Research article

Effects of NPK Fertilizer on Growth, Phytochemical Content and Antioxidant Activity of Purslane (*Portulaca grandiflora*)

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Abstract

Purslane (*Portulaca grandiflora*) is a succulent plant that contains phytochemicals including flavonoids, carotenoids, polyphenolic acids, sterols, and reducing agents. The pharmacological properties of this plant include antioxidant activity, and the plant is used in sore throat and skin rashes medications, and for detoxification purposes. The plant's secondary metabolite content is influenced by mineral nutrition. The types and amounts of plant secondary metabolites are determined by soil nutrients. Therefore, this research aimed to observe and analyze the NPK fertilizer effect on plant growth, total phenolics, and antioxidant activity in purslane. Purslane planting was carried out by applying NPK fertilizer (doses of 0, 100, 200 and 300 kg/ha) in August-October 2022 at the Green House of the Department of Biochemistry, IPB University, Indonesia. The total number of leaves and branches was found to be highest with 200 kg/ha dose of NPK fertilizer treatment. The highest total phenolic content, 0.7346 mg GAE/g FW, was found for the treatment with 100 kg/ha dose of NPK fertilizer. The highest increase in antioxidant activity was observed in extracts treated with 100 kg/ha (FRAP, CUPRAC) and 200 kg/ha (DPPH, ABTS) of NPK fertilizer. Therefore, applying NPK fertilizer at optimal doses can increase the plant growth, total phenolic content, and antioxidant activity of purslane. From the research, the recommended doses was 100 kg/ha, which gave the highest total phenolic, and the highest single electron transfer antioxidant activity (FRAP, CUPRAC). Moreover, there was no significant difference in growth parameters at higher doses.

Keywords

antioxidant;
NPK fertilizer;
phenolic compound;
plant growth;
Portulaca grandiflora

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1. Introduction

Purslane (*Portulaca grandiflora* Hook.), also known as Moss Rose, is a succulent plant native to the United States. Hooker discovered purslane in Mendoza, Argentina. This plant is also known as Bombay silk, portulaca, Moss Rose, and sunplant. Purslane grows to a height of about 10-30 cm with round, blackish-brown seeds. Purslane has purplish-brown round stems and leaves that are 12-35 mm long and 1-4 mm wide, which are subulate linear, thick fleshy, and regular spiral. Purslane has colorful flowers 2-3 cm in diameter and has showy stamens. Purslane can grow well despite climate and poor soil composition, so it is classified as a weed plant. Purslane is commonly used as an ornamental plant as well as a medicinal plant. Purslane used as traditional medicine can treat sore throats, skin rashes, and assist in detoxification. The phytochemical contents of purslane are polyphenolic acids, flavonoids, carotenoids, sterols, and reducing agents [1, 2].

Free radicals, such as reactive oxygen species, contribute to various human diseases, including neurodegenerative disease, cardiovascular disease, aging, and cancer. Antioxidants bind to free radicals and prevent them from oxidizing compounds in the body. Endogenous antioxidants are enzymes produced by the body that function as antioxidants. Examples include glutathione peroxidase, catalase, and superoxide dismutase. However, the work of these enzymes may not be enough to keep cells functioning under oxidative stress, so dietary antioxidants are required. Phenolic compounds have antioxidant properties because they stimulate the synthesis of endogenous antioxidant molecules, and their hydroxyl groups can directly capture free radicals [3]. Purslane plants have a high content of polyphenolic compounds, so they have the potential to be a natural source of antioxidants [4].

Mineral nutrition affects the content of secondary metabolites in plants. The soil nutrients determine the types and amounts of plant secondary metabolites. The amount of nitrogen in the soil affects the types of secondary metabolites that accumulate in plants. A lack of nitrogen promotes the accumulation of non-nitrogenous metabolites, such as phenols and terpenoids. In contrast, enough nitrogen fertilizer encourages nitrogenous secondary metabolite accumulation, such as cyanogenic glycosides and alkaloids [5]. Soil factors such as macroelements (N, P, K) and microelements (Fe, Zn, and Mn) influence bioactive substance synthesis in plants. Soil NPK content has been shown to significantly impact plant growth and development, as well as the accumulation of compounds with drug potential [6]. Kamaluddin *et al*. [7] showed that NPK fertilizer application increased plant growth and production in *Kalanchoe blossfeldiana*. To date, there has been limited research on the effects of NPK fertilizer on purslane (*P. grandiflora*). This research aimed to observe and analyze the effect of the application of NPK fertilizer on plant growth, total phenolics, and antioxidant activity in purslane (*P. grandiflora*).

