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Short Communication: Differences in phytochemical compounds and antioxidant activity of *Portulaca oleracea* and *Portulaca grandiflora*

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Abstract. *Aisyah SI, Oktavia AWP, Ayuningtyas AA, Putra RP, Prassiska S, Jamilah S , Nurcholis W. 2023. Short Communication: Differences in phytochemical compounds and antioxidant activity of* Portulaca oleracea *and* Portulaca grandiflora. *Biodiversitas 24: 1385-1390. Portulaca oleracea* and *Portulaca grandiflora* are medicinal ornamental plants used in traditional and modern medicine. Therefore, this study aimed to analyze the phytochemical compounds and antioxidant activity in two purslane plants, *P. oleracea*, and *P. grandiflora*. The purslane ethanol extract was obtained by sonication and maceration methods. The phytochemical screening included the examination of secondary plant metabolites, while antioxidant activity was analyzed using the 2,2-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) and cupric ion reducing antioxidant capacity (CUPRAC) methods. The results showed that both extract samples contain alkaloids, phenol hydroquinones, flavonoids, saponins, tannins, triterpenoids, and steroids. The highest ABTS antioxidant activity was found in *P. grandiflora* extract at 26.93 µmol TE/g FW compared to *P. oleracea* extract at 15.98 µmol TE/g FW. The highest CUPRAC antioxidant activity was found in the *P. oleracea* extract at 33.22 µmol TE/g FW, while in the *P. grandiflora* extract, it was 18.55 µmol TE/g FW. Based on the results, it was concluded that the chemical complexity of various purslane plants causes differences in the antioxidant capacity.

Keywords: Antioxidant activity, phytochemistry, *Portulaca grandiflora*, *Portulaca oleracea*

INTRODUCTION

The Portulacaceae family contains over 100 species, including *Portulaca oleracea* and *Portulaca grandiflora* (Christenhusz and Byng 2016). In Indonesia, *Portulaca* has several local names such as *krokot, sutra bombai,* and *bunga pukul sembilan* (Amirul et al. 2014). *P. oleracea* L., purslane, is a plant found worldwide, particularly in tropical and subtropical areas (Ocampo and Columbus 2012). The stems are round, purplish-brown in color, grow upright, and have a single leaf, thick, fleshy, and oval. Furthermore, the leaves are generally curved inward, while the upper and lower surfaces are dark-green and red with short stems and rounded ends (Karlina et al. 2013). *P. oleracea* has been a common medicine and traditional food in many parts of the world since ancient times (Chugh et al. 2019; Uddin et al. 2020). Several ethnobotanical studies showed that indigenous peoples use the plant to treat diabetes, urinary tract infections, kidney and cardiovascular diseases, diarrhea, headaches, boils, and snake and insect bites (Faruque et al. 2019; Nemzer et al. 2020). Furthermore, *P. oleracea* is used as a febrifuge, wound healer (Zhou et al. 2015), anti-inflammatory (Uddin et al. 2014), and antioxidant (Chen et al. 2012; Uddin et al. 2014). The World Health Organization (WHO) labeled *P. oleracea* as "Global Panacea" because it is widely used as a medicinal plant (Wu et al. 2012).

Portulaca grandiflora, a member of the Portulacaceae family, is a South American ornamental plant with a succulent habitat and has spread widely worldwide. It is a popular landscape plant that grows and reproduces quickly (Jia et al. 2017). Despite being considered a weed in some areas due to its rapid growth and adaptation, *P. grandiflora* has beautiful flowers and attractive shapes, making it a potential ornamental plant with economic value (Setiawan et al. 2016). The plant has a reddish-green leaf color, short rounded leaf shapes, lengths ranging from 2-4 cm, flower size of 3-5 cm, and plant lengths of 7-15 cm (Aisyah et al. 2022). Furthermore, *P. grandiflora* has various uses, including as an ornamental plant, and can be consumed as a vegetable, spice, and medicine since ancient Egypt up to England. This plant contains various compounds, such as flavonoids, alkaloids, polysaccharides, terpenoids, sterols, vitamins, proteins, minerals, and omega-3 fatty acids, which are antioxidants (Uddin et al. 2014; Kumar et al. 2021).

