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Effect of potassium fertilizer application on total phenolic level, flavonoids, and the antioxidant activity of the Purslane (*Portulaca oleracea*)

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Abstract The study investigated the impact of K fertilizer on the antioxidant properties of purslane (*Portulaca oleracea* L.) ethanol extract. The total phenolic content was measured using the Folin-Ciocalteu method with gallic acid standards, peaked in the F3 treatment (0.15 g potassium per plant) at 1.4929 mg GAE/g. Total flavonoid levels, assessed with quercetin as the standard, were highest in the F3 treatment at 0.1602 mg QE/g. Antioxidant activity was evaluated through FRAP, CUPRAC, and ABTS methods, with Trolox standards, revealing the highest values of 3.7644 µmol TE/g in the control (F0), 2.1197 µmol TE/g in F2 (0.1 g potassium per plant), and 6.4251 µmol TE/g in F2, respectively. Potassium fertilizers were significantly increased the total phenolic and flavonoid content, particularly in the F3 treatment, which applied 0.15 grams of fertilizer per sample over nine repetitions. Despite this increase, the highest antioxidant activity was not observed in the F3 treatment, due to variables such as sample storage time, dilution, and pre-test handling. The findings highlighted a complex relationship between potassium fertilization and antioxidant activity in purslane, suggesting potential variables influencing antioxidant outcomes beyond phenolic and flavonoid content.

Keywords: Purslane, *Portulaca oleracea*, Antioxidants, Potassium

Introduction

Purslane plant (*Portulaca oleracea* L.) is a type of wild plant that can grow in environments with poor climate and soil composition. Purslane has a plant

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height of up to 35 cm with ovate b-shaped leaves of green, old stems of reddish-brown color and young stems of light green color (Sicari *et al*., 2018). Purslane is usually only considered as a weed or wild plant that has no benefits and is generally only used as animal feed. The use of purslane as a food ingredient is still minimal in the community, even though purslane contains fatty acids, flavonoids, carotene, phenolic, vitamins E and C and various other beneficial compounds. The content of vitamins in purslane has the potential to be an antioxidant (Yuniastri *et al*., 2020).

Planting environmental conditions affect the level of components of secondary metabolite compounds (Farida *et al.,* 2019). The environmental conditions of planting can be improved using fertilizing using organic and inorganic fertilizers. One of the inorganic fertilizers that support plant growth is Potassium fertilizer. Potassium is necessary for plants for various physiological functions, such as carbohydrate metabolism, enzyme activity, osmotic regulation, eficiency of water used, absorption of nitrogenous elements, synthesis of proteins, and the translocation of assimilates (Rahmawan *et al*., 2019).

Potassium (K) is a macronutrient that plays an important role in ensuring optimal plant growth. K is an enzyme activator important in plant metabolism such as protein synthesis, sugar transport, N and C metabolism, and photosynthesis. These minerals also play an important role in producing results and improving quality. However, the appropriate potassium concentration varies in each plant, and too high potasium concentrations in soil solutions can inhibit the absorption of Mg minerals and can lead to Mg deficiency in plants (Xu *et al.,* 2020).

Recent reports have shown that the addition of potassium can optimize growth, production of secondary metabolites osmolitic, and plant photosynthesis activity (Sardans and Peñuelas, 2021). Additional potassium can also affect the quantity and composition of metabolite compounds in plants. Recent reports have shown that the addition of potassium can optimize growth, production of secondary metabolites osmolitic, and plant photosynthesis activity. Additional potassium can also affect the quantity and composition of metabolite compounds in plants. Primary metabolites such as starch and soluble sugars usually decrease in potassium deficiency situations, whereas amino acids accumulate during excessive potassium addition in tobacco, rice, and nuts. Secondary metabolites such as blackberry total phenolic and sunflower scopolin were also affected by the addition of potassium (Ibrahim *et al.,* 2012).

