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Effect of Temperature and Extraction Time on the Total Phenolic Content of 70% Ethanol Extract of Temu Giring Rhizome (*Curcuma heyneana* Valeton & Zijp.)

Dwi Susiloningrum[1*], Yanulia Handayani[2], Luthfiana Nurulin Nafi'ah[3]

[1,2,3)] Pharmacy Faculty, Institut Teknologi Kesehatan Cendekia Utama Kudus, Jawa Tengah, Indonesia

e-mail:
dsusiloningrum@gmail.com

ABSTRACT

Temu giring (*Curcuma heyneana* Valeton & Zijp.) is a type of medical plant in the form of rhizomes. The plant is from the Zingiberaceae family. Intersection giring rhizome has various activities including antioxidant, antiviral, antiaging, and antimicrobial. Temu giring rhizome has various activities including antioxidant, antiviral, phenolic, saponin, and essential oil. The powder of temu giring rhizome (*Curcuma heyneana Valeton* & Zijp) was dissolved in a ratio of 1:10 with a sample weighing 20 grams and then added with 200 mL of 70% ethanol solvent each. Then it was placed in an ultrasonic bath (with temperature variations of 30℃ 40℃and 50℃and time of 10, 20, and 30 minutes) with a frequency of 47 kHz. The extract obtained was then concentrated using a water bath at 40°C, because phenolic compounds are compounds that cannot withstand heating. The purpose of this study was to determine the effect of temperature and time of extraction with UAE (Ultrasonic Assited Extraction) on the total phenolic content of the 70% ethanol extract of the rhizome of teemu giring by spectrophotometry. Based on the results of determining the total phenolic content of 70% ethanol extract of ginger rhizome (*Curcuma heyneana Valeton* & Zijp), the highest phenolic content was at 40°C for 20 minutes, at 2.33% GAE. The results showed that the highest total phenolic content was at a temperature of 40°C with a time 20 minutes namely 2,33%GAE, while the lowest total phenolic content was at a temperature of 30°C with a time of 10 minute namely 1,24%GAE.

Keywords : UAE, total phenolic, *Curcuma heyneana* Valeton & Zijp, temperature

Introduction

The rhizome of temu giring (*Curcuma heyneana* Valeton & Zijp) is a plant from the Zingiberaceae family. The rhizome of temu giring has a bright yellow color and has various activities, including antioxidant, antiviral, antiaging, and

antimicrobial properties. Based on research by Jalil (2019), temu giring rhizomes contain secondary metabolites flavonoids, phenolics, saponins, and essential oils.

Phenolics are compounds that have one or more hydroxyl groups (-OH) that bind to the aromatic ring of phenol. Phenolic compounds are found in many types of plants, especially in plants containing aromatic compounds with a characteristic structure of benzene rings and hydroxyl groups (Hakim *et al*., 2020). Secondary metabolites are biomolecules that can be used as lead compounds in the discovery and development of new drugs (Ergina *et al*., 2014).

Phenolics are polar compounds, and the solvents used to extract these secondary metabolites are usually polar solvents such as ethanol, methanol, and acetone (Rifai *et al*., 2018). Secondary metabolites in a plant are influenced by the type of solvent and extraction method. The solvent used is adjusted to the polarity of the targeted compound. According to the principle of like dissolves like, a solvent will tend to dissolve compounds that have the same level of polarity. Using 70% ethanol as a solvent because ethanol is a polar solvent that can attract polar secondary metabolites (Rachmawati *et al*., 2020). One way to extract secondary metabolites is by extraction. One of the extraction methods is Ultrasonic Assisted Extraction.

UAE (Ultrasonic Assisted Extraction) method can also increase the yield of extracts with faster extraction times and lower amounts of solvent compared to conventional extraction methods (Langat, 2011). The UAE mechanism occurs through increasing the intensity of mass transfer and accelerating solvent diffusion in penetrating and dissolving active compounds in the material (Endarini, 2016).

According to research from Wayan *et al*., (2017) the increase in temperature in the extraction process needs to be considered, too high an extraction temperature and too long an extraction time or exceeding the optimum limit can cause the loss of compounds in the solution due to the oxidation process.

