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Antioxidant Activity Test of Ethanol Extract of White Ginger (*Zingiber officinale* var. *Amarum*) Using the DPPH Method

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Abstract
Antioxidants are compounds that can inhibit, decelerate, or prevent oxidative processes induced by free radicals. This study aims to identify the phytochemical constituents and evaluate the antioxidant activity of fresh white ginger and ginger powder (*Zingiber officinale*) using the DPPH assay. Both samples were extracted using the maceration technique with ethanol as the solvent. The ethanol extract of fresh white ginger yielded 70.042%, whereas that of ginger powder yielded 59.984%. Phytochemical screening of the ethanol extracts of fresh white ginger and ginger powder indicated the presence of flavonoids, phenolic compounds, and saponins, while steroids were absent. The antioxidant activity was measured using the DPPH method with a UV-Vis spectrophotometer. The ethanol extract of fresh white ginger exhibited an IC_{50} value of 19.85 ppm, whereas the extract of ginger powder demonstrated an IC_{50} value of 31.58 ppm. Although the IC_{50} value of the powdered white ginger extract was higher than that of the fresh white ginger extract, both extracts are classified within the strong antioxidant category.

INTRODUCTION

Indonesia, as a country rich in biodiversity, especially in agricultural and spice production, benefits greatly from its tropical climate. One of the widely distributed and beneficial spices in Indonesia is ginger, which comes in three main varieties: red ginger, small white ginger, and large white ginger. The morphological differences among them lie in the size and color of their rhizome skin (Fathiah, 2022). White ginger, particularly the small white ginger variety (*Zingiber officinale* var. *Amarum*), plays a crucial role in Indonesian society. Apart from being used as a culinary spice, ginger is utilized in traditional medicine, herbal concoctions, cosmetics, and various food and beverage products. However, despite ginger being a long-standing export commodity, optimal cultivation development is still lacking, leading to low productivity and quality. In many regions of Indonesia, ginger is predominantly utilized as a cooking ingredient, believed to have benefits in relieving bloating, providing body warmth, and healing irritation (Siregar *et al*., 2022).

This research focuses on white ginger in its fresh and commercial powder forms for antioxidant activity testing using ethanol extraction. While previous studies have examined the antioxidant activity of fresh red ginger. Red ginger rhizomes are known to contain various bioactive compounds that function as antioxidants (Bonita Yunpayani & Leni Sri Mulyani, 2024). These phenolic compounds, including gingerols, shogaols, zingerones, and flavonoids,

play an important role as powerful antioxidants (Herawati & Saptarini, 2020a). There has been no research on fresh white ginger and its powder (commercial) counterpart. The choice of solvent is crucial in extracting compounds, and ethanol is selected for its polar nature that can extract secondary metabolites, such as flavonoids, found in ginger. Bioactive compounds in ginger, such as gingerol, shogaol, zingeron, gingerdiol, and zingiberene, have proven antioxidant activity due to the presence of benzene rings and hydroxyl groups (Widowati *et al*., 2022).

Antioxidants play a vital role in protecting tissues from damage caused by free radicals, which can lead to oxidative reactions in cells and tissues, contributing to the aging process (Chandimali *et al*., 2025). Antioxidant compounds can neutralize these oxidation reactions by binding to and preventing the damage caused by free radicals (Erviana *et al*., 2016). The assessment of antioxidant activity can be performed qualitatively through phytochemical tests to observe changes in color, sedimentation, and gas formation, as well as quantitatively using the DPPH (1,1-diphenyl-2-picrylhydrazyl) method (Herawati & Saptarini, 2020b; Munadi & Arifin, 2022; Siregar *et al*., 2022).

RESEARCH METHODS

This study used fresh white ginger (*Zingiber officinale*) and commercial white ginger powder as the main samples. The study began with the calculation of water content, namely 10.4 g of ginger samples dried at room temperature and weighed, then weighed and dried until it reached a constant weight. The next study was the extraction process. Commercial ginger powder extract of fresh white ginger was made by weighing 15 g of fresh white ginger powder, putting it in an Erlenmeyer flask, and adding 300 mL of ethanol. Extraction was carried out by the maceration method with a sample to solvent (ethanol) ratio of 1:20 for 2 days (48 hours) at room temperature.

