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Research articles

Effect of nitrogen and potassium application on growth, total phenolic, flavonoid contents, and antioxidant activity of *Eleutherina palmifolia*

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Abstract: *Eleutherine palmifolia* is one of the medicinal plants widely used by the Dayak and Kutai tribes in Borneo island, Indonesia as traditional medicines that can treat various diseases. Identification of the phytochemical content of *E. palmifolia* is very important to determine its potential as a medicinal plant. A significant factor in the success of the growth of *E. palmifolia* is the amount of fertilizer applied as a source of nutrients. In this study, the influence of various amounts of nitrogen (N) and potassium (K) fertilizers on the growth, total phenolic and flavonoid content (TPC and TFC), and antioxidant activity was investigated in different extracts of *E. palmifolia*. The treatments included 0, 100, or 200 kg/ha of N combined with 25 kg/ha of K, and 0, 100, or 200 kg/ha of K combined with 100 kg/ha of N. Using water, ethanol, or n-hexane as solvents, each sample was extracted with sonication method. TPC and TFC of the bulb extracts were quantified using Folin-Ciocalteu and aluminum chloride assays, respectively. Antioxidant activity was analyzed using 2,2′-diphenyl-1-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) assays. Results showed that combining N and K had no significant adverse effects on plant height, number of tillers, leaves, or bulbs, and bulb diameter, while the chlorophyll reading value, total plant weight, and fresh bulb weight were significantly increased. The results indicated that availability of N and K has a substantial effect on TPC, TFC, and antioxidant activity. The aqueous extract had the highest TPC and DPPH scavenging activity, whereas the ethanol extract exhibited the highest TFC and antioxidant FRAP activity. Therefore, to improve fresh bulb weight, phenol and flavonoid content, and antioxidant properties of *E. palmifolia* aqueous and ethanol extracts, supplementation of 100 kg/ha N and 25 kg/ha K is

recommended.

Keywords: Antioxidants; DPPH; flavonoid; FRAP; Iradaceae; phenolic

1. Introduction

Eleutherine palmifolia (L.) Merr., in Indonesia called as "bawang dayak", is a medicinal plant that is grown in Borneo Island, Kalimantan, Indonesia. *E. palmifolia* is one of the species of Iridaceae family and is considered as a synonym of *E. americana, E. plicata*, and *E. bulbosa* [1, 2]. This species is widely cultivated in regions of Africa, South America, and Asia for its beneficial chemical compounds as traditional medicine [2, 3]. *E. palmifolia* is traditionally used to treat diabetes, stroke, hypertension, breast cancer, fertility, and hypertensive diseases [4]. Numerous studies reported the beneficial effects of *E. palmifolia* on human health, including its anti-cancer [5], anti-fungal [6], anti-malarial [7], antioxidant [8], and anti-melanogenesis [9] activities. Bioactive chemicals of *E. palmifolia* have a large variety of pharmacological targets. For example, antidiabetic activity of *E. palmifolia* is strongly connected with its eleutherinoside A content [4], whereas its antioxidant activity is associated to polyphenols [8]. Polyketide compounds in *E. palmifolia* have been linked to its antimicrobial activity [10]. With regard to investigations on *E. palmifolia* as a medicinal plant, it is very important to associate the amount of active compounds in the plant extracts to its pharmacological activity. Therefore, in this study, total phenolic and flavonoid content (TPC and TFC) and antioxidant activity were assessed as parameters to evaluate the effect of nitrogen (N) and potassium (K) fertilizers on *E. palmifolia*.

Plant nutrition is a critical aspect in increasing plant productivity. An important factor in plant growth and development is N [11]. N fertilizers have an impact on the yield and productivity of many plants, such as onions [12, 13], rice [14], and milk thistle [15]. On the other hand, K is one of the most critical elements in physiological functions, such as photosynthetic capacity, ionic homeostasis, carbohydrate metabolism [16], enzymatic activation [17], water relations [18], osmotic adjustment [19], or resistance against abiotic stress [20, 21] of plants. Secondary metabolites and their biological activity in plants may be influenced by N and K levels. For example, combination of N and K increased the polyphenol and vitamin C content and antioxidant activity of sweet fennel [22]. Typically, *E. palmifolia* is planted as a side plant by farmers and is not cultivated at optimal conditions. Numerous fertilizers can influence the flavonoid content, growth, and yield of *E. palmifolia*. Application of NPK 16:16:16 fertilizer at 2.5 g/L increased the production of *E. palmifolia*, and a concentration of 1.25 g/L increased its flavonoid content [23], chicken manure [24], and vinasse liquid organic [25]; however, our knowledge is limited about the effect of N and K on the growth, polyphenol content, and antioxidant activity of *E. palmifolia*. In this study, we investigated the effect of N and K combination on morphological characters, polyphenol content and antioxidant activity of different extracts of *E. palmifolia*. The application of nitrogen and potassium fertilizers with optimum concentrations may have a positive impact on increasing the growth and yield of E. palmifolia, as well as improving the quality of its important chemical compounds.

2. Materials and Methods

2.1. Experimental treatments

Eleutherine palmifolia seedlings were obtained from Faculty Agriculture of Bengkulu University. The research was conducted from February to June 2020 in Bengkulu province, Indonesia (3°45’44” S; 102°16’45” E; altitude of 15 m). Urea (46% N) and KCl (50% K_2O) were used as the N and K source fertilizer, respectively. The treatments of N and K combinations are presented in Table 1. A three-replication completely randomized design was used. K fertilizer was applied at the time of planting, while N fertilizer was given in two times, once at the time of planting and again 21 days after planting.

The sixth-month bulbs of *E. palmifolia* were used as planting material. The bulbs were selected with relatively uniform shape and size. The top 1/4 of bulbs were cut horizontally using a cutter to enhance shoot growth. The bulbs were immersed in a PGPR (*plant growth promotion rhizobacteria*) solution with a concentration of 10 ml/L for 15 min. Bulbs were planted in polybags with a diameter of 45 cm containing 10 kg of growing medium (mixed of soil, manure, and rice husk in a ratio of 2: 1: 1). Each polybag was planted with three shallot bulbs. The irrigation, fertilization, weed, and diseases control were manually performed for maintenance of plants. Plant height, number of tillers, number of leaves, chlorophyll reading value (SPAD), total plant weight, number of bulbs, the diameter of the bulb, and fresh bulb weight were observed as morphological characteristics. Selected plants in each unit were used to collect data. Randomly selected ten plants from each unit were used to provide the best possible accuracy. Four months after planting, the bulb of *E. palmifolia* was harvested and further analyses were carried out.

Table 1. Treatment combination of N and K fertilizers

Treatment combination	Urea (kg/ha)	KCl (kg/ha)
N_0K_{25}	0	25
$N_{100}K_{25}$	100	25
$N_{200}K_{25}$	200	25
$N_{100}K_0$	100	0
$N_{100}K_{50}$	100	50
$N_{100}K_{100}$	100	100

2.2. Sample preparation and extraction

The bulbs of each treatment were cleaned, cut, dried, and then reduced into powdered form. Samples were extracted using aqueous, ethanol, and n-hexane as solvent extraction using the procedure by [26] with modifications. Briefly, sample (2 g) was extracted twice with solvent (10 mL) in the dark room and then sonicated and stirred for 30 min. The mixtures were centrifugated at 10,000 x g and 4°C (Kitman-T24, Tomy Tech USA Inc.) for 15 min, and the supernatants were concentrated using rotary vacuum evaporator (HAHNVAPOR, Korea) until the volume reached 10 mL. The final extract of *E. palmifolia* (0.2 g/mL) was obtained and then used for further analysis.

2.3. Total phenolic content (TPC) and total flavonoid content (TFC)

In accordance with previously published assays, TPC and TFC were determined using spectrophotometric techniques [27]. TPC was measured in the extract obtained of each sample with Folin-Ciocalteu reagent and expressed as mg gallic acid equivalents per g of dry weight (mg GAE/g DW). Meanwhile, TFC was measured using aluminium chloride reagent and expressed as mg quercetin equivalents per g of dry weight (mg QE/g DW).

2.4. Antioxidant analysis

Antioxidant activity was determined using two in-vitro techniques. The free radical scavenging and reducing power antioxidant activities were determined using the 2,2-diphenyl picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) assays [28]. Results of antioxidant activity expressed in µmol Trolox equivalent per g of dry weight (µmol TE/g DW).

2.5. Antioxidant analysis

Three replicates were used to get mean data ± SD. ANOVA was determined using the ExpDes packages in R, and then a Scott-Knott test was performed [29]. When $p < 0.05$ is used, there are significant differences.

3. Results and Discussion

3.1. Growth and yield of E. palmifollia

Nitrogen is a macroelement that all plants, including *E. palmifolia*, require in substantial amounts to enhance their growth and productivity [13]. The results of the study showed that the combination of N and K fertilizer significantly ($p < 0.05$) affected chlorophyll reading value and total plant weight, which all attained maximum values at a combination of 100 kg/ha N and 25 kg/ha K (Table 2). The results showed that the combination treatment of N and K fertilizers has no significant effect on the growth parameters of plant height, number of tillers, and leaves of *E. palmifolia*. The role of nitrogen during plant growth can be observed through the chlorophyll reading value in plants to increase the efficiency of nitrogen use according to plant needs and reduce the risk of environmental pollution.

Table 2. The growth characters of *E. plamifolia* as a response to the application of different fertilizers

Treatment combination	Plant height (cm)	Number of tillers	Number of leaves	Chlorophyll reading value (SPAD)	Total plant weight (g)
N_0K_{25}	36.32 ± 2.14a	8.00± 5.29a	27.00±5.29a	46.03±6.56a	78.00±6.55b
$N_{100}K_{25}$	40.58 ± 3.64a	13.67±7.37a	40.00±9.98a	47.65±1.43a	95.33±7.50a
$N_{200}K_{25}$	41.95 ± 1.72a	8.00± 2.64a	25.00±2.64a	47.80±11.60a	78.67±8.08b
$N_{100}K_0$	38.92 ± 1.59a	10.17±10.15a	31.67±10.15a	37.33± 4.73b	94.83±14.25a
$N_{100}K_{50}$	39.90 ± 2.03a	10.83±6.33a	36.83± 6.33a	37.00±4.73b	106.33±12.66a
$N_{100}K_{100}$	40.62 ± 2.27a	10.67±9.98a	39.33±7.37a	35.33±1.53b	102.83±10.34a

Note: Each value is expressed as the mean plus standard deviation; The Scott-Knott test indicates that the mean values in each column denoted by different letters differ significantly at $p < 0.05$; For the treatment combination, see Table 1.

The results recorded that treatment of N (100-200 kg/ha) combined with K (25 kg/ha) increased the chlorophyll reading value of *E. palmifolia* compared to other combination treatments (Table 2). This result is similar to the report presented by Lee et al., [30] that the highest of plant height was obtained in shallot plants fed with nitrogen fertilizer of 180 kg/ha. As reported by Skudra and Ruza [31], nitrogen is significantly affects the chlorophyll reading value in leaves and stems of wheat (*Triticum aestivum*). The result indicated the importance of the presence of N for plant growth of *E. palmifolia*, especially in the chlorophyll formation.

A higher total plant weight parameter was shown in the combination treatment of 100 kg/ha N with 25 kg/ha K, which was not significantly different from the combination treatment of 50 kg/ha N with 50-100 kg/ha K (Table 2). These results indicate that the application of nitrogen fertilizer combination of 50-100 kg/ha N with 25-100 kg/ha K was required for *E. palmifolia* to increase growth and development, which was shown by higher plant weights.

The effect of N and K fertilizers on the yield parameters of *E. palmifolia* is presented in Table 3. The results showed that the combination of N and K fertilizers has no significantly different effect on the number and diameter of a bulb of *E. palmifolia*. However, the combination of N and K fertilizers significantly impacted fresh bulb weight. The highest fresh bulb weight (66.01-71.14 g/clump) was obtained in the combination treatment of N fertilizer (50-200 kg/ha) with K fertilizer (0-100 kg/ha). Similar results were previously reported by Gebretsadik and Dechassa [32] that increasing the rate of nitrogen from 50 to 100 kg/ha increased the onion marketable fresh bulb yield by a different percentage of 30%. The result showed that the treatment of K fertilizer (25 kg/ha) without N fertilizer produced the lowest tuber weight (58.68 g/clump). The result indicates the importance of nitrogen nutrients in increasing the yield of *E. palmifolia*. Similar results were reported by Uher et al., [33] that the yield depended on the increase in nitrogen dose, and the highest nitrogen dose obtained the highest broccoli yield. The deficiency of nitrogen may decrease chlorophyll biosynthesis, which leads to yield reduction.

