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Purple Cleome (*Cleome rutidosperma*) Growth, Yield, and Total Flavonoid Under Different Media Compositions and Harvesting Frequencies

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Abstract

Purple cleome (*Cleome rutidosperma*) is a medicinal plant known for its flavonoid content, which confers multiple pharmacological benefits. However, cultivation methods to optimize both biomass production and bioactive compound accumulation remain underdeveloped. This study aimed to assess the influence of planting media composition and harvesting frequency on the growth, yield, and total flavonoid concentration of purple cleome. The experiment was conducted in Gunung Batu, Bogor, Indonesia, from January to April 2025, employing a two-factor randomized block design. The first factor was planting media composition, with four treatments: 100% soil (0) and soil mixed with cow manure at ratios of 1:1, 1:2, and 1:3 (v/v). The second factor was harvesting frequency, with two levels: A single harvest at 8 weeks after planting (WAP) and two harvests at 4 and 8 WAP. Results indicated that all tested media compositions supported plant growth effectively. Notably, total flavonoid concentration was significantly higher at 4 WAP compared to 8 WAP. Moreover, cumulative flavonoid yield from two harvests was significantly greater, reaching 5,639.80 mg quercetin equivalents (QUE) per plant dry weight, compared to 1,456.50 mg QUE per plant from a single harvest. These findings suggest that harvesting at multiple intervals enhances flavonoid yield in purple cleome cultivation.

Keywords: functional vegetables, organic, phytochemicals, traditional medicine

Introduction

Herbal medicine has been an integral component of health systems since ancient times, with the consumption of vegetables and medicinal plants/herbs practiced by diverse cultures, ethnic groups, and societies across the world. Plants serve as valuable sources of secondary metabolites with significant medicinal properties, contributing to well-documented pharmacological effects such as antioxidant and anti-inflammatory activities. One notable species with potential therapeutic value is purple cleome (*Cleome rutidosperma*). Native to tropical Africa, this species is recognized both as a weed and as a medicinal plant rich in various bioactive compounds (Ghosh et al., 2019).

Extracts from *C. rutidosperma* leaves have demonstrated efficacy in alleviating ear irritation and itching. Among the indigenous Ogoni people of Nigeria, it is regarded as a more accessible alternative to conventional hospital treatments due to its therapeutic benefits. Additionally, in Ghana, the leaf sap is traditionally applied to treat earaches and hearing impairments (Kalu et al., 2017; Shilla et al., 2019).

Although commonly perceived as a weed, *C. rutidosperma* possesses tangible benefits, including its powder form, which is a source of essential macrominerals. Furthermore, its leaves serve as an organic pesticide, owing to metabolite compounds such as propanoate, phenol, and isopropyl myristate (Jamiołkowska, 2020). Phytochemical analyses reveal that *C. rutidosperma* contains a diverse array of

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bioactive constituents, including alkaloids, tannins, glycosides, terpenoids, saponins, and flavonoids. Flavonoids present in purple cleome exhibit multiple pharmacological effects, including analgesic, antipyretic, anti-inflammatory, locomotor-enhancing, wound-healing, antimicrobial, antioxidant, anticonvulsant, antidiabetic, diuretic, and laxative activities (Akinsola et al., 2022; Ikhwan et al., 2021; Nguyen et al., 2023). Besides its pharmacological potential, purple cleome meets human nutritional requirements by providing substantial amounts of protein, carbohydrates, and other nutrients (Akinsola et al., 2022).

Enhancing the growth and concentration of bioactive compounds in purple cleome can be achieved through optimized cultivation practices. The composition of the planting medium, particularly the inclusion of organic fertilizers, plays a crucial role in supplying nutrients that support plant development. Cow manure is an organic fertilizer that contains a balanced nutrient profile, including approximately 0.40% nitrogen (N), 0.20% phosphorus (P), and 0.10% potassium (K). Its application has been shown to improve growth and essential oil concentration in related species such as red ginger and to enhance soil fertility (Han et al., 2023; Saputri et al., 2018).

