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Contents

Effect of Extraction Solvent on Extraction Yield, Cytotoxic Activity and Bioactive Compound in Zingiber officinale Roscoe var rubrum

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Abstract. Red ginger (Zingiber officinale Roscoe var rubrum) is a commonly used spice in Indonesia. It contains various chemical constituents, such as phenolic compounds, terpenes, polysaccharides, lipids, organic acids, and fiber. The health benefits of ginger are mainly related to its phenolic compounds, such as gingerols and shogaols. 6-Gingerol has various biological properties including anticancer, antioxidant, anti-inflammatory, anti-platelet aggregation, and antifungal. The method used to obtain the red ginger extract was macerated using 30%, 70%, and 96% ethanols, n-hexane, ethyl acetate, and water. The research was started by testing the phytochemicals and water content of the simplicia. Furthermore, toxicity tests were carried out on each extract on shrimp larvae, and the determination of gingerol and shogaol levels was by High-Performance Liquid Chromatography. The results showed that the highest yield of red ginger extract was 12.1% in a water solvent. Phytochemical testing of red ginger simplicia contains saponins. The 96% ethanol extract had the highest cytotoxic activity with an LC50 value of -79.516 ppm. The highest contents of 6-gingerol, 8-gingerol, 10-gingerol, and 6-shogaol were found in n-hexane solvents, respectively 123.07 mg/g; 26.39 mg/g; 61.40 mg/g and 22.41 mg/g.

Keywords: Extraction Solvent, Cytotoxic Activity, Bioactive Compound, Zingiber officinale Roscoe var rubrum

1 Introduction

Ginger is one of the most widely used herbal plants in Asian countries across generations since hundreds of years ago in the medicinal and culinary fields. This plant belongs to the Zingiberaceae family and the Zingiberales order. The Zingiberaceae family has 50 genera with 1,300 species, some of which are widely found and used in Indonesia. The main ginger-producing countries in the world are India, China, Indonesia, and Nigeria. The scientific name for red ginger is Zingiber officinale Roscoe var. Rubrum,

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known as a variant of ginger is different from other varieties, especially in its rhizome which is layered in orange to red [1].

It is common to use spice plants as medicine in Indonesia because they are easy to obtain, easy to process, and have been used for generations. One of the most used spices is a ginger rhizome. Red ginger extraction is a separation process that is carried out to obtain certain desired components from the starting material. The selection of methods, solvents, and steps greatly affects the quality of the resulting product. The bioactive compounds in red ginger have certain biological activities when red ginger is consumed or extracted [2]. Extraction is the process of soluble chemical compounds withdrawal, so that they are separated from materials that are insoluble in liquid solvents. Different solvents type will affect secondary metabolite compound produced from red ginger rhizome extraction process. Secondary metabolite is an inessential for organism growth, which can be found in different or unique types on each species. The function of secondary metabolite is to defend the plants from endangering environmental condition, for example to defend themselves from pests or diseases, to attract pollinators, and as signaling molecule [3]. The identification of secondary metabolite is an important initial stage to discover bioactive compound from natural materials, which can be made as precursor for novel synthetic medication or certain activity drug prototype generation [4]. The chemical content of ginger rhizome is volatile components (camphene, ß-phellandrene, curcumene, cineole, geranyl acetate, terpineol, borneol, geraniol, limonene, ß-elemene, zingiberol, linalool, a-zingiberene, ß-sesquiphellandrene, ß-bisabolene, zingiberenol and a-Farnesene) and non-volatile components which consists of biologically active components namely gingerols (6, 8 and 10), shogaol (6, 8, and 10), paradol and zingerone [5]. Ginger contains pungent phenolic compounds known as gingerols. One of them, 6-gingerol (1-[4'-hydroxy-3'-methoxyphenyl]-5-hydroxy-3-decanone), is the main pharmacologically active component of ginger [6, 7], and the active part of the molecule is part of the aliphatic chain containing the hydroxyl [8]. 6-Gingerol has been reported to have various biological properties including anticancer, antioxidant, anti-inflammatory, anti-platelet aggregation, and anti-fungal [9–11].