Table 3. The yield components of *E. plamifollia* as response to fertilizer application

Treatments	Number of bulbs	Diameter of bulb (cm)	Fresh bulb weight (g)
N_0K_{25}	14.67 ± 1.3 a	1.75 ± 0.06 a	58.68 ± 6.11 b
$N_{100}K_{25}$	16.17 ± 2.4 a	1.76 ± 0.08 a	71.14 ± 5.29 a
$N_{200}K_{25}$	13.83 ± 1.5 a	1.75 ± 0.02 a	67.06 ± 2.64 a
$N_{100}K_0$	11.50 ± 2.6 a	1.81 ± 0.06 a	65.55 ± 8.75 a
$N_{100}K_{50}$	11.50 ± 0.9 a	1.86 ± 0.09 a	66.70 ± 5.76 a
$N_{100}K_{100}$	14.67 ± 4.5 a	1.75 ± 0.11 a	66.01 ± 7.28 a

Note: Each value is expressed as the mean plus standard deviation; The Scott-Knott test indicates that the mean values in each column denoted by different letters differ significantly at $p < 0.05$; For the treatment combination, see Table 1.

The results showed that without the addition of K fertilizer, the *E. palmifolia* produced good yields (Table 3). Under conditions of low K availability, plants have a strategy to maintain K needs. For example, the plant increased K uptake from the soil redistributes K ions between cytosolic and vacuolar pools, cytosolic homeostasis, and modifications in root system development and architecture [34]. Supplying optimum N and K level was proved to be essential for plant growth and production of high yield and improving the quality of *E. palmifolia*.

3.2. Growth and yield of E. palmifollia

TPC in the *E. palmifolia* extracts strongly influenced the combination of N and K fertilizer and the extraction solvents. TPC ranged from 2.192-3.688 GAE /g DW in aqueous extract, 1.474-2.815 GAE/g DW in ethanol extract, and 0.219-0.304 GAE/g DW in n-hexane extract (Table 4). Among the combination treatments of N and K fertilizers observed, the combination treatment of 100 kg/ha N and 50 kg/ha K fertilizers produced the highest TPC (aqueous extract, 3.688 mg GAE/g DW) compared to other treatments. These results indicated that nitrogen as an essential element strongly affects growth and polyphenol metabolism of *E. palmifolia*. Similar results were recorded that boosting N and K fertilization increased the total phenolic content in sweet fennel [22] and coloured potato [35]. Meanwhile, TPC in aqueous extract of *E. palmifolia* presented higher than ethanol and n-hexane extract. This finding contrasted with Febrinda et al., [36], who discovered that TPC concentrations in ethanolic extracts were higher than those in aqueous extracts. The results of this study are intriguing since the combination of N and K fertilizers can increase the phenolic content that is soluble in aqueous solvents of *E. palmifolia*. As a result, herbal medicine will be safer than utilizing ethanol or hexane extracts.

Table 4. Total phenolic content in aqueous, ethanol and n-hexane extracts of *E. palmifolia*

Treatments	Total phenolic content (mg GAE /g DW)		
	Aqueous	Ethanol	n-Hexane
N_0K_{25}	2.694 ± 0.00 d A	2.740 ± 0.01 a A	0.253 ± 0.01 c B
$N_{100}K_{25}$	3.688 ± 0.00 a A	2.815 ± 0.00 a B	0.304 ± 0.01 a B
$N_{200}K_{25}$	2.192 ± 0.01 e A	2.192 ± 0.01 c A	0.288 ± 0.00 b B
$N_{100}K_0$	3.032 ± 0.00 c A	1.474 ± 0.00 d B	0.251 ± 0.01 c C
$N_{100}K_{50}$	2.624 ± 0.01 d A	2.381 ± 0.00 b B	0.286 ± 0.01 b C
$N_{100}K_{100}$	3.150 ± 0.01 b A	1.940 ± 0.00 b B	0.219 ± 0.00 d C

Note: Each value is expressed as the mean plus standard deviation; a-d, the Scott-Knott test indicates that the mean values in each column denoted by different letters differ significantly at $p < 0.05$; A-C, the Scott-Knott test indicates that the mean values in each row denoted by different letters differ significantly at $p < 0.05$; For the treatment combination, see Table 1.

TFC of *E. palmifolia* was measured using different extraction solvents, namely in aqueous, ethanol, and n-hexane, as shown in Table 5. TFC in the *E. palmifolia* extracts depended on the combination of N and K fertilizer and the extraction solvents. Among the treatment of N and K fertilizer combinations investigated, TFC ranged from 0.398 to 0.529 QE/g DW in aqueous extract, 0.616 to 0.861 QE/g DW in ethanol extract, and 0.573 to 0.676 QE/g DW in n-hexane extract. These results indicated that the TFC in the combination of 100 kg/ha N and 25 kg/ha K has the highest TFC content. TFC was low found in the application of nitrogen-free with 50 kg/ha K extracted using aqueous as a solvent. The results showed that plants produced low amounts of flavonoid compounds without nitrogen fertilizer. Results indicate that nitrogen is an essential element in increasing plant metabolism. In solvent extraction, ethanol extract was presented higher TFC than aqueous and n-hexane extracts. Febrinda et al., [36] also found that TFC in the ethanolic extract were higher than aqueous extract. This study shows that the combination of 100 kg/ha N and 25 kg/ha K fertilizers has increased TFC in ethanol extract of *E. palmifolia*, which is likely to be a dose target for the production of flavonoid compounds.

Table 5. Total flavonoid content in aqueous, ethanol and n-hexane extracts of *E. palmifolia*

Treatments	Total flavonoid content (mg QE /g DW)		
	Aqueous	Ethanol	n-Hexane
N_0K_{25}	0.398 ± 0.00 c C	0.779 ± 0.00 b A	0.651 ± 0.01 b B
$N_{100}K_{25}$	0.529 ± 0.01 a C	0.861 ± 0.02 a A	0.676 ± 0.01 a B
$N_{200}K_{25}$	0.409 ± 0.00 c C	0.842 ± 0.02 a A	0.573 ± 0.01 e B
$N_{100}K_0$	0.464 ± 0.01 b B	0.616 ± 0.01 d A	0.617 ± 0.00 d A
$N_{100}K_{50}$	0.448 ± 0.01 b C	0.711 ± 0.01 c A	0.629 ± 0.00 c B
$N_{100}K_{100}$	0.399 ± 0.01 c C	0.722 ± 0.00 c A	0.572 ± 0.01 e B

Note: Each value is expressed as the mean plus standard deviation; a-e, the Scott-Knott test indicates that the mean values in each column denoted by different letters differ significantly at $p < 0.05$; A-C, the Scott-Knott test indicates that the mean values in each row denoted by different letters differ significantly at $p < 0.05$; For the treatment combination, see Table 1.

3.3. Growth and yield of E. palmifollia

This study examined the antioxidant capacity of different extract of *E. palmifolia* using DPPH and FRAP assays. Based on the antioxidant activities determined using the DPPH (Table 6) and FRAP (Table 7) methods, it is possible to demonstrate that the antioxidant capacity of *E. palmifolia* extracts found using the FRAP method is more significant than that determined using the DPPH method. This effect is caused by distinct reaction processes of antioxidant activity, including power reduction for FRAP and free radical scavenging for DPPH [28]. Antioxidant activity differed amongst sample of *E. palmifolia* extracts, which can be explained by the varying forms or concentrations of antioxidant compounds contained inside [26, 37]. The ability to absorb and neutralize free radicals, to quench single and triple oxidants, or to degrade peroxides is referred to as antioxidant activity [38]. Antioxidant-rich plants may be beneficial as possible therapeutic herbs. The plant's antioxidant properties can help scavenge free radicals. Polyphenol (flavonoid and phenolic) chemicals found in plants act as natural antioxidants, scavenging free radicals—the higher the flavonoids or total phenols content, the better the antioxidant ability in vitro. The antioxidant activity of *E. bulbosa* extract demonstrated its unique nutritional value, indicating that it has the potential to serve as a natural source of antioxidants [8]. Antioxidant activity differed amongst *E. palmifolia* extracts, which can be explained by the varying forms or concentrations of antioxidant components contained inside.

DPPH radical scavenging activity of *E. palmifolia* extracts was affected by N and K fertilizer and solvent extraction, as presented in Table 6. Among the treatment of N and K fertilizer investigated, DPPH scavenging activity ranged from 0.097 to 0.361 µmol TE/g DW. The highest antioxidant scavenging activity (0.361 µmol TE/g DW) was recorded at 100 kg/ha N followed by 200 kg/ha N and 25 kg/ha K combined (0.339 µmol TE/g DW) and 100 kg/ha N and 25 kg/ha K combined (0.318 µmol TE/g DW). These results are agreement with Barzegar et al., [22], who reported that applying N and K fertilizer affected increasing of DPPH scavenging activity in sweet fennel extract. Ma et al., [39] found that the N management increased antioxidant activity in wheat grain. Meanwhile, DPPH scavenging activity in different solvent extracted was ranged from 0.198 to 0.361 µmol TE/g DW in aqueous extract, 0.204 to 0.305 µmol TE/g DW in ethanol extract, and 0.097 to 0.153 µmol TE/g DW in n-hexane extract. Results indicated that aqueous extracts of *E. palmifolia* were higher radical scavenging activity than ethanol and n-hexane extract. Attractive, these results are in line with the data of total phenolic content (Table 4). Antioxidant properties caused by phenolic compounds have been proven in a number of research in the last several decades [40–42].

Table 6. DPPH radical scavenging activity in aqueous, ethanol, and n-hexane extracts of *E. palmifolia*

Treatments	DPPH scavenging activity (µmol TE/g DW)		
	Aqueous	Ethanol	n-Hexane
N_0K_{25}	0.232 ± 0.01 b A	0.221 ± 0.00 c C	0.143 ± 0.01 b A
$N_{100}K_{25}$	0.318 ± 0.01 a A	0.305 ± 0.01 a B	0.153 ± 0.01 a B
$N_{200}K_{25}$	0.339 ± 0.00 a A	0.253 ± 0.00 b C	0.105 ± 0.01 c B
$N_{100}K_0$	0.361 ± 0.01 a A	0.208 ± 0.00 d B	0.073 ± 0.01 d C
$N_{100}K_{50}$	0.215 ± 0.00 b A	0.219 ± 0.00 c A	0.097 ± 0.01 c B
$N_{100}K_{100}$	0.198 ± 0.01 b A	0.204 ± 0.00 d B	0.143 ± 0.01 b A

Note: Each value is expressed as the mean plus standard deviation; a-e, the Scott-Knott test indicates that the mean values in each column denoted by different letters differ significantly at $p < 0.05$; A-C, the Scott-Knott test indicates that the mean values in each row denoted by different letters differ significantly at $p < 0.05$; For the treatment combination, see Table 1.

Ferric reducing antioxidant power (FRAP) value of E. palmifolia extracts is showed in Table 7. FRAP value varied from 61.598 to 123.810 µmol TE/g DW among the treatments of N and K fertilizer studied. The highest reducing antioxidant power value was identified at 100 kg/ha N and 25 kg/ha K. These findings are in line with those of Michalska et al., [35], who found that FRAP value of coloured potato increased when N and K fertilizer was applied. Besides fertilization treatments, the solvent used to extract antioxidants can also have an effect on how effective they are. FRAP value was varied from 87.545 to 104.757 µmol TE/g DW in aqueous extract, 103.393 to 123.810 µmol TE/g DW in ethanol extract, and 61.598 to 72.151 µmol TE/g DW in n-hexane extract. Results indicated that ethanol extracts of E. palmifolia were higher reducing antioxidant power activity than aqueous and n-hexane extracts. According to the total flavonoid content as presented in Table 5, these results are in line with them. These results indicated that flavonoid compounds are responsible for antioxidants activity of E. palmifolia, especially through the mechanism of reducing antioxidant power. There has been a lot of research in the last few decades that shows that flavonoid compounds have antioxidant properties [43–45].

