The Effect of Various Guano Fertilizer Doses on *Physalis angulata* Growth, Flavonoid Content, and Saponin Content

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ABSTRACT

Physalis angulata L. is a valuable medicinal and nutritional plant. The plant's phytochemicals have been associated with various health benefits, making it a subject of increasing interest in pharmaceutical and dietary industries. However, the cultivation practices to optimize its growth and phytochemical composition remain relatively unexplored. Guano fertilizer is known for its nutrient-rich properties and has been successfully used in enhancing the growth of various crops. This study aims to bridge the knowledge gap by investigating the influence of different guano fertilizer doses on *P. angulata*, with a focus on growth parameters, flavonoid content, and saponin content. This study aims to know ability of guano fertilizer on several parameters. The research design used a completely randomized design with 6 doses treatments and 4 replications. The results showed that differences in the dose of guano fertilizer significantly affected plant height, number of leaves, number of fruits, stem diameter, and root length. But no significant effect on flowers, wet weight, and dry weight of stover. The highest levels of flavonoids and saponins were shown by 9 tons ha^{-1} treatment with a value of 4.16% in leaves, 0.192% flavonoids, and 0.28% saponins in fruit. The findings of this study provide valuable insights and offering a practical approach for optimizing growth and enhancing the phytochemical composition of *P. angulata*. The novelty of this research lies in its comprehensive investigation of the dose-dependent effects of guano fertilizer on *P. angulata* and contribute to the development of innovative agriculture.

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1. INTRODUCTION

The cultivation of *Physalis angulata* L., a versatile plant known for its pharmacological and nutritional significance, has gained substantial attention in recent years [1]-[3]. This perennial herbaceous plant has garnered interest due to its rich content of flavonoids and saponins, compounds with potential health-promoting properties [3]-[5]. The chemical content of *P. angulata* plants has at least 8 secondary metabolites, namely alkaloids, flavonoids, saponins, steroids, triterpenoids, monoterpenes, and sesquiterpenoids [6]. In addition, substances contained in *P. angulata* are vitamin C, sugar, malic acid, folacin, alkaloids, chlorogenic acid, elaidic acid, and tannins [5]. This plant can be used as a diuretic, and cough medicine, neutralizes poisons, has an analgesic effect, and activates the body's glandular functions. Specifically, flavonoid glucoside in *P. angulata* plants is a drug for diabetes mellitus because it can improve regulation in the blood and eliminate side effects (complications) of diabetes mellitus in which the sufferer's body cannot automatically control the level of sugar (glucose) in his blood [7]. Flavonoids are renowned for their antioxidant and anti-inflammatory effects [8], [9], while saponins exhibit a range of biological activities, including antimicrobial and anti-inflammatory properties [5], [10], [11]. Consequently, the demand for Phytochemical-rich extracts from *Physalis angulata* has surged in the pharmaceutical and nutraceutical industry [3], [5], [12].

Despite its potential, the cultivation practices and strategies to optimize *P. angulata* growth while enhancing its phytochemical content remain relatively underexplored. This dearth of research knowledge necessitates a comprehensive investigation into the factors that influence its growth and phytochemical composition. Among the various approaches to enhance plant growth, the use of guano fertilizer has demonstrated remarkable efficacy in promoting the growth of several crops [13]-[16]. Guano fertilizers contain a lot of nutrients for plants because they contain seeds derived from plants [17]. Nutrients that are mostly contained in guano fertilizers such as nitrogen 8.0-13%, phosphorus 5-12%, potassium 1.5-2.5%, calcium 7.5-11%, magnesium 0.5-1.0%, and sulfur 2.0-3.5% [16]. Guano contains phosphorus in the form of P_2O_5 by 20%. Its high nutrient content, including nitrogen, phosphorus, and potassium, makes it an attractive option for enhancing plant productivity [19].

While there is a growing body of literature on the cultivation and phytochemical composition of various medicinal plants, the specific focus on *P. angulata* remains somewhat limited. Existing research predominantly highlights the plant's phytochemical properties and their potential health benefits [2], [3], [12]. However, few studies have comprehensively addressed the factors affecting its growth and phytochemical content, especially in the context of agricultural practices. Consequently, there is a notable gap in knowledge concerning the optimization of *P. angulata* cultivation for enhanced phytochemical yield.