The composition of chemical compounds from plants (phytochemicals) is essential for determining the quality of herbal products because it determines their safety and effectiveness. The effectiveness of an extraction process of a compound by solvent is highly depended on the solubility of the compound with the solvent, according to the principle of like dissolve like, where a compound will be dissolved on solvent with similar natures, based on the polarity of the compound in the solvent during extraction process. Polar compound can only be dissolved on polar solvents such as ethanol, methanol, butane, and water. Non-polar compounds can only be dissolved on non-polar solvents such as ether, chloroform, and n-hexane [12]. This study is aimed to acknowledge the influence of different types of solvents (water, ethanol 30%, ethanol 70%, ethanol 96%, n-hexane, and ethyl acetate) on yield rate, cytotoxic activity, and bioactive compound content (6-gingerol, 8-gingerol, 10-gingerol and 6-shagol) on red ginger rhizome. Also to identify the most suitable solvent for the extraction process to isolate bioactive component and to gain cytotoxic activity of red ginger rhizome.

2 Method

2.1 Place, Time of Sampling, and Determination of Samples

The research material was the fruit of the red ginger plant taken from the Biopharmaca Cultivation and Conservation Unit (BCCU) of the Tropical Biopharmaceutical Research Center, LPPM IPB University which was obtained from Java and harvested at the age of 9 months. The determination was carried out at the Biopharmaca Cultivation and Conservation Unit (BCCU).

2.2 Making Simplicia

Three kilograms of fresh red ginger rhizome were weighed and then washed and dried in direct sunlight for 4-5 days. Then, the dry sorting was done and mashed with a blender. The simplicia powder obtained was sieved using an 80 mesh sieve and then weighed. After that, it was stored in a clean, dry container and protected from sunlight for the next extraction process.

2.3 Preparation of Red Ginger Extract

Extract preparation and testing were carried out at the Laboratory of the Tropical Biopharmaca Research Center, LPPM IPB University. Samples of red ginger simplicia were weighed for extraction with 10 grams of various solvents for each, then solvents were added namely 96%, 70%, 30% ethanol, 500 mL of water, ethyl acetate, and n-hexane. Maceration was carried out 2 x 24 hours with several times of stirring, then filtering. The collected filtrate was concentrated using a vacuum rotary evaporator at 45-50˚C to obtain a viscous ethanol extract of 96%, 70%, 30%, water extract, ethyl acetate extract, and n-hexane extract.

2.4 Water content

Two grams of red ginger simplicia were weighed in a container with a constant weight. Then, red ginger simplicia was heated in an oven at a temperature of +105 degrees Celsius for 3 hours. After being heated and then cooled in a desiccator, it was weighed until it reached a constant weight [13].

2.5 Analysis of Red Ginger Extract Yield

The yield of red ginger extract was calculated by comparing the weight of the red ginger extract with the weight of the red ginger simplicia used for extraction.

2.6 Phytochemical Screening

Phytochemical screening in this study includes the tests for alkaloids, flavonoids, tannins, saponins, triterpenoids, and steroids based on the method of Harborne 1987.

2.7 Alkaloids

0.5 grams of condensed extract or 1 gram of simplicia was dripped with 3-5 drops of ammonia. Then, 5 mL of chloroform was added. After that, it was homogenized and filtered. The filtrate obtained was added with 2M Sulfuric acid reagent, then it was homogenized. The top layer was taken and used as an experimental solution which was treated as follows: 1) Experimental solvent 1 was added 2 drops of Mayer's reagent, a positive result with the formation of a white precipitate. 2) Experimental solvent 2 was added 2 drops of Dragendorf reagent, positive result with the formation of an orange or orange precipitate. 3) Experimental solvent 3 was added 2 drops of Wagner's reagent, positive result with the formation of a brown precipitate.

2.8 Flavonoids, Tannins, and Saponins

0.5 grams of condensed extract or 5 grams of simplicia was dissolved in distilled water and then heated for 5 minutes. Then, the solvent was filtered and divided into 3 parts. To test the flavonoid filtrate, Mg and HCl powder:Ethanol (1:1) were added, then amyl alcohol was added. The color formed on the amyl alcohol layer was observed, if a yellow, orange or red color is formed then it contains flavonoids. For the tannin filtrate test, 3 drops of 10% FeCl3 were added, if a greenish-black color is formed then it contains tannins. For the saponin test, the filtrate was shaken for 10 seconds. A positive result is indicated by the formation of stable foam for over 2 minutes.

