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Original Research Article

Phytochemical Profiling and Antioxidant Activities of Red Ginger (*Zingiber officinale* var. rubrum) Cultivated Eco-Farming

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ABSTRACT

Red ginger (*Zingiber officinale* var. rubrum) is useful as an antioxidant, immunomodulator, antibacterial, and anti-inflammatory. Eco-enzymes can enhance ginger growth and increase phytochemical content and antioxidant activity. This study aimed to identify the composition of phytochemical compounds and antioxidant activity. The study was designed in a completely randomized design in 3 replications. This study consisted of 4 treatments: without eco-enzymes, 0.1% eco-enzymes, 0.3% eco-enzymes and 0.5% eco-enzymes. The phytochemical content of the ginger rhizome eight months after planting and its antioxidant activity were measured using various methods, such as the *1,1-diphenyl-2-picrylhydrazyl* (DPPH) method and the spectrophotometric method for measuring total flavonoid content (TFC) and total phenolic content (TPC). The study showed that ginger rhizome contained flavonoids, phenolic and terpenoid compounds in all treatments. Eco-enzymes significantly increased ginger rhizome's flavonoid and phenolic content ($p<0.05$). The results obtained are non-linear because the higher the concentration of eco-enzyme, both the flavonoid, phenolic, and antioxidant activity values are non-linear. The highest flavonoid content was obtained from ginger, with an eco-enzyme concentration of 0.3%, which was 0.11%, while the highest phenolic content was found at a concentration of 0.5%, 53.54%. In contrast, the percentage of inhibition in the DPPH test was 73.65%. Red ginger with 0.5% eco-enzyme has potent antioxidant activity with an IC_{50} value of 4.238 ppm. With the help of eco-enzymes, red ginger can be transformed into antioxidant-rich food products that are environmentally friendly.

Keywords: Antioxidant, Eco-enzyme, Natural products, Phytochemistry, *Zingiber officinale* var. rubrum.

Introduction

Ginger is a pure local (*Zingiber officinale*) active *phytocompound* successfully used for human health. This rhizome plant is a powerful antioxidant that either decreases or inhibits the formation of free radicals. Red ginger's biological actions may be attributed to these chemicals' synergistic or cumulative effects. Polyphenolic compounds in red ginger exhibit antioxidant properties and their ability to modulate various enzyme activities.[1] Many flavonoids are more potent antioxidants than vitamins C and E. [1] The total number of phenolic and flavonoids in red ginger is higher than in common ginger.[2] The ginger extract showed antioxidant effects in human chondrocyte cells, with oxidative stress mediated by interleukin-1β (IL-1β).[3] Several experimental studies on the therapeutic activity of ginger have pharmacological effects such as cold, indigestion, analgesic, osteoarthritis, anti-inflammatory, antidiabetic, primary dysmenorrheal, antioxidant, boost immunity, and as an antiviral in SARS-CoV-2.[4-10]

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The main active compounds responsible for pharmacodynamic actions are alkaloids, flavonoids, polyphenols, tannins, terpenoids, and glycosides. Fresh red ginger's most abundant phenolic compounds are gingerols, shogaols, and flavonoids. It also contains many terpene components, which are necessary components of essential oils.[11,12] Research by Purwaningsih *et al.* showed that feeding red ginger with an eco-enzyme (homemade by the researchers) once a week, for up to 200 mL for four months, can increase very high levels of flavonoids (0.12%) at an eco-enzyme concentration of 0.75%.[13]

Organic cultivation as a raw material for herbal medicines can be carried out using organic fertilizers that are free of contaminants; one of the organic fertilizers is eco-enzymes, which can meet nutrient needs and prevent pest attacks. Eco-enzymes are environmentally friendly products that are fermented from fruit or vegetable residues. The production of eco-enzymes is one way to process waste into valuable products.[14]

The average harvest time for red ginger is 7-8 months for optimal secondary metabolite content. The optimal harvest age of ginger dramatically determines ginger seed rhizomes' quality and shelf life. Ginger rhizome content consists of two components: volatile and non-volatile, which begin to form when the ginger is 4-8 months old.[15]

The purpose of adding eco-enzymes is to improve the quality of the active substance content of red ginger so that it meets the qualifications of raw material for traditional medicine. In addition, this research will also produce an organic cultivation technology of red ginger that can become a standard for the organic cultivation of red ginger so that red ginger farmers can apply it. This study aims to determine the composition of phytochemical compounds and antioxidant activity.