Cow manure is specifically favoured for its higher water and fiber content relative to other manure types and has demonstrated efficacy in augmenting growth and secondary metabolite production in species like *Adenostemma madurense* (Mursiani et al., 2022; Nurfalah et al., 2024). Beyond growing medium composition, harvesting frequency is a critical determinant influencing both yield and bioactive compound concentrations, including flavonoids (Raisawati, 2020; Yao et al., 2020).

Despite its potential, *C. rutidosperma* lacks a standardized cultivation protocol. This study, therefore, aims to evaluate the effects of planting medium composition and harvest frequency on growth performance, yield, and total flavonoid content in purple cleome. The objective is to establish optimal cultivation parameters to maximize both productivity and phytochemical concentration in this species

Materials and Methods

Field trials were conducted at Gunung Batu, Bogor, West Java (6.5936° S, 106.7721° E). Laboratory analyses were conducted at the AGH-IPB Testing Laboratory, the AGH-IPB Post-Harvest Laboratory, the Tropical Biopharmaca Research Center Laboratory in Bogor, and the Regional Health Laboratory in Jakarta. The trials were conducted from May to September 2025.

The study used a randomized block design with two factors. The first factor was the composition of the planting medium in the form of a ratio of soil volume to cow manure (v/v), consisting of four levels, namely 0, 1:1, 1:2, and 1:3. The second factor was the harvest frequency, consisting of two levels, namely one harvest at 8 weeks after planting and two harvests 4 and 8 WAP. There were eight treatment combinations with three replications, resulting in 24 experimental units. Each experimental unit consisted of 7 plants, for a total of 168.

Experimental Procedures

Purple cleome seedlings are transferred to polybags at 2 WAP or when they have 3 fully opened leaves. Planting is done by placing one seedling in each polybag filled with the appropriate growing medium. Maintenance of purple cleome plants includes watering, weeding, pest and disease control, and weeding. Pest control is carried out using organic pesticides. Harvesting is done once in the 8 WAP, while harvesting twice is done in the 4 and 8 WAP, as per the treatment. Harvesting is done by cutting the plant stem 5 cm above ground.

Soil analysis was conducted by taking composite soil samples from each growing media treatment before planting. Samples were taken from several polybags, combined in a single plastic bag, and analyzed for pH, organic carbon, total nitrogen, total phosphorus, phosphorus, total potassium, and potassium.

Cow manure was analyzed for pH, organic carbon, nitrogen, phosphorus, and potassium

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before planting (Tables 1 and 2). Vegetative character observations included plant height, leaf number, primary branch number, and stem diameter at 5 cm above the soil surface, recorded weekly starting in the second week after planting. Generative character observations included the number of purple cleome plants counted. The fruit number was calculated by counting perfectly grown fruits. Observations of fresh and dry weight were conducted at 4 and 8 WAP.

The analysis of total flavonoid concentration was carried out using the aluminum chloride colorimetric method of Vongsak et al. (2013) with modifications; 0.01 g of leaf powder was added to 4 ml of ethanol, then macerated for 72 hr at room temperature in the dark. The solution was then diluted to 10 ml with 70% ethanol and shaken. The sample solution was then centrifuged at 5000 rpm for 7 min. Measurement of flavonoid levels was carried out by taking 2 ml of supernatant with a micropipette, adding 2 ml of 2% $AlCl_3$, homogenizing, and incubating for 10 min at room temperature in the dark. After incubation, absorbance was measured at 415 nm using a spectrophotometer with a quercetin standard.

