DETERMINATION OF UAE(Ultrasonic Assisted Extraction) TEMPERATURE AND TIME OF CONTENT TOTAL PHENOLIC % RHIZOME TEMU GIRING (*Curcuma Heyneana* Valeton & Zijp)

Dwi Susiloningrum*, Kharismatul istiqomah

Health Technology Institute Cendekia Utama Kudus, Indonesia

*Corresponding Author: dsusiloningrum@gmail.com:

Abstract. Temu giring (*Curcuma heyneana* Valeton & Zijp.) is a type of medicinal 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. Intersection giring rhizome has secondary metabolites of flavonoids, phenolics, saponins, and essential oils. The study aimed to determine the effect of temperature and time of extraction with UAE (Ultrasonic Assisted Extraction) on the total phenolic content of 70% ethanol extract of temu giring (Curcuma heyneana Valeton & Zijp) rhizome by spectrophotometry. The results showed that the highest total phenolic content was at 40°C for 20 minutes, namely 2.33%GAE, while the lowest total phenolic content was at 30°C for 10 minutes, namely 1.24%GAE.

Keywords: [Extraction time temperature, UAE, total phenolic content, 70% ethanol, temu giring rhizome]

INTRODUCTION

Intersection giring rhizome (*Curcuma heyneana* Valeton & Zijp) is a plant that comes from the Zingiberaceae family. Intersection giring rhizome which has a bright yellow rhizome color and has a variety of activities including antioxidant, antiviral, antiaging, and antimicrobial. Based on Jalil's research (2019) the rhizome of Intersection Giring has secondary metabolites of flavonoids, phenolics, saponins, and essential oils.

Phenolics are compounds that have one or more hydroxyl groups (OH) attached to a phenolic aromatic ring. Phenolics have 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 like dissolves like, namely a solvent will tend to dissolve compounds that have the same level of polarity. Using 70% ethanol solvent because ethanol is a polar solvent that can attract polar secondary metabolites (Rachmawati et al., 2020).

The UAE (Ultrasonic Assisted Extraction) method can also increase the yield of extracts with a faster extraction time and lower amount of solvent compared to conventional extraction methods (Langat, 2011). According to research from Wayan et al (2017) an increase in temperature in the extraction process needs attention, an extraction temperature that is too high and an extraction time that is too long and exceeds the optimum limit can cause the loss of compounds in solution due to the oxidation process.

Based on the research of Andriani et al., (2019) the phenolic compounds of starfruit leaves with a temperature variation of 40°C with a time of 20 minutes, namely 437.79 mg GAE/g, optimal phenol yield. Because the high temperature causes the phenolic compounds in the solvent to increase so that the extraction process is easier.

Based on the above background, this study aims to determine the effect of temperature and extraction time with Ultrasonic Assisted Extraction (UAE) on the total phenolic content of 70% ethanol extract of temu giring (Curcuma heyneana Valeton & Zijp) rhizome by spectrophotometry.

METHODS

Types of research

The type of research used was quantitative experimental research, namely the effect of temperature and time of extraction with Ultrasonic Assisted Extraction (UAE) on the total phenolic content of ethanol extract 70% of the rhizome of Curcuma heyneana Valeton & Zijp by spectrophotometry.

Tool

Analytic (Ohaus), pipette, measuring cup (Pyrex), Erlenmeyer (pyrex), ultrasonic *bath (Branson 2002)*, *rotary vacuum evaporator (IKA Laborthechnik)*, analytical balance (Ohaus), blender (Philips), cuvette, pipette, beaker glass (pyrex), volumetric flask (pyrex), oven, porcelain cup, filter paper, label paper, sieve no.44 mesh, UV-Vis spectrophotometry (UV Mini SHIMADZU), volume pipette (pyrex) (scorex), analytical balance (Ohaus), cuvette, pipette, glass beaker (pyrex), spatula, glass funnel, porcelain cup, label paper.

Material

Intersection giring rhizome (Curcuma heyneana Valeton & Zijp), 70% ethanol, magnesium powder, 2N HCl, concentrated HCl, 10% NaOH, drag drof reagent, $FeCl_3$3%, aqua dest, ethanol pa, gallic acid, $Na_2CO_3$7.5%, Folin-Ciocalteu reagent.