Materials and Methods

Materials and Chemicals reagents

The ingredients used were ginger rhizome powder, aquadest, eco-enzyme, Folin-Ciocalteu's (FC) phenol reagent obtained from Merck (Darmstadt, Germany), $AlCl_3$, gallic acid, and 1,1-diphenyl-2-picrylhydrazyl (DPPH) were purchased from Sigma (Steinheim, Germany), Electrical food blender (Panasonic MX-V310KSR, Japan), and ultraviolet-visible spectrophotometer (UV-Vis) (Thermo Genesys model 10 S UV–VIS double-beam spectrophotometer-Thermo Fisher, Netherlands).

The protocol for preparing plant materials for red ginger rhizome was determined using the modified method of Okti *et al*.[16] The plant materials, Rhizome *Zingiber officinale* var. rubrum, were collected from a village in Samigaluh, Kulonprogo, Yogyakarta, Indonesia on December 11, 2021. Geographically, it is located between 7°38'42" - 7°59'3" South latitude and 110° 1'37" - 110°16'26" East longitude.[17] The rhizomes were washed with pure water and soaked in an eco-enzyme solution for 60 minutes. Following this, they were planted in 15 x 15 cm growing pots filled with peat moss, weighing around 1 kg, and left to germinate for two weeks. During this time, they were kept under glasshouse conditions with the buds facing upwards. After two weeks, the seedlings were transplanted into 35 x 30 cm polybags filled with a soil-less mixture of burnt rice husk and coco peat in a 1:1 ratio. Each polybag weighed approximately 5 kg. Once a week, 200 mL of eco-enzyme was applied to each polybag at the appropriate concentration. Harvesting occurred eight months after the plantation, and the rhizome, leaf, and stem were separated and washed with pure water. Observations were made on the phytochemical content of the rhizome, namely flavonoids, phenolic, and terpenoids.

Eco-enzyme preparation

The organic materials used to manufacture eco-enzymes are sweet orange peel, pear, dragon fruit, watermelon, apples, papaya, lemongrass, carrots, spinach, kale, cucumber, tobacco leaves, moringa leaves, neem leaves, and molasses. All the fresh organic materials are mixed. These ingredients are mixed with molasses and water using a ratio of 1:3:10 (1 part molasses, three parts the remaining vegetables and fruit, and 10 water). After that, put it in an airtight plastic container and tightly close it. The maximum volume of water is 60% of the volume of the container. Fermentation is carried out for at least four months. The characteristics of a good eco-enzyme are pH <4 with a fresh sour aroma typical of fermentation. [16] The eco-enzyme concentrations applied include without eco-enzyme (control), 0.1% eco-enzyme, 0.3% eco-enzyme, and 0.5% eco-enzyme. Eco-enzyme was applied to ginger plants at intervals of 1 week, as much as 200 mL/plant.

Red ginger preparation

Fresh red ginger rhizomes harvested in UPY's Agroshop garden were eight months old and then cleaned of roots and dirt using running pure water (Figure 1). After that, it was cut into thin strips with a thickness of 3 mm transversely and longitudinally. Red ginger is dried in an oven at 90-100^{0}C for three days. Drying was carried out until the water content of red ginger was <10%—dried red ginger mashed with a blender.