Research on the phytochemical content of purslane conducted by Khursheed and Jain (2021) shows that purslane ethanol extract contains phenolic life, flavonoids, alkaloids, steroids, terpenoids, and anthraquinones. In addition, research on the antioxidant activity of purslane plants has been widely carried

out (Uddin *et al*., 2012; Lolo *et al*., 2017; Yuniastri *et al*., 2020), but no one has observed differences in antioxidant activity and the content of phenolic compounds and flavonoids of purslane plants based on the application of variations in the dose of K fertilizer. This study aimed to measure the content of phytochemical compounds of purslane plants with the treatment of variations in the dose of K fertilizer, as well as determine the antioxidant activity of purslane extract.

Materials and methods

The research was conducted at the Research Laboratory Department of Biochemistry, Dramaga, Bogor. Research starts from August to December 2022. This research used purslane plants (*Portulaca oleraceae*) obtained from the Collection of the IPB Biopharmaceutical Study Center, Bogor Agricultural University, West Java, Indonesia. Planting material covers the soil and potash fertilizers. The analysis used ethanol solvent 70%, Follin Ciocelteau reagent 10%, Na2CO3 10%, gallic acid, aluminum chloride (AlCl3¬) 10%, ABTS (2,2'-azino-bis-(3 ethylbenzothiazolin)-6 sulfonate acid), potassium persulfate (K2S2O8), CuCl2, neuchopranine, ammonium acetate, potassium acetate 1 M, aqueous, absolute ethanol, quercetin, Trolox, glacial acetic acid, CH3COONa, TPTZ, FeCl3.6H2O, 2,2-diphenyl-1-picrylhydrazyl (DPPH). The tools used are glass and glass utensils, Whatman filter paper, analytical balance sheets, pipettes, micropipettes, microplates 96 well, microplate readers, and nano spectrophotometers (Biorad model 680 XR).

Planting treatment

Purslane was planted in polybags and placed in direct sunlight. It was planted for three months and divided into four treatments of potassium fertilizer concentrations: F0 (control) (0 g potassium), F1 (0.05 g potassium), F2 (0.1 g potassium), and F3 (0.15 g potassium). Each treatment was repeated three times for each test of three plants.

Sample preparation

Ethanol extract of purslane was prepared which based on the procedure of Nurcholis *et al*. (2022) with modification. 2 grams of sample from each treatment were extracted using 10 mL 70% ethanol. Sample was macerated in the dark for 48 hours. Filter paper is used to filter the sample and the filtrate is taken, while the pulp is removed.

Total phenolic levels

Total phenolic content was measured which based on a method by Nurcholis *et al*. (2022). Total phenolic levels were calculated using reagents of Follin Coiceltau solution and Na2CO3 solution. 20 µL extract purslane was mixed with 120 µL of 10% Follin Ciocalteau, and 10% Na2CO3 (80 µl) on a 96-well clear polystyrene microplate. The mixture is incubated for 30 minutes at room temperature. A nano spectrophotometer was used to measure the absorbance of the mixture at a wavelength of 750 nm. Total phenolic levels were calculated based on the standard curve of gallic acid using a concentration variation of 0; 20; 60; 100; 140; 180; 220; 260; 300 µg/mL, and marked equivalent mmol equivalent of gallic acid ((mg GAE)/g).

Total flavonoid levels

Measurement of total flavonoid levels was carried out on a method by Nurcholis *et al*. (2022). A total of 10 µL of purslane extract was pipetted into a 96-well clear polystyrene microplate, then 120 µL of aqueous, 10% aluminum chloride (10 µL), 1 M (10 µL) of potassium acetate was added, and p.a ethanol (50 µL), the mixture was incubated on room temperature for 30 minutes. Absorption was measured at 415 nm using a microplate reader spectrophotometer. Quercetin standards were created for calibration curves with a concentration of 0; 25; 50; 100; 150; 200; 250; 300; 350; 400; 450; 500 µg/mL.

