

Antioxidant activity and total phenolic content of different varieties of *Portulaca grandiflora*

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Abstract

Crude extracts from the whole plant of five different varieties (white, purple red, orange, pink and red) of *Portulaca grandiflora* were screened for their *in vitro* antioxidant and total phenolic content. These plants were extracted by methanol, acetone and ethanol, respectively. Their total phenolic content as determined by Folin-Ciocalteu reagent, were ranging from 46.39-82.19 mg GAE/100 g, 36.72-56.45 mg GAE/100 g, and 41.46-85.70 mg GAE/100 g for methanolic, acetone and ethanolic extract, respectively. The antioxidant activity of plant extracts as determined by DPPH scavenging assay were ranging from 0.69-2.14 mg/mL, 1.40-4.38 mg gallic acid/g, and 7.32-29.21 mg ascorbic acid/g when expressed as IC₅₀, GEAC and AEAC, respectively. The highest antioxidant activity was observed in acetone extract of orange variety (PG 3). Therefore, each part of the PG 3, i.e. leaf, stem and flower was separated and further evaluated. The results showed that the leaf of PG 3 contained the highest phenolic content and antioxidant activity. The present study suggests that extracts from *P. grandiflora* could be utilised as natural sources for antioxidant.

Keywords: Antioxidant, Total Phenolic Content, *Portulaca grandiflora*, DPPH, Folin-Ciocalteu

1. Introduction

Numerous physiological and biochemical processes in the human body like breathing, energy production, breakdown of macromolecule and others depend on oxidation reaction¹. This reaction can produce oxygen-centered free radicals, more generally known as reactive oxygen species (ROS) along with reactive nitrogen species (RNS) as by products^{2,3}. Overproduction of such free radicals can contribute to many chronic diseases, such as cancer, atherosclerosis, aging, immuno-suppression, and degenerative diseases in humans^{2,4,5,6}. Plants including fruits, vegetables and medicinal herbs contain a variety of antioxidants, such as phenolic compounds, nitrogen compounds, vitamins, and terpenoids². However, more attentions have been focused on phenolics which may have higher antioxidant activities than those of conventional vitamins C, E and β -carotene⁷. Plant phenolics comprise a great diversity of compounds. It is estimated that over 8000 naturally occurring phenolic compounds are known, and it can be divided into 15 classes⁸. The antioxidant activity of phenolics is mainly attributed to their redox properties which allow them to function as reducing agents, hydrogen donors, singlet oxygen quenchers and metal chelators^{9,10}.

Portulaca grandiflora is a small herbaceous annual plant belongs to the family Portulacaceae. Common names include Moss Rose, Rose Moss and Sun plant¹¹. Previously, the water extract of *P. grandiflora* was employed to study its toxicity on Wistar rats¹², *in vitro* anti-herpes simplex viruses and anti-adenoviruses activities¹³. In addition, the water extract of *P. grandiflora* was found to enhance lymphocyte proliferation *in vitro*, suggesting a role in immunomodulation¹⁴. Compared to its close relative *P. oleracea*^{15,16,17,18}, studies on the health benefits and detailed characterisation of *P. grandiflora* are small and limited. To the best of our knowledge, the effects of different varieties of *P. grandiflora* on total phenolic content and their antioxidant activity have not been reported. The objectives of this study are therefore, to evaluate the effects of various solvents of different polarities on the extraction yields, total phenolic content and antioxidant activity of different varieties of *P. grandiflora*.

2. Materials and Methods

2.1 Plant materials: *P. grandiflora* or moss rose was obtained from Sungai Siput (U), Perak, Malaysia and was identified with reference to literature¹¹. The plants were harvested at maturity, and during or prior to their flowering period. Five varieties (white, purple red, orange, red, and pink) of the same species were used in this study. These five varieties, as shown in Figure 1 were named as PG 1- PG 5. Total fresh weight of 300

g from each variety was gathered. The fresh plant samples were air dried under shade for 1 week and followed by complete drying at 40 °C oven until constant weight was obtained.

