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Effect of cow manure fertilizer on growth, polyphenol content, and antioxidant activity of purslane plants

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Abstract

Purslane plants have been known as herbs that have many health benefits because they are known to contain many secondary metabolites, such as phenolics and flavonoids. The content of purslane plant secondary metabolites can be influenced by various factors such as fertilization, environmental conditions, and the genetics of the plant itself. This study aims to determine the effect of cow manure on growth, phenolic content, flavonoids, and antioxidant activity in purslane plants. This study used four dosage levels of cow manure, namely doses of 0 (control), 10 g/polybag (20 tons/ha), 20 g/polybag (40 tons/ha), and 30 g/polybag (60 tons/ha). The highest phenolic, flavonoid and antioxidant activity content was produced by purslane plants fed with cow manure at a dose of 10 g/polybag. Meanwhile, purslane plants that were not given fertilizer (control plants) produced the lowest value for the content of phenolic, flavonoid contents, and antioxidant activity. Treatment of cow manure doses did not significantly affect the growth of branches and leaves of purslane plants. According to the findings, the polyphenol antioxidant levels in the purslane plant were raised after being treated with cow manure.

Keywords: Purslane plant, cow manure, plant growth, polyphenol content, antioxidant activity

Full length article **Corresponding Author*, e-mail: wnurcholis@apps.ipb.ac.id

1. Introduction

Indonesia is rich in natural resources, with biodiversity spread throughout its territory. This biodiversity includes various types of flora and fauna. The public does not widely know most of the flora that can be used as herbal medicines, so they live as wild plants and are considered weeds [1]. One example of this plant is *Portulaca grandiflora*, commonly known as purslane, which is a wild plant species. Purslane is known to have antioxidant, diuretic, hypolipidemic, anti-inflammatory, anticonvulsant, and antimicrobial properties [2]. The purslane plant is a plant that comes from the Portulacaceae family with the genus Portulaca. According to Zhou et al. [3], purslane plants have various phytochemical contents. These phytochemicals include sterols, carotenoids, flavonoids, polyphenolic acids, and polysaccharides. Purslane is a type of wild plant and is often considered a weed that can live and thrive mainly in sandy and clay areas [4]. This plant is characterized by a round stem with a single leaf that is thick and fleshy [5]. The many properties and phytochemical content of purslane plants are classified as many, so it is necessary to propagate by cultivation. Plant cultivation is an effort to develop a plant with various techniques or specific nurseries [6].

Plant cultivation can be done optimally by using the right fertilizer. The use of fertilizers can be done by using synthetic fertilizers and organic fertilizers. One type of fertilizer that is often used in cultivation is manure. Manure is a type of fertilizer from livestock pens consisting of manure, food waste, and livestock urine [7]. One of the manures that is often used is cow manure [8]. Cow manure is a solid fertilizer containing a lot of water and mucilage with a nutrient composition of 0.40% N, 0.20% P_2O_5, and 0.1% K_2O [9]. Research by Wangiyana and Ngawit [10] stated that

the application of cow manure could increase the growth of corn plants by increasing the number of green leaves, accelerating the ageing of panicles and cob hairs, thus increasing the yield and index of corn harvest.

The use of fertilizers must pay attention to the dosage and needs of the plants themselves to optimize cultivation. This statement is supported by research conducted by Clark et al. [11], who obtained optimal results at adequate doses of fertilizer use. The proper dosage of fertilizer can also increase the cultivation of purslane plants to obtain optimal yields, so research is needed to examine the correct dose of cow manure for purslane plants. This study analyses the growth, phenolic and flavonoid contents, and antioxidant activity of purslane plants with varying doses of cow manure.

2. Materials and methods

The samples used in this study were the aerial parts of the purslane plant which included stems, leaves and flowers from cuttings from the Tropical Biopharmaceutical Research Center, IPB University, Bogor, Indonesia. This research was conducted from August to December 2022. The materials used in this study were cow manure, 70% technical ethanol, distilled water, $AlCl_3$, CH_3COOK, Folin ciocalteu, Na_2CO_3, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,4,6-Tri(2-pyridyl)-s-triazine (TPTZ), $FeCl_3.6H_2O$, glacial acetic acid, sodium acetate, HCl, 2,2'-azino-bis (3-ethylbenzothiazoline-6- sulphonic acid) (ABTS), $K_2S_2O_8$, $CuCl_2.2H_2O$, neocuproine, and ammonium acetate. The standards used were gallic acid, quercetin, and trolox.

Cultivation and Fertilization Treatment Methods

This study was designed utilizing a randomized complete block layout with three replicates of each of the following cow manure fertilizer doses: 0, 10, 20, and 30 g/polybag [12]. The study was conducted in 4 treatment combinations, with each treatment carried out in 3 replications, so there were 12 experimental units. Each experimental unit contained 9 plants in 3 polybags, so a total of 108 purslane plants; then, 3 sample plant was taken from each experimental unit for observation.

