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Agronomic characteristics, polyphenol content, and antioxidant capacity of rose purslane (*Portulaca grandiflora* Hook.) against nitrogen fertilizer

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Abstract The result showed no significant difference between the application of urea at various concentrations (0.5, 0.1, and 1.5 (g/polybag)) on the variable number of leaves and branching. Still, there were significantly differed in the varying number of leaves during the six weeks of measurement. The phytochemical of total phenolic content (TPC) also showed significance. differences at a dose of 1.5 g/polybag of 1.26 (mg GAE/g DW). On the other hand, there were no significantly differed for total flavonoids. In addition, the antioxidant capacity of DPPH also significantly differed at a dose of 1.5 g/polybag of 5.59 (µmol TE/g DW). There is no significant difference in the capacity of ABTS and FRAP antioxidant activities.

Keywords: Agromorphological, Antioxidant capacity, Rose purslane, Phytochemical

Introduction

Rose purslane (*Portulaca grandiflora* Hook.) is a plant originating from South America (Sari *et al.*, 2017). This plant grows wild and is often considered a weed (Husein *et al.*, 2021). *P. grandiflora* belongs to the class Magnoliopsida, order Caryophyllales, family Portulacaceae, genus *Portulaca* L., and species *Portulaca grandiflora* Hook. (Mane *et al.*, 2022). Rose purslane has a round purplish-brown stem with branches growing up to 10-30 cm. Rose purslane leaves generally have a length of 12-35 mm with a width of 1-4 mm. In addition, this plant is *linear-subulate*, regular spiral, fleshy, and thick. *P. grandiflora* have a diameter of 2-3 cm with striking stamens and have various

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colours (Sari *et al.,* 2017). Rose purslane is widely used by the community as an herbal medicine to treat various ailments such as cardiovascular, cancer, neurodegenerative, liver problems, kidney disease, and anti-oxidative stress. In addition, in some place's purslane can also be used as a vegetable (Husein *et al.,* 2021). This plant contains phytochemicals in the form of flavonoids, alkaloids, omega-3 fatty acids, phenolics, sterols, polysaccharides, and sterols (Husnawati *et al.,* 2019; Husein *et al.,* 2021).

Oxidative stress, generated by high concentrations of free radicals, is a vital factor in the induction of several degenerative diseases such as cancer, aging, rheumatoid arthritis, autoimmune disorders, neurodegenerative disorders, and cardiovascular diseases, and cataracts (Nurcholis *et al.,* 2021*).* Free radicals in the body are obtained from the body's metabolism, air pollution, food contamination, and sunlight (Husnawati *et al.,* 2019). Those found in the body can come from the body's metabolism, air pollution, food contamination, and sunlight. The human body has many pathways to combat oxidative stress by producing antioxidant compounds, either produced naturally by the body or provided externally by supplements or food. Therefore, antioxidant compounds can increase and decrease the immune response to the risk of degenerative diseases.

Rose purslane is known to have antioxidant activity due to the phytochemical compounds it contains (Husein *et al.,* 2021). The phytochemical content of purslane herbs are carotenoids, flavonoids, and polyphenolic acids that have potential as an antioxidant (Zhou *et al.,* 2015). Kemit *et al.* (2017) stated that the content of flavonoids correlated positively with antioxidant activity. The higher the flavonoid content, the higher the antioxidant activity. Flavonoid compounds in purslane that can act as antioxidants are epigenin, kaempferol, and quercetin (Zhou *et al.,* 2015). Each type of soil has different characteristics that affect the nutrient content contained therein (Bodake *et al.,* 2018). The availability of nutrients in the form of nutrients in plants is needed to maintain the normal physiological function of cells (Sharma and Chetani, 2018). One of the nutrients needed is nitrogen (N) which plays an important role for plant growth, thereby affecting crop yields. This element has a major role in the plant life cycle where it acts as the main mineral nutrient needed for the production of chlorophyll and other components of plant cells such a secondary metabolite (Muñoz-Huerta *et al.,* 2013). Nitrogen is often found in inadequate levels for plants, so it requires the addition of N during its growth period (Fagard *et al.,* 2014).