The resulting maceration extracts of each sample were then identified through phytochemical tests, including flavonoid testing, phenolic testing, steroid testing, and saponin testing. Subsequently, the extract samples were subjected to antioxidant activity testing using the DPPH method with a concentration of 40 ppm. The absorbance was measured using a UV-Vis spectrophotometer at a wavelength of 514 nm. The percentage of inhibition of DPPH radicals at each sample concentration was calculated using the following equation:

$$I = \frac{A_0 - A_1}{A_0} \text{X } 100\% \quad (1)$$

Where A_0 = absorbance of the control (DPPH solution without sample), A_1 = absorbance of the sample solution (Ruiz-Ruiz et al., 2017). This calculation determines the radical scavenging activity of the extract based on the decrease in absorbance at the maximum wavelength of DPPH, as measured by a UV-Vis spectrophotometer

RESULTS AND DISCUSSION

Sample Preparation

Fresh white ginger was thoroughly cleaned to prevent contamination during the extraction process. The ginger samples were then finely chopped and air-dried at room temperature, followed by a determination of their moisture content. Moisture content analysis is an essential chemical test in food science, as it provides an indicator of product quality, resistance to spoilage, and shelf stability. The experimental results showed that the moisture content of fresh white ginger was 24.22%. Therefore, to enhance the shelf life of ginger, drying

is necessary. According to Nasihin et al. (2024), a moisture content exceeding 10% can promote enzymatic activity and microbial deterioration.

Extraction

Extraction is performed to separate the components present in the material using a specific solvent (Syarifah *et al*., 2023) he extraction method used in this study is maceration. Maceration is chosen because it is easy and simple; besides, it can extract active compounds well through soaking without high heating, thus avoiding component damage. In this study, samples of fresh white ginger extract and powdered ginger extract were extracted using a polar solvent, namely ethanol. Each sample, consisting of 15 g of fresh and powdered ginger, was extracted by the maceration method for 48 hours. The ethanolic extract of fresh white ginger exhibited a yellowish-brown color, while that of powdered ginger appeared orange-brown. The color difference between the two samples was influenced by the types of secondary metabolites present in the samples. The extracts were subsequently filtered, and the resulting filtrates were evaporated using a rotary evaporator to remove the solvent and obtain concentrated extracts. The percentage yields of the ethanol extracts of fresh white ginger and powdered ginger are presented in Table 1.

Table 1. Extract Yield Percentage Results

Sample Type	Ethanol Solvent Percentage Yield (%)
Fresh White Ginger	70.042
Powdered Ginger	59.984

Based on the data in Table 1, the percentage of fresh white ginger extract was 70.042 %, which is higher than the powdered ginger extract at around 59.984 %. This difference is because fresh white ginger still contains more chemical compounds compared to powdered ginger, which has undergone various processes such as heating, drying, etc (Tapalina *et al*., 2022).

Phytochemical Test

Phytochemical analysis is a qualitative assessment aimed at identifying secondary metabolite compounds. Extracts derived from natural sources generally contain various secondary metabolites that contribute to their biological activities. These compounds can be detected using specific reagents that produce characteristic reactions for each metabolite group (Asworo & Widwiastuti, 2023). Both ethanol and aqueous extracts of fresh ginger and powdered ginger were subjected to phytochemical screening for the presence of flavonoids, phenolics, steroids, and saponins.

Flavonoid Test

The presence of flavonoid compounds in the ethanol extracts of fresh white ginger and powdered ginger was determined using the Shinoda test with 1% HCl and magnesium powder. Each extract was subjected to flavonoid analysis to observe color changes resulting from the reaction.

