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Phytochemical Tests and Antioxidant Activities of the Rhips Ginger (*Zingiber Officinale Var Amarum.*)

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Abstract

Ginger is a traditional plant that thrives and has essential functions, including an antioxidant. This study aimed to determine the phytochemical content and test the antioxidant activity of fresh white ginger and powdered ginger (Zingiber officinale var. Amarum.). Two methods are used in this research: the phytochemical test and the antioxidant activity test using DPPH. Phytochemical tests, such as flavonoid, phenolic, steroid, and saponin, were conducted qualitatively. The antioxidant activity test was carried out using a UV-Vis spectrophotometer. Fresh white ginger and powdered ginger were extracted using the maceration method with water solvent. The study results showed that the effects of fresh white ginger extract obtained a yield of 64.08%. Meanwhile, powdered ginger extract was 58.68%. Phytochemical test results of fresh white ginger extract and powdered ginger (commercial) showed positive results for flavonoid, phenolic, and saponin compounds while adverse effects for steroids. Antioxidant activity test on fresh white ginger extract with water solvent showed an IC_{50} value of 25.41 ppm. The powdered ginger extract (commercial) has an IC_{50} value of 36.70 ppm. The results showed fresh white ginger and powdered ginger had relatively high antioxidant activity.

Keywords: Antioxidants, DPPH, fresh white ginger, powdered ginger, phytochemicals, UV-Vis spectrophotometer.

INTRODUCTION

The ginger plant (Zingiber officinale var. Amarum.) is one of the most important plants finds and is widely used by Indonesians. Ginger is a multifunctional plant because apart from being a cooking spice, ginger is also used as a raw material for medicines, traditional herbal medicine, cosmetics, and various kinds of processed food and beverage products (Pradita et al., 2022). Ginger has long been cultivated as an export commodity, but the wide-scale development of ginger has not been supported by optimal and sustainable cultivation, resulting in low productivity and quality. Almost all regions in Indonesia generally use ginger only as a cooking ingredient, which is believed to have many benefits as a remedy for bloating, warming the body, and curing irritation. Ginger rhizome has many uses in traditional use, including for headaches, colds, and to increase appetite, a stimulant (Redi Aryanta, 2019)

In this study, white ginger was used in the form of fresh ginger and powder (commercial). An antioxidant activity test was conducted by extracting fresh and powdered white ginger using water as a solvent. In previous studies, antioxidant activity tests have been carried out using fresh red ginger. Still, there has been no research on antioxidant activity tests on fresh white ginger and powdered (commercial) ginger. One of the determinants of success in extracting a compound is the type of solvent. The solvent used is a solvent that can remove most of the desired secondary metabolites from simplicia (Verdiana et al., 2018). Water is a type of organic solvent that is polar. One of the polar bioactive compounds is flavonoid, so a polar solvent is needed in the extraction process (Fajriaty et al., 2018). Ginger has phenolic bioactive compounds such as: gingerol, shagoal, zingeron, gingerdiol and zingebren, which are proven to have antioxidant activity. Gingerol and shagoal have antioxidant activity because they contain benzene rings and hydroxyl groups (Srikandi et al., 2020).

Antioxidants are compounds whose role is to protect compounds or tissues from the destructive effects of oxygen tissue. Antioxidants are compounds that have a molecular structure that can give electrons to free radical molecules and can break the chain reaction of free radicals. Antioxidants act as nutritional and non-nutritional substances contained in foodstuffs, which are able to prevent or slow down the occurrence of oxidative damage in the body. Antioxidants also act as electron donors or reductants/reducing agents. So, it can be concluded that antioxidants are substances that can delay, slow down, and prevent the oxidation process due to free radicals (Hasanela & Souhoka, 2022).

Free radicals are a form of compounds that have unpaired electrons (Suhesti et al., 2021). The presence of unpaired electrons causes the mixture to be very reactive, looking for a partner. These free radicals will seize electrons from other molecules around them to stabilize themselves. Free radicals are closely related to cell damage, tissue damage, and aging. Free radicals can also change a molecule into a radical (Arbi & Ma.ruf, 2016).

