

Research article

Effect of nitrogen and potassium application on growth, total phenolic, flavonoid contents, and antioxidant activity of *Eleutherine 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; Iridaceae; 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 eleutherinose 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 method

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% K₂O) 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 *E. palmifolia* 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 ₂ O per ha
N ₀ K 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 × 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 μ mol Trolox equivalent per g of dry weight (μ mol TE/g DW).

2.5. Antioxidant analysis

Three replicates were used to get mean data \pm 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. palmifolia*

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].

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.

Table 2. The growth characters of *E. palmifolia* 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 ₀ K ₂₅	36.32 ± 2.14 ^a	8.00 ± 5.29 ^a	27.00 ± 5.29 ^a	46.03 ± 6.56 ^a	78.00 ± 6.55 ^b
N ₁₀₀ K ₂₅	40.58 ± 3.64 ^a	13.67 ± 7.37 ^a	40.00 ± 9.98 ^a	47.65 ± 1.43 ^a	95.33 ± 7.50 ^a
N ₂₀₀ K ₂₅	41.95 ± 1.72 ^a	8.00 ± 2.64 ^a	25.00 ± 2.64 ^a	47.80 ± 11.60 ^a	78.67 ± 8.08 ^b
N ₁₀₀ K ₀	38.92 ± 1.59 ^a	10.17 ± 10.15 ^a	31.67 ± 10.15 ^a	37.33 ± 4.73 ^b	94.83 ± 14.25 ^a
N ₁₀₀ K ₅₀	39.90 ± 2.03 ^a	10.83 ± 6.33 ^a	36.83 ± 6.33 ^a	37.00 ± 4.73 ^b	106.33 ± 12.66 ^a
N ₁₀₀ K ₁₀₀	40.62 ± 2.27 ^a	10.67 ± 9.98 ^a	39.33 ± 7.37 ^a	35.33 ± 1.53 ^b	102.83 ± 10.34 ^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 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.

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*.

Table 3. The yield components of *E. plamifolia* 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.

3.2. Growth and yield of *E. palmifolia*

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, the TPC content obtained in *E. palmifolia* with aqueous extract was higher than using ethanol and n-hexane extracts. Our finding is in contrast 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 ₀ K ₂₅	2.694 ± 0.00 ^{dA}	2.740 ± 0.01 ^{aA}	0.253 ± 0.01 ^{cB}
N ₁₀₀ K ₂₅	3.688 ± 0.00 ^{aA}	2.815 ± 0.00 ^{aB}	0.304 ± 0.01 ^{aB}
N ₂₀₀ K ₂₅	2.192 ± 0.01 ^{eA}	2.192 ± 0.01 ^{cA}	0.288 ± 0.00 ^{bB}
N ₁₀₀ K ₀	3.032 ± 0.00 ^{cA}	1.474 ± 0.00 ^{dB}	0.251 ± 0.01 ^{cC}
N ₁₀₀ K ₅₀	2.624 ± 0.01 ^{dA}	2.381 ± 0.00 ^{bB}	0.286 ± 0.01 ^{bC}
N ₁₀₀ K ₁₀₀	3.150 ± 0.01 ^{bA}	1.940 ± 0.00 ^{bB}	0.219 ± 0.00 ^{dC}

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 ^{cC}	0.779 ± 0.00 ^{bA}	0.651 ± 0.01 ^{bB}
N ₁₀₀ K ₂₅	0.529 ± 0.01 ^{aC}	0.861 ± 0.02 ^{aA}	0.676 ± 0.01 ^{aB}
N ₂₀₀ K ₂₅	0.409 ± 0.00 ^{cC}	0.842 ± 0.02 ^{aA}	0.573 ± 0.01 ^{eB}
N ₁₀₀ K ₀	0.464 ± 0.01 ^{bB}	0.616 ± 0.01 ^{dA}	0.617 ± 0.00 ^{dA}
N ₁₀₀ K ₅₀	0.448 ± 0.01 ^{bC}	0.711 ± 0.01 ^{cA}	0.629 ± 0.00 ^{cB}
N ₁₀₀ K ₁₀₀	0.399 ± 0.01 ^{cC}	0.722 ± 0.00 ^{cA}	0.572 ± 0.01 ^{eB}

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. palmifolia*

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 antioxidant 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].

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 ₀ K ₂₅	0.232 ± 0.01 ^{bA}	0.221 ± 0.00 ^{cC}	0.143 ± 0.01 ^{bA}
N ₁₀₀ K ₂₅	0.318 ± 0.01 ^{aA}	0.305 ± 0.01 ^{aB}	0.153 ± 0.01 ^{aB}
N ₂₀₀ K ₂₅	0.339 ± 0.00 ^{aA}	0.253 ± 0.00 ^{bC}	0.105 ± 0.01 ^{cB}
N ₁₀₀ K ₀	0.361 ± 0.01 ^{aA}	0.208 ± 0.00 ^{dB}	0.073 ± 0.01 ^{dC}
N ₁₀₀ K ₅₀	0.215 ± 0.00 ^{bA}	0.219 ± 0.00 ^{cA}	0.097 ± 0.01 ^{cB}
N ₁₀₀ K ₁₀₀	0.198 ± 0.01 ^{bA}	0.204 ± 0.00 ^{dB}	0.143 ± 0.01 ^{bA}

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.

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].

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 ₀ K ₂₅	91.030 ± 0.00 ^{cB}	122.219 ± 0.00 ^{bA}	64.242 ± 0.00 ^{cC}
N ₁₀₀ K ₂₅	104.757 ± 0.00 ^{aB}	123.810 ± 0.01 ^{aA}	65.477 ± 0.00 ^{bC}
N ₂₀₀ K ₂₅	101.992 ± 0.00 ^{bB}	114.621 ± 0.00 ^{cA}	72.151 ± 0.00 ^{aC}
N ₁₀₀ K ₀	90.712 ± 0.00 ^{cB}	103.393 ± 0.00 ^{fA}	61.856 ± 0.00 ^{dC}
N ₁₀₀ K ₅₀	88.401 ± 0.01 ^{dB}	106.166 ± 0.00 ^{eA}	65.174 ± 0.00 ^{bC}
N ₁₀₀ K ₁₀₀	87.545 ± 0.00 ^{dB}	109.681 ± 0.00 ^{dA}	61.598 ± 0.00 ^{dC}

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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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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