Leaf NPK analysis was conducted at 4 and 8 WAP, by taking mature leaves and drying them in an oven at 60 °C for 12 hr. The analysis included determining nitrogen (N) concentration using the Kjeldahl method, and phosphorus (P) and potassium (K) using the wet extraction method with 65% HNO_3 and $HClO_4$ solutions, then measuring them by spectrophotometry (Sulaeman et al., 2005). Antioxidant activity analysis was conducted at the 8 WAP harvest using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method. The procedure for this analysis began by preparing 100 mg of concentrated purple cleome leaf extract, dissolving it in 1 ml of DMSO, and diluting it to various concentrations (125-8000 μg/ml). 100 μl of sample extracts at different concentrations was added to a 96-well microplate containing 100 μl of DPPH (125 μM). The extract solution was incubated in a dark room for 30 minutes. The absorbance was measured at 517 nm. Ascorbic acid was used as a standard at concentrations of 0.3125–20 μg/ml in methanol. Gas Chromatography-Mass Spectrometry (GC-MS) analysis was performed to determine the initial chemical compounds present in the leaves. This analysis aims to identify and quantify volatile and semi-volatile chemical compounds in the samples.

Data Analysis

Data was analyzed using the *F*-test at the 5% level, followed by the Duncan Multiple Range Test (DMRT). Statistical analysis was performed using Microsoft Excel and RStudio version 4.3.1.

Results and Discussion

General Conditions of the Experimental Land

Soil at the experimental site showed a slightly acidic pH and low organic C and total N contents (Table 1). Potential P and K levels were very high (Table 1). Supplementing cow manure can increase soil organic C, available P, soil pH, and total N availability for plants (Saifulloh & Suntari, 2022).

The analysis of cow manure (Table 2) shows that the organic C concentration of 42% is very high and can improve soil structure and fertility. Cow manure also provides macronutrients N, P, and K. The macronutrient concentration, from the results of the analysis of cow manure, is total N (1.59%), total P_2O_5 (1.69%), and total K_2O (1.78%). According to Whalen et al. (2002), cow manure can help the availability of several macronutrients, especially N in the soil. This shows that adding organic fertilizer effectively provides nutrients for purple cleome plants. The analyzed cow manure exhibits chemical characteristics consistent with its organic nature. According to Al-suhaibani et al. (2020), cow manure has sufficient N, P, and K elements to increase soil fertility. However, the total N, total P_2O_5, and total K_2O concentrations are lower than the standard (≥2%). Meanwhile, the organic C, pH, Ca, and Mg concentrations meet the established criteria.

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Vegetative Phase of Purple Cleome Plant

At 6 WAP, the composition of soil and cow manure (1:2) increased plant height by 43.48% compared to soil without cow manure (Table 3), likely due to improvements in the soil's physical, chemical, and biological properties. The effects of planting media composition on stem diameter is presented in Table 3. Significant differences were observed with cow manure application at 6 and 8 WAP. Notably, at 8 WAP, a soil-to-cow manure ratio of 1:2 resulted in a 24.69% increase in stem diameter relative to soil alone. This finding suggests that higher proportions of cow manure elevate organic matter content in the growing medium, thereby stimulating soil

Table 1

Chemical Properties of Cow Manure

Parameter	Cow manure	Standard*
Water content	73.44	8.20
C-organic	42.0	Minimum 15
pH	8.66	4-9
Total N	1.59	Minimum 2
Total P_2O_5	1.69	Minimum 2
Total K_2O	1.78	Minimum 2
C/N	26.4	≤25
Ca	2.69	≥1
Mg	0.25	≥2

Note. *Standard criteria based on the Soil and Fertilizer Instrument Standards Testing Center (2023).

