

## Antioxidant Activities of Iranian Corn Silk

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**Abstract:** Traditionally corn silk (CS) has been used as diuretic, antilithiasic, uricosuric, and antiseptic. It is used for the treatment of edema as well as for cystitis, gout, kidney stones, nephritis, and prostatitis. In the present study, the antioxidant properties of ethanol-water extract from CS were estimated by different methods. Also phenol and flavonoid content of the extract were measured by Folin Ciocalteu and  $AlCl_3$  assays. CS extract contained a significant amount of phenol and flavonoids. The percentage of DPPH radical scavenged by CS extract was 92.6 at a concentration of  $1.6 \text{ mg ml}^{-1}$ .  $IC_{50}$  of the extract and the standard compounds butylated hydroxytoluene (BHA) and quercetin was 0.59, 0.053, and  $0.025 \text{ mg ml}^{-1}$ , respectively. Iron chelating activity of the extract was less than the standard compounds. CS extract showed nitric oxide-scavenging effect less than the reference agent (quercetin). The extract showed a high reducing ability. According to ferric thiocyanate (FTC) method, the extract showed more than 88% inhibition of linoleic acid peroxidation. It might be concluded that some of the properties of CS in traditional medicine is due to its antioxidant ability.

**Key Words:** Antioxidant, corn silk

### İran Mısır Püskülünün Antioksidant Aktivitesi

**Özet:** Mısır püskülü (MP) diüretik, antilithiasik, urikosurik ve antiseptik şeklinde geleneksel olarak kullanılmaktadır. Ödemin tedavisinde olduğu kadar sistitis, gout, böbrek taşları, nefritis ve prostat gibi hastalıklarda da kullanılır. MS'nin etanol-su ekstresinin antioksidant özellikleri farklı yöntemlerle çalışılmıştır. İlave olarak fenol ve flavonit muhtevası Folin Ciocalteu ve  $AlCl_3$  yöntemi ile çalışılmıştır. MP önemli miktarda fenol ve flavonit ihtiva eder. MP özütünün DPPH radikal süpürücü etkisi  $1.6 \text{ mg ml}^{-1}$  de 92.6, özütün, bütüle hidroksi toluen ve quersetin  $IC_{50}$  değeri 0.59, 0.053 ve  $0.025 \text{ mg ml}^{-1}$  bulunmuştur. Demir şelatlama aktivitesi standart maddeden daha azdır. MP özütü referansdan (quersetin) daha az nitrik oksit süpürücü etkiye sahiptir. Özüt yüksek indirgeme yeteneğine sahiptir. Ferrik tiosiyanat yöntemine göre linoleik asit peroksidasyonunda % 86 inhibisyona neden olur. MP nin geleneksel tıp alanında kullanılmasının nedeni antioksidant yeteneği olabilir.

**Anahtar Sözcükler:** Antioksidant, mısır püskülü

### Introduction

The potentially reactive derivatives of oxygen, attributed as reactive oxygen species (ROS), are continuously generated inside the human body as a result of contact with excess of exogenous chemicals in our ambient environment and/or due to a number of endogenous metabolic processes involving redox enzymes. Under normal circumstances, the ROS generated are detoxified by the antioxidants present in the body and there is equilibrium between the ROS generated and the antioxidants present. However, owing to ROS overproduction and/or inadequate antioxidant defense, this equilibrium is interfered favoring the ROS upsurge that terminates in oxidative stress. The ROS

easily affect and persuade oxidative damage to various biomolecules including proteins, lipids, lipoproteins, and DNA (1). This oxidative damage is a critical etiological factor implicated in several chronic human diseases such as diabetes mellitus, cancer, atherosclerosis, arthritis, and neurodegenerative diseases and also in the ageing process. Based on the growing interest in free radical biology and the lack of effective therapies for most chronic diseases, the usefulness of antioxidants in protection against these diseases is supported. Epidemiological studies have found that the intake of antioxidants, such as Vitamin C, reduce the risk of coronary heart disease and cancer (2). The antioxidants may mediate their effect by directly reacting with ROS,

