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IN-VITRO ANTIOXIDANT POTENTIAL OF *POLYGONUM CONVOLVULUS* EXTRACTS

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Abstract

The main objective of this study is to determine the antioxidant activity and total phenolic content of *Polygonum convolvulus* extracts. Three solvents consists of methanol, dichloromethane and ethylacetate have been used as solvents for extraction. DPPH assay revealed that the methanolic extract was the most effective one since its IC_{50} (0.57 mg/ml) is less than ethylacetate (IC_{50}=5.77 mg/ml) and dichloromethane (IC_{50}=17.06 mg/ml). In addition, FRAP assay showed that the most powerful antioxidant is methanolic extract which scavenged the free radical more than ethylacetate and dichloromethane, respectively.

Key words : *Polygonum convolvulus*, DPPH, FRAP, IC_{50}.

Introduction

The worldwide nearly 80% population depends on traditional medicines for primary health problems. Most of the researchers focused to investigate various traditional medicines for their scientific value (Grover and Yadav, 2004). Recently, the demand for plant antioxidants has risen remarkably for use as food additives (Herrero *et al*., 2005). The constraint in the use of synthetic antioxidants in the food industry is due to their toxicological effects (Se˜norans *et al*., 2000). Natural antioxidants are products with non-synthetic origin which prevent or retard the onset of oxidation without changing the food products quality (Crego *et al*., 2004; Iba˜nez *et al*., 2000). The oxidative environment presents a range of free radicals including superoxide, hydroxyl radical, nitric oxide and peroxynitrite, for living organisms to deal with. There are a number of concrete evidences about the role of free radicals in the development of various diseases including cancer, neurodegeneration and some inflammatory diseases; antioxidants have therefore gained importance for their capacity to neutralize free radicals (Halliwell, 2006, 2007; Ferguson, 2010). Phenolic compounds including flavonoid and phenolic acids are secondary plant metabolites that play a key role in the sensory and nutritional quality of fruits, vegetables and other plants (Ignat *et al*., 2011). Polyphenols, protect cell constituents against destructive oxidative damages, thus they limit the risk of various degenerative diseases associated with oxidative stress. Their ability to act as antioxidants is due to their chemical structure and their ability to donate or accept electrons within the aromatic structure (Rasineni *et al*., 2008; Ross and Kasum, 2002).

Polygonaceae family has several genuses, for example, Fallopia, Fagopyrum, Rheum, Rumex, Fagop, Polygonum, etc. Polygonum is a medicinal large genus of Polygonaceae, it falls into about 300 species widely distributed around the world. This genus contains variety of medicinal plants, such as *P. multiforum*, *P. cuspidatum*, *P. bistorta*, *P. aviculare*, *P.* tinctorium, etc (Wang *et al*., 2006). Most of the genus Polygonum have antioxidant and clears the body of excess free radicals (Wang *et al*., 2006). 5, 6-dihydropyranobenzopyronean and 5, 6 dihydropyranobenzopyrone isolated from *P. amplexicaule* had a strong ability to scavenge oxygen free radicals (Mudasir *et al*., 2012). Similarly, hydropiperoside B, vanicode A and vanicode E isolated from *P. hydropiper* L also exhibited antioxidant activity (Kiem *et al*., 2008).

P. convolvulus L. Black bindweed is an herbaceous annual climbing plant with a thin, spindle-formed, deep root and it is often profusely branched. The stem is slender and 2–100 inches long with long internodes. Leaves are alternate, long-petiole, elongate-ovate, pointed and heart or arrow shaped. *P. convolvulus* prefers full to partial

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sun with loamy soil, but is often found in poor soils along railways, roadsides and in old farm pastures and waste grounds (Gleason *et al*., 1991, Gleason 1963).

The objective of this study is to evaluate the antioxidant activity and total phenolic content of *P. Convonvulus* extract by DPPH free radical scavenging and FRAP method.