Observation of Agro-Morphological Characters

Agro-morphological characteristics measured on the growth of purslane include the number of leaves and the number of branches based on Lestari et al. [13] with modifications. Measurement of agro-morphological characters was carried out every 2 weeks after planting (WAP) for 8 weeks. Measurement of the total number of leaves and the number of branches was measured both for new and old leaves and branches.

Sample Preparation and Extraction

The test material was prepared by taking the aerial parts of the purslane plant, including the stems, branches and leaves. The plant parts were washed, dried and homogenized using a blender. With modifications, the purslane sample extraction method was carried out based on Calvindi et al. [14]. In short, after the sample was homogenized using a blender, 8 g of sample for each treatment was extracted using 70% ethanol (20 ml). Samples were extracted using a microwave (SHARP Low watt R21DO(S)IN), then macerated at 125 rpm for 3 × 24 hours in a dark room using a water bath shaker. Then the samples were centrifuged at 5000 rpm for 5 minutes. The sample filtrate was filtered using filter paper, and an extract with a concentration of 0.4 g/mL was obtained.

Total Phenolics Analysis

The total phenolic content was analyzed based on the modification of Khumaida et al. [15]. 20 µL of sample extract was added with 120 µL of Folin-Ciocalteu (10%) (v/v), put into a microplate, and incubated for 5 minutes in the dark room. After that, 80 µL of Na_2CO_3 solution (10%) (w/v) was added to the microplate and incubated for 30 minutes in the dark room. The absorbance of the solution was measured using a nano spectrophotometer (SPECTROstarNano BMG LABTECH) at a wavelength of 750 nm. The standard is gallic acid with a concentration of 0-300 ppm.

Analysis of Total Flavonoids

The total content of flavonoids was analyzed based on the modification of Calvindi et al. [14]. A total of 10 µL of sample extract was added with 50 µL of ethanol Pro analysis, 10 µL of aluminium chloride ($AlCl_3$) 10% (w/v), 10 µL of glacial acetic acid (CH_3COOH), and 120 µL of distilled water were put into a 96 well microplate. The solution was incubated for 30 minutes in the darkroom and at room temperature. Then the absorbance of the sample extract was measured using a nano-spectrophotometer (SPECTROstarNano BMG LABTECH) at a wavelength of 415 nm. The standard used was quercetin with a concentration of 0-500 ppm.

DPPH Antioxidant Activity Measurement

Testing of antioxidant activity with the DPPH method based on Calvindi et al. [14]. 100 µL of purslane extract added with 125 µM DPPH reagent as much as 100 µL was put into a microplate and incubated at room temperature in the dark for 30 minutes. The absorbance of the solution was measured at 517 nm using a nano spectrophotometer (SPECTROstarNano BMG LABTECH). The standard used was trolox with a concentration of 0-50 µmol.

FRAP Antioxidant Activity Measurement

Testing of antioxidant activity with the FRAP method is based on a modification of Benzie and Strain [16]. The FRAP reagent consisted of 10 mM TPTZ in 40 mM HCl, 20 mM $FeCl_3.6H_2O$ in distilled water, and acetate buffer at pH 3.6 in the respective ratio (1:1:10). Prior to use, the FRAP reagent was stored at 37°C for 30 minutes. After that, 10 µL aliquots of the extract were mixed with 300 µL FRAP reagent and then incubated at 37°C for 30 minutes in the dark. The absorbance was read at a wavelength of 593 nm using a nano spectrophotometer (SPECTROstarNano BMG LABTECH) at 734 nm. The standard used was trolox with various concentrations of 0-600 µmol.

ABTS Antioxidant Activity Measurement

Measurement of antioxidant activity using 2, 2-azinodi-(3-ethyl benzo thialo zone sulphonic acid (ABTS) refers to Re et al. [17] with modification. A total of 20 µL of purslane extract was added to 280 µL of ABTS reagent and then put into a microplate. The solution was incubated for 6 minutes, and the absorbance was measured at a wavelength of 734 nm using the SPECTROstarNano BMG LABTECH nano spectrophotometer). The standard used was trolox with various concentrations of 0-500 µM.

CUPRAC Antioxidant Activity Measurement

Testing of antioxidant activity with the CUPRAC method is based on a modification of Öztürk et al. [18]. A total of 50 µL of the sample was mixed with 50 µL of neocuproine (7.5 × 10-3 M), copper (II) chloride (10-2 M), and ammonium acetate buffer solution (pH 7). After that, the mixed solution was incubated in a dark room for 30 minutes. The absorbance of the solution was measured at a wavelength of 450 nm using a nano spectrophotometer (SPECTROstarNano BMG LABTECH). Standard using trolox with various concentrations of 0-500 µM.

Data analysis

Data is expressed in the average test using the One-Way ANOVA method and Tukey's follow-up test using IBM SPSS Statistics 25. Data calculations are performed using Microsoft Office Excel 2019 software. Data are presented in tables and graphs using Graphpad Prism 8 software.