quenching them and/or chelating the catalytic metal ions (3). Several synthetic antioxidants, such as butylated hydroxyanisole and butylated hydroxytoluene, are commercially available but are quite unsafe and their toxicity is a problem of concern. Natural antioxidants, especially phenolics and flavonoids, are safe and also bioactive. Therefore, in recent years, considerable attention has been directed towards the identification of plants with antioxidant ability that may be used for human consumption. Diuretic, as well as antilithiasic, uricosuric, and antiseptic, properties are traditionally attributed to CS, stigma/style of *Zea mays* Linne (Poaceae/Gramineae), which has been used in many parts of the world for the treatment of edema as well as for cystitis, gout, kidney stones, nephritis, and prostatitis (4, 5, 6, 7, 8). CS contains proteins, vitamins, carbohydrates,  $\text{Ca}^{2+}$ ,  $\text{K}^+$ ,  $\text{Mg}^{2+}$  and  $\text{Na}^+$  salts, volatile oils, and steroids such as sitosterol and stigmasterol, alkaloids, saponins, tannins, and flavonoids (5, 7, 8). Phenolic compounds present in CS are anthocyanins, p-coumaric acid, vanillic acid, protocatechuic acid, derivatives of hesperidin and quercetin, and bound hydroxycinnamic acid forms composed of p-coumaric and ferulic acid (10). There are also reports about antioxidant activity of CS (11,12). The constituents in the volatile extract and petroleum ether, ethanol, and water extract of CS exhibited clear antioxidant activities (13). There are not enough records about antioxidant activity of CS extracts by different antioxidant assay methods. In Iran, CS is used as a traditional remedy for several maladies. In this study we carried out an antioxidant survey by different methods to present a reason for the use of CS in herbal medicine.

## Materials and Methods

### Chemicals

Gallic acid, DPPH, quercetin, BHA, BHT, Vitamin C, and EDTA were purchased from Merck and Fluka companies. All other chemicals and reagents used were of the highest commercially available purity.

### Plant material

CS (dried cut stigmata of *Zea mays* L, *Poaceae* female flowers) used for this investigation was collected in January 2006 and authenticated by P. Hojjat. A voucher specimen, number 280, was deposited at the herbarium section of the faculty. CS was dried at room temperature and an ethanol-water (1:1) extraction was performed

using maceration method by soaking in the solvent mixture (14). The extract was collected after removing the solvent and lyophilization.

### Total flavonoid determination

Colorimetric aluminum chloride method was used for flavonoid determination (15). CS extract (0.5 ml of 1:10  $\text{g ml}^{-1}$ ) in methanol was separately mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 M potassium acetate, and 2.8 ml of distilled water. The extract remained at room temperature for 30 min; the absorbance of the reaction mixture was measured at 415 nm with a double beam Perkin Elmer UV/Visible spectrophotometer (USA). The calibration curve was prepared by preparing quercetin solutions at concentrations 12.5 to 100  $\text{mg ml}^{-1}$  in methanol.

### Total phenol determination

Total phenol content was determined by Folin Ciocalteu reagent (16). A dilute solution of CS extract (0.5 ml of 1:10  $\text{g ml}^{-1}$ ) or gallic acid (standard phenolic compound) was mixed with Folin Ciocalteu reagent (5 ml, 1:10 diluted with distilled water) and aqueous  $\text{Na}_2\text{CO}_3$  (4 ml, 1 M). The mixture was allowed to stand for 15 min and the phenols were determined by colorimetry at 765 nm. The standard curve was prepared by 0, 50, 100, 150, 200, and 250  $\text{mg ml}^{-1}$  solutions of gallic acid in methanol:water (50:50, v/v). Total phenol values are expressed in terms of gallic acid equivalent ( $\text{mg g}^{-1}$  of dry mass), which is a common reference compound.