2.2 Sample preparation and extraction: Sample preparation and extraction were carried out as described by Saha *et al.* with slight modifications¹⁹. The whole dried plants (stem, leaf and flower) were homogenised, and about 25 g of the sample from each variety was extracted separately with 250 mL of three different solvents: methanol, acetone and ethanol for 48 h in an orbital shaker at 200 rpm and temperature 30 °C. The extracts were filtered and filtrates were concentrated in a rotary evaporator (BüchiRotavapor R-200, Switzerland) at 45 °C. Extracts were kept at 4 °C until the bioassay analyses.

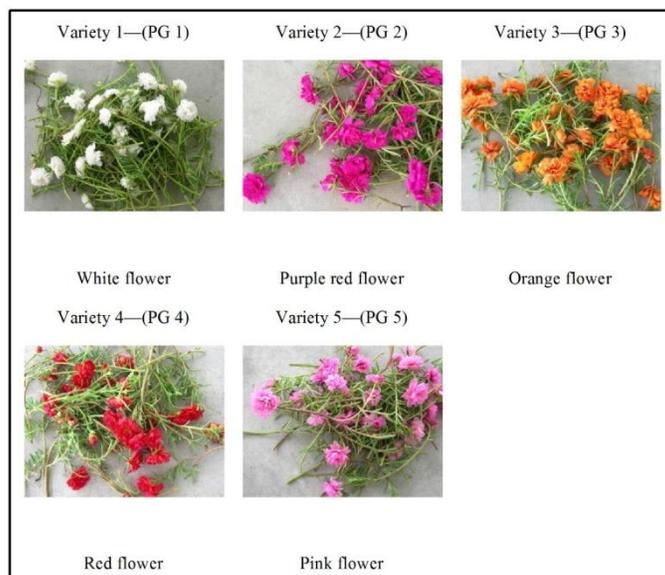


Figure 1. The different varieties of *Portulaca grandiflora*. The common characteristics of the varieties are: double layered petal, needle shaped leaves, green colour leaves and alternate leaf arrangement. PG 1-5 denotes different flower colour varieties, ranging from white, purple red, orange, red, and pink.

2.3 Total phenolic content: Total phenolic content was determined using Folin-Ciocalteu method as described by Miliauskas *et al.*²⁰. Gallic acid was used for the preparation of calibration curve. Volumes of 100 µL aliquots of 30, 60, 90, 150 and 180 mg/L gallic acid solutions were added to test tubes followed by 1 mL of 10% (v/v) Folin-Ciocalteu reagent. The mixture was mixed and incubated for 5 min before addition of 0.8 mL 7.5% (w/v) sodium carbonate. The resulting mixture was further incubated for 1 h in dark at room temperature before absorbance was measured at 765 nm with a UV-VIS spectrophotometer (Genesys 20, Thermo Spectronic, USA). For the test samples, 1 mg/mL plant extracts were prepared and analysed as described above. Results were expressed as mg Gallic Acid Equivalent (GAE) per 100 g dry weight of plant.

2.4 Antioxidant activity: The antioxidant activity of the plant extracts was determined by assessing their ability to scavenge 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical according to the procedures described by Abas *et al.*²¹ with some modifications, whereby the scavenging of a DPPH free radical was monitored at a characteristic wavelength in the presence of the sample. The assay was carried out in 96-well microtiter plates, which contained 100 µL of test solution (extracts dissolved in methanol) and 5 µL of 300 µM DPPH. Reaction mixture was incubated in dark for 30 min at room temperature and absorbance was then measured with a microplate reader (Bio-RAD Laboratories, Inc., Japan) at 520 nm. Control was prepared by replacing the test solution with 100 µL methanol. Test solution without DPPH was prepared as a blank to eliminate any colour interference caused by the plant crude extracts.

The IC₅₀ denoted the amount of plant crude extract (mg/mL) required to reduce initial concentration of DPPH radicals by 50% was determined. Gallic acid and ascorbic acid were used as the standards and IC₅₀ values were expressed in Gallic Acid Equivalent Antioxidant Activity (GEAC) and Ascorbic Acid Equivalent Antioxidant Activity (AEAC) as well, i.e. the quantity of gallic acid / ascorbic acid required to produce the same scavenging activity as the extract per g of sample¹⁵.