2. Materials and Methods

2.1 Preparation of the research group

The research was done over the period of August-October 2022 at the Green House of the Department of Biochemistry, IPB University, Dramaga, Bogor, West Java, Indonesia under the conditions described in Table 1. *Portulaca grandiflora* stem cuttings from Tropical Biopharmaca Research Center, IPB University were grown in polybags (20x20) for two months (8 weeks) using soil media: NPK chemical fertilizer. The NPK chemical fertilizer used was a mixture of urea (N, total nitrogen ≥ 46%), diphosphorus pentaoxide (P_2O_5, total phosphorus ≥ 36%, and potassium chloride (KCl, total potassium 60%). NPK fertilizer was used because it provided the primary

nutrients needed by the plants to grow and develop [6, 8]. The research consisted of four treatments: (T1) Control: without NPK fertilizer, (T2) NPK fertilizer 100 kg/ha, (T3) NPK fertilizer 200 kg/ha, and (T4) NPK fertilizer 300 kg/ha. Each treatment was given the same irrigation as the control. Watering was done once every two days, but the plants were not watered if it rained. The experiments were done in a three-replicate and used randomized complete block design for statistical analysis.

Table 1. Experiment environment conditions [9-11]

Month	Rainfall (mm)	Humidity (g/m^2)	Temperature ($^\circ$C)
August	384.9	83.00	26.10
September	353.7	84.00	26.00
October	492.3	86.00	26.00

2.2 Growth measurements

Measurement of plant growth parameters in the form of the number of branches and leaves was carried out at 2, 4, 6, and 8 weeks after planting (WAP). Measurements were taken by counting the total number of branches and leaves, including shoots, on all the plant (counted in triplicate for each dose of NPK fertilizer).

2.3 Sample preparation and extraction

Sample preparation method refers to Calvindi *et al.* [12]. The aerial parts of *P. grandiflora* that were 8 WAP (week after planting) were harvested. Furthermore, the aerial parts were cut into small pieces and ground using a mortar until smooth.

Extraction of aerial parts of *P. grandiflora* refers to Nurcholis *et al.* [13] with modifications. First, 4 grams of refined sample was added to 40 mL of 70% ethanol (Merck) in an erlenmeyer flask. Ethanol with this concentration is used to dissolve polar and semipolar bioactive compounds [14]. Each sample was then microwaved (Sharp R-21D0(S)-IN) at 135W for 3 min. The microwave samples were then cooled, filtered using filter paper, and concentrated using a rotary evaporator (LabTech. Ltd.) to produce a filtrate with a concentration of 0.2 g/mL.

2.4 Total phenolic content

Measurement of total phenolic content refers to Batubara *et al.* [15] with modifications. Extract (20 µL) of *P. grandiflora* and 120 µL of Folin Ciocalteu 10% (v/v) were reacted on a 96-well microplate (BiologiX) for 5 min. Then 80 µL of Na_2CO_3 10% (w/v) was added to the solution and incubated for 30 min at room temperature. The absorbance of the solution was then measured at a wavelength of 750 nm using a nano spectrophotometer (SPECTROstarNano BMG LABTECH). Gallic acid at concentrations of 0-225 ppm with an R^2 value of 0.9987 was used as the measurement standard. Meanwhile, the measured values of samples were expressed as milligram gallic acid equivalent per gram fresh weight (mg GAE/g FW).

2.5 Antioxidant measurements

The antioxidant activity of the samples was measured using four methods: DPPH, ABTS, FRAP, and CUPRAC. These four antioxidant methods were used to evaluate the mechanism of action of

antioxidants extracted from *P. grandiflora*. In general, the mechanism of action of antioxidants consists of hydrogen atom transfer (DPPH and ABTS) and single electron transfer (FRAP and CUPRAC). Standard trolox preparation was carried out by dissolving 0.01 grams of trolox powder (Merck) in 10 mL ethanol 70% followed by dilution according to the antioxidant measurement method.