Free radicals attack important biomacromolecules in the body, such as the building blocks of cells, namely proteins, nucleic acids, lipids, and polysaccharides. The main targets of free radicals are proteins, unsaturated fatty acids, lipoproteins, and DNA, including their polysaccharides. Unsaturated fatty acids are the most vulnerable. Free radicals will damage the polyunsaturated fats in the cell membrane so that the cell walls become brittle, damage blood vessels, and cause atherosclerosis. Free radicals also harm DNA bases, disrupting the genetic information system and forming cancer cells. Free radical compounds will also damage lipid tissue to form peroxides and cause degenerative diseases (Amin et al., 2016)

Antioxidants can inhibit oxidation reactions by binding to radicals and highly reactive molecules to prevent cell damage. This compound has a small molecular weight but can inactivate oxidation reactions by preventing the formation of free radicals Antioxidant activity tests can be done qualitatively and quantitatively. Qualitative tests can be carried out with phytochemical tests to see changes in color, residue, and gas formed. While free radical activity test can be done quantitatively using the DPPH (1,1-diphenyl-2-picryhydrazil) method.

METHODOLOGY

Materials and Instrumentals

The materials used were fresh white ginger (Zingiber Officinale Var Amarum.), commercial white ginger (powder), distilled water, aluminum foil, $FeCl_3$ p.a (E. Merck), Mg powder p.a (E. Merck), HCl p.a (E. Merck), glacial acetic acid p.a (E. Merck), H_2SO_4 p.a (E. Merck), 1,1-diphenyl-2-picryhydrazil p.a (E. Mrck), ethanol p.a (E. Merck).

The instrumentals used were knife, analytical balance (Denver Instrument XP-3000), a set of glassware (Pyrex), hot plate (Cimarec 2), UV-Vis spectrophotometer (Apel PD-3000 UV), rotary evaporator (Rotavapor R-215 Buchii), micropipette (Eppendorf).

Sample Preparation

Ginger sample was cleaned of dirt, cut into small pieces, then the sample was put into a 1000 mL beaker to be dried at room temperature.

Calculation of Water

Content Fresh ginger as much as 10.4094 g is put into a porcelain cup which has been dried and the weight is known. Then, fresh ginger that has been dried at room temperature is weighed. The drying and weighing process is carried out until it reaches a constant weight. Water content determined using Equation 1.

$$\% \text{ Water content} = \frac{w-w1}{w} \text{ x } 100\% \qquad (1)$$

Preparation of Fresh Ginger

Water extract Fresh ginger was prepared by weighing 15 g of fresh white ginger powder, putting it into an Erlenmeyer, and adding 300 mL water at 50 ^{o}C. Then, the Erlenmeyer containing the sample was covered with aluminum foil, and the sample was macerated for two days (48 hours) at room temperature. Macerated fresh white ginger extract was then filtered to obtain ginger extract.

Reparation of Powdered Ginger

Water extract of powdered ginger is carried out by weighing 15 g of powdered ginger powder stored in a chemical glass. Then, put it in Erlenmeyer and add 300 mL of distilled water. The Erlenmeyer was covered using aluminum foil, and the mixture was macerated for two days (48 hours) at room temperature. The mixture was filtered to obtain a thick extract.

Flavonoid Test

Ginger water extract (2 mL) was put into a test tube and then added a little Mg powder and 1 mL of 1m HCl. The test is positive if it produces a deep blue color (Rumagit et al., 2015).

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Phenolic Test

Ginger water extract (2 mL) was put into a test tube and added 2 drops of 1% $FeCl_3$ and then shaken. The test is positive if it produces a dark green color.

Steroids Test

Ginger water extract (2 mL) was put into a test tube and added 10 drops of acetic anhydrous and 3 drops of concentrated sulfuric acid. The test is positive if it produces a blue or green color.

Saponin Test

Ginger water extract (2 mL) is put into a test tube and 2 mL of distilled water is added, then shaken until homogeneous. After that, it is heated for 2-3 minutes. Then it is cooled and shaken vigorously. Positive test if it produces foam.