### DPPH assay

The stable 1, 1-diphenyl-2-picryl hydrazyl radical (DPPH) was used for determination of free radical-scavenging activity of the extract (17). Different concentrations of CS extract were added, at an equal volume, to methanolic solution of DPPH (0.15 mM). After 15 min at room temperature, the absorbance was recorded at 517 nm. The experiment was repeated 3 times. BHT, Vitamin C, and quercetin were used as standard controls.  $\text{IC}_{50}$  value denotes the concentration of a sample, which is required to scavenge 50% of DPPH free radicals.

### Metal chelating activity

The chelation of ferrous ions by CS extract was estimated by the method of Dinis et al. (18). Briefly, 50  $\mu\text{l}$  of 2 mM  $\text{FeCl}_2$  was added to 1 ml of different concentrations of the extract (0.2, 0.4, 0.8, 1.6, and 3.2

mg ml<sup>-1</sup>). The reaction was initiated by the addition of 0.2 ml of 5 mM ferrozine solution. The mixture was vigorously shaken and left to stand at room temperature for 10 min. The absorbance of the solution was thereafter measured at 562 nm. The percentage inhibition of ferrozine-Fe<sup>2+</sup> complex formation was calculated as  $[(A_0 - A_s) / A_s] \times 100$ , where A<sub>0</sub> is the absorbance of the control and A<sub>s</sub> is the absorbance of the extract/standard. Na<sub>2</sub>EDTA was used as positive control.

#### Assay of nitric oxide-scavenging activity

The procedure was performed based on the method by Sreejayan & Rao (19). Sodium nitroprusside (10 mM), in phosphate-buffered saline, was mixed with different concentrations of CS extract dissolved in water and incubated at room temperature for 150 min. Griess reagent (0.5 ml), containing 1% sulfanilamide, 2% H<sub>3</sub>PO<sub>4</sub> and 0.1% N-(1-naphthyl) ethylenediamine dihydrochloride, was added to the mixture after incubation time. The absorbance of the chromophore formed was read at 546 nm. Quercetin and the same mixture of the reaction without CS extract were employed as positive and negative control.

#### Reducing power determination

Fe (III) reduction is often used as an indicator of electron-donating activity, which is an important mechanism of phenolic antioxidant action (20). The reducing power of CS was determined according to the method of Yen and Chen (21). Different amounts of the extract (0.025-0.4 µg/ml) in water were mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide [K<sub>3</sub>Fe(CN)<sub>6</sub>] (2.5 ml, 1%). The mixture was incubated at 50 °C for 20 min. A portion (2.5 ml) of trichloroacetic acid (10%) was added to the mixture to stop the reaction, which was then centrifuged at 3000 rpm for 10 min. The upper layer of the solution (2.5 ml) was mixed with distilled water (2.5 ml) and FeCl<sub>3</sub> (0.5 ml, 0.1%), and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power. Vitamin C was used as positive control.

#### Antioxidant Activity by ferric thiocyanate (FTC) method

The FTC method was adopted from Osawa and Namiki (22). Two ml of 0.4 mg ml<sup>-1</sup> CS extract was mixed with 2.88 ml of linoleic acid (2.51%, v/v in 4 ml of

99.5% (w/v) ethanol), 0.05 M phosphate buffer pH 7.0 (8 ml), and distilled water (3.9 ml) and incubated at 40 °C for 96 h. To 0.1 ml of this solution, 9.7 ml of 75% (v/v) ethanol and 0.1 ml of 30% (w/v) ammonium thiocyanate were then added. Precisely 3 min after the addition of 0.1 ml of 20 mM ferrous chloride in 3.5% (v/v) hydrochloric acid to the reaction mixture, the absorbance at 500 nm of the resulting red solution was measured, and it was recorded again every 24 h until the day when the absorbance of the control reached the maximum value. The percent inhibition of linoleic acid peroxidation was calculated as: (%) inhibition = 100 - [(absorbance increase of the sample / absorbance increase of the control) × 100]. All tests were run in duplicate and analyses of all samples were run in triplicate and averaged. Vitamin C and BHA were used as positive control.

