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Growth, Phenolic, and Flavonoid Production of *Adenostemma platyphyllum* at Different Dosages of Cow Manure

Pertumbuhan, Produksi Fenolik, dan Flavonoid *Adenostemma platyphyllum* pada Dosis Pupuk Kandang Sapi Berbeda

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ABSTRACT

Adenostemma platyphyllum is a weed plant containing various secondary metabolites, such as phenolics and flavonoids, making it essential to cultivate properly. This study aims to determine the optimal cow manure dosage for plant growth, focusing on phenolic and flavonoid production in the cultivation of A. platyphyllum. The experiment employed a randomized complete block design (RCBD) with a single factor: cow manure doses of 0.5, 5, 10, 15, and 20 ton/Ha. Phenolic content was measured using the Folin-Ciocalteu reagent, while flavonoid content was assessed using the $AlCl_3$ reagent. Results indicate that the highest phenolic and flavonoid production occurred in plants treated with 20 ton/Ha manure. This dosage also resulted in the best plant growth, yielding the highest crop, number of leaves, and branches compared to other treatments.

Keywords: *Adenostemma platyphyllum*, phenolic, flavonoid, cow manure, plant growth

ABSTRAK

Legetan warak (*A. platyphyllum*) merupakan tumbuhan gulma yang mengandung berbagai metabolit sekunder antara lain fenolik dan flavonoid, sehingga perlu dibudidayakan dengan baik. Penelitian ini bertujuan untuk mendapatkan dosis pupuk kandang sapi yang terbaik untuk pertumbuhan tanaman, produksi fenolik, dan produksi flavonoid pada budidaya *A. platyphyllum*. Percobaan menggunakan rancangan acak kelompok 1 faktor: pupuk kandang sapi dosis 0, 5, 10, 15 dan 20 Ton Ha^{-1}. Kadar produktivitas fenolik ditentukan dengan menggunakan pereaksi *Folin-Ciocalteu* dan produktivitas flavonoid menggunakan pereaksi $AlCl_3$. Produktivitas fenolik dan produktivitas flavonoid tertinggi terdapat pada tanaman yang diberi pupuk (20 Ton Ha^{-1}). Dosis pupuk kandang sapi sebanyak 20 Ton Ha^{-1} juga merupakan dosis terbaik untuk pertumbuhan tanaman karena menghasilkan hasil panen, jumlah daun, dan jumlah cabang paling tinggi dibandingkan tanaman dengan perlakuan lainnya.

Kata Kunci: *Adenostemma platyphyllum*, fenolik, flavonoid, pupuk kandang, pertumbuhan tanaman

INTRODUCTION

Indonesia is known for its diversity of medicinal plants that have been used for generations as herbal or traditional medicine. Most plants that can be used as medicines are not yet known for their efficacy by the general public, so they have never been properly maintained and live as wild plants (Hariana., 2018). One example of these plants is *Adenostemma platyphyllum. A. platyphyllum* (legetan warak), is a type of medicinal plant that is widely used to treat diarrhea, dysentery, and burns and is known to be effective in dealing with swollen eyes (Chen et al., 2011).

The legetan warak is a plant that comes from the Asteraceae family in the *Adenostemma* genus. According to Fauzan et al., (2018), this genus contains various secondary metabolite compounds, such as alkaloid compounds, terpenoids, and the main compounds from the phenolic group, including flavonoids. *A. platyphyllum* has a height of about 30 - 55 cm. This plant is classified as a herb or weed that lives in moist and open places such as forests and shrubs (Chen et al., 2011). The shape of the stem of *A. platyphyllum* is hard, straight, and stiff, and the stem of this plant has branches at the top. *A. platyphyllum* plant whose (**Figure 1**) leaves are 4 – 12 cm long, 2 – 5 cm wide, green in color with a bare surface, thin, oval in shape, and serrated leaf edges (Najibufahmi et al., 2019). The benefits of this plant are so many that it needs to be reproduced by way of cultivation. Plant cultivation is a technique to develop a type of plant or nursery in certain ways (Nurmayulis & Hermita, 2015).