Table 2

Chemical Properties of the Soil and Different Planting Media Compositions

Parameter	Soil	S: CM (1:1)	S: CM (1:2)	S: CM (1:3)
pH H_2O	6.94 (SA)	8.12 (N)	7.92 (N)	7.76 (N)
pH KCl	5.52 (SA)	7.33 (A)	7.36 (A)	7.24 (A)
Organic C	1.19 (L)	1.25 (H)	1.82 (H)	1.35 (H)
Total N	0.17 (L)	0.38 (S)	0.69 (H)	0.63 (H)
Avail. P	73.60 (VH)	520.00 (VH)	890.00 (VH)	824.00 (VH)
CEC	16.30 (M)	25.40 (H)	23.80 (M)	29.90 (H)
Exch. Mg	1.11 (M)	2.88 (M)	6.30 (M)	0.44 (M)
Exch. Ca	6.94 (M)	10.51 (H)	11.06 (H)	12.32 (H)
Exch. K	0.82 (H)	9.24 (H)	11.87 (H)	11.20 (H)
Exch. Na	0.07 (L)	0.61 (M)	5.28 (H)	5.39 (H)
Exch. Al	0.19 (S)	0.28 (M)	0.29 (M)	0.27 (M)
Exch. H	0.27 (L)	0.40 (M)	0.29 (M)	0.27 (M)
Potential P	195.00 (VH)	279.00 (VH)	673.00 (VH)	734.00 (VH)
Potential K	43.70 (H)	479.00 (VH)	1293.00 (VH)	1196.00 (VH)

Note. S = soil, CM = cow manure, SA = slightly acidic, A = acidic, N = neutral, R = low, H = high, M = medium, VH = very high according to the Soil and Fertilizer Instrument Standards Testing Center (2023).

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microbial activity. Enhanced microbial activity supports soil fertility and nutrient availability, which in turn promotes plant growth, including stem and branch development (Andharesta & Sundahri, 2024; Shao et al., 2024; Sofian et al., 2024).

The effect of planting media composition on primary branch number and leaf number is shown in Table 4. The application of cow manure on the primary branch and leaf number is significant at 2-8 WAP. At 8 WAP, the composition of soil and cow manure (1:2) increased the primary branch number by 46.93% compared to soil. The effect of the planting medium composition on leaf number is shown in Table 4. The application of cow manure showed significant differences at 2, 6, and 8 WAP. At 8 WAP, the composition of soil and cow manure (1:3) increased leaf number by 76.28% compared to the soil alone. This is in line with research by Mantovani et al. (2017), which shows that organic fertilizer in planting media increases nutrient availability and leaf growth during the vegetative phase.

The growth of the vegetative phase of purple cleome, which includes plant height, stem diameter, primary branch number, and leaf number, increased with the addition of cow manure (Figure 1). Media composition alters microbial community and metabolic functions, and soil microbial enrichment influences plant secondary metabolite biosynthesis (Schütz et al., 2021).

Generative Phase of Purple Cleome

The effects of planting media composition on flower number and fruit number are shown in Table 5 and Figure 1. The provision of cow manure does not significantly affect in flower number. However, increase the flower number

Table 3

Plant Height and Stem Diameter with Different Planting Media Compositions

Planting media compositions	Plant height (cm)				Stem diameter (mm)			
	2 WAP	4 WAP	6 WAP	8 WAP	2 WAP	4 WAP	6 WAP	8 WAP
Soil	10.44	13.64	18.58b	21.85	3.30	2.28	2.44b	2.47b
Soil: CM (1:1)	12.62	18.35	25.65a	27.40	2.21	2.72	2.89a	2.87ab
Soil: CM (1:2)	13.47	18.47	26.66a	28.44	2.19	2.72	3.02a	3.08a
Soil: CM (1:3)	11.87	17.61	23.71a	26.84	2.16	2.67	2.82ab	2.84ab
Anova	ns	ns	*	ns	ns	ns	*	*

Notes. ns = not significant, * = significant at α=0.05. The values followed by the same letter in the same column indicate no significant difference according to DMRT at 5%. WAP = weeks after planting, CM = cow manure.

Table 4

Primary Branch and Leaf Number with Different Planting Media Compositions

Planting media compositions	Primary branch number				Leaf number			
	2 WAP	4 WAP	6 WAP	8 WAP	2 WAP	4 WAP	6 WAP	8 WAP
Soil	3.7b	4.8b	4.8b	4.9b	19.4b	20.0	38.6b	50.6b
Soil: CM (1:1)	5.9a	6.4a	6.8a	6.6a	30.9a	36.4	65.1a	77.5a
Soil: CM (1:2)	6.0a	6.7a	6.7a	7.2a	27.0ab	39.0	65.1a	83.6a
Soil: CM (1:3)	4.5b	5.6ab	6.3a	6.4a	25.9ab	34.5	71.2a	89.2a
Anova	*	*	*	*	*	ns	*	*

Notes. ns = not significant, * = significant at the 5% level. The values in the same column that share the same letter indicate no significant difference according to DMRT at the 5% level. WAP = weeks after planting, CM = cow manure.