Figure 1. Flavonoid test

As shown in Figure 1, the flavonoid test yielded positive results, indicated by an orange coloration in the fresh ginger extract and a brown coloration in the powdered ginger extract (Ramonah, 2023). In this test, flavonoid compounds are reduced by magnesium and hydrochloric acid, producing red or orange hues as confirmation of their presence. The flavonoid reaction can be written in the chemical reaction equation (Yuda *et al*., 2017).

$$Mg(s) + 2HCl\ (l) \rightarrow MgCl_2\ (aq) + H_2 \quad (2)$$

$$MgCl_2\ (aq) + 6\ ArOH\ (s) \rightarrow Mg\ (OAr)6]^{-4} + 6H^+ + 2\ Cl^- \quad (3)$$

Phenolic Test

The phenolic test will show a positive result if a strong green color forms when reacted with $FeCl_3$, as phenolic compounds have 32 hydroxyl (-OH) functional groups that interact with Fe^{3+} ions from $FeCl_3$, producing a complex compound that imparts a green and dark green color to the test solution (Nofita *et al*., 2023). The tested samples displayed positive results, indicated by a dark green color in the fresh white ginger extract and a green color in the ginger powder extract.

Figure 2. Phenolic test

Phenolic compounds typically exhibit easy solubility in water since, in general, these compounds establish bonds with sugars in the form of glycosides and are commonly present in the cellular vacuole (Jabbar *et al*., 2024). This aligns with the phenolic compound test reaction as depicted (Yuda *et al*., 2017).

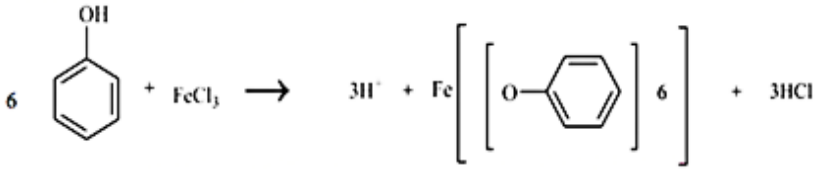

Figure 3. Chemical reaction of phenolic

Steroid Test

The presence of steroid compounds was evaluated using a qualitative test involving acetic anhydride and concentrated sulfuric acid, which produces a blue or green coloration as a positive indication (Fasya *et al*., 2020). The results demonstrated that both the ethanol extracts of fresh white ginger and powdered ginger showed negative reactions, indicating the absence of steroid compounds. This conclusion was supported by the lack of any color change to blue or green during the test. The steroid test is shown in Figure 4.

Figure 4. Steroid test

Saponin Test

The presence of saponin compounds in the extracts was determined through a qualitative test involving the addition of warm water, followed by vigorous agitation (Suleman *et al.*, 2022). The results of the saponin test for both the ethanol extracts of fresh white ginger showed positive reaction, and powdered ginger showed negative reaction, as evidenced by the formation of stable and persistent foam. The saponin test is shown in Figure 5.

Figure 5. Saponin test

Saponin is a substance with active surface compounds easily detectable through its ability to form foam. When agitated, the hydrophilic groups bind with water, while the hydrophobic groups bind with air, resulting in foam formation. The saponin compound reaction can be written in the chemical reaction equation (Herawati & Saptarini, 2020b).

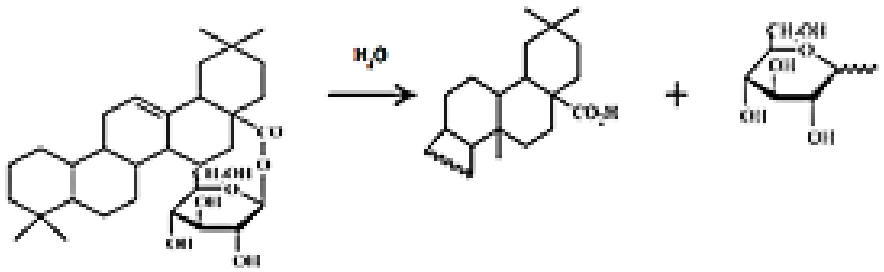

Figure 6. Chemical reaction of saponin

Phytochemical Test Results for Fresh White Ginger and Powdered Ginger Extracts Using Ethanol Solvent are Presented in Table 2.

