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COMPARISON OF PHENOLIC CONTENT OF RED GINGER (*Zingiber officinale var. rubrum* Theilade) AT DIFFERENT GROWING LOCATIONS

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ABSTRACT
Red ginger (*Zingiber officinale* var. *rubrum* Theilade) as a traditional medicine can be used as anti-inflammatory, analgesic, antipyretic, lower cholesterol, prevent impotence, depression, and others. The rhizome of the red ginger plant has different phenolic levels in each region. The locations were chosen due to differences such as altitude, soil type, rainfall, temperature at the planting site, and harvest age of red ginger. To find out the comparison of the levels of these compounds, an examination was carried out with UV-Vis's spectrophotometry. The purpose of this research was to determine the comparison of phenolic content of red ginger from different growing locations, namely from Wonosobo Regency and Karanganyar Regency. This research used samples of red ginger extract (*Zingiber officinale* var. *rubrum* Theilade), as much as 500 grams of red ginger simplisia extracted using maceration method with 96% ethanol solvent in ratio of 1:5. Quantitative analysis phenolic content using UV-Vis's spectrophotometry methode. The results of quantitative tests using UV-Vis's spectrophotometry obtained a significant comparison of the total phenolic content of red ginger (*Zingiber officinale* var. *rubrum* Theilade) from Wonosobo Regency of 0.49% v/b and red ginger from Karanganyar Regency of 0.47% v/b.

Keywords: Red ginger; Phenolic; Maceration; UV-Vis Spectrophotometry

1. INTRODUCTION

Red ginger (*Zingiber officinale* var. *rubrum* Theilade) is a Zingiberaceae family that has a role in various aspects of Indonesian society because it is used as traditional medicine. Red ginger rhizome can also be used to reduce fever, overcome indigestion, colds, as an analgesic, anti-inflammatory, lower cholesterol, prevent impotence, impotence, and others (Pakpahan, 2015). Red ginger (*Zingiber officinale* var. *rubrum* Theilade) contains many active phenolic components, besides that it also has a very sharp distinctive aroma with a very spicy taste (Wiendarlina & Sukaesih, 2019).

Phenolic compounds are known as antioxidants and antidotes to free radicals associated with oxidative damage. Phenolics are gaining traction at the moment because of their in vitro studies showing that they have various beneficial biological properties such as anti-inflammatory, anti-tumor, and anti-microbial activities. Studies have attributed that the antioxidant properties are due to the presence of phenols and flavonoids. The antioxidant activity of phenolic compounds is based on their ability to donate hydrogen atoms to the antioxidant (Nishanthini et al., 2012; Türkoğlu et al., 2007).

Phenolic compounds have important roles in human health. Red ginger contains phenolic compounds gingerol, shagaol, and zingeron. The role of phenolics in red ginger as a source of immunoregulators by affecting the regulation of pro-inflammatory cytokine synthesis, immune

cells, and gene expression (Luhurningtyas et al., 2021). Active components in red ginger contribute to its biological compounds. The levels of active components depend on several other factors such as the altitude of the growing location and the harvesting time of red ginger (Styawan et al., 2022). Climate, which includes rainfall, growing location, temperature and humidity, affects the development and production of ginger plants. Rainfall greatly influences the growth of ginger, requiring approximately 2500 to 4000 mm per year with a dry period of approximately 5 months per year. Sunlight is needed in the growth and planting of ginger to produce good rhizomes, especially at the age of 2.5 to 7 months of growth. The air temperature for ginger growth is between 25 and 35 °C. Temperatures of more than 35 °C will cause the leaves to burn and dry, while temperatures too low will cause the ginger to live longer. A good growing location is between 300 and 900 meters above sea level (Hapsoh et al., 2008).

Wonosobo Regency has an altitude of 200-2,250 meters above sea level and Karanganyar Regency has an altitude of 90-2,000 meters above sea level. Soil types in Wonosobo Regency are mostly regosols, which are soils resulting from volcanic eruptions that are gray, brown or yellowish brown in color. The average daily air temperature is between 14.3-26.5 °C (Pemkab Wonosobo, 2017). Soil types in Karanganyar Regency are mostly andosols, which are black or dark brown soils at the bottom. The average daily air temperature is between 18-31°C (Pemkab Karanganyar, 2013).