A total of 100 g of the dry powder from the rhizome was soaked in 500 mL of absolute ethanol in Becher for 72 h at room temperature for 72 hours. After 72 hours, the whole mixture was filtered to remove residue. Extracts were evaporated under vacuum to dryness and stored in dried bottles at 4°C.[18]

Total Flavonoid Content

One millilitre of the Simplicia sample was dissolved in 10 mL water and filtered. The filtrate is put into a test tube. Add with 1% $AlCl_3$ reagent and shake rigorously. If the mixture appears intensely yellow, it suggests the presence of flavonoid compounds. The extract samples were assessed at a concentration of 0.1 mg/mL. A calibration curve was established using quercetin as the standard at 5-100 mg/L. The total flavonoid content was then expressed in terms of quercetin (mg/g).

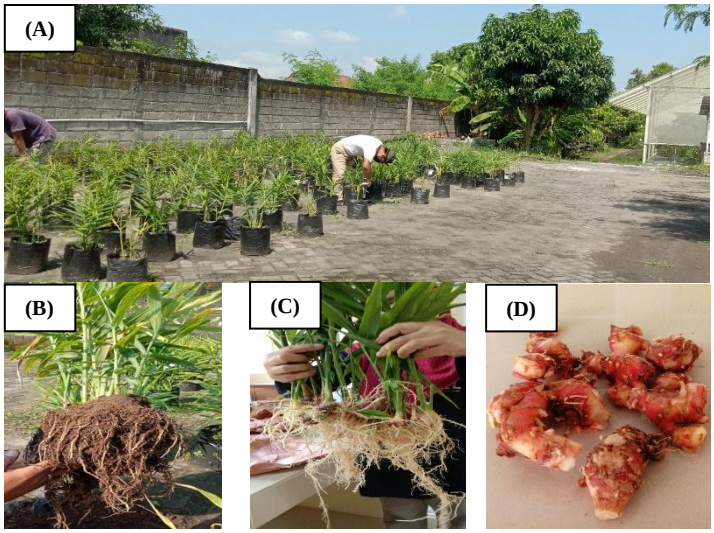

Figure 1: (A) Cultivation and care of red ginger plants with eco-enzymes, (B) harvesting red ginger (4 months old), (C) washing process, and (D) young red ginger rhizomes.

Total Phenolic Content (TPC)

Total phenolic content was determined by the Folin–Ciocalteu method with some modification by Ali *et al*.[19] The extract (1 mg/mL) was mixed with 5 mL Folin–Ciocalteu reagent diluted with water 1:10 v/v and 4 mL of 7.5% sodium carbonate. Absorbance was then measured at 765 nm using a Thermo Genesys model 10 S UV-Vis double beam spectrophotometer. A calibration curve was prepared using gallic acid (25–250 mg/L) as standard and used to calculate the total phenolic compound. The total phenolic contents were expressed as gallic acid equivalents (mg/g) using the following equation based on the calibration curve: y = 0.00934x - 0.00621.

Evaluation of Antioxidant Activity (1.1-Diphenyl-2-picrylhydrazyl (DPPH) Assay

A freshly prepared 100 µM methanolic solution of DPPH was mixed with 5 mL of ginger extract. The solutions were incubated at 25^{0}C for 30 minutes in the dark, and the absorbance was read at 517 nm against a blank (methanol and DPPH solution without sample). Gallic acid was used as a standard control, and samples were assayed three times.[20] The percentage of inhibition was calculated using a specific formula:

% inhibition = [(A-B) /A)] x 100%

The results were expressed as mean ± SD, and the IC_{50} values were obtained from the linear regression plots.

Results and Discussion

Analysis of the phytochemical content of red ginger rhizome aged eight months after planting

In this study, ginger plants were dismantled eight months after planting (Voucher number: 13/07/2022.), and the ginger rhizome was analyzed for its phytochemical content. The results of the analysis of the phytochemical content qualitatively can be seen in Table 1. The analysis of the phytochemical content of ginger rhizome aged eight months showed the presence of terpenoid, phenolic, and flavonoid compounds. The gingerol and shogaol compounds found in ginger rhizome are polyphenolic compounds, so positive reactions to flavonoids, phenolics, and terpenoids indicate the results of the phytochemical test.