Figure 1. *Adenostemma platyphyllum* plant

Various types of fertilizers can be used to optimize plant cultivation, one of which is organic fertilizer or manure. This research focuses on using organic fertilizers as an optimal driver for the growth of *A. platyphyllum* plants. Organic fertilizers are derived from all types of organic materials from plants or animals that undergo engineering processes and provide nutrients for plants (Roidah, 2013). Organic fertilizers have a role in soil chemical properties, including providing macronutrients (N, P, K, Ca, Mg, and S) and micro (Zn, Cu, Mo, Co, B, Mn, and Fe), increasing Cation Exchange Capacity (CEC), soil, and can form complex compounds with toxic metal ions such as Al, Fe, and Mn so that these metals are not toxic (Hartatik et al., 2015).

Even though applying fertilizer is a treatment that can optimize plant cultivation, its use must be based on rules or regulations and pay attention to the plants' needs. Providing sufficient and not excessive fertilizer doses, namely 5 tons Ha^{-1}, can increase the growth and crop yield of eggplant and Napier grass (Evanita et al., 2014). Based on previous studies in this study and supported by related explanations, researchers wanted to examine the effect of organic fertilizers on optimizing the cultivation of legetan warak plants (*A. platyphyllum*) to obtain the highest levels of phenolics and flavonoids. Fertilization with hydroponic system a can increase the phenolic and flavonoid productivity of *A. platyphyllum* (Tamsin et al., 2023). Researchers want to study the *A. platyphyllum* plant because this plant is one of the plants with many properties and is in great demand by the public. The research was conducted by measuring the levels of phenolics and flavonoids in optimizing the cultivation of *A. platyphyllum* plant growth using one factor. One-factor randomized complete block design (RCBD) by giving different treatments related to 5 levels of treatment on plants. The results of this research are expected to provide information regarding the optimal treatment effect of different doses of cow manure to produce the highest phenolic and flavonoid productivity from *A. platyphyllum.*

MATERIALS AND METHODS

1. Plant Cultivation

The location used as a place for our research is the Biopharmaca Cultivation Conservation Unit, Tropical Biopharmaca Research Center IPB University, which is located at S-6°54'74,7", E106°71'57,9", and an altitude of 140 meters above sea level. This experiment used a one-factor randomized complete block design (RCBD). The treatment consisted of 5 dosage levels of cow manure, namely 0, 5, 10, 15, and 20 Ton Ha^{-1}. Each treatment was repeated three times so that there were 15 experimental units. Each experimental unit consisted of 33 plants, so there were 495 plants. From each experimental unit, 8 sample plants were taken for observation.

The nursery was prepared with *A. platyphyllum* seedlings propagated by stem cuttings with a cutting length of about 10 cm. The cutting material used comes from mother plants that are already available in the garden and the plants are 2 months old. Stem cuttings are taken from the tip of the plant 10 cm by removing the shoot. Stem cuttings were planted in polybags measuring 10 cm × 10 cm with a planting medium mixed with soil, manure, and fresh husks at a ratio of 1:1 v/v. Seedlings are stored under 50% shade for 1.5 months. Furthermore, the plant seeds are transferred to land that has been determined for the mineral content of the soil, given 50% shade, and cow manure according to the treatment.

2. Plant Measurement

The plant growth variables observed during treatment until harvest included plant height, number of leaves, and number of branches. Observations included non-destructive observations at weekly intervals starting 1 week after planting (week) for 6 weeks. Observations were made for each of the 8 plants in each treatment and recorded. Using a ruler, plant height was measured from the base of the stem above the ground to the tip of the highest branch. The number of leaves was measured based on the number of leaves on each of the 8 plants once a week during the study starting at 1 week. The number of branches is measured based on the primary branch, which is measured once a week. Simplicia fresh weight, dry weight, water content, phenolic and flavonoid content at harvest was measured at 6 weeks.

3. Moisture Content Determination

The water content of simplicia was determined according to (AOAC et al., 2016). A porcelain cup was cleaned and dried at ±105°C for 30 minutes using an oven, then cooled in a desiccator for 30 minutes. This stage was repeated twice to obtain a constant weight from the porcelain cup. After constant weight, 5 g of *A. platyphyllum* leaf simplicia was put into a porcelain cup and in the oven at ±105°C for 3 hours. The porcelain cup containing the simplicia was then removed and cooled in a desiccator for 30 minutes. After it cooled, the porcelain cup containing the simplicia was then weighed. This process was repeated 3 times to obtain a constant weight.