Determination of phenolic content using UV-Vis Spectophotometry method because phenol absorbs in the short UV region and can be detected with the best reagent, namely Folin-Ciocalteu to determine phenol with catechol or hydroquinone core which produces a blue spot color after spraying the reagent (Harborne, 1996). The Folin-Ciocalteu method has the principle that phenolic ions reduce phosphomolybdic acid-phosphotungstic acid under alkaline conditions into a blue molybdenum-tungsten complex compound. Phenolic ions are formed through proton dissociation under alkaline conditions obtained from an alkaline compound. The higher the content of phenol compounds, the greater the phenolic ions, so that more phenolic ions reduce phosphomolybdate-phosphotungstate, causing the blue color to form more intense (Andriani & Murtisiwi, 2018).

2. RESEARCH METHODS

2.1. Plant Determination

Determination of red ginger rhizome (*Zingiber officinale* var. *rubrum* Theilade) was conducted at the Plant Systematics Laboratory, Center for Research and Development of Medicinal Plants and Traditional Medicines (B2P2TOOT), Tawangmangu, Central Java.

2.2. Simplisa Manufacturing

Red ginger (*Zingiber officinale* var. *rubrum* Theilade) from Wonosobo was harvested at 10 months old and red ginger from Karanganyar was harvested at 12 months old. 5 kg of red ginger rhizomes were taken and washed thoroughly with running water. Red ginger rhizomes were chopped with a thickness of 2-3 mm and then heated at 60 °C. After the oven, it was pulverized with a blender and then sieved using a 40 mesh sieve (Luhurningtyas et al., 2021). Red ginger simplisia is carried out non-specific observations of the powder. As much as 2 grams of simplisia powder was placed on a cup and then dried in an oven for 30 minutes at 105°C. Cooling using a desiccator for 15 minutes then weighing the constant weight and calculating the water content. Water content is formulated as follow Eq. (1) (Handayani et al., 2019).

$$Water\ content = \frac{b-(c-a)}{b} x100\% \quad (1)$$

2.3. Extract Preparation

Extraction using the maceration method. The maceration method can avoid damage to compound components to heating, so this method was chosen because phenol compounds have

conjugated aromatic systems that are easily damaged at high temperatures. The solvent used is 96% ethanol because it can attract phenolic compounds optimally (Ramadhani et al., 2020). The purpose of maceration is to attract active compounds (Suhendar et al., 2020). Solvents used in extraction must have properties such as selectivity, ability to extract, not contain toxins, easy to evaporate, and affordable (Yunita & Khodijah, 2020). The solvent used in maceration extraction is 96% ethanol because it has a level of safety and ease when evaporated and its properties are able to dissolve almost all substances, both polar, semi-polar, and non-polar. This solvent can also attract phenolic compounds optimally (Ramadhani et al., 2020).

Red ginger (*Zingiber officinale* var. *rubrum* Theilade) simplisia powder was taken as much as 500 grams each and soaked in 96% ethanol solvent in a ratio of 1:5, macerated with 96% ethanol as much as 1800 ml, covered with alumuniom foil, and allowed to stand for 1 x 24 hours at room temperature (20°C-25°C). In order for the active substance to be extracted perfectly, repeated shaking is carried out. After 24 hours the extract was filtered with flannel cloth, the pulp was macerated again using 700 ml of 96% ethanol solvent for 2 x 24 hours then filtered with flannel cloth. The filtrate obtained was collected and then concentrated using a water bath at 70°C to produce a thick extract. The thick extract obtained was then weighed to determine the weight of the extract and the calculation of the extract weight yield was carried out (Luhurningtyas et al., 2021). The calculation is formulated in Eq. (2) (Sani et al., 2013).

$$\%\ yield = \frac{weight\ of\ the\ extract\ produced}{initial\ weight} x100\% \quad (2)$$