Determination of DPPH Free Radical Scavenging Activity

As much as 0.1 g of the extract was made into a 1000 ppm solution, then diluted to a concentration of 5, 10, 15, 20, 25 ppm. As much as 1 mL was taken from each test solution/sample that had been prepared, put into a test tube and added 1 mL of DPPH solution, then allowed to stand for 30 minutes at room temperature. Absorption measurements were carried out at the maximum wavelength (514 nm) using a UV-Vis spectrophotometer. The test was carried out with two measurements, the same procedure was also carried out on the quercetin standard. The percentage of inhibition/inhibition against DPPH radicals from each concentration of the sample solution can be calculated using the formula (Equation 2):

$$I = \frac{A_0 - A_1}{A_0} \text{X } 100\% \quad (2)$$

After obtaining the percentage of inhibition from each concentration, the antioxidant activity was determined using the line equation of % inhibition as the Y-axis and the sample concentration as the X-axis (µg/mL). Antioxidant activity is expressed by IC_{50}, namely the sample concentration that can reduce DPPH radicals by 50%. The IC_{50} value is calculated by entering the value of 50 into the line equation as the Y axis and then calculating the X value as the IC50 concentration.

Data analysis

Data were obtained from the results of measuring the absorbance of fresh ginger aqueous extract and dried ginger aqueous extract (storage time) using UV-Vis spectrophotometry and statistical data analysis was made using linear regression. The calculation used uses the following formula (Equation 3):

$$y = bx + a \quad (3)$$

RESULTS AND DISCUSSION

Fersh Ginger Morphology

Ginger is a plant with pseudo or soft stems and has a height of 30 cm until 75 cm. Ginger has a root with a branching shape and smells good with a yellow or orange color and is fibrous. Ginger lives in clumps, reproduces, produces rhizomes and flowers and is included as an annual plant (Sari et al., 2021). Ginger is shown in Figure 1.

Figure 1. Fresh Ginger

Ginger contains many phytochemical compounds and phytonutrients. The substances contained are secondary metabolites from flavonoids, phenolics, terpenoids, and essential oils. Gingerol and shogaol are bioactive compounds found in ginger and can be used as antioxidants. Ginger has bioactive components that can protect fat or membranes from the oxidation process, inhibit cholesterol oxidation, and increase immunity (Sari et al., 2021).

Powdered Ginger

Powdered ginger is a type of commercial white ginger in the form of fine powder which can be obtained from traditional markets and supermarkets. White ginger powder is brown and has a powerful ginger odour. In the market, powdered ginger is sold under various trademarks. In this study, the commercial ginger used was white ginger, which is the same as fresh ginger, as shown in Figure 2

Figure 2. White Ginger (Powder)

Fresh white ginger is cleaned so that it is not contaminated during extraction. Fresh white ginger was then cut into small pieces, dried at room temperature and then tested for water content. Measurement of moisture content is one of the most critical chemical laboratory test methods in the food industry to determine the quality of food resistance to damage and shelf life. From the research results, the percentage of water content for fresh white ginger was obtained at 24.22%. To maintain the shelf life of ginger, it must be done by drying it. According to (Manoi, 2006), if the water content is more significant than 10%, it will cause enzymatic processes and damage by microbes.

Extraction Result

Extraction is carried out to separate the components present in the material using certain solvents. The extraction method used in this research is maceration. The maceration method was chosen because it is easy and simple, but it can also extract active compounds properly by immersion without high heating to avoid component damage (Souhoka et al., 2019). In this study, samples of fresh ginger extract and powdered ginger were extracted using two polar solvents, namely water. Each 1000 g of fresh and powdered ginger was extracted using the maceration method for two days (48 hours). The results showed that the water extract of fresh ginger was blackish brown. Meanwhile, the water extract of ginger powder is blackish yellow. Several factors that affect the success of the extraction process are temperature, stirring speed, particle size, and type of solvent used (Anggista et al., 2019). Fresh ginger extract and powdered ginger are shown in Figure 3.

Furthermore, the extract results were filtered using a filter. The filtrate from each extraction was evaporated using a rotary evaporator to remove the solvent so that a thick extract was obtained. The yield percentage results for fresh white ginger extract and water powder ginger extract can be shown in Table 1.

Table 1. Extract yield %

Sample type	Rendamen Water solvent (%)
Fresh Ginger	64.08
Powder Ginger	58.68

Figure 3. Fresh ginger extract and powdered ginger

Based on the data in Table 1, it is obtained that fresh ginger extract has a more significant percentage of yield than powdered ginger extract. This is because fresh ginger is still pure, and there has been no treatment in the production process, such as powdered ginger. In the production process, powdered ginger undergoes a drying and grinding process, which can reduce the presentation of powdered ginger residue. According to Sari *et al*, 2021, determining the yield determines the levels of secondary metabolites carried by the solvent but cannot choose the type of compound.