Table 7. Ferric reducing antioxidant power (FRAP) in aqueous, ethanol, and n-hexane extracts of *E. palmifolia*

Treatments	Ferric reducing antioxidant power (µmol TE/g DW)		
	Aqueous	Ethanol	n-Hexane
N_0 + ½ K	91.030 ± 0.00 c B	122.219 ± 0.00 b A	64.242 ± 0.00 c C
N_1 + ½ K	104.757 ± 0.00 a B	123.810 ± 0.01 a A	65.477 ± 0.00 b C
N_2 + ½ K	101.992 ± 0.00 b B	114.621 ± 0.00 c A	72.151 ± 0.00 a C
K_0 + ½ N	90.712 ± 0.00 c B	103.393 ± 0.00 f A	61.856 ± 0.00 d C
K_1 + ½ N	88.401 ± 0.01 d B	106.166 ± 0.00 e A	65.174 ± 0.00 b C
K_2 + ½ N	87.545 ± 0.00 d B	109.681 ± 0.00 d A	61.598 ± 0.00 d C

Note: Each value is expressed as the mean plus standard deviation; a-d, the Scott-Knott test indicates that the mean values in each column denoted by different letters differ significantly at $p < 0.05$; A-C, the Scott-Knott test indicates that the mean values in each row denoted by different letters differ significantly at $p < 0.05$; For the treatment combination, see Table 1.

4. Conclusions

The results of our study indicated that fertilization application of N and K improved chlorophyll reading value, total plant weight, and fresh bulb weight of *E. palmifolia*. In addition, the *E. palmifolia* treated with N and K exhibited higher total phenolic content, total flavonoid content, and antioxidant activity in aqueous and ethanol extracts. These results suggest that commercial application of N 100 kg/ha and K 25 kg/ha can be proposed to improving *E. palmifolia* growth, polyphenol content, and antioxidant activities.

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Conflict of interest

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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Major Revisions—Agri-500

1 message

agriculture@aimspress.org <agriculture@aimspress.org> Thu, Jun 23, 2022 at 2:05 PM
Reply-To: qiqi.li@aimsciences.org
To: Waras Nurcholis <wnurcholis@apps.ipb.ac.id>
Cc: Marlin Marlin <marlin@unib.ac.id>, Marulak Simarmata <marulak_simarmata@yahoo.com>, Umi Salamah <umisalamah@unib.ac.id>

Dear Dr. Nurcholis,

Thank you for submitting the following manuscript to AIMS Agriculture and Food.

Manuscript ID: Agri-500
Type of manuscript: Research article
Title: Effect of nitrogen and potassium application on growth, total phenolic, flavonoid contents, and antioxidant activity of Eleutherina palmifolia
Authors: Waras Nurcholis *, Marlin Marlin, Marulak Simarmata, Umi Salamah
Received: 12 May 2022
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Active components and biological functions of food

It has been reviewed by experts in the field and we request that you make major revisions before it is processed further. Please find your manuscript and the review reports at the following link:
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Please revise the manuscript according to the reviewers' comments and upload the revised file within 14 days. Use the version of your manuscript found at the above link for your revisions, as the editorial office may have made formatting changes to your original submission. Any revisions should be clearly highlighted, for example using the "Track Changes" function in Microsoft Word, so that changes are easily visible to the editors and reviewers. Please provide a cover letter to explain point-by-point the details of the revisions in the manuscript and your responses to the reviewers' comments. Please include in your rebuttal if you found it impossible to address certain comments. The revised version will be inspected by the editors and reviewers.

Do not hesitate to contact us if you have any questions regarding the revision of your manuscript. We look forward to hearing from you soon.

Kind regards,

Best wishes,
Qiqi Li
Editorial Assistant
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Review Reports

Reviewer 1	Review Report (Round 1) You did not reply to the comments yet.
Reviewer 2	Review Report (Round 1) You did not reply to the comments yet.
Reviewer 3	Review Report (Round 1) You did not reply to the comments yet.

Please reply to the above reviewers comments before resubmission of your manuscript.

Upload options will appear for the corresponding author once all comments have been replied to.

Academic Editor Comments for Major revisions

Academic Editor Comments Please continue to peer review

Authors Response

Response authors to reviewer 1

The overall language of the paper needs extensive revision and the discussion part looks very weak. I would suggest you to justify your results in a proper way and adding slight details of the work done by others too which will make it easier to have a comparitive view. There are many errors in the manuscript some of which are as mentioned below:
Author response:
We are trying to improve and fix the writing style of this article, starting from an Abstract to Conclusion, especially fixing the structure of writing English and the content of the article itself.
Improvements to the content of article and grammar as well as references have been done as suggested by the first reviewer.
Overall corrections are highlight in the article.

Line 63: Any study explaining the impact of N & K on secondary metabolites?
Author response: another study with reference [23] has been added to explaining the impact of N & K on polyphenol secondary metabolites.

You can merge lines 93 & 94 : Randomly selected ten plants from each unit were used for data collection.
Author response: corrected.

Do add as of How many plants were grown per set up? As you mentioned 10 were used for data collection, bulbs from all rest of the plants were used for extract preparation?
Author response: corrected. Yes bulbs collected from randomly ten selected plants. Ten plants were grown

Line 101: Add the authors name before reference (26). Like protocol of Lowry et al (26) was used for protein estimation
Author response: corrected.

Line 102: 10,000 X g at (not and)
Author response: corrected.

Line 105: How did you estimate the final conc of E. palmifolia as 0.2 g/ml? Did you maintain same concentration of all extracts?
Author response: The determination of the concentration that we do is based on the dry weight of the plants that we do (2 g dry weigh per 10 mL final volume, thus 0.2 g/mL of concentration sample. This is in accordance with research as in the following literature:
https://www.sciencedirect.com/science/article/pii/S0254629918315758

2.1.1. Sample preparation

Sample was prepared according to the methods proposed by Koley et al., (2018a) with slight modification. The pod of each genotype was cut into small pieces, and after removal of seeds, homogenized in a domestic blender for 2 min. Two gram of homogenized samples were extracted twice with 10 mL of ethanol (80%), by stirring and sonicating for 30 min in the dark. The homogenate was centrifuged for 15 min at 10000 × *g* at 4 °C (model 5427R, Eppendorf, Hamburg, Germany). The supernatant was concentrated up to the volume 10 mL (Final concentration = 0.2 g/mL), stored at – 20 °C, and used as the extract for further estimation of total phenolics, total flavonoids, and antioxidant activity.

Yes, all extracts were same prepared with same concentration.

Line 129-130: What makes you reach the conclusion that increase in chlorophyll content reduces the risk of environmental pollution? Explain
Author response: Thank you for your input. These statements are so irrelevant to the existing data, that we delete them.

What is the significance of enhanced chlorophyll if there is a decrease in the no of tillers as well as leaves on increasing the conc of N upto 200 ??
Author response: The important parameters in the plants that we studied were tubers and plant weight, so for growth parameters these were more important than number of tillers. Our results show a correlation between chlorophyll and total plant weight and bulb data.

Line 171: Your statement doesnot make sense, N & K affects TPC and not the other way round. Write *** extracts "is" strongly influenced "by" the****
Author response: corrected.

Line 177: Rewrite
Author response: corrected.

Line 179: The language is v poor and needs to be rechecked as well as rewritten as most sentences donot make sense.
Author response: corrected.

Line 180: Our finding is in contrast with the findings of………………..
Author response: corrected.

Line 183-184: Explain your justification in detail? What was the work done by Febrinda et al? Did they study different exxtracts? Was any external nutrient application was given by them?
Author response: corrected. Febrinda et al compare TPC contained in ethanol and aqueous extracts of *E. palmifolia* which collected from traditional market in East Kalimantan Indonesia. No information nutrien applied in *E. palmifolia* sample. Therefore, our result compared with extract from Febrinda.

Line 196: rewrite !!!!! Lower content of TFC was obtained after the application of …………….
Author response: corrected.

Line 212: methods, we could conclude that the FRAP method was better than DPPH in analysing the anti-oxidant activities.
Author response: Yes, corrected.

Line 222: Only in vitro? This is in general a fact and applicable to both in vivo as well as in vitro conditions.
Author response: Yes, corrected with in vitro deleded.

Line 225: repeated. Same as line 216
Author response: Yes, corrected with deled the sentence.

Whatever your results are, make them crisp and clear with due reasoning. Also give a clear conclusion as what do you suggest is the best combination of fertilisation.
Author response: Thank you for the input given so that our manuscript deserves to be published.

Response authors to reviewer 2
In my opinion, it is difficult to understand the results because the substrate's chemical characteristics are not presented. The authors must present at least the values of N and K content, the C/N ratio, and the pH of growing media. On the other hand, throughout the text, there is often a certain confusion between nitrogen and nitrogen fertilizer.
Author response:
The properties chemical of growing media is added in experimental treatments section.

As in your growing media, one of the components is manure, which is subject to mineralization is important to add to the material and methods a sentence about climatic conditions during the experiment.
Author response:
The climatic conditions were added in text.

"-replication completely randomized design was used. K fertilizer was applied at the time of planting, while N fertilizer was given in two times, once at the time of planting and again days after 84 planting." Was the amount of urea used in both applications the same?
Author response:
Yes same dose, 0.5 dose in first and o.5 dose at secondly. In manuscript was corrected.

growing medium (mixed of soil, manure, and rice husk in a ratio of 2: 1: 1).
Please insert chemical analysis of the soil used, compost?
What kind of manure?
Ratio 2:1:1 V/V or w/w?
Author response:
The properties chemical of growing media is added in experimental treatments section. Cow manure was used in this study. Ratio was v/v/v. All recommendation was corrected in manuscript.

Table –
Please, instead of kg of Urea, use kg of nitrogen.
Please, instead of kg of KCl, use kg of K or K20. Also is important to take in consideration the amount Cl-.
Author response:
Table 1 corrected. The KCl that we use does not provide information on the Cl content. Thus, we are sorry that we cannot provide this data. In some studies, because Cl is a micronutrient element, the amount is not given. Examples in the following research:

Int. J. Mol. Sci. 2012, 13(11), 15321-15342; https://doi.org/10.3390/ijms131115321

3.1. Experimental Location, Plant Materials and Treatments

The experiment was carried out in growth houses at Field 2, Faculty of Agriculture Greenhouse Complex, Universiti Putra Malaysia (longitude 101°44′ N and latitude 2°58′ S, 68 m above sea level) with a mean atmospheric pressure of 1.013 kPa. Three-month old *L. pumila* seedlings of var. *alata*, were left for a month to acclimatize in a nursery until ready for the treatments, then they were fertilized with four rates of potassium applied in the form of muriate of potash (MOP), *viz*. 0 kg K/ha (0.0 g per plant), 90 kg K/ha (0.25 g per plant), 180 kg K/ha (0.51 g per plant) and 270 kg K/ha (0.76 g per plant). The potassium was split into three fertilization phases, and each phase was about 33.3% of total potassium fertilizer. Every potassium treatment received urea (46% N; 0.72 g per plant) and Triple Super Phosphate, TSP (60% K; 0.51 g per plant) at standard rates of 180 kg /ha during the studies there were no indication of K deficiency in all the pl[illegible] K/ha. The seedlings were planted in soilless medium containing coco-peat and well composted chicken [illegible]:1 (*v*/*v*) ratio in 25 cm diameter polyethylene bags. The medium properties are presented in **Table 7**. Da[illegible] temperatures in the greenhouse were maintained at 27–30 °C and 18–21 °C, respectively, and relative humidity from 50% to 60%. All the seedlings were irrigated using overhead mist irrigation given four times a day or when necessary. Each irrigation session lasted for 7 min [85]. The experiment was based on a Randomized Complete Block Design (RCBD) with four replicates. The factor was four levels of potassium fertilization (0, 90, 180 and 270 kg K/ha). Each combination treatment consisted of 10 plants totaling a sum of 160 plants used in the experiment. Plants were harvested at 12 weeks after planting.

"The role of nitrogen during plant growth can be observed through the chlorophyll reading value in plants to increase the efficiency of nitrogen use according to plant needs and reduce the risk of environmental pollution." I did not understand the previous sentence. In my opinion, you do not have data that permit you to say that was an increase in nitrogen use efficiency.
Author response: The result and discussion were improved with added reference and corrected sentence.

Line 136 The results recorded that treatment of N (100-200 kg/ha) combined with K (25 kg/ha) increased chlorophyll reading value of E. palmifolia compared to other combination treatments (Table 2). Please compare with N0K25
Author response: Corrected.

"fed with nitrogen fertilizer of 180 kg/ha". This sentence is ambiguous. Because the nitrogen concentration varies greatly depending on the fertilizer, I believe you should use kg of nitrogen.
Author response: We think the sentence no related with chlorophyl reading value, therefore this sentence was deleded. Discussion was improved, please see in text.