4. Phenolics Productivity

According to Budiarti et al., (2019), the total phenolic content was determined. 1 g of *A. platyphyllum* leaf simplicia was dissolved in 7 mL of methanol in a 25 mL vial. The sample was then homogenized using shakers for 3 hours, and the sample was then filtered using filter paper to obtain the filtrate. The residue recovered was extracted using solvent 2 times until the volume reached 20 mL. 20 µL of sample extract was added with 110 µL of Folin-Ciocalteu reagent (15%), and 80 µL of NaOH (1M), then incubated at room temperature for 30 minutes in the dark. The absorbance of the mixture was measured at a wavelength of 730 nm. Productivity can be determined by multiplying the total phenolic content by the dry weight of the yield per plant. Gallic acid was used as standard. The concentration of gallic acid used is 0; 0.092; 0.184; 0.368; 0.735; 1.471; 2.941 µmol/mL solution.

5. Flavonoids Productivity

According to Moncayo et al. (2021), the total flavonoid content was carried out. A total of 10 µL of the extract was mixed with 60 µL of methanol, 10 µL of aluminum chloride (10% w/v), 10 µL of CH_3COOK (1 M), and 110 µL of distilled water on a 96-well micro-plate and incubated at room temperature for 30 minutes in a room dark. Absorbance was measured at a wavelength of 415 nm. Productivity can be determined by multiplying the total flavonoid content by the dry weight of the yield per plant. Quercetin was used as standard. The quercetin concentration was 0; 0.092; 0.184; 0.368; 0.735; 1.471; 2.941 µmol/mL solution.

6. Data Analysis

The data obtained were analyzed using a one-factor randomized complete block design (RCBD) method with three replications. The statistical test used is the variance test or the F test at the 5% confidence level. If the test results are significantly different, then the data is continued with the Duncan Multipic Range Test to see which data causes the difference. Our analysis was performed using the SAS program (https://welcome.oda.sas.com/).

RESULTS AND DISCUSSION

1. Cultivation Condition

The type of soil used is latosol soil. Latosol soils are generally soils that have undergone heavy weathering so that many alkaline cations are leached, making the pH of this type of soil slightly acidic (Saptiningsih & Haryanti, 2015). The soil analysis results showed a low soil fertility level marked by low total N, but organic C, P, CEC, and BS content was found in medium-category soils (**Table 1**).

The cow manure used in this study and its composition can be seen in **Table 2**. The table shows that the pH of the fertilizer is in the acidic category, the organic C is in the moderate category, the total N is low, the C/N ratio is according to standards, while the P_2O_5 and K_2O are in the low category. At the beginning of the cultivation period, some *A. platyphyllum* plants experienced stress because they had adapted to the new planting media, but after 1 week, the *A. platyphyllum* plants grew well (**Figure 2a**). At the age of 5 weeks, the plants began to enter the generative phase marked by the appearance of flowers. Because the available P element in the soil is in the medium-high category, thus spurs the growth of flowers on plants to be faster. Some attacks by parasites and pests experienced during the cultivation process included attacks by female cord weeds, which grew and spread so that the host plants were covered and died (**Figure 2b**), and plants attacked by caterpillar pests (**Figure 2c**).

The dominant weeds during the growth process of *A. platyphyllum* are bandotan (*Ageratum conyzoides*) and tali putri (*Cassyta filiformis*). Control of these two weeds is done manually. The weed *A. conyzoides* was controlled by removing it from the soil and keeping it away from the research area. The *C. filiformis* weed was controlled by removing the coils from the *A. platyphyllum* plants and keeping them away from the research area. The *C. filiformis* weed is a bit annoying because it can cause *A. platyphyllum* plants to die due to its entanglement. Pests that attack the leaves, in the form of caterpillars and grasshoppers, begin to appear from the third to sixth week. Control is carried out manually by picking from leaves and keeping them away from the research area. Weed and pest attacks do not have a significant effect on crop yield growth. During the research process weed and pest control did not use chemical herbicides and pesticides.