Secondary metabolites play a crucial role in interacting with plants and their environment for adaptation and defense. They are synthesized in higher plants from primary metabolites such as carbohydrates, lipids, and amino acids to adapt to environmental pressures and defend against herbivores or pathogens (Ramakrishna and Ravishankar 2011). Meanwhile, plants high in phytochemicals help supplement the human body's needs

by acting as natural antioxidants (Altemimi et al. 2017). That includes purslane plants which can grow in various soil conditions, while the differences in the environmental conditions might affect the levels of antioxidants contained. In addition, genetic, environmental factors, post-harvest storage, and processing conditions influence plants' synthesis and accumulation of phytochemicals. Therefore, plants' phytochemical content and composition changes can affect their bioavailability and biological activity (Li et al. 2012). Advances in quantitative phytochemical analysis have led to the identification of metabolites from various parts of the purslane plant (Negi 2018). Several ethnobotanical studies in vitro and in vivo have tested the efficacy of purslane as a medicinal plant due to its pharmacological potential (Al-Sheddi et al. 2016; Rahimi et al. 2019). This study aims to analyze the chemical compounds in purslane plants through phytochemical tests and observation with the 2,2-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and cupric ion reducing antioxidant capacity (CUPRAC) methods as well as different mechanisms of the antioxidant activity. It is expected to provide scientific information about *P. oleracea* and *P. grandiflora*.

MATERIALS AND METHODS

Study area

This study was conducted in August-September 2022 at the Research Laboratory, Department of Biochemistry, IPB University, Bogor, West Java, Indonesia. The primary materials used were two purslanes, *P. oleracea* and *P. grandiflora*, from Tropical Biopharmaca Research Center, IPB University at 6E32’25.47" N and 106E42’53.22" E.

Preparation and extraction

The purslane simplicia was prepared according to the study by Nurcholis et al. (2021) with slight modifications. First, the samples were sorted by separating the roots and aerial parts; then, the aerial parts were dried at 50ºC for 2-3 days. Finally, the dried purslane was mashed and sieved using a 60-mesh sieve and stored at room temperature.

The extraction of purslane simplicia referred to Makkiyah et al. (2022) with slight modifications. First, 4 g purslane simplicia was weighed and dissolved in 40 mL of solvent, namely 70% ethanol, with a ratio of 1:10. Next, the samples were sonicated using a sonicator (Decon F5 Major, Decon Laboratories, US) for 30 minutes. Next, the products obtained were macerated at 30°C for 180 minutes using a water bath shaker (WiseBath). Furthermore, filter paper filtered the filtrate, and a liquid extract was obtained. The solvent in the extract was further evaporated using a rotary evaporator (LabTech Ltd.) to obtain a concentration of 0.2 g/mL. Finally, then the extract was used to determine the phytochemical compounds and the antioxidant activity.

Phytochemical analysis

Alkaloid test

With slight modifications, the alkaloid compounds were analyzed following Imra et al. (2022). First, 1 mL extract was added to 5 mL of chloroform and two drops of NH_4OH (ammonia), then the mixture was placed into a test tube and covered with a lid. Next, the chloroform extract was shaken with 6 mL of 2M H_2SO_4, and the acid layer was separated into another test tube. Next, the acid layer was dripped onto the drip plate, then Mayer, Wagner, and Dragendorf reagents were added, giving rise to white, brown, and orange-red precipitates, respectively.

Hydroquinone phenolic test

The hydroquinone phenolic compounds analysis was conducted according to Imra et al. (2022). First, about 1 mL of the extract was added with three drops of 5% $FeCl_3$ solution, followed by a change in color to blackish purple, indicating the presence of phenolic compounds.

Flavonoid test

The flavonoid compounds in the extract were tested and aligned with a study by Rahmania et al. (2018). About 1 mL of each extract was added with Mg powder, 2N hydrochloric acid and then heated over a water bath. Afterward, amyl alcohol was added and shaken until well blended. A positive result was obtained, as indicated by the yellow-red discoloration of the alcohol coating.

