Antioxidant properties and Flavonoids-Phenolic content of citrullus colocynthis (L.) schrad growing in Khuzestan, Iran

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Original Article

Abstract

Introduction: Free radicals cause several human diseases. Antioxidants decrease the risk of cardiovascular diseases and heart attack, and prevent the development of cancers through neutralization of free radicals. This study set out to determine the antioxidative properties of Citrullus colocynthis growing in Khuzestan. This plant is traditionally used for the treatment of type II diabetes.

Methods: In this study, 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and beta-carotene linoleic acid assays were used for the evaluation of antioxidative properties. In addition, the measurement of flavonoids and phenolic compounds was done based on the Gallic acid and quercetin reagents.

Results: In the DPPH assay, the maximum and minimum scavenging activity of free radicals were found in the extractions of stem (IC_{50}=12.4µg/ml) and leaves (IC_{50}=5.46 µg/ml), respectively. With respect to the beta-carotene linoleic assay, the maximum and minimum antioxidative activities were observed in the extractions of leaves (79.78%) and fruits (47.08%), respectively. Moreover, the maximum content of flavonoids and phenolic compound were 22.15µg/ml in stem, and 4.09µg/ml in fruit.

Conclusion: The methanol extracts from different organs of citrullus colocynthis (C. colocynthis) showed antioxidative activities at different levels. Therefore, they can be used as antioxidants in food industries and adjuvant treatments.

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Introduction:

A free radical is an atom or molecule that has unpaired valence electrons. It is capable of inflicting irreversible damages to the molecules in biologic systems such as nucleic acids, carbohydrates, and connective tissue macromolecules. Fortunately, during the evolution of biological systems, nature protected living creatures against the potentially harmful effects of free radicals through the design and fabrication of antioxidants. In natural conditions, there is usually a balance between the production of free radicals on the one hand and antioxidative defense system on the other hand. In case of overproduction of free radicals or weakness

of antioxidative defense system, this balance is disturbed, causing an oxidative stress (1).

Citrullus colocynthis with many common names including "bitter cucumber," "wild gourd," is a Cucurbitales plant species in the family Cucurbitaceae, which grows [in the interdunal flats] between the dunes. *C. colocynthis* is an ivy-like herbaceous perennial vine with smooth twinning stems, yellow flowers, alternate leaves and toothed lobes up to 5-10 cm long and 3-7 cm width. Each plant yields 15-30 fruits up to 7-10cm thickness. Almost 75% of the plant weight is because of its seeds (5).

The main compounds of C. colocynthis pulp are "Colocynthin," and "pectin," which have bitter taste. The oil and albumin contents of the seeds are more than 17% and 6%, respectively (5). A study on the effect of C. colocynthis on blood glucose of a diabetic rabbit showed that the extracts from the pulp and seeds reduced serum glucose level (7). Another study was conducted on the effects of different concentrations of powdered fruit of this plant on some biochemical factors of blood among diabetic male rats, revealing it reducing effect on blood sugar, cholesterol, and triglyceride levels [4]. It is a plant with useful properties and traditional application (as medicine). The antioxidative properties of this plant have been less addressed in Iran. As a result, this experimental study was conducted to investigate and compare antioxidative effects of the extracts from leaves, stems, and fruits of *C. colocynthis* through the measurement of its flavonoids and phenolic content and capability to detoxifying free radicals.

Methods:

First, a plant specimen of C. colocynthis was collected, after fruiting stage, from Chah Salem Plains in Omidiyeh City, Khuzestan Province, Iran. The plant was then recognized by the Research Center of Agricultural and Natural Resources.

Materials: In this study, substances like quercetin, folin-ciocaltue, beta-carotene, linoleic acid, methanol, tween 40, Gallic acid, aluminum chloride, sodium carbonate, potassium acetate, chloroform (Merck, and DPPH (Sigma) were used.

A. Preparation of methanol extract: First, 40 g leaf, stem, and fruit of the plant were soaked in 200 mL methanol and then underwent the extraction process for 48 hours. The obtained extract was filtered with a filter paper #1, and concentrated by the rotary device at 50°C.

B. Measurement of phenolic compounds: To this end, phenol content was measured through a method, in which Folin-ciocaltue was used as reagent and Gallic acid as standard. First, 0.02% methanol concentration of leaf, fruit, and stem was prepared. Then, three test tubes were provided for each specimen. After that, 100 µL of the specimen extract was diluted by 1.500µL Folin-ciocalteu (0.1%) and 1.000µL distilled water in each tube. After remaining at room temperature for 1 minute, 1.500µL sodium carbonate (20%) was added, and the solution was left in dark at room temperature for 2 hours. Finally, the absorbance of the solution was measured at 760nm, using a spectrophotometer (Jenway 6405, England). The same method was used for all standard Gallic acid solutions and preparation of the standard curve (3).