FRAP antioxidant activity

Testing antioxidant activity using the FRAP method is based on the method of Nurcholis *et al*. (2022). CH3COONa 1.55 g dissolved in 8 mL of glacial acetic acid and then stamped with aqueous to reach 500 mL. a pH 3.6 acetate buffer solution is added HCl or NaOH to regulate its pH. A 10mM TPTZ solution was weighed as much as 31.2 mg TPTZ (C18H12N6, BM 312.33) and dissolved with 100 mL HCl 40 mM. A total of 135.25 mg of FeCl3.6H2O powder in 25 mL of aqueduct to make a 20 mM solution of FeCl3.6H2O. The FRAP reagent was created by mixing 10 mL of 3.6 p H acetate buffer, 1 mL of 10 mM TPTZ in 40 mM HCl, and 1 mL 20 mM FeCl3.6H2O (10:1:1). Incubation of FRAP reagents was carried out for 30 minutes at 37 °C. The reagent must be used before 3 hours. 25 mg Trolox (C4H18O4) dissolved with 100 mL of ethanol p.a so that stock solution of Trolox 1000 is µmol/L. Trolox stock diluted to a concentration of 0; 100; 200; 400; 500; 600 µmol/L. 10 µL of standard or sample solution is inserted into a microplate and then 150 µL of FRAP reagent is added. The mixture was

incubated in a dark state for 4 min at 37 °C. Absorption was measured using a microplate spectrophotometer at a wavelength of 593 nm.

Antioxidant activity of ABTS

Testing of antioxidant activity using the ABTS method is based on the method of Nurcholis *et al*. (2022). ABTS powder weighing 90 mg was dissolved with 25 ml of aqueous, so that a concentration of ABTS solution of 7.7 Mm was obtained. A solution of potassium persulfate ($K_2S_2O_8$) (2.4 Mm) is prepared by dissolving 66,289 mg of K2S2O8 into 100 ml of aqueous. ABTS reagents are made by adding ABTS reagents with $K_2S_2O_8$ in a ratio of 2:1. ABTS reagent preparation was carried out by varying the addition of aqueous so that an absorbance of 0.7 ± 0.02 was obtained at a wavelength of 734 nm. Trolox as much as 0.0025 g was dissolved into ethanol p.a 100 ml, so a solution of Trolox with a concentration of 1000 ppm was obtained. Trolox stock diluted to a concentration of 0; 100; 200; 300; 400; 500 µmol/L. The test was performed by adding 20 µl of sample with 180 µl of ABTS reagent and then incubation for 6 min. Absorbance is read at a wavelength of 734 nm.

CUPRAC antioxidant activity

Testing antioxidant activity using the CUPRAC method is based on the method of Nurcholis *et al*. (2022). A total of 0.0161 g of CuCl2 was dissolved into 12 ml of aqueous. The neucoprin reagent was prepared by applying 0.018 grams of neucoprin into 12 ml of aqueous, and ammonium acetate buffer was created by dissolving 1.92 grams of ammonium acetate powder into 25 ml of aqueous. Trolox as much as 0.0025 g was dissolved into ethanol p.a 100 ml, so a solution of Trolox with concentration of 1000 ppm was obtained. Trolox stock diluted to a concentration of 0; 100; 200; 300; 400; 500 µmol/L. A total of 50 µl of the sample was added with 50 µl of CuCl2 solution (0.01M), 50 µl of neucoprin reagent (0.0075 M), and 50 µl of ammonium acetate buffer solution (pH 7). Absorbance is read at a wavelength of 450 nm.

Data analysis

The resulting values and statistical analysis were processed using IBM SPSS Statistics 26 and GraphPad Prism 9.0. Analysis of variance (ANOVA) and Duncan test was employed to determine statistical significance of the treatment ($\alpha = 0.05$).

Results

Total phenolic content

Based on the results of the calculation of the total phenolic content in various treatments of applying potassium on different concentrations showed the order of total phenolic content of the highest, namely F3 of 1.4929, F2 of 0.4815, and F1 of 0.3944 mg of equivalent acid per gram of sample weight. Meanwhile, the control treatment produced a total phenolic content of 0.3954 mg GAE/g. Total phenolic levels are shown in Figure 1.