#### Statistical analyses

All values are expressed as mean ± S.E. Statistical analyses were performed by Student's t-test. The values of P lower than 0.05 were considered statistically significant.

## Results and Discussion

#### Flavonoid and total phenol content of the extracts

It has been recognized that flavonoids show antioxidant activity and their effects on human nutrition and health are considerable. The mechanisms of the actions of flavonoids are through scavenging or chelating processes (23, 24). Phenolic compounds are a class of antioxidant agents acting as free radical terminators (25). The flavonoid content of the extract in terms of quercetin equivalent was 58.22 ± 1.34 mg g<sup>-1</sup>. The total phenol content was measured by Folin Ciocalteu reagent in terms of gallic acid equivalent. The total obtained phenol was 118.94 ± 2.78 mg g<sup>-1</sup>. The amount of polyphenolic compounds in CS different extracts is dependent on its origin. In comparison with previous reports, polyphenolic content of CS extract was observable in our study (26, 27). The compounds, such as flavonoids, which contain hydroxyl groups, are responsible for the radical scavenging effect in the plants (28, 29). According to our study, the contents of these phytochemicals in CS extract can explain its antioxidant activity.

### Antioxidant activity

The stable free radical DPPH method is an easy, rapid, and sensitive way to survey the antioxidant activity of a specific compound or plant extracts (17). The results of previous studies with different antioxidant assay on CS acetone-water extract suggest that polyphenol content should be considered as an important feature of CS, as some of its effects, such as antioxidant activity, could be attributed to the presence of these constituents (27). The capacity of CS extract to scavenge DPPH was measured and the results are shown in Figure 1. The antioxidants react with DPPH, a purple colored stable free radical, and convert it into a colorless  $\alpha$ - $\alpha$ -diphenyl- $\beta$ -picrylhydrazine. The amount of reduced DPPH could be quantified by measuring the decrease in absorbance at 517 nm. CS extract reduced DPPH radicals in a dose dependent manner.  $IC_{50}$  of the standard compounds, BHA, Vitamin C, and quercetin were  $0.05 \mu\text{g ml}^{-1}$ ,  $0.005 \mu\text{g ml}^{-1}$ , and  $0.005 \mu\text{g ml}^{-1}$ , respectively (Figure 1). As can be seen in Figure 2, the extract at  $800 \text{ mg ml}^{-1}$  scavenged

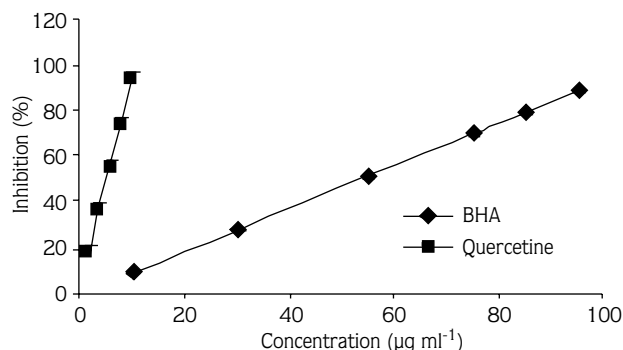


Figure 1. Free radical scavenging activity of BHA and Quercetine as reference compounds.  $IC_{50}$  of BHA and Quercetine was  $0.05 \text{ mg ml}^{-1}$  and  $0.005 \text{ mg ml}^{-1}$ , respectively. Each value is presented as mean  $\pm$  S.E. ( $n = 5$ ).

