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The addition of vermicompost and biostarter affects the growth, total phenolic and antioxidant activity of *Echinacea purpurea*

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Abstract. *Choirunnisa LF, Widiayastuti Y, Solichatun, Yunus A. 2022. The addition of vermicompost and biostarter affects the growth, total phenolic and antioxidant activity of* Echinacea purpurea. *Nusantara Bioscience 14: 148-154. Echinacea purpurea* (L.) Moench or purple coneflower is a medical plant that originated in North America and contained various bioactive compounds, one of which is phenolic. Applying organic fertilizer like vermicompost has been reported to increase plants' bioactive compounds' components and antioxidant activity. The purpose of this study was to determine the addition of vermicompost and biostarter on the growth, total phenolic and antioxidant activity of *E. purpurea*. Split-Plot Randomized Complete Block Design was used with dosages of vermicompost 0, 40, 60, and 80 g/plant and different types of biostarter from Banana peel waste and effective microorganisms (EM). The results showed that treatment of 80 g/plant vermicomposts and EM highest resulted in the growth rate parameters (plant high, leaf numbers, leaf area, roots volume, plant fresh and dry weight) and total phenolic content with 1.802%. On the other hand, the herb extracts had the highest result with the treatment of 40 g/plant vermicomposts and EM (4.96%). The antioxidant activity was tested using the DPPH method with TLC and showed that all treatments indicated positive antioxidant activity.

Keywords: Antioxidant, biostarter, *Echinacea purpurea*, phenolic, vermicompost

INTRODUCTION

Echinacea purpurea (L.) Moench or purple coneflower is a medical plant that originated in North America and contained various bioactive compounds, one of which is phenolic. This plant is widely cultivated as medicinal because it increases the human body's immunity. The morphology characteristics of *E. purpurea* turned out to have changed after being developed and cultivated in Indonesia. The clear morphological difference is the flower.

Bioactive compounds were most commonly found in *E. purpurea*, such as alkamides, polysaccharides, lipoproteins, betaine, sesquiterpenes, polyacetylenes, saponins, and phenolic compounds (echinacoside and caffeic acid). Various components of bioactive compounds found in medicinal plants can be obtained with different results due to various internal and external factors. The *E. purpurea* were reported to have the most efficient free radical scavenging activity than another genus *Echinacea*. The antioxidant activity comes from bioactive compounds such as flavonoids, phenolic acids, or phenolic diterpenes (Sharifi-Rad et al. 2018; Oniszczuk et al. 2019; Coelho et al. 2020).

Adding organic fertilizer is very important in supporting growth and development because it provides essential nutrients needed by plants. Humic acid or humic substances contained in vermicompost can increase plants' phenolic compounds and antioxidant activity. In addition, humic acid increases the biosynthesis of phenolics such as flavonoids and anthocyanins. Other than that, humic acid can increase the number of microorganisms in the soil and the availability of plants' nitrogen uptake (Gholami et al. 2018; Hosseinzadeh et al. 2018).

Biostarter can be made by mixing organic waste (fruits or vegetables) with melted sugar and water and fermenting for about 2-3 weeks. Sugar is used to accelerate the growth of microorganisms so that microorganisms are obtained (Wiryanti 2014). The biostarter used in this study is EM or an effective microorganism. According to Mayer et al. (2010), EM is a combination of various types of beneficial microorganisms selected and isolated from various environments. Microorganisms contain in EM are the populations of lactic acid bacteria, yeasts, smaller populations of phototropic bacteria, filamentous fungi, and actinomycetes.

Organic materials such as vermicompost and biostarter can be a way to help increase the growth rate, the accumulation of total phenolic content, and the antioxidant

activity of *E. purpurea*. However, since *E. purpurea* is an introduced species from a subtropical country, a proper method is needed to improve this species to adjust and cultivate in a tropical area like Indonesia. Therefore, the purpose of this study was to determine the addition of vermicompost and biostarter on the growth, total phenolic, and antioxidant activity of *E. purpurea* cultivated in a lowland area (± 300 meters above mean sea level) in Experimental Garden Faculty of Agriculture, Universitas Sebelas Maret, Sukosari, Jumantono, Karanganyar, Central Java, Indonesia.

