Phytochemical screening and antioxidant properties of ethanolic crude extract of *Portulaca grandiflora*

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Abstract:

Portulaca grandiflora is a flowering plant in the Portulaca oleracea family, native to southern Brazil, Argentina, and Uruguay and seen in south Asia. The entire plant has been reported to have medicinal benefits. The present study aimed to study phytochemical screening, total phenolic, total flavonoid contents and the antioxidant activity of the ethanolic crude extract from *Portulaca grandiflora*. The phytochemical screening of three crude extracts from different organs, including flowers, leaves and stems of *Portulaca grandiflora* showed that all of the extracts consisted of phenolic and flavonoid compounds. Total phenolic and flavonoid contents were measured by Folin-Ciocalteu and aluminum chloride assays. The results showed that crude extract from the flowers of the *Portulaca grandiflora* yielded the highest total amount of phenolic and flavonoid compounds of 767.86±0.38 mgGAE/g extract and 439.18±0.18 mgQE/g extract, respectively. The antioxidant efficiency of the extract was studied by 2 assays: DPPH and FRAP. In the DPPH assay, it was found that the crude extract from the flowers of *Portulaca grandiflora* in ethanol solvent gave the IC_{50} value equal to 184.96±0.67 mg/L. In contrast, ascorbic acid gave the IC_{50} equal to 168.12±0.57 mg/L. The flower extracts gave the FRAP value equal to 24.11±0.01 µM Fe^{2+}E/g extract in the FRAP assay. These results suggested that phenols and flavonoids may function as antioxidant agents in *Portulaca grandiflora*.

1. Introduction

Cellular metabolism in the human body typically produces free radicals as a by product. In addition, free radicals may be made by exposure to external stimuli, including pollution, tobacco smoke, heavy metals, transition metals, an industrial solvents, pesticides, ultraviolet light, some specific drugs, or even poor diet[1]. Free radicals are atoms or molecules containing an unpaired electron in a valency shell or outer orbit[2]. They are highly reactive and unstable molecules. They can abstract electrons from other compounds to stabilize themselves, making the attacked molecule lose its electron and become a free radical. Although free radicals have a short lifespan, their reactive process can cause damage to the DNA of human cells, sometimes resulting in mutations that can lead to various diseases, including cellular aging, heart disease, cancer, diabetes and neurodegenerative infections. The significant free radicals in the human body are oxygen and nitrogen free radicals such as singlet oxygen, hydrogen peroxide, superoxides, hydroxyl anions, peroxynitrite, nitric oxide and hypochlorous acid[1]. The availability of these free radicals creates oxidative stress in the body. Antioxidants are compounds that can prevent or minimize the oxidation of oxidizable products by scavenging the free radicals and reducing oxidative stress. In the human body has many endogenous enzymatic antioxidant defenses that protect the cells against oxidative damage. However, most human cells do not generate adequate amounts of antioxidants to protect them against oxidative reactions of the produced free radicals[3]. Therefore, there is a need to look for more dietary sources of antioxidants to decrease the risk of these free radicals[4].

Plants in nature are rich sources of bioactive secondary metabolites, such as flavonoids, glycosides, saponins, terpenes, sterols, tannins, alkaloids and other metabolites[5]. It has been reported that the most of these groups have antioxidant activity. Therefore, currently, attention is paid to the phytochemical screening and antioxidant activity of the various plant extracts.

Portulaca grandiflora is a flowering plant in the Portulaca oleracea family, native to southern Brazil, Argentina, and Uruguay and also seen in south Asia. The entire plant has been reported to have medicinal benefits. The entire plant is depurative [6]. It is used to treat of hepatitis, cirrhosis of the liver with ascites, swelling, and pain in the pharynx[6]. The fresh juice of the leaves and stems is applied externally as a lotion to snake and insect bites, burns, scalds, and eczema [6-7].

The objectives of this study were to perform the phytochemical screening, to determine the total phenolic and flavonoid contents as well as antioxidant activities of *Portulaca grandiflora* extracts.

2. Materials and Methods

2.1 Plant materials

Portulaca grandiflora was collected from self grown plant. It was grown from seeds purchased from local market in Bangkok, Thailand. The seed were germinated for two weeks in small pots and then transferred to outdoor garden. The plants were

harvested after nine weeks, with the leaves, stems, and flowers separated.