Measurement of DPPH antioxidant activity refers to Nurcholis *et al.* [16] with modifications. Extract of *P. grandiflora* (100 µL) was reacted with 100 µL 125 µM DPPH reagent in ethanol on a 96-well microplate (BiologiX) and then incubated for 30 min at room temperature without light. After incubation, the absorbance of the solution was measured at a wavelength of 517 nm using a nano spectrophotometer (SPECTROstarNano BMG LABTECH). Trolox at concentrations of 0-90 µM with an R^2 value of 0.9819 was used as a standard and the measured experimental sample values were expressed as µmol Trolox equivalent per gram fresh weight (µmol TE/g FW).

ABTS antioxidant activity measurement refers to Nurcholis *et al.* [13] with modifications. Extract of *P. grandiflora* (20 µL) was reacted with 180 µL of ABTS reagent on a 96-well microplate (BiologiX) and then incubated for 6 min at room temperature without light. After incubation, the absorbance of the solution was measured at a wavelength of 734 nm using a nano spectrophotometer (SPECTROstarNano BMG LABTECH). Trolox at concentrations of 0-500 µM with an R^2 value of 0.9984 was used as a standard and the measured sample values were expressed as µmol Trolox equivalent per gram fresh weight (µmol TE/g FW).

Measurement of FRAP antioxidant activity refers to Nurcholis *et al.* [17] with modifications. Extract of *P. grandiflora* (10 µL) was reacted with 300 µL of FRAP reagent on a 96-well microplate (BiologiX) and then incubated for 30 min at room temperature without light. After incubation, the solution was measured for its absorbance at a wavelength of 593 nm using a nano spectrophotometer (SPECTROstarNano BMG LABTECH). Trolox at concentrations of 0-600 µM with an R^2 value of 0.9994 were used as a standard and the measured sample values were expressed as µmol Trolox equivalent per gram fresh weight (µmol TE/g FW).

Measurement of CUPRAC antioxidant activity refers to Nurcholis *et al.* [13] with modifications. Extract of *P. grandiflora* (50 µL) was reacted with 50 µL $CuCl_2.6H_2O$ 10^{-2} M, 50 µL neocuproine 0.0075 M, and 50 µL ammonium acetate buffer (pH 7.0) on a 96-well microplate (BiologiX) and then incubated for 30 min at room temperature without light. After incubation, the absorbance of the solution was measured at a wavelength of 450 nm using a nano spectrophotometer (SPECTROstarNano BMG LABTECH). Trolox at concentrations of 0-500 µM with an R^2 value of 0.9893 were used as a standard and the measured sample values were expressed as µmol Trolox equivalent per gram fresh weight (µmol TE/g FW).

Trolox is used as a standard for calculating antioxidant activity because it is an analog of vitamin E and is universal; that is, it has polar (-OH) and non-polar (C-H) properties to represent polar and non-polar antioxidant compounds. In addition, trolox is a compound that has strong antioxidant activity [18]. The limitation or challenge of this research was the length of time for purslane to grow, resulting in a small sample weight. Therefore, purslane fresh weight was measured in this study.

2.6 Data analysis

The results of measurements of total phenolic and antioxidant activity were analyzed qualitatively using OneWay Analysis of Variance (ANOVA) and Tukey's test with a confidence level of $\alpha = 5\%$ for determining the effect of NPK fertilization doses. Meanwhile, agromorphological data were analyzed using TwoWay Analysis of Variance (ANOVA) and Tukey's test with a confidence level of $\alpha = 5\%$ to determine the effect of NPK fertilization doses. Analysis and visualization of data were performed using GraphPad Prism 9.0 by Dotmatics software.