Saponin test

Furthermore, Imra et al. (2022) analyzed the extract's saponin compounds. A total of 1 mL of extract was placed into a test tube, added with 10 mL of hot water, cooled, and shaken vigorously for 10 seconds. A stable froth 1 cm to 10 cm high formed for not less than 10 minutes indicates a positive reaction. However, after adding one drop of 2N hydrochloric acid, the foam did not disappear.

Tannin test

The tannin compounds in the extract were tested according to Rahmania et al. (2018). First, about 2 mL of the extract was added with three drops of iron (III) chloride ($FeCl_3$) reagent; the solution turned blue or black after a positive reaction. Then, gelatin was added to the sample to ensure the presence or absence of tannins until a white precipitate was formed.

Triterpenoid and steroid test

The triterpenoid and steroid compounds were analyzed based on Herawati et al. (2021). Identification was conducted using a mixture of acetic anhydride and concentrated sulfuric acid, commonly known as the Liebermann-Burchard reagent. In this test, ten drops of acetic anhydride and two drops of concentrated sulfuric acid were added sequentially to 1 mL of the test sample dissolved in acetone. Furthermore, the test sample was shaken and left for a few minutes. A change follows the reaction in color; red and purple indicate the presence of triterpenoids, while green and blue are characteristic of steroids.

Antioxidant assays

2,2-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay

The antioxidant activity was assessed using the ABTS method by Nurcholis et al. (2022) with slight modifications. First, a 7,7 mM ABTS stock solution was prepared by adding 90 mg of ABTS with aquabides up to 25 mL in a 25 mL volumetric flask, then collected in a dark bottle, wrapped in aluminum foil, and placed in the refrigerator. Next, potassium persulfate was prepared by adding 66,289 mg $K_2S_2O_8$ with aquabides up to 100 mL to produce 2.4 mM $K_2S_2O_8$, then collected in a dark bottle, wrapped in aluminum foil, and placed in the refrigerator. The ABTS reagent was also prepared by mixing 7 mM ABTS and 2,4 mM $K_2S_2O_8$ in a ratio of 2:1. Aquabides variations were added, and the absorbance was measured with a nano spectrophotometer at 734 nm to obtain A=0.7±0,02. Next, about 20 µL of the sample was added with 180 µL of ABTS reagent and incubated for 6 minutes in the dark at room temperature. Finally, the absorbance was measured with a nano spectrophotometer at 734 nm. This test was expressed in µmol TE/g wet weight, with the Trolox standard of 0-350 µM, and each test was carried out in triplicate.

Cupric Ion Reducing Antioxidant Capacity (CUPRAC) assay

The antioxidant activity was tested with the CUPRAC method referred to by Nurcholis et al. (2022) with slight modifications. First, the test was carried out by adding 50 µL of the sample with 50 µL of 0.01 M $CuCl_2$ in distilled water, then 50 µL of 0.0075 M neokuproin was added with distilled water, 50 µL of ammonium acetate buffer solution (pH 7.0) and placed into a microplate. The solution was further incubated for 30 minutes at room temperature and in the dark. After incubation, the absorbance was measured with a nano spectrophotometer at 450 nm. This test was expressed in µmol TE/g wet weight with the Trolox standard of 0-800 µmol, and each test was carried out in triplicate.

Data analysis

According to Husein et al. (2021), the phytochemical screening data were analyzed qualitatively. In addition, the antioxidant activity analysis was performed quantitatively using One-Way ANOVA (IBM SPSS 25.0) with trust value α=0.05 to compare the data of the two purslanes.

RESULTS AND DISCUSSION

Morphology and phytochemical compounds

Figure 1 shows that the two purslanes examined had different morphological characteristics, including leaf, stem shape, and color variations. Based on the morphological observations of *Portulaca oleracea*, it has aerial herbaceous stems that grow upright, with a round shape, fleshy, glabrous, smooth, reddish-brown, cylindrical, and branches at the base which intersects with soil. The leaves are single, ciliated (short-stemmed), succulent (fleshy and watery), ovate breech (obovatus), alternate or scattered as also reported by (Uddin et al. 2014). Meanwhile, *P. grandiflora* purslane generally has the characteristics of round and solid stems that grow upright and fine thin hair at the boundaries of the segments. It also has single leaves, non-stemmed, shiny green color, thick fleshy, watery and relatively soft, linear or cylindrical leaf shape resembling pine needles, spread and alternately arranged leaves 1-3 cm long. The flowers are bisexual and compound, located at the branches' ends like roses. These morphological observations are the same as those reported by several previous studies (Setiawan et al. 2016).