2.2 Reagents

DPPH (2,2-diphenyl-1-picrylhydrazyl), 2,4,6-Tris (2-pyridyl)-s-triazine (TPTZ), Folin-Ciocalteu reagent, gallic acid, quercetin, ascorbic acid, iron(III) chloride, aluminum chloride hexahydrate, ethanol, hydrochloric acid, bromine, gelatin, vanillin, zinc dust and magnesium ribbon were purchased from Sigma–Aldrich (St. Louis, USA). Propanol and ammonium hydroxide were supplied from Merck (Darmstadt, Germany). All reagents were prepared using ultrapure water (Milli-Q, USA) in all experiments.

2.3 Preparation of crude extracts

Extraction of *Portulaca grandiflora* leaves, stems and flowers was conducted by maceration using an electric stirrer. Each fresh organ was shade dried and crushed to powder. A total of 2 g of each powered organ was extracted in 400 ml of ethanol for 5 days. The extracts were filtered and evaporated using rotary evaporator (40^{o}C) under reduced pressure to obtain the crude extracts. These crude extracts were used for the phytochemical analysis, total phenolic and flavonoid contents and antioxidant activity.

2.4 Phytochemical screening

The qualitative assay consisted of tests for phenolic compounds, tannins and flavonoids using the previously reported methods[8-10] with some modifications. Control negative containing no *Portulaca grandiflora* extract was performed in each test to ensure that the coloration change in the test was caused by *Portulaca grandiflora* extract. Each test, along with negative control, was replicated three times.

2.4.1 Test for tannins and phenolic compounds

1 ml of the extract was added with 25 ml of hot water and stirred until cool. 1 ml of 10% sodium chloride was then added and filtered the precipitate from the solution. The same amount of filtrate obtained was transferred into six test tubes. Finally, each tube except for the control tube was filled with 5 drops of different reagents. These reagents were 1% gelatin solution, iron(III)chloride, bromine water, lime solution and vanillin-HCl. Positive test results from using 1% gelatin solution, bromine water and lime solution are the formation of white precipitation, buff precipitate and blue-gray precipitation, respectively. The appearance of a bluish-green color of the test solution indicates the positive test results when iron (III) chloride is used as a reagent while the crimson color of the test solution is obtained when using vanillin-HCl.

The criteria for concluding the experimental results were as follows:

1) Negative results when tested with iron(III) chloride reagents indicate the absence of tannins or phenolic compounds in the extract.
2) Positive results when tested with iron(III) chloride (appearance of a bluish-green or a blackish green colors of the test solution) and gelatin indicate the presence of catechol-type tannins.
3) Positive results when tested with iron(III) chloride (appearance of a dark blue color of the test solution) and gelatin indicate the presence of pyrogallol or gallic-type tannins.
4) Positive results when tested with iron (III) chloride (appearance of green or blue of the test solution) and negative results when tested with gelatin indicate the absence of tannins but presence of other phenolic compounds.
5) Negative results when tested with gelatin and iron (III) chloride indicate the absence of tannins and polyphenols in the extract.
6) Positive results when tested with gelatin, iron (III) chloride (appearance of a green color of the test solution), bromine water and vanillin-HCl and negative results when tested with lime solution indicate the appearance of condensed tannins in the extract.
7) Positive results when tested with gelatin, iron (III) chloride (appearance of blue Blue-black of the test solution) and lime solution and negative results with bromine water and vanillin-HCl indicate the appearance of hydrolyzable tannins in the extract.
8) Positive results when tested with all reagents and the appearance of a greenish blue color of the test solution when tested with iron (III) chloride indicate the appearance of condensed and hydrolyzable tannins in the extract.

2.4.2 Test for flavonoids

2.4.2.1 Shinoda test

Few fragments of magnesium ribbon were added to 1 ml of the extract. Then 10 drops of concentrated hydrochloric acid were added dropwise. Observe the color appearance within 2-3 minutes by comparing with the color of the original extract. If the resulting color of the test solution is orange to red, it indicates that flavones are present. If it is a red to dark red color, it indicates the presence of flavonols. If it is a dark red to reddish-purple color, it indicates the presence of flavanone.

2.4.2.2 Pew test

0.5 g of zinc dust and 2 drops of 2 N hydrochloric acid was added to 1 ml of the extract. After shaken the solution for 1 min, 10 drops of concentrated hydrochloric acid were added dropwise. If a dark red color of the test solution appears within 2-5 min, it indicates the presence of flavanonols. If it is a faded red color, it shows the presence of flavanones or flavonols.