Research procedure

1. Production of dry simplicia and powder

   The rhizomes of Intersection Giring (*Curcuma heyneana* Valeton & Zijp) which had been cleaned were then finely chopped to facilitate the drying process. Intersection giring (Curcuma heyneana Valeton & Zijp) rhizomes that had been cut were then weighed as much as 5 kg and sorted wet, then dried using a simplicia drying cabinet at 50℃, then sorted dry. The dried Simplicia was then mashed using a blender and sieved using a No.40 mesh sieve to obtain smooth and homogeneous simplicia.
2. Extract Manufacturing

   Intersection giring rhizome powder (*Curcuma heyneana* Valeton & Zijp) was dissolved in a ratio of 1:10, the sample was weighed 20 grams and then added with each solvent 70% ethanol as much as 200 mL. Then 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 filtered with filter paper (Whatman-50). The filtrate obtained was concentrated in a water bath at 40℃.
3. Phytochemical Screening
   a. Identification of Flavonoids

      Identification of flavonoids in the rhizome of Intersection giring *(Curcuma heyneana* Valeton & Zijp) used 3 reagents namely Wilstatter reagent, Bate-Smite reagent, and 10% NaOH reagent.

      The Wilstatter test can be carried out by taking 1 ml of temu giring leaf extract and then putting it in a test tube, then adding 2-4 drops of concentrated HCl and shaking vigorously. Added a little magnesium powder and shake vigorously. Positive results are indicated by the presence of foam and the solution turns yellow/orange (Rahayu et al., 2015).

      The Bate-Smite test can be carried out by taking 1 mL of extract into a test tube, then adding a few drops of concentrated HCl. Then the mixture was heated for 15 minutes in the bath. The formation of red color indicates the presence of the anthocyanidin class of flavonoids (Rahayu et al., 2015).

      The 10% NaOH test can be carried out by putting as much as 1 mL of the extract in a test tube, then adding a few drops of 10% NaOH solution. The occurrence of color changes indicates the presence of flavonoids because they are classified as phenolic compounds (Rahayu et al., 2015).

   b. Phenolic Identification

      Identification of phenolics was carried out by taking a sample and putting it in a test tube. Then 3% $FeCl_3$ reagent was added in ethanol solvent as much as 3 drops. Then observe the color change. Positive results indicate the presence of green color. Red, purple, blue, or black (Mukhriani et al., 2019).
4. Determination of Total Phenolic Content
   a. Preparation of gallic acid as parent acid

      The 1000 ppm gallic acid mother liquor was carried out by weighing 100 mg of standard gallic acid dissolved in 1 mL of ethanol pa and diluted with 100 mL of distilled

water. Furthermore, the 1000 ppm gallic acid mother liquor is diluted to 100 ppm by taking 10 ml of 1000 ppm gallic acid mother liquor and then dissolving it with distilled water to a volume of 100 mL (Ryanata, 2015).

b. Maximum wavelength setting

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

c. Determination of operating time (OT)

0.3 mL of gallic acid solution with a concentration of 30 ppm was used, put into a 5 mL volumetric flask, then 1.5 mL of Folin-Ciocalteu reagent was added. 1.3 mL of 7.5% Na2CO3 solution was added to the solution, then shaken until homogeneous. The absorption of the solution was observed at the maximum wavelength for 1 hour with an interval of 1 minute until a stable absorption was known (Alfian & Susanti, 2012).

d. Determination of gallic acid standard curve

The gallic acid solution was taken as much as 0.3 mL at concentrations of 10, 20, 30, 40, and 50 ppm. Each concentration was added to a 5 mL volumetric flask and 1.5 ml of Folin-Ciocalteu reagent was added, then shaken and added at room temperature and during the operating time. The absorbance of the solution was measured at the maximum wavelength of gallic acid and a standard gallic acid curve was made between concentration and absorbance (Alfian & Susanti, 2012).

e. Determination of Total Phenolic Content of 70% ethanol extract of temu giring rhizome (Curcuma heyneana Valeton & Zijp)

Determination of the total phenolic content of the 70% ethanol extract of the rhizome of temu giring (Curcuma heyneana Valeton & Zijp) was carried out by weighing 10 mg of the extract and dissolving it to a volume of 10 mL with ethanol pa and then homogenizing. Then 0.3 mL of the solution was pipetted and 1.5 ml of Folin-Ciocalteu reagent was added, shaken, then 1.2 mL of 7.5% Na2CO3 solution was added. The solution left the operating time.