2.5 Statistical analyses: The experimental data was analysed by using one-way ANOVA and post hoc Tukey HSD test (SPSS, version 11.5). The values obtained were presented as means ± SD of triplicate analyses.

3. Results & Discussion

3.1 Extraction yield: The extraction yields for whole plants of PG 1 to PG 5 varied from 2.70-3.30%, 1.51-2.88% and 0.93-3.54%, when extracted with methanol, acetone and ethanol, respectively (Figure 2). These results suggested that the variation in extraction yields might be attributed by the properties of the extracting solvents, such as polarity, that allowed extraction of different types of phenolic compounds^{22,23}. This is in agreement with previous studies^{24,25} that reported the polarity of extracting solvents significantly affected the yields, phenolic content and antioxidant activity of plant extracts. In general, methanol extraction gave the highest yield among all the varieties of *P. grandiflora* studied.

The study by Lim and Quah showed that methanol gave the highest extraction efficiency in *P. oleracea* and mainly this was due to the ability of the solvent to inhibit action of polyphenol oxidase that caused the oxidation of phenolics¹⁵.

3.2. Total phenolic content: Total phenolic content in different solvent extracts varied widely, ranging from 46.39-82.19 mg GAE/100 g in methanol extracts; acetone extracts from 36.72-56.45 mg GAE/100 g and ethanol extracts from 41.46-85.70 mg GAE/100 g in all varieties assessed (Figure 3). The discrepancy observed might be caused by the types of compound extracted by the solvent. Only crude extracts were used in this study, so the higher yield might be contributed by other polar compounds besides phenolics, namely polysaccharides, plant debris and so on²⁶.

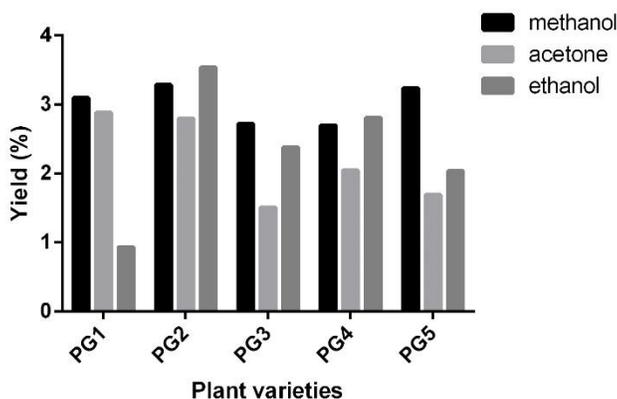


Figure 2. Percent yield of different *Portulaca grandiflora* varieties extracted with different solvent systems. Yield is defined as total polar compounds extracted by using the respective solvent with respect to the dry plant materials. Yields for each variety were obtained from a pool of three biological replicates. PG 1-5 denotes different flower colour varieties, ranging from white, purple red, orange, red, and pink.

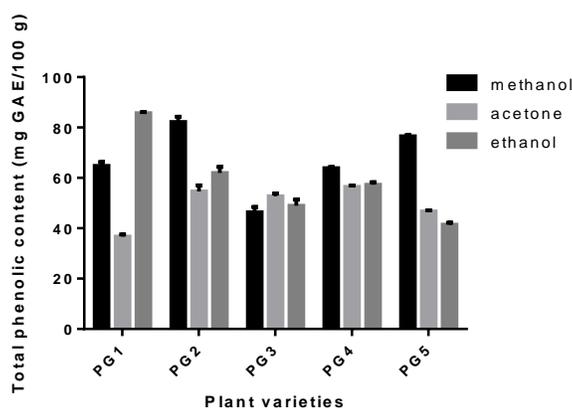


Figure 3. Total phenolic content of different *Portulaca grandiflora* varieties extracted with different solvent systems. The phenolic content was assessed by the Folin-Ciocalteu method and expressed as gallic acid equivalents (mg GAE/100 g). Values were expressed as means \pm SD (n=3). PG 1-5 denotes different flower colour varieties, ranging from white, purple red, orange, red, and pink.