Furthermore, phytochemical screening was carried out to determine the class of active compounds in the plants (Figure 1). It is a simple way to conduct a qualitative analysis of the compounds present in plants. This study's screening includes alkaloid, hydroquinone phenol, flavonoid, saponin, tannin, triterpenoid, and steroid tests, which represent several plant compounds. The phytochemical screening results of *P. oleracea* and *P. grandiflora* in Table 1 showed positive (+) reactions for all compounds tested, which aligns with Husein et al. (2021).

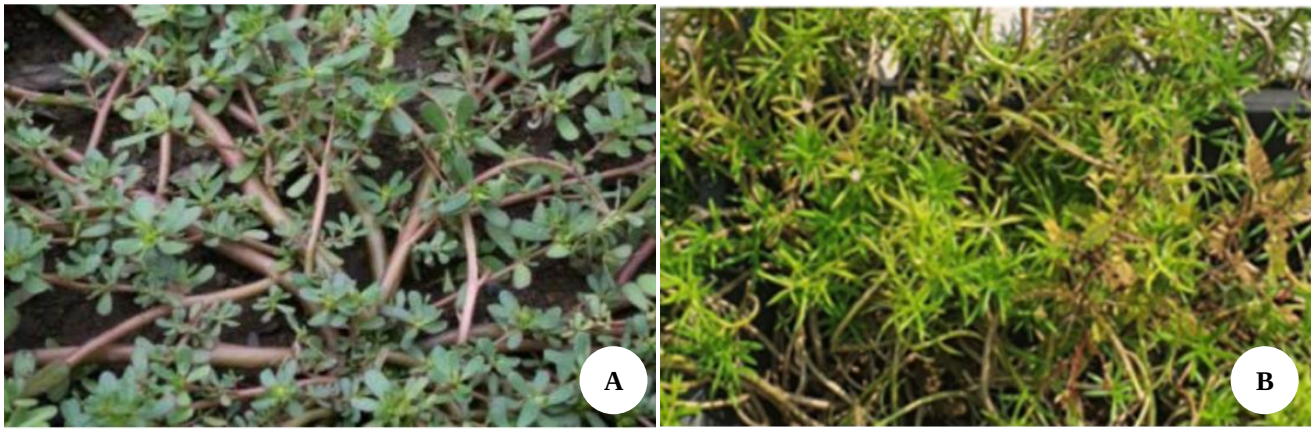

Figure 1. Morphology of the two types of purslane species used in this study. A. *Portulaca oleracea* (Yuniastri et al. 2020). B. *P. grandiflora*

Table 1. The phytochemical screening results of *Portulaca oleracea* and *P. grandiflora* regarding Husein et al. (2021)

Phytochemical compounds	*P. oleracea*		*P. grandiflora*	
	Husein et al. (2021)	Present	Husein et al. (2021)	Present
Alkaloid	+	+	+	+
Phenol hydroquinone	+	+	+	+
Flavonoid	+	+	+	+
Saponin	+	+	+	+
Tannin	+	+	+	+
Triterpenoid	+	+	+	+
Steroid	+	+	+	+

Note: +: identified; -: not identified

Table 2. Antioxidant activity of *Portulaca oleracea* and *P. grandiflora*

Purslane	ABTS (µmol TE/g FW)	CUPRAC (µmol TE/g FW)
P. oleracea	15.98	33.22
P. grandiflora	26.93	18.55

Note: ABTS: 2,2-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid), CUPRAC: cupric ion reducing antioxidant capacity, TE: Trolox equivalent, FW: fresh weight