Nitrogen elements in plants can be added through the provision of fertilizers, one of which is urea. Urea is a synthetic fertilizer with the highest nitrogen content (Jariah *et al.,* 2013). However, previous studies have yet to be

conducted regarding the effect of varying nitrogen fertilizer applications on agronomic parameters, total phytochemical content, and antioxidant capacity of rose purslane plants. The purpose of this research was to analyze the impact of nitrogen fertilization on the agronomic parameters, polyphenol levels, and antioxidant potential of *P. grandiflora*.

Materials and methods

Preparation of the research group

This research was conducted in August – December 2022 at the Biopharmaceutical Conservation and Cultivation Unit Garden, Tropical Biopharmaca Research Center, Cikabayan, Bogor Agricultural University (6°32′25.47″ N, 106°42′53.22″ E, 142.60 m asl) and Instrumentation Laboratory of the Department of Biochemistry, Bogor Agricultural University, West Java, Indonesia. This research used a randomized block design, with one factor being the concentration of urea fertilizer dose. The dose of urea fertilizer used was 0.00 g/polybag (control), 0.05 g/polybag, 0.10 g/polybag and 0.15 g/polybag. Each treatment was repeated 3 times.

Observation of agromorphological characters

Observation of agromorphological characteristics was carried out using the modified Rini *et al.* (2022) method. Observation of agromorphological characters includes the number of branches and number of leaves. The number of leaves is calculated by adding up all the leaves in one branch. The number of branches is calculated by adding up all the branches in the polybag. Measurement of agromorphological characteristics was carried out every 2 weeks after planting.

Microwave assisted extraction

Ethanol extract of rose purslane was prepared using the microwave assisted extraction (MAE) method based on the procedure of Nurcholis *et al.* (2022). As much as 4 g of purslane was put into Erlenmeyer and added 40 mL of ethanol. The Erlenmeyer was put in the microwave for 3 minutes at 135 W. Then the extract was filtered using filter paper and the volume was measured.

Determination of total phenolic content

Total phenolic content was measured on a modified method by Nurcholis *et al.* (2022); Li and Wah (2017). Measurement of total phenolic content was initiated by preparing 10% Folin-Ciocalteu reagent and 10% Na_2CO_3. Pipette 5 mL of Folin-Ciocalteu reagent 100% and dissolve it in 50 mL of distilled water. Na_2CO_3 was weighed as much as 5 grams and dissolved in 50 mL of distilled water. As much as 20 µL of purslane ethanol extract was added to 120 µL of 10% Folin-Ciocalteu reagent on the microplate, then the solution was incubated for 5 minutes in a dark room. The solution that had been incubated was then added with 80 µL of 10% Na_2CO_3 and incubated again in the dark for 30 minutes. The absorbance of the solution was read at a wavelength of 750 nm with a microplate reader. The standard curve used in the measurement of total phenolic is gallic acid with a concentration of 20 ppm to 300 ppm. Gallic acid 1000 ppm was prepared with 25 mg gallic acid in 25 mL ethanol. The total phenolic content of purslane extract is expressed as mg gallic acid equivalent (GAE)/g dry weight.

Determination of total flavonoid content

Measurement of total flavonoid levels was carried out based on a modified method by Nurcholis *et al.* (2022) and Mukhriani *et al.* (2019). Measurement of total flavonoid levels was initiated by preparing $AlCl_3$ 10%$_3$ in 50 mL of distilled water. 120 µL of distilled water was added with 10 µL of purslane extract, 10 µL of $AlCl_{3,}$10 µL of glacial acetic acid, and 50 µL of pro-analyzed ethanol, then incubated for 30 minutes in a dark room. Absorbance was read at a wavelength of 415 nm with a microplate reader. The standard curve used in this test is quercetin with a concentration of 25 ppm to 500 ppm. Quercetin 1000 ppm was prepared by 25 mg quercetin in 25 mL ethanol. Total flavonoid levels are expressed in mg quercetin equivalent (QE)/g dry weight.

Determination of free radical scavenging activity (DPPH)

Testing for antioxidant activity using the DPPH method is based on the method of Nurcholis *et al.* (2022). Pipette 100 µL of purslane extract and add 100 µL of DPPH 125 µM reagent. The solution was incubated for 30 minutes in a dark room. Antioxidant activity can be seen by changing the colour from dark purple to yellow. The standard curve used in this assay is Trolox 20 µM to 90 µM. The absorbance was measured with a microplate reader at a wavelength of 515 nm.