3. Results and Discussions

Agro-Morphological Characteristics of Purslane Plants

Each plant is characterized by the agro-morphological characteristics found in that plant. Agro-morphological characteristics in plants can generally be used to distinguish the genotype of a plant [19]. The agro-morphological characteristics of this plant include the number of branches, number of leaves, plant height, leaf shape, and flower colour. Several factors, both the planting medium and the type of plant genotype, can influence these characteristics. The agro-morphological characteristics measured in this study included the number of branches and the number of leaves produced by purslane plants treated with cow manure. According to the research by Abdullahi et al. [20], combined fertilizer treatment on plants will affect the branches and leaves produced.

The number of purslane branches measured is presented in Table 1. The purslane plants planted were given three treatments of growing media (besides the control), namely the difference in fertilizer doses of 10 g/polybag, 20 g/polybag, and 30 g/polybag. The number of purslane branches was measured at 2 weeks after planting (WAP), 4 weeks after planting (WAP), 6 weeks after planting (WAP), and 8 weeks after planting (WAP). Control plants produced the highest number of purslane branches at 2 WAP with 7.22 ± 0.60 branches. Meanwhile, the highest number of branches produced after 2 WAP varies. Fertilization 10 g/polybag produced the highest number of branches aged 4 WAP and 8 WAP, respectively, 8.00 ± 0.58 branches and 8.00 ± 0.62 branches. In comparison, 20 g/polybag fertilization produced the highest number of branches at the age of 6 WAP, 8.56 ± 0.88 branches. Tukey's other test results showed no significant difference in the fertilization treatment on the number of purslane plant branches. These results indicate that the dose of cow manure does not affect the number of branches produced. However, this result is inversely proportional to the research of Turuko and Mohammed [21], which states that fertilizer doses affect the number of branches. This difference in yield is caused by differences in the types of plants fed with cow manure. Cow manure contains more carbon (C) and cellulose than other manure. The high content of cellulose and carbon in cow manure inhibits plant growth [22]. Thus, branch growth becomes stunted or less influential. Measurement of the number of purslanes leaves also yielded various data (Table 2). Just like measuring the number of branches, the number of leaves measured on purslane plants starts at the age of 2 WAP to 8 WAP. Control plants produced the highest leaves at 2 WAP of 117.33 ± 11.94. In contrast to the control plants, the plants that were given 30 g/polybag fertilizer produced the highest number of leaves at the age of 4 WAP and 8 WAP, namely 146.33 ± 12.01 leaves and 148.56 ± 5.38 leaves. Meanwhile, at the age of 6 WAP, the highest number of leaves was produced by a fertilizer dose of 20 g/polybag, namely 157.22 ± 14.04 leaves.

Variations in fertilizer doses given to purslane plants also showed results similar to Tukey's advanced test. These results indicate no effect of cow manure on the number of leaves produced. Fertilizer application does not always increase plant growth; it can be influenced by the type of plant, growing medium, environment, and regulation of the plant. This statement is supported by Esteban et al. [23], who state that excessive fertilizer application can cause decreased plant growth. However, the type of fertilizer given to plants also affects the agronomic character of these plants. Prasetya's research [24] revealed that the application of cow manure did not significantly affect the agronomic characteristics of curly red chilli plants.

On the other hand, his research also revealed a very significant effect of pearl NPK fertilizer on the growth of red curly chilli plants. In addition, the type of plant also plays an essential role in the absorption of cow manure. This statement is supported by research by Purbajanti et al. [25], which stated that cow manure had a significantly affected the plant height, ANR and total chlorophyll contents of peanuts (*Arachis hypogaea* L.). These results prove that there is an influence of plant species on fertilizer absorption.

Table 1: The number of purslane plant branches based on the age of the plant as a response to the cow manure application

Cow Manure Treatment	Number of Branches			
	2 WAP	4 WAP	6 WAP	8 WAP
Control (No cow manure)	**7.22 ± 0.60**	6.89 ± 0.51	7.89 ± 0.77	6.89 ± 0.61
10 g/polybag	7.11 ± 0.39	**8.00 ± 0.58**	8.11 ± 0.74	**8.00 ± 0.62**
20 g/polybag	6.44 ± 0.65	6.11 ± 0.51	**8.56 ± 0.88**	7.78 ± 0.85
30 g/polybag	6.33 ± 0.37	6.33 ± 0.37	7.11 ± 0.56	7.00 ± 0.50

[1]Note: Each value is presented as the mean of three replicates ± standard deviation (SD); WAP: weeks after planting

Table 2: The number of purslane plant leaves based on the age of the plant as a response to the cow manure application