2.4.2.3 Chalcones and aurones test

This method is used to test for samples that give a negative result on Shinoda test. 10 drops of concentrated hydrochloric acid were added to 1 ml of the extract. If a red color of the test solution appears immediately, it indicates the presence of chalcone and aurone.

2.4.2.4 Alkaline reagent test

Ammonia TS (28% NH_4OH 35.7 ml and adjust the volume to 100 ml) was added dropwise to 1 ml of the extract. If the color of the test solution is yellow, it indicates the presence of flavone, flavonol and xanthone. If it is red-orange, it indicates the presence of flavonone. If a purple color of the test solution appears immediately, it indicates the presence of chalcone and aurone. The appearance of a brown orange of the test solution shows the presence of flavanonol.

2.4.2.5 Anthocyanins test

The presence of anthocyanins has been demonstrated by adding 1 ml of the extract with 1 ml of 2 N hydrochloric acid. The appearance of a pink-red color indicates the presence of flavonoids. After adding ammonium TS dropwise, if a red color of the test solution turns purplish blue, it shows the presence of anthocyanins.

2.4.2.6 Leucoanthocyanin test

The presence of leuco anthocyanin has been demonstrated by adding 1 ml of plant extract with 10 drops of 2 N hydrochloric acid and 10 drops of 1-propanol. The solution was heated in the water bath for 15-30 minutes. The appearance of a yellow-brown color of the test solution indicates the presence of catechin whereas a yellow-brown color shows the presence of leucoanthocyanin.

2.5 Determination of total phenolic content

The total phenolic content was determined with Folin-Ciocalteu method according to the method of Zhou, K. et al. with some modifications[11]. In brief, 2 ml of the extract (1 g/L) or standard solution of gallic acid was mixed with 1 ml of 20% w/v sodium carbonate then the mixture was allowed to stand at room temperature for 15 min. After addition of 1 mL of Foline–Ciocalteu reagent and 5 ml of pure water, the mixture was left in the dark for 30 min at room temperature. The absorbance was measured at 765 nm using a spectrophotometer (Jasco V-730 spectrophotometer, Japan). The analysis was performed in triplicate. The concentration of phenolic compounds was expressed as mg of gallic acid equivalents per gram of extract (mgGAE/g) by comparison with standard curve of gallic acid.

2.6 Determination of total flavonoid content

The total flavonoid content was determined according to the method of Ramamoorthy et.al with some modifications[12]. One milliliter of 2% $AlCl_3$ was mixed with 2 mL of the extract (1 g/L) or standard solution of quercetin and 1 ml of pure water. The mixture was incubated for 10 min at room temperature. The absorbance was measured using a spectrophotometer (Jasco V-730 spectrophotometer, Japan) at 415 nm against a prepared reagent blank. The analysis was performed in triplicate. Total flavonoid content was expressed as quercetin equivalent per gram of the extract (mg QE/g).

2.7 Determination of antioxidant activity

2.7.1 DPPH radical scavenging assay

Free radical scavenging capacity of the extracts was determined by using the scavenging of the stable 2,2-diphenyl-1-picryhydrazyl (DPPH) free radical according to the method of Mensor, L.L. et al. with some modifications[13]. Two microliters of each sample were added to 1 mL of DPPH in EtOH (20 mM). After incubation for 30 min in the dark, the absorbance of each sample was measured at 520 nm spectro-photometrically (Jasco V-730 spectrophotometer, Japan). The DPPH solution was used as a negative control. Ascorbic acid was used as the reference standard. Triplicate determinations were performed. The percentage of DPPH scavenging activity was calculated using the following formula:

$$\% \text{ antiradical activity} = [(A_c - A_s)/ A_c] \times 100$$

where A_c is the absorbance of the control and A_s is the absorbance of the sample. The IC_{50} values denote the concentration of the sample which is required to scavenge 50% of DPPH free radicals.

2.7.2 Ferric-reducing antioxidant power (FRAP) assay

The FRAP assay was done according to Benzie and Strain[14] with some modifications. The stock solution was prepared by mixing 300 mM acetate buffer pH 3.6, 10 mM TPTZ (2, 4, 6- tripyridyl-s-triazine) solution in 40 mM HCl, and 20 mM $FeCl_3$ $6H_2O$ solution in the proportion of 10:1:1 (*v*/*v*/*v*) at 37°C. The freshly prepared working reagent (2 ml) and the extract (1 ml) were mixed thoroughly. The absorbance was taken at 593 nm (Jasco V-730 spectrophotometer, Japan) after 30 min incubation at 37°C. Standard curve was prepared using different concentrations of $FeSO_4 7H_2O$ (concentration 0.3-0.8 mM). The results were expressed as µM equivalents Fe^{2+} per gram of the extract (µM Fe^{2+}E/g extract). Triplicate determinations were performed and the mean values were calculated.

