Impact of Eco-Enzyme Fertilizer on Phytochemical Content and Antioxidant Activity of Turmeric Rhizomes at Early Growth Stage

Rahmat A Hi Wahid[1*], Okti Purwaningsih[2]

Abstract

Background: Turmeric (Curcuma longa L.) is widely recognized for its bioactive compounds, including phenolics, flavonoids, and curcumin, which exhibit significant antioxidant, anti-inflammatory, and antimicrobial properties. These phytochemicals are secondary metabolites that contribute to turmeric's therapeutic potential. However, the influence of plant age and organic fertilization, such as eco-enzyme, on turmeric's phytochemical content is not fully understood. This study evaluates the phytochemical profile of turmeric rhizomes harvested four months after planting and assesses the impact of eco-enzyme fertilization on secondary metabolite production. Methods: Turmeric rhizomes were collected from plants treated with different concentrations of eco-enzyme fertilizer (0.5%, 1%, and 1.5%) and a control group (no fertilizer). Qualitative and quantitative analyses were performed to determine the presence and concentration of phenolic compounds, flavonoids, and curcumin. Antioxidant activity was assessed using the DPPH assay. Data were statistically analyzed to compare phytochemical content across treatments. Results: Qualitative analysis revealed the presence of phenolics, flavonoids, and curcumin in all treatments. Quantitative results showed that plants treated with 0.5% eco-enzyme had significantly higher flavonoid content (0.12%) and phenolic content (4.911 mg GAE/g extract) compared to other treatments. Curcumin levels were low across all samples, likely due to the young age of the rhizomes. Antioxidant activity, measured via the DPPH assay, was weak, with IC50 values exceeding 200 ppm, consistent with previous findings on immature turmeric rhizomes. The observed differences in phytochemical content may be attributed to eco-enzyme's enzymatic activity, which enhances secondary metabolism by breaking down complex nutrients into simpler forms. Conclusion: The application of eco-enzyme fertilizer, particularly at a concentration of 0.5%, enhances the phytochemical content of young turmeric rhizomes, notably flavonoids and phenolic compounds. However, the relatively low levels of curcumin and weak antioxidant activity suggest that rhizomes harvested at four months may not have fully developed their secondary metabolites. Further research is needed to investigate phytochemical profiles at later growth stages and optimize eco-enzyme application to maximize bioactive compound production. These findings highlight eco-enzyme's potential as an organic fertilizer for improving the quality of medicinal plants.

Keywords: Turmeric rhizomes, Phytochemical content, Eco-enzyme fertilizer, Antioxidant activity, Secondary metabolites

Significance | Eco-enzyme fertilizer might enhance secondary metabolites in young turmeric rhizomes, offering insights into sustainable agriculture and medicinal compound optimization.

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1. Introduction

Turmeric (*Curcuma longa* L.) is a widely cultivated medicinal plant

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known for its diverse therapeutic applications and active phytochemicals. Originating from South and Southeast Asia, it has been extensively domesticated in regions such as Indonesia, where it is integral to traditional medicine. For centuries, turmeric has been employed in Indian and Chinese systems of medicine to treat jaundice, liver diseases, and various inflammatory conditions (Aggarwal, 2011).

The phytochemical composition of *C. longa* is dominated by polyphenols, particularly curcuminoids, which are renowned for their potent pharmacological properties. Among these, curcumin is the most studied due to its anti-inflammatory, antioxidant, and antimicrobial activities. Numerous studies have highlighted curcumin's broad-spectrum effects, including its hepatoprotective (Lee et al., 2017), antifungal, antibacterial (Khan et al., 2012), antihypertensive, and neuroprotective capabilities (Xu et al., 2019). These properties have cemented turmeric's status as a functional food with significant therapeutic potential (Amalraj et al., 2017).

Despite its medicinal promise, the cultivation of turmeric often relies on chemical fertilizers and pesticides, which pose risks to human health and the environment (Ak & Gülçin, 2008). To address these challenges, sustainable agricultural practices emphasizing the use of organic inputs, such as eco-enzyme, are gaining traction. Eco-enzyme, derived from the fermentation of fruit and vegetable waste, has shown potential in enhancing the growth and phytochemical content of medicinal plants. Previous research demonstrated its efficacy in increasing flavonoid and phenolic levels in ginger, with notable antioxidant activity (Wahid et al., 2023; Purwaningsih et al., 2023).