Why did you use in table 4 two letters, one uppercase and one lowercase? aA must be a?
Author response:
aA compared different data, a for same column comparing mean data of treatment fertilizer, and A for same row comparing mean data of extracts different. Note in table gave detail information about aA.

"These results suggest that commercial application of N 100 kg/ha and K 25 kg/ha can be proposed to improving E. palmifolia growth, polyphenol content, and antioxidant activities." If I understand well, 100 kg/ha of urea were applied, that is, 46 kg of nitrogen, the same reasoning for K. On the other hand, this is only true if the substrate used is the same.
Author response:
Corrected. Our conclusions were obtained from our results, therefore this study condition was needed if use dose fertilization our recommendation.

Response authors to reviewer 3

Its a good study, no doubt, with respect to medicinal plant. According to the journal information, some of the URLs are missing. The author should insert the URLs in the references section. Generally, in overall rsults dissuction, Urea and Kcl both are synthetic fertilizers, please give the proper logic, " how nitrogen increases the total phenolic and total flavonoid contents" as it can be suggested that the phenolics concentration in plants can get decreased at high nitrogen availability and vice versa (Haukioja et al., 1998). Recently, Hakkinen and Torronen (2000) noticed maximum phenolic compounds in organically grown strawberry in comparison with other strawberry cultivars cultivated inorganically.

If author gives the proper logic for increasing the total phenolic and total falvonoid contents by the application of nitrogen through Urea, then paper may be accepted for publication in the journal otherwise rejected in my point of view.

Author response:

Our manuscript has been improved based on reviewer comment. The manuscript revision presented with gray-25% highlight.

3.2. Growth and yield of E. palmifollia

TPC in the *E. palmifolia* extracts is strongly influenced by the combination of N and K fertilizer. TPC ranged from 2.192-3.688 GAE /g DW in aqueous extract, 1.474-2.815 GAE/g DW in ethanol extract, and 0.219-0.304 GAE/g DW in n-hexane extract (Table 4). The phenolic content of several plants has been shown to be affected by environmental factors and crop management [41, 42]. Phenolics are antioxidant compounds in stressed plant tissues [43], especially those with nutritional deficiencies [44]. Previous research revealed that the concentration of phenolics varies in response to N and K fertilization. Nitrogen is the substrate for the biosynthesis of phenylalanine, whereas phenylalanine is the substrate for polyphenol chemicals [45]. In most cases, increasing the application of nitrogen reduces the polyphenol content of plants [46]. It is possible, due to conflict between the utilization of nitrogen for growth and the formation of secondary metabolites; if growth increases, polyphenolic compound production will decrease [42]. In general, the administration of N and K fertilizers has been regarded as a technique for enhancing the phenolic content of plant tissue [47]. Among the combination treatments of N and K fertilizers observed, the combination treatment of 46 kg/ha N and 12.5 kg/ha K fertilizers produced the highest TPC (aqueous extract, 3.688 mg GAE/g DW) compared to other treatments. These results indicated that N and K fertilizers strongly affected the

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Effect of nitrogen and potassium application on growth, total phenolic, flavonoid contents, and antioxidant activity of *Eleutherina palmifolia*

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Abstract: *Eleutherine palmifolia* is one of the medicinal plants widely used by the Dayak and Kutai tribes in Borneo Island, Indonesia as traditional medicines that can treat various diseases. Identification of the phytochemical content of *E. palmifolia* is very important to determine its potential as a medicinal plant. A significant factor in the success of the growth of *E. palmifolia* is the amount of fertilizer applied as a source of nutrients. In this study, the influence of various amounts of nitrogen (N) and potassium (K) fertilizers on the growth, total phenolic and flavonoid content (TPC and TFC), and antioxidant activity was investigated in different extracts of *E. palmifolia*. The treatments included 0, 46, or 92 kg/ha of N combined with 12.5 kg/ha of K, and 0, 25, or 50 kg/ha of K combined with 46 kg/ha of N. Using water, ethanol, or n-hexane as solvents, each sample was extracted with sonication method. TPC and TFC of the bulb extracts were quantified using Folin-Ciocalteu and aluminum chloride assays, respectively. Antioxidant activity was analyzed using 2,2′-diphenyl-1-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) assays. Results showed that combining N and K had no significant effects on plant height, the number of tillers, leaves, or bulbs, and bulb diameter, while the chlorophyll reading value, total plant weight, and fresh bulb weight were significantly increased. The results indicated that the availability of N and K has a substantial effect on TPC, TFC, and antioxidant activity. The aqueous extract had the highest TPC and DPPH scavenging activity, whereas the ethanol extract exhibited the highest TFC and antioxidant FRAP activity. Therefore, to improve fresh bulb weight, phenol and flavonoid content, and antioxidant properties of *E. palmifolia* aqueous and ethanol extracts, supplementation of 46 kg/ha N and 12.5 kg/ha K is recommended.

Keywords: Antioxidants; DPPH; flavonoid; FRAP; Iradaceae; phenolic

1. Introduction

Eleutherine palmifolia (L.) Merr., in Indonesia called "bawang dayak", is a medicinal plant that is grown in Borneo Island, Kalimantan, Indonesia. *E. palmifolia* is one of the species of Iridaceae family and is considered a synonym of *E. americana, E. plicata*, and *E. bulbosa* [1, 2]. This species is widely cultivated in regions of Africa, South America, and Asia for its beneficial chemical compounds as traditional medicine [2, 3]. *E. palmifolia* is traditionally used to treat diabetes, stroke, hypertension, breast cancer, fertility, and hypertensive diseases [4]. Numerous studies reported the beneficial effects of *E. palmifolia* on human health, including its anti-cancer [5], anti-fungal [6], anti-malarial [7], antioxidant [8], and anti-melanogenesis [9] activities. Bioactive chemicals of *E. palmifolia* have a large variety of pharmacological targets. For example, the antidiabetic activity of *E. palmifolia* is strongly connected with its eleutherinoside A content [4], whereas its antioxidant activity is associated with polyphenols [8]. Polyketide compounds in *E. palmifolia* have been linked to its antimicrobial activity [10]. With regard to investigations on *E. palmifolia* as a medicinal plant, it is very important to associate the number of active compounds in the plant extracts with its pharmacological activity. Therefore, in this study, total phenolic and flavonoid content (TPC and TFC) and antioxidant activity were assessed as parameters to evaluate the effect of nitrogen (N) and potassium (K) fertilizers on *E. palmifolia*.

Plant nutrition is a critical aspect of increasing plant productivity. An important factor in plant growth and development is N [11]. N fertilizers have an impact on the yield and productivity of many plants, such as onions [12, 13], rice [14], and milk thistle [15]. On the other hand, K is one of the most critical elements in physiological functions, such as photosynthetic capacity, ionic homeostasis, carbohydrate metabolism [16], enzymatic activation [17], water relations [18], osmotic adjustment [19], or resistance against abiotic stress [20, 21] of plants. Secondary metabolites and their biological activity in plants may be influenced by N and K levels as reported by Barzegar et al [22], who stated that combination of N and K increased the polyphenol and vitamin C content and antioxidant activity of sweet fennel. Another study found that fertilizing N and K combination on *Berberis microphylla* increased the polyphenol secondary metabolite contents and antioxidant activities [23]. Typically, *E. palmifolia* is planted as a side plant by farmers and is not cultivated under optimal conditions. Numerous fertilizers can influence the flavonoid content, growth, and yield of *E. palmifolia*. Application of NPK 16:16:16 fertilizer at 2.5 g/L increased the production of *E. palmifolia*, and a concentration of 1.25 g/L increased its flavonoid content [24], chicken manure [25], and vinasse liquid organic [26]; however, our knowledge is limited about the effect of the N and K on the growth, polyphenol content, and antioxidant activity of *E. palmifolia*. In this study, we investigated the effect of N and K combination on morphological characters, polyphenol content and antioxidant activity of different extracts of *E. palmifolia*. The application of nitrogen and potassium fertilizers with optimum concentrations may have a positive impact on increasing the growth and yield of E. palmifolia, as well as improving the quality of its important chemical compounds.

2. Materials and Methods

2.1. Experimental treatments

Eleutherine palmifolia seedlings were obtained from the Faculty of Agriculture of Bengkulu University. The research was conducted from February to June 2020 in Bengkulu province, Indonesia (3°45'44" S; 102°16'45" E; altitude of 15 m). Bengkulu University station is located at 15 m above sea level with range of humidity of 78.6% to 85.9%, temperature of 25.5°C to 28.5°C, and 4.5 mm/year of rainfall. Urea (46% N) and KCl (50% K_2O) were used as the N and K source fertilizer, respectively. The treatments of N and K combinations are presented in Table 1. The experiment was organized in a completely randomized design with 3 replications. K fertilizer was applied at the time of planting, while N fertilizer was given in two times, once at the time of planting (0.5 dose) and again 21 days after planting (0.5 dose).

The sixth-month bulbs of *E. palmifolia* were used as planting material. The bulbs were selected with relatively uniform shape and size. The top 1/4 of bulbs were cut horizontally using a cutter to enhance shoot growth. The bulbs were immersed in a PGPR (*plant growth promotion rhizobacteria*) solution with a concentration of 10 ml/L for 15 min. Bulbs were planted in polybags with a diameter of 45 cm containing 10 kg of growing medium (mixed of soil, cow manure, and rice husk in a ratio of 2: 1: 1 (*v/v/v*)). The chemicals properties of growing medium were consisted of pH 4.43, C 3.99%, N 0.31%, P 6.10 ppm, and K 0.20 (me/100). Each polybag was planted with three shallot bulbs. The irrigation, fertilization, weed, and diseases control were manually performed for maintenance of plants. Plant height, number of tillers, number of leaves, chlorophyll reading value (SPAD), total plant weight, number of bulbs, the diameter of the bulb, and fresh bulb weight were observed as morphological characteristics. Randomly selected ten plants from each unit were used for data collection. Four months after planting, the bulb of *E. palmifolia* (n = 10 plants) randomly selected plant was harvested and further analyses were carried out.

Table 1. Treatment combination of N and K fertilizers

Treatment combination	kg of N per ha	kg of K per ha
N_0K_{25}	0	12.5
$N_{100}K_{25}$	46	12.5
$N_{200}K_{25}$	92	12.5
$N_{100}K_0$	46	0
$N_{100}K_{50}$	46	25
$N_{100}K_{100}$	46	50

2.2. Sample preparation and extraction

The bulbs collected of randomly selected ten plants of each treatment were cleaned, cut, dried, and then reduced into powdered form. Samples were extracted using aqueous, ethanol, and n-hexane as solvent extraction using the procedure by Nurcholis et al [27] with modifications. Briefly, sample (2 g) was extracted twice with solvent (10 mL) in the dark room and then sonicated and stirred for 30 min. The mixtures were centrifugated at 10,000 x g at 4°C (Kitman-T24, Tomy Tech USA Inc.) for 15 min, and the supernatants were concentrated to the volume of 10 mL using a rotary vacuum evaporator (HAHNVAPOR, Korea). The final extract concentration of *E. palmifolia* that used for further analysis was 2 g/10 mL or 0.2 g/mL. The final extract of *E. palmifolia* was obtained and then used for further analysis total phenolic, flavonoid contents, and antioxidant activity.

2.3. Total phenolic content (TPC) and total flavonoid content (TFC)

In accordance with previously published assays, TPC and TFC were determined using spectrophotometric techniques [28]. TPC was measured in the extract obtained from each sample with Folin-Ciocalteu reagent and expressed as mg gallic acid equivalents per g of dry weight (mg GAE/g DW). Meanwhile, TFC was measured using an aluminium chloride reagent and expressed as mg quercetin equivalents per g of dry weight (mg QE/g DW).

2.4. Antioxidant analysis

Antioxidant activity was determined using two in-vitro techniques. The free radical scavenging and reducing power antioxidant activities were determined using the 2,2-diphenyl picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) assays [29]. Results of antioxidant activity expressed in µmol Trolox equivalent per g of dry weight (µmol TE/g DW).

2.5. Antioxidant analysis

Three replicates were used to get mean data ± SD. ANOVA was determined using the ExpDes packages in R, and then a Scott-Knott test was performed [30]. When $p < 0.05$ is used, there are significant differences.