Cow Manure Treatment	Number of Leaves			
	2 WAP	4 WAP	6 WAP	8 WAP
Control (No cow manure)	**117.33 ± 11.94**	120.11 ± 7.85	139.78 ± 9.60	122.56 ± 9.06
10 g/polybag	108.89 ± 11.85	135.78 ± 8.84	142.44 ± 11.00	133.33 ± 9.94
20 g/polybag	114.33 ± 14.35	122.22 ± 8.20	**157.22 ± 14.04**	147.78 ± 10.83
30 g/polybag	106.56 ± 9.27	**146.33 ± 12.01**	141.33 ± 6.07	**148.56 ± 5.38**

[2]Note: Each value is presented as the mean of three replicates ± standard deviation (SD); WAP: weeks after planting

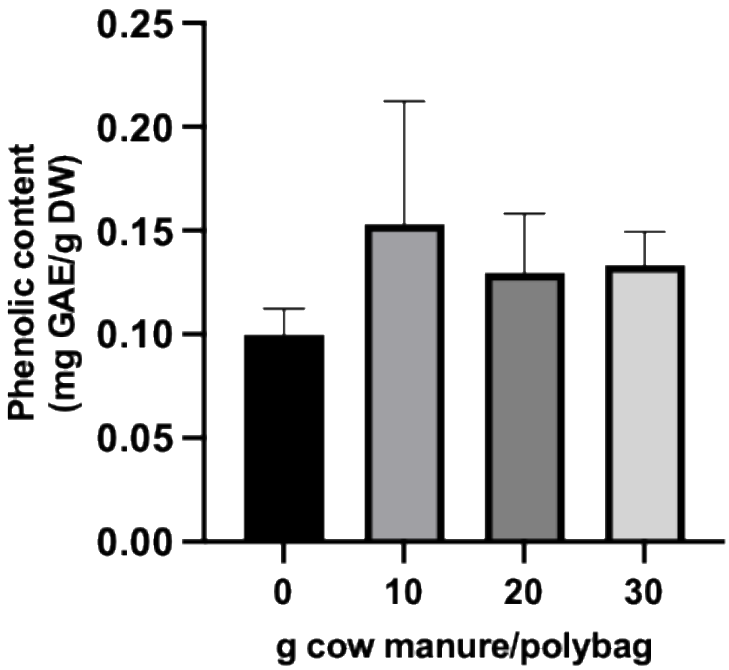

Figure 1. Total phenolic content of purslane plants as a response to the cow manure application. Each value is presented as the mean of three replicates ± standard deviation (SD).

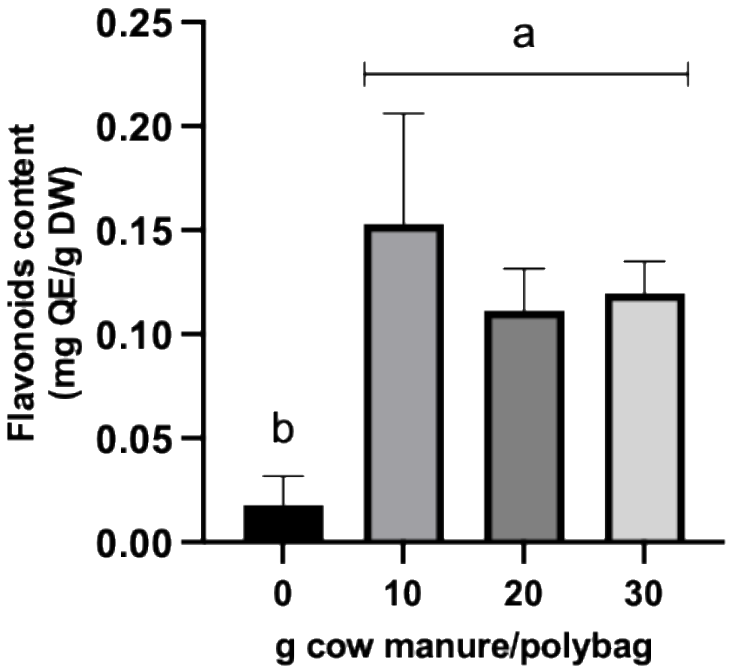

Figure 2. Total flavonoids content of purslane plants as a response to the cow manure application. Each value is presented as the mean of three replicates ± standard deviation (SD). The mean value in each column marked with different letters differs significantly at $p < 0.05$ and Tukey test results 5%.