This study explores the application of eco-enzyme in turmeric cultivation to develop an environmentally friendly approach for producing high-quality rhizomes. Utilizing locally sourced raw materials, eco-enzyme enhances nutrient availability and reduces the dependency on synthetic inputs. The objectives of this study were to extract and analyze the phytochemical profile of *C. longa* rhizomes cultivated at Universitas PGRI Yogyakarta (UPY), with a specific focus on curcumin content determined via spectrophotometry. Additionally, antioxidant activity was evaluated using the Folin-Ciocalteu (FC) and DPPH assays. To the best of our knowledge, this represents the first comprehensive analysis of turmeric cultivated with eco-enzyme in Indonesia, potentially establishing a sustainable model for turmeric production that aligns with global demands for green agricultural practices.

2. Materials and Methods

2.1 Chemicals and Reagents

The chemical reagents used in this study included curcuma extract, distilled water (aquadest), and eco-enzyme. Additional reagents, such as Folin-Ciocalteu (FC) phenol reagent (Merck, Darmstadt, Germany), aluminum chloride ($AlCl_3$), gallic acid, and 1,1-diphenyl-2-picrylhydrazyl (DPPH), were sourced from Sigma-Aldrich (Steinheim, Germany). All chemicals were of analytical grade and used without further purification.

2.2 Plant Materials

Turmeric (*Curcuma longa* L.) rhizomes were obtained from local farms in Kulonprogo, Yogyakarta, Indonesia. The rhizomes were carefully washed with pure water to remove dirt and soaked in a 1% eco-enzyme solution for 60 minutes to prepare them for germination. The germination process was conducted in 15 × 15 cm pots containing 1 kg of peat moss as the growth medium. Rhizomes were planted 7 cm deep with the buds oriented upward and incubated under controlled glasshouse conditions.

After two weeks, when seedlings developed young leaves approximately 5 cm in height, they were transplanted into 35 × 30 cm polybags filled with a soil-less medium comprising burnt rice husk and coco peat in a 1:1 ratio (5 kg per polybag). This cultivation process was carried out at the Agroshop garden of Universitas PGRI Yogyakarta (UPY) from December 2023 to July 2024. Eco-enzyme was applied weekly at a dose of 200 mL per polybag. Rhizomes were harvested eight months after planting. Post-harvest, the rhizomes, leaves, and stems were separated, washed with pure water, and analyzed for phytochemical content, including flavonoids, phenolics, and curcumin.

2.3 Eco-Enzyme Preparation

Eco-enzyme was prepared using a combination of organic fruit and vegetable waste, including sweet orange peel, pear, dragon fruit, watermelon, apples, papaya, lemongrass, carrots, spinach, kale, cucumber, tobacco leaves, moringa leaves, and neem leaves. These materials were mixed with molasses and water in a 1:3:10 ratio (1 part molasses, three parts organic waste, and ten parts water). The mixture was placed in airtight plastic containers, ensuring that the liquid occupied no more than 60% of the container's volume. The fermentation process was carried out for at least four months. A high-quality eco-enzyme was characterized by a pH of <4 and a sour, fermentation-specific aroma. Different concentrations of eco-enzyme (0%, 0.5%, 1%, and 1.5%) were used in weekly applications of 200 mL per plant.

2.4 Turmeric Rhizome Preparation

Fresh turmeric rhizomes, eight months post-plantation, were cleaned thoroughly under running water to remove roots and dirt. The cleaned rhizomes were cut into thin strips (3 mm thickness) in both transverse and longitudinal sections. These strips were dried in an oven at 90–100°C for three days, ensuring a final water content of less than 10%. The dried turmeric was ground into powder using a blender, producing the turmeric extract used in subsequent analyses.

2.5 Determination of Total Flavonoid Content

To determine flavonoid content, 1 mL of turmeric extract was dissolved in 10 mL of distilled water and filtered. The filtrate was transferred to a test tube, and 1% $AlCl_3$ reagent was added. A yellow coloration indicated the presence of flavonoids.