C. Measurement of flavonoids compounds: First, 0.02% methanol concentration was prepared from leaf, fruit, and stem. Then, three test tubes were provided for each specimen. To each tube, 0.5mL of the prepared extracts was diluted by 1.5mL methanol (0.1%) and aluminum chloride (10%), as well as 1 M potassium acetate (0.1 mL) and 2.8mL distilled water. After remaining at room temperature for 1 hour, the absorbance of the solution was measured at 760nm using a spectrophotometer (Jenway 6405, England). The same method was used for all standard quercetin solutions and preparation of the standard curve.

D. Antioxidative activity: The antioxidative activity was assessed through following methods.

i. DPPH: in this method, 50µL of the prepared extracts (0.02%) was mixed with methanol solution (3 mL) containing DPPH radicals (0.004%,

w/w). After 30 minutes, the absorbance of the specimens was measured at 517nm, using the spectrophotometer (Jenway 6405, England). The inhibition of free radicals was measured through following equation:

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

Where, A_{blank} is the absorbance of the control reaction (including all reagents, except the defined concentration of the given extract). The IC_{50} represents the concentration of the extracts that causes 50% inhibition of the radical (3).

ii. β-carotene/linoleic acid: In this method, the antioxidative potential of the extracts was measured through plotting decolorization of the β-carotene/linoleic acid assay. To prepare the β-carotene/linoleic acid solution, 1.5 mgβ-carotene was mixed with 3mL chloroform, and then 75μL linoleic and 600mg Tween-40 were added. The chloroform was completely evaporated using a vacuum evaporator. In the next stage, 100mL oxygen-saturated distilled water was added and the container was vigorously shaken. Then, 4.500μL reaction mixture and 1 mL of the obtained extracts (0.02%) were added to the test tube. The absorbance of the specimens was measured at 490nm immediately after and 24-hour after the preparation of the test tubes, using the pectrophotometer (Jenway 6405, England). The antioxidative capacity of the extract was compared with positive and negative case and control specimens, and presented in percentage (3).

Statistical analysis: Results were expressed as mean±standard deviation. Statistical significance and between-groups difference were evaluated using MiniTab and Dunnett.

Results:

Evaluation of DPPH: The activities of hydrogen and oxygen atoms of the extracts, as well as some pure compounds were measured through plotting the decolorization of DPPH purple color of methanol extracts. This method uses the pectrophotometery of DPDH free radicals as the reagent. According to Table 1, DPPH free radical is a free radical with the central atom of nitrogen, which undergoes a color change from purple to yellow after reduction. The inhibition concentration (50%) in the methanol extract of fruit, leaf, and stem was 5.04±0.17, 5.46±0.57, and 5.46±0.57 μg/mL, respectively (P<0.001); whereas, IC_{50} for

Table 1. Results from antioxidative effects of methanol extract of *C. colocynthis*

Methodology Specimen	IC_{50} (μg/ml)	β -Carotene/linoleic acid (% inhibition rate)
Methanol extract of fruit	5.04±0.17c	47.08±5.26
Methanol extract of leaf	5.46±0.57c	79.78±4.77D
Methanol extract of stem	4.12±0.4b	54.13±8.38c
BHT (control)	4.12±0.4b	32±1.46A

Figures are in form of mean±standard deviation with three repetitions; Means with completely distinctive letters have significant difference at probability level of 0.001.

Table 2. Results from antioxidative effects of methanol extract of *C. colocynthis*

Different extract of fruit	Flavonoids compounds(μg/ml)	Phenolic compounds (μg/ml)
Methanol extract of fruit	5.04±0.17c	47.08±5.26
Methanol extract of leaf	5.46±0.57c	79.78±4.77D
Methanol extract of stem	4.12±0.4b	54.13±8.38c
BHT (control)	4.12±0.4b	32±1.46A

Figures are in form of mean±standard deviation with three repetitions; Means with completely distinctive letters have significant difference at probability level of 0.001.

BHT was 12.4±0.4 μ/mL (P<0.001). These findings suggest that the methanol extracts of *C. colocynthis* have significant antioxidative potentials, and are capable of reducing DPPH free radicals. According to Diagram 1, the methanol extract of C. colocynthis from stem is more efficient than that from fruit in terms of DPPH scavenging activity; however, the antioxidative activity of the extracts are, in total, weaker than the synthetic antioxidative activity of butylated hydroxytoluene (BHT).

Evaluation of β-carotene linoleic: According to Table 1, the inhabitation abilities of the methanol extracts from fruit, leaf, and stem were 47.08±5.26, 79.78±4.77, and 54.13±8.38m

respectively; whereas, it was 32±1.46 for the BHT (P<0.001). Results of this study showed that the antioxidative activity of methanol extracts was higher in the experimental organs as compared to the control organs (Chart 1).