Antioxidant activity

The antioxidant activity measurement aims to determine the total antioxidant capacity of the compounds in the aerial parts of the purslane plant extract. This parameter was measured in the two purslanes using the ABTS and CUPRAC methods. The ANOVA measurement showed significant results with $p<0.05$ as shown in Table 2. The highest CUPRAC antioxidant activity was found in *P. oleracea* at 33.22 µmol TE/g FW, while the lowest was found in *P. grandiflora* at 18.55 µmol TE/g FW. Furthermore, the highest ABTS antioxidant activity was found in *P. grandiflora* at 26.93 µg TE/g FW, while the lowest was in *P. oleracea* at 15.98 µmol TE/g FW. The chemical complexity of various purslane plants causes differences in the antioxidant capacity of each species.

Discussion

Purslane (*Portulaca*) plants were collected and subjected to drying and extraction processes to obtain simplicia. The samples were pollinated after drying in an oven, while extraction was carried out with the combined sonication-maceration method. The solvent used was 70% ethanol which can extract both polar and non-polar organic compounds from the sample (Permadi et al. 2021). After obtaining the extract, phytochemical screening was conducted to determine the class of active compounds from the plants.

Based on the tests, samples of *P. oleracea* and *P. grandiflora* contained saponins, as evidenced by the firm foam produced. In addition, both samples were found to have tannins, as indicated by the white precipitate. That is in line with Husein et al. (2021), which stated that the components of chemical compounds discovered in *P. oleracea* include phenolics, flavonoids, fatty acids, alkaloids, organic acids, vitamins, terpenoids, sterols, saponins, tannins, minerals, and volatile compounds. In contrast, information about the chemical compounds in purslane plants of the *P. grandiflora* type is limited. However, several chemical compounds reported in this species include phenolics, flavonoids, alkaloids, fatty acids, terpenoids, polysaccharides, and sterols.

Portulaca oleracea and *P. grandiflora* samples contained hydroquinone phenol, as indicated by the change in color to blackish purple. That aligns with Fernández-Poyatos et al. (2021), which stated that *P. oleracea* has a predominant component of phenolics. These phenolics include caffeoylglucaric acid, caffeic acid glucuronide isomers, ferulic acid, ferilic acid derivative, and sinapoyl hexoside. While in *P. grandiflora*, only specific phenolics, namely caffeic and chlorogenic acids, were found. Furthermore, both samples contain flavonoids, as evidenced by a reddish color on the alcohol layer. According to Sicari et al. (2018), several flavonoids have been isolated from purslanes such as apigenin, kaempferol, luteolin, quercetin, isorhamnetin, oleracone C, and oleracone D.

Alkaloids were also found in samples of *P. oleracea* and *P. grandiflora*, as evidenced by the presence of white, brown, and orange-red colors with Mayer's, Wagner's, and Dragendorf's reagents, respectively. That is in line with Kumar et al. (2021), which reported alkaloids as one of the essential chemical elements of this plant. Several alkaloids analyzed and isolated in *P. oleracea* include oleracein A, oleracein B, oleracein C, oleracein D, oleracein E, scopoletin, and aurantiamide. Meanwhile, in *P. grandiflora*, the report on alkaloid analysis was limited to only phytochemical screening through specific chemical reactions, which proved that this plant contains alkaloids.

The steroid test yielded positive results for both samples, meaning that *P. oleracea* and *P. grandiflora* contain steroids, as evidenced by a green coloration. That is consistent with Netala et al. (2014), which stated that *P. oleracea* and *P. grandiflora* contain sterol compounds. Both samples were also found to contain triterpenoids, as evidenced by the change to a red color. According to Husein et al. (2021), beta-carotene is the most widely analyzed class of terpenoids in purslane plants. In *P. oleracea*, the beta-carotene in leaves was twice higher than in stems and flowers. Meanwhile, for *P. grandiflora*, the carotenoid content found in the ethyl ether extract was much less than that of *P. oleracea*.