2.6 Determination of Total Phenolic Content (TPC)

The total phenolic content was measured spectrophotometrically using the Folin-Ciocalteu reagent. A standard curve was prepared using gallic acid solutions at concentrations of 10, 20, 40, 60, 80, and 100 ppm. The same procedure was applied to the turmeric samples, and measurements were performed in triplicate to ensure accuracy.

2.7 Curcumin Quantification

Curcumin content was quantified using a spectrophotometric method. A standard curve was created by dissolving 1 mg of curcumin in 95% ethanol at concentrations of 0, 2, 4, 6, 8, 10, and 12 ppm. The absorbance of these solutions was measured at a wavelength of 420 nm. Turmeric extract (1 mg dissolved in 95% ethanol) was similarly analyzed, and the curcumin content was determined by comparing the sample absorbance to the standard curve.

2.8 Evaluation of Antioxidant Activity

The antioxidant activity of turmeric extract was evaluated using the DPPH assay (Yan-Hwa et al., 2000). A 100 μM methanolic solution of DPPH was freshly prepared and protected from light. Turmeric extract (5 mL) was mixed with 3 mL of the DPPH solution, and the mixture was incubated at room temperature in the dark for 30 minutes. Absorbance was measured at 517 nm, with gallic acid serving as the standard. All experiments were conducted in triplicate.

The percentage of radical scavenging activity (RSA) was calculated using the formula:

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

where A is absorbance of control; B is absorbance of sample.

3. Results and Discussion

3.1 Phytochemical Content of Turmeric Rhizomes at Four Months of Growth

This study analyzed the phytochemical content of turmeric rhizomes harvested four months after planting. The qualitative analysis revealed the presence of phenolic compounds, flavonoids, and curcumin, consistent across all treatments, including plants fertilized with eco-enzyme and the control group. The results of the phytochemical analysis are summarized in Table 1.

Phenolic compounds are a major group of secondary metabolites in plants, known for their antioxidant, anti-inflammatory, and antimicrobial properties. These compounds can influence transcription factors, such as nuclear factor-κB (NF-κB) and nuclear factor-erythroid 2-related factor 2 (Nrf2), modulating their activity through antioxidant pathways (Rahmat et al., 2020). By inhibiting enzymes associated with human diseases, phenolic compounds have shown potential in managing conditions such as hypertension, metabolic disorders, infections, inflammation, and neurodegenerative diseases. For example, the inhibition of angiotensin-converting enzyme (ACE) by phenolic compounds has proven effective in treating hypertension (Ghayur et al., 2005).

Phenolic compounds also play a critical role in suppressing reactive oxygen species (ROS) and reactive nitrogen species (RNS), which are known to cause oxidative stress and damage to macromolecules like DNA and proteins. Disruption of antioxidant balance can lead to aging, disease progression, and cell death (Kruk et al., 2022). The biological activities of phenolics, including their antioxidant and anti-inflammatory effects, underscore their therapeutic potential.

3.2 Flavonoids and Their Antioxidant Activity

Flavonoids, another group of secondary metabolites detected in this study, serve as potent antioxidants. They scavenge free radicals, protecting cells from oxidative damage, and have been reported to exhibit antibacterial and anti-inflammatory properties. Flavonoids interact with metal ions, such as iron, to form stable complexes, enhancing their antioxidant efficacy (Braakhuis, 2019; Lesjak & Srai, 2019).

The application of eco-enzyme fertilizer influenced flavonoid content in turmeric rhizomes. Plants treated with 0.5% eco-enzyme had significantly higher flavonoid levels than other treatments, as shown in Table 1. This increase may be attributed to the enzymes (amylase, lipase, and protease) present in eco-enzyme, which enhance plant metabolism and stimulate secondary metabolite production. Flavonoids have been linked to improved health outcomes, acting as anticoagulants, anticancer agents, and antioxidants (Ghasemzadeh et al., 2016).

Eco-enzyme also contains iron, which may regulate flavonoid activity via the Nrf2 pathway. Bayele et al. (2015) reported that quercetin, a type of flavonoid, activates the Nrf2 pathway, enhancing the expression of antioxidant enzymes. This finding aligns with Purwaningsih et al. (2021), who demonstrated that eco-enzyme's high iron content correlates with increased flavonoid levels in plants.