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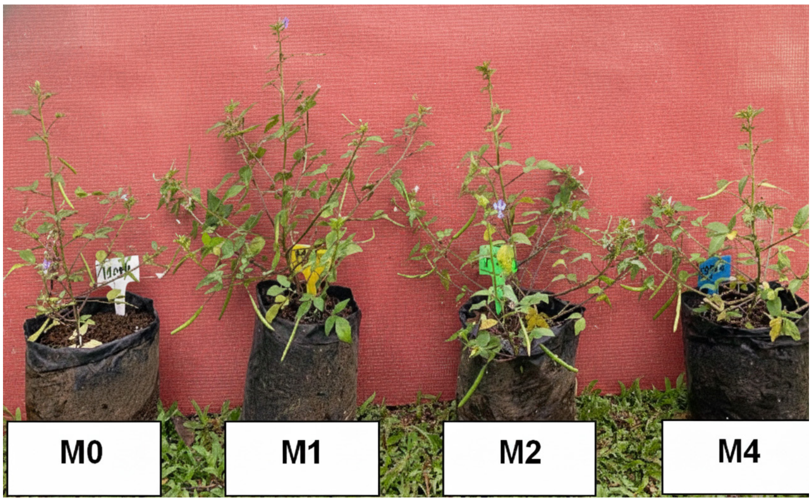
Figure 1

Generative Phase Growth of Purple Cleome under Different Soil Planting Media Compositions



Notes. M0 = soil, M1 = soil: cow manure (1:1), M2 = soil: cow manure (1:2), M3 = soil: cow manure (1:3).

by 38% compared to soil alone. The increase in the number of flowers is related to the improvement of soil fertility due to the addition of cow manure containing macro nutrients N, P, and K. Phosphorus has an important role in flowering (Ali et al., 2025) and can improve strawberry fruit quality (Bai et al., 2025).

Meanwhile, the effect of planting medium composition on fruit number is shown in Table 5. The application of soil and cow manure (1:1) increased the fruit number by 48.53% compared to the soil alone.

The effects of planting media composition and harvest frequency did not significantly affect fresh, dry, or total harvest weights (Tables 6 and 7). Still, cow manure tended to increase plant fresh and dry weight. The addition of cow manure, which provides macro nutrients such as nitrogen (N), phosphorus (P), and potassium (K), can support the growth and yield of purple cleome plants. This is in line with the research of Karimuna et al. (2015), which reported that organic fertilizer can increase the fresh and dry leaf weight of orange jessamine.

Leaf NPK Concentration

The effects of planting media composition and harvest frequency on N, P, and K levels in purple cleome plants are shown in Table 8. The average levels of N, P, and K with the provision of cow manure did not differ significantly with different composition of the planting media, except for P concentration in the 8 WAP, which showed a significant difference. The treatment with soil: manure 1:1 increase the P concentration by 15.21% in the 8 WAP compared to the soil alone. Meanwhile, the harvest frequency treatment showed a significant difference, with nutrient levels generally higher in younger plants than in older plants. According to research by Houdegbe et al. (2022), the nutrient adequacy range in *Gynandropsis gynandra* L. or *Cleome gynandra* leaves was in the range of 2.0%–5.0% for nitrogen (N), 0.25%–0.60% for phosphorus (P), and around 1.5% for potassium (K). The effects of planting medium composition and harvest frequency on N, P, and K levels were greater at 4 WAP than at 8 WAP. This is thought to be due to strong sinks in the generative phase, which led to nutrients being transferred to the reproductive organs rather than to the buds in the vegetative phase.