2.4. Determination of Phenol Content

2.4.1. Gallic Acid Standard Solution

Gallic acid was weighed as 10 mg with 0.5 ml of ethanol pro analysis to make 1000 ppm gallic acid solution. Aquabides was added up to the limit mark and homogenized. The solution was pipetted as much as 1, 2, 3, 4, 5 ml and added ethanol pro analysis to 5 ml. The resulting solutions were 30, 40, 50, 60, 70, 80, and 100 ppm. Folin-Ciocalteu reagent was added as much as 1.5 ml at each concentration of 30, 40, 50, 60, 70, 80, and 100 ppm then shaken and allowed to stand for 3 minutes. Add with 7% Na_2CO_3 as much as 1.2 ml and shaken until homogeneous (Luhurningtyas et al., 2021).

2.4.2. Determination of Operating time

1000 ppm gallic acid standard solution was taken as much as 0.25 ml and put into a 5 ml volumetric flask and aquabidest was added to the limit. Gallic acid solution 0.3 ml was taken and added with Folin-Ciocalteu reagent as much as 1.5 ml. Sodium carbonate (Na_2CO_3) 7.5% was added as much as 1.2 ml. The absorbance of the solution was read every 5 minutes using a UV-Vis Spectophotometer with a maximum lamda of 747 nm for 60 minutes (Luhurningtyas et al., 2021).

2.4.3. Maximum Wavelength Determination

Gallic acid solution of 1000 ppm was taken as much as 0.25 ml and put into a 5 ml volumetric flask, aquabidest was added to the limit mark. Gallic acid solution was taken 0.3 ml and added with Folin-Ciocalteu reagent as much as 1.5 ml. Sodium carbonate (Na_2CO_3) 7.5% was added as much as 1.2 ml. Left for operating time then the wavelength in the range of 600-800 nm was read with 3 replicates (Luhurningtyas et al., 2021).

2.4.4. Phenolic Content of Extract

The extract solution was made by weighing the sample as much as 0.3 ml and adding Folin-Ciocalteu reagent as much as 1.5 ml then shaken and allowed to stand for 3 minutes. 1.2 ml of 7% Na_2CO_3 was added and shaken until homogeneous and incubated for 60 minutes at room temperature. The maximum wavelength absorption at 747 nm was measured with replication 3

times so that the phenol content was obtained as gallic acid equivalents per extract (Luhurningtyas et al., 2021).

3. RESULT AND DISCUSSION

3.1. Plant Determination

This study used the Red Ginger plant (*Zingiber officinale* var. *rubrum* Theilade). The determination results show that the plants used in this study are true red ginger plants (*Zingiber officinale* var. *rubrum* Theilade). Plant determination was carried out at the Plant Systematics Laboratory, Center for Research and Development of Medicinal Plants and Traditional Medicines, Tawangmangu, Central Java. Red ginger rhizome (*Zingiber officinale* var. *rubrum* Theilade) samples are presented in **Figure 1**.

Figure 1. Red ginger rhizomes (*Zingiber officinale* var. *rubrum Theilade*) from Wonosobo Regency and Karanganyar Regency

Plant determination was carried out to state that the plants used in this study were red ginger plants (*Zingiber officinale* var. *rubrum* Theilade). The determination results stated that the plants used in the study were true red ginger plants (*Zingiber officinale* var. *rubrum* Theilade).

3.2. Preparation of Red Ginger Rhizome Simplisia

The plant part utilized is the rhizome of red ginger. Wet sorting, washing, chopping, drying, and dry sorting were carried out. The chopped ginger rhizomes were then dried using an oven at 60°C for 2 days. Simplified dry sorted and pulverized with a blender then sieved using a 40 mesh sieve.

Simplisia was tested for moisture content using the gavimetric method. The water content of red ginger simplisia from Wonosobo Regency was 8% and red ginger simplisia from Karanganyar Regency was 7.5%. Test the moisture content of simplisia according to (Depkes RI, 2017) Indonesian Herbal Pharmacopoeia second edition 2017 that the moisture content of good ginger simplisia is < 10%.