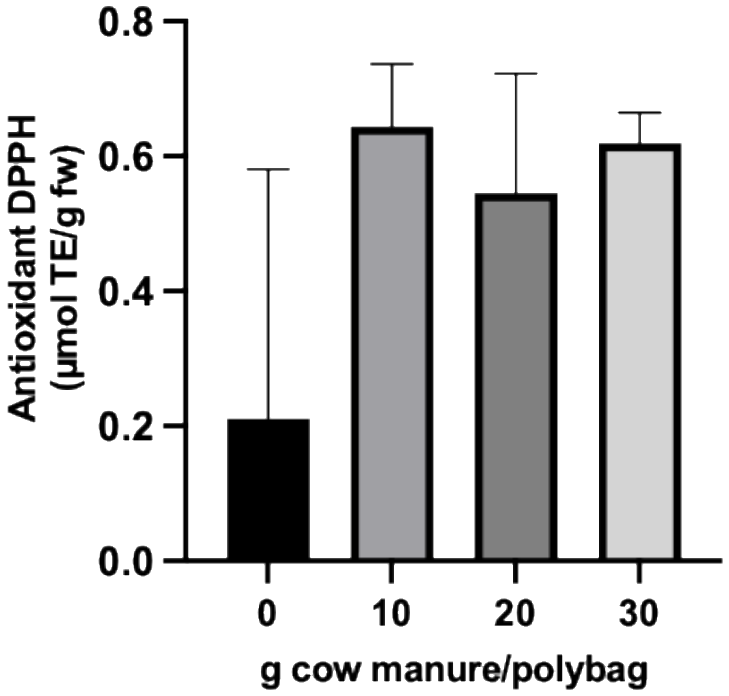

Figure 3. DPPH antioxidant activity of purslane plants as a response to the cow manure application. Each value is presented as the mean of three replicates ± standard deviation (SD). The mean value in each column marked with different letters differs significantly at $p < 0.05$ and Tukey test results 5%.

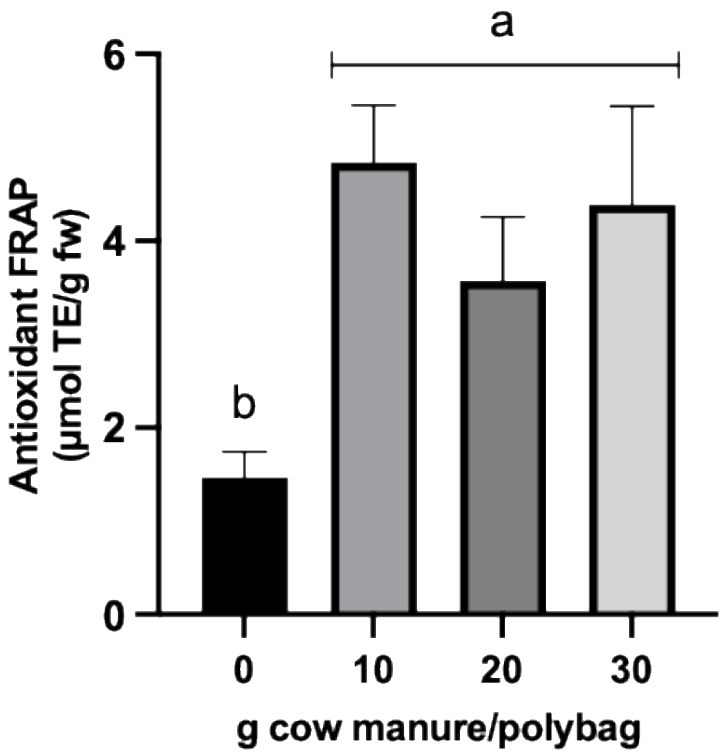

Figure 4. FRAP antioxidant activity of purslane plants as a response to the cow manure application. Each value is presented as the mean of three replicates ± standard deviation (SD). The mean value in each column marked with different letters differs significantly at $p < 0.05$ and Tukey test results 5%.

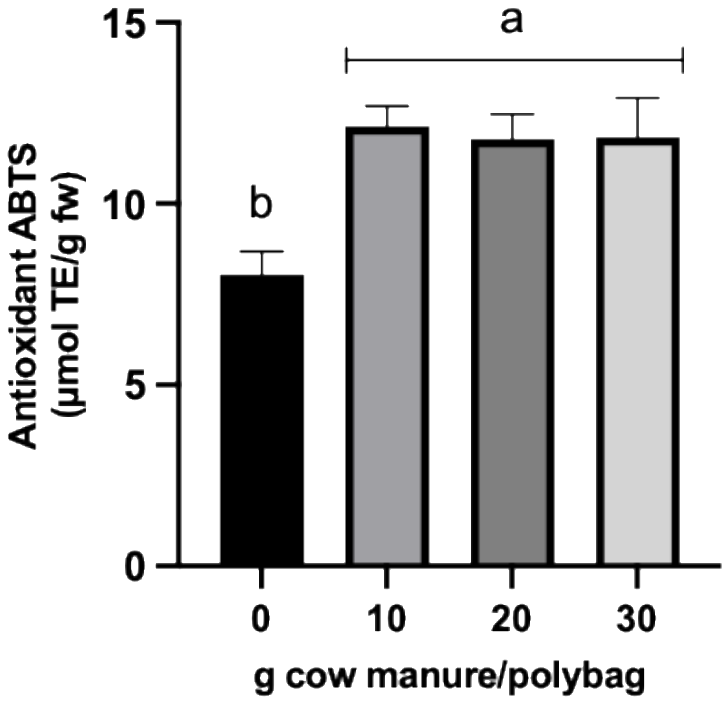

Figure 5. ABTS antioxidant activity of purslane plants as a response to the cow manure application. Each value is presented as the mean of three replicates ± standard deviation (SD). The mean value in each column marked with different letters differs significantly at $p < 0.05$ and Tukey test results 5%.