MATERIALS AND METHODS

Experimental design

The *E. purpurea* is cultivated in lowland areas (± 300 meters above mean sea level), in Experimental Garden Faculty of Agriculture, Universitas Sebelas Maret, Sukosari, Jumantono, Karanganyar, Central Java, Indonesia (7º37'829" S, 110º56'901" W). Split-Plot Randomized Complete Block Design was used with dosages of vermicompost 0, 40, 60, and 80 g/plant and different types of biostarter from Banana peel waste and EM with the trademark "EM4". The treatments were done twice when the plants were 6 and 10 weeks after transplanting in the experimental garden.

Procedures

Collection of seeds

The seeds were from the collection of the Research Center of Medical Plants and Traditional Medicines (B2P2TOOT), Tawangmangu, Karanganyar, Central Java, Indonesia. We used and cultivated accession 4 in the field.

Planting and harvesting

There were 12 combinations of treatments with a total of 270 plants of *E. purpurea*. The plants were transplanted into beds consisting of 10 per bed, with a total of 27 beds. The temperature was captured between 27°C to 33°C, and the humidity was around 50-60%. The plants were cultivated during the 2021 rainy season. The plant's watering was done every day (morning or afternoon). Harvesting was done after the plants were 80% flowering or about 12 weeks after transplanting. The qualitative observation was the antioxidant activity using the DPPH method with TLC. In contrast, quantitative observations measured growth rate parameters (plant high, leaf numbers, leaf area, roots volume, fresh and dry weight), total phenolic content, and herb extract. The leaf area measurement was done with non-destructive models by combining width length and maximum width. Based on Aminifard et al. (2016), leaf area estimation on *E. purpurea* can be done by measuring leaf dimension and resulting in the most accurate formulation [LA = 0,575 (Length x width) – 0,934]. The length and width of leaves were measured manually by a ruler.

Analysis of herb extract

The plant parts that are extracted are the aerial parts (stems, leaves, and flowers). The drying method was directly exposed to the sun and carried out for about 14 days. After completely drying, it was ground into a powder and weighed 5 grams for herb extract residue. Next, the powder was put into a bottle and macerated with 50 mL of 70% ethanol for three days. Then, it was filtered, and the filtrate was put into a cup that had been weighed previously as the empty weight of the cup. After that, it dried in the oven at 50ºC. The herb extract calculation was done by subtracting the weight of the herb extract and the initial sample weight (Choirunnisa et al. 2021; Ferdyana et al. 2022). Then, the percentage of the herb extract was done using the formula:

$$r\,(\%) = \frac{x}{y} \times 100\%$$

Where:
r: Herb extract content (%)
x: Weight of herb extract (g)
y: Initial sample weight (g)

Analysis of total phenolic content

The accumulation of phenolic content was using the Folin-Ciocalteau (FC) method with modifications from the Research Center of Medical Plants and Traditional Medicines (B2P2TOOT) based on the research of Sidhiq et al. (2020). The analysis of phenolic content using this method is based on the reaction of phenol with the Folin-Ciocalteau reagent. The indication of this reaction was a changed color turned into complex blue.

For the preparations, the sample extract of *E. purpurea* weighed 15 mg in 10 mL of 50% ethanol solution per treatment, then sonicated at 40 Hz for 15 minutes at 60ºC and left for 1 night. After that, prepare the gallic acid stock solution and Na_2CO_3 solution. First, the stock solution was prepared by dissolving 1 g of gallic acid with 1 L of aquadest in a liter measuring cup. Next, the solution was shaken and poured into a bottle covered with aluminum foil to prepare the Na_2CO_3 solution by dissolving 10.5 g of Na_2CO_3 in 100 mL of aquadest in a measuring cup.

Various concentrations of 40, 60, 80, 100, and 120 ppm were made from gallic acid solutions. In the sample solution, 500 mL of each concentration of the gallic acid solution was taken, 500 mL of FC reagent and 8 mL of aquadest were added, then waited for 5 minutes. After that, 1 mL of 20% Na_2CO_3 solution was added. The solution was transferred into a test tube and incubated for 30 minutes. Each solution concentration was read at a wavelength of 760 nm with a UV-Vis Spectrophotometer, and the results were calculated by the equation $y = bx – a$.

The total phenol content contained in the extract of *E. purpurea* was calculated based on the absorbance value using the standard curve obtained previously, $y = 0.0041x + 0.0296$ and $R^2 = 0.9971$. Then, the percentage of the total phenolic was calculated with the following formulation:

$$Total\ phenolic\ content\ (\%) = c\ \frac{V}{m} \times 100\%$$

Where:
c: concentration of gallic acid (mg/mL)
V: volume of extract (mL)
m: mass of extract (mg)

Analysis of antioxidant activity

The antioxidant activity was tested using DPPH (*1,1-diphenyl-2-picrylhydrazyl*) with TLC or thin-layer chromatography modifications from B2P2TOOT. The spot that turned yellow with a purple background indicates the extract positively has antioxidant activity.