The success or failure of cultivating a plant is largely determined by the care and method of planting. Plant cultivation requires good growing media and standard care that must be met. This is done so the plants get the nutrients and other necessities for their growth. In addition to the availability of nutrients in the soil, plants also need other supporting factors that can accelerate and optimize their growth. This is because the physical factors of the soil will affect the biological and chemical aspects. This factor can be in the form of the physical state of the soil as a planting medium. According to (Lawenga et al., 2015), in maintaining the physical condition of soil which is often neglected because it focuses on its chemical fertility, fertilization is needed to maintain the physical condition of the soil.

The soil used has a pH of 4.69 which is acidic, affecting the availability of nutrients for plants, especially N, K, and Ca. N, K, and Ca availability tends to decrease with decreasing pH, while elements P and B do not directly affect pH (Handayanto et al., 2017). Soil analysis on P measurement was carried out using a Bray I extractor and 25% HCl (**Table 1**). The P levels found in the soil are following the literature according to (Aprianto et al., 2020) that is, the P content using the 25% HCl extractor produces a higher P level than using the Bray I extractor because the 25% HCl extractor can dissolve almost all the available P, while the Bray I extractor only dissolves some of the P that is in the soil.

Table 1. Soil composition used in the Biopharmaca Cultivation Conservation Unit

Test Parameters	Results	Criteria
pH (pH 1:5)	4.69	Acid
Organic C (%) (Walkley&Black)	2.05	Medium
Total N (%) (Kjeldahl)	0.2	Low
P-Bray (ppm) (Bray I)	8.0	Medium
P-HCl 25% (ppm) (HCl 25%)	466.4	High
CEC (cmol/kg) (*N* NH_4OAc pH7.0)	17.17	Medium
Base Saturation (%)	45.98	Medium

Note: ITSL Laboratory (2021)

Table 2. Composition of cow manure used

Parameters	Results*	Standard*	Criteria
pH	5.24	4-9*	Standard Compliant
Water Content	2.68	8-20*	Not Standard
Organic C (%)	23.48	≥15*	Standard Compliant
Total N (%)	1.60	≥2*	Not Standard
C/N	14.675	≤25*	Standard Compliant
P_2O_5 (%)	0.64	≥2*	Not Standard
K_2O (%)	0.23	≥2*	Not Standard

Note: ITSL Laboratory (2021) (Results), (Keputusan Menteri Pertanian, 2019)

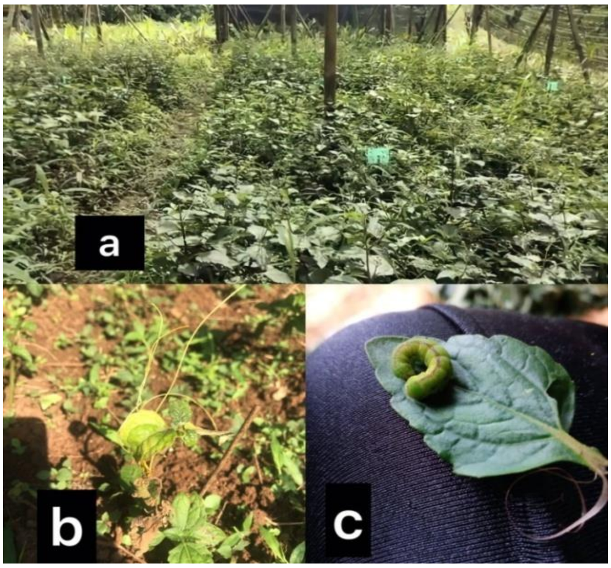

Figure 2. Conditions during the observation of the plants: (a) Cultivation of *A. platyphyllum*, (b) Tali putri (*Cassyta filiformis*) (c) caterpillars

According to (Andayani & Sarido, 2013), a deficiency of N and P nutrients can cause plant metabolism and development disturbances. Based on the criteria above, it indicates that the soil used in this experiment has a moderate level of fertility and organic C content. One alternative that can be used to overcome this problem is the use of organic materials such as manure.