Plant material

The plant material was collected from Baba Aman mountains located in North Khorasan province in Iran. The plant was identified and confirmed by Natural Products and Medicinal Plants Research Centre of North Khorasan University of Medical Sciences (Iran) and Voucher specimen (No. 50-1-2) was deposited in herbarium of the Natural Products & Medicinal Plants Research Centre. The aerial parts of plant were air-dried at room temperature in the shade. The plant material was then chopped and ground to fine powder using a mechanical blender. Due to extraction kinetics of this study is controlled by the kernel particle size, a sieving step was carried out to achieve reproducible extraction yield. The samples were passed through a sieve with mesh sizes between 20 and 30 (particle diameters ranging over 0.60-0.85 mm). Finally, the dried samples were kept within a sealed bag in the cold and dry place until they were used (Hernandez *et al*., 2009).

Preparation of *Polygonum convolvulus* extracts

Three solvents including methanol, dichloromethane and ethyl acetate were added to 30 gr of dried powdered plant headed 2 cm above them. It was left to be shaked for 2 days in room temperature. The obtained extracts were isolated from dried plant and fresh solvents were added to them and left for 1 day to increase the yield of extraction. After that, the extracts were dried in reduced pressure using rotary apparatus at 40°C.

DPPH free radical scavenging assay

100 µl of each extract at various dilutions, were mixed with 100 µl of 0.16 mM DPPH methanolic solution. The mixture was vortexed for 1 min, kept for 30 min in dark and then, the absorbance was read at 517 nm in an automated microplate reader (Sunrise-Elisa Reader, Tecan, Swiss) (Zhang *et al*., 2007). The antioxidant capacity was calculated using the following equation:

$$\% \text{ Inhibition} = ((A_{blank} - A_{sample})/A_{blank}) \times 100$$

Ferric reducing antioxidant power (FRAP) assay

The ferric reducing antioxidant power (FRAP) reagent contained 10 mM TPTZ solution in 40 mM HCl, 20 mM $FeCl_3 \cdot 6H_2O$ and 0.3 M acetate buffer with pH 3.6 (1:1:10) (Xu *et al*., 2010). 3mL freshly prepared FRAP reagent mixed with 100µL of each sample (16mg/ml) was incubated at 37^0C for 10 min in a water bath. After incubation, the absorbance was measured at 593 nm. Aqueous solutions of $FeSO_4 \cdot 7H_2O$ (0–1 mM) were used for calibration. FRAP values were expressed as mean ± standard error (SE) mmol Fe (II) per gram of extract.

Total phenolic content Determination

The total phenolic content was determined by Folin-Ciocalteu method. 100 µL of three extracts (1000 mg/L) were added to 500 µL of diluted Folin-Ciocalteu reagent (1/10). After 1 min, Sodium carbonate (Na_2CO_3) (20%, 1.5 ml) was added to each tube. Tubes were vortexed and incubated for 30 min at 25°. After that, the absorbance was read at 760 nm. Finally, the standard curve was prepared using 0.03 to 0.22 mg/ml solutions of Gallic acid in methanol. The analyses were done in triplicates. Total phenolic contents were expressed as Gallic acid equivalents (mg Gallic acid: (GA) per dried weight of extract (Hayouni *et al*., 2007).

Results and Discussion

The yield of extraction varied from 3.65% to 15.69% and methanol extract had highest yield of extraction. The total phenolic content of aerial parts of methanolic, dichloromethane and ethyl acetate extracts of *P. convolvulus* was determined by Folin-Ciocalteu method. The results for total phenolic content have been reported as mg Gallic acid: (GA) per dried weight of extract (table 1).

Plants which are rich in secondary metabolites including phenols, flavonoids and carotenoids usually show

Table 1 : Total phenolic content of three solvent extracts of *P. convolvulus*.