3.3 Antioxidant activity: Antioxidant activity was evaluated in term of free radical scavenging activity by DPPH assay, and the result was expressed as IC_{50} , GEAC and AEAC (Table 1). Overall in all varieties, the IC_{50} obtained ranging from 0.69-2.14 mg/mL, 1.40-4.38 mg gallic acid/g, and 7.32-29.21 mg ascorbic acid/g when expressed as GEAC and AEAC, respectively. The highest antioxidant activity (IC_{50} = 0.69 mg/mL), GEAC (4.38 mg gallic acid/g) and AEAC (29.21 mg ascorbic acid/g) were observed in the acetone extract of orange variety, PG 3. PG 3, being the variety that showed the highest antioxidant activity among the five varieties studied (Table 1), was selected for further evaluation whereby the antioxidant activity and total phenolic content of stem, leaf and flower were analysed separately (Table 2). PG 3 leaves shown to have the highest total phenolic content (66.95 mg GAE/100 g), GEAC (4.92 mg gallic acid/g), AEAC (32.82 mg ascorbic acid/g) and antioxidant activity (IC_{50} = 0.61 mg/mL), compared to other part of the plants.

DPPH assay (Table 1) showed that *P. grandiflora* could act as free radical scavengers to a certain extent. However, the free radical scavenging efficiency of each species differed depending on the solvents used and the varieties studied. As suggested in previous literature⁴, polar solvents are commonly used for extraction of antioxidants, therefore generally higher antioxidant activity could be observed in varieties that were extracted with methanol and ethanol than acetone. The highest antioxidant activity was observed in the acetone extract of PG 3. This might due to the antioxidants present in PG 3 were less polar and only able to be extracted by less polar solvent like acetone. The result obtained also showed that acetone extract of PG 3 had comparable free radical scavenging activity to its close relative, *P. oleracea* (IC_{50} = 0.89 \pm 0.07 mg/mL) as reported by Lim and Quah¹⁵. Also, the variation of antioxidant activity obtained from *P. grandiflora* of different varieties and different solvent systems might be affected by types and structures of phenolic compound. It had been reported that antioxidant activity is greatly influenced by number and configuration of hydroxyl group, as well as glycosylation^{2,7}. For instance, flavonoids without hydroxyl group (e.g. isoflavone, flavanone) tend to have reduced antioxidant activity.

As shown in Table 2, leaves of PG 3 contained the highest total phenolic content and antioxidant activity compared to its stems or flowers. Similarly, the leaf samples of *P. oleracea*, a close relative of *P. grandiflora*, also reported to have higher antioxidant activity than stems²⁷. Similar findings were observed by Simpson, for peanut leaves²⁸ and Chan *et al.* for leaves of *Etilingera elatior*²⁹. It is believed that light induction could increase phenolic production in leaf, particularly flavonol synthesis in the chloroplasts and cytoplasm^{27,28,29}.

Previous studies have shown that betalains are major compounds present in *P. grandiflora*^{30,31}. Betalains are water-soluble nitrogenous (alkaloid) pigments that provide the colours in a wide variety of flowers and fruits. Betalains cannot be found in plants containing anthocyanin

pigments, and their distribution is restricted to certain families of plants³². Similar results also reported betalain pigments are responsible for the purple colour of beet root, *Beta vulgaris*, and this is the major compound contributed to high antioxidant activity in beet root^{32,33}. The production of betalains via biotechnological approach is a subject of interest due its medicinal and pharmacological activities³⁴. Using plant tissue culture technique, *P. grandiflora* can be used as an alternative source for the production of betalains in future³⁵.

The anti-viral activity and immunomodulation properties of the extract of *P. grandiflora* have been described^{12,13,14}. A clinical trial showed that oral administration of *P. grandiflora* aqueous extract did not show toxicity effects to healthy volunteers³⁶, suggesting this plant is safe to use as a source of dietary antioxidant.