3. Results and Discussion

3.1. Growth and yield of E. palmifollia

Nitrogen is a macroelement that all plants, including *E. palmifolia*, require in substantial amounts to enhance their growth and productivity [13]. The results of the study showed that the combination of N and K fertilizer significantly ($p < 0.05$) affected chlorophyll reading value and total plant weight, which all attained maximum values at a combination of 46 kg/ha N and 12.5 kg/ha K (Table 2). The results showed that the combination treatment of N and K fertilizers has no significant effect on the growth parameters of plant height, number of tillers, and leaves of *E. palmifolia*. An increase in chlorophyll reading value with growth parameters was also found in strawberries treated with fresh chicken manure [31]. The role of nitrogen during plant growth can be observed through the chlorophyll reading value in plants to increase the efficiency of N and K use according to plant needs [32].

Table 2. The growth characters of *E. plamifolia* as a response to the application of different fertilizers

Treatment combination	Plant height (cm)	Number of tillers	Number of leaves	Chlorophyll reading value (SPAD)	Total plant weight (g)
N_0K_{25}	36.32 ± 2.14a	8.00± 5.29a	27.00±5.29a	46.03±6.56a	78.00±6.55b

$N_{100}K_{25}$	40.58 ± 3.64a	13.67±7.37a	40.00±9.98a	47.65±1.43a	95.33±7.50a
$N_{200}K_{25}$	41.95 ± 1.72a	8.00± 2.64a	25.00±2.64a	47.80±11.60a	78.67±8.08b
$N_{100}K_{0}$	38.92 ± 1.59a	10.17±10.15a	31.67±10.15a	37.33± 4.73b	94.83±14.25a
$N_{100}K_{50}$	39.90 ± 2.03a	10.83±6.33a	36.83± 6.33a	37.00±4.73b	106.33±12.66a
$N_{100}K_{100}$	40.62 ± 2.27a	10.67±9.98a	39.33±7.37a	35.33±1.53b	102.83±10.34a

Note: Each value is expressed as the mean plus standard deviation; The Scott-Knott test indicates that the mean values in each column denoted by different letters differ significantly at $p < 0.05$; For the treatment combination, see Table 1.

The results recorded that treatment of N (0-92 kg/ha) combined with K (12.5 kg/ha) increased the chlorophyll reading value of *E. palmifolia* compared to other combination treatments (Table 2). This data indicated that the K (12.5 kg/ha) produced the highest chlorophyll reading value. The obtained data of this work were in line with the previous study on cotton photosynthetic capacity [33]. As reported by Naciri et al. [34], potassium significantly affects the chlorophyll content of tomato. The result indicated the importance of the presence of K for plant growth of *E. palmifolia*, especially in the chlorophyll formation.

A higher total plant weight parameter was shown in the combination treatment of 46 kg/ha N with 12.5 kg/ha K, which was not significantly different from the combination treatment of 46 kg/ha N with 0-50 kg/ha K (Table 2). These results indicate that the application of nitrogen fertilizer combination of N and K was required for *E. palmifolia* to increase growth and development, which was shown by higher plant weights. In different plants, N and K enhances the growth performance in rice [35], strawberry [36], and rainfed wheat [37] plants.

The effect of N and K fertilizers on the yield parameters of *E. palmifolia* is presented in Table 3. The results showed that the combination of N and K fertilizers has no significantly different effect on the number and diameter of a bulb of *E. palmifolia*. However, the combination of N and K fertilizers significantly impacted fresh bulb weight. The highest fresh bulb weight (66.01-71.14 g/clump) was obtained in the combination treatment of N fertilizer (46-92 kg/ha) with K fertilizer (0-50 kg/ha). Similar results were previously reported by Gebretsadik and Dechassa [38] that increasing the rate of nitrogen from 50 to 100 kg/ha increased the onion marketable fresh bulb yield by a different percentage of 30%. The result showed that the treatment of K fertilizer (12.5 kg/ha) without N fertilizer produced the lowest tuber weight (58.68 g/clump). The result indicates the importance of nitrogen nutrients in increasing the yield of *E. palmifolia*. Similar results were reported by Uher et al., [39] that the yield depended on the increase in nitrogen dose, and the highest nitrogen dose obtained the highest broccoli yield. The deficiency of nitrogen may decrease chlorophyll biosynthesis, which leads to yield reduction.

Table 3. The yield components of *E. plamifollia* as response to fertilizer application

Treatments	Number of bulbs	Diameter of bulb (cm)	Fresh bulb weight (g)
$N_{0}K_{25}$	14.67 ± 1.3 a	1.75 ± 0.06 a	58.68 ± 6.11 b
$N_{100}K_{25}$	16.17 ± 2.4 a	1.76 ± 0.08 a	71.14 ± 5.29 a
$N_{200}K_{25}$	13.83 ± 1.5 a	1.75 ± 0.02 a	67.06 ± 2.64 a
$N_{100}K_{0}$	11.50 ± 2.6 a	1.81 ± 0.06 a	65.55 ± 8.75 a

$N_{100}K_{50}$	11.50 ± 0.9 a	1.86 ± 0.09 a	66.70 ± 5.76 a
$N_{100}K_{100}$	14.67 ± 4.5 a	1.75 ± 0.11 a	66.01 ± 7.28 a

Note: Each value is expressed as the mean plus standard deviation; The Scott-Knott test indicates that the mean values in each column denoted by different letters differ significantly at $p < 0.05$; For the treatment combination, see Table 1.

The results showed that without the addition of K fertilizer, *E. palmifolia* produced good yields (Table 3). Under conditions of low K availability, plants have a strategy to maintain K needs. For example, the plant increased K uptake from the soil redistributes K ions between cytosolic and vacuolar pools, cytosolic homeostasis, and modifications in root system development and architecture [40]. Supplying optimum N and K levels was proved to be essential for plant growth and production of high yield and improving the quality of *E. palmifolia*.

3.2. Growth and yield of E. palmifollia

TPC in the *E. palmifolia* extracts is strongly influenced by the combination of N and K fertilizer. TPC ranged from 2.192-3.688 GAE /g DW in aqueous extract, 1.474-2.815 GAE/g DW in ethanol extract, and 0.219-0.304 GAE/g DW in n-hexane extract (Table 4). The phenolic content of several plants has been shown to be affected by environmental factors and crop management [41, 42]. Phenolics are antioxidant compounds in stressed plant tissues [43], especially those with nutritional deficiencies [44]. Previous research revealed that the concentration of phenolics varies in response to N and K fertilization. Nitrogen is the substrate for the biosynthesis of phenylalanine, whereas phenylalanine is the substrate for polyphenol chemicals [45]. In most cases, increasing the application of nitrogen reduces the polyphenol content of plants [46]. It is possible, due to conflict between the utilization of nitrogen for growth and the formation of secondary metabolites; if growth increases, polyphenolic compound production will decrease [42]. In general, the administration of N and K fertilizers has been regarded as a technique for enhancing the phenolic content of plant tissue [47]. Among the combination treatments of N and K fertilizers observed, the combination treatment of 46 kg/ha N and 12.5 kg/ha K fertilizers produced the highest TPC (aqueous extract, 3.688 mg GAE/g DW) compared to other treatments. These results indicated that N and K fertilizers strongly affected the growth and polyphenol metabolism of *E. palmifolia*. Similar results were recorded that boosting N and K fertilization increased the total phenolic content in sweet fennel [22] and colored potato [48]. Meanwhile, theTPC content obtained in *E. palmifolia* with aqueous extract was higher than using ethanol and n-hexane extracts. Our finding is in contras with Febrinda et al., [49], who discovered that TPC concentrations in ethanolic extract from traditional market at East Kalimantan, Indonesia was higher than those in aqueous extract. The results of this study are intriguing since the combination of N and K fertilizers can increase the phenolic content that is soluble in aqueous solvents of *E. palmifolia*. As a result, herbal medicine will be safer than utilizing ethanol or hexane extracts.

Table 4. Total phenolic content in aqueous, ethanol and n-hexane extract of *E. palmifolia*

Treatments	Total phenolic content (mg GAE /g DW)		
	Aqueous	Ethanol	n-Hexane
N_0K_{25}	2.694 ± 0.00 d A	2.740 ± 0.01 a A	0.253 ± 0.01 c B

$N_{100}K_{25}$	3.688 ± 0.00 a A	2.815 ± 0.00 a B	0.304 ± 0.01 a B
$N_{200}K_{25}$	2.192 ± 0.01 e A	2.192 ± 0.01 c A	0.288 ± 0.00 b B
$N_{100}K_{0}$	3.032 ± 0.00 c A	1.474 ± 0.00 d B	0.251 ± 0.01 c C
$N_{100}K_{50}$	2.624 ± 0.01 d A	2.381 ± 0.00 b B	0.286 ± 0.01 b C
$N_{100}K_{100}$	3.150 ± 0.01 b A	1.940 ± 0.00 b B	0.219 ± 0.00 d C

Note: Each value is expressed as the mean plus standard deviation; a-d, the Scott-Knott test indicates that the mean values in each column denoted by different letters differ significantly at $p < 0.05$; A-C, the Scott-Knott test indicates that the mean values in each row denoted by different letters differ significantly at $p < 0.05$; For the treatment combination, see Table 1.

TFC of *E. palmifolia* was measured using different extraction solvents, namely aqueous, ethanol, and n-hexane, as shown in Table 5. TFC in the *E. palmifolia* extracts depended on the combination of N and K fertilizer and the extraction solvents. Among the treatment of N and K fertilizer combinations investigated, TFC ranged from 0.398 to 0.529 QE/g DW in aqueous extract, 0.616 to 0.861 QE/g DW in ethanol extract, and 0.573 to 0.676 QE/g DW in n-hexane extract. These results indicated that the TFC in the combination of 46 kg/ha N and 12.5 kg/ha K has the highest TFC content. Lower content of TFC with aqueous extracts as a solvent was obtained after the application of the nitrogen-free with K of 25 kg/ha. The results showed that plants produced low amounts of flavonoid compounds without nitrogen fertilizer. Results indicate that nitrogen is an essential element in increasing plant metabolism. In solvent extraction, ethanol extract was presented higher TFC than aqueous and n-hexane extracts. Febrinda et al., [49] also found that TFC in the ethanolic extract were higher than aqueous extract. This study shows that the combination of 46 kg/ha N and 12.5 kg/ha K fertilizers has increased TFC in ethanol extract of *E. palmifolia*, which is likely to be a dose target for the production of flavonoid compounds.

Table 5. Total flavonoid content in aqueous, ethanol and n-hexane extract of *E. palmifolia*

Treatments	Total flavonoid content (mg QE /g DW)		
	Aqueous	Ethanol	n-Hexane
$N_{0}K_{25}$	0.398 ± 0.00 c C	0.779 ± 0.00 b A	0.651 ± 0.01 b B
$N_{100}K_{25}$	0.529 ± 0.01 a C	0.861 ± 0.02 a A	0.676 ± 0.01 a B
$N_{200}K_{25}$	0.409 ± 0.00 c C	0.842 ± 0.02 a A	0.573 ± 0.01 e B
$N_{100}K_{0}$	0.464 ± 0.01 b B	0.616 ± 0.01 d A	0.617 ± 0.00 d A
$N_{100}K_{50}$	0.448 ± 0.01 b C	0.711 ± 0.01 c A	0.629 ± 0.00 c B
$N_{100}K_{100}$	0.399 ± 0.01 c C	0.722 ± 0.00 c A	0.572 ± 0.01 e B

Note: Each value is expressed as the mean plus standard deviation; a-e, the Scott-Knott test indicates that the mean values in each column denoted by different letters differ significantly at $p < 0.05$; A-C, the Scott-Knott test indicates that the mean values in each row denoted by different letters differ significantly at $p < 0.05$; For the treatment combination, see Table 1.

3.3. Growth and yield of E. palmifollia

This study examined the antioxidant capacity of different extracts of *E. palmifolia* using DPPH and FRAP assays. Based on the antioxidant activities determined using the DPPH (Table 6) and FRAP (Table 7) methods, we concluded that FRAP method was better than DPPH in analyzing the anti-oxidant activities. This effect is caused by distinct reaction processes of antioxidant activity, including power reduction for FRAP and free radical scavenging for DPPH [29]. Antioxidant activity differed amongst a sample of *E. palmifolia* extracts, which can be explained by the varying forms or concentrations of antioxidant compounds contained inside [27, 50]. The ability to absorb and neutralize free radicals, to quench single and triple oxidants, or to degrade peroxides is referred to as antioxidant activity [51]. Antioxidant-rich plants may be beneficial as possible therapeutic herbs. The plant's antioxidant properties can help scavenge free radicals. Polyphenol (flavonoid and phenolic) chemicals found in plants act as natural antioxidants, scavenging free radicals—the higher the flavonoids or total phenols content, the better the antioxidant ability. The antioxidant activity of *E. bulbosa* extracts demonstrated its unique nutritional value, indicating that it has the potential to serve as a natural source of antioxidants [8].