Variations in temperature and extraction duration significantly affected the total phenolic content, antioxidant activity, total solids, color, and pH of ginger juice beverages. Organoleptic tests on taste, color, and appearance parameters showed a significant interaction between treatments at a 5% significance level. The most optimal treatment based on the physicochemical characteristics of ginger juice beverages was achieved at an extraction temperature of 95°C with a time of 25 minutes, in accordance with the physicochemical parameters reported (Ibrahim *et al*., 2015).

Based on the research of Andriani & Murtisiwi, (2018) the phenolic compounds of starfruit leaves with a temperature variation of 40℃with a time of 20 minutes, namely 437.79 mg GAE / g optimal phenol yield. Because high temperatures cause phenolic compounds in the solvent to increase so that the extraction process is easier. Based on the background above, this study aims to determine the effect of temperature and time of extraction with Ultrasonic Assisted Extraction (UAE) on the total phenolic content of 70% ethanol extract of temu giring rhizome (*Curcuma heyneana* Valeton & Zijp).

Methodology

Tools and Materials

Analytical scales (Ohaus), simplicia blender, sieve mesh No. 40, filter paper, measuring cup (Pyrex), beaker glass (Pyrex), Erlenmeyer (Pyrex), water bath (DHH - 4), test tube (Pyrex), glass funnel (Pyrex), dropper pipette, test tube rack, drying cabinet, crucible porcelain, spatula, rotary vacuum evaporator (RE 100 - Pro), moisture balance, micropipette, ultrasonic bath (Brason 2002), label paper, measuring flask (Pyrex), spatula, cuvette, and UV - Vis spectrophotometry (Shimadzu).

Curcuma heyneana Valeton & Zijp rhizome, 70% ethanol, magnesium powder, 2N HCl, concentrated HCl, 10% NaOH, dragendrof reagent, 3% $FeCl_3$, distilled water, p.a. ethanol, gallic acid, 7.5% Na_2CO_3, Folin-Ciocalteu reagent. The research materials used to determine the total phenolic Rimpang temu giring (*Curcuma heyneana* Valeton & Zijp), etanol 70%, serbuk magnesium, HCl 2N, HCl pekat, NaOH 10%, reagen dragendrof, $FeCl_3$ 3%, aquadest, etanol p.a, asam galat, Na_2CO_3 7,5%, reagen Folin-Ciocalteu.

Research Procedures

1. Making dry and powdered simplicial

   The cleaned rhizome of temu giring (*Curcuma heyneana* Valeton & Zijp) is then thinly sliced to facilitate the drying process. The cut rhizome of temu giring (*Curcuma heyneana* Valeton & Zijp) is then weighed as much as 5 kg and sorted wet, then dried using a simplicia drying cabinet at a temperature of 50℃ then sorted dry. The dried simplicia is then mashed using a blender and sieved using a No. 40 mesh sieve to obtain a smooth and homogeneous simplicia.
2. Extraction

   The powder of temu giring rhizome (*Curcuma heyneana* Valeton & Zijp) was dissolved in a ratio of 1:10 with a sample weighing 20 grams and then added with 200 mL of 70% ethanol solvent each. Then it was placed in an ultrasonic bath (with temperature variations of 30℃, 40℃and 50℃and time of 10, 20, and 30 minutes) with a frequency of 47 kHz. Then it was filtered with filter paper (whatman-50). The filtrate obtained was concentrated in a water bath at a temperature of 40℃
3. Phytochemical Screening
   a. Flavonoid Identification

      Identification of flavonoids in ginger rhizomes (*Curcuma heyneana* Valeton & Zijp) using three reagents: Wilstatter's reagent, Bate-Smite's reagent, and 10% NaOH reagent. The Wilstatter test can be performed by taking 1 ml of ginger leaf extract, placing it in a test tube, adding 2-4 drops of concentrated HCl and shaking vigorously. A small amount of magnesium powder is added and shaken vigorously. A positive result is indicated by the appearance of foam and the solution turning yellow/orange (Rahayu *et al*., 2015).

      The Bate-Smite test can be performed by taking 1 mL of extract into a test tube and adding a few drops of concentrated HCl. The mixture is then

heated for 15 minutes on a water bath. The formation of a red color indicates the presence of anthocyanidin flavonoids (Rahayu *et al*., 2015).