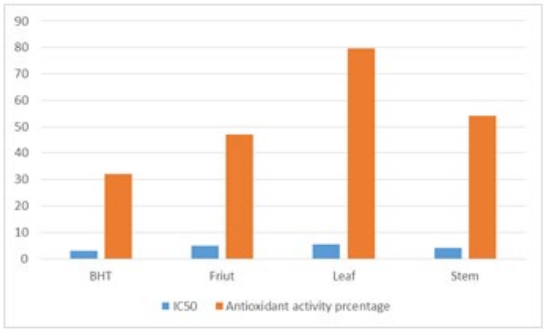

Figure 1. IC_{50} and antioxidant activity from DPPH and β-caroten-linoleic acid assays methods

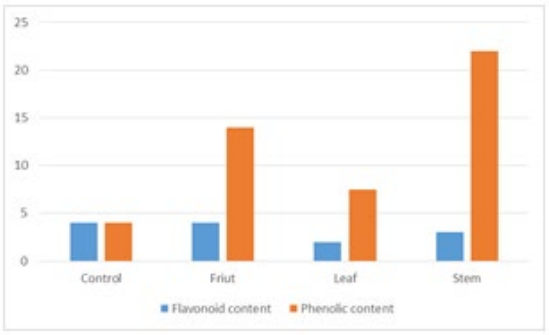

Figure 2. Flavonoid and phenolic contents of different extracts of C.colocynthis

Evaluation of Flavonoids and Phenolic Compounds: the Dunnett test (Diagram 2) shows a significant difference in the content of flavonoids and phenolic compounds for different parts of C.colocynthis (at significance level of 0.001). The means and standard deviations of flavonoids and phenolic compounds for different parts of *C. colocynthis* are presented in Table 2. In this study, the maximum contents of flavonoids and phenolic compounds were observed in fruits and stems, respectively. In addition, the flavonoids contents of the ethanol extracts of fruit, leaf, and stem were 4.09±0.11, 1.88±0.7, and 3.09±0.11µg/mL, respectively (P<0.001). The contents of phenolic compounds of methanol extracts from fruit, leaf, and stem were 14.37±0.26, 7.69±0.36, and 22.15±1.86µg/mL, respectively. Difference between them is significant at the probability level of 0.001 (P<0.001).

Conclusion:

This study investigated the antioxidative traits of *C. colocynthis* growing in Khuzestan. Although, there is no study on antioxidative trait, and flavonoids and phenolic contents of this plant in Iran, there are some separate studies in neighboring countries (e.g. Turkey) that have been mostly conducted on the root and fruit of this plant. This study set out to concurrently measure flavonoids and phenolic contents, and antioxidative properties of some of the main organs of *C. colocynthis*. Moreover, results from different studies show differences in flavonoids and phenolic contents, as well as antioxidative properties of different parts of this plant.

Fenina et al. in a study on the antioxidative and free radical reduction properties of *C. colocynthis* observed that the extraction from seeds had the highest free radical reduction capability than the extracts of other parts (stem, root, and fruit); whereas, in the present study, the stem extract with IC_{50}=4.12µg/mL had the highest capability in free radical inhibition (8).

Gurudeenban et al. showed that the fruit of *C. colocynthis* contained the highest contents of tannins and flavonoids. This finding was also observed in our study with flavonoid content of 4.09µg/mL.

These compounds cause the eradication of free radical and may be regarded as a pharmaceutical supplement. Abdullah et al. conducted a study in Pakistan on antioxidative activity and flavonoids contents of different extracts of *C. colocynthis*, and observed that the contents of total phenol (3.07-18.6 mg/g) and total flavonoid (0.51-13.9 mg/g) were maximum in ethanol extracts from the root, leaf, and fruit. Moreover, the free radicals inhibition rates in ethanol extracts of root and leaf were 56.8-67.2% and 5.97-6.42 µg/mL. Among the ethanol extracts, leaf had the highest free radicals inhibition activity (IC_{50}=2.97, 67.2%). The IC_{50} in hexane extracts from the root, leaf, and fruit was 16.7, 7.25, and 15.9 µg/mL, respectively. Therefore, in above study, the hexane extract from root showed the highest free radicals inhibitory activity; whereas, the present study found that the extract from stem

(IC50=4.12 µg/ml) had the highest free radicals inhibitory activity (2).

Other studies showed that the fruit of *C. colocynthis* had antibacterial, antidiabetic, and anticancer traits. This plant was also used as traditional medicine to treat constipation and for abortion (6).

Vashishta et al. conducted a study on the antioxidative and free radicals inhibition activities of the methanol extract of *C. colocynthis*. They found that there existed a great content of flavonoids 13 m/m%) and phenolic (74 m/m%) compounds. This finding is consistent with the present study (9,10).

Concerning the findings of this and other studies, *C. colocynthis* is highly capable of reducing free radicals and thus can be used as a suitable antioxidant.

Among the complications of this plant is diarrhea, abortion caused by having direct skin contact, and even death (at high dosage).

Findings of this study suggest that *C. colocynthis* growing in Khuzestan Province, Iran, has a significant antioxidative ability. In addition, it is used in some areas of this province as a traditional medicine to reduce or treat diabetes, which may be due to its antioxidative effects. Therefore, performing further studies on the identification of its compounds and usage for treating diseases with oxidative stress (diabetes, cardiovascular disease, and cancer in lab animal is significant) is very important.

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