The 2, 2-azino-di-3-ethylbenzothialozinesulphonic acid (ABTS) assay

Testing of antioxidant activity using the ABTS method is based on the method of Nurcholis *et al.* (2022). A total of 20 µL of purslane extract was added to 180 µL of ABTS reagent, then incubated for 6 minutes in a dark room. The standard curve used in this assay is Trolox 100-500 µM. Trolox standard was prepared with 25 mg of Trolox dissolved in 100 mL of ethanol. The absorbance was measured with a microplate reader at a wavelength of 734 nm.

Cupric reducing antioxidant capacity (CUPRAC) assay

Testing antioxidant activity using the CUPRAC method is based on the method of Nurcholis *et al.* (2022). To 50 µL of purslane extract was added $CuCl_2$ 0,01 M, 50 µL buffer NH_4CH_3COO (pH 7), and 50 µL of neokuproin 7.5 $\times 10^{-3}$ M on a microplate. The microplate was incubated for 30 minutes in a dark room and the absorbance was measured at 450 nm using a microplate reader. The standard curve used is Trolox with a concentration series of 100-500 µM.

Ferric reducing antioxidant power (FRAP) analysis

Testing antioxidant activity using the FRAP method is based on the method of Nurcholis *et al.* (2022). Purslane extract 10 µL was added with FRAP reagent (acetate buffer, 2,4,6-Tripyridyl-S-triazine, and $FeCl_3$ ratio 10:1:1) 300 µL. The solution was incubated for 30 minutes in a dark room, then the absorbance was measured with a microplate reader at a wavelength of 593 nm. Trolox with a concentration series of 100-500 µM is the standard curve used in this assay.

Data analysis

Agronomic data, total phytochemicals, and antioxidant capacity obtained were analyzed by The Analysis of Variance (ANOVA) based on randomized completely block design with significant difference analysis (α = 0.05) and continued with The Duncan Multiple Range Test (DMRT) using SPSS 25 and GrapPad Prism 9.0.0. Analysis of the correlation of phytochemical and antioxidant parameters using the Pearson correlation found in the *Performance Analytics package* R. Studio 4.2.2.

Results

Agronomic character of rose purslane

Agronomic characteristics include the number of leaves and branching observed each week after planting (WAP) using ANOVA followed by DMRT. The results showed that the application of urea fertilizer concentrations of 0.5, 0.1, and 1.5 g/poybag did not show agronomic significance in plants (Table 1). The application of urea fertilizer showed an increase in the number of leaves every 2 weeks of observation. However, it is not significant to the number of stems.

Tabel 1. Effect of urea fertilizer on the number of leaves and germination of rose purslane

Urea Treatment (gram/polybag)	**2 WAP**		**4 WAP**		**6 WAP**	
	leaves	branches	leaves	branches	leaves	branches
Control	77[a]	7[a]	82[a]	7[a]	120[a]	9[a]
0.5	72[a]	7[a]	78[a]	6[a]	64[a]	4[a]
1	79[a]	5[a]	74[a]	4[a]	83[a]	5[a]
1.5	69[a]	5[a]	64[a]	5[a]	94[a]	5[a]
Mean	74	6	75	6	90	6
Significance	ns	ns	ns	ns	ns	ns

Each value represents the average of three sample repetitions. The same letter in the same column shows the results are not significantly different from the results of the 5% DMRT test (ns = not significant at P>0.05).

Phytochemical analysis

The results of the phytochemical analysis of the ethanol extract of rose purslane treated with urea at various doses are presented in Table 2. The samples used in the analysis of the overall antioxidant capacity using plants within six weeks after planting. The total phenolic content was measured by the equivalent of gallic acid (GAE), while the total flavonoid by the equivalent of quercetin (QE). The total phenolic content of *P. grandiflora* had significant differences ranging from 0.75 mg GAE/g DW to 1.26 mg/GAE/g DW. The total flavonoid content did not vary significantly and ranged from 0.007 mg QE/g DW to 0.042 mg QE/g DW.