3.3. Extraction of Red Ginger Rhizomes

Extraction using maceration method. Damage to compound components against heating can be avoided by maceration method, this method was chosen because phenol compounds have conjugated aromatic systems that are susceptible to damage at high temperatures. The solvent used is 96% ethanol because it can optimally attract phenolic compounds (Ramadhani et al., 2020). The maceration container uses a dark glass bottle to avoid direct sunlight, because it can affect the results of maceration and the solvent used does not evaporate at room temperature.

Simplisia of red ginger (*Zingiber officinale* var. *rubrum* Theilade) from Wonosobo Regency and Karanganyar Regency were each taken as much as 500 grams, macerated with 1800 ml of 96% ethanol for 1x24 hours then remacerated with a new 96% ethanol solvent as much as 700 ml. The resulting extract is brown in color and has a distinctive aroma of red ginger. The result of red ginger extract from Wonosobo Regency was 106.723 grams with a yield value of 21.3% b/w. The yield of red ginger extract from Karanganyar Regency was 92.662 grams with a

yield value of 18.5% b/w. The yield results are in accordance with (Depkes RI, 2017) Indonesian Herbal Pharmacopoeia edition II which states that the extract yield is not < 17%.

3.4. Total Phenolic Content Determination

Phenol is a compound that has an aromatic ring and one or two hydroxyl groups. Phenol compounds with more than two hydroxyl groups are called polyphenols (Hanani et al., 2014). Phenolic compounds have a role in antioxidant activity in the form of polyphenols or phenolic acids they contain (Haryoto & Ardiyani, 2021). The working principle of UV-Vis Spectrophotometer is when monochromatic light passes through a solution, some of the light is absorbed, reflected, and emitted (Yanlinastuti & Fatimah, 2016). Determination of phenolic content using the Folin-Ciocalteu method where the absorbance is measured at a wavelength of 747 nm. The standard solution used is gallic acid, because gallic acid is one of the stable and natural phenolic compounds. Gallic acid that reacts with Folin-Ciocalteu reagent produces a yellow color indicating the presence of phenol compounds. Na_2CO_3 solution is added as an alkaline atmosphere giver (Sari & Ayuchecaria, 2017).

Gallic acid standard solution was measured by UV-Vis Spectrophotometer method to determine the maximum wavelength. The maximum wavelength obtained was 747 nm with an absorbance of 0.802. The absorption reading of gallic acid solution was carried out to determine the operating time at a maximum wavelength of 747 nm. Determination of operating time in this study is 60 minutes.

Concentration and absorbance of phenolic standard curve solution using 9 concentration series. To find out the difference in absorbance, the concentration variation is carried out, where the higher the concentration, the greater the absorbance value. The concentrations used were 5,000 ppm; 15,000 ppm; 30,000 ppm; 40,000 ppm; 50,000 ppm; 60,000 ppm; 70,000 ppm; 80,000 ppm; 100,000 ppm.

The linear regression equation is used to determine the standard curve of the relationship between gallic acid content as the x-axis and Folin-Ciocalteu reagent with gallic acid absorbance as the y-axis. The relationship value (R^2) obtained from the determination of the standard curve of gallic acid is 0.9966 close to 1, it can be said that there is a linear relationship between the concentration of gallic acid and its absorbance results. The results of the standard curve graph are presented in **Figure 2**.