3. Results and Discussion

3.1 Plant growth

In this research, the plant growth parameters observed were the number of leaves and branches of the purslane plants and observations were carried out every 2 weeks after planting (WAP) for 8 weeks. The total number of leaves during the planting period tended to increase as the planting season progressed (Figure 1A). The highest increase in the number of leaves, which were from 60 to 184 leaves at 4 WAP, occurred in plants that were given 300 kg/ha treatment of NPK fertilizer, while the lowest increase, from 71 to 86 leaves, occurred in plants that had not been given NPK fertilizer. The increase in the total number of leaves also continued to appear at 6 WAP. Plants with the highest total number of leaves at this time were those that had been given 200 kg/ha treatment of NPK fertilizer (228 leaves), followed by plants that had been given 300 kg/ha treatment of NPK fertilizer (220 leaves) (Table 2). No significant increase was seen in all plants after 8 weeks. Plants that were not given fertilizer, or were given fertilizer at 200 kg/ha, and 300 kg/ha did not show an increase in the total number of leaves. In comparison, plants that were given fertilizer 100 kg/ha showed an increase in the total number of leaves, although the increase was not significant. The results of the two-way ANOVA statistical test showed a significant value ($p < 0.05$) between plants that were given fertilizer and those that were not, but between plants that were given fertilizer at different doses, it did not show a significant value ($p > 0.05$).

The measurement of the total number of branches showed results that were not different from the measurement of the total number of leaves (Figure 1B). An increase in the total number of branches occurred for all plants at 4 WAP, with plants given 200 kg/ha and 300 kg/ha dose of NPK fertilizer having the highest number of branches, namely 8 branches. The total number of branches for each plant continued to increase, except for plants with 300 kg/ha dose of NPK fertilizer. The plants with the highest total number of branches at 6 WAP, which was 9 branches, were those given a dose of 200 kg/ha, while the plants with the lowest total number of branches were those that had not been given fertilizer and those that had been given 100 kg/ha treatment of NPK fertilizer, namely 6 branches. The total number of branches on plants that were given 200 kg/ha treatment of NPK fertilizer continued to increase to 10. This made it the group of plants with the highest total number of branches at 8 WAP, while plants that had not been given fertilizer had the least total number of branches, which was 6 branches. Based on the results of the TwoWay ANOVA statistical test obtained, the total number of branches on plants that were not given fertilizer and plants that were given fertilizer produced a significant value ($p < 0.05$), but the difference in NPK fertilizer doses did not have a significant value ($p > 0.05$) for the total number of branches the plants produced.

The results of this research are in line with the findings of Arab *et al*. [19], who stated that the addition of NPK manure significantly affected the development of the number of leaves and branches of marigold (*Calendula officinalis* L.) when compared with the zero NPK. However, Budiarto *et al*. [20] found that adding NPK with different concentrations did not cause any significant changes in the total number of leaves of chrysanthemum (*Dendranthema grandiflora* Twelve) plants. NPK fertilizer has been shown to increase plant height, total number of leaves, branches, and other growth parameters. Nitrogen, phosphorus, and potassium are important elements in plant metabolic processes and are included in plant macronutrients. Nitrogen is an important macronutrient that gives plants a dark green color, is a component of chlorophyll, nucleic acid, protein, and protoplasm and can boost the vegetative growth of cultivated plants. Phosphorus is essential macronutrient for photosynthesis and cell division process. Phosphorus deficiency can inhibit plant growth. Meanwhile, potassium plays an important role in nutrient absorption and nutrient transportation, water absorption, and plant growth [21]. Plant growth is presumed to