Extract powder weighed 1 g and dissolved with 10 mL of methanol in a bottle. After that, it was sonicated for 30 minutes, filtered, and evaporated until a thick extract was obtained. As the stationary phase, G plate F254 nm was used, which had previously been activated on the oven at 100ºC for 1 hour. Then, mark the G plate with a distance of 1 cm from the bottom and top sides as the elution limit. Afterward, prepare a solution used in the mobile phase by mixing 25 mL of ethyl acetate; 15 mL of n-butanol; 5 mL of formic acid; 5 mL of aquadest and pouring it into a bottle.

Took 5 µL of the prepared extract for each treatment using a micropipette and dropped it slowly on the plate. Each treatment was spaced 1.5 cm on the plate. Next, prepare a TLC vessel filled with a mixed solution that has been made to saturate the plate. The TLC vessel was then tightly closed using silicon grease and waited until the elution limit reached 1 cm from the top side of the plate. After the mobile phase was complete, the plate dried and was observed on a TLC visualizer with UV light at 245 nm and 366 nm. Then, the dried plate was sprayed with DPPH solution using a sprayer in LAF.

Data analysis

The quantitative data from growth rate parameters were analyzed using ANOVA (Analysis of Variance) and continued with the Duncan Multiple Range Test (DMRT) at the 5% level to know the significant effect of the treatments. In addition, the herbs extract and total phenolic content were analyzed from the average of each observation based on the available graphs. Meanwhile, the qualitative data as antioxidant activity were analyzed descriptively.

RESULTS AND DISCUSSION

Growth rate

The growth rate is important to observe and indicates the plant's physiological function. This measurement is frequently used to know the effect of each treatment. In this study, we measured the growth rate parameters such as plant high, leaf numbers, leaf area, root volume, and fresh and dry weight. The quantitative analysis of growth rate parameters using ANOVA and continued with the DMRT at the 5% level among all treatments significantly showed different results (Table 1). The whole plant of *E. purpurea* used in this study is shown in Figure 1.

The results showed that the combination treatment of 80 g/plant vermicomposts and EM highest resulted in the growth rate parameters such as plant high, leaf numbers, leaf area, roots volume, and fresh and dry weight compared to the other treatments. Using organic fertilizer like vermicompost can increase plant growth properties. Based on Choirunnisa et al. (2022), plant regulators such as auxin can also increase cell walls and longitudinal growth in the plant. On the other hand, the macro and microelements on vermicompost increase the plant's nutrition status and nourish the leaf and leaf morphology. Amiri et al. (2017) stated that plant growth regulators such as auxin and cytokinin play an important role in increasing the microorganism activity in the soil. The humic acid found in vermicompost has many elements for improving its growth development properties.

On the other hand, effective microorganisms result in many beneficial microorganisms introduced, which enhance the decomposition of organic materials, release nutrients for plant uptake, and improve soil's physical and chemical characteristics. Therefore, EM was applied in combination with organic fertilizer, which can be attributed largely to the activity of the introduced beneficial microorganisms, which enhanced the decomposition of organic materials and the release of nutrients for plant uptake (Joshi et al. 2019; El-Mageed et al. 2020).

Figure 1. The whole plant of *E. purpurea was* used in this study (A-L). A: T_{11} (vermicompost 80 g/plant with EM). B: T_8 (vermicompost 60 g/plant with EM). C: T_5 (vermicompost 40 g/plant with EM). D: T_2 (vermicompost 0 g/plant with EM). E: T_{10} (vermicompost 80 g/plant with banana peel waste). F: T_7 (vermicompost 0 g/plant with banana peel waste). G: T_4 (vermicompost 40 g/plant with banana peel waste). H: T_1 (vermicompost 0 g/plant with banana peel waste. I: T_9 (vermicompost 80 g/plant without biostarter). J: T_6 (vermicompost 60 g/plant without biostarter). K: T_3 (vermicompost 40 g/plant without biostarter). L: T_0 (vermicompost 0 g/plant without biostarter or control)

Table 1. The addition of vermicompost and biostarter on the growth rate of *E. purpurea*