According to Amir et al., (2017), manure has few nutrients but has several advantages, including encouraging the life of microorganisms, adding nutrients, increasing humus, and improving soil structure. Manure is slower to react than artificial fertilizers because it takes a long time to give good results in soil fertilized with manure. If the availability of nutrients from manure is sufficient, the growth results of plants will be good. Conversely, if the availability of nutrients from manure is insufficient, it will provide less growth results.

Nutrients N, P, and K are elements that function as the formation of new cells and certain proteins in plants (Andayani & Sarido, 2013b). Nutrient N is an important element for spurring plant vegetative growth, namely the formation of new cells such as leaves, and branches and replacing damaged cells (Sitorus & Tyasmoro, 2019). Generally, nitrogen nutrients plants absorb nitrate (NO_3^-) and ammonium (NH_4^+). N elements (nitrate and ammonium) adsorption ability is generally regulated complexly.

Plants absorb element P generally in the form of primary orthophosphate ($H_2PO_4^-$) and, to a lesser extent, in the form of hydrogen phosphate ions or secondary orthophosphate (HPO_4^{2-}) (Dianoor et al., 2022). Element P plays a role in capturing sunlight and converting it into biochemical energy. Element P is a constituent component of enzymes, a constituent of plant cell membranes, and plays a role in nucleotides as a building block for nucleic acids. The protein synthesis process, especially found in green tissue, stimulates the formation of flowers, and the synthesis of carbohydrates is also a function of the P element (Hafizah & Mukarramah, 2017). In addition to element P, potassium (K_2O) also plays an important role in plant growth. The functions of potassium include forming and transporting carbohydrates, increasing the growth of meristem tissue, as a catalyst in protein formation, and regulating stomata movement. The K content in cow manure is also low, so it is suspected that it cannot meet the nutritional needs of plants (Hafizah & Mukarramah, 2017).

2. Plant Growth

The observed plant growth was plant height, number of leaves, and number of branches. Plant height, number of leaves, and number of branches are supported by the availability of N, P, and K in the soil. **Figure 3** shows the growth of *A. platyphyllum* at the age of 5 week.

Plant height is a variable of plant growth. The analysis of variance showed that the effect of cow manure on plant height was not significantly different, but it was seen that the cow manure produced higher *A. platyphyllum* plants compared to the treatment without cow manure. The highest plants were in the treatment with a dose of 20 Ton Ha^{-1} at 6 weeks with a height of 35.18 cm, while the lowest was at a dose of 0 Ton Ha^{-1} with the same age, namely 6 week of 29.21 cm (**Figure 4**). With the increasing age of the plant, the need for nutrients, especially Nitrogen (N), is greater but cannot be fully met by the soil where it grows, so the application of cow manure can increase the availability and absorption of N elements which can help plant vegetative growth.

Figure 3. *A. platyphyllum* plants at various doses of cow manure at 5 weeks
Note: P0 = 0 Ton Ha^{-1}, P5 = 5 Ton Ha^{-1}, P10 = 10 Ton Ha^{-1}, P15 = 15 Ton Ha^{-1}, P20 = 20 Ton Ha^{-1}