The 10% NaOH test can be performed by adding 1 mL of extract to a test tube and adding a few drops of 10% NaOH solution. A color change indicates the presence of flavonoids, as they are classified as phenolic compounds (Rahayu *et al*., 2015).

b. Identification of Phenolics

Phenolic identification is performed by taking a sample and placing it in a test tube. Then, 3 drops of 3% $FeCl_3$ reagent in ethanol solvent are added. The color change is then observed. A positive result is indicated by the presence of green, red, purple, blue, or black (Mukhriani *et al*., 2015).

4. Determination of Total Phenolic Content

a. Determination of maximum wavelength

Determination of the maximum wavelength of gallic acid with Folin-Ciocalteu reagent. A 0.3 mL standard gallic acid solution with a concentration of 30 ppm was put into a 5 mL volumetric flask, 1.5 mL of Folin-Ciocalteu reagent was added and then left for 3 minutes. After that, 1.2 mL of 7.5% Na2CO3 solution was added. The next step was to shake the mixture until homogeneous and leave it at room temperature for the operating time range. Then the solution was measured for absorbance at a wavelength of 600-800 nm. The wavelength that shows the highest absorption is the maximum wave (Alfian & Susanti, 2012).

b. Determination of operating time (OT)

0.3 mL gallic acid solution with a concentration of 30 ppm was placed in a 5 mL volumetric flask, followed by 1.5 mL of Folin-Ciocalteu reagent. 1.3 mL of 7.5% Na_2CO_3 solution was added to the solution and then shaken until homogeneous. The solution was observed for absorbance at its maximum wavelength for 1 hour at 1-minute intervals until a stable absorbance level was reached (Alfian & Susanti, 2012).

c. Determination of total phenolic content of 70% ethanol extract of temu giring rhizome (*Curcuma heyneana* Valeton & Zijp)

Determination of the total phenolic content of a 70% ethanol extract of ginger rhizome (*Curcuma heyneana* Valeton & Zijp) was performed by weighing 10 mg of the extract and dissolving it to a volume of 10 mL with ethanol p.a., then homogenizing it. Then, 0.3 mL of the solution was pipetted and 1.5 mL of Folin-Ciocalteu reagent was added, shaken, and then 1.2 mL of 7.5% Na_2CO_3 solution was added. The mixture was allowed to stand for the operating time. The absorbance of the solution was measured at the maximum wavelength in three repetitions (Alfian & Susanti, 2012).

Result and Discussion

Extraction is the process of extracting soluble active compounds from insoluble compounds using a liquid solvent. Factors affecting extraction rate include extraction time, sample size, temperature, and solvent type. During the extraction

process, the active ingredient will be dissolved by a solvent that matches its polarity (Depkes RI, 2000).

This ultrasonic extraction equipment consists of an extraction vessel equipped with an ultrasonic generator and a water bath for temperature control. This extraction method has the advantage of removing organic and inorganic compounds from the plant matrix (Endarini, 2016). This study used the Ultrasonic Assisted Extraction (UAE) extraction method.

The UAE mechanism involves intensified mass transfer and accelerated solvent access to the active compounds contained within plant cells. This ultrasonic extraction equipment consists of an extraction vessel equipped with an ultrasonic generator and a water bath for temperature control. This extraction has the advantage of removing organic and inorganic compounds from the plant matrix (Endarini, 2016).

The extraction process using the UAE method was carried out by weighing 20 grams of temu giring rhizome powder (*Curcuma heyneana* Valeton & Zijp) and then adding 200 mL of 70% ethanol solvent. Extraction was carried out at temperatures of 30°C, 40°C, 50°C and for 10, 20, and 30 minutes, with three replications. After that, it was filtered and the extract was separated from the residue. The extract obtained was then concentrated using a water bath at 40°C, because phenolic compounds are compounds that cannot withstand heating. To obtain a thick extract, it was then weighed and the yield weight was calculated (Andriani *et al*., 2019). Yield is the ratio between the extract obtained and the initial simplicial.

Table 1. Results of the Extract Yield of Temu Giring Rhizome

Handling	Time (minute)			
Temperature°C	10	20	30	Average
30	15%	17%	15,5%	15,8%
40	15,5%	18%	17%	16,8%
50	16%	17,5%	16,5%	16,6%
Average	15,5%	17,5%	16,3%	

(Source: Personal Data, 2024)

The highest yield was obtained at a temperature of 40°C, namely 16.8% and at a time of 20 minutes, namely 17.5%. This is in accordance with the research of (Andriani *et al*., 2019). The longer the time and the higher the temperature until it reaches the optimum point, namely a temperature of 40°C with a time of 20 minutes, the yield of the ginger rhizome extract produced will be higher, whereas if it exceeds the optimum point, the yield produced will decrease.