Characters	Treatments											
	T_0	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	T_9	T_{10}	T_{11}
Plant high (cm)	72.60c	73.60b	77.00a	73.20c	77.20b	82.60a	76.60c	80.40b	87.60a	78.60c	82.40b	88.80a
Leaf numbers	45.40b	55.20a	63.20a	46.33b	70.80a	72.60ab	47.75b	74.00a	75.20a	54.50b	68.40a	82.60a
Leaf area (cm^2)	59.34a	65.21a	67.02a	69.80ab	64.42ab	69.84ab	70.39b	72.82b	78.93b	79.67c	87.21c	83.83c
Roots volume (ml)	18.80c	20.20b	22.10d	21.20c	22.20b	24.40a	25.60c	28.40b	29.80b	27.80c	31.20a	34.50a
Plant fresh weight (g)	201.48c	243.49b	280.89a	215.49c	247.38c	303.13a	262.73b	288.43b	340.27b	319.98a	340.27a	392.21a
Plant dry weight (g)	53.35c	70.20b	73.66a	63.73b	75.85b	85.20b	68.67a	86.33a	88.20a	71.20c	89.43a	91.40a

Note: Numbers followed by different letters in the same row show a significant difference in DMRT at the 5% level. T_0 (vermicompost 0 g/plant without biostarter or control). T_1 (vermicompost 0 g/plant with banana peel waste). T_2 (vermicompost 0 g/plant with EM). T_3 (vermicompost 40 g/plant without biostarter). T_4 (vermicompost 40 g/plant with banana peel waste). T_5 (vermicompost 40 g/plant with EM). T_6 (vermicompost 60 g/plant without biostarter). T_7 (vermicompost 0 g/plant with banana peel waste). T_8 (vermicompost 60 g/plant with EM). T_9 (vermicompost 80 g/plant without biostarter). T_{10} (vermicompost 80 g/plant with banana peel waste). T_{11} (vermicompost 80 g/plant with EM)

Herb extract

The maceration method carried out the herb extraction process of E. purpurea. Before the extraction, this plant needed to be dried and powdered because the texture of the aerial parts (stems, leaves, and flowers) that we used was quite hard, so it must be grounded until the texture became smooth. This method prevented the growth of microbial activity and fungi so that they could be stored for quite longer. The maceration process is a simple extraction method that uses more solvents and is easy to obtain.

Figure 2 presents the graph of the percentage of herb extract among all treatments. The results showed the best result on the combination treatment of 40 g/plant vermicomposts and EM with 4.96%. The treatment of vermicompost 80 g/plant without biostarter showed the lowest result of herb extract percentages with 4.16%. The higher value of herb extract is correlated to the more active compounds attached to the solvent or indicates many bioactive compounds in the plant's extract. But according to Choirunnisa et al. (2021), the high value of herb extract does not correlate to the specific amount of bioactive compounds found in the plant because this is an accumulation of all the bioactive compounds contained in plants.

Total phenolic content

The accumulation of phenolic content was done using the Folin-Ciocalteau (FC) method. The analysis of phenolic content using this method is based on the reaction of phenol with FC reagent using gallic acid as the standard. The indication of this reaction was changed color and turned into complex blue. The absorbance values obtained at different concentrations (40, 60, 80, 100, and 120 ppm) were used to construct the calibration curve. The data were analyzed from the average of each observation based on the available graphs.

Figure 3 presents the percentage of total phenolic content among all treatments. The results showed the best result on the combination treatment of 80 g/plant vermicomposts and EM with 1.802%. The lowest result of total phenolic content was on the combination treatment of vermicompost 0 g/plant and EM with 0.808%. Extracts of *E. purpurea* may contribute significantly to the antioxidant properties. Because of these properties, this plant has been used in several herbal medications and is known for increasing the human body's immunity. In addition, some interference may arise from other chemical components in the extract.

The accumulation of total phenolic content in the plant is related to antioxidant activity. Phenolic compounds act as reducing agents and hydrogen donors and can scavenge free radicals. Phenolic compounds are important plant constituents because of their scavenging ability on free radicals due to their hydroxyl groups. The antioxidant response of phenolic compounds varies remarkably, depending on their chemical structure. Several investigations of the antioxidant activity of plant extracts have confirmed a correlation between total phenolic content and antioxidant activity (Ahmed et al. 2019; Phuyal et al. 2020).