3.3 Phenolic Content and Its Implications

The phenolic content of turmeric rhizomes in this study was relatively low, with the highest concentration observed at 4.911 mg GAE/g extract in the 0.5% eco-enzyme treatment. This is consistent with findings that young rhizomes may not have fully developed their secondary metabolites. Phenolic compounds are highly sensitive to environmental factors, including extraction conditions. Studies by Antony and Farid (2022) indicate that high extraction temperatures and water presence can degrade phenolic structures, reducing their concentration.

In comparison, higher phenolic concentrations have been reported in other studies. Yusuf (2022) documented a phenolic content of 87.24 ± 2.75 mg GAE/g extract, while Marliani et al. (2017) reported

Figure 1. A) Cultivation and care of curcuma longa plants with eco-*enzyme*es, B) Harvesting curcuma longa (4 months old), C) Washing process, and D) Young curcuma longa.

Table 1. Phytochemical screening of *Curcuma longa* L. Rhizoma at various concentrations of eco-enzyme

Treatment	**Secondary Metabolite Test**			***p-value***
	Phenolic (mg GAE /gr extract)	**Flavonoid (mg GAE /gr extract)**	**Curcumin (ppm)**	
0 (control)	3.316 ± 0.004	4.236 ± 0.008	2.312 ± 0.009	<0.001
0,5% (eco-enzyme)	4.911 ± 0.004	8.126 ± 0.004	4.524 ± 0.002	<0.001
1% (eco-enzyme)	3.451 ± 0.001	7.449 ± 0.003	4.135 ± 0.008	<0.001
1,5% (eco-enzyme)	2.594 ± 0.005	5.585 ± 0.002	3.005 ± 0.001	<0.001

84.15 ± 4.142 mg GAE/g extract. These discrepancies highlight the impact of extraction methods and environmental conditions on phenolic stability and recovery.

Phenolic compounds contribute significantly to the antioxidant activity of turmeric. Their ability to delay lipid peroxidation and stabilize degradation products underscores their role in mitigating oxidative stress and promoting cellular health (Panche et al., 2016; Rudrapal et al., 2022).

3.4 Curcumin Content and Antioxidant Properties

Curcumin, a bioactive compound in turmeric, is renowned for its antioxidant and anti-inflammatory properties. It neutralizes free radicals, thereby preventing oxidative damage. The curcumin content in this study was relatively low, potentially due to the young age of the rhizomes. Research by Malahayati et al. (2021) suggests that curcuminoid levels are higher in mature rhizomes and vary depending on solvent extraction methods. Ethyl acetate, for example, yields higher curcumin concentrations compared to ethanol or hexane.

3.5 Antioxidant Activity (DPPH Assay)

The antioxidant activity of turmeric rhizomes was evaluated using the DPPH assay, which measures free radical scavenging ability. The IC50 values in this study indicated weak antioxidant activity, with results exceeding 200 ppm. These findings are consistent with research by Kusumaningrum et al. (2022) and Iqbalunnajih et al. (2023), who reported similar IC50 values.

Several factors may influence antioxidant activity, including extraction methods, solvent type, temperature, and pressure during processing. Semuel et al. (2019) noted that heating and drying processes might degrade active compounds, thereby reducing antioxidant potential.

3.6 Impact of Eco-Enzyme on Secondary Metabolite Production

The application of eco-enzyme fertilizer enhanced the production of secondary metabolites, particularly flavonoids. Eco-enzyme's enzymatic activity facilitates the breakdown of complex molecules into simpler forms, which plants utilize to synthesize secondary metabolites. This process underscores the importance of optimizing eco-enzyme concentrations to maximize phytochemical production.

3.7 Comparison with Other Studies

While this study observed moderate levels of phytochemicals, higher concentrations have been reported in mature turmeric rhizomes. For instance, Malahayati et al. (2021) documented total phenolic content of 193.26 mg GAE/kg and curcuminoid levels of 8.13 mg/L in turmeric extracted with ethyl acetate. These variations highlight the influence of plant age, extraction methods, and environmental conditions on phytochemical profiles.