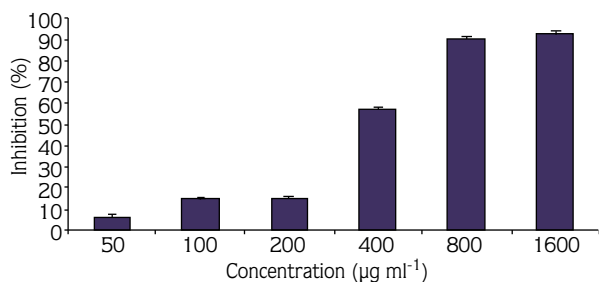


Figure 2. Free radical scavenging activity of different concentrations of CS ethanol-water extract with  $IC_{50} = 590 \text{ mg ml}^{-1}$ . Each value is presented as mean  $\pm$  S.E. ( $n = 5$ ).

about 91% of DPPH radicals and had an  $IC_{50}$  value of  $590 \text{ mg ml}^{-1}$ . So the extract showed less potency than the controls in this study. The DPPH scavenging ability of the extract may be attributed to its hydrogen donating ability.

### Metal chelating activity

Ferrozine can quantitatively form complexes with  $\text{Fe}^{2+}$ . However, in the presence of chelating agents, the complex formation is disrupted with the result that the red color of the complex is decreased. Measurement of color reduction, therefore, allows the estimation of the chelating activity of the coexisting chelator. The transition metal ion  $\text{Fe}^{2+}$  possesses the ability to move single electrons by virtue of which it can allow the formation and propagation of many radical reactions, even starting with relatively nonreactive radicals (30). The main strategy to avoid ROS generation that is associated with redox active metal catalysis involves chelating of the metal ions. The effect of CS extract on  $\text{Fe}^{2+}$  and ferrozine complex formation is shown in Figure 3. CS extract interferes with the formation of ferrous and ferrozine complex, suggesting that it has chelating activity, and captures ferrous ion before ferrozine.  $IC_{50}$  of the extract for chelating activity was  $2 \text{ mg ml}^{-1}$ , which is lower than the positive standard EDTA ( $IC_{50} = 17.4 \text{ mg ml}^{-1}$ ).

### Assay of nitric oxide-scavenging activity

The procedure is based on the principle that sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions that can be estimated using Griess reagent. CS extract as a scavenger of nitric oxide competed with oxygen, leading to reduced production of nitrite ions. The  $IC_{50}$  of the extract and quercetin were  $552$  and  $17 \text{ mg ml}^{-1}$ . CS extract showed a weaker potency than quercetin in this

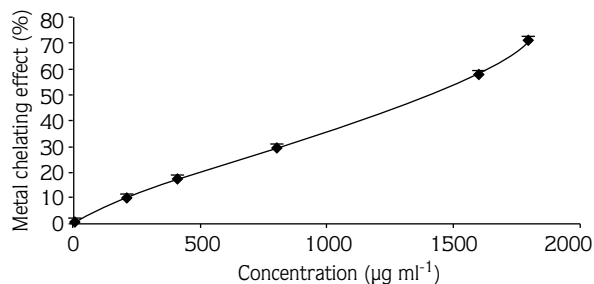


Figure 3. Metal chelating effect of different concentrations of CS extract. Each point represents the mean  $\pm$  S.E. ( $n = 5$ ).

study. In addition to reactive oxygen species, nitric oxide is also a factor involved in inflammation, cancer, and other pathological conditions (31). Natural extracts may have the property to counteract the effect of NO formation and, in turn, may be of considerable interest in preventing the ill effects of excessive NO generation in the human body. Furthermore, the scavenging activity may also help to arrest the chain of reactions initiated by excess generation of NO that are detrimental to human health.

### Reducing power

Fe (III) reduction is often used as an indicator of electron-donating activity, which is an important mechanism of phenolic antioxidant action (20). The reducing power was determined according to the method of Yen and Chen (21). In the reducing power assay, the presence of antioxidants in the samples would result in the reducing of  $\text{Fe}^{3+}$  to  $\text{Fe}^{2+}$  by donating an electron. Amount of  $\text{Fe}^{2+}$  complex can then be monitored by measuring the formation of Perl's Prussian blue at 700 nm. Increasing absorbance at 700 nm indicates an increase in the reductive ability. Figure 4 shows the dose-response curves for the reducing power of the extract from CS. The extract exhibited a good reducing power at 0.8 and 1.6  $\text{mg ml}^{-1}$  that was comparable with Vitamin C ( $P > 0.05$ ). Because the reductive ability of the extract was significantly comparable to Vitamin C, it was evident that the extract showed reductive potential and could serve as electron donor, terminating the radical chain reaction.