This study seeks to address this research gap by exploring the effects of different guano fertilizer doses on *P. angulata*. Specifically, the varying concentrations of guano fertilizer impact the plant's growth parameters, flavonoid content, and saponin content [16]. In doing so, the aim of this study is to shed light on the optimal fertilizer conditions for cultivating this valuable plant, with implications for both agronomic and phytochemical content. This research offers a novel perspective by investigating the dose-dependent effects of guano fertilizer on *P. angulata*. By evaluating the plant's growth parameters, flavonoid content, and saponin content, this research contributes to providing practical insights into the cultivation of this valuable herb. Other potential contributions of this study are twofold. Firstly, it offers practical recommendations for optimizing the growth of *P. angulata*, which may have significant implications for agricultural practices. Secondly, it explores the prospect of enhancing the phytochemical content of this plant, thereby bolstering its appeal to industries focused on pharmaceuticals and functional foods.

2. MATERIAL AND METHODS

The research was carried out in the experimental farm of the Major of Agroecotechnology, Faculty of Agriculture, Universitas Trunojoyo Madura with a height of ± 5 m above sea level from February to April 2018.

2.1. Materials

The tools used include a nursery, stationery, labels, buckets, rulers, calipers, analytical scales, and ovens. The materials used include *P. angulata* seeds, guano fertilizer, cow manure, pot tray, polybags measuring 35 x 35 cm, and mediterranean soil. *P. angulata* seeds come from the Batang-Batang, Sumenep Regency [4]. Nursery media and planting media in the form of a mixture of mediterranean soil and cow manure with a ratio of 1:1.

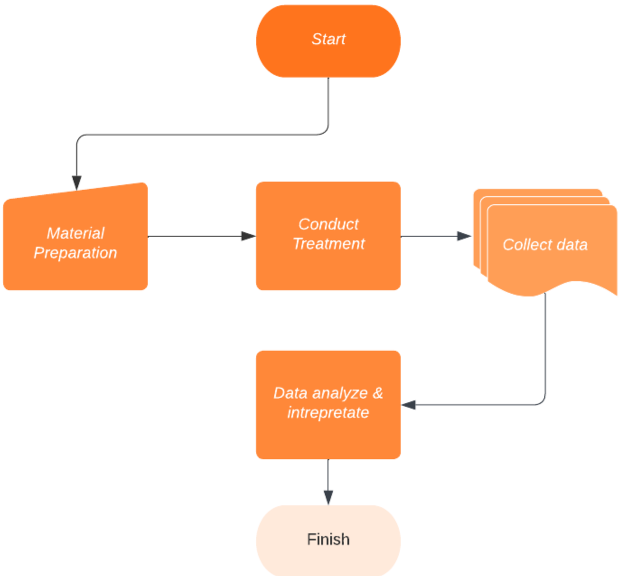

Fig. 1. Flowchart this research

2.2. Research design and flowchart

The study design uses a completely randomized design (CRD) with a single treatment factor, namely the dose of guano fertilizer with six dosage levels as follows. There were six treatments and four replications. So 24 experimental units were obtained. Each experimental unit consisted of three samples, so there were a total of 72 plant samples. The research flow follows the flow chart (Fig. 1).

P0=No guano fertilizer (control)
P1=3 tons ha^{-1} of guano fertilizer or equivalent to 13.24 g per polybag
P2=6 tons ha^{-1} of guano fertilizer or equivalent to 26.48 g per polybag
P3=9 tons ha^{-1} of guano fertilizer or equivalent to 39.72 g per polybag
P4=12 tons ha^{-1} of guano fertilizer or equivalent to 52.92 g per polybag
P5=15 tons ha^{-1} of guano fertilizer or equivalent to 66.20 g per polybag

2.3. Phytochemical measure

Determination of flavonoid levels was carried out using a spectrophotometric tool using aluminum chloride reagent. A total of 2 mL of extract solution with a concentration of 10 µg mL^{-1}, was added with 2 mL of 2% aluminum chloride and 1 M sodium hydroxide which has been dissolved in ethanol. The next process is by vortexing for 20 minutes. Solution mixture incubation process for 24 minutes and measure the absorbance at 415 nm. Make an average calculation of 3 measurements (triple repetition) and flavonoid content is expressed using standard comparison equivalents [17]. Saponin tests were carried out using the methods described by Lim et. al. (2020) [18].

2.4. Data analysis

Data obtained from observations were analyzed using analysis of variance (ANOVA). If there is a treatment effect on the observed parameters, then a further test is carried out using the Duncan multiple range test at the 5% confidence level by the R program[19].