2.9 Triterpenoids and Steroids

A sample of 0.5 grams of condensed extract or 1 gram of simplicia was dissolved in ethanol and heated for 5 minutes, then the sample was filtered into a porcelain dish. Then, the filtrate was heated to dryness, and 1 mL of diethyl ether,1 drop of acetic anhydrous, and 1 drop of concentrated sulfuric acid were added. A positive reaction is indicated by the formation of a red/purple solution for triterpenoids and blue or green for steroids.

2.10 Quinone

0.5 gram of condensed extract or 1 gram of simplicia was added with methanol and then heated, after that it was filtered. The filtrate results are added 3 drops of 10% NaOH. A positive reaction is indicated by the formation of a red color for hydroquinone [14].

2.11 Cytotoxic Activity Test on Shrimp Larvae

The cytotoxic activity test by determining the LC50 value was carried out using Artemia salina shrimp eggs. A. salina used for the toxicity test was obtained from hatching using seawater with the help of an aerator to meet dissolved oxygen levels. The extract toxicity test was carried out using A. salina shrimp larvae. The shrimp larvae used were aged 48 hours after the shrimp larvae hatched. A. salina cysts of as much as ± 50 mg were put into a container containing seawater that had been filtered and equipped with an aerator. The cysts were left for 48 hours under light to hatch completely. After hatching, 10 A. salina larvae were put into a 2 ml vial, then it was added a stock extract solution with a concentration of 4000 ppm and adjusted the volume with seawater so that the final concentration of the extract was 0, 10, 100, and 1000 ppm. After 24 hours, the number of dead larvae was counted. The lethal concentration (LC) value was determined by the probit analysis method with a 95% confidence interval [15].

2.12 Determination of Gingerol content with High-Performance Liquid Chromatography (HPLC)

Standard and sample preparation. Standards of 6.8 and 10 gingerols and 6 shogaols dissolved in methanol were made at concentrations of 50 ppm, 25 ppm, 50 ppm, and 50 ppm respectively. Each extract was weighed as much as 0.1 gram and then added 8 mL of methanol solvent, was then sonication was done for 1 hour. Then the sample solvent was filtered into a 10 mL flask and then calibrated with methanol up to 10 mL, then filtered with 0.45 micrometer Whatman filter paper and then 20 µL injected into the HPLC.

Identification by HPLC. The mobile phase used was Acetonitrile and aqua bides with a composition comparison in Table 1. The wavelength used was 280 nm, while the flow of the mobile phase was 1 mL/minute. Table 1. Comparison of the composition of Acetonitrile and Aqua bides:

Table 1. The composition of the mobile phase in the determination of gingerol content

Time (minutes)	Acetonitrile (%)	Aqua bides (%)
0	40	60
10	40	60
40	10	90
40.5	0	100
45	0	100
45.5	60	40
50	60	40

3 Result and Discussion

3.1 Plant Determination

The test sample was identified at the Biopharmaca Conservation & Cultivation Station (BCCS) Tropical Biopharmaceutical Study Center, Institute for Research and Community Service (LPPM) IPB University, showing a sample of Red Ginger (Zingiber Officinale Roscoe var rubrum) from the Zingiberaceae tribe.

3.2 Phytochemical compounds

Tests for the content of phytochemical compounds were carried out on simplicia, which contained saponin compounds. The positive reactions in the flavonoids, saponins, and tannins indicate the presence of phenol groups.

3.3 Water Content and Extraction

The water content of red ginger simplicia obtained was 10%, fulfilling the quality requirements so that it can be used for further analysis. Removing the water content up to a certain amount is useful for extending the durability of simplicia. Water content that is too high will become a medium for the growth of microorganisms that cause damage to the simplicia [16]. The viscous extract obtained is blackish-brown in color, and has a distinctive aroma of ginger. The color of red ginger extract produced in n-hexane, ethyl acetate, and ethanol solvents is a dark brown liquid and slightly viscous.

3.4 Impact of Extraction Solvents on the Yield of the Red Ginger Extract

The yield of the red ginger extract can be seen in Table 2. The highest yield was found in an aqueous extract. This extract was then tested for its toxicity to shrimp larvae, and a test was carried out to determine the levels of 6-gingerol, 8-gingerol, 10-gingerol, and 6-shogaol by HPLC.