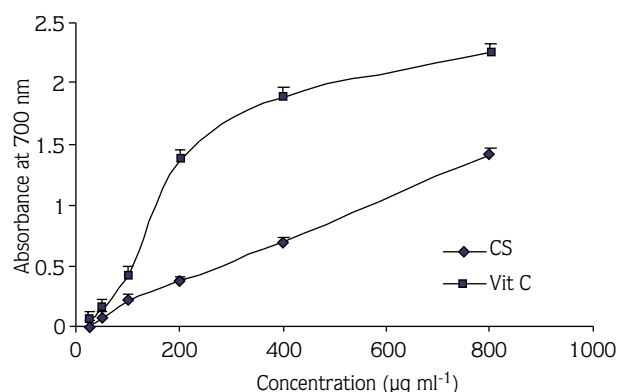


Figure 4. Dose-response curve of CS extract in reducing power method (reducing  $\text{Fe}^{3+}$  to  $\text{Fe}^{2+}$ ) compared to Vitamin C as a reference standard compound ( $P > 0.05$ ). Each value is presented as mean  $\pm$  S.E. ( $n = 3$ ).

### FTC Method

Membrane lipids are rich in unsaturated fatty acids that are most susceptible to oxidative processes. Especially, linoleic and arachidonic acid are targets of lipid peroxidation (30). The inhibition of lipid peroxidation by antioxidants may be due to their free radical-scavenging activities. Antioxidant activity of 0.4  $\text{mg ml}^{-1}$  of CS extract, determined according to ferric thiocyanate method, can be observed in Figure 5. CS extract displayed a comparable antioxidant activity to the reference standards.

### Conclusion

Using plants as a good source of antioxidants have been examined by many researchers. In our previous studies, we found several plants showing potent antioxidant activity (14, 33). In the present study, CS extract bears comparable antioxidant activity to the standard compounds. Its constituents scavenge free radicals, chelate the catalytic metal ions, and may exert a protective effect against oxidative damage induced to cellular macromolecules. Free radicals are often generated as by-products of biological reactions or from exogenous factors. The involvements of free radicals in the pathogenesis of a large number of diseases are well documented. A potent scavenger of free radicals may serve as a possible preventative intervention for the diseases (34). The preliminary chemical examination of alcoholic-water extract has shown the presence of phenols and flavonoids, which may be responsible of the antioxidant and lipid peroxidation inhibitory activities. The high scavenging

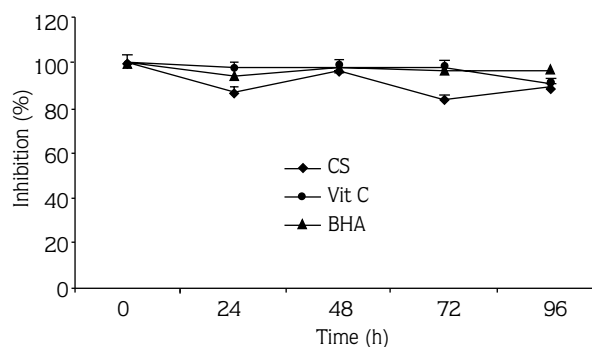


Figure 5. Antioxidant activity of CS extract determined according to FTC method. CS extract at 4  $\text{mg ml}^{-1}$  showed almost the same pattern of activity as Vitamin C. Each value is presented as mean  $\pm$  S.E. ( $n = 3$ ).

property of CS may be due to hydroxyl groups existing in the phenolic compounds' chemical structure that can provide the necessary component as a radical scavenger. Further studies on the isolation of these compounds are in progress. CS extract activity may be related to the high amount of flavonoid and phenolic compounds leading to an antioxidant activity in the extract.

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