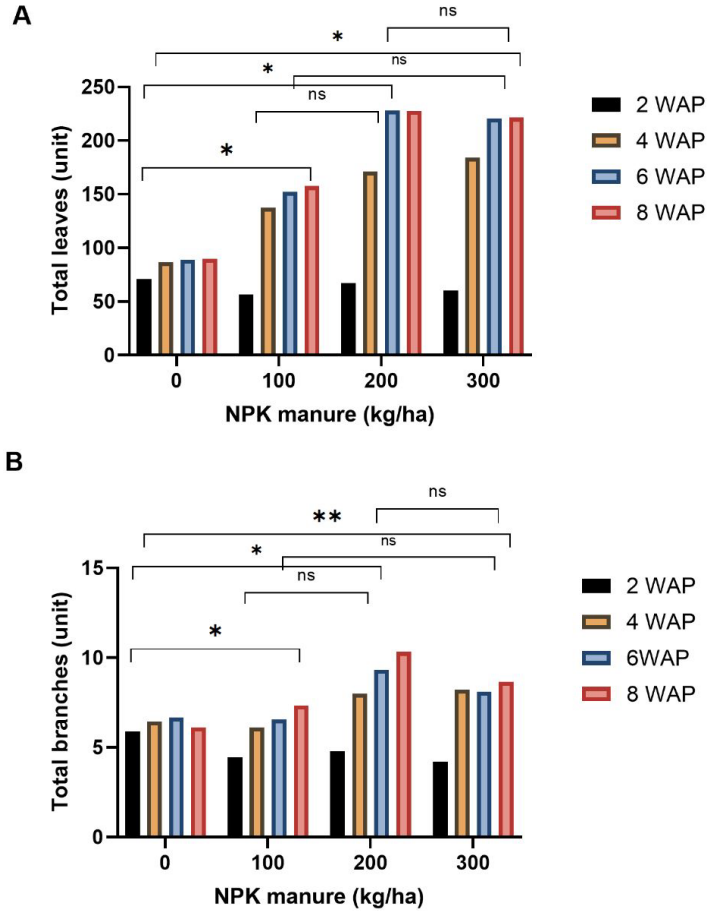

Figure 1. Total leaves (A) and total branches (B) of different concentrations of NPK fertilizer in *Portulaca grandiflora*. Post hoc Tukey's test was used in multiple comparison of means. * $p < 0.05$, ** $p < 0.01$, ns: non-significant, WAP: week after planting, kg: kilograms, ha: hectare.

Table 2. Effects of NPK fertilizer in growth of *P. grandiflora*

Doses of NPK Fertilizer (kg/ha)	Total Leaves				Total Branches			
	2 WAP	4 WAP	6 WAP	8 WAP	2 WAP	4 WAP	6 WAP	8 WAP
0	71	86.67	89	89.67	5.89	6.44	6.67	6.11
100	56.77	137.33	152.33	157.56	4.44	6.11	6.55	7.33
200	67.22	171.11	228.11	227.33	4.78	8.00	9.33	10.33
300	60.11	184.11	220.56	221.44	4.22	8.22	8.11	8.67

Description: WAP: week after planting, kg: kilogram, ha: hectare

increase along with increasing dosage of NPK fertilizer until an optimum dose is reached. However, excessive doses generate insignificant results in plant growth [22].

3.2 Total phenolic content

Secondary metabolites produced by the pentose phosphate, shikimic acid, and phenylpropanoid pathways are known as phenolic compounds. These compounds have a benzene ring with one or more hydroxyl substituents in their structure and can range from simple phenolic molecules to complex compounds [23]. Plant phenolic compounds can be measured using spectrophotometric techniques. The Folin-Ciocalteu test is one method for determining total phenolic content. This method produces a blue complex using the chemical reduction principle between tungsten and molybdenum reagents with hydroxyl groups [24]. The plant variety, growing media conditions, maturity level, weather conditions, and method of extraction and refining determine the total phenolic content of a plant [25].

Measurement of total phenolic content showed that the addition of NPK fertilizer only had a significant increasing effect ($p < 0.05$) at a dose of 100 kg/ha (Figure 2) with a total phenolic content of 0.7346 mg GAE/g FW. An increase also occurred for plants that were given different doses of NPK fertilizer but there was no significant different value ($p > 0.05$) observed for plants that were not given fertilizer or for plants that were given a fertilizer dose of 100 kg/ha. Meanwhile, the lowest total phenolic content (0.5520 mg GAE/g FW) was in plants that were not given fertilizer (Table 3). These results are in line with research conducted by Vasca-Zamfir *et al*. [26] regarding the use of NPK manure on Murraya (*Murraya exotica* L.) plant, where the addition of NPK manure increased the total phenolic content but the increase was not significant compared with the non-fertilizer plant. Nguyen and Niemeyer [27] reported that total phenolic content in basil (*Ocimum basilicum* L.) became elevated only at the lowest dosage of nitrogen manure. This could have happened because under limited nitrogen nutrition conditions, plants tend to increase the accumulation of polyphenolic components [28].