Gingerol and shogaol compounds are the main components that form the spicy taste of red ginger. These three compounds are also natural antioxidants that work by reducing free radicals depending on the number of hydroxy groups in their molecular structure. Phenolic compounds can benefit health by reducing the risk of metabolic

disorders, such as type 2 DM.[21–23] Several previous studies reported that red ginger has flavonoid and terpenoid compounds with antioxidant activity due to their capacity to donate either electrons or hydrogens to free radicals and effectively suppress NO production in LPS-stimulated cells. This is achieved by inhibiting iNOS J774.1 has reported on this.[24,25,26] In this study, we prefer terpenoids over alkaloids because the direction of this research was prepared for the use of ginger in the cosmetic, anti-inflammatory, and antioxidant fields. Red ginger has a high content of essential oils (EOs) in the terpenoid group, namely monoterpenes (81.9%) and sesquiterpenes (16.86%).[24] Terpenoids are a class of phytochemicals that are incredibly diverse and have a variety of functions. They can act as pigments for absorbing light, hormones, phytoalexins, and semi-chemicals. Many terpenoids are shown to have anti-diabetic, antimicrobial and anti-inflammatory properties.[27] They also have limited antioxidant activities.[28] Previous studies by Mot *et al*. revealed that sage essential oil was found to have limited antioxidant capacity (33.61% and 84.50% inhibition as determined by DPPH and ABTS assays, respectively). However, when inhaled by hospitalized patients, a sage essential oil with a high borneol content improved satisfaction due to its sedative and hypnotic effects. [28]

In the qualitative test, all treatments fertilized with eco-enzymes and the control (not fertilized) had the same type of phytochemical compounds. Flavonoid and phenolic compounds also function as antioxidants, anti-inflammatories, and antibacterials, while terpenoids have functioned as antiseptics and antimicrobial effects. According to research by Wang *et al.*, triterpenoids, specifically meroterpenoids, have potent antioxidant properties.[29] Additionally, Cai *et al.* discovered that Sanghuangporus sanghuang triterpenoids possess antioxidant activity due to their ability to eliminate hydroxyl radicals, superoxide anions, DPPH free radicals, and ABTS (2,2′-azino-bis) free radicals. Their findings indicate that the clearance of free radicals increased from 10% to 90% when the concentration of Sanghuangporus sanghuang triterpenoids ranged from 18.75–350 µg/mL. [30]

There was a significant difference between the treatments in the quantitative tests conducted on the content of flavonoids and phenolic compounds. The results of statistical testing can be seen in Tables 2 and 3.

Table 2 shows that the ginger plants fertilized with eco-enzymes at a concentration of 0.3% had the most flavonoids and were significantly different at concentrations of 0.1%, 0.5%, and without eco-enzymes. In our research results (Tables 2 and 3), the total content is not linear; this might be due to inappropriate sample preparation techniques, extraction methods, drying, harvesting time, and small sample size.

Table 1: Phytochemical content of ginger rhizome at various concentrations of eco-enzymes.

Eco-enzyme concentration	Flavonoids	Phenolic	Terpenoid
0 (control)	+	+	+
0.1% (eco-enzyme)	+	+	+
0.3% (eco-enzyme)	+	+	+
0.5% (eco-enzyme)	+	+	+

Description: (+): detected, (-): not detected

Table 2: Average of ginger rhizome flavonoid content at various concentrations of eco-enzymes

Eco-enzyme concentration	Mean (%)	Std. deviation	*p-value*
0 (control)	0.065	0.004	
0.1% (eco-enzyme)	0.042	0.004	<0.001
0.3% (eco-enzyme)	0.110	0.005	
0.5% (eco-enzyme)	0.086	0.007	

The increase in ginger rhizome flavonoid content due to the administration of eco-enzymes will improve the quality of ginger rhizome. As a medicinal plant, ginger has health benefits because it contains many secondary metabolites in its rhizomes. Secondary metabolites in plants are generally flavonoids, alkaloids, steroids, saponins, terpenoids, and tannins. Ginger has also been pharmacologically beneficial as an antiseptic, antioxidant, anti-inflammatory, anticoagulant, anticancer, and antibiotic.[31]