3. RESULTS AND DISCUSSION

3.1. The effect of guano fertilizer on *P. angulata* growth

The results showed that the different dosages of guano fertilizer had different effects on all observational parameters. In the treatment of 15 tons ha^{-1} (P5) guano fertilizer dosage tends to have a good effect on the parameters of plant height, number of leaves, stem diameter, and root length at 2-8 WAP. Similarly, the amount of flower, the number of fruits, and stover weight. The P0 treatments differed from P1, P2, P3, and P4 where the results were generally lower. This is because, at the beginning of growth, plant roots are still small so fewer nutrient needs are low. However, entering the generative phase at week 6, more plant nutrients are needed. Plant height is one of the parameters that is often observed as an indicator of growth and as a parameter to measure environmental influences because plant height is a measure of growth that is easily seen [20]. Based on the results in Table 1, it can be seen that the treatment of P5 has the highest average height of plants with values of 20.79, 38.56, 60.24, and 67.24 cm respectively. Each at the age of observation 2, 4, 6, and 8 weeks after Planting (WAP). The provision of organic fertilizer requires a high volume because the nutrients contained in organic fertilizer are generally lower compared to the nutrient content found in inorganic fertilizers [21], [22]. Plant growth was strongly influenced by the increasing age of observation and the availability of sufficient nutrients in the soil. Thus, it can be seen the increase in plant height at each age of observation [23]. Guano reported high nitrogen content and release base on solution, pH, and temperature [24].

Table 1. Average *P. angulata* height due to provision of guano fertilizer in various ages of observation.

Treatment	Age of plant (WAP)			
	2	4	6	8
P0	13.73 a	25.48 a	51.81 a	56.03 a
P1	16.98 b	33.88 b	54.20 ab	56.54 bc
P2	19.21 cd	35.66 bc	56.18 abc	56.46 ab
P3	18.76 c	36.63 cd	57.94 bc	64.60 d
P4	17.98 bc	36.44 c	57.75 bc	65.09 de
P5	20.79 d	38.56 d	60.24 c	67.24 f
Duncan Test 5 %	*	*	*	*

Note: Numbers followed by the same letter are not significantly different based on the 5% Duncan test, *: significant effect.

The leaves are generally one of the plant organs which is considered as the main photosynthetic producer. So leaf observation is needed in addition to being an indicator of growth as well as supporting data to explain the growth process that occurs in the formation of plant biomass [25]. This is following the characteristics of good plants. The results of the analysis of variance in the parameters of the number of leaves showed that the dose of guano fertilizer had a significant effect on observations of ages 2 and 4 WAP. But no significant effect on observations of ages 6 and 8 WAP. The average number of leaves in each observation can be seen in Table 2. The highest average number of leaves were found in the P5 treatment at the age of observation (2 to 6 WAP), namely 15.88, 55.63, and 85.25 strands. While the lowest average number of leaves was found in the control treatment (P0). However, the number of leaves on plants decreased at 8 WAP [26]. The most influential nutrient in the growth and development of plant leaves is nitrogen, especially in the vegetative phase. The higher concentration of nitrogen will produce a greater number of leaves [27]. Guano fertilizer is one of the organic fertilizers which releases nutrients more slowly whereas nutrients are released slowly and continuously in a certain time [24].

Table 2. The average number of *P. angulata* leaves due to the provision of guano fertilizer in various ages

Treatment	Age of plant (WAP)			
	2	4	6	8
P0	8.50 a	27.38 a	72.50	76.38
P1	10.13 ab	49.25 b	76.38	72.25
P2	11.00 bc	52.25 bc	78.50	72.38
P3	12.25 cd	58.00 cd	77.13	73.00
P4	14.00 de	62.38 d	83.25	67.88
P5	15.88 e	55.63 bcd	85.25	75.00
Duncan Test 5%	*	*	ns	ns

Note: Numbers followed by the same letter are not significantly different based on the 5% Duncan test, *: significant effect.

Stem diameter is one of the main parameters for the estimation of annual plant biomass, but is rarely used as a growth parameter for annual crops [20]. In several studies of medicinal plants, stem diameter is one of the observation parameters to determine plant growth. Measurements were made using calipers. The results of the analysis of variance showed the lowest average value in the treatment (P0) without giving guano fertilizer with values of 0.06, 1.21, 1.46, and 1.55 cm, respectively (Table 3).

After the blossoming occurs, the petals will shrink, dry out, and eventually fall. Furthermore, the tip of the flower petal has locking and the middle part is bulging and over time, the part will become a fruit which will then become a fruit [28]. Determination of the number of fruits is done by counting the fruits that are ripe perfectly. Results of the analysis of variance showed that the treatment of guano fertilizer had a significant effect on all ages of observation. The average number of fruits in each observation can be seen in Table 4. The average value of *P. angulata* fruit in observations 4, 6, and 8 WAP increased. However, the highest value was at the time of observation at the age of 6 WAP contained in the treatment of 12 tons guano fertilizer dose (P4) with a value of 80.38. While the lowest average value is in the control (P0). But when the plant is 8 WAP the number of fruits decreases because the fruit begins to fall.

