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Original Article

Testing the Antioxidant and Characteristics of *6-Shogoal* and *6-Gingerol* in Ginger (*Zingiber Officinale*)

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Abstract: Ginger is a medicinal plant. The ingredients used in this study included young Ginger, old Ginger, and bent Ginger. This study examined the results of calculating the antioxidant activation test method in Ginger's DPPH, FRAP, and TPC. When comparing antioxidant test methods based on the type of reaction mechanism, antioxidant samples are chosen based on the polyhydroxy or polyphenol structure, the fundamental constituent of natural antioxidants. The FRAP test method yielded 904.27 ± 152.95 for Bentung Ginger, 858.22 ± 6.58 for young Ginger, and 741.88 ± 29.92 for old Ginger. The TPC method yielded 488.17 ± 39.41 for old Ginger, 443.42 ± 22.41 for old Ginger Bentung, and 432.61 ± 53.83 for young Ginger. The DPPH levels in young Ginger are 89.50 ± 0.31, bent Ginger is 89.45 ± 0.50, and old Ginger is 89.45 ± 0.50. The results showed that old Ginger had the highest 6-gingerol compound content (10.37), while young Ginger had the highest shogaol content (0.35). The characteristics show that the yield of 6-Ginger for old Ginger is higher than that of other Ginger, namely 10.37, for young ginger 7.95, and for bentung ginger 8.07. Meanwhile, young Ginger has a higher Shogaol yield than others, with 0.34, old ginger 0.32, and Bentung ginger 0.22.

Keywords: Antioxidant; Ferric Reducing Antioxidant Power; Ginger; Total Plate Count; Gingerol and Shogaol.

1. Introduction

Ginger is a medicinal plant. Ginger is a traditional medicine in Indonesia that prevents and treats various diseases. Ginger (*Zingiber officinale*) is antioxidant-rich in phenolic compounds, such as gingerol, shagaol, paradol, and gingerdion. It also contains alkaloids, ascorbic acid, terpenoids, beta-carotene, and polyphenols, specifically flavonoids (Sururi et al., 2022). In Indonesia, three types of Ginger are recognized based on the size and color of their rhizomes: large white Ginger, small white Ginger, and red Ginger. Small white and red Ginger are predominantly used in the beverage industry as refreshing drinks and raw materials for IOT (Traditional Herbal Medicines), standardized herbal medicines, and phytopharmaceuticals. The National Agency of Drug and Food Control (Badan POM) has been promoting research towards isolating

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identity compounds (marker compounds) from superior plants like Ginger to support the development of phytopharmaceuticals. Phenols are the main components in Ginger that contain active substances, including [6]-gingerol, which possesses pharmacological effects and imparts flavor. These compounds can inhibit the formation of tumors in the skin of experimental mice and suppress the proliferation of human cancer cells by inducing apoptosis, including in leukemia blood cancer cells, colon cancer, and others. Small white Ginger and red Ginger have been designated by Badan POM as one of the phytopharmaceutical industry materials for cancer treatment (Gardjito et al., 2021).

Antioxidants are compounds that, at low concentrations, can significantly prevent substrate oxidation during chain reactions (Choe & Min, 2009; Shebis et al., 2013). They can also protect cells in the body from damage caused by free radicals (Gulcin, 2020). Ginger contains several antioxidants, including 6-gingerol and shogaol. 6-Gingerol in Ginger is an anticoagulant that works to prevent blood clots in humans, thereby preventing blockage of blood vessels, which is the leading cause of stroke and heart attack. Aside from that, 6-gingerol and shogaol exhibit antirheumatic properties. Because the 6-gingerol compound is found in Ginger, a drying process is required to ensure the activity of the 6-gingerol compound in the ginger extract using the maceration extraction method with methanol solvent concentrations of 30%, 80%, and 100% (Pathiassana et al., 2020). This study used the maceration method, which is appropriate for extracting gingerol and shogaol because it does not use high temperatures, which can affect gingerol levels. Multilevel maceration is a gradual extraction method that uses various solvents to produce increasing amounts of extract.