The use of fruits and vegetables in the manufacture of eco-enzymes in this study is based on research conducted by Okti *et al*. that eco-enzymes with these ingredients have good content for plants such as N, P, K, Fe, etc.[16] In line with this research, Fadlilla *et al*. reported that eco-enzymes contain organic N, P, K, and C compounds that stimulate plant growth.[32] The increased flavonoid content in ginger rhizome indicates the increased content of gingerol and shogaol in ginger. The quality of ginger rhizome can be seen from the content of gingerol and shogaol in the rhizome. The results of this study indicate that the application of eco-enzymes can improve the quality of ginger rhizomes, as indicated by the increased content of flavonoids in the rhizome.[33]

On the other hand, when flavonoids interact with metal ions, such as iron, they form stable and strong bonds that can enhance their antioxidant effects.[34,35,36] Recent evidence shows that iron may affect the activity of flavonoids by regulating their expression and activity and regulation.

Bayele *et al.* reported that quercetin[24] increased hepcidin expression (the primary iron regulatory hormone), thus possibly involving the Nrf2 antioxidant pathway.[37] Quercetin was used to compare DPPH free radical scavenging activity against red ginger extract. Quercetin is a flavonoid aglycone obtained from routine hydrolysis, and it is known that quercetin has high antioxidant activity. Quercetin can activate the Nrf2 pathway by translocation into the nucleus and activity in the transcription process. In our previous research, Purwaningsih *et al.* found that eco-enzymes had high Fe content. Hence, the quantitative test results obtained relatively high levels of flavonoids, up to (0.12%)[13], in contrast to the research conducted by Herawati and Saptarini, who received flavonoid levels of red ginger rhizome of 0.0068%.[38]

The antioxidant properties of plants can be derived from phenolic compounds, which are plant secondary metabolites. Phenolic compounds have a polyphenolic structure in which several hydroxyl groups (–OH) are bonded to two or more benzene rings. Phenolic compounds can delay, inhibit or prevent lipid peroxidation, thereby reducing or stabilizing the number of degradation products as precursors of oxidative stress. Biological activity often occurs together with antioxidant properties due to the polyphenolic compounds of the plant itself. [36,39,40] The highest phenolic content in this study was obtained at 53.15% at a 0.5% eco-enzyme concentration of ginger. In several studies, the total phenolic content was 434.7 and 698.1 mg/100 g dry weight (DW) in Korean and Ethiopian samples [41], 37% [33], 21.90 mg GAE/g[42] and 10-13%.[2] This could be due to structural damage to phenol due to the high extraction temperature. Phenolics are thermosensitive compounds that allow hydrolysis and reduction of phenol levels at high temperatures.[43]

Analysis of the antioxidant activity of red ginger rhizome aged eight months after planting

The results of previous studies indicate that the antioxidant activity of herbs/plants is closely related to the phytochemical content.[6,44] In this study, the antioxidant activity obtained was relatively high at 73.65%, presumably due to the high content of flavonoids and phenolics. This result is higher than the study by Ghasemzadeh *et al.* found the antioxidant activity of freeze-dried and fresh red ginger in the DPPH test at 48.3% and 22.5%, respectively.[31] Mao *et al.* also conducted a test and found that dried ginger exhibited the most potent antioxidant activity due to its higher phenolic compound content compared to fresh, stir-fried, and carbonized ginger.[45]

Phenolic compounds can affect transcription factors such as *nuclear factor kappa* B (NF-κB) or *nuclear factor erythroid-related factor-2* (Nrf-2),[46] which can increase or decrease the elements of these factors through the antioxidant pathway. Phenolic compounds can also inhibit

enzymes involved in human disease development and have been used to treat various human ailments, including hypertension, metabolic disorders, infections, inflammation, and neurodegenerative diseases. For example, phenolic compounds' inhibition of angiotensin-converting enzyme (ACE) has been used to treat hypertension.[47]