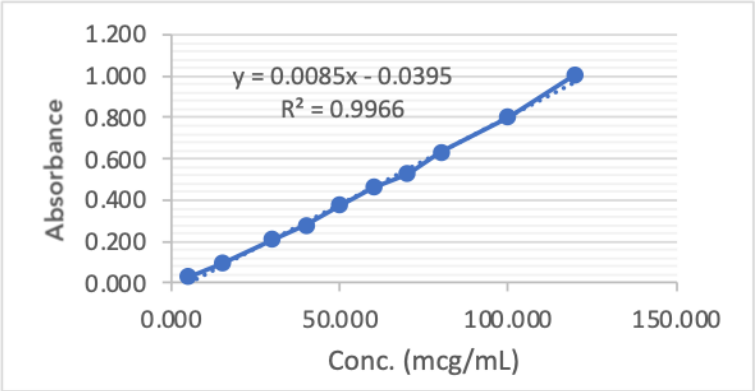

Figure 2. Standard curve graph

The results of the determination of total phenolic content of red ginger extract (*Zingiber officinale* var. *rubrum* Theilade) obtained from different growing locations using the UV-Vis Spectrophotometry method were replicated 3 times on each sample for accurate data acquisition. The results of the determination of total phenolic content of red ginger extract (*Zingiber officinalle* var. *rubrum* Theilade) from wonosobo district are presented in **Table 1**.

Table 1. Determination of phenolic content of red ginger from Wonosobo Regency

Replication	Sample mass (mg)	Sample volume (mL)	ppm concentration	Abs	Content (%)
1	50.15	0.3	81.884	0.802	0.4898345
2	50.15	0.3	82.151	0.802	0.4914317
3	50.15	0.3	82.344	0.802	0.4925862
		$\bar{x}$ ± SD			0.4912841

Based on the results of this study, the total phenolic content of red ginger extract (*Zingiber officinalle* var. *rubrum* Theilade) obtained from Wonosobo Regency was 0.49% b/b, and the results of the determination of total phenolic content of red ginger extract (*Zingiber officinalle* var. *rubrum* Theilade) from Karanganyar district are presented in **Table 2**.

Table 2. Determination of phenolic content of red ginger from Karanganyar Regency

Replication	Sample mass (mg)	Sample volume (mL)	ppm concentration	Abs	*X*±SD
1	50.27	0.3	78.411	0.802	0.4703023
2	50.27	0.3	78.529	0.802	0.4710005
3	50.27	0.3	78.647	0.802	0.4717346
$\bar{X}$ SD					0.4710130

Based on the results of this study, the total phenolic content of red ginger extract (*Zingiber officinalle* var. *rubrum* Theilade) obtained from Karanganyar Regency was 0.47% b/w. Phenolic levels obtained from each sample were then analyzed for normality with the Shapiro-Wilk Normality Test and continued using the Independent T-Test statistical analysis. Data analysis of the normality of phenolic levels tested with Shapiro-Wilk is presented in **Table 3**.

Table 3. Data analysis of normality of phenolic levels in red ginger extract of Wonosobo Regency and Karanganyar Regency with shapiro-wilk test

Sample	Normality
Wonosobo	0.991
Karanganyar	1.000

Based on **Table 3**, the normality value of the red ginger samples obtained from Wonosobo Regency and Karanganyar Regency is 0.991 and 1.000, indicating that both samples have normality> 0.05 so that the results are said to be normal and then conducted an *Independent T-Test.* Analysis of normality data with the *Independent T-Test* is presented in **Table 4**.

Table 4. Data analysis of normality of phenolic levels in red ginger extracts Wonosobo Regency and Karanganyar Regency with independent t-test and normality

Sample	P value/ sign
Wonosobo	0.000
Karanganyar	0.000

Based on **Table 4** the significant value (p) of red ginger samples obtained from Wonosobo Regency and Karanganyar Regency is 0.000 and 0.000 which both samples have a p value <0.05 so that H0 is rejected, then Ha is accepted. This means there is a significant difference between the phenolic content of red ginger obtained from Wonosobo Regency and Karanganyar Regency. Statistical data analysis of phenolic content of red ginger from Wonosobo Regency and Karanganyar Regency is presented in **Table 5**.

The phenolic content of red ginger from Wonosobo Regency has a significant value (p) of 0.000 which means p < 0.05. This proves that there is a significant difference in phenolic content of red ginger from Wonosobo Regency and Karanganyar Regency.