DPPH radical scavenging activity of *E. palmifolia* extracts was affected by N and K fertilizer and solvent extraction, as presented in Table 6. Among the treatment of N and K fertilizer investigated, DPPH scavenging activity ranged from 0.097 to 0.361 µmol TE/g DW. The highest antioxidant scavenging activity (0.361 µmol TE/g DW) was recorded at 46 kg/ha N followed by 92 kg/ha N and 12.5 kg/ha K combined (0.339 µmol TE/g DW) and 46 kg/ha N and 12.5 kg/ha K combined (0.318 µmol TE/g DW). These results are in agreement with Barzegar et al., [22], who reported that applying N and K fertilizer affected increasing of DPPH scavenging activity in sweet fennel extract. Ma et al., [52] found that the N management increased antioxidant activity in wheat grain. Meanwhile, DPPH scavenging activity in different solvents extracted ranged from 0.198 to 0.361 µmol TE/g DW in aqueous extract, 0.204 to 0.305 µmol TE/g DW in ethanol extract, and 0.097 to 0.153 µmol TE/g DW in n-hexane extract. Results indicated that aqueous extracts of *E. palmifolia* were higher radical scavenging activity than ethanol and n-hexane extract. Attractive, these results are in line with the data of total phenolic content (Table 4). Antioxidant properties caused by phenolic compounds have been proven in a number of research in the last several decades [53–55].

Table 6. DPPH radical scavenging activity in aqueous, ethanol, and n-hexane extracts of *E. palmifolia*

Treatments	DPPH scavenging activity (µmol TE/g DW)		
	Aqueous	Ethanol	n-Hexane
N_0K_{25}	0.232 ± 0.01 b A	0.221 ± 0.00 c C	0.143 ± 0.01 b A
$N_{100}K_{25}$	0.318 ± 0.01 a A	0.305 ± 0.01 a B	0.153 ± 0.01 a B
$N_{200}K_{25}$	0.339 ± 0.00 a A	0.253 ± 0.00 b C	0.105 ± 0.01 c B
$N_{100}K_0$	0.361 ± 0.01 a A	0.208 ± 0.00 d B	0.073 ± 0.01 d C
$N_{100}K_{50}$	0.215 ± 0.00 b A	0.219 ± 0.00 c A	0.097 ± 0.01 c B
$N_{100}K_{100}$	0.198 ± 0.01 b A	0.204 ± 0.00 d B	0.143 ± 0.01 b A

Note: Each value is expressed as the mean plus standard deviation; a-e, the Scott-Knott test indicates that the mean values in each column denoted by different letters differ significantly at $p < 0.05$; A-C, the Scott-Knott test indicates that the mean values in each row denoted by different letters differ significantly at $p < 0.05$; For the treatment combination, see Table 1.

Ferric reducing antioxidant power (FRAP) value of *E. palmifolia* extracts is showed in Table 7. FRAP value varied from 61.598 to 123.810 µmol TE/g DW among the treatments of N and K fertilizer studied. The highest reducing antioxidant power value was identified at 46 kg/ha N and 12.5 kg/ha K. These findings are in line with those of Michalska et al., [48], who found that the FRAP value of colored potato increased when N and K fertilizer was applied. Besides fertilization treatments, the solvent used to extract antioxidants can also have an effect on how effective they are. The FRAP values varied from 87.545 to 104.757 µmol TE/g DW in aqueous extract, 103.393 to 123.810 µmol TE/g DW in ethanol extract, and 61.598 to 72.151 µmol TE/g DW in n-hexane extract. Results indicated that ethanol extracts of *E. palmifolia* were higher in reducing antioxidant power activity than aqueous and n-hexane extracts. According to the total flavonoid content as presented in Table 5, these results are in line with them. These results indicated that flavonoid compounds are responsible for the antioxidant activity of *E. palmifolia*, especially through the mechanism of reducing antioxidant power. There has been a lot of research in the last few decades that shows that flavonoid compounds have antioxidant properties [56–58].

Table 7. Ferric reducing antioxidant power (FRAP) in aqueous, ethanol, and n-hexane extracts of *E. palmifolia*

Treatments	Ferric reducing antioxidant power (µmol TE/g DW)		
	Aqueous	Ethanol	n-Hexane
N_0K_{25}	91.030 ± 0.00 c B	122.219 ± 0.00 b A	64.242 ± 0.00 c C
$N_{100}K_{25}$	104.757 ± 0.00 a B	123.810 ± 0.01 a A	65.477 ± 0.00 b C
$N_{200}K_{25}$	101.992 ± 0.00 b B	114.621 ± 0.00 c A	72.151 ± 0.00 a C
$N_{100}K_0$	90.712 ± 0.00 c B	103.393 ± 0.00 f A	61.856 ± 0.00 d C
$N_{100}K_{50}$	88.401 ± 0.01 d B	106.166 ± 0.00 e A	65.174 ± 0.00 b C
$N_{100}K_{100}$	87.545 ± 0.00 d B	109.681 ± 0.00 d A	61.598 ± 0.00 d C

Note: Each value is expressed as the mean plus standard deviation; a-d, the Scott-Knott test indicates that the mean values in each column denoted by different letters differ significantly at $p < 0.05$; A-C, the Scott-Knott test indicates that the mean values in each row denoted by different letters differ significantly at $p < 0.05$; For the treatment combination, see Table 1.

4. Conclusions

The results of our study indicated that fertilization application of N and K improved chlorophyll reading value, total plant weight, and fresh bulb weight of *E. palmifolia*. In addition, the *E. palmifolia* treated with N and K exhibited higher total phenolic content, total flavonoid content, and antioxidant activity in aqueous and ethanol extracts. These results suggest that commercial application of N 46 kg/ha and K 12.5 kg/ha can be proposed to improve *E. palmifolia* growth, polyphenol content, and antioxidant activities.

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determination.

Conflict of interest

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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Research articles

Effect of nitrogen and potassium application on growth, total phenolic, flavonoid contents, and antioxidant activity of *Eleutherina palmifolia*

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Abstract: *Eleutherine palmifolia* is one of the medicinal plants widely used by the Dayak and Kutai tribes in Borneo Island, Indonesia as traditional medicines that can treat various diseases. Identification of the phytochemical content of *E. palmifolia* is very important to determine its potential as a medicinal plant. A significant factor in the success of the growth of *E. palmifolia* is the amount of fertilizer applied as a source of nutrients. In this study, the influence of various amounts of nitrogen (N) and potassium (K) fertilizers on the growth, total phenolic and flavonoid content (TPC and TFC), and antioxidant activity was investigated in different extracts of *E. palmifolia*. The treatments included 0, 46, or 92 kg/ha of N combined with 12.5 kg/ha of K, and 0, 25, or 50 kg/ha of K combined with 46 kg/ha of N. Using water, ethanol, or n-hexane as solvents, each sample was extracted with sonication method. TPC and TFC of the bulb extracts were quantified using Folin-Ciocalteu and aluminum chloride assays, respectively. Antioxidant activity was analyzed using 2,2′-diphenyl-1-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) assays. Results showed that combining N and K had no significant effects on plant height, the number of tillers, leaves, or bulbs, and bulb diameter, while the chlorophyll reading value, total plant weight, and fresh bulb weight were significantly increased. The results indicated that the availability of N and K has a substantial effect on TPC, TFC, and antioxidant activity. The aqueous extract had the highest TPC and DPPH scavenging activity, whereas the ethanol extract exhibited the highest TFC and antioxidant FRAP activity. Therefore, to improve fresh bulb weight, phenol and flavonoid content, and antioxidant properties of *E. palmifolia* aqueous and ethanol extracts, supplementation of 46 kg/ha N and 12.5 kg/ha K is recommended.

Keywords: Antioxidants; DPPH; flavonoid; FRAP; Iradaceae; phenolic

1. Introduction

Eleutherine palmifolia (L.) Merr., in Indonesia called "bawang dayak", is a medicinal plant that is grown in Borneo Island, Kalimantan, Indonesia. *E. palmifolia* is one of the species of Iridaceae family and is considered a synonym of *E. americana, E. plicata*, and *E. bulbosa* [1, 2]. This species is widely cultivated in regions of Africa, South America, and Asia for its beneficial chemical compounds as traditional medicine [2, 3]. *E. palmifolia* is traditionally used to treat diabetes, stroke, hypertension, breast cancer, fertility, and hypertensive diseases [4]. Numerous studies reported the beneficial effects of *E. palmifolia* on human health, including its anti-cancer [5], anti-fungal [6], anti-malarial [7], antioxidant [8], and anti-melanogenesis [9] activities. Bioactive chemicals of *E. palmifolia* have a large variety of pharmacological targets. For example, the antidiabetic activity of *E. palmifolia* is strongly connected with its eleutherinoside A content [4], whereas its antioxidant activity is associated with polyphenols [8]. Polyketide compounds in *E. palmifolia* have been linked to its antimicrobial activity [10]. With regard to investigations on *E. palmifolia* as a medicinal plant, it is very important to associate the number of active compounds in the plant extracts with its pharmacological activity. Therefore, in this study, total phenolic and flavonoid content (TPC and TFC) and antioxidant activity were assessed as parameters to evaluate the effect of nitrogen (N) and potassium (K) fertilizers on *E. palmifolia*.

Plant nutrition is a critical aspect of increasing plant productivity. An important factor in plant growth and development is N [11]. N fertilizers have an impact on the yield and productivity of many plants, such as onions [12, 13], rice [14], and milk thistle [15]. On the other hand, K is one of the most critical elements in physiological functions, such as photosynthetic capacity, ionic homeostasis, carbohydrate metabolism [16], enzymatic activation [17], water relations [18], osmotic adjustment [19], or resistance against abiotic stress [20, 21] of plants. Secondary metabolites and their biological activity in plants may be influenced by N and K levels as reported by Barzegar et al [22], who stated that combination of N and K increased the polyphenol and vitamin C content and antioxidant activity of sweet fennel. Another study found that fertilizing N and K combination on *Berberis microphylla* increased the polyphenol secondary metabolite contents and antioxidant activities [23]. Typically, *E. palmifolia* is planted as a side plant by farmers and is not cultivated under optimal conditions. Numerous fertilizers can influence the flavonoid content, growth, and yield of *E. palmifolia*. Application of NPK 16:16:16 fertilizer at 2.5 g/L increased the production of *E. palmifolia*, and a concentration of 1.25 g/L increased its flavonoid content [24], chicken manure [25], and vinasse liquid organic [26]; however, our knowledge is limited about the effect of the N and K on the growth, polyphenol content, and antioxidant activity of *E. palmifolia*. In this study, we investigated the effect of N and K combination on morphological characters, polyphenol content and antioxidant activity of different extracts of *E. palmifolia.* The application of nitrogen and potassium fertilizers with optimum concentrations may have a positive impact on increasing the growth and yield of E. palmifolia, as well as improving the quality of its important chemical compounds.

2. Materials and Methods

2.1. Experimental treatments

Eleutherine palmifolia seedlings were obtained from the Faculty of Agriculture of Bengkulu University. The research was conducted from February to June 2020 in Bengkulu province, Indonesia (3°45'44" S; 102°16'45" E; altitude of 15 m). Bengkulu University station is located at 15 m above sea level with range of humidity of 78.6% to 85.9%, temperature of 25.5°C to 28.5°C, and 4.5 mm/year of rainfall. Urea (46% N) and KCl (50% K_2O) were used as the N and K source fertilizer, respectively. The treatments of N and K combinations are presented in Table 1. The experiment was organized in a completely randomized design with 3 replications. K fertilizer was applied at the time of planting, while N fertilizer was given in two times, once at the time of planting (0.5 dose) and again 21 days after planting (0.5 dose). The fertilizers were given according to the treatments as presented in Table 1.