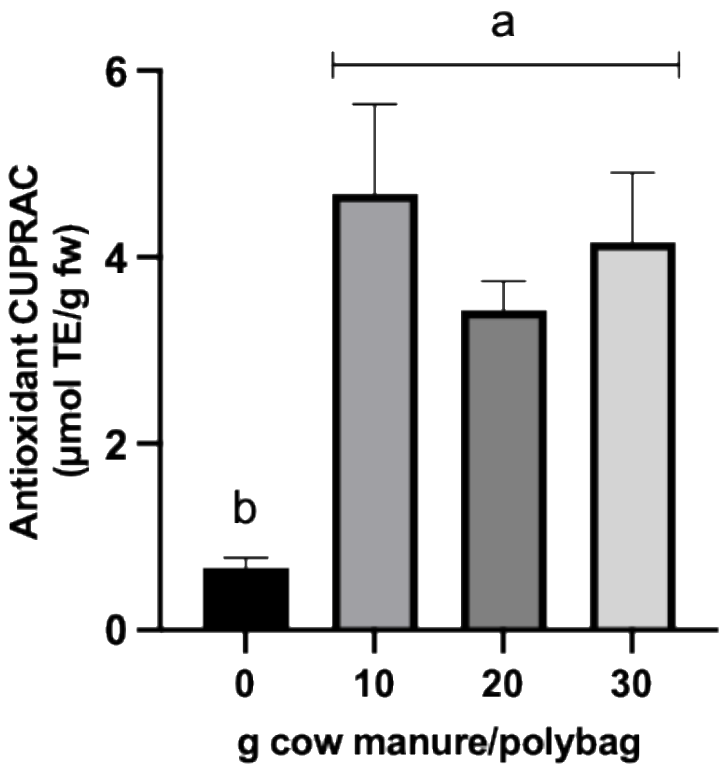

Figure 6. CUPRAC antioxidant activity of purslane plants as a response to the cow manure application. Each value is presented as the mean of three replicates ± standard deviation (SD). The mean value in each column marked with different letters differs significantly at $p < 0.05$ and Tukey test results 5%.

Total Phenolics Content

Phenolic compounds are secondary metabolite compounds produced by plants and act as natural antioxidants. Phenolic compounds protect against UV radiation and cell death [26]. The total phenolic content was determined using the Folin-Ciocalteu reagent with gallic acid as standard. The use of gallic acid as a standard is because gallic acid is classified as a simple phenolic acid which is stable and pure [27]. The phenolic compound will react with the Folin-Ciocalteu reagent to form a blue complex. The darker the blue colour produced, the higher the absorbance value obtained [28]. Measurement of phenolic compounds in purslane plants was carried out after the age of 8 weeks after planting (8 WAP). The phenolic compounds produced by the purslane plant are thought to function as protection against excess water loss [29]. This statement follows the conditions where purslane grows in areas exposed to direct sunlight. The phenolic content produced based on the fertilizer dose treatment showed differences. The content of phenolic compounds obtained can be seen in Figure 1. The analysis showed that the effect of cow manure dose on the purslane plants' total phenolic content was not significantly different (Figure 1). There were four doses of cow manure treatments: control (without cow manure), 10 g/polybag, 20 g/polybag, and 30 g/polybag. The highest total phenolic content was obtained in plants treated with a 10 g/polybag of 0.1532 mg GAE/g. This result is due to several factors, such as proper processing and storage (temperature, light, and oxygen). Meanwhile, 20 g/polybag and 30 g/polybag fertilization resulted in a total phenolic content of 0.1297 mg GAE/g and 0.1335 mg GAE/g, respectively. Meanwhile, the lowest total phenolic content was obtained in plants treated with a dose of 0 g/polybag (control) of 0.0998 mg GAE/g.

Fertilization using cow manure does not significantly affect the phenolic content produced. This result is thought to be caused by the condition of the plants not experiencing environmental stress so that the phenolic content in purslane plants tends to be stable. The environmental conditions where the purslane grows were analyzed without drought stress or lack of nutrition. Drought stress is affected by ambient temperature. Environmental conditions with high temperatures will cause an increase in free radicals in the form of reactive oxygen species (ROS) in plants which can trigger stress and damage cells [30]. So that plants carry out self-defence by producing secondary metabolites [31]. In addition, this insignificant result is thought to be due to improper sample storage. It is known that it is better to store samples for analysis of phenolic compounds under low-temperature conditions so that the phenolic compounds in the samples are not damaged [32]. The highest phenolic content in the 10 g/polybag fertilizer

dose treatment should be in line with this treatment's high flavonoid content and antioxidant activity.