Table 2. The yield of red ginger rhizome extract

	ETOH 30%	ETOH 70%	ETOH 96%	Water	Ethyl acetate	N hexane
Extract (g)	11.02	10.06	9.1	12.1	5.9	2.5

Impact of Extraction Solvents on Shrimp Larvae Cytotoxic Activity Test. Shrimp larvae cytotoxic activity tests were carried out to observe the potential bioactivity and toxicity of each extract so that a safe extract concentration could be determined for further testing. A plant extract will be bioactive if it has an LC50 value of less than 1000 ppm [15]. Based on Table 3 it can be seen that all red ginger rhizome extracts have the potential as bioactive compounds and can be used as medicine. It was because each extract produced an LC50 of less than 1000 ppm so that at low concentrations it

was able to kill 50% of the population of A. salina shrimp larvae. The extract that has the highest bioactive potential and is toxic was 96% ethanol extract. It is because the 96% ethanol extract of red ginger rhizome has the lowest LC50 value, namely -79.516 ppm, which means that at a small concentration, this extract can kill half the population of A. salina shrimp larvae. The LC50 value is the highest concentration limit for determining various extract concentrations in subsequent tests.

Table 3. LC50 value of red ginger rhizome extract against A. Salina larvae

	ETOH 30%	**ETOH 70%**	**ETOH 96%**	**Water**	**Ethyl acetate**	**N hexan**
LC_{50} (ppm)	208.62	365.39	-79.516	290.16	191.47	365.89

3.5 Impact of Extraction Solvents on Content of Gingerol and Shogaol.

The results of calculating the concentration of gingerol and shogaol compounds in each extract can be seen in Figure 1-4. The highest 6-gingerol compound is found on n-hexane solvent, followed by ethyl acetate, water, and ethanol 96% respectively at 123.07 mg/g, 81.79 mg/g, 31.15 mg/g, and 12.34 mg/g. The highest 10-gingerol content was discovered on n-hexane solvent, followed by ethyl acetate, and ethanol 96% respectively at 61.40 mg/g, 36.28 mg/g, and 5.10 mg/g. The highest 8-gingerol content was discovered in n-hexane solvent, followed by ethyl acetate, and ethanol 96% respectively at 26.39 mg/g, 16.70 mg/g, and 2.68 mg/g. The highest 6-shagol content was discovered on n-hexane solvent, followed by ethyl acetate, and ethanol 96% respectively at 24.41 mg/g, 14.70 mg/g, and 2.73 mg/g. It shows that the compounds 6-gingerol, 8-gingerol, 10-gingerol, and 6-shogaol can be extracted higher with non-polar solvents. In general, the bioactive components in red ginger are non-polar, characterized by more bioactive components that dissolve in hexane, then ethyl acetate and ethanol [17]. It is also caused by ethyl acetate, which is a semi polar solvent that has the ability to attract both polar and non-polar compounds, meanwhile ethanol 96% is a universal solvent that can attract polar, non-polar, or semi polar compounds [18].

The content of 6-gingerol, 8-gingerol, and 10-gingerol in each red ginger extract in this study was higher than in previous studies [19, 20]. The difference in the high content of gingerol in this study and the previous study could be due to the different ecological conditions of the ginger plant, the variety of ginger used, the age of ginger harvest, the method of making ginger simplicia, and the extraction method used.