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Table 5

Flower Number and Fruit Number with Different Planting Media Compositions

Planting media compositions	Flower number				Fruit number			
	2 WAP	4 WAP	6 WAP	8 WAP	2 WAP	4 WAP	6 WAP	8 WAP
Soil	1.9b	1.7	2.3	1.3	1.9	2.8	4.4	3.8b
Soil: CM (1:1)	2.9a	2.7	3.1	1.7	2.6	4.3	6.6	5.6a
Soil: CM (1:2)	2.1b	2.6	2.7	1.6	3.1	4.3	5.7	4.5ab
Soil: CM (1:3)	1.7b	2.8	2.6	1.8	2.6	4.6	6.8	5.3a
Anova	*	ns	ns	ns	ns	ns	ns	*

Notes. ns = not significant, * = significant at the 5% level. Values within a media composition column that share the same letter do not differ significantly according to DMRT at the 5% level. WAP = weeks after planting, CM = cow manure.

Table 6

Fresh Weight, Dry Weight, and Total Harvest Weight with Different Planting Media Compositions

Planting media compositions	2x Harvest (g per plant)				Total harvest weight (g per plant)			
					2x Harvest		1x Harvest	
	FW	DW	FW	DW	FW	DW	FW	DW
	4 WAP		8 WAP		8 WAP			
Soil	7.62	1.33	12.48	1.99	20.10	3.32	9.00	1.92
Soil: CM (1:1)	10.56	1.84	14.51	2.32	25.07	4.16	13.49	3.02
Soil: CM (1:2)	10.53	1.50	21.30	3.42	31.83	4.90	14.53	2.85
Soil: CM (1:3)	9.44	1.39	14.35	2.42	23.79	3.81	13.47	2.55
Anova	ns	ns	ns	ns	ns	ns	ns	ns

Notes. ns = not significant, * = significant at the 5% level. Values within a media composition column that share the same letter do not differ significantly according to DMRT at the 5% level. FW = fresh weight; DW = dry weight; WAP = weeks after planting.

Table 7

Fresh weight, dry weight, and total harvest weight at different harvest frequencies

	FW (g per plant)		DW (g per plant)		Total FW (g per plant)		Total DW (g per plant)	
	4 WAP	8 WAP	4 WAP	8 WAP	1x Harvest	2x Harvest	1x Harvest	2x Harvest
	9.54	15.66	1.51	2.54	12.62	20.76	2.58	4.11
Anova	ns		ns		ns		ns	

Notes. ns = not significant, * = significant at the 5% level. Values within the same row for fresh or dry weight, followed by the same letter, are not significantly different according to DMRT at the 5% level. FW = fresh weight; DW = dry weight; WAP = weeks after planting.

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Secondary Metabolite Profile

The effects of planting medium composition and harvest frequency on total flavonoid concentration and leaf production are shown in Table 9 and the secondary metabolite shown in Table 10. The average flavonoid concentration and leaf production did not differ significantly with cow manure application across planting media. Meanwhile, in the young plant (4 WAP) harvest, the leaf total flavonoid concentration was 125% higher than in the old plant (8 WAP) leaf NPK concentration at 4 WAP was much higher than at 8 WAP (Table 8). Harvesting frequency affects plant regrowth and stress response, which can trigger secondary metabolite production (Table 9).

Table 8

Nitrogen, Phosphorus, and Potassium Levels of Purple Cleome at Different Planting Media Compositions and Harvest Frequencies

Planting media compositions	Leaf N (%)		Leaf P (%)		Leaf K (%)	
	4 WAP	8 WAP	4 WAP	8 WAP	4 WAP	8 WAP
Soil	3.87	2.07	1899.47	3195.29	5094.76	822.52
Soil: CM (1:1)	4.15	2.10	2981.34	3718.93	6700.27	1561.30
Soil: CM (1:2)	3.52	1.98	1979.87	5482.22	7462.09	1550.96
Soil: CM (1:3)	3.53	2.12	2260.98	3889.92	6150.90	1891.28
Anova	ns	ns	ns	ns	ns	ns
Harvest frequencies	3.77a	2.05b	0.54a	0.49b	3.23a	1.65b
t test	*		*		*	

Notes. ns = not significant, * = significant at the 5% level. Values with the same letter within the same media composition and nutrient column indicate no significant difference according to DMRT at 5% level. Values with the same letter within the same harvesting row and nutrient type did not differ significantly according to t test at 5%. WAP = week after planting, CM = cow manure.