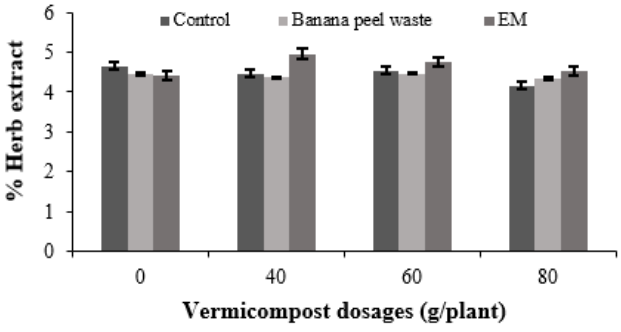

Figure 2. Percentage of herb extract *E. purpurea* on each treatment

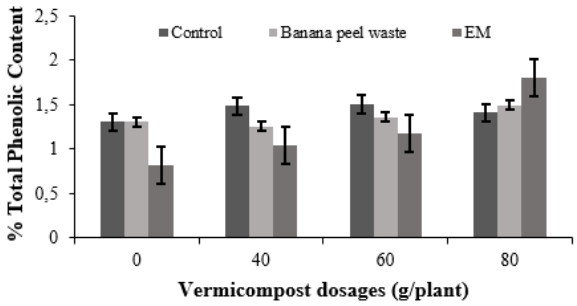

Figure 3. Percentage of the total phenolic content of *E. purpurea* in each treatment

Antioxidant activity

The DPPH method is based on the presence of an electron donor or hydrogen radical (H), which produces antioxidant compounds. This method is selected because it is simple, easy, fast, and sensitive and requires only a small number of samples. Qualitative antioxidant activity analysis is done using TLC (thin-layer chromatography).

The antioxidant activity test of *E. purpurea* showed that all treatments indicated positive antioxidant activity (Figures 4, 5, and 6). After the plate was sprayed with DPPH solution, the spot turned yellow with a purple background, as shown on the picture's left side. TLC visualizer detects UV light at 245 nm and 366 nm. The detection with a UV light at 245 nm showed on the middle side of the picture, while UV light at 366 nm showed on the right side.

Based on Pratiwi et al. (2013), the detection using a UV light was to find the spot that can fluoresce so that it can be seen visually due to the interaction between UV rays with a chromophore group attached to an auxochrome at that spot. Visible light fluorescence results from the emission of light emitted by the component when electrons are transferred to a higher energy level and then come back again while releasing energy.

The TLC plate was eluted using eluents in the form of n-butanol, ethyl acetate, and formic acid. The eluents were used, so the color's appearance and the distance of the stain were quite clear on the sample spotted on the TLC plate. According to Handayani et al. (2014), based on its polarity, the eluent is more nonpolar, so separated compounds are also nonpolar, like on the principle of TLC, "like dissolves like." After the TLC plate was eluted and then sprayed with DPPH, spots that changed color to yellow indicated the presence of antioxidant activity. Antioxidant compounds will react with DPPH radicals through the mechanism of hydrogen atom donation and cause color decay from purple to yellow.

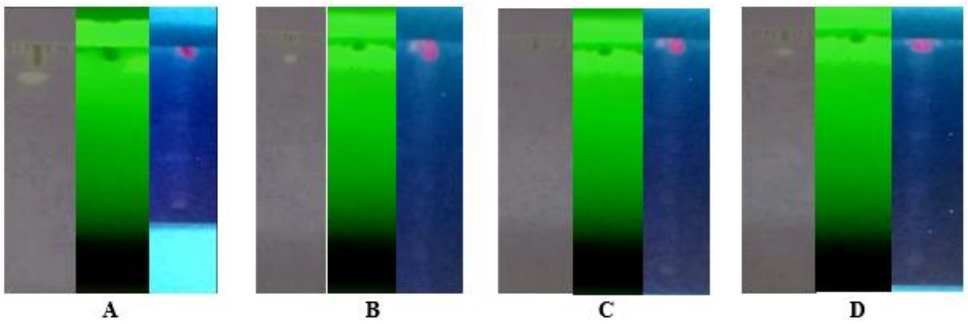

Figure 4. Antioxidant activity using the DPPH method with TLC visualizer at 245 and 265 nm. A: T_0 (vermicompost 0 g/plant without biostarter or control). B: T_1 (vermicompost 0 g/plant with banana peel waste). C: T_2 (vermicompost 0 g/plant with EM). D: T_3 (vermicompost 40 g/plant without biostarter)