Fresh Ginger and Powder Ginger Phytochemical Test

Phytochemical testing is a qualitative analysis of secondary metabolite compounds. The water extract of fresh ginger and powdered ginger was tested for phytochemicals consisting of flavonoid, phenolic, steroid, and saponin tests. Flavonoid test from fresh ginger extract and powdered ginger showed positive results marked with a red color (Ergina et al., 2014), flavonoid compounds will be reduced with Mg and HCl to produce red and orange colors.

The steroid test shows a positive result if it is blue or green when reacted with acetic anhydrate and concentrated sulfuric acid. The test results showed that fresh ginger extract and powdered ginger with water solvents showed negative effects or did not contain steroids; this was indicated by the absence of a change in color to green or blue. This is because the steroid content is very small, so it is difficult to detect with coloring reagents. In addition, the type of solvent factor also influence (Telussa et al., 2022).

The results of the saponin test using warm water on the ethanol extract of fresh ginger and powdered ginger obtained positive results, namely the formation of visible or stable foam. Saponins have surface active compounds that are easily detected through their ability to form foam. Saponins are generally glycosides, so they tend to be polar (Ergina et al., 2014). The phytochemical test can be seen in Figure 4. Results of the phytochemical test of fresh ginger and water-solvent ginger powder at Table 2.

Table 2. Results of the phytochemical test of fresh ginger and water-solvent ginger powder

Test phytochemical	Extract	Water solvent
	Fresh Ginger	Powder Ginger
Flavanoid	+	+
Fenolic	+	+
Steroid	-	-
Saponin	+	+

Phytochemical test for aqueous extract of fresh ginger and powder with water solvent gave positive results for flavanoid, phenolic and saponin compounds. While negative results for steroid compounds. Negative results on steroids can be caused because water, which is a polar solvent, is unable to extract the steroids present in ginger. Steroid extracts can be produced with semi-polar solvents such as chloroform and ethyl acetate (Kurniawan et al., 2022)

(a) (b) (c) (d)

Figure 4. Color changes that occur in thephytochemical test (a) flavanoid test, (b) tanin test, (c) steroid test, and (d) saponim test.

Determination of Free Radical Antidote Activity

In determining free radical activity, DPPH is expressed by the IC_{50} value, which is the ability of a solution to reduce free radical activity by 50%. The 1,1-diphenyl-1-picrylhydrzil (DPPH) method was used to measure antioxidant activity because it is stable at room temperature (Molyneux, 2004). Antioxidant activity was measured in ginger extract, namely fresh ginger and powder extracted using a water solvent. The antioxidant activity produced from the crude extract of ginger, both fresh ginger and powdered ginger, is influenced by the solvent used to extract the bioactive compounds and secondary metabolites. The antioxidant activity of fresh ginger extract with water solvent was tested using a UV-Vis spectrophotometer at concentrations of 5, 10, 15, 20 and 25 ppm. Test of free radical scavenging activity of DPPH fresh ginger extract with water solvent can be shown in Table 3.

Table 3. Test results for free radical scavenging activity of DPPH fresh ginger extract

Fresh Ginger Extract		
Concentration (ppm)	Water solvent	
	Absorbance (nm)	Inhibition (%)
5	0.124	45.61
10	0.122	46.49
15	0.119	47.80
20	0.117	48.68
25	0.114	50

Antioxidant activity was determined using the electron transfer method with DPPH as a free radical. This is because measurement with DPPH has advantages, namely: simple, fast and does not require a lot of reagents (Hasanela & Souhoka, 2022). The concentration and absorbance data calculated the inhibition presentation for fresh ginger extract using water as a solvent. Based on the calculation, results show that the determination of DPPH free radical scavenging activity in water-solvent fresh ginger with the regression equation $y = 0.2194x + 44.425$ has an IC_{50} value of 25.41 ppm, including antioxidants. The high antioxidant activity of fresh ginger extract is influenced by several things, one of which is the freshness and purity of ginger, which has not undergone a production process such as heating.

Fresh ginger extract with water solvent successfully extracted secondary metabolites and bioactive compounds as precursors of antioxidant compounds. One of the active components in fresh ginger is oleoresin, which can be removed by water solvent. Oleoresin extracts are generally removed with organic solvents (Oktora & Sudaryanto, 2007). The free radical scavenging antioxidant activity curve of DPPH fresh ginger extract with water solvent can be shown in Figure 5.