Table 2. Total phenolic and flavonoid content of ethanol extract rose purslane

Dose of Urea (g/polybag)	TPC (mg QE/g DW)	TFC (mg QE/g DW)
0	0.75	0.007
0.05	1.10	0.027
0.10	1.21 *	0.038
0.15	1.26 **	0.042

Each value represents the average of three sample repetitions. * and ** indicated significance at $p<0.05$ and <0.01 in the ANOVA analysis

Antioxidant analysis

Free radical scavenging capacity of the ethanol extract of rose purslane was determined by the DPPH and ABTS methods. The samples used in the analysis of the overall antioxidant capacity using plants within six weeks after planting. The free radical scavenging capacity by the DPPH and ABTS methods is presented in Table 3. The free radical scavenging capacity by the DPPH method of *P. grandiflora* with varying doses of urea treatment had significant differences ranging from 2.24 µmol TE/g DW to 5.59 µmol TE/g DW, ethanol extract ranged from 84.99 µmol TE/g DW to 87.00 µmol TE/g DW.

Table 3. Radical scavenging capacity of rose purslane ethanol extract

Dose of Urea (g/polybag)	DPPH (µmol TE/g DW)	ABTS (µmol TE/g DW)
0	2.24	84.99
0.05	3.24	86.28
0.10	3.32	86.26
0.15	5.59 **	87.00

Each value represents the average of three sample repetitions. * and ** indicated significance at $p<0.05$ and <0.01 in the ANOVA analysis

Reduction capacity of rose purslane ethanol extract using the FRAP method. The reduction capacity of the ethanol extract of rose purslane to the various doses of urea is presented in Table 4. The capacity of reduction with

FRAP on the ethanol extract of rose purslane ranged from 7.26 µmol TE/g DW to 20.23 µmol TE/g DW.

Tabel 4. Reduction capacity of rose purslane ethanol extract

Dose of Urea (g/polybag)	FRAP (µmol TE/g DW)
0	7.26
0.05	17.19
0.10	19.51
0.15	20.23

Each value represents the average of three sample repetitions in the ANOVA analysis

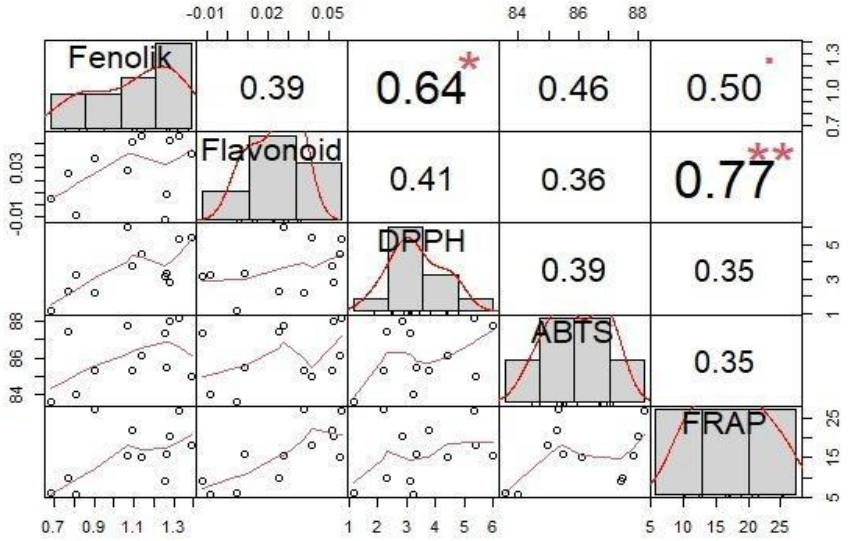

Figure 1. Correlation matrix of total phytochemical content and antioxidant capacity. The upper diagonal shows the Pearson correlation coefficient while the lower diagonal shows the scatter plot. *,**,*** showed significance at $p<0.05$, <0.01, and <0.001

Correlation matrix of test parameters

The basis for statistically significant Pearson correlations is determined based on the p-value generated from the analysis. A significant relationship occurs when the p-value is smaller than the significance level (α) used ($p<0.05$), but this value does not indicate a correlation value between variables.

Correlation is shown by the value of the correlation coefficient (r) with the correlation value divided into five groups, namely very strong correlation (0.90-1.00), strong correlation (0.70-0.89), moderate correlation (0.40-0.69), weak correlation (0.10-0.39), and negligible correlation (0.00-0.10). Result showed the correlation matrix of total phytochemical content and antioxidant activity of the ethanol extract of rose purslane treated with the addition of urea fertilizer (Figure 1). The total phenolic results showed a significant positive correlation with the antioxidant capacity of DPPH (r = 0.64) and FRAP (r = 0.50). Furthermore, flavonoids were significantly correlated with the antioxidant capacity of FRAP (r = 77).