Variety	*Solvent	IC ₅₀ (mg/mL)	GEAC (mg gallic acid/g)	AEAC (mg ascorbic acid/g)
PG 1	M	0.81 ± 0.24 ^a	3.87 ± 1.15 ^a	25.83 ± 7.67 ^a
	A	1.44 ± 0.23 ^{bd}	2.11 ± 0.33 ^{bd}	14.06 ± 2.21 ^{bd}
	E	0.93 ± 0.01 ^a	3.24 ± 0.02 ^a	21.62 ± 0.17 ^a
PG 2	M	1.63 ± 0.04 ^c	1.84 ± 0.05 ^c	12.27 ± 0.32 ^c
	A	1.39 ± 0.12 ^{bd}	2.17 ± 0.19 ^{bd}	14.50 ± 1.26 ^{bd}
	E	1.29 ± 0.09 ^{bd}	2.34 ± 0.17 ^{bd}	15.60 ± 1.12 ^{bd}
PG 3	M	1.22 ± 0.45 ^{bd}	2.64 ± 0.98 ^{bd}	17.60 ± 6.53 ^{bd}
	A	0.69 ± 0.02 ^a	4.38 ± 0.14 ^a	29.21 ± 0.90 ^a
	E	0.76 ± 0.08 ^a	3.99 ± 0.41 ^a	26.63 ± 2.74 ^a
PG 4	M	1.55 ± 0.10 ^{bc}	1.94 ± 0.12 ^{bc}	12.93 ± 0.83 ^{bc}
	A	1.20 ± 0.02 ^{bd}	2.51 ± 0.04 ^{bd}	16.74 ± 0.30 ^{bd}
	E	1.82 ± 0.02 ^{ce}	1.65 ± 0.02 ^{ce}	11.02 ± 0.13 ^{ce}
PG 5	M	1.54 ± 0.10 ^{bc}	1.96 ± 0.10 ^{bc}	10.22 ± 0.52 ^{bc}
	A	2.14 ± 0.10 ^c	1.40 ± 0.04 ^c	7.32 ± 0.19 ^c
	E	1.77 ± 0.02 ^{ce}	1.70 ± 0.02 ^{ce}	8.88 ± 0.11 ^{ce}

Table 1. Antioxidant activity of the whole plant of five varieties (PG 1-5) of *Portulaca grandiflora* extracted with methanol, acetone and ethanol. Values were expressed as means ± SD (n=3). Values with different letters denote the values are significantly different using one way ANOVA and Tukey HSD tests (p<0.01). Values bearing the same letter are not significantly different from each other. Statistical analyses for IC₅₀, GEAC and AEAC were performed separately. * M = Methanol, A = Acetone, E = Ethanol. PG 1-5 denotes different flower colour varieties, ranging from white, purple red, orange, red, and pink.

Plant part	TPC (mg GAE/100 g)	IC ₅₀ (mg/mL)	GEAC (mg gallic acid/g)	AEAC (mg ascorbic acid/g)
Leaves	66.95 ± 0.95 ^a	0.61 ± 0.03 ^a	4.92 ± 0.23 ^a	32.82 ± 1.52 ^a
Stems	58.79 ± 1.19 ^{bc}	1.09 ± 0.10 ^{bc}	2.76 ± 0.25 ^{bc}	18.42 ± 1.67 ^{bc}
Flowers	56.57 ± 2.81 ^c	1.31 ± 0.04 ^c	2.30 ± 0.06 ^c	15.33 ± 0.42 ^{bc}

Table 2. Total phenolic content, IC₅₀, GEAC and AEAC of different plant parts of the orange variety (PG 3) of *Portulaca grandiflora* extracted with acetone. Values were expressed as means ± SD (n=3). Values with different letters denote the values are significantly different using one way ANOVA and Tukey HSD tests (p<0.05). Values bearing the same letter are not significantly different from each other. Statistical analyses for total phenolic content, IC₅₀, GEAC and AEAC were performed separately.

4. Conclusion

For the first time, the present work provides brief insights on the total phenolic content and antioxidant activity of different colour varieties of *P. grandiflora*. This study suggests that *P. grandiflora* could be used as a source of natural antioxidant and is merit for detailed studies. In future, the antioxidant properties of *P. grandiflora* can be validated with other assays such as ABTS[•] or NO radical scavenging activities. Also, the phenolic compounds that contributed to the antioxidant activity of this plant can be characterised in future.

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