2. Materials and Methods

The materials used in this study are young Ginger, old Ginger, and Bentung variant of Ginger, which comes from Pahang, Malaysia, near Kuala Lumpur, 80% Methanol, 100% Alcohol, Aquadesh, Buffer pH 3.6, FeCl3, FeSO4, Acid, Soduim, Carbonate, Folin, Garlic Acid, and DPPH. This study's tools include digital scales, blenders, ovens, measuring cups, Erlenmeyer, spatulas, funnels, filter paper, spectrometers, HPLC, knives, gloves, vortex, micropipette tubes, Eppendorf tubes, drop pipettes, micropipettes, fume hood, shaker water bath, shaker, and tube. Next, several research procedures as follows:

- Sample Making
  Samples of young ginger fruit, old Ginger, and bentung Ginger were obtained from cultivation in Pahang, Malaysia (Kuala Lumpur). The samples are now in the laboratory for testing. Ginger samples were washed, sliced, and oven-dried for 12 hours to reduce water content. They were then blended into powder and stored at room temperature for further testing.
- Sample Extraction
  The sample obtained in the previous step creates a methanol extract. For each sample, 3 grams of ginger powder were taken, and three replications were performed on each sample to achieve the best results. Then, 30 ml of 80% methanol was mixed and placed in a shaker for two hours at 150 rpm. After thorough mixing, it is filtered through filter paper (Filter) and put in an Eppendorf Tube. The extract's activity as an antioxidant will then be assessed.
- Antioxidant Activity Test with the FRAP Method
  This test developed a calibration curve with Trolox as a comparison compound. The FRAP array was prepared using 0.03 grams of TPTZ and 0.054 grams of $FeC1_3.6H_20$, and each sample was dissolved in 10 mL of 40 mM HCl and mL of distilled water, followed by 50 mL of pH 3.6 acetic acid derivative pads. Mix 5 mL of TPTZ and $FeC1_3$.composition. H_2O to 5 milliliters, then fill the measuring flask with 100 mL of distilled water. Guaranteed cell strengthening movement of 0.1 mL in each composition of free phenolic concentrate and bound phenolic extract by treating with ethanol remover (ET), pure water separation (AQ), and untreated concentrate (TP) plus 3 mL of FRAP Reagent. Then vortexing, and finally a saw with the highest frequency of 596 nm.
- TPC Method
  TPC was determined using the Folin-Ciocalteau method. The Total Phenolic Content (TPC) method employs a solution of sodium carbonate, foline, and gallic acid. Make a 7.5 g sodium carbonate solution and 100 mL distilled water, then homogenize. To make the folin solution, combine 10 mL of folin and 1000 mL of distilled water. Make a gallic solution of 10 mg gallic acid in 100 mL H_2O. To begin, add 300 µL of ginger sample to an Eppendorf tube. Then, add 1500 µL of follin's karutan and 1200 µL of sodium carbonate solution. Finally, homogenize briefly and let stand for 30 minutes. The absorbance will be measured using UV-vis at 765 nm.
- Antioxidant Activity Test. Using the DPPH method
  Make a methanol solution with DPPH for analysis. This method prepares ginger samples in various ways, including concentrations (20, 40, 60, 80). To prepare the DPPH method solution, dissolve 0.0075 gr of DPPH in 50 ml of MeOH, then store it in a dark place. The DPPH method involves adding 1 ml of ginger sample to 2 ml of DPPH solution, storing the mixture in a dark place for 30

minutes, and measuring the absorbance at a wavelength of 517 nm with a UV-Vis spectrophotometer.

- Content of 6-gingerol and 6-shogaol
  In ginger samples was determined using HPLC (Shimadzu model Class VP, Japan). When a sample is prepared and analyzed using HPLC, 1 gram is mixed with 50 ml of 100% methanol, extracted, and placed in a special HPLC tube before being analyzed. A single sample HPLC analysis can take up to 40 minutes.

3. Results

This study used young, old, and bent Ginger. Organic fruit tissue is separated using the maceration method. Methanol accounts for 80 per cent of the solvent used in this study. It is hoped that the polar, non-polar, and semi-polar compounds in the ginger samples will be perfectly extracted. Table 1 shows the consequences of ginger extraction.

Table 1. Extraction Results of Ginger (Zingiber officinale)

Sample	Method	Solvent	Sample Weight (gr)	Extraction Weight	Yield (%)
Ginger	Maceration	Methanol 80%	3.00 gr	30.00 gr	90

Table 1 displays the extraction of 3.00 grams of ground ginger fruit with 80% methanol solvent yielded 30.00 grams. The methanol extract has a clean yellow colour and a soaking value of 90%. The soaking value corresponds to the amount of secondary metabolites in the plant. According to Jones & Kinghorn (2005), the soaking value is proportional to the amount of secondary metabolite extracted during extraction.