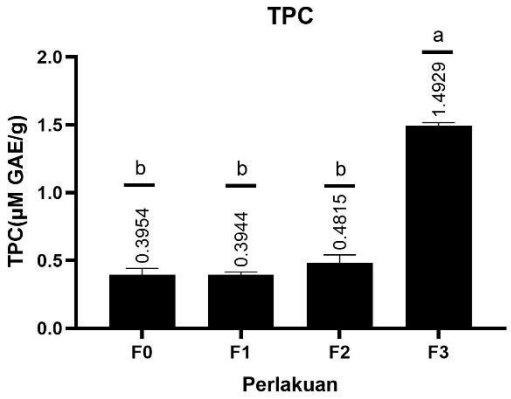

Figure 1. Graph of total phenolic levels of purslane extract. F0 = 0 g K, F1 = 0.05 g K, F2 = 0.1 g K, F3 = 0.15 g K. The same letter indicates the sample does not differ significantly

Total flavonoid content

The results of the analysis of total flavonoid levels of purslane extract with different K fertilizer application treatment concentrations showed samples of F1, F2, and F3 were 0.1243, 0.1407, and 0.1602 mg respectively in quercetin equivalent per gram of sample weight. Samples of F0 or purslane plants without treatment showed a total flavonoid content of 0.0980 mg QE/g. Total flavonoid levels are shown in Figure 2.

Antioxidant activity

ABTS

The results of antioxidant activity assay using ABTS showed that F2 produced the highest value of 6.4251 µmol Trolox equivalent per gram of sample weight (Figure 3). The F1 treatment produced antioxidant activity of 3.8588

µmol Trolox equivalent per gram sample showed much smaller than the control sample of 5.0294 µmol Trolox equivalent per gram to l. Meanwhile, the antioxidant activity value of treatment 3 (F3) was greater than the control, which was 5,892 µmol Trolox equivalent per gram of sample.

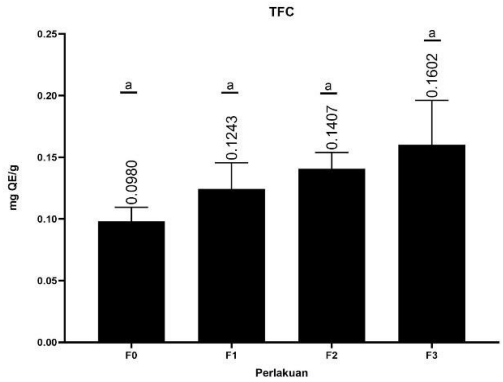

Figure 2. Total flavonoid content of purslane extract. F0 = 0 g K, F1 = 0.05 g K, F2 = 0.1 g K, F3 = 0.15 g K. The same letter indicates the sample does not differ significantly

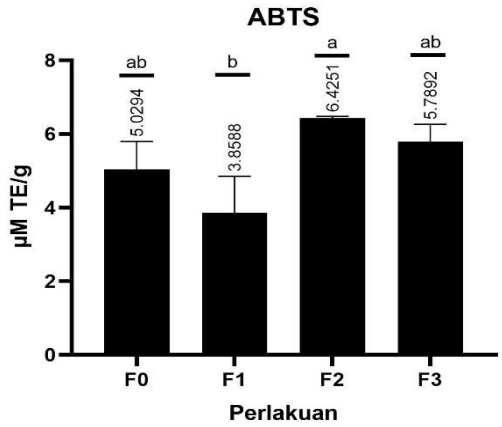

Figure 3. Graph of antioxidant activity of ABTS purslane extract. F0 = 0 g K, F1 = 0.05 g K, F2 = 0.1 g K, F3 = 0.15 g K. The same letter indicates the sample does not differ significantly

FRAP

The reducing power of purslane extract in various treatments of K fertilizer application with concentrations different from the FRAP method is shown in Figure 4.

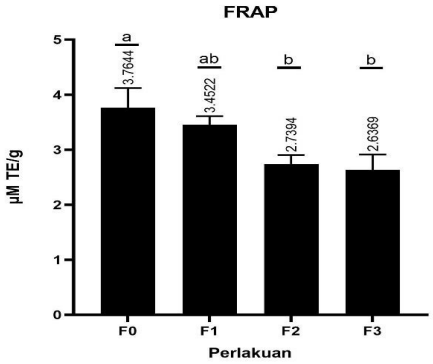

Figure 4. Graph of antioxidant activity of FRAP purslane extract. F0 = 0 g K, F1 = 0.05 g K, F2 = 0.1 g K, F3 = 0.15 g K. The same letter indicates the sample does not differ significantly

The greatest reducing ability was demonstrated by the sample without treatment or F0 (control), which was 3.7644 µmol per gram of sample. Treatment of F1, F2, and F3 sequentially produces antioxidant activity of 3.4522, 2.7394, and 2.6369 µmol TE/g.