The formula for determining the total phenolic content

$$\text{TPC} = \frac{Y \times N \times V}{W} \times 100$$

Information:

TPC = *Total Phenolic Content*
Y = Phenol concentration (mg/L)
N = dilution factor
V = Extraction volume (ml)
W = Weight fraction (mg)

RESULTS AND DISCUSSION

Extraction is a withdrawal activity carried out to obtain the soluble active compound content so that it is separated from the insoluble material using a liquid solvent. The factors that affect the rate of extraction are extraction time, number of samples, temperature, and type of solvent. During the extraction process, the active ingredient will be dissolved by the solvent according to its polarity (Depkes RI, 2000). This ultrasonic wave extraction equipment consists of an extraction vessel equipped with an

ultrasonic generator and a water bath as a temperature regulator. This extraction has the advantage of removing inorganic and inorganic compounds from the matrix of plant parts (Endarini, 2016).

Table 1. Yield of Temu Giring Rhizome Extract

Treatment	Time (minutes)			
°C temperature	**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	

The highest yield results were obtained at 40°C, namely 16.8%, and at 20 minutes of treatment, namely 17.5%, this is by the research of Andriani et al. (2019). The longer the time and the higher the temperature until it reaches the optimum point, which is 40°C for 20 minutes, the yield of the rhizome extract of the Intersection Giring produced will be higher, whereas if it exceeds the optimum point, the yield produced will decrease.

Table 2. Phytochemical Screening Result

Secondary Metabolites	Reactor	Literature	Screening Results	Conclusion
Phenolic	FeCl 3%	Green or red	Green	+
Flavonoids	*Wilstate r test*: Concentrated HCl + Mg	Yellow or orange	Yellow	+
	Battery Test – smith: concentrated HCl + heated	Red	Red	+
	10% NaOH Test: NaOH 10%	Brown or tawny	Yellow brown	+

Based on the results of the identification of compounds carried out on the ethanol extract of the rhizome of Intersection giring positive (+) containing phenolic which is indicated by the appearance of green. In addition, the sample also tested positive (+) for containing flavonoids as evidenced by several Willstarter Tests, Bate-Smite Tests, and 10% NaOH Tests.

*Willstarter test*on the phytochemical screening of 70% ethanol extract of temu giring rhizome (Curcuma heyneana Valeton & Zijp) showed that the extract contained flavonoid compounds of the flavones group because the extract formed froth and a yellow color (Rahayu et al., 2015).

*Bate-Smite Test*on the phytochemical screening of 70% ethanol extract of temu giring rhizome (Curcuma heyneana Valeton & Zijp) showed that the extract contained flavonoid compounds of the anthocyanin type with the formation of red color.

The 10% NaOH test on the phytochemical screening of the 70% ethanol extract of temu giring rhizome (Curcuma heyneana Valeton & Zijp) showed that the extract contained flavonoid compounds of the phenol group, marked by a brownish-yellow color change.

Determination of Total Phenolic Content

Determination of the total phenolic content of 70% ethanol extract of temu giring rhizome was carried out using UV-Vis spectrophotometry. The maximum wavelength of gallic acid obtained was 759 nm with an absorbance of 1.049 at a concentration of 30 ppm. The operating time in this study using a standard of 30 ppm gallic acid showed a stable absorption at 31 minutes with an absorbance of 1.049.

Table 3. Results of Gallic Acid Absorbance Measurement

Concentration	absorbance
10	0.247

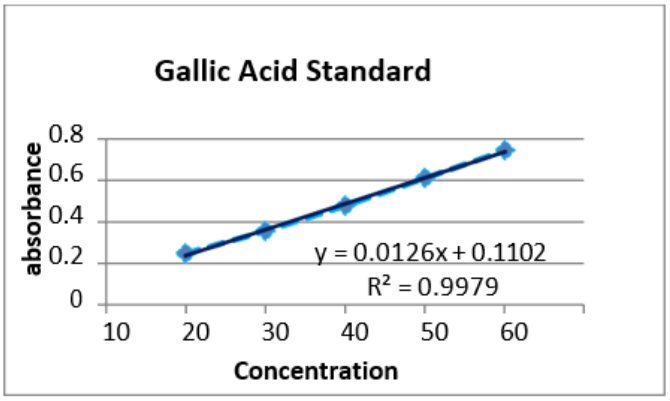

Figure 1. Gallic Acid Curve

Based on the results in Table 3, it can be concluded that the higher the concentration used, the higher the absorbance obtained. Based on the results of the gallic acid standard curve test, we obtained a linear regression y = 0.0126x + 0.1102 with a value of r = 0.9979. In the standard curve equation for gallic acid, a linear relationship between absorbance and concentration is obtained with a correlation coefficient (r) > 0.98 which indicates that the regression equation is linear. The linear regression equation obtained was used to calculate the total phenolic content of the 70% ethanol extract of the rhizome of the temu giring rhizome.

