



RESEARCH PAPER

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Phenolic compounds and antiradical properties of methanolic extracts of *Citrullus colocynthis* and *Plantago major* in Iran

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Key words: Total phenolic compounds, radical scavenging, DPPH, *Citrullus colocynthis*, *plantago major*.

<http://dx.doi.org/10.12692/ijb/4.3.224-228>

Article published on February 14, 2014

Abstract

Citrullus colocynthis and *plantago major* are popular in folk medicine in Iran. In this research Methanolic extracts of *Citrullus colocynthis* and *plantago major* from plants grown in Jiroft city were evaluated for their phenolic compounds and antioxidant antiradical. After drying the plant materials in shade, extracts were obtained by methanol 80% solvent, phenolic content was measured by Folin–Ciocalteu method and antiradical activity was measured by DPPH method. results of this study showed the extract of *plantago major* contained highest amount phenolic compounds (4.104 mg Gallic acid/1 g sample dry weight) and *Citrullus colocynthis* contained lowest amount phenolic compounds (3.696 mg galic acid/1 g sample dry weight). Extracts of *plantago major* and *Citrullus colocynthis* respectively contained lowest and highest amount of IC₅₀ (*plantago major* IC₅₀:1369.31 ppm, and *Citrullus colocynthis* IC₅₀:5538.25 ppm, Gallic acid IC₅₀:22.64). The obtained results showed that extracts of two plants of *Citrullus colocynthis* and *plantago major* possess antiradical activity and could be used as a natural antioxidant ingredient in food and drug industries.

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Introduction

Plants have been a rich source of medicines because they produce wide array of bioactive molecules, most of which probably evolved as chemical defense against predation or infection and antioxidant compounds (Cosimir and Min, 2008). Generally, dietary plants and plant products are rich sources for natural phytochemical antioxidants including vitamins) ascorbic acid, vitamin A and α -tocopherols), carotenoids and phenolic compounds (Rice-Evans *et al.*, 1997; Demmig- Adams and Adams 2002).

Plantago major L. is a perennial plant that belongs to the Plantagina'ceae family. It can become about 15 cm high, but the size varies a lot depending on the growth habitats. *P. major* is a plant that many people know only as a weed, but *P. major* is also an old medicinal plant that has been known for centuries. In Scandinavia this plant is mostly known for its wound healing properties. Antioxidant capacity by bleaching of the absorbance of pre-formed 2,2%-azinobis (3-ethylbenzthiazolinesulfonic acid) radical cation in the presence of infusions made from *P. major* herbal tea bags and *P. major* leaves were determined. The infusion of *P. major* tea contained small amounts of free radical scavengers compared to black tea. The antioxidant capacity of the green leaves was higher than that of the *P. major* tea indicating that processing can lead to significant loss of activity (Campos and Lissi, 1995).

Citrullus colocynthis (Cucurbitaceae), locally known as Sherry or Handal, is used in folk medicine in rural areas as a purgative, ant rheumatic, and a remedy for skin infections. This plant contains cucurbitacins A, B, C, and D, α -elaterin, and probably other constituents. The fruits of *Citrullus colocynthis*, commonly known as bitter apple, are bitter, acrid, cooling, cathartic, carminative, antipyretic, antihelmintic and are useful in hypoglycemia, tumors, ascites, leucoderma, ulcers, asthma, bronchitis, urethrorrhea, jaundice, dyspepsia, constipation, elephantiasis and splenomegaly (Delazar *et al.*, 2006). Tannin-Spitz *et al.* (2007) studied the antioxidant

properties of cucurbitacin B + E glucosides (from *Citrullus colocynthis*) and their direct free-radical scavenging properties, using ESR spectroscopy. Their results showed that cucurbitacin B + E glucosides exhibits antioxidant properties, probably through the involvement of a direct scavenging effect on several free radicals. Additionally, several studies indicated that different species of *Citrullus colocynthis* and *plantago major* exhibit the antioxidant and antiradical effects. Since no data exists on polyphenol content and antiradical activity of *Citrullus colocynthis* and *plantago major* from Jiroft city, present study was conducted in order evaluation of the potential antioxidant activity and also estimation of the phenolic content of these plants.

Materials and methods

Chemicals and reagents

DPPH, Gallic acid, Folin-Ciocalteu's phenol reagent, methanol, sodium carbonate were purchased from Merck (Darmstadt, Germany); all chemicals were of reagent grade.

Plant material

The plants, *Citrullus colocynthis* and *plantago major* were collected from Jiroft local area at range in spring and summer.

Plants material extraction

The aerial parts of plant were collected, shade dried for seven days and ground. The dried powder of *Citrullus colocynthis* and *plantago major* (1g) was soaked in 10 ml methanol-acetic acid (85:15 v/v). Extraction carried out at freezer for 24 h then, samples were transferred to ultrasonic system for 15 min. Each extract was centrifuged (1000rpm at 0° C) and above liquid was separated for later experiments (Elizabeth & Kelly, 2007)

Estimation of total phenolics

Total phenolic content of each extract was determined by the Folin-Ciocalteu micro-method (Slinkard and Singleton, 1977). Briefly, 20 μ l of extract solution were mixed with 1.16 ml distilled water and 100 μ l of Folin-Ciocalteu reagent, followed by addition of 300

μl of Na_2CO_3 solution (20%) after 1 min and before 8 min. Subsequently, the mixture was incubated in a shaking incubator at 40°C for 30 min and its absorbance was measured at 760 nm. Gallic acid was used as a standard for calibration curve. The phenolic content was expressed as Gallic acid equivalents using the following linear equation based on the calibration curve:

$$A = 0.98 C + 9.321 \times 0.001 \quad R^2 = 0.9965$$

Where A is the absorbance and C is concentration as Gallic acid equivalents (mg/g).