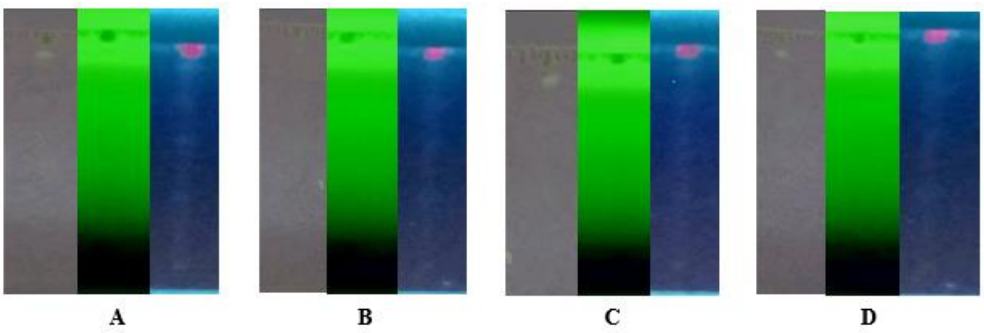

Figure 5. Antioxidant activity using the DPPH method with TLC visualizer at 245 and 265 nm. A: T_4 (vermicompost 40 g/plant with banana peel waste). B: T_5 (vermicompost 40 g/plant with EM). C: T_6 (vermicompost 60 g/plant without biostarter). D: T_7 (vermicompost 0 g/plant with banana peel waste)

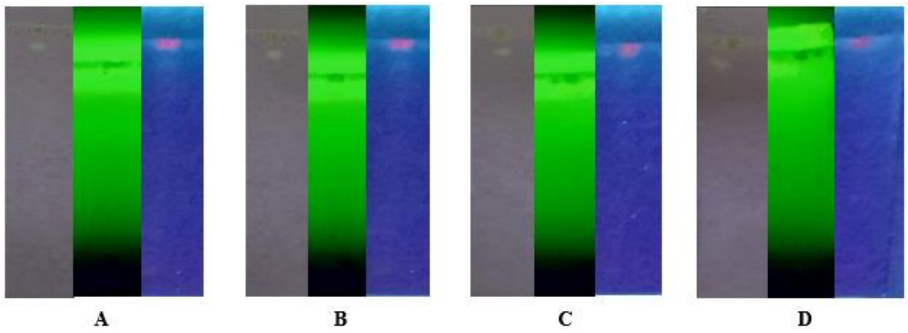

Figure 6. Antioxidant activity using the DPPH method with TLC visualizer at 245 and 265 nm. A: T_8 (vermicompost 60 g/plant with EM), B: T_9 (vermicompost 80 g/plant without biostarter). C: T_{10} (vermicompost 80 g/plant with banana peel waste). D: T_{11} (vermicompost 80 g/plant with EM)

The antioxidant activity of *E. purpurea* extract has the highest antioxidant capacity (relative to ascorbic acid and gallic acid). The antioxidant activity of *E. purpurea* extract was measured using the DPPH method test. The high ability to scavenge free radicals from the extract was found to be related to the increase in antioxidant concentration. The *E. purpurea* were reported to have the most efficient free radical scavenging activity compared to the other genus of *Echinacea* (Oniszczuk et al. 2019; Banica et al. 2020).

Using fertilizer added to *E. purpurea* increases the antioxidant activity in counteracting hydroxyl radicals. This antioxidant mechanism is described as eliminating free radicals and metal ions. The antioxidant activity is considered from bioactive compounds such as polyphenol components (flavonoids, phenolic acids, or phenolic diterpenes). Applying organic fertilizer is crucial in supporting growth and development because it provides essential nutrients plants need (Ahmadi et al. 2020).

This study concluded that there is an effect on the growth, total phenolic, and antioxidant activity of *E. purpurea* by adding vermicompost and biostarter. The combination treatment of 80 g/plant vermicomposts and EM highest resulted in the growth rate parameters (plant high, leaf numbers, leaf area, roots volume, plant fresh and dry weight) and total phenolic content of 1.802%. The herb extracts highest resulted in the combination treatment of 40 g/plant vermicomposts and EM (4.96%). The antioxidant activity was tested using the DPPH method with TLC and showed that all treatments indicated positive antioxidant activity. Further studies are needed to promote the integration between vermicompost and biostarter, especially given *E. purpurea* in Indonesia. A good adaptation and better improvement to increase the bioactive compounds are important because this plant is promising for medicinal uses and products.

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