3.1. FRAP

According to Benzie & Strain (1996), the FRAP method is one way to test antioxidants in plants. The FRAP technique has the advantage of being simple regarding strategy, reagent planning, and speed (Benzie & Strain, 1996). The FRAP method can determine a material's total antioxidant content based on the ability of antioxidant compounds to convert Fe_{3+} ions into Fe_{2}^{+} (Maryam et al., 2015). The result of liquefaction 20 times; the FRAB results were read using a spectrophotometer; however, the dark blue result was too high to be read by a spectrophotometer, so it will be liquefied again using serial delusion.

Table 2. Tabel FRAP mg FeSO4 Eq/100gr

Sample	FRAP mg FeSO4 Eq/100gr
Young Ginger (HM)	858.22 ± 6.58 [a]
Old Ginger (HT)	741.88 ± 29.92 [a]
Bentung Ginger (HB)	904.27 ± 52.92 [a]

Table 2 shows that Bentung Ginger has the highest antioxidant content (904.27 ± 52.95 a), followed by young Ginger (858.22 ± 6.58 a) and old Ginger (741.88 ± 29.92 a). This can be proven through FRAP tests. The ferric-reducing antioxidant power (FRAP) method is the only way to measure antioxidants directly in plants. It is inexpensive, quick, and uses simple reagents rather than specialized equipment to calculate total antioxidant levels (Mangkasa, 2018). The antioxidant activity in Ginger varies significantly depending on several factors that influence the content of active compounds in its rhizome. It causes the highest and lowest antioxidant activity in each type of Ginger (large white Ginger, small white Ginger, and red Ginger). Large white Ginger typically has a lower essential oil content than red Ginger and small white Ginger. These essential oils contain compounds like gingerol and shogaol, known as potent antioxidants. The lower concentration of the compounds in large white Ginger has lower antioxidant activity than the other types of Ginger. The extraction method can greatly influence the amount of active compounds extracted from Ginger. Extraction using organic solvents tends to produce extracts with higher antioxidant activity than water extraction because more phenolic compounds and key antioxidants dissolve in organic solvents.

3.2. Total Plate Count (TPC)

The Total Plate Count (TPC) method was used with 30 minutes of thawing followed by serial delusion. Absorbent solutions used in the TPC method include sodium carbonate, follin, and gallic acid. 300 mL ginger sample, 1500 mL follin solution, and 1,200 mL sodium carbonate solution.

Table 3. Total Plate Count (TPC)

Sample	TPC mg GA Eq/100g
Young Ginger (HM)	432.61 ± 53.83
Old Ginger (HT)	488.17 ± 39.41
Bentung Ginger (HB)	443.42 ± 22.41

Table 3 indicates that old Ginger is taller than young Ginger and bent Ginger. Old Ginger produces 488.17 ± 39.41, bent Ginger 443.42 ± 22.41, and young Ginger 432.61 ± 53.83.

3.3. DPPH

The antioxidant activation test was performed quantitatively using the DPPH (2,2-diphenyl-1-picrylhidrazyl) method. The wavelength measured to create a standard curve follows the "Lambert-beer" law, which states that the concentration and absorbance graph results form a straight line. The DPPH method is a simple, cost-effective measure of antioxidant activation (Prasetyo, 2020). The DPPH Antioxidant Activity Test in this study was performed on ginger extract samples containing 80% methanol. To achieve the best results, 1 ml of sample is added to an Eppendorf tube, followed by 2 ml of DPPH, which is then homogenized and stored in a dark place. The standards for using the DPPH method are as follows:

Table 4. DPPH % Inhibition

Sample	DPPH % Inhibition
Young Ginger (HM)	89.50 ± 0.31 [a]
Old Ginger (HT)	84.64 ± 0.28 [b]
Bentung Ginger (HB)	89.45 ± 0. 50 [a]

Table 4 shows that young Ginger has a higher antioxidant compound content, namely 89.50 ± 0.31 han other Ginger. Meanwhile, Bentung Ginger was 89.45 ± 0.50 and old Ginger had the lowest results, 89.45 ± 0.50.

3.4. Contains 6-Gingerol and Shogaol

Gingerol is a synthetic compound found in red Ginger that is widely used as a pain reliever. In general, gingerol extraction from ginger tubers uses a Soxhlet extraction strategy. However, this procedure has disadvantages, including a long procedure and unsatisfactory results.