Table 2. Phytochemical Test Results for Fresh White Ginger and Powdered Ginger Extracts Using Ethanol Solvent

Phytochemical Test	Ethanol Extract	
	Fresh White Ginger	Ginger Powder
Flavonoid	+	+
Phenolic	+	+
Steroid	-	-
Saponin	+	-

The determination of antioxidant activity with the DPPH

Free radical was conducted for samples of fresh white ginger and ginger powder extracts using ethanol as the solvent. In this assessment, absorbance was measured using a UV-Vis spectrophotometer at a wavelength of 514 nm (Baliyan *et al*., 2022), as shown in Table 3.

Table 3. Free Radical from Fresh White Ginger and Powdered Ginger Extracts Using Ethanol Solvent

Samples	C (ppm)	Abs	% I
Fresh Ginger Extract	5	0.109	46.49
	10	0.115	47.36
	15	0.119	47.80
	20	0.120	49.56
	25	0.122	52.19
Ginger Powder Extract	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

Determination of Free Radical Scavenging Activity (DPPH) in Fresh Ginger

The free radical scavenging activity of DPPH is expressed as the IC_{50} value, which represents the concentration of a sample required to inhibit 50% of free radical activity (Kusumorini *et al*., 2022). The 2,2-diphenyl-1-picrylhydrazyl (DPPH) method was used to evaluate antioxidant activity due to its stability at room temperature (Fajobi *et al*., 2017). Antioxidant activity was assessed in extracts of fresh white ginger and powdered ginger obtained using ethanol as a solvent. The antioxidant potential of both crude extracts was influenced by the type of solvent employed, which affects the extraction efficiency of bioactive and secondary metabolite compounds. The determination of antioxidant activity for ethanol extracts of fresh ginger was performed using a UV-Vis spectrophotometer at concentrations of

5, 10, 15, 20, and 25 ppm. The results of the DPPH free radical scavenging activity for ethanol and water extracts of fresh ginger are presented in Table 3.

Antioxidant activity was determined using the electron transfer method with DPPH as the free radical source. The DPPH assay offers several advantages, including simplicity, rapid execution, and minimal reagent requirements (Hasanela & Souhoka, 2022). Based on the concentration and absorbance data, the percentage of inhibition was calculated for fresh ginger extracts obtained using ethanol and water as solvents. The calculation results indicated that the determination of free radical scavenging activity for fresh ginger extracted with ethanol followed the regression equation y = 0.272x+44.6, with an IC_{50} value of 19.85 ppm, classifying it as a substance with strong antioxidant potential. The high antioxidant activity observed in the ethanol extract of fresh ginger is influenced by several factors, particularly the type of solvent used during the extraction process (Popova & Bankova, 2023).

The ethanol extract of fresh ginger effectively extracts various secondary metabolites and bioactive compounds that serve as precursors to antioxidant activity. One of the major active constituents in fresh ginger is oleoresin, which can be efficiently extracted using ethanol as a solvent. Oleoresin extraction is typically carried out with organic solvents, among which ethanol is recognized as an effective and safe option. The antioxidant activity curve representing the DPPH free radical scavenging ability of the ethanol extract of fresh ginger is presented in Figure 7.

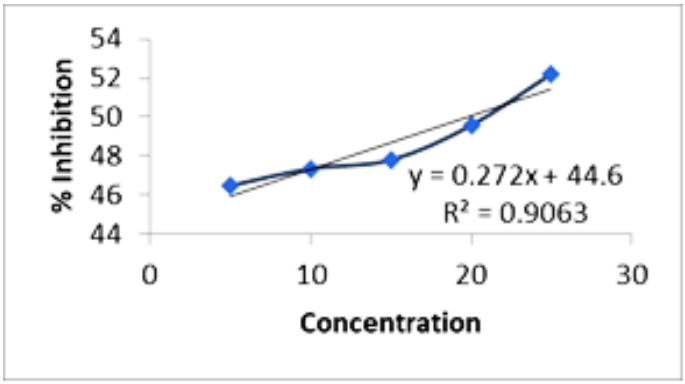

Figure 7. Antioxidant Activity Curve against DPPH Free Radicals of Fresh Ginger Extract with Ethanol Solvent

Determination of Free Radical Scavenging Activity of DPPH in Ginger Powder

The antioxidant activity of the powdered ginger extract was determined using ethanol as the solvent, with concentrations: 5, 10, 15, 20, and 25 ppm. The results of the DPPH free radical scavenging assay for the ethanol extract of powdered ginger are presented in Table 3. Based on the calculated data, the free radical activity of the ethanol extract of powdered ginger followed the regression equation y = 0.307x + 40.303, yielding an IC_{50} value of 31.58 ppm. According to the classification criteria, the antioxidant activity of the powdered ginger extract with ethanol solvent is categorized as very strong, since its IC_{50} value is below 50 ppm. The antioxidant activity curve representing the DPPH free radical scavenging ability of the ethanol extract of powdered ginger is shown in Figure 8.