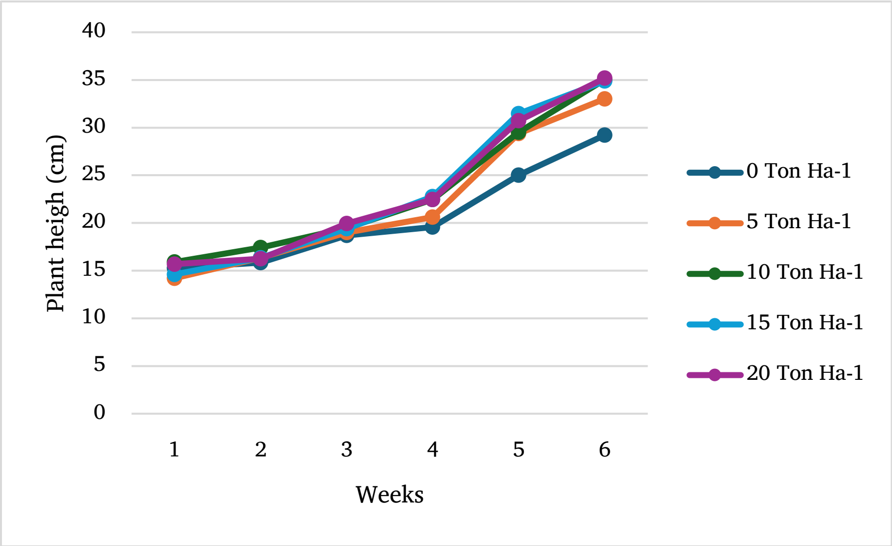

Figure 4. Plant height of *A. platyphyllum* at doses of cow manure

Another plant growth variable that plays an important role besides plant height is the number of leaves. **Table 3** shows the results of the analysis of variance for the number of leaves at different doses of cow manure for 6 weeks. Based on the table, the response of cow manure application to the number of leaves of *A. platyphyllum* was significantly different at 3 week and 4 weeks ($P < 0.5$). Plants without cow manure produced the least number of leaves compared to the other 4 treatments, namely 18 leaves at 3 week and 22 leaves at 4 weeks, while the highest number of leaves was found in the treatment dose of 20 Ton Ha^{-1} at 5 weeks of 75 strands.

In addition to plant height and number of leaves, the number of branches is also an important variable in plant growth. **Table 4** shows that the response between cow manure and the number of branches of *A. platyphyllum* up to 5 weeks was significantly different ($P < 0.5$). The highest number of branches were at the age of 5 weeks with a dose of cow manure 20 Ton Ha^{-1} with 9 branches, while the lowest number of branches were found in plants without cow manure treatment with 4 branches.

Table 3. Number of leaves of *A. platyphyllum* at various doses of cow manure

Treatment Cow manure (Ton Ha^{-1})	Number of plant leaves at age (strands)					
	1 week	2 weeks	3 weeks	4 weeks	5 weeks	6 weeks
0	13	16	18^{b}	22^{c}	34	29
5	16	22	26^{a}	33^{ab}	50	39
10	14	20	27^{a}	35^{ab}	74	50
15	15	19	27^{a}	32^{b}	59	46
20	15	20	34^{a}	43^{a}	75	62
F test	ns	ns	**	**	ns	ns
Pr>F	0.92	0.53	0.01	0.008	0.25	0.12

Note: ns = non-significant * = significantly different on α = 5% ** = significantly different on α = 1%

Table 4. Number of branches of *A. platyphyllum* on various doses of cow manure

Treatment Cow manure (Ton Ha^{-1})	Number of plant branches at age (branches)					
	1 week	2 weeks	3 weeks	4 weeks	5 weeks	6 weeks
0	3	3	3	3	4^{b}	3
5	3	3	3	5	7^{a}	5
10	2	3	3	6	9^{a}	5
15	3	3	4	5	8^{a}	5
20	3	3	4	5	9^{a}	7
F Test	ns	ns	ns	ns	*	ns
Pr>F	0.71	0.39	0.16	0.30	0.003	0.18

Note: ns = non-significantly * = significantly different on α = 5% ** = significantly different on α = 1%

The cultivation process and observations were made on the growth of *A. platyphyllum* plants for 6 weeks, then they were harvested, and the fresh and dry weights of the leaves were weighed to see the optimum treatment dose of cow manure which produced the highest biomass. The results of the analysis of variance showed that there was no significant difference between the cow manure and the fresh weight of *A. platyphyllum* plants, but it can be seen in **Table 5** that the highest leaves fresh weight was in the treatment dose of 20 Ton Ha^{-1} (20.923 g/plant), while the lowest fresh weight dose was found at a dose of 0 tons/ha (20.92 g/plant) (**Table 5**). In addition to the fresh weight, **Table 5** shows cow manure's response with a significantly different dry weight (P<0.01). The dry weight of *A. platyphyllum* plants without treatment produced the lowest dry weight compared to the other four treatments, namely 1.30 g/plant. While the highest dry weight was obtained at a dose of 20 Ton Ha^{-1} of 2.88 g/plant.