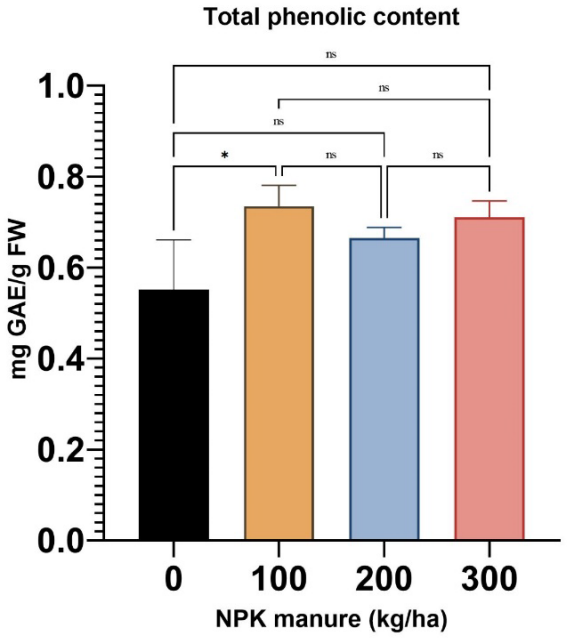

Figure 2. Total phenolic content of different concentrations of NPK fertilizer in *Portulaca grandiflora* extracts. Post hoc Tukey's test was used in multiple comparison of means. * $p < 0.05$, ns: non-significant, GAE: gallic acid equivalent, FW: fresh weight, kg: kilogram, ha: hectare.

Table 3. Effects of NPK fertilizer on total phenolic content and antioxidant activity of *P. grandiflora*

Doses of NPK fertilizer (kg/ha)	TPC (mg GAE/g FW)	DPPH (µmol TE/g FW)	FRAP (µmol TE/g FW)	ABTS (µmol TE/ FW)	CUPRAC (µmol TE/g FW)
0	0.5520	2.2837	1.9810	7.3548	1.3614
100	0.7346	2.3028	2.2940	6.8694	1.4969
200	0.6652	2.5740	1.5867	7.9004	0.8335
300	0.7106	2.3493	2.0867	7.0294	1.2125

Description: TPC: total phenolic content, DPPH: 2,2-Diphenyl-1-picrylhydrazyl, FRAP: ferric reducing antioxidant power, ABTS: 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid), CUPRAC: cupric ion reducing antioxidant capacity, GAE: gallic acid equivalent, FW: fresh weight, ha: hectare, kg: kilogram, TE: Trolox equivalent

3.3 DPPH scavenging activity

DPPH (2,2-Diphenyl-1-picrylhydrazyl) is a radical that receives a proton or hydrogen to develop a stable diamagnetic molecule. Due to the radical's delocalization in the aromatic ring, this radical is well-known for its extraordinary durability [29]. The DPPH method is a free radical potential determination method commonly used to evaluate antioxidant properties [30]. Because of its straightforward, affordable, and quick, along with producing repeatable results, the DPPH method is widely used. DPPH radicals are neutralized in tests by accepting hydrogen atoms or electrons from antioxidants, and these radicals are converted into reduced forms (DPPH-H) at the end of the reaction [31].

The results showed that all *P. grandiflora* extracts could stabilize the DPPH radical compound (Figure 3A). These results were indicated by the change of the purple color of DPPH to yellow after adding *P. grandiflora* extract. The highest DPPH free radical scavenging (2.5740 µmol TE/g FW) was obtained in *P. grandiflora* extract by applying NPK fertilizer at 200 kg/ha. In contrast, the lowest activity (2.2837 µmol TE/g FW) was found in extracts without NPK fertilizer. The difference in NPK fertilizer doses was insignificant ($p > 0.05$) (Figure 1A). These results agree with the research conducted by Vasca-Zamfir *et al.* [26], where the application of NPK (EC50 of 6.25 mg/mL) on Murraya (*Murraya exotica* L.) resulted in higher antioxidant activity than without the application of NPK manure (EC50 of 6.87 mg/mL).

3.4 ABTS scavenging activity

The ABTS assay uses the ABTS radical (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)), which is created by oxidizing ABTS with potassium persulfate. Since ABTS radicals are soluble in organic solvents and water, they are usually used to assess the antioxidant capacity of hydrophilic and lipophilic compounds [32]. One of the main disadvantages of this assay is that the radicals formed are unstable, and the results need to be repeatable. However, this assay has also been generally reported to measure antioxidant activity [33].