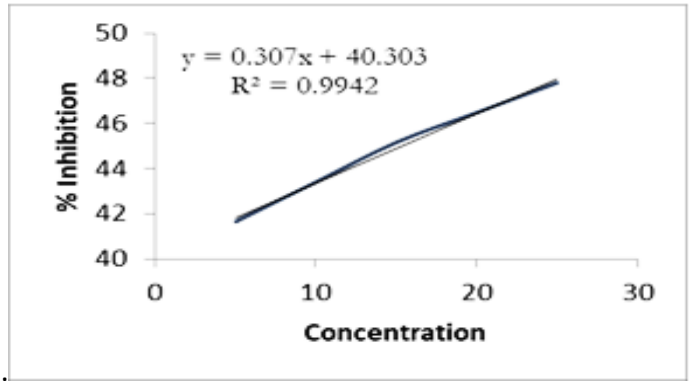

Figure 8. Antioxidant Activity Curve against DPPH Free Radicals of Ginger Powder Extract with Ethanol Solvent

The antioxidant activity of the ethanol extract of powdered ginger exhibited a higher IC_{50} value compared to that of the fresh ginger extract, although both extracts fall within the category of strong antioxidants. This difference may be attributed to the various processing treatments undergone by the powdered ginger, particularly the drying process involving heat exposure. Excessive heat can cause a reduction in the concentration of bioactive compounds and secondary metabolites present in ginger. Such degradation is mainly due to oxidation processes induced by heat, light, and air (Hasanela *et al*., 2023).

The results of the determination of DPPH free radical scavenging activity for both fresh white ginger and commercial powdered ginger are presented in Table 4.

Table 4. DPPH Free Radical Scavenging Activity on Ginger Ethanol Extract

Sample Type	Antioxidant Activity (ppm)
Fresh White Ginger	19,85
Powdered Ginger	31,58

Based on the data in Table 4, it can be observed that the highest free radical scavenging activity against DPPH in fresh white ginger and ginger powder is found in fresh white ginger with ethanol solvent. with a value of 19.85 ppm. This is because ethanol has a lower polarity compared to water solvent (Lee *et al*., 2024). The ethanol extract of fresh ginger is capable of dissolving both polar and moderately nonpolar secondary metabolites. Furthermore, the extract of fresh white ginger contains relatively purer compounds compared to the commercial powdered ginger extract. The powdered ginger extract, being a processed commercial product, tends to have a less pure composition due to the inclusion of additives such as preservatives and other substances.

CONCLUSION

Based on the results of this study, it can be concluded that the phytochemical analysis of ethanol extracts from fresh white ginger and powdered white ginger revealed the presence of flavonoids, phenolics, and saponins, while steroids were not detected. The ethanol extract of fresh white ginger demonstrated an IC_{50} value of 19.85 ppm, whereas the ethanol extract of powdered white ginger exhibited an IC_{50} value of 31.58 ppm. Although the IC_{50} value of the powdered white ginger extract was higher than that of the fresh white ginger extract, both extracts are classified within the strong antioxidant category.

DECLARATIONS

Author Contributions

The first author designs the research, then, together with co-authors, conducts the research in the laboratory and collects the research data. The first author writes the report and manuscript for submission to the journal.

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Independent research

Ethics Statement

The data in this article represent the results of genuine research and contain no indication of plagiarism.

Declaration of Interests

This research is not for the benefit of any party and is purely a lecturer's research as part of the Tri Dharma (Three Pillars of Excellence).

Data Sharing Statement

Not willing

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