The biological properties of phenolic compounds are diverse, and their specific mechanisms of disease-preventive effects are unknown. However, their antioxidant effect plays a significant role in reducing Reactive Oxygen Species (ROS) levels. ROS and Reactive Nitrogen Species (RNS) can cause damage to critical biological macromolecules such as proteins and DNA.[40] Phenolic compounds have been shown to have anti-inflammatory, anti-ageing, antiproliferative, and antioxidant effects in several studies. Antioxidant enzymes play a crucial role in preventing oxidative damage at the molecular level. As ROS and RNS are continuously produced under normal cell conditions, disruption of antioxidant activity can lead to cell damage, ageing, disease, and cell death.[40,39]

Mustafa and Chin reported that the ethanol extract of ginger showed higher antioxidant activity than the ethanol-water extract, resulting in a DPPH antioxidant activity of 95%.[48] The same thing was reported by Stoilova *et al.,* who also researched the antioxidant activity of the alcoholic extract of Vietnamese ginger and found that the DPPH radical inhibition reached up to 90.1%. Thus, a perfect correlation exists between total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activity in ginger. [49] Sample preparation techniques, extraction methods, drying, harvesting time, and instrumentation methods may cause a contradiction with our research. Nevertheless, there is an exciting thing in our research: the higher the concentration of eco-enzymes given to the red ginger plant, the stronger the antioxidant activity is, namely the IC_{50} of 4,238 ppm. According to Putri and Hidajati, the level of antioxidant strength is divided into four levels, namely extreme (IC50 < 50 ppm), strong (IC_{50} : 50-100 ppm), medium (IC_{50} :100-250 ppm, and weak (IC_{50} : 250-500 ppm). [50] In Ali *et al*. study, red ginger containing 6-gingerol and 6-shogaol showed high antioxidant activity in both tests with IC_{50} 4.85 + 0.58DPPH and 5.35 ± 0.33ABTS µg/mL for the first and IC_{50} 7.61±0.81DPPH and IC_{50} 7.05±0.23ABTS µg/mL. [34] In contrast to research by Widyapuspa *et al*., who reported that red ginger ethyl acetate extract had an IC_{50} value of 51.36 ± 0.05. [51]

Red ginger's antioxidant activity is linked to its total flavonoid and phenolic content. This activity is attributed to the ability of these compounds to donate electrons or hydrogen to free radicals. Flavonoids and phenolic compounds are plant secondary metabolites that have aromatic rings with at least one hydroxyl group, which directly impacts their antioxidant activity.[2]

Table 3: Average of ginger rhizome Phenolic content at various concentrations of eco-enzymes

Eco-enzyme concentration	Mean (%)	Std. deviation	*p-value*
0 (control)	39.74	0.012	
0.1% (eco-enzyme)	22.70	0.055	<0.001
0.3% (eco-enzyme)	22.30	0.013	
0.5% (eco-enzyme)	53.15	0.116	

Table 4: IC_{50} of ginger rhizome antioxidant activity test at various concentrations of eco-enzymes

Eco-enzyme concentration	IC_{50} (ppm)
0 (control)	11.633 ± 0.064
0.1% (eco-enzyme)	6.088 ± 0.002
0.3% (eco-enzyme)	4.924 ± 0.012
0.5% (eco-enzyme)	4.238 ± 0.011

Conclusion

The phytochemical content detected in red ginger rhizome-given eco-enzymes is flavonoid, phenolic, and terpenoid compounds. Eco-enzyme concentration of 0.3% increased the highest level of flavonoids by 0.11%, and Eco-enzyme concentration of 0.5% increased the level of phenolics with the highest level of 53.54%. At the same time, the antioxidant activity in the DPPH test was 73.65% and had a potent antioxidant activity with an IC_{50} value of 4.238 ppm. Therefore, giving eco-enzymes to ginger plants can improve the quality of the phytochemical content of ginger rhizomes.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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