Table 9

Treatment of Planting Media Composition and Harvest Frequency on the Leaf Total Flavonoid Concentration of Purple Cleome

Treatment	Leaf total flavonoid concentration (mg QUE per plant dry weight)		Total leaf flavonoid production (mg QUE per plant dry weight)			
Planting media compositions	4 WAP	8 WAP	2x Harvest			1x Harvest
			4 WAP	8 WAP	Total harvest 2x	8 WAP
Soil	1368.09	775.81	1899.47	3195.29	5094.76	822.52
Soil: CM (1:1)	1547.62	530.40	2981.34	3718.93	6700.27	1561.30
Soil: CM (1:2)	1305.98	537.75	1979.87	5482.22	7462.09	1550.96
Soil: CM (1:3)	1601.33	734.55	2260.98	3889.92	6150.90	1891.28
Anova	ns	ns	ns	ns	ns	ns
Harvest frequencies	1455.75a	644.63b	2280.40	3359.40	5639.80	1456.50
t test	*		*		*	

Notes. ns = not significant, * = significant at the 5% level. Values followed by the same letter within the same planting media composition and harvest frequency column and within the same harvesting frequency row indicate no significant difference according to t test at the 5% level. WAP = week after planting, CM = cow manure.

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Table 10

Secondary Metabolite Compounds Found in Purple Cleome Plants

No	Compound name	Remarks	Content (%)
Fatty acid			
1	n-Hexadecanoic acid	N-hexadecanoic acid is a fatty acid that has been identified as an anti-inflammatory compound, primarily due to its ability to inhibit Phospholipase A2 (Bermúdez et al., 2022)	22.45
2	Octadecadienoic acid	Included in the fatty acid compounds, which have several health benefits as anti-atherogenic, anti-inflammatory, and anti-oxidative (Virsangbhai et al., 2020)	3.16
3	Phytyl linolenate	It is an α-linolenic acid compound that has shown potential in suppressing the proliferation and invasion of cancer cells (Fan et al., 2022)	8.37
4	Tetramethyl	Included in various groups of Tetramethylsilane, its benefits are treating pulmonary hypertension. (Chen et al., 2020)	4.30
5	Hexadecenyl ester	Compounds derived from hexadecanoic acid, which are useful as antifungals (Abubacker and Deepalakshmi, 2013)	4.30
6	Hexadecenoic acid	Included in the group of fatty acid compounds, it has benefits in managing metabolic diseases such as diabetes and cardiovascular conditions (Aparna et al., 2012)	4.30
Diterpenoid			
7	Neophytadiene	Included in these are diterpene compounds that have been identified in methanolic extracts of certain plants that function to treat seizure disorders (Bhardwaj et al., 2020)	8.09
8	Phytol	Phytol is a diterpene alcohol derived from chlorophyll metabolism in plants, which has benefits as an antioxidant (Cells et al., 2022)	2.29
Steroid			
9	Stigmasta-3,5-diene	Included in the steroid compounds, this compound is characterized by its tetracyclic triterpene structure, which is common among plant sterols. It includes anti-inflammatory and anticancer properties (Bakrim et al., 2022)	5.13
Vitamin E			
10	γ-Tocopherol	belongs to a group of compounds known as vitamin E, which is a family of eight fat-soluble compounds called tocochromanols, which are beneficial as antioxidants (Mathur et al., 2015)	2.37
Fitosterol			
11	Campesterol	Included in the group of compounds known as phytosterols, it has the benefit of lowering cholesterol (Demonty, 2007)	2.38
12	Stigmasterol	Belonging to a group of compounds known as phytosterols, it has anti-inflammatory benefits (Valitova et al., 2024)	1.87
13	γ-Sitosterol	Included in the group of compounds known as phytosterols, it has the benefit of lowering blood cholesterol (Poli et al., 2021)	4.85