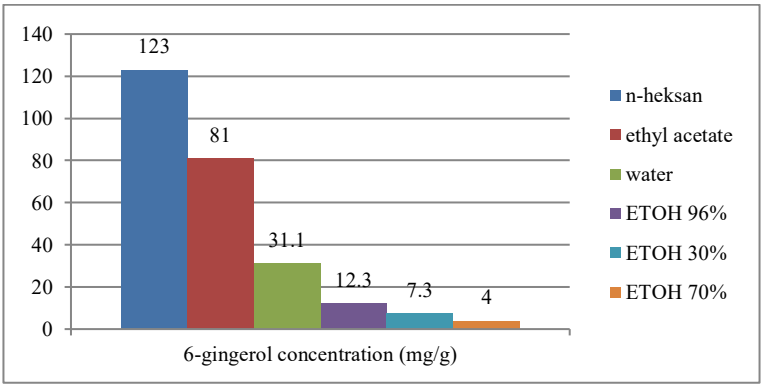

Fig. 1. Graph of 6-gingerol levels of various red ginger extracts

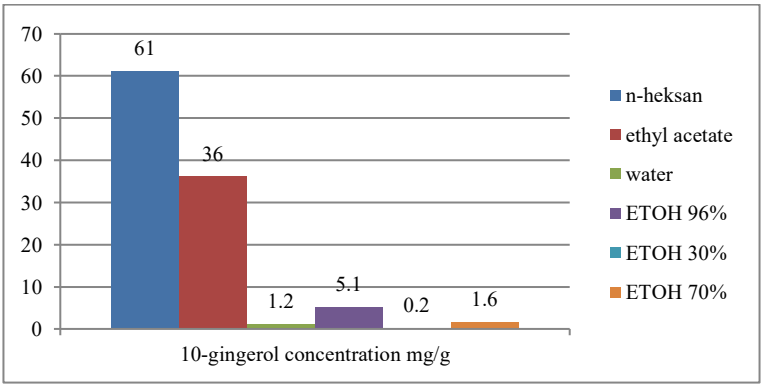

Fig. 2. Graph of 10-gingerol levels of various red ginger extracts

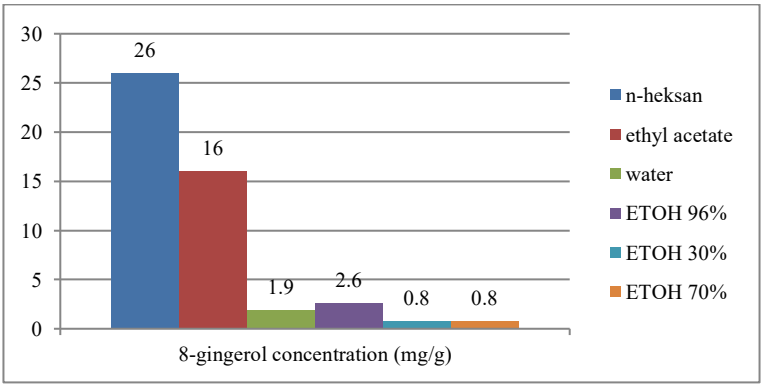

Fig. 3. Graph of 8-gingerol levels of various red ginger extracts

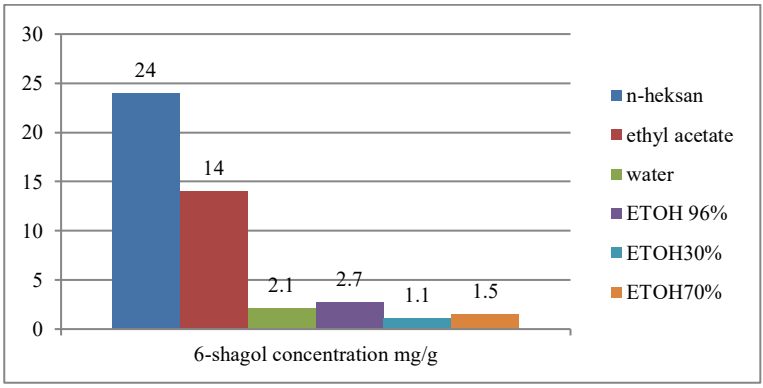

Fig. 4. Graph of 6-shogaol levels of various red ginger extracts

4 Conclusion

The different type of solvent will produce different amount of rhizome, cytotoxic activities, and gingerol and shagol bioactive compounds. The highest yield of red ginger extract was 12.1% in a water solvent. The secondary metabolites in red ginger simplicia are saponins. The extract that has the highest bioactive potential and cytotoxic activity is 96% ethanol extract. The highest content of 6-gingerol was found in n-hexane solvent of 123.07 mg/g. The highest content of 8-gingerol was found in the n-hexane solvent of 26.39 mg/g. The highest content of 10-gingerol was found in n-hexane solvent of 61.40 mg/g. The highest content of 6-shogaol was found in n-hexane solvent of 22.41 mg/g.

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