Table 4. Results of the Total Phenolic Value of Ethanol Extract 70% of Temu Giring Rhizomes

Treatment	Time (minutes)		
°C temperature	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

Based on the results of the determination of the total phenolic content of the 70% ethanol extract of temu giring rhizome (Curcuma heyneana Valeton & Zijp) in Table 2. the lowest was at 30°C and 10 minutes, namely 1.24% GAE. This is to the research of Andriani et al. (2019) that the optimum temperature for simplicial extraction to produce phenolic content is 40°C with a time of 20 minutes. So if the temperature and extraction time exceed the optimum limit, the phenolic content produced will decrease.

CONCLUSION

1. 70% ethanol extract of temu giring rhizome (Curcuma heyneana Valeton & Zijp) containing secondary metabolites are flavonoids and total phenolics.
2. The optimal value of total phenolic content of 70% ethanol extract of temu giring rhizome (Curcuma heyneana Valeton & Zijp) was found at 40°C for 20 minutes, with a value of 2.33% GAE.
3. The results of the statistical analysis of the F test of the ethanol extract of the rhizome of Intersection Curcuma with variations in temperature (30,40,50) and time (10,20,30) show that the calculated F value is 4.508 > 3.39 F table and the significance value is 0.000 <0.05, so it can be concluded that the temperature and time variables simultaneously affect the phenolic content**.**

REFERENCES

The bibliography and citation follow the author-year APA style and we recommend using references, such as Mendeley, Zotero, EndNote, etc. Make sure the number of references is at least 20 and all are cited in the

text
Alfian, R., & Susanti, H.(2012). Determination of the total phenolic content of the methanol extract of red rosella calyx (*Hibiscum sab dariffa* Linn) with variations in the place of growth by spectrophotometry. Pharmaceutical Scientific Journal, 2(1), pp. 73–80.
Andriani, M., Permana, I., & Widarta, I. (2019). Effect of temperature and time of extraction of starfruit leaves (*Averrhoa bilimbi* L.) on antioxidant activity using the ultrasonic-assisted extraction (UAE) method. Journal of Food Science and Technology, 8(3), 330–340.
Republic of Indonesia Ministry of Health. 2000. General Standard Parameters of Medicinal Plant Extracts (1st ed.).Ministry of Health of the Republic of Indonesia.
Endarini, LH (2016). Pharmacognosy and phytochemistry. Jakarta: Pusdik HR Kesehatan.
Hendri & Setiawan, R. 2017. Effect of work motivation and Compensation on employee performance at PT. Main Ocean. Journal of Agora, 5(1).
Jalil, M. 2019. Temu giring (*Curcuma heyneana* Valeton & Zijp): a morphological, phytochemical, and pharmacological review. Educational Scientific Journal, 2(2), pp. 104–116.
Langat, MK 2011. Chemical constituents of east European Forest species. Book of extended extracts, Kenya: Napreca 77-78.
Mukhriani, Sugiarna, R., Farhan, N., Rusdi, M., & Arsul, MI 2019. Total phenolic and flavonoid levels of the ethanol extract of grape leaves (*Vitis vinifera* L). Ad-Dawaa' J. Pharm. Sci, 2(2).
Rachmawati, RA, Wisaniyasa, N. Wayan, & Suter., IK 2020. The effect of the type of solvent on the antioxidant activity of meniran extract (*Phyllanthus niruri* L.). Journal of Technology and Food, 9(4), pp. 458–467.
Rahayu, S., Urniasih, N., & Malia, D. (2015). Onion skin waste is a natural antioxidant. Journal of al-Kimiya, 2(1).
Rifai, G., Widarta, IWR, & Nocianitri, KA (2018). The influence of the type of solvent and the ratio of materials to solvents on the content of phenolic compounds and the antioxidant activity of avocado (*Persea americana* Mill.) seed extract. ITEPA Journal, 7(2), 22–32.
Ryanata, E. (2015). Determination of tannins and rind of a ripe banana (*Musa paradisiaca* L.) by spectrophotometry and permanganometric. The University of Surabaya Student Scientific Journal, 4(1).
Wayan, N., Yuliantari, A., & Permana, IDGM.(2017).The effect of temperature and extraction time on the flavonoid content and antioxidant activity of soursop leaves (*Annona muricata* L.) using ultrasonics. Journal of Scientific Media Food Technology, 4(1), p. 35–42.
Almukhambetov, B. M. T., & Nebessayeva, Z. (2015). The Application Of Figuratif Arts Capabilities In The Art-Pedagogical Activity Of A Teacher". *Journal Procedia Social and Behavioral Science*, *197*, 1525–1529.