DPPH radical scavenging activity

The ability of extracts to scavenge DPPH radicals was determined according to the method of Blois (1958). Briefly, 1 ml of a 1 mM methanol solution of DPPH was mixed with 3 ml of extract solution in methanol (containing 50–400 μg of dried extract). The mixture was then vortexed vigorously and left for 30 min at room temperature in the dark place. The absorbance was measured at 517 nm and activity was expressed as percentage DPPH scavenging relative to control using the following equation:

$$\text{DPPH scavenging activity (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

Gallic acid was used as the free radical scavenger reference compound.

Statistical analysis

All these experiments were replicated three times, and the average values are reported. The effect of varieties on antioxidant activity of two plants were determined using the analysis of variance (ANOVA) method, and significant differences of means were compared using Duncan's test at 5% significant level using the SAS software (2001) program.

Result and discussion

Total phenol compound (TPC)

The averages of total phenolic compound of *Citrullus colocynthis* and *plantago major* based on Folin-Ciocalteu method was shown in table 1. As can be seen from table 1 two plants do not have significant differences ($P < 0.05$) in total phenolic content. Among studied plants, *plantago major* contained the highest amount of total phenolic, followed by *Citrullus colocynthis*.

Table 1. Total Phenol compound of *Citrullus colocynthis* and *plantago major*.

Total phenolic)mg/g(compound	sample	Row
3.696	<i>Citrullus colocynthis</i>	1
4.104	<i>plantago major</i>	2

DPPH radical scavenging activity

The results of DPPH radical scavenging activity of extracts of two plants were shown in figures 1 and 2. Free radicals which are involved in the process of lipid per-oxidation are considered to play a major role in numerous chronic pathologies, such as cancer and cardiovascular diseases among others (Dorman *et al.*, 2003). The DPPH radical has been widely used to evaluate the free radicals scavenging ability of various natural products and has been accepted as a model compound for free radicals originating in lipids (Porto *et al.*, 2000).

Citrullus colocynthis ($\text{IC}_{50} = 5538.25$) extracts showed that this plant didn't have significant antiradical activity and there were high differences between IC_{50} of this plant extracts and Gallic acid standard ($\text{IC}_{50} = 22.64$). Against our results, Maheshwar Reddy *et al.* (2010) didn't observe any antiradical activity in *Citrullus colocynthis*. These differences can be due to various factors such as variety, growing condition, maturity, season, and geographic origin between the two countries, fertilizers, soil type, amount of sunlight received and experimental conditions (storage, extraction).

The results of DPPH radical scavenging activity of

As can be seen from figure 2, *plantago major* extracts

exhibited potential antioxidant activity. The methanol extract of *plantago major* scavenged 50% DPPH free radical at the lowest inhibitory concentration (IC₅₀: 1369.31µg/ml) that showed significant difference with Gallic acid standard (IC₅₀= 22.64).

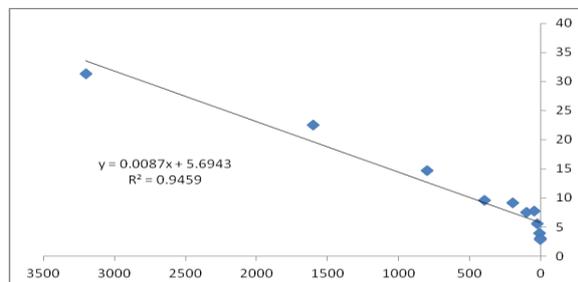


Fig. 1. Standard curve of gallic acid in *Citrullus colocynthis* (IC₅₀= 5538.25).

Phenolic compounds are widely distributed in plants which have been reported to exert multiple biological effects, including antioxidant, free radical scavenging abilities, anti-inflammatory, anticarcinogenic, etc (Gao *et al.*, 2000). As it can be seen *plantago major* that contained the highest amount of total phenol, was found to be the most active radical scavenger followed by *Citrullus colocynthis*. A high correlation between free radical scavenging and the phenolic contents has been reported for fruits (Arabshahi Delouee and Urooj, 2006; Jimenez-Escrig, 2001; Gao *et al.*, 2000; Benzie and Szeto, 1999). So less Antioxidant activity may be due to less phenolic compounds in *Citrullus colocynthis* fruit but further work should be done on the isolation and identification of other antioxidant components of *Citrullus colocynthis*.

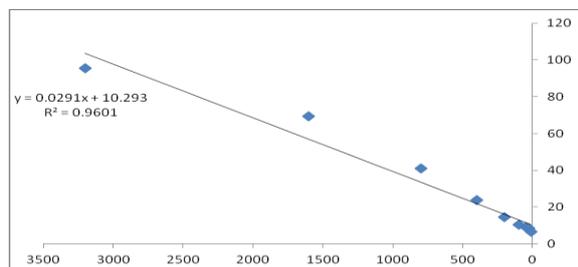


Fig. 2. Standard curve of gallic acid in *plantago major* (IC₅₀= 1369.31).

Conclusion

The phenolic compounds and antioxidant activities (AA) of *Citrullus colocynthis* and *plantago major* was determined and presented in this paper. Among two

selected plants, *plantago major* contained the higher amount of phenolic compounds and also exhibited the stronger antiradical activity than *Citrullus colocynthis*. There is a great promise for development of novel drugs and antiradical compounds from two plants of *Citrullus colocynthis* and *plantago major* for dreadful human diseases and oxidation prevention.

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