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Total flavonoid production from two harvests was significantly higher by 287% compared to a single harvest. The flavonoid production is calculated by multiplying the plant dry weight by the total flavonoid concentration. In Tables 6 and 7, the dry weight from two harvests is significantly higher than that from a single harvest, thereby increasing total flavonoid production. Flavonoids are secondary metabolites from the phenolic group that act as antioxidants to protect plant cells from damage caused by free radicals. This is related to plant physiological processes, particularly photosynthesis. During the vegetative phase, leaves are still actively photosynthesizing with optimal chlorophyll concentration, thus increasing the plant's ability to absorb light energy and produce high levels of photosynthate (Murakami & Matsuda, 2016).

GC-MS analysis yielded 14 metabolites, as shown in Table 10. Purple cleome leaves contain various secondary metabolite compounds, such as fatty acids (n-hexadecanoic acid, octadecadienoic acid, phytyl linolenate), which are known to have anti-inflammatory and antioxidant activities, and health benefits (Aparna et al., 2012). Diterpenoid compounds such as neophytadiene and phytol were also detected and function as antioxidants and antimicrobials (Kim et al., 2022). In addition, a steroid compound (stigmasta-3,5-diene) has potential as an anti-inflammatory and anticancer agent (Bakrim et al., 2022). The concentration of vitamin E (γ-tocopherol) adds to the antioxidant potential of this plant (Mathur et al., 2015). Purple cleome also contains phytosterols (campesterol, stigmasterol, γ-sitosterol), effectively lowering blood cholesterol levels (Demonty, 2007; Poli et al., 2021; Valitova et al., 2024).

The effect of planting media composition on antioxidant activity is presented in Table 11. Antioxidant activity analysis in this study was conducted only at the 8 WAP harvest due to limited sample availability. This condition prevented measurement of antioxidant activity at the 4 WAP harvest, even though total flavonoid concentration at 4 WAP was higher than at 8 WAP. Therefore, the antioxidant activity results presented in this study represent the condition of the plants at the 8 WAP harvest, in accordance with the available test material. The average antioxidant activity with cow manure application showed no significant difference; however, the antioxidant activity of purple cleome at 8 WAP increased with a higher proportion of cow manure in the planting media. The highest value was obtained in the Soil: CM (1:3) medium at 91.58%, while the lowest was found in the soil without cow manure medium at 73.71%. The increased antioxidant activity is associated with improved media fertility and enhanced synthesis of secondary metabolites, such as flavonoids and phenolics, which function as natural antioxidants (Kumar & Pandey, 2013).

Table 11

Antioxidant Activity in Different Planting Media Compositions

Planting media compositions	% Inhibition at 8 WAP
Soil	73.71
Soil: CM (1:1)	79.89
Soil: CM (1:2)	82.12
Soil: CM (1:3)	91.58
Anova	ns

Notes. ns = not significant, * = significant at α 5%. Values followed by the same letter in the same column indicate no significant difference in the 5% DMRT test, WAP = weeks after planting, CM = cow manure.

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Conclusions

The results showed that all soil and cow manure compositions can be used as planting media for the purple cleome (*Cleome rutidosperma)*. The total flavonoid concentration at 4 WAP was significantly higher than at 8 WAP. The total flavonoid production over two harvests was considerably higher than that in a single harvest. Two harvests produced a total flavonoid yield of 5,639.80 mg QUE per plant dry weight, significantly higher than one harvest, which only produced 1,456.50 mg QUE per plant dry weight. This research significantly advances organic cultivation practices for purple cleome and provides a valuable reference for subsequent studies and scientific progress in agronomy and phytochemistry.

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