The root is an organ that has an important role in plant growth which serves to provide nutrients and water needed in plant metabolism [20]. Plant root systems are more controlled by the genetic characteristics of plants and other things that affect the root system, namely the condition of plants and growing media[29], [30]. Factors that influence root spread patterns include temperature, aeration, water availability, and nutrient availability [31]. Based on the results of the analysis of variance in differences in the guano fertilizer dosage on root length parameters showed a significant effect. The results of the analysis of variance showed that the dose of guano fertilizer significantly affected the root length parameters. The average length of the *P. angulata* root can be seen in Table 5. Based on Table 5, it turns out that the highest average root length was found in the P5 treatment which was 52.05 cm, and the lowest mean in the P0 treatment was 38.44 cm. Besides containing high N and P elements, guano fertilizer also contains potassium (K). The element phosphorus (P) is a fertilizer that can stimulate root growth and flowering. While serves to strengthen plant tissue, especially in the stem of the plant [32]. In addition, potassium (K) and phosphorus (P) are affected by accelerating root growth, accelerating and strengthening young plants to mature, phosphorus nutrients (P) also accelerate the flowering and ripening process [33], [34]. The use of guano fertilizer can directly affect the availability of P in the soil, where P in the soil is in the form of a solution and some will be absorbed by the soil and plants [20].

In other results, the treatment did not significantly affect the parameters of the fresh weight and dry weight of plants. There is no difference between the treatment of the parameters of the wet and dry weight of plants (Table 5). However, the tendency of the highest average value of wet weight was found in treatment P1 (184.66 g) and lowest in treatment P2 (150.76 g). While the highest average dry weight was found in treatment P5 (29.97 g) and the lowest in treatment P0 (19.74 g). Although the results of the analysis of variance did not show any significant effect, the highest tendency of wet weight was found in the P1 treatment and the lowest was in the P2 treatment with values of 184.66 g and 150.76 g, respectively. While the highest dry weight tends to be found in treatment P5 and the lowest in treatment P0 (control). Besides the treatment, one of the influencing factors that affect the wet weight of plants is the water content contained in plant tissue [20]. The more amount of water contained, the higher the wet weight of the plant. The dry weight of the plant is a balance of the process of taking up carbon dioxide (CO_2) and the process of releasing oxygen (O_2) which is clearly shown by the fresh weight of the plant Another result also showed that plants was obtained guano fertilizer can increase the growth [25].

Table 3. Average stem diameter of *P. angulata* due to provision of guano fertilizer in various ages.

Treatment	Age of plant (WAP)			
	2	4	6	8
P0	0.06 a	1.21 a	1.46 a	1.55 a
P1	0.14 b	1.38 bc	1.64 b	1.69 b
P2	0.18 de	1.37 b	1.65 b	1.72 bc
P3	0.14 bc	1.39 bc	1.66 b	1.75 cd
P4	0.18 cd	1.38 b	1.67 b	1.75 d
P5	0.26 f	1.41 c	1.75 c	1.80 e
Duncan Test 5%	*	*	*	*

Note: Numbers followed by the same letter are not significantly different based on the 5% Duncan test, *: significant effect.

Table 4. The average number of *P. angulata* fruits due to the provision of guano fertilizer in various ages.

Treatment	Age of plant (WAP)		
	4	6	8
P0	9.88 a	47.00 a	43.50 e
P1	12.00 ab	55.13 ab	33.75 ab
P2	15.63 bc	63.25 bc	35.75 d
P3	17.50 cd	77.63 de	34.63 c
P4	21.25 de	80.38 ef	33.63 a
P5	22.88 ef	72.38 cde	34.50 bc
Duncan test 5%	*	*	*

Note: Numbers followed by the same letter are not significantly different based on the 5% Duncan test, *: significant effect.

Table 5. The average of root length and weight of *P. angulata* due to the provision of guano fertilizer

Treatment	**Root length**	**Weight (g)**	
		Wet	**Dry**
P0	38.44 a	155.54	19.74
P1	45.75 b	184.66	25.37
P2	46.95 bc	150.76	25.10
P3	48.39 bcd	151.96	25.40
P4	51.49 cd	166.66	24.96
P5	52.05 d	168.61	29.97
Duncan test 5%	*	ns	ns

Note: Numbers followed by the same letter are not significantly different based on the 5% Duncan test, *: significant effect. ns: Not significant

3.2. The effect of guano fertilizer on flavonoid and saponin content

The results of the test analysis of *P. angulata* leaf flavonoid simplicial at various doses of guano fertilizer as shown in Figure 2, showed that the P3 treatment showed the highest flavonoid levels of 4.16% compared to other treatments. While the lowest value of flavonoid levels was found in the P0 treatment which is 3.02%. The form of the relationship between the levels of flavonoids (%) in leaves with the dose of guano fertilizer (ton ha^{-1}) follows the following equation: $Y = 0.0007X_3 - 0.0275X_2 + .3064X + 3.0525$ with the value R^2 = 0.9557, where Y = levels of flavonoids (%) and X = dosage of guano fertilizer (0-15 tons ha^{-1}). Fertilization can affect the flavonoid content [35].