3.8 Therapeutic Potential of Phytochemicals

The therapeutic properties of turmeric's phytochemicals extend beyond their antioxidant effects. Phenolic compounds have been shown to inhibit enzymes involved in disease progression, offering potential treatments for conditions like hypertension and inflammation (Ghayur et al., 2005). Similarly, flavonoids contribute to cardiovascular health by scavenging free radicals and enhancing antioxidant enzyme activity (Braakhuis, 2019).

Curcumin, in particular, has garnered attention for its role in preventing metabolic and cardiovascular disorders. Its ability to modulate transcription factors, such as NF-κB and Nrf2, underscores its potential as a therapeutic agent (Araya-Sibaja et al., 2021; Kotha & Luthria, 2019).

3.9 Limitations and Future Directions

This study highlights the potential of eco-enzyme fertilizer in enhancing phytochemical content in turmeric rhizomes. However, the relatively low levels of phenolics, flavonoids, and curcumin observed in this study may be attributed to the young age of the rhizomes. Future research should focus on analyzing phytochemical profiles at later growth stages and optimizing eco-enzyme application methods.

Additionally, advanced extraction techniques, such as ultrasound-assisted or microwave-assisted extraction, could improve the recovery of bioactive compounds. Investigating the impact of eco-enzyme on other medicinal plants may further validate its efficacy as an organic fertilizer.

4. Conclusion

This study investigated the phytochemical content of turmeric rhizomes harvested four months after planting, with a focus on phenolic compounds, flavonoids, and curcumin. The results revealed the presence of these bioactive compounds across all treatments, with the highest flavonoid and phenolic content observed in rhizomes treated with 0.5% eco-enzyme fertilizer. The application of eco-enzyme significantly enhanced the production of secondary metabolites, likely due to its enzymatic components, which promote plant metabolism and biosynthesis. However, the levels of phytochemicals and antioxidant activity were relatively low, likely because the rhizomes were harvested at an immature stage when secondary metabolite production was incomplete.

The bioactive compounds detected in this study—phenolics, flavonoids, and curcumin—are known for their antioxidant, anti-inflammatory, and antimicrobial properties, which contribute to the therapeutic potential of turmeric. Phenolic compounds, in particular, exhibit strong antioxidant activity by neutralizing reactive oxygen and nitrogen species, thus reducing oxidative stress and preventing cellular damage. Similarly, flavonoids interact with metal ions to enhance antioxidant efficacy and play a critical role in regulating oxidative pathways, such as the Nrf2 signaling cascade. Curcumin further contributes to the bioactivity of turmeric by scavenging free radicals, mitigating oxidative damage, and supporting cardiovascular and metabolic health.

While the findings underscore the potential of eco-enzyme fertilizer as an organic enhancer of secondary metabolite production, the study highlights certain limitations. The young age of the rhizomes and the associated immaturity of secondary metabolites likely contributed to the lower observed levels of phenolics and flavonoids compared to those reported in other studies with mature turmeric. Moreover, the extraction methods, temperature sensitivity of phenolic compounds, and the presence of water during processing may have further influenced the phytochemical yields and antioxidant activity.

Future research should explore the impact of eco-enzyme on turmeric rhizomes at later growth stages and evaluate the efficacy of alternative extraction techniques, such as ultrasound-assisted or solvent-specific extractions, to maximize bioactive compound recovery. Additionally, expanding the scope of eco-enzyme application to other medicinal plants could validate its broader applicability and potential for sustainable agriculture.

In conclusion, the study demonstrates the promising role of eco-enzyme in enhancing the phytochemical profile of turmeric rhizomes, contributing to their value as a functional food and medicinal resource. However, optimizing growth conditions, harvesting time, and processing methods will be essential to fully realize the therapeutic potential of turmeric's bioactive compounds.

Author contributions

R.A.H.W. conceptualized the study, supervised the research process, and contributed to data analysis and manuscript drafting. O.P. conducted data collection, performed data analysis, and contributed to manuscript writing and revisions. Both authors reviewed and approved the final version of the manuscript.

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Competing financial interests

The authors have no conflict of interest.

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