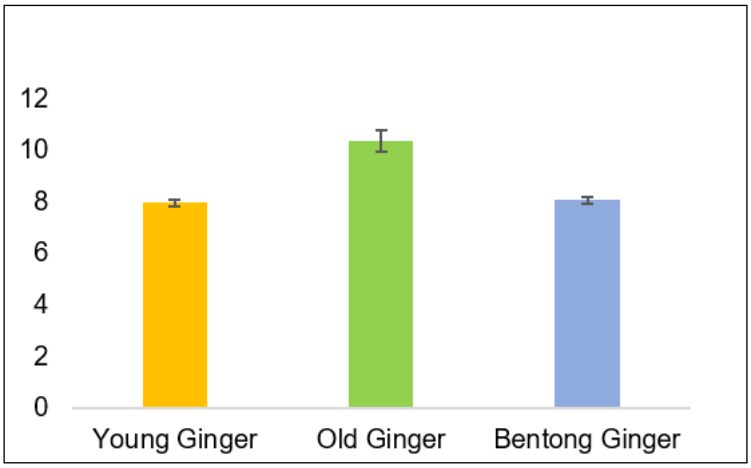

Figure 1. Bar Diagram of 6-Gingerol in Ginger

Figure 1 shows that old Ginger has the highest 6-Gingerol content. Old Ginger contains more gingerol than other types of Ginger. Ginger's distinct odour and aroma result from a combination of zingerin compounds. Shogaol and essential oils range from 1-3% in fresh Ginger. Zingeron is less spicy and tastes sweeter (Green & Hayes, 2004). Meanwhile, Ginger's spiciness comes from the presence of non-volatile phenylpropanoid compound derivatives like gingerol and shogaol. This phenolic component contributes to Ginger's spicy taste (Green & Hayes, 2004). According to Gao et al. (2024) and Vichakshana et al. (2022),

gingerol is very unstable in the presence of sunlight and, at high temperatures, changes to shogaol. Shogaol, a ginger constituent, shares a chemical structure with gingerol.

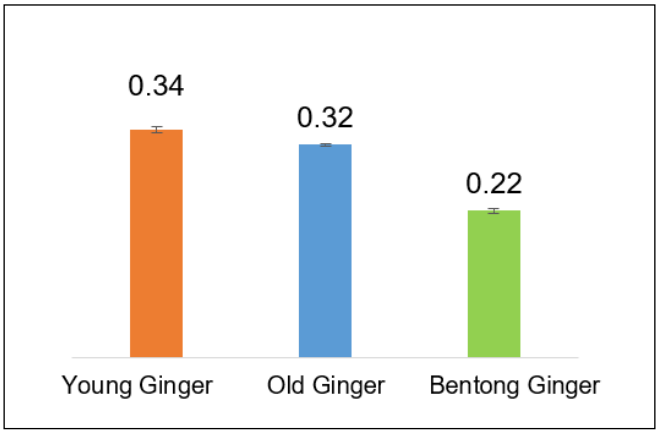

Figure 2. Shogaol Bar Diagram in Ginger

Figure 2 shows that young Ginger has the highest concentration of 6-shogaol. Young Ginger also has a higher gingerol content than Ginger. Gingerol and shogaol give Ginger its spicy taste, commonly used to relieve nausea.

Table 5. Characteristics of 6 Gingerol and Shogaol

Sample	6-Gingerol (mg/g dw)	Mean	Std. Dev	Shogaol (mg/g dw)	Mean	Std. Dev
1A	7.83			0.35		
1B	7.94			0.34		
1C	8.09	7.95	0.12	0.34	0.34	0.00
2A	10.26					
2B	10.81					
2C	10.03	10.37	0.40	0.32	0.32	0.00
3A	7.92			0.21		
3B	8.09			0.22		
3C	8.19	8.07	0.14	0.22	0.22	0.00

Table 5 captures the average results of the three specimens, showing that the 6-Gingerol yield for old Ginger is higher than for other Ginger, namely 10.37, 7.95, and 8.07. Meanwhile, young Ginger has a higher Shogaol yield than others, with 0.34, old ginger 0.32, and bentung ginger 0.22.

4. Conclusions

The compounds found in Ginger were discovered using the HPLC method. Also, bentong Ginger contains the most antioxidants of any ginger. The 6-gingerol content demonstrates the unique characteristics of bentong Ginger. Aside from that, 6-shogaol content is only found in dried ginger samples. The active compounds discovered varied over time, with the highest content found in the sixth and seventh months of ginger harvest for fresh and dried Bentong ginger samples.

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