Table 5. Comparison of phenolic content of red ginger from Wonosobo Regency and Karanganyar Regency with test independent t-test normality

Sample	N	Mean (%)	Standard Deviation	P value
Wonosobo	3	0.4912841	0.0013817725	0.000
Karanganyar	3	0.4710130	0.0007162250	

Phenolic content was determined using the Folin-Ciocalteu method because phenolic compounds can react with the Folin-Ciocalteu reagent to form a solution whose absorbance can be measured, where the absorbance is measured at a wavelength of 747 nm. Gallic acid is the standard solution used, because gallic acid includes stable and natural phenolic compounds. The yellow color that results from the gelling of gallic acid with Folin-Ciocalteu reagent indicates the presence of phenolic compounds, as a giver of alkaline atmosphere added with sodium carbonate. Hydroxyl groups in phenolic compounds react with Folin-Ciocalteau to form a blue molybdenum-tungsten complex that can be detected by spectrophotometer (Andriani & Murtisiwi, 2018).

Determination of the maximum wavelength aims to determine the wavelength required for gallic acid solution to reach maximum absorption. The maximum wavelength obtained was 747 nm with an absorbance value of 0.802. The difference in total phenolic content in red ginger from Wonosobo Regency and Karanganyar Regency in this study shows that the phenolic content of red ginger from Wonosobo Regency is higher. This difference is influenced by altitude, air temperature, soil, and rainfall which are different from each region.

The air temperature at the time of planting red ginger until harvesting can change due to the long planting period of ginger. Ginger cultivation lasts between 8 to 12 months. Air temperature affects the moisture content of red ginger rhizomes. The average temperature at planting time in Wonosobo Regency is 14.3-26.5 °C (Pemkab Wonosobo, 2017), while the average air temperature in Karanganyar Regency is 18-31 °C (Pemkab Karanganyar, 2013).

Harvesting of both red ginger samples was done in the morning. Harvesting in the morning is done because the ambient temperature is still low enough to increase harvesting efficiency and can reduce the effects of plant respiration. Air temperature has an important influence on the growth and development of ginger in physiological processes such as photosynthesis, transpiration, water and nutrient absorption, and flower primodia formation.

The soil used for red ginger growth in Wonosobo Regency is regosol soil while the soil in Karanganyar Regency is andosol soil. Regosol soils are soils resulting from volcanic eruptions that are gray, brown or yellowish brown in color. Andosol soil is brown or dark brown soil and is at the bottom. The types of soil commonly used for ginger planting media are brownish-red latosols, brownish-red latosol-andosol mixes, and soils in new forest clearings (Hapsoh et al., 2008).

The altitude of the red ginger growing location in Kalibawang District, Wonosobo Regency is 626 meters above sea level, while the red ginger growing location in Jatiyoso District, Karanganyar Regency is 875 meters above sea level. The altitude of the area in both locations is a good ginger growing location according to the Ministry of Agriculture (Hapsoh et al., 2008) that the best growing location for ginger is between 300-900 meters above sea level.

Rainfall in the process of planting red ginger is needed around 2500 to 4000 mm per year with dry months less than 5 months per year. Rainfall in Wonosobo Regency with high intensity in the first 3 months of planting with a dry month length of 5 months, while rainfall in Karanganyar Regency with high intensity in the first 5 months with a dry month length of 7 months.

According to Pakpahan (2015) Red ginger contains phenolics that are useful for analgesics, so it is necessary to develop research on the formulation of herbal medicinal preparations using red ginger taken from Wonosobo Regency. The herbal preparation in question is anti-pain herbal

syrup. The preparation of syrup is recommended because syrup is more quickly absorbed by the body and is already in dissolved form.

4. CONCLUSION

Red ginger extract from Wonosobo Regency has a phenolic content of 0.49% b/w and red ginger extract from Karanganyar Regency has a phenolic content of 0.47% b/w. There is a significant difference between phenolic content in red ginger of Wonosobo Regency and Karanganyar Regency with a significant value of 0.000 which means $p < 0.05$.

5. AUTHOR DECLARATION

Authors' Contributions and Responsibilities

The authors made substantial contributions to the conception and design of the study. The authors took responsibility for data analysis, interpretation, and discussion of results. The authors read and approved the final manuscript.

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Availability of Data and Materials

All data are available from the authors.

Competing Interests

The authors declare no competing interest.

Additional Information

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