2.8 Statistical analysis

All work was done in triplicates and the data presented are means ± S.D. of three independent determinations. Significance was accepted at $p>0.05$.

3. Results & Discussion

3.1 Crude extracts

Three plant parts of *Portulaca grandiflora* including leaves, stems, and flowers were separately extracted with ethanol. The percentage yields reported by the ratio

of crude extract and weight of the sample (w/w) were presented in Table 1. As can be seen in Table 1, the flower yielded the highest amount of crude extract of 3.97% whilst leaves and stems yielded 3.13% and 2.03%, respectively.

Table 1. The percentage (%) yield of crude extracts obtained from different plant parts of *Portulaca grandiflora*.

Extracts	Percent yield*
Leaves	3.13±0.54
Stems	2.03±0.04
Flowers	3.97±0.74

[a] percent yield obtained from 5 days extraction time.

The influence of extraction time of 3,5,7 days on the percent yield of the flower extracts was also studied. It was found that the extraction time of 5 days resulted in the highest percent yield of 3.97±0.74, while the 3 days of extraction time yielded the lowest percent yield of 3.63±0.14. The extraction time of up to 7 days resulted in an even lower percent yield (3.4%) than the extraction time of 5 days.

3.2 Phytochemical screening test

As phenolic compounds have been related to antioxidant activity, some studies have emphasized specific classes such as tannins and flavonoids. Therefore, phytochemical screening tests in this study are focused only on those three compounds. The results of the phytochemical screening test of the extracts from leaves, stems and flowers of *Portulaca grandiflora* were shown in Tables 2-3.

From the phytochemical screening test of tannin and phenolic compounds, it was found that the crude extract from leaves, stems and flowers of *Portulaca grandiflora* consisted of phenolic compounds due to their positive results with iron (III) chloride and negative results with gelatin. In addition, the phytochemical screening test of flavonoids in the three crude extracts of *Portulaca grandiflora* revealed the presence of flavonoids such as xanthones due to the positive results with the alkaline reagent test and negative results with the Shinoda test and Pew test.

Table 2. The results of the phytochemical screening test for tannin and phenolic compounds.

Tube No	Test	Result	Appearance[a]
1	Gelatin	-	-
2	Fe(III)chloride	+	Green
3	Br water	-	-
4	Lime solution	-	-
5	Vanillin-HCl	-	-

* leaves, stems and flowers extracts showed the same results

Table 3. The results of the phytochemical screening test for flavonoids.

Tube No	Test	Result	Appearance[a]
1	Shinoda test	-	-
2	Pew test	-	-
3	Chalcones and Aurones test	-	-
4	Alkaline reagent test	+	Yellow
5	Anthocyanins test	-	-
6	Leucoanthocyanin test	-	-

[a] the extracts from leaves, stems and flowers showed the same results

3.3 Total phenolic and flavonoid contents

The total phenolic content of the crude extract of *Portulaca grandiflora* is presented in Table 4.

Table 4. Total phenolic acid and flavonoid contents of the extracts of *Portulaca grandiflora* from various parts.

Extracts	**Total phenolic (mgGAE/g extract)**	**Total flavonoids (mgQE/g extract)**
Leaves	679.69±0.20	157.40±0.21
Stems	619.54±0.33	31.55±0.55
Flowers	767.86±0.38	439.18±0.18

The amounts of total phenolic acids and flavonoids from different parts of *Portulaca grandiflora* were different. The values of phenolic acid contents varied from 619.54±0.33 to 767.86±0.38 mg GAE/g of the dry weight of plant material as measured by the Folin-Ciocalteau method. The flavonoid contents values ranged from 31.55±0.55 to 439.18±0.18 mg QE/ g of the dry plant material as measured by the $AlCl_3$ method. The extracts of *Portulaca grandiflora* from flowers part found to have the highest phenolic acid and flavonoid content values, followed by the extracts from leaves and stems. In addition, the total phenolic acids contents obtained were much higher than the flavonoids contents in all the extracts studied.