Discussion

Fertilizer treatment with various concentrations of rose purslane showed a correlation between agronomic variables, such as several leaves, at six weeks of observation, although it did not show significance. This is because all plants utilize nitrogen (N) in the form of NO_{3-} and NH_{4+}. Nitrogen is an essential element for the proper growth and development of plants, significantly increasing their yield and quality through its role in regulating plant biochemical mechanisms. In addition, nitrogen is the backbone for forming protoplasm in plants in various mechanisms such as differentiation, catalysis of different chemical compounds, photosynthesis, and other tools in plants, thus triggering the development and growth of plant organs (Lillo *et al.*, 2008; Anas *et al.*, 2020; Zhao *et al.*, 2021). In addition, research by Kubar *et al.* (2022) also showed that applying nitrogen fertilizers affected aspects of plant physiology, such as the rate of photosynthesis, cellular carbon dioxide levels, and stomatal conductance in wheat plants. Selassie (2015) stated that adding nitrogen fertilizers can increase yield and biomass gain in corn cultivation plants.

Furthermore, using various doses of nitrogenous fertilizers (0.5, 1.0, and 1.5 (g/polybag)) increased phytochemical content such as total phenolic and flavonoids and agronomical parameters such as biomass and development of plant parts. This study showed that a dose of 1.5 (g/polybag) is the best dose for increasing the total phytochemical content and agronomic aspects. Manipulating nitrogen levels in plants had various benefits, including controlling the growth and production of secondary metabolites, thereby increasing crop quality (Buetow *et al.*, 2017). One of the metabolic pathways regulated by nitrogen is the shikimic pathway. The role of nitrogen in this pathway is that, together with carbon, it provided a framework for the aromatic amino acids L-tryptophan, L-tyrosine, and L-phenylalanine (Tohge *et al.*, 2013). The shikimate pathway is one of the vital pathways in plants. In this

pathway, the biosynthesis mechanism of flavonoid group metabolites occurs (such as flavanones, flavones, isoflavones, flavonols, flavan-3-ol, and tannin derivatives) (Jaakola, 2013). Various enzymes that play a role in the shikimate pathway work by catalyzing various precursors in the biosynthesis of flavonoids. This is regulated by post-translational control with feedback from various aromatic amino acids (Lillo *et al.,* 2008). Research by Arista *et al.* (2023) stated that the addition of nitrogen fertilizer doses at concentrations of 0.9 and 1.3 (g/polybag) increased several parameters, such as the number of leaves, plant height, fresh weight, and dry weight of Java cardamon (A. compactum Soland ex. Mato) plants. In addition, research conducted by Yousaf *et al*. (2021) showed an increase in yield and biomass of nitrogen fertilizer application from 0.00 soil to 0.30 g N. kg^{-1} in Radish (*Raphanus sativus* L.). Zhao *et al.* (2021) also mentioned significant differences in the content of phytochemicals and antioxidants in applying nitrogen fertilizers with a concentration of 0 to 260 kg. ha^{-1}.

The increase in total phytochemicals in applying nitrogen fertilizers correlated with the increase in antioxidant capacity, especially DPPH and FRAP correlated significantly in this study. The total phenolic content correlated well with the capacity of DPPH and FRAP, while the total flavonoids showed a significant effect on the capacity of FRAP. This phenomenon occurs because DPPH is less effective against flavonoid compounds due to steric hindrance. Besides that, DPPH is also less reactive to hydrophilic antioxidants (Platzer *et al.,* 2021). On the other hand, FRAP has better specificity than DPPH. FRAP has good potential against various flavonoid and hydrophilic compounds (Sadeer *et al.,* 2020).

Treatment of nitrogenous fertilizers with varying concentrations affects the growth and increases secondary metabolite product in rose purslane plants. This aligns with the function of nitrogenous components in the biosynthesis of amino acid molecules as building blocks and regulators of various metabolic pathways in plants (The *et al.,* 2020). Nitrogen fertilizers increased agronomic aspects such as plant height, number of leaves, and branching of plants, as well as biochemical aspects such as phytochemical (TPC and TFC) and antioxidant capacity content in rose purslane. Nitrogen fertilizer concentration of 0.15 fram/polybag showed the highest yield in agronomic and biochemical characteristics on rose purslane.

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