The antioxidant activity measurement using the ABTS method on *P. grandiflora* extract with different doses of NPK fertilizer produced a significant value ($p > 0.05$). The antioxidant activity of *P. grandiflora* treatment with NPK fertilizer at a dose of 0 kg/ha resulted in a significant value ($p < 0.05$) compared to *P. grandiflora* fed with NPK fertilizer at a 200 kg/ha dose. In addition, the applications of NPK fertilizer doses of 100, 200 and 300 kg/ha produced more significant values

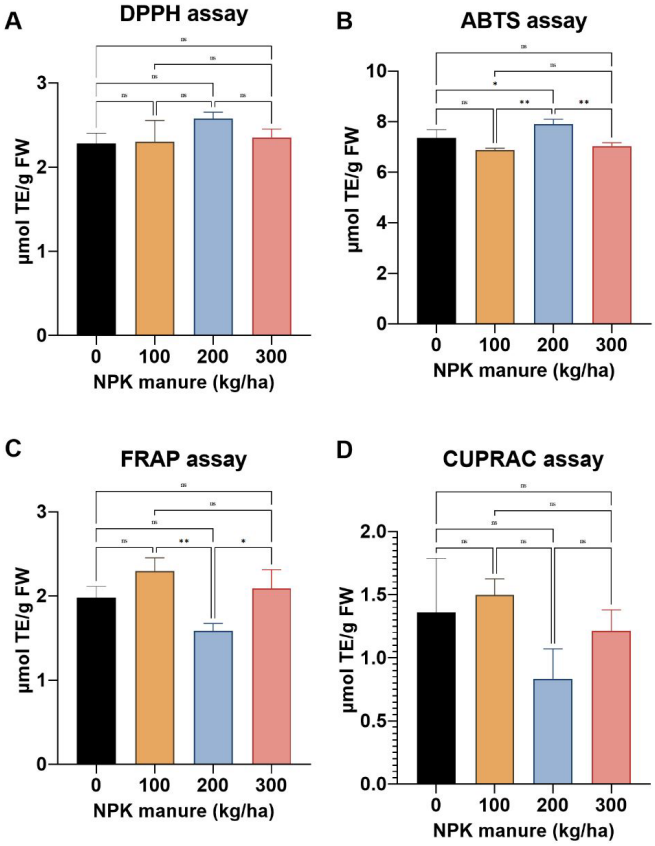

Figure 3. DPPH assays (A), ABTS assays (B), FRAP assays (C), and CUPRAC assays (D) of different concentrations of NPK fertilizer in *Portulaca grandiflora* extracts. Post hoc Tukey's test was used in multiple comparison of means. * $p < 0.05$, ** $p < 0.01$, ns: non-significant, TE: Trolox equivalent, FW: fresh weight, kg: kilogram, ha: hectare.

($p < 0.01$) (Figure 3B). The activities of ABTS radical inhibition were 7.3548, 6.8694, 7.9004, and 7.0294 µmol TE/g FW for doses of 0, 100, 200, and 300 kg/ha of NPK, respectively. These results were consistent with previous research by Skywarylo-Bednarz and Krzepilko [34], where NPK fertilizer application increased the activity of radical inhibition of ABTS in spinach (*Amaranthus cruentus* L.) leaves by up to 52% compared to spinach without NPK application. Oleyede *et al.* [35] also informed that antioxidant activity of *Cucurbita pepo* seeds with 0, 50, and 100 kg/ha NPK levels produced more remarkable results than at the 150, 200, and 250 kg/ha NPK levels. The lowering in antioxidant activity at high NPK levels is due to high levels of nutrients that can reduce the synthesis of secondary metabolites in plants, one of which is the phenolic group which has antioxidant properties. The total phenolic components in this research tended to decrease at the highest dosage of NPK manure (Figure 2), which explains why the same trend happened in antioxidant activity. Amarowicz *et al*. [36] also found a positive correlation between the total phenolic components and the antioxidant activity of Jerusalem artichoke (*Helianthus tuberosus* L.) using the ABTS method.