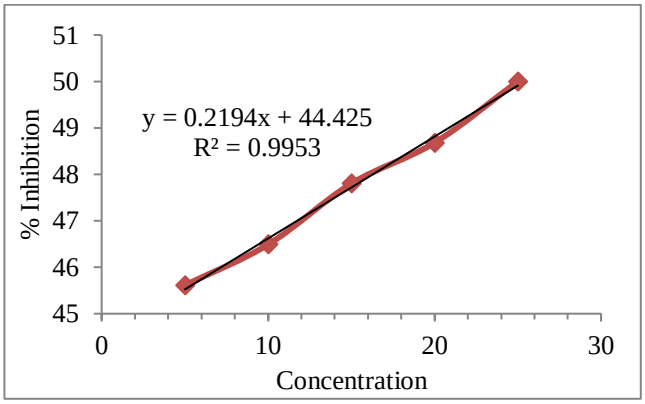

Figure 5. Antioxidant activity curve of DPPH free radical scavenger of fresh ginger extract

The calculation of the antioxidant activity in powdered ginger extract was carried out with water as a solvent, which has the same concentration as fresh ginger extract, namely 5, 10.15, 20 and 25 ppm. The test of the free radical scavenging activity of DPPH powdered ginger extract with water solvent can be shown in Table 4.

Table 4. Test results for free radical scavenging activity of DPPH powder ginger extract

Powder Ginger Extract		
Concentration (ppm)	Water solvent	
	Absorbance (nm)	Inhibition (%)
5	0.133	41.66
10	0.129	43.42
15	0.125	45.17
20	0.122	46.49
25	0.119	47.80

Based on the calculation results. it was obtained that the free radical scavenging activity of DPPH water-solvent ginger powder extract with the regression equation y = 0.4124x + 34.864 had an IC_{50} value of 36.70 ppm. The free radical scavenging antioxidant activity curves of DPPH powdered ginger extract with water solvent can be shown in Figures 6. The antioxidant activity of the DPPH free radical scavenger of powdered ginger extract with water solvent has a smaller IC_{50} value compared to the water solvent fresh ginger extract sample. Still. both are in the high antioxidant range. This is because powdered ginger extract has undergone many treatments. including heat drying. Excessive heat will cause ginger's bioactive compounds and secondary metabolites to become less good. This is due to the oxidation process by heating. light. and air (Hasanela & Souhoka. 2022). Determination of DPPH free radical scavenging activity on fresh white ginger and powdered ginger (commercial) can be shown in Table 5.

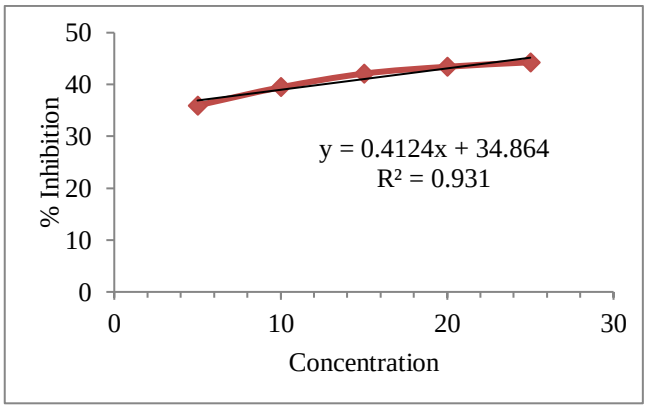

Figure 6. Antioxidant activity curve for DPPH free radical scavenger of powdered ginger extract

Table 5. Antioxidant activity of ginger

Sample type	Antioxidant Activity Water Solvent (ppm)
Fresh Ginger	25.41
Powder Ginger	36.70

Based on the data in Table 5. it can be seen that the highest free radical scavenging DPPH activity was found in fresh white ginger at 25.41 ppm. compared to ginger powder. which was 36.70 ppm. This is because fresh white ginger extract contains pure compounds compared to powdered ginger extract. Besides that. ginger powder is also a commercial product with an impure composition because there is already a mixture of additional ingredients such as preservatives and others.

CONCLUSION

Based on the study results. the antioxidant activity test on fresh white ginger extract with water solvent showed an IC_{50} value of 25.41 ppm. While powdered ginger extract (commercial) has an IC_{50} value of 36.70 ppm. This indicates that both fresh white ginger and powdered ginger have high antioxidant activity.

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