CUPRAC

The results of calculating the antioxidant activity of the Cuprac method to purslane showed treatment 2 (F2) had the highest antioxidant activity of 2.1197, followed by treatment 3 (F3) of 2.1142 µmol TE/g (Figure 5). Meanwhile, treatment 1 (F1) showed antioxidant activity of 1.5308 µmol TE/g, which not much differed from the antioxidant activity of the control sample extract of 1.3281 µmol TE/g.

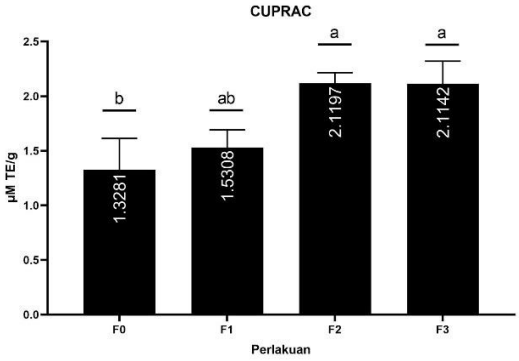

Figure 5. Graph of antioxidant activity of FRAP purslane extract. F0 = 0 g K, F1 = 0.05 g K, F2 = 0.1 g K, F3 = 0.15 g K. The same letter indicates the sample does not differ significantly

Discussion

Phenolic compounds are one of the secondary metabolites of plants that act as antioxidant agents. In general, purslane type *P. oleracea* contains phenolic compounds that dominate, namely caffeic acid, p-cumaric acid, scopoletin, ferulic acid, chlorogenic and coloenate (Barnhart *et al*., 2015; Handayani *et al*., 2017; Anggraini *et al*., 2018). Purslane leaves of *Portulaca oleracea* L. with different drying methods affect its phytochemical components and antioxidant activity. The highest total phenolic was obtained in fresh leaves without drying treatment with a content of 1447.59 mg/100 g dry weight. While the solvent also affects the phytochemical components of purslane plants with methanol solvent as a better potential in attracting phenolic compounds than ethanol and water solvents with the highest acquisition of 142.2±3.83 mg/g extract (Habibian *et al*., 2017). The total phenolic content depends on the polarity of the solvent used in the extraction. The solubility of total phenolics will be high in polar solvents and give high concentrations in extracts obtained with polar solvents during extraction (Indra *et al*., 2019).

Potassium has the potential to increase plant resistance to disease, playing a role in strengthening plant cell walls, thereby increasing resistance to certain diseases. Phenol compounds also have a relationship with plant resistance to pathogens (Habibi *et al*., 2017). Based on the results of the analysis, the highest total phenolic content was F3 at 1,4929 mg GAE/g with K fertilizer treatment of 0.15 grams for each repetition of 9 times. This is in accordance with the statement of Habibi *et al*. (2017), high phenol content is obtained in the application of potassium fertilizer which also increases plant resistance to disease. The total phenolic content of purslane leaf extract is expressed as Gallic Acid Equivalent (GAE) because the chemical structure of the compounds in purslane leaf extract is unknown.

Flavonoids are phenolic compounds with a phenyl benzopyran structure and are the components with the highest bioactivity (Yahia and Lopez, 2018). Measurement of total flavonoid content using colorimetric-$AlCl_3$ method with quercetin standard curve. The analysis showed the highest flavonoid content in F3, which is the treatment of potassium fertilizer 0.15 grams each repetition of 9 times. The total flavonoid content of F3 is 0.1602 mg QE/g. Research conducted by Alam *et al*. (2014) showed that *Portulaca oleracea* varieties contain total flavonoids between 0.13±0.19 mg/g to 1.44±0.08 mg/g. The results of the F3 treatment are in accordance with the results of the study by Alam *et al*. (2014) because it falls into the range of total flavonoid content. The difference in the results obtained is due to differences in growing places that affect the levels of purslane secondary metabolites (Husnawati *et al*., 2020).

