in extraction temperature induces the rupture of plant cell walls, which facilitates the dispersion of plant constituents into the water medium. Additionally, an increase in temperature reduces the surface tensions of the solvent, which promotes the formation of cavitation. Therefore, as the temperature increases, so does the compound's solubility in the plant.[27,28]

Effect of Extraction Method on Total Phenolic Content

Before quantifying the phenolic compound concentrations in leaf extract samples from Ketapang, the incubation time is ascertained to ascertain the optimal reaction time and the maximal wavelength necessary to obtain the highest possible concentrations.[29] The incubation time for the total phenolic content's operation was determined to be 45 minutes, during which the maximum wavelength (λ max) observed was 782 nm. The standard curve typically illustrates the linear correlation between absorbance and standard concentrations (10-50 µg/ml). The linear regression equation was derived as y = 0.007x - 0.0776, yielding an R2 value of 0.9854 (Figure 1).

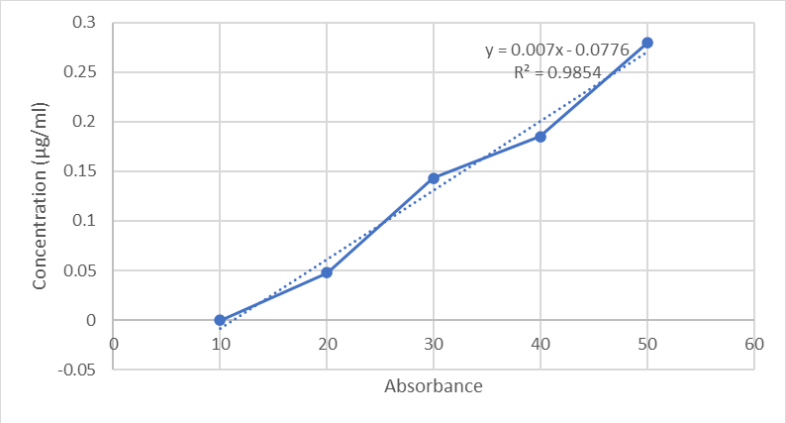

Figure 1. Standard curve of gallic acid for total phenolic content calculation

Table 1 show total phenolic content of Ketapang leaf extract which extracted with maceration, SAE, UAE, and MAE were 167.943±0,655, 218.038±5.918, 251.705±2.970, and 205.943±5.826 mgGAE/g DE respectively. The yellow hue of phosphotungstic and phosphomolybdic acids' anionic derivatives can be changed to blue by antioxidants. The extent of the color change, which is directly related to the reducing activity of phenolic compounds, indicates that the reaction has been completed. The antioxidant ability of an extract from Terminalia catappa is commonly quantified as gallic acid equivalents (GAE). Specifically, the movement of electrons from phenolic compounds to phosphomolybdic/phosphotungstic acid complexes in an alkaline solution generates blue complexes that may be identified via spectroscopy at approximately 782 nm. Polyphenols undergo a reaction with the F-C reagent exclusively in alkaline conditions with a pH of 10, which is achieved by adding a sodium carbonate solution.

In contrast to maceration, the SAE, UAE, and MAE procedures yield a greater quantity of total phenolic content. This finding substantiates the notion that elevated temperatures can influence the overall quantity of phenolic content that is extracted. Additionally, SAE is subjected to an agitating treatment in which the turbulence generated by the increased stirring speed intensifies the contact between the solid and the solvent. Consequently, the mass transfer coefficient increases, leading to a corresponding rise in the diffusion of phenolic compounds from the surface of

ketapang leaf powder into the solvent. This ultimately results in a larger quantity of phenolic compounds being extracted.[30]

Ultrasound on ultrasound-assisted extraction show unique propagation characteristics in fluid media and possess energy that can break down leaf matrices to facilitate the extraction of valuable bioactive compounds. Furthermore, ultrasound has the potential to induce a rise in temperature. In a study conducted by Golmohamadi et al., (2013), ultrasound at a frequency of 20 kHz for a duration of 30 minutes caused a temperature increase of 21^{o}C. As a consequence, the extraction temperature is elevated in the UAE method as opposed to SAE. Consequently, the UAE method yields an extract with a greater total phenolic content than SAE.[31]