Based on the results of compound identification conducted on the ethanol extract of temu giring rhizome, it was positive (+) for containing phenolics, indicated by the appearance of green. In addition, the sample was also positive (+) for containing flavonoids, as proven by several Willstater Tests, Bate-Smite Tests, and 10% NaOH Tests.

The Willstater test for phytochemical screening of a 70% ethanol extract of ginger rhizome (*Curcuma heyneana* Valeton & Zijp) indicated that the extract contained flavonoids of the flavone group, as foam and a yellow color were formed (Rahayu *et al*., 2015)

The Bate-Smite test for phytochemical screening of a 70% ethanol extract of ginger rhizome (*Curcuma heyneana* Valeton & Zijp) indicated that the extract contained anthocyanin flavonoids, resulting in a red color.

The 10% NaOH test for phytochemical screening of a 70% ethanol extract of ginger rhizome (*Curcuma heyneana* Valeton & Zijp) indicated that the extract contained flavonoids of the phenol group, indicated by a brownish-yellow color change.

Determination of Total Phenolic Content

Determination of the total phenolic content of a 70% ethanol extract of ginger rhizome was performed using UV-Vis spectrophotometry. The maximum wavelength of gallic acid obtained was 759 nm, and the OT showed a stable time at 31 minutes.

The principle of phenolic content determination is the formation of a blue complex compound that can be measured at a wavelength of 759 nm. Phenolic compounds react with Folin-Ciocalteu reagent only under alkaline conditions to dissociate protons in the phenolic compounds into phenolate ions. To create the alkaline conditions, 7.5% Na2CO3 was used. The hydroxyl groups in phenolic compounds react with Folin-Ciocalteu reagent to form a blue molydenum tungsten complex that can be detected spectrophotometrically (Alfian & Susanti, 2012).

Table 2. Results of Total Phenolic Value of 70% Ethanol Extract of Temu Giring Rhizome

Handling		Time (minute)	
Temperature °C	10	20	30
30	1,24 ± 0,01	1,84 ± 0,01	1,39 ±0,01
40	1,34 ± 0,01	2,33 ± 0,01	1,9 ± 0,01
50	1,31 ± 0,01	2,13 ± 0,01	1,76 ± 0,01

(Source: Personal Data, 2024)

The principle of determining phenolic levels is the formation of a blue complex compound that can be measured at a wavelength of 759 nm. Phenolic compounds react with Folin-Ciocalteu reagent only in alkaline conditions to cause proton dissociation in phenolic compounds into phenolate ions. 7.5% Na_2CO_3 is used to create alkaline conditions. The hydroxyl group in phenolic compounds reacts with Folin-Ciocalteu reagent to form a blue molydenum tungsten complex that can be detected spectrophotometrically (Alfian & Susanti, 2012).

Based on the results of determining the total phenolic content of 70% ethanol extract of ginger rhizome (*Curcuma heyneana* Valeton & Zijp) in Table 2, the highest phenolic content was at 40°C for 20 minutes, at 2.33% GAE. The lowest total phenolic content was at 30°C for 10 minutes, at 1.24% GAE. This is consistent with research by Andriani *et al*., (2019) that found the optimum temperature for extracting medicinal plants to produce phenolic content is 40°C for 20 minutes. Therefore, if the temperature and extraction time exceed the optimum limit, the resulting phenolic content will decrease.

According to research by Wayan *et al*., (2017), increasing the temperature during the extraction process requires careful attention. Excessively high temperatures and long extraction times, or exceeding the optimum limit, can cause the loss of compounds in the solution due to oxidation. An extraction temperature that is too short will result in the bioactive components extracted from the material not being optimal, so that the bioactive components obtained will be low.

Conclusion

1. The 70% ethanol extract of ginger rhizome (*Curcuma heyneana* Valeton & Zijp) contains secondary metabolites, namely flavonoids and total phenolics.
2. The optimal total phenolic content of the 70% ethanol extract of ginger rhizome (*Curcuma heyneana* Valeton & Zijp) is found at a temperature of 40°C for 20 minutes, with a GAE value of 2.33%

Declaration of Competing Interest

No Conflict Interest

Reference

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