Content of Total Flavonoids

Flavonoids are compounds of the polyphenol group, secondary metabolites found naturally in plants. This compound is also a derivative of phenolic compounds. Flavonoids are classified based on their chemical structure and biosynthesis [33]. The biosynthetic pathway for flavonoid compounds consists of two pathways: the polyketide pathway and the phenylpropanoid pathway [34]. These compounds have functions similar to phenolics. Flavonoids in plants act as self-protectors from diseases caused by the surrounding environment [35]. Measurement of total flavonoid levels can use aluminium chloride reagent ($AlCl_3$) with quercetin as a standard for flavonoid compounds [36].

The total flavonoid content measured in this study showed different results between the control and fertilized plants. Control plants gave the lowest flavonoid content, which was 0.0178 mg GAE/g. While the highest flavonoid content was produced by plants with a fertilizer dose of 10 g/polybag, amounting to 0.1529 mg GAE/g. Then the lower flavonoid content was followed by purslane plants with fertilizer doses of 30 g/polybag and 20 g/polybag, respectively 0.1196 mg GAE/g and 0.1113 mg GAE/g. A comparison of the flavonoid content obtained can be seen in Figure 2.

These results indicate a significant difference in Tukey's follow-up test. So the treatment of fertilizer doses has a significant effect on the content of flavonoids. Giving cow manure can increase total levels of flavonoids in purslane plants. This increase was due to the fulfilment of the need for plant nutrient elements to form flavonoid compounds obtained from cow manure. The results obtained in this study are in line with the research of Fonseca et al. [37], who found a significant difference between the treatment of cow manure and the resulting flavonoid content. The flavonoid content of a plant is also influenced by the genetics of the plant [38]. The genetics of a plant will perform its role optimally if the adequacy of nutrients is adequately met. Adequacy of nutrients can be obtained from fertilization on plants. The results were also in line with the phenolic content of purslane plants, plants treated with a fertilizer dose of 10 g/polybag also produced the highest flavonoid content. These results also indeed indicate a relationship between phenolic compounds and flavonoids because it is known that flavonoids are phenolic derivatives.

Antioxidant Activity

Antioxidants are compounds that play a role in counteracting free radicals to defend the body in plants. Antioxidants in plants come from phenolic compounds and flavonoids, which are produced as plant secondary metabolites. These compounds act as reducing agents and free radical scavengers [39]. The antioxidant activity contained in plants usually varies depending on the type of plant. Measurement of antioxidant activity in plants can be carried out using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method, FRAP (Ferric Reducing Antioxidant Power), CUPRAC (Cupric ion reducing antioxidant capacity), and ABTS (2,2'-Azinobis-(3-ethyl benzo thiazoline-6-sulfonic acid)). Measurement of antioxidants using these four methods using Trolox as a standard. Trolox is a synthetic compound that can act as an antioxidant commonly used in laboratories [40]. The four methods previously mentioned have different working principles from each other [41].

Measurement of antioxidant activity using the DPPH method has the principle of reducing DPPH reagents by antioxidants contained in the sample. Sample antioxidants reduced DPPH compounds by capturing hydrogen from antioxidant compounds. This reaction causes the purple colour of the DPPH solution to decrease so that the absorbance of the solution can be measured [42, 43]. Antioxidant testing using the DPPH method is classified as effective for determining the antioxidant activity of a sample. However, antioxidant testing using DPPH compounds must be carried out in a dark room because these compounds are susceptible to light [41]. The results of measuring the antioxidant activity of DPPH on purslane plants treated with fertilizer doses are shown in Figure 3.

The antioxidant activity of the four fertilizer dose treatments showed different values. Purslane plants produced the highest antioxidant activity with a fertilizer dose of 10 g/polybag, which was 0.6440 µmol TE/g fw. At the same time, the lowest antioxidant activity was produced by control plants with an antioxidant activity of 0.2103 µmol TE/g fw. Fertilizer doses of 20 g/polybag and 30 g/polybag produced antioxidant capacities close to that of purslane plants at a dose of 10 g/polybag. Fertilizer doses of 20 g/polybag and 30 g/polybag produced an antioxidant activity of 0.5450 µmol TE/g fw and 0.6192 µmol TE/g fw. Although the highest antioxidant activity was produced by plants treated with 10 g/polybag fertilizer, Tukey's further tests revealed no significant difference between the doses of fertilizer treatment and the DPPH antioxidants produced. These results indicate that the dose of fertilizer treatment does not significantly affect the antioxidant activity of the DPPH method. However, purslane plants that were given a fertilizer dose of 10 g/polybag were also known to have the highest DPPH antioxidant activity. These results indeed illustrate the relationship between phenolic content, flavonoids, and the antioxidant activity of DPPH. Thus, the treatment dose of 10 g/polybag should also produce the highest antioxidant activity in the FRAP, ABTS and CUPRAC methods.