3.5 FRAP assay

The FRAP technique quantifies the ability of an antioxidant to reduce the Fe^{3+}-TPTZ complex to Fe^{2+}-TPTZ under an acidic condition. Fe^{3+}-TPTZ is an oxidizing compound that may present in the body and injure body cells, although Fe^{2+}-TPTZ is a harmless compound. The greater the concentration of reduced Fe^{3+}-TPTZ is, the more significant the activity of the sample antioxidant can be found [37]. FRAP is a non-specific method but can determine the amounts of phenolic compounds from the reduction process. The weakness of this assay is that it cannot measure the activity of antioxidants with thiol group (-SH) and is limited to water-soluble antioxidants [38].

All treatments with varying concentrations of NPK fertilizer on *P. grandiflora* showed significantly different results ($p < 0.05$) in the activities of antioxidants tested using the FRAP method. Antioxidant activity ranged from 1.5867 µmol TE/g FW at 200 kg/ha treatment to 2.2940 µmol TE/g FW at 100 kg/ha treatment (Figure 3C). The 200 kg/ha dose treatment had significantly different values from 100 kg/ha ($p < 0.01$) and 300 kg/ha ($p < 0.05$) NPK levels. The treatment that produced the best antioxidant activity was the 100 kg/ha dose treatment, which showed no significant difference between the 300 kg/ha dose treatment and the control ($p > 0.05$). These results agreed with Ibrahim *et al.* [39], who studied the effects of various concentrations (0, 90, 180, and 270 kg N/Ha) of NPK fertilizer application in *Labisia pumila*. It was found that the highest antioxidant activity of 814.21 µm Fe(II)/g dry weight occurred at 90 kg N/Ha treatment. This antioxidant activity was reduced at the concentrations of 180 and 270 kg N/Ha, which produced 621.71 and 511.78 µm Fe(II)/g DW, respectively.

Applying high doses of NPK fertilizer to *P. grandiflora* only sometimes produces the best activity of antioxidant. In accordance with the C/N (carbon/nitrogen) proportion hypothesis, plants will prioritize the formation of high-N compounds, such as proteins, when N is available. Nevertheless, when N availability is restricted, metabolism will be redirected to forming high-C compounds, for example, secondary metabolites (phenolics and terpenoids) and starch [40]. This was also proven by Salahas *et al.* [41], who observed that N deficiency stimulated the biosynthesis of plant secondary metabolites such as total phenolics and betacyanin. Variations in N concentration affect the accumulation of secondary metabolites, which play a role in antioxidant activity [33]. This explains why the antioxidant activity decreased upon adding NPK manure at 200 and 300 kg/ha compared with 100 kg/ha.

3.6 CUPRAC assay

The CUPRAC (cupric ion reducing antioxidant capacity) method has several advantages. It involves short reaction time, utilizes stable reagents, can be performed at physiological pH, and can be used to evaluate hydrophilic and lipophilic antioxidants [41]. Measurement of antioxidant activity using this method generated a significant value ($p < 0.05$), but did not show significant values ($p > 0.05$) for the correspondence between treatments. The 100 kg/ha dose treatment had the highest activity of antioxidant at 1.4969 µmol TE/g FW, while the 200 kg/ha dose treatment had the lowest activity at 0.8335 µmol TE/g FW (Figure 3D). The differences in antioxidant activities between treatments were probably due to differences in the amounts of secondary metabolites produced by the plants. NPK manure at optimal doses increases secondary metabolites in plants. However, adding NPK manure above its optimum dosage will decrease the accumulation of secondary metabolites because the plant will allocate most of the nutrition for its growth and development [5].

4. Conclusions

NPK fertilizer treatment with the right dose can increase the number of leaves, branches, total phenolic content and antioxidant activity of *Portulaca grandiflora*. The results of this research can benefit the agricultural sector, especially in terms of *P. grandiflora* cultivation. The highest growth of *P. grandiflora* was found in the treatment with 200 kg/ha dose of NPK fertilizer. The total phenolics in the treatment with 100 kg/ha dose of NPK fertilizer had the highest content, 0.7346 mg GAE/g FW. The most increased antioxidant activity of *P. grandiflora* was found in 100 kg/ha (FRAP, CUPRAC) and 200 kg/ha (DPPH, ABTS) of NPK fertilizer treatment. In this research, the recommended dose of 100 kg/ha produced the highest total phenolics, and dominant mechanism of single electron transfer antioxidant activity (FRAP, CUPRAC). Moreover, plant growth parameters were not significantly different at higher doses.

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