In this study, the antioxidant activity was measured using two methods because their characteristic depends on the mechanism of antioxidant metabolites in the extract. Therefore, different methods are needed to evaluate the nature of the antioxidant capacity. ABTS and CUPRAC were used to measure the antioxidant capacity of these two purslanes. The ABTS method measures the scavenging activity by providing hydrogen atoms to free radicals, making free radical compounds non-radical. Meanwhile, the CUPRAC method is used to determine the reducing power of free radicals by donating one electron. Free

radicals are a form of reactive compounds generally known to have unpaired electrons in their outermost shell. They are formed when molecules that lose electrons become unstable (Rubio et al. 2016).

The ABTS method determines the antioxidant activity based on the oxidation of potassium persulfate with ABTS diammonium salt. The blue color disappearance indicated the presence of antioxidant activity in a sample in the ABTS reagent. This method has high sensitivity and can be used to analyze antioxidants in food. It works based on the ability of compounds to stabilize free radicals by donating protons (Magalhaes et al. 2008; Shah and Modi 2015). Meanwhile, the CUPRAC (Cupric Ion Reducing Antioxidant Capacity) method is used to determine the presence of activity and measure the antioxidant capacity of iodine leaves against free radicals whose absorbance is measured on a UV-Vis spectrophotometer with a wavelength of 450 nm (Apak et al. 2010). It measures antioxidant activity using bis(neokuproin) copper (II) ($Cu(Nc)_2^{2+}$) as a chromogenic reagent. The blue-colored $Cu(Nc)_2^{2+}$ reagent will experience a reduction to $Cu(Nc)^{2+}$ which is yellow.

Furthermore, CUPRAC is a simple and low-cost antioxidant testing method (Sadeer et al. 2020) that uses a selective reagent with a low reduction potential value. Therefore, it has several advantages compared to other antioxidant measurement methods. For example, the CUPRAC reagent is fast oxidizing thiol types of antioxidants and is selective due to its lower redox potential. In addition, the reagent is more stable and accessible than other chromogenic types, such as ABTS and DPPH. Moreover, this method is easy and applicable in conventional laboratories using standard colorimeters that do not require sophisticated equipment and qualified operators. It can also measure hydrophilic and lipophilic antioxidants, such as β-carotene and α-tocopherol (Sadeer et al. 2020).

Based on the results, the antioxidant activity of *P. oleracea* extracts tested by the CUPRAC method showed higher activity than ABTS, while *P. grandiflora* extract tested by the ABTS method was higher than CUPRAC. The antioxidant analysis quantitatively measures a component's ability to act as a reducing agent. The analysis is divided into two methods, namely HAT (Hydrogen Atom Transfer) and SET (Single Electron Transfer), which have different mechanisms. The HAT method measures the ability of antioxidants to neutralize free radicals by donating H atoms and has been effectively employed for testing phenolic components. In that test, the presence of antioxidant activity is indicated by a change in color from colored to colorless. Meanwhile, SET is a method based on redox reactions in which the tested antioxidant will react with the oxidizing agent, a fluorescence compound. It is conducted using a spectrophotometer to measure the color change that correlates with the concentration of antioxidants in the sample. That is influenced by the pH and the solvent used (Sadeer et al. 2020). The results indicated that *P. oleracea* extract predominantly uses a single electron transfer (SET) mechanism, while *P. grandiflora* extract uses hydrogen atom transfer (HAT).

The difference in the ability of SET and HAT in the samples is influenced by several factors, such as dilution and the type of solvent used in the extraction process (Purwanto et al. 2017). In addition, a sample might have different antioxidant activity levels due to compound constituents' variation. Differences in the levels of a compound are caused by several factors, including the geographical location of plants, climatic factors, which include temperature, air and humidity, essential factors such as light, water, and soil nutrients, as well as pest or disease and weed disturbance (Ramakrishna and Ravishankar 2011). In this study, there were differences in the dilution factor for the two test samples, where the value for *P. oleracea* was 15 while that of *P. grandiflora* was 10.

Based on the results, *P. oleracea* and *P. grandiflora* contain phytochemicals in alkaloids, phenol hydroquinones, flavonoids, saponins, tannins, triterpenoids, and steroids which can produce pharmacological effects such as antioxidants. The antioxidant activity of the compounds in *P. oleracea* tends to use the SET mechanism, while those in *P. grandiflora* use the HAT mechanism.

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