The sixth-month bulbs of *E. palmifolia* were used as planting material. The bulbs were selected with relatively uniform shape and size. The top 1/4 of bulbs were cut horizontally using a cutter to enhance shoot growth. The bulbs were immersed in a PGPR (*plant growth promotion rhizobacteria*) solution with a concentration of 10 ml/L for 15 min. Bulbs were planted in polybags with a diameter of 45 cm containing 10 kg of growing medium (mixed of soil, cow manure, and rice husk in a ratio of 2: 1: 1 (*v/v/v*)). The chemicals properties of growing medium were consisted of pH 4.43, C 3.99%, N 0.31%, P 6.10 ppm, and K 0.20 ~~(~~meq of K/100 g of soil). Each polybag was planted with three shallot bulbs. The irrigation, fertilization, weed, and diseases control were manually performed for maintenance of plants. Plant height, number of tillers, number of leaves, chlorophyll reading value (SPAD), total plant weight, number of bulbs, the diameter of the bulb, and fresh bulb weight were observed as morphological characteristics. Randomly selected ten plants from each unit were used for data collection. Four months after planting, the bulb of *E. palmifolia* (n = 10 plants) randomly selected plant was harvested and further analyses were carried out.

Table 1. Treatment combination of N and K fertilizers

Treatment combination	kg of N per ha	kg of K_2O per ha
N_0K_{25}	0	12.5
$N_{100}K_{25}$	46	12.5
$N_{200}K_{25}$	92	12.5
$N_{100}K_0$	46	0
$N_{100}K_{50}$	46	25
$N_{100}K_{100}$	46	50

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2.2. Sample preparation and extraction

The bulbs collected of randomly selected ten plants of each treatment were cleaned, cut, dried, and then reduced into powdered form. Samples were extracted using aqueous, ethanol, and n-hexane as solvent extraction using the procedure by Nurcholis et al [27] with modifications. Briefly, sample (2 g) was extracted twice with solvent (10 mL) in the dark room and then sonicated and stirred for 30 min. The mixtures were centrifugated at 10,000 x g at 4°C (Kitman-T24, Tomy Tech USA Inc.) for 15 min, and the supernatants were concentrated to the volume of 10 mL using a rotary vacuum evaporator

(HAHNVAPOR, Korea). The final extract concentration of *E. palmifolia* that used for further analysis was 2 g/10 mL or 0.2 g/mL. The final extract of *E. palmifolia* was obtained and then used for further analysis total phenolic, flavonoid contents, and antioxidant activity.

2.3. Total phenolic content (TPC) and total flavonoid content (TFC)

In accordance with previously published assays, TPC and TFC were determined using spectrophotometric techniques [28]. TPC was measured in the extract obtained from each sample with Folin-Ciocalteu reagent and expressed as mg gallic acid equivalents per g of dry weight (mg GAE/g DW). Meanwhile, TFC was measured using an aluminium chloride reagent and expressed as mg quercetin equivalents per g of dry weight (mg QE/g DW).

2.4. Antioxidant analysis

Antioxidant activity was determined using two in-vitro techniques. The free radical scavenging and reducing power antioxidant activities were determined using the 2,2-diphenyl picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) assays [29]. Results of antioxidant activity expressed in µmol Trolox equivalent per g of dry weight (µmol TE/g DW).

2.5. Antioxidant analysis

Three replicates were used to get mean data ± SD. ANOVA was determined using the ExpDes packages in R, and then a Scott-Knott test was performed [30]. When $p < 0.05$ is used, there are significant differences.

3. Results and Discussion

3.1. Growth and yield of E. palmifollia

Nitrogen is a macroelement that all plants, including *E. palmifolia*, require in substantial amounts to enhance their growth and productivity [13]. The results of the study showed that the combination of N and K fertilizer significantly ($p < 0.05$) affected chlorophyll reading value and total plant weight, which all attained maximum values at a combination of 46 kg/ha N and 12.5 kg/ha K (Table 2). The results showed that the combination treatment of N and K fertilizers has no significant effect on the growth parameters of plant height, number of tillers, and leaves of *E. palmifolia*. An increase in chlorophyll reading value with growth parameters was also found in strawberries treated with fresh chicken manure [31]. The role of nitrogen during plant growth can be observed through the chlorophyll reading value in plants to increase the efficiency of N and K use according to plant needs [32].

Table 2. The growth characters of *E. plamifolia* as a response to the application of different fertilizers

Treatment combination	Plant height (cm)	Number of tillers	Number of leaves	Chlorophyll reading value (SPAD)	Total plant weight (g)

N_0K_{25}	36.32 ± 2.14a	8.00± 5.29a	27.00±5.29a	46.03±6.56a	78.00±6.55b
$N_{100}K_{25}$	40.58 ± 3.64a	13.67±7.37a	40.00±9.98a	47.65±1.43a	95.33±7.50a
$N_{200}K_{25}$	41.95 ± 1.72a	8.00± 2.64a	25.00±2.64a	47.80±11.60a	78.67±8.08b
$N_{100}K_0$	38.92 ± 1.59a	10.17±10.15a	31.67±10.15a	37.33± 4.73b	94.83±14.25a
$N_{100}K_{50}$	39.90 ± 2.03a	10.83±6.33a	36.83± 6.33a	37.00±4.73b	106.33±12.66a
$N_{100}K_{100}$	40.62 ± 2.27a	10.67±9.98a	39.33±7.37a	35.33±1.53b	102.83±10.34a

Note: Each value is expressed as the mean plus standard deviation; The Scott-Knott test indicates that the mean values in each column denoted by different letters differ significantly at $p < 0.05$; For the treatment combination, see Table 1.

The results recorded that treatment of N (0-92 kg/ha) combined with K (12.5 kg/ha) increased the chlorophyll reading value of *E. palmifolia* compared to other combination treatments (Table 2). This data indicated that the K (12.5 kg/ha) produced the highest chlorophyll reading value. The obtained data of this work were in line with the previous study on cotton photosynthetic capacity [33]. As reported by Naciri et al. [34], potassium significantly affects the chlorophyll content of tomato. The result indicated the importance of the presence of K for plant growth of *E. palmifolia*, especially in the chlorophyll formation.

A higher total plant weight parameter was shown in the combination treatment of 46 kg/ha N with 12.5 kg/ha K, which was not significantly different from the combination treatment of 46 kg/ha N with 0-50 kg/ha K (Table 2). These results indicate that the application of nitrogen fertilizer combination of N and K was required for *E. palmifolia* to increase growth and development, which was shown by higher plant weights. In different plants, N and K enhances the growth performance in rice [35], strawberry [36], and rainfed wheat [37] plants.

The effect of N and K fertilizers on the yield parameters of *E. palmifolia* is presented in Table 3. The results showed that the combination of N and K fertilizers has no significantly different effect on the number and diameter of a bulb of *E. palmifolia*. However, the combination of N and K fertilizers significantly impacted fresh bulb weight. The highest fresh bulb weight (66.01-71.14 g/clump) was obtained in the combination treatment of N fertilizer (46-92 kg/ha) with K fertilizer (0-50 kg/ha). Similar results were previously reported by Gebretsadik and Dechassa [38] that increasing the rate of nitrogen from 50 to 100 kg/ha increased the onion marketable fresh bulb yield by a different percentage of 30%. The result showed that the treatment of K fertilizer (12.5 kg/ha) without N fertilizer produced the lowest tuber weight (58.68 g/clump). The result indicates the importance of nitrogen nutrients in increasing the yield of *E. palmifolia*. Similar results were reported by Uher et al., [39] that the yield depended on the increase in nitrogen dose, and the highest nitrogen dose obtained the highest broccoli yield. The deficiency of nitrogen may decrease chlorophyll biosynthesis, which leads to yield reduction.

Table 3. The yield components of *E. plamifollia* as response to fertilizer application

Treatments	Number of bulbs	Diameter of bulb (cm)	Fresh bulb weight (g)
N_0K_{25}	14.67 ± 1.3 a	1.75 ± 0.06 a	58.68 ± 6.11 b
$N_{100}K_{25}$	16.17 ± 2.4 a	1.76 ± 0.08 a	71.14 ± 5.29 a
$N_{200}K_{25}$	13.83 ± 1.5 a	1.75 ± 0.02 a	67.06 ± 2.64 a

$N_{100}K_0$	11.50 ± 2.6 a	1.81 ± 0.06 a	65.55 ± 8.75 a
$N_{100}K_{50}$	11.50 ± 0.9 a	1.86 ± 0.09 a	66.70 ± 5.76 a
$N_{100}K_{100}$	14.67 ± 4.5 a	1.75 ± 0.11 a	66.01 ± 7.28 a

Note: Each value is expressed as the mean plus standard deviation; The Scott-Knott test indicates that the mean values in each column denoted by different letters differ significantly at $p < 0.05$; For the treatment combination, see Table 1.

The results showed that without the addition of K fertilizer, *E. palmifolia* produced good yields (Table 3). Under conditions of low K availability, plants have a strategy to maintain K needs. For example, the plant increased K uptake from the soil redistributes K ions between cytosolic and vacuolar pools, cytosolic homeostasis, and modifications in root system development and architecture [40]. Supplying optimum N and K levels was proved to be essential for plant growth and production of high yield and improving the quality of *E. palmifolia*.

3.2. Growth and yield of E. palmifollia

TPC in the *E. palmifolia* extracts is strongly influenced by the combination of N and K fertilizer. TPC ranged from 2.192-3.688 GAE /g DW in aqueous extract, 1.474-2.815 GAE/g DW in ethanol extract, and 0.219-0.304 GAE/g DW in n-hexane extract (Table 4). The phenolic content of several plants has been shown to be affected by environmental factors and crop management [41, 42]. Phenolics are antioxidant compounds in stressed plant tissues [43], especially those with nutritional deficiencies [44]. Previous research revealed that the concentration of phenolics varies in response to N and K fertilization. Nitrogen is the substrate for the biosynthesis of phenylalanine, whereas phenylalanine is the substrate for polyphenol chemicals [45]. In most cases, increasing the application of nitrogen reduces the polyphenol content of plants [46]. It is possible, due to conflict between the utilization of nitrogen for growth and the formation of secondary metabolites; if growth increases, polyphenolic compound production will decrease [42]. In general, the administration of N and K fertilizers has been regarded as a technique for enhancing the phenolic content of plant tissue [47]. Among the combination treatments of N and K fertilizers observed, the combination treatment of 46 kg/ha N and 12.5 kg/ha K fertilizers produced the highest TPC (aqueous extract, 3.688 mg GAE/g DW) compared to other treatments. These results indicated that N and K fertilizers strongly affected the growth and polyphenol metabolism of *E. palmifolia*. Similar results were recorded that boosting N and K fertilization increased the total phenolic content in sweet fennel [22] and colored potato [48]. Meanwhile, theTPC content obtained in *E. palmifolia* with aqueous extract was higher than using ethanol and n-hexane extracts. Our finding is in contras with Febrinda et al., [49], who discovered that TPC concentrations in ethanolic extract from traditional market at East Kalimantan, Indonesia was higher than those in aqueous extract. The results of this study are intriguing since the combination of N and K fertilizers can increase the phenolic content that is soluble in aqueous solvents of *E. palmifolia*. As a result, herbal medicine will be safer than utilizing ethanol or hexane extracts.

Table 4. Total phenolic content in aqueous, ethanol and n-hexane extract of *E. palmifolia*

Treatments	Total phenolic content (mg GAE /g DW)		
	Aqueous	Ethanol	n-Hexane

N_0K_{25}	2.694 ± 0.00 d A	2.740 ± 0.01 a A	0.253 ± 0.01 c B
$N_{100}K_{25}$	3.688 ± 0.00 a A	2.815 ± 0.00 a B	0.304 ± 0.01 a B
$N_{200}K_{25}$	2.192 ± 0.01 e A	2.192 ± 0.01 c A	0.288 ± 0.00 b B
$N_{100}K_0$	3.032 ± 0.00 c A	1.474 ± 0.00 d B	0.251 ± 0.01 c C
$N_{100}K_{50}$	2.624 ± 0.01 d A	2.381 ± 0.00 b B	0.286 ± 0.01 b C
$N_{100}K_{100}$	3.150 ± 0.01 b A	1.940 ± 0.00 b B	0.219 ± 0.00 d C

Note: Each value is expressed as the mean plus standard deviation; a-d, the Scott-Knott test indicates that the mean values in each column denoted by different letters differ significantly at $p < 0.05$; A-C, the Scott-Knott test indicates that the mean values in each row denoted by different letters differ significantly at $p < 0.05$; For the treatment combination, see Table 1.