Extract	Yield %	Total phenolic content (mgr)/per gr of dried extract	Mmol of ferric chloride/ gr of extract	IC_{50} (mg/ml)
Methanol	15.69%	31.11±0.028	81.79±0.087	0.57±0.038
Dichloro methane	3.65%	5.37±0.030	2.8±0.068	17.06±0.041
ethylacetate	4.38%	5.95±0.023	16.93±0.079	5.77±0.028
Vitamin C		—	—	0.003±0.017
BHT		—	—	0.013±0.014

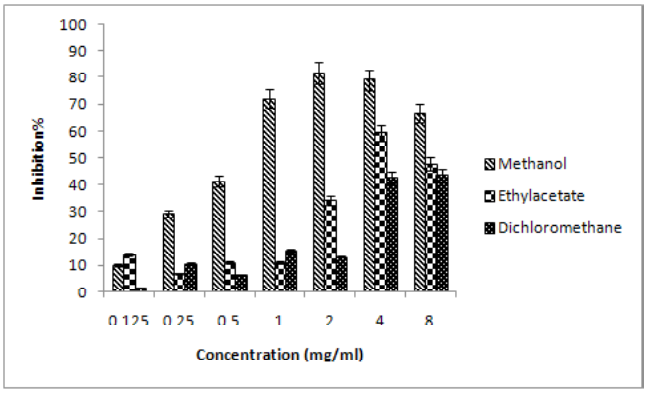

Fig. 1 : The DPPH scavenging activity of three extracts of *P. convolvulus* in different concentration.

high antioxidant activity because of their redox properties and chemical structures. The antioxidant property of the methanolic, dichloromethane and ethylacetate extracts were determined by various biochemical assays such as DPPH and FRAP assays. The methanolic extract of aerial parts demonstrated comparatively stronger antioxidant activity as compared to the ethylacetate and dichloromethane extract. The DPPH scavenging activity of three extracts with different concentration is compared in fig. 1. Metabolites which show antioxidant and reductant properties in the plant extracts can lead to the reduction of Fe^{3+} ferricyanide complex to the ferrous form (Fe^{2+}). The results showed that the methanolic extract of this plant was able to reduce the Fe^{3+} to Fe^{2+} more than two other extracts. The consequences of two antioxidant activity assay showed that methanolic extract is the most effective one and ethylacetate and dichloromethane extracts have lower antioxidant properties, respectively. The results of these two antioxidant assays have been shown in table 1. Many members of Polygonum genus have antioxidant properties and clear the body of excess free radicals; various researches have been done on different sub-genus of polygonum showed that most of them have high antioxidant activity (Hsu *et al*., 2007; Wang *et al*., 2005). In addition they almost always are rich in phenolic compounds which make them powerful antioxidants (Wang *et al*., 2005). A recent research showed that flavonones and anthraquinones of flower of *P. sachalinensis* extracts are good antioxidant compounds and those phenylpropanoid glycosides from the rhizomes exhibit β-glucosidase inhibitory activity (Kawai *et al*., 2006). While seeds and leaves of *P. tinctorium* have antioxidant and anticancer properties (Yu *et al*., 2005). 5, 6-dihydropyranobenzopyrone isolated from *P. amplexicaule* had a strong ability to scavenge oxygen free radicals (Mudasir *et al*., 2012). Similarly, hydropiperoside B, vanicode A and vanicode E isolated from *P. hydropiper* L also exhibited antioxidant activity (Kiem *et al*., 2008). *P. aviculare* L extracts strongly exhibited antioxidant effects by free radical scavenging assays. *P. minus* extracts exhibited gastro protective activities. The mechanisms were attributed to the synthesis of antioxidant (Qader *et al*., 2012). In presented study the extracts of aerial parts of *P. convolvulus* have been tested for antioxidant activity. Three solvent extracts including methanolic, dichloromethane and ethylacetate have been compared for their antioxidant activity. Methanolic extract showed the highest antioxidant activity superior to dichloromethane and ethylacetate extracts in both DPPH free radical scavenging and FRAP assays. Additionally, among these extracts the methanolic one showed the highest phenolic content, which may lead to high antioxidant activity.

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