Table 5. Fresh and dry weight of *A.* platyphyllum leaves at harvest

Treatment Cow manure (Ton Ha^{-1})	Fresh Weight (g/plant)	Dry Weight (g/plant)
0	8.65	1.30^{a}
5	14.71	1.99^{b}
10	14,21	1.96^{b}
15	15.37	2.06^{b}
20	20.92	2.88^{c}
F test	ns	**
Pr>F	0.14	0.0001

Note: ns = non-significantly * = significantly on α = 5% ** = significantly on α = 1%

Plant height will affect the increasing age of the plant, so the need for nutrients, especially Nitrogen (N), is greater but cannot be completely met by the soil where it grows, so the application of cow manure can increase the availability and absorption of N elements which can help plant vegetative growth. Giving the right dose of cow manure will provide the N nutrients plants need. Based on research that has been done, a dose of 20 Ton Ha^{-1} cow manure is the right dose for *A. platyphyllum* plant growth because it produces the highest plants compared to other plants. Sriyanto et al. (2015) suggested that the element N is needed to stimulate plants' vegetative growth, such as stems, roots, leaves, and branches.

Another plant growth variable that plays an important role besides plant height is the number of leaves. According to Masruhing et al., (2019), the part of the plant that contributes the most to plant growth and development is the leaves. The more the number of leaves on a plant, the more light is absorbed by the plant for the photosynthesis process, thereby helping to increase the primary metabolites produced. Furthermore, primary metabolites will form secondary metabolites that support the protection and adaptability of the plant itself.

The vegetative phase of plant growth of *A. platyphyllum* only reached the age of 5 week, while at 6 weeks, it had entered the generative phase. This is indicated by the appearance of flowers at the age of 5 week (**Figure 3**) because flowers are a response to generative growth (Hana et al., 2020), so the number of leaves decreases at 6 week. Apart from the fact that the *A. platyphyllum* plant has entered the generative phase at the age of 6 week, the decrease in the number of leaves is also since nutrients, especially N in the fertilizer, can only help the process of plant growth until the age of 5 week. There are disturbances caused by the weed *C. filiformis* and caterpillars, but it did not have a significant effect on reducing the number of leaves and branches of *A. platyphyllum* (**Figures 2b** and **2c**).

In addition to plant height and number of leaves, the number of branches is also an important variable in plant growth. The results in the table follow those carried out by Kurniawan et al. (2014), namely increasing the dose of fertilizer can stimulate lateral root meristem activity and nutrient uptake, especially N, because high N is needed to increase vegetative growth, such as the formation of new branches. However, the uptake of nutrients that contain available N elements can only help the growth process until the plant is 5 weeks old. In comparison, at 6 weeks, the plants have entered the generative phase because the available N element is limited, so the number of branches has decreased. The results of fresh and dry weight are also in line with the research of Kurniawan et al. (2014), which states that applying organic fertilizer will increase the fresh and dry weight of a plant. Also, all of this growth parameters related to research of Tamsin et al. (2023), which also states using different concentration of hydroponic solutions apply in *A. platyphyllum* with higher concentrations make the overall growth of *A. platyphyllum* optimum. Plant growth in the form of height, number of leaves, and number of branches resulting from organic cultivation are still relatively the same when compared with the results of research (Nurfalah et al., 2023) which combines organic and inorganic fertilizers on *Adenostemma madurense* plants.

3. Water Content, Phenolics Productivity, and Flavonoids Productivity

The water content was repeated three times for each simplicia. The water content produced in *A. platyphyllum* leaves were not significantly different, ranging from 8 - 9% (**Table 6**). The results of phenolics productivity variance showed that the effect of cow manure application on phenolics productivity content was significantly different, and the highest phenolics productivity content was found in *A. platyphyllum* plants with cow manure of 20 Ton Ha^{-1} application of 82.8 μmol GAE/g plant while the lowest phenolics productivity content was in without treatment of 42.1 μmol GAE/g plant (**Table 6**). Determination of the flavonoid productivity content of the methanol extract of *A. platyphyllum* leaves was carried out in triplicate. Flavonoid productivity levels at each dose did show significantly different results. The highest flavonoid productivity levels were obtained in *A. platyphyllum* plants with a dose of 20 Ton Ha^{-1} cow manure of 35.8 μmol QE/g plant, while the lowest flavonoid productivity content was found without treatment of 16.4 μmol QE/g plant (**Table 6**).