Potassium fertilizer treatment affects the total flavonoid content of purslane plants. Research conducted by Gaaluche *et al*. (2019), the antioxidant activity of a plant has increased and is directly proportional to the increase in total flavonoid levels with potassium fertilization of 3g/liter. Another study conducted by Yaldiz (2017) showed an increase in total flavonoid levels from the medicinal plant *Silybum marianum* which correlated with an increase in its antioxidant activity.

Antioxidant compounds are electron donor compounds and biologically, antioxidants are defined as compounds that can counteract or reduce the negative effects of oxidants or free radicals. Antioxidants work by donating one electron to compounds that are oxidant so that the activity of the oxidant compound can be inhibited. Several methods are used to determine the antioxidant activity of a substance, including the ABTS method, FRAP method, and CUPRAC method (Sayuti and Yenrina, 2015). Research conducted by Zaini (2021) showed an increase in the antioxidant activity value of the medicinal plant kencur (*Kaempferia galanga* L.) by applying potassium fertilizer.

The mechanism of testing antioxidant activity with ABTS method is the decolorization of ABTS cation to measure the antioxidant capacity that directly reacts with ABTS cation radicals (Setiawan *et al*., 2018). The results of the analysis with the ABTS method showed the highest antioxidant content in F2, which is the treatment of potassium fertilizer 0.1 gram each repetition of 9 times and produced a value of 6.4251 µmol trolox equivalent per gram sample weight. Antioxidant activity that was conducted by Popova *et al*. (2014) showed that *Portulaca oleracea* varieties with ABTS method have antioxidant activity of 40.47±1.54 to 173.54±0.50 µmol TE/g sample weight. The value of antioxidant content in F2 of purslane plants showed discrepancies with previous studies. This difference in value may be influenced by agronomic and environmental conditions, including the cropping system and type of management including the type of fertilizer applied (Yuniastri *et al*., 2020).

The FRAP or Ferric Reducing Antioxidant Power method is a spectrophotometric determination of antioxidant content based on the reduction of the ferroin analog, Fe^{3+} complex of tripyridyltriazine Fe $(TPTZ)^{3+}$ to an intensive blue Fe^{2+}, $Fe(TPTZ)^{2+}$ complex by antioxidants in an acidic condition (Yefrida *et al*., 2015). The results of analysis by FRAP method showed the highest antioxidant content in F0, which is without potassium fertilizer and produces a value of 3.7644 µmol per gram of sample. The antioxidant value is different from the results of antioxidant activity analysis conducted by Alam *et al*. (2014) which showed that *Portulaca oleracea* varieties with the FRAP method had antioxidant activity of 7.39 ± 0.08 to 104.2 ± 6.34 µmol TE/g. Differences in the results obtained can be caused by differences in the environment where plants grow, such as water availability, temperature, pollution levels, and fertilization (Yuniastri *et al*., 2020).

CUPRAC (Cupric ion Reducing Antioxidant Capacity) assay, using Cu (II)-neocuproine reagent (Cu (II)-$(Nc)_2$) as a chromogenic oxidizing agent because the reduction of Cu (II) ions can be measured (Maryam *et al*., 2015). The results of the analysis with the CUPRAC method showed the highest antioxidant content in F2, which is the treatment of potassium fertilizer 0.1 gram each repetition of 9 times and produced a value of 2,1197 µmol trolox equivalent per gram sample weight. Antioxidant activity analysis conducted by Popova *et al*. (2014) showed that *Portulaca oleracea* varieties with the CUPRAC method have antioxidant activity of 4.52±0.05 to 128.22±1.1 µmol TE/g sample weight. There are differences in results caused by environmental influences.

The application of potassium fertilizer has been shown to increase the total phenolic content, total flavonoid content, and antioxidant activity of purslane plants. The highest total phenolic and flavonoid contents were obtained in the F3 treatment with the application of 0.15 grams of potassium fertilizer. The increase in total phenolic and flavonoid levels is directly proportional to the levels of antioxidant, but in these result, the highest antioxidant activity is not found in F3. This is influenced by the sample storage time, dilution, and the treatment of the sample before testing.

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