3.4 Antioxidant activities

Antioxidant activities of the extracts of *Portulaca grandiflora* were assessed to identify potential sources of substances possibly useful against the effects of free radicals by two methods: DPPH and FRAP assays.

DPPH is a free radical, stable at room temperature, which produces a violet solution in ethanol. The DPPH assay is based on the ability of the stable DPPH free radical to react with hydrogen donors. The DPPH• radical displays an intense UV-VIS absorption spectrum. In this test, a solution of radical is decolorized after reduction with an antioxidant (AH) or a radical (R•) in accordance with the following scheme:

$$DPPH\bullet + AH \rightarrow DPPH\bullet\text{-}H + A\bullet,$$
$$DPPH\bullet + R\bullet \rightarrow DPPH\bullet\text{-}R.$$

The results from DPPH assay are presented in Table 5.

Table 5. DPPH radical scavenging ability of the extracts from *Portulaca grandiflora* and ascorbic acid.

Extracts	IC_{50} (mg/L)
Leaves	202.51±1.20
Stems	250.25±0.75
Flowers	184.96±0.67
Ascorbic acid	168.12±0.57

The half-maximal inhibitory concentration (IC_{50}) values shown in Table 5 indicated that the DPPH free radicals were scavenged by the plant extracts and ascorbic acid with different capacities. The lower the value of half-maximal inhibitory concentration, the higher the effectiveness of antioxidant capacity. Therefore, the order of free radical scavenging ability of the extracts/fractions was found to be as follows: ascorbic acid > flower extracts >leaf extracts >stem extracts. In addition, it was found that the DPPH free radical scavenging was antioxidant concentration dependent. Table 6 shows example data of this dependency characteristic. The percentage of antiradical activity of the flower extracts was increased from 47.19±1.04 to 94.58±0.01 when the flower extracts concentration was increased from 200 to 1,000 mg/L. In fact, the higher the concentrations of the plant extracts used, the higher the %inhibition values obtained.

Ferric reducing antioxidant power (FRAP) is an alternative method for investigating antioxidant activities. The assay was based on the reducing power of an antioxidant. A potential antioxidant will reduce the ferric ion (Fe^{3+}) to the ferrous ion (Fe^{2+}); the latter forms a blue complex (Fe^{2+}/TPTZ), which increases the absorption at 593 nm. The results from FRAP assays are shown in Table 7.

Table 6. Antioxidant concentration dependent on DPPH free radical scavenging.

Extracts	concentration (mg/L)	%inhibition
Flowers	200	47.19±1.04
	400	64.22±0.04
	600	79.12±0.91
	800	90.44±0.03
	1000	94.58±0.01

Table 7. The FRAP value of the extracts from *Portulaca grandiflora* and gallic acid.

Extracts	FRAP value (μM Fe^{2+}E/g extract)
Leaves	20.22±0.30
Stems	10.15±0.74
Flowers	24.11±0.01
Gallic acid	28.58±0.01

The FRAP values in Table 7 indicated that the order of antioxidant capacity of the extracts/fractions was found to be as follows: gallic acid > flower extracts > leaf extracts > stem extracts. In fact, higher FRAP value gives higher antioxidant capacity because FRAP value is based on reducing ferric ion, where antioxidants are the reducing agent. Antioxidants are compounds capable of donating a single electron or hydrogen atom for reduction.

4. Conclusion

The present study confirms that extracts from leaves, stems and flowers of *Portulaca grandiflora* contained phenolic and flavonoid compounds in different proportions. Phenolic components are antioxidants, and exhibit a wide range spectrum of medicinal properties such as anti–cancer, anti–inflammatory and diabetic effects. Flavonoids are one of the most diverse groups of natural components that have been shown to possess a broad spectrum of chemical and biological activities including radical scavenging properties, anti–allergenic, antiviral and anti–inflammatory effects. The results from the DPPH radical scavenging ability and the FRAP assays indicated that all the extracts of *Portulaca grandiflora* exhibited antioxidant activity. The order of free radical scavenging ability of the extracts/fractions was found to be as follows: flower extracts >leaf extracts >stem extracts. Therefore, the extracts of this plants could be used as a natural antioxidant in both food preservation and human health to some extent.

Acknowledgements

The authors would like to thank the department of science, faculty of science and technology, the Rajamangala University of Technology Krungthep for instrument facilities.

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