Determination of the water content in a simplicia is carried out to determine the quality and maximum limit of the water content of the simplicia. The method used in analyzing the water content is the gravimetric method using the help of an oven. Determination of water content is done to determine the quality of the materials used. The results in this study are close to the water content value according to the literature, which is 7% (Fauzan et al., 2018).

Phenolic compounds are a class of secondary metabolite compounds generally found in plants and have many specific benefits, including anti-inflammatory, anticancer, antimicrobial, and several other benefits in disease prevention (Vuolo et al., 2019). The Folin-Ciocalteu method is one of the most frequently used methods to determine the total phenolic content in plants using the gallic acid standard curve as a standard; this is because gallic acid is a derivative of hydroxybenzoate which comes from a simple phenolic acid which is pure and stable. In determining the total phenolic content, the darker the blue color produced, the higher the absorbance obtained (Khadijah et al., 2017).

The research results (**Table 6**) show that cow manure can increase phenolic and flavonoid productivity. This is in line with the research results by

(Liwanda et al., 2023) which states that cow manure can increase phenolic, flavonoid, and antioxidant activity in purslane plants.

The study's results (**Table 6**) show that the difference is that plants that grow in an environment lacking in nutrients will experience stress, thereby increasing the production of the metabolite compounds produced by these plants (Montgomery & Biklé, 2021). Plants tend to produce higher secondary metabolites because these plants will increase their resistance and defense against disease by sacrificing plant growth.

Table 6. Water content, phenolics productivity, and flavonoids productivity of *A. platyphyllum* simplicia

Treatment Cow mature (Ton Ha^{-1})	Water Content (%)	Phenolics Productivity (μmol GAE/g plant)	Flavonoids Productivity (μmol QE/g plant)
0	9.01	42.1^{e}	16.4^{e}
5	8.22	58.0^{d}	23.8^{c}
10	8.39	50.8^{c}	23.4^{d}
15	9.18	63.2^{b}	28.4^{b}
20	8.60	82.8^{a}	35.8^{a}

Note: Different letters in the same column indicate a significant difference at $\alpha = 0.05$

Flavonoid content was determined using a colorimetric technique using aluminum chloride reagent, which is based on the formation of a stable quercetin-aluminum chloride complex, resulting in a shift in wavelength to the visible direction, which is indicated by the formation of a more yellow-colored solution. This technique for determining the flavonoid content using aluminum chloride is one of the most commonly used procedures in evaluating medicinal plant samples (Pękal & Pyrzynska, 2014). The compound used as a standard for determining the total flavonoid content is quercetin, which is included in the flavonoid group of flavonols that reacts with $AlCl_3$ to form a stable complex. The wavelength used in this study is 415 nm. In determining the levels of flavonoids, adding potassium acetate to detect the presence of the 7-hydroxyl group and maintaining the wavelength in the visible area, while the 30-minute incubation treatment carried out before the measurement is intended to make the reaction run perfectly, thus providing maximum color intensity (Azizah et al., 2014). Based on previous research on plants that are in the same genus as *A. platyphyllum*, namely *A. lavenia*, the total flavonoid content produced by *A. lavenia* is 0.54 μmol QE/g simplicia (Budiarti et al., 2019), so the results of the research that have been carried out contained higher flavonoids than the literature. The productivity of phenolics and flavonoids from organic cultivation is still relatively low, namely 82.8 μmol GAE/g plant and 35.8 μmol QE/g plant respectively when compared with the results of combined organic and inorganic fertilizer cultivation carried out by (Nurfalah et al., 2023) respectively 739.50 μmol GAE/g plant and 97.37 μmol QE/g plant in plants of the genus Adenostemma.

CONCLUSION

This research was carried out using organic cultivation to improve the quality and safety of medicinal plants. The use of different cow manure is to get the best dose based on growth and secondary metabolites. Plant height, number of leaves, number of branches, wet weight, dry weight, phenolic productivity, and flavonoid productivity were highest in the 20 Ton Ha^{-1} cow manure treatment. The 20 Ton Ha^{-1} cow manure treatment was the best dose for plant growth, phenolic productivity, and flavonoid productivity compared to other treatments.

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