The FRAP method, or Ferric Reducing Antioxidant Power, is a method for determining antioxidant content commonly used in laboratories. This method has the principle of reducing the Fe^{3+} complex from tripiridyltriazine

$Fe(TPTZ)^{3+}$ to Fe^{2+} complex; $Fe(TPTZ)^{2+}$ in an acidic medium will turn blue, and the intensity of the resulting blue colour will be measured using a spectrophotometer [44]. This method also uses a trolox standard to measure antioxidant activity so that the results obtained are expressed in trolox equivalents. The results of measuring the antioxidant activity of the FRAP method for the four fertilizer dose treatments are shown in Figure 4.

The antioxidant activity of FRAP in plants treated with fertilizer doses also showed different values. Plants still produced the highest antioxidant activity at a dose of 10 g/polybag, which was 4.8358 µmol TE/g fw. In comparison, the lowest antioxidant activity was still produced by control plants with a value of 1.4640 µmol TE/g fw. For comparison, a fertilizer dose of 20 g/polybag produces an antioxidant activity of 3.5730 µmol TE/g fw, while a fertilizer dose of 30 g/polybag produces an antioxidant activity of 4.3871 µmol TE/g fw. These results indicate a significant difference in Tukey's follow-up test. These results also align with the phenolic content, flavonoids, and antioxidant activity that have been analyzed previously.

Measurement of antioxidant activity using the ABTS method has the same principles as the DPPH method. The ABTS compound is a radical with a nitrogen centre with a characteristic blue-green colour. It will be reduced to a colourless non-radical form when it reacts with antioxidants [45]. After reacting with antioxidants in the sample, the reduced colour intensity can be measured using a spectrophotometer. The results of measuring the antioxidant activity of the ABTS method for purslane plants treated with fertilizer doses are shown in Figure 5.

The highest antioxidant activity was obtained from purslane plants at a dose of 10 g/polybag with a value of 12.1276 µmol TE/g fw. As a comparison, the lowest antioxidant activity was produced by control plants with a value of 8.0378 µmol TE/g fw. Purslane plants with fertilizer doses of 20 g/polybag and 30 g/polybag produced antioxidant capacities of 11.7868 µmol TE/g fw and 11.8281 µmol TE/g fw, respectively. The results showed a significant effect between the doses of fertilizer treatment on the antioxidant activity of purslane plants. These results also prove a strong relationship between polyphenolic compounds and the antioxidants they produce. Of course, the resulting antioxidant activity showed the highest content at a fertilizer dose of 10 g/polybag.

The CUPRAC (Cupric ion reducing antioxidant capacity) method of antioxidant capacity testing has principles similar to the FRAP method of antioxidant testing. The antioxidant compounds in the sample will reduce the Cu^{2+} complex to Cu^{+} complex [46]. This reaction is indicated by a change in the colour of the solution from blue to yellow. The intensity of the resulting colour can then be measured for its absorbance using a spectrophotometer. The results of measuring the antioxidant activity of the CUPRAC method for various fertilizer dose treatments are shown in Figure 6.

Purslane plants that were given fertilizer at a dose of 10 g/polybag still produced the highest antioxidant activity, amounting to 4.6839 µmol TE/g fw. While the control plants also still produced the lowest activity, 0.6672 µmol TE/g fw. The antioxidant activity resulting from fertilizer doses of 20 g/polybag and 30 g/polybag still follows the same pattern as in the DPPH, FRAP, and ABTS methods. Purslane plants with a fertilizer dose of 20 g/polybag produced an antioxidant activity of 3.4339 µmol TE/g fw, while a fertilizer dose of 30 g/polybag produced an antioxidant activity of 4.1617 µmol TE/g fw. These results are by order of antioxidant activity in the previous method, which obtained antioxidant activity at a dose of 10 g/polybag > 30 g/polybag > 20 g/polybag > control. Furthermore, the fertilizer dose treatment had a significant effect on the antioxidant activity of CUPRAC. This statement was reinforced by Tukey's further test, which stated that there was a significant difference between the fertilizer dose treatment on the CUPRAC antioxidant activity of purslane plants.

The antioxidant activity from all the methods used showed the highest at the fertilizer dose of 10 g/polybag. In contrast, the lowest antioxidant activity of all methods resulted from control plants that were not given fertilizer. The fertilizer itself can cause this result. Appropriate fertilizer application can increase the nutritional content of plants and the secondary metabolites produced. However, excessive application of fertilizers can reduce the ability to synthesize essential compounds in plants, including the secondary metabolites of these plants [10, 11].

4. Conclusions

The application of cow manure to purslane plants did not significantly affect the growth of the number of branches and the number of leaves produced. Purslane plants fed cow manure at a dose of 10 g/polybag produced the highest phenolic, flavonoid and antioxidant activity. Meanwhile, plants not given fertilizer treatment produced the lowest phenolic, flavonoid, and antioxidant content. Giving cow manure can affect polyphenol content and antioxidant activity but does not affect the growth of branches and leaves of purslane plants.

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