Following the rapid generation of heat and pressure by microwave irradiation in MAE method, the physical properties of the plant cells are further altered, and the porosity of the biological matrix is increased. This phenomenon results in a greater yield of phenolic compounds from the sample than maceration.[32] In the present investigation, the duration of microwave exposure was set at 10 minutes, leading to a corresponding elevation in the temperature of the extract solution to a range of 80-87^{o}C. Previously, Mokrani and Madani, (2016) has reported that TPC was actually decreased with increasing temperatures up to 70^{o}C. For this reason, the TPC MAE is diminished in comparison to the SAE and UAE.[33]

Effect of Extraction Method on Antioxidant Activity

The evaluation of the Ketapang leaf extract's antioxidant capacity was conducted by observing the color change of 2,2-diphenyl-1-picrylhydrazyl (DPPH). During the incubation period of 40 minutes, the DPPH radical, which initially generates a purple solution, undergoes a reduction reaction with any antioxidant capable of donating a hydrogen atom. This results in the formation of yellow diphenylpicrylhydrazine. At 516 nm, the absorbance of the DPPH-containing mixed solution is detected.[34] The free-radical DPPH absorption spectrum indicates that the highest level of absorption occurs at a wavelength of 516 nm, resulting in a purple tint. A free-radical scavenger antioxidant reacts with DPPH to produce DPPHH, which has a lower absorbance than DPPH due to a smaller amount of hydrogen. Compared to the DPPH-H state, this radical variant induces decolorization (resulting in a yellow color) as the amount of accumulated electrons rises.

Free radical scavenger capacity of meceration, SAE, UAE, and MAE is 68.495%$\pm$2.891, 75.709%$\pm$0.106, 84.269%$\pm$0.159, and 65.065%$\pm$3.965 respectively. While the value of DPPH antioxidant activity (IC50) is 20.891, 17.569, 15.427, and 21.353 µg/mL respectively (Table 1). These results show that all extraction methods produce extracts with very strong antioxidant categories.

Based on the obtained results, it is evident that ultrasound-assisted extraction exhibits the highest value of free radical inhibition, specifically 84.269%$\pm$0.159. The utilization of ultrasonic extraction is a straightforward, expeditious, and environmentally sustainable method of extraction. The duration needed to execute this method is reduced in comparison to alternative extraction techniques. Ultrasound-

assisted extraction is therefore extensively implemented in numerous industries, including the food and chemical sectors.[35] Nevertheless, time and temperature must be accounted for in this procedure. Extended periods of time and elevated temperatures can accelerate the extraction of polyphenols, leading to the degradation of phenolic compounds and a subsequent reduction in the obtained antioxidant value.[36]

The findings of the study indicated a correlation between the total phenolic compounds and the level of antioxidant activity. There exists a positive correlation between the phenolic content and the antioxidant activity, whereby an increase in phenolic content is associated with a corresponding increase in antioxidant activity.[37] Phenolics exhibit antioxidant properties through several mechanisms. Firstly, they demonstrate a high reactivity towards hydrogen, functioning as electron donors. Additionally, phenolics act as chain-breaking agents, as the radical derived from phenolics can stabilize and distribute the unpaired electron. Furthermore, phenolics are capable of terminating the Fenton reaction by chelating transition metal ions.[38]

Table 1. Yield Extract, Total Phenolic Content, and Antioxidant Activity of Ketapang Leaf Extract

Method	Yield extract (%)	TPC[a] (mg GAE/g DE)	Inhibition capacity[b] (%)	IC_{50} (µg/mL)
Maceration	17.05	167.943 ± 0.655	68.495+2.891	20.891
SAE	21.67	218.038 ± 5.918	75.709+0.106	17.569
UAE	22.14	251.705 ± 2.970	84.269+0.159	15.427
MAE	32.04	205.943 ± 5.826	65.065+3.965	21.353

[a]mean ± standard deviation
[b]mean ± standard deviation inhibition capacity at 50µg/mL concentration

4. CONCLUSION

Based on the findings of this study, it can be inferred that extraction method has a significant impact on the yield extract obtained as well as the total phenolic contents and antioxidant activity of ketapang leaf extract. The microwave-assisted extraction approach yielded the highest yield extract (32.04%), whereas the ultrasound-assisted extraction method resulted in the greatest total phenolic content (251.705 ± 2.970 mg GAE/g) and antioxidant activity (15.427 µg/mL).

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