TFC of *E. palmifolia* was measured using different extraction solvents, namely aqueous, ethanol, and n-hexane, as shown in Table 5. TFC in the *E. palmifolia* extracts depended on the combination of N and K fertilizer and the extraction solvents. Among the treatment of N and K fertilizer combinations investigated, TFC ranged from 0.398 to 0.529 QE/g DW in aqueous extract, 0.616 to 0.861 QE/g DW in ethanol extract, and 0.573 to 0.676 QE/g DW in n-hexane extract. These results indicated that the TFC in the combination of 46 kg/ha N and 12.5 kg/ha K has the highest TFC content. Lower content of TFC with aqueous extracts as a solvent was obtained after the application of the nitrogen-free with K of 25 kg/ha. The results showed that plants produced low amounts of flavonoid compounds without nitrogen fertilizer. Results indicate that nitrogen is an essential element in increasing plant metabolism. In solvent extraction, ethanol extract was presented higher TFC than aqueous and n-hexane extracts. Febrinda et al., [49] also found that TFC in the ethanolic extract were higher than aqueous extract. This study shows that the combination of 46 kg/ha N and 12.5 kg/ha K fertilizers has increased TFC in ethanol extract of *E. palmifolia*, which is likely to be a dose target for the production of flavonoid compounds.

Table 5. Total flavonoid content in aqueous, ethanol and n-hexane extract of *E. palmifolia*

Treatments	Total flavonoid content (mg QE /g DW)		
	Aqueous	Ethanol	n-Hexane
N_0K_{25}	0.398 ± 0.00 c C	0.779 ± 0.00 b A	0.651 ± 0.01 b B
$N_{100}K_{25}$	0.529 ± 0.01 a C	0.861 ± 0.02 a A	0.676 ± 0.01 a B
$N_{200}K_{25}$	0.409 ± 0.00 c C	0.842 ± 0.02 a A	0.573 ± 0.01 e B
$N_{100}K_0$	0.464 ± 0.01 b B	0.616 ± 0.01 d A	0.617 ± 0.00 d A
$N_{100}K_{50}$	0.448 ± 0.01 b C	0.711 ± 0.01 c A	0.629 ± 0.00 c B
$N_{100}K_{100}$	0.399 ± 0.01 c C	0.722 ± 0.00 c A	0.572 ± 0.01 e B

Note: Each value is expressed as the mean plus standard deviation; a-e, the Scott-Knott test indicates that the mean values in each column denoted by different letters differ significantly at $p < 0.05$; A-C, the Scott-Knott test indicates that the mean values in each row denoted by different letters differ significantly at $p < 0.05$; For the treatment combination, see Table 1.

3.3. Growth and yield of E. palmifollia

This study examined the antioxidant capacity of different extracts of *E. palmifolia* using DPPH and FRAP assays. Based on the antioxidant activities determined using the DPPH (Table 6) and FRAP (Table 7) methods, we concluded that FRAP method was better than DPPH in analyzing the anti-oxidant activities. This effect is caused by distinct reaction processes of antioxidant activity, including power reduction for FRAP and free radical scavenging for DPPH [29]. Antioxidant activity differed amongst a sample of *E. palmifolia* extracts, which can be explained by the varying forms or concentrations of antioxidant compounds contained inside [27, 50]. The ability to absorb and neutralize free radicals, to quench single and triple oxidants, or to degrade peroxides is referred to as antioxidant activity [51]. Antioxidant-rich plants may be beneficial as possible therapeutic herbs. The plant's antioxidant properties can help scavenge free radicals. Polyphenol (flavonoid and phenolic) chemicals found in plants act as natural antioxidants, scavenging free radicals—the higher the flavonoids or total phenols content, the better the antioxidant ability. The antioxidant activity of *E. bulbosa* extracts demonstrated its unique nutritional value, indicating that it has the potential to serve as a natural source of antioxidants [8].

DPPH radical scavenging activity of *E. palmifolia* extracts was affected by N and K fertilizer and solvent extraction, as presented in Table 6. Among the treatment of N and K fertilizer investigated, DPPH scavenging activity ranged from 0.097 to 0.361 µmol TE/g DW. The highest antioxidant scavenging activity (0.361 µmol TE/g DW) was recorded at 46 kg/ha N followed by 92 kg/ha N and 12.5 kg/ha K combined (0.339 µmol TE/g DW) and 46 kg/ha N and 12.5 kg/ha K combined (0.318 µmol TE/g DW). These results are in agreement with Barzegar et al., [22], who reported that applying N and K fertilizer affected increasing of DPPH scavenging activity in sweet fennel extract. Ma et al., [52] found that the N management increased antioxidant activity in wheat grain. Meanwhile, DPPH scavenging activity in different solvents extracted ranged from 0.198 to 0.361 µmol TE/g DW in aqueous extract, 0.204 to 0.305 µmol TE/g DW in ethanol extract, and 0.097 to 0.153 µmol TE/g DW in n-hexane extract. Results indicated that aqueous extracts of *E. palmifolia* were higher radical scavenging activity than ethanol and n-hexane extract. Attractive, these results are in line with the data of total phenolic content (Table 4). Antioxidant properties caused by phenolic compounds have been proven in a number of research in the last several decades [53–55].

Table 6. DPPH radical scavenging activity in aqueous, ethanol, and n-hexane extracts of *E. palmifolia*

Treatments	DPPH scavenging activity (µmol TE/g DW)		
	Aqueous	Ethanol	n-Hexane
N_0K_{25}	0.232 ± 0.01 b A	0.221 ± 0.00 c C	0.143 ± 0.01 b A
$N_{100}K_{25}$	0.318 ± 0.01 a A	0.305 ± 0.01 a B	0.153 ± 0.01 a B
$N_{200}K_{25}$	0.339 ± 0.00 a A	0.253 ± 0.00 b C	0.105 ± 0.01 c B
$N_{100}K_0$	0.361 ± 0.01 a A	0.208 ± 0.00 d B	0.073 ± 0.01 d C
$N_{100}K_{50}$	0.215 ± 0.00 b A	0.219 ± 0.00 c A	0.097 ± 0.01 c B
$N_{100}K_{100}$	0.198 ± 0.01 b A	0.204 ± 0.00 d B	0.143 ± 0.01 b A

Note: Each value is expressed as the mean plus standard deviation; a-e, the Scott-Knott test indicates that the mean values in each column denoted by different letters differ significantly at $p <$ 0.05; A-C, the Scott-Knott test indicates that the mean values in each row denoted by different letters differ significantly at $p < 0.05$; For the treatment combination, see Table 1.

Ferric reducing antioxidant power (FRAP) value of *E. palmifolia* extracts is showed in Table 7. FRAP value varied from 61.598 to 123.810 µmol TE/g DW among the treatments of N and K fertilizer studied. The highest reducing antioxidant power value was identified at 46 kg/ha N and 12.5 kg/ha K. These findings are in line with those of Michalska et al., [48], who found that the FRAP value of colored potato increased when N and K fertilizer was applied. Besides fertilization treatments, the solvent used to extract antioxidants can also have an effect on how effective they are. The FRAP values varied from 87.545 to 104.757 µmol TE/g DW in aqueous extract, 103.393 to 123.810 µmol TE/g DW in ethanol extract, and 61.598 to 72.151 µmol TE/g DW in n-hexane extract. Results indicated that ethanol extracts of *E. palmifolia* were higher in reducing antioxidant power activity than aqueous and n-hexane extracts. According to the total flavonoid content as presented in Table 5, these results are in line with them. These results indicated that flavonoid compounds are responsible for the antioxidant activity of *E. palmifolia*, especially through the mechanism of reducing antioxidant power. There has been a lot of research in the last few decades that shows that flavonoid compounds have antioxidant properties [56–58].

Table 7. Ferric reducing antioxidant power (FRAP) in aqueous, ethanol, and n-hexane extracts of *E. palmifolia*

Treatments	Ferric reducing antioxidant power (µmol TE/g DW)		
	Aqueous	Ethanol	n-Hexane
N_0K_{25}	91.030 ± 0.00 c B	122.219 ± 0.00 b A	64.242 ± 0.00 c C
$N_{100}K_{25}$	104.757 ± 0.00 a B	123.810 ± 0.01 a A	65.477 ± 0.00 b C
$N_{200}K_{25}$	101.992 ± 0.00 b B	114.621 ± 0.00 c A	72.151 ± 0.00 a C
$N_{100}K_0$	90.712 ± 0.00 c B	103.393 ± 0.00 f A	61.856 ± 0.00 d C
$N_{100}K_{50}$	88.401 ± 0.01 d B	106.166 ± 0.00 e A	65.174 ± 0.00 b C
$N_{100}K_{100}$	87.545 ± 0.00 d B	109.681 ± 0.00 d A	61.598 ± 0.00 d C

Note: Each value is expressed as the mean plus standard deviation; a-d, the Scott-Knott test indicates that the mean values in each column denoted by different letters differ significantly at $p < 0.05$; A-C, the Scott-Knott test indicates that the mean values in each row denoted by different letters differ significantly at $p < 0.05$; For the treatment combination, see Table 1.

4. Conclusions

The results of our study indicated that fertilization application of N and K improved chlorophyll reading value, total plant weight, and fresh bulb weight of *E. palmifolia*. In addition, the *E. palmifolia* treated with N and K exhibited higher total phenolic content, total flavonoid content, and antioxidant activity in aqueous and ethanol extracts. These results suggest that commercial application of N 46 kg/ha and K 12.5 kg/ha can be proposed to improve *E. palmifolia* growth, polyphenol content, and antioxidant activities.

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determination.

Conflict of interest

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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Waras Nurcholis <wnurcholis@apps.ipb.ac.id>

Accepted for Publication—Agri-500

1 message

agriculture@aimspress.org <agriculture@aimspress.org> Wed, Jul 13, 2022 at 12:23 PM
Reply-To: qiqi.li@aimsciences.org
To: Waras Nurcholis <wnurcholis@apps.ipb.ac.id>
Cc: Marlin Marlin <marlin@unib.ac.id>, Marulak Simarmata <marulak_simarmata@yahoo.com>, Umi Salamah <umisalamah@unib.ac.id>

Dear Dr. Nurcholis,

We are pleased to inform you that the following paper has been officially accepted for publication:

Manuscript ID: Agri-500
Type of manuscript: Research article
Title: Effect of nitrogen and potassium application on growth, total phenolic, flavonoid contents, and antioxidant activity of Eleutherina palmifolia
Authors: Waras Nurcholis *, Marlin Marlin, Marulak Simarmata, Umi Salamah
Received: 12 May 2022
E-mails: wnurcholis@apps.ipb.ac.id, marlin@unib.ac.id, marulak_simarmata@yahoo.com, umisalamah@unib.ac.id
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Waras Nurcholis <wnurcholis@apps.ipb.ac.id>

Final Proofreading Before Publication

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agriculture@aimspress.org <agriculture@aimspress.org> Wed, Jul 20, 2022 at 11:43 AM
Reply-To: qiqi.li@aimsciences.org
To: Waras Nurcholis <wnurcholis@apps.ipb.ac.id>
Cc: marlin@unib.ac.id, marulak_simarmata@yahoo.com, umisalamah@unib.ac.id

Dear Dr. Nurcholis,

We invite you to proofread your manuscript prior to publication:

Manuscript ID: Agri-500
Type of manuscript: Research article
Title: Effect of nitrogen and potassium application on growth, total phenolic, flavonoid contents, and antioxidant activity of Eleutherina palmifolia
Authors: Waras Nurcholis *, Marlin Marlin, Marulak Simarmata, Umi Salamah
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5 messages

Qiqi Li <qiqi.li@aimsciences.org> Mon, Jul 18, 2022 at 9:37 AM
To: Waras Nurcholis <wnurcholis@apps.ipb.ac.id>

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Date: 2022-07-14 14:21
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Subject: Our payment Manuscript ID: Agri-500

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Best regards,
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Waras Nurcholis <wnurcholis@apps.ipb.ac.id> Tue, Jul 19, 2022 at 3:56 PM
To: Qiqi Li <qiqi.li@aimsciences.org>

Dear Qiqi Li

In attached our final article.

Best regards,
Waras Nurcholis
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Dr Waras Nurcholis, SSi, MSi

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Qiqi Li <qiqi.li@aimsciences.org> Wed, Jul 20, 2022 at 3:30 AM
To: Waras Nurcholis <wnurcholis@apps.ipb.ac.id>

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Waras Nurcholis <wnurcholis@apps.ipb.ac.id> Thu, Jul 21, 2022 at 9:26 AM
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agriculture@aimspress.org <agriculture@aimspress.org> Fri, Jul 22, 2022 at 5:23 AM
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