Saponin is one of the secondary metabolites of terpenoids which is synthesized through mevalonic acid from the respiratory pathway [18], [36], [37]. Figure 3 shows the relationship between the levels of flavonoids and saponins in *P. angulata* fruits with a dose of guano fertilizer. Based on this figure it appears that the highest value of flavonoids and saponins in *P. angulata* fruit is found in the P3 treatment which is 0.192% (flavonoids) and 0.280% (saponins). While the lowest value in the treatment (P0) with a value of 0.091% (flavonoids) and 0.114% (saponins). Other metabolites were determined until 27 compounds [36]. Flavonoid content is highly dependent on the extraction method. Other *Physalis* species, *Physalis minima* L, was known that flavonoid content (Quercetin) in the ethyl acetate highest than others [38], [39]. Flavonoid compounds are compounds that are widely found in plants. Flavonoid compounds are flavonol compounds and this type of compound is mostly contained in *P. angulata* plants [3].

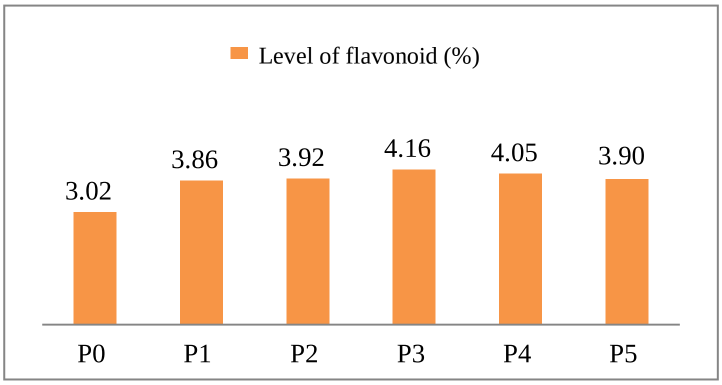

Fig. 2. The effect of various guano fertilizer doses on flavonoid content in *P. angulata* leaves

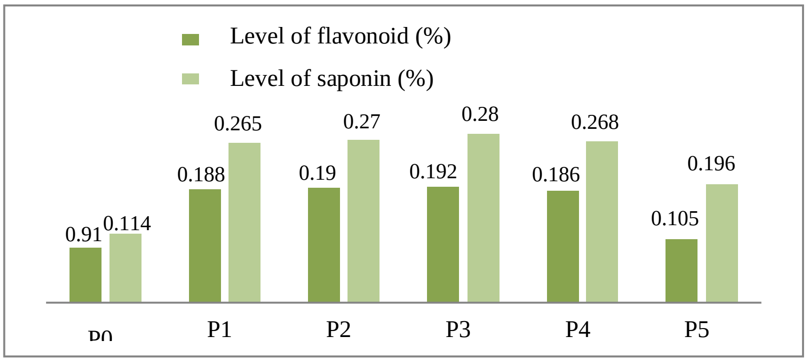

Fig. 3. The effect of various guano fertilizer doses on flavonoid and saponin content in *P. angulata* fruit

4. CONCLUSION

Guano fertilizer doses have a significant effect on plant height, number of leaves, number of fruits, stem diameter, and root length at ages 2-8 WAP of *P. angulata*. But no significant effect on the parameters of the number of flowers, wet weight, and dry weight of stover. P3 treatment (guano fertilizer dosage of 9 tons ha^{-1}) tended to produce the highest flavonoid levels of 4.16% in leaves, 0.192% in fruit, and 0.28% in saponin levels in fruit. The results showed that fertilizing guano at a dose of 15 tons ha^{-1} (G5) gives the best results for parameters of plant height, number of leaves, leaf area, total wet weight, and total dry weight of plants.

Author Contribution

S and LM considered and planned the experiment. AD and ES carried out the preparation of guano. LM and SK collected data and also performed analysis data. S and SK interpreting the data and preparing the manuscript. All authors have read and agreed to the published version of the manuscript.

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Conflict of Interest

The authors declare no competing interest.

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