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Research Article
Antioxidant Activities of Beetroot (*Beta vulgaris* L.) Extracts

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Abstract

Background and Objective: Beetroot (*Beta vulgaris* L.) is one of the most commonly produced vegetables around the world, recently, plant extracts containing phenolic compounds have been screened to find new natural food ingredients. The antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play an important role in their ability to chelate and neutralize free radicals, quench singlet and triplet oxygen and decompose peroxides. This study was designed to determine the antioxidant activity of the aqueous and ethanolic extracts of beetroot by using two different methods. **Methodology:** The samples of beetroot were locally obtained, cleaned and ground. Twenty grams of the ground material was extracted with 250 mL of distilled water or 95% ethanol at reflux for 1 h. The extract was filtered and concentrated at 50°C for dryness. **Results:** The phenolic compounds and flavonoids in the ethanolic extract of beetroot were 16.88 and 10.80%, respectively, of the mass of the extract and their contents in the aqueous extract were 7.812 and 4.77%, respectively. The reducing powers of the ethanolic and aqueous extracts were also determined. The reducing power was increased by increasing the sample concentration and reached 88.89% for the ethanolic and 80.89% for the aqueous extract. The aqueous extract showed a lower chelating capacity than EDTA (absorbances of 53.98 and 93.00, respectively) and a lower capacity than that of the ethanolic extract, which had an absorbance of 68.11. **Conclusion:** It is concluded that natural antioxidants from edible sources are considered safer alternatives to synthetic antioxidants for food preservation.

Key words: *Beta vulgaris* L., antioxidants, phenolic, flavonoids, aqueous extract, ethanolic extract, reducing power, chelating capacities

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Bioactive compounds commonly observed in fruits, vegetables, herbs and other plants exhibit possible health benefits, such as antioxidative, anticarcinogenic, atherosclerosis, antimutagenic and angiogenesis inhibitory activities[1,2]. Interestingly, many herbs are known to contain large amounts of vitamin C, vitamin E and carotenoids.

Phenolic antioxidants in herbs mainly consist of phenolic acids[1], flavonoids[3] and catechine[4,5]. The antioxidant activities of phenolic compounds are mainly due to their redox properties, which play an important role in the abilities of these compounds to adsorb and neutralize free radicals, quench singlet and triplet oxygen and decompose peroxides[5].

Beetroot (*Beta vulgaris* L.), locally known as Shamandar, is a vegetable plant belonging to the family Amaranthaceae. Beetroot have long been used in traditional Arab medicine to treat a wide variety of diseases and it has been used for its carminative, emmenagogue and hemostatic and renal protective properties and for the treatment of cardiovascular diseases[6]. Recent reports have indicated that extracts from the root of *Beta vulgaris* L. possess antihypertensive, hypoglycemic[7], anti-inflammatory and hepatoprotective activities[8-10]. Previous studies have shown that red beetroot extract is an effective multi organ tumor suppressing agent in laboratory animals[11-13]. Beetroot is known to be a powerful antioxidant and antimicrobial[14].

In ancient times, beetroot was believed to help enhance human sex hormones and was consumed as an aphrodisiac. The juice of beetroot was also consumed as a natural remedy for sexual weakness and to expel kidney and bladder stones[15]. In recent years, beetroot has gained popularity among athletes as natural food for boosting energy[16,17].

Recently, plant extracts containing phenolic compounds have been screened to find new natural food ingredients. In these evaluations beetroot (*Beta vulgaris* L.) extracts, especially peel extracts, have shown relatively strong antioxidant activity in comparison to other vegetables[13,18,19], which has resulted in increased interest in the compounds present in beetroot extracts. The phenolic antioxidants in beetroot mainly consist of phenolic acids[20], flavonoids[21] and catechins[22]. Beetroot is a potential source of valuable water-soluble nitrogenous pigments, called betalains, which can be divided into two main groups, the red betacyanins and the yellow betaxanthins[14] and these compounds are widely used as additives in the food industry because of their natural colorant properties and absence of toxicity[23].

Another nutritional feature of beets is beet juice, which is a convenient alternative for vegetal consumption and it contains compounds, such as potassium, magnesium, folic acid, iron, zinc, calcium, phosphate, sodium, niacin, biotin, the B6 vitamin and soluble fiber, which have various health benefits[24].

In the food industry, synthetic antioxidants, such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), have long been widely used as antioxidant additives to preserve and stabilize the freshness, nutritive value, flavor and color of foods and animal feed products. However, at least one study has revealed that BHT could be toxic, especially at high doses[25]. Currently, there is increasing interest in the substitution of synthetic food antioxidants for natural compounds.

The objectives of this study were: (1) To determine the phenolic and flavonoid contents of the ethanolic and aqueous extracts of beetroot and (2) To determine their antioxidant activities based on their reducing power and chelating capacity.

MATERIALS AND METHODS

The samples of beetroot were obtained from a local market in Baghdad, Iraq, cleaned and ground. Twenty grams of the ground material was extracted using 250 mL of distilled water or 95% ethanol (from Sigma, St. Louis, MO, USA) at reflux for 1 h. The extract was filtered through a Whatman 1 filter (Whatman Clifton, NJ, USA) and concentrated at 50°C to complete dryness.

Determination of the total phenolic compounds: The Folin-Ciocalteu (from Sigma, St. Louis, MO, USA) calorimetric method was used as described by Biglari *et al.*[26]. To 0.5 mL of (1 mg mL^{-1}) extract, 2.5 mL of a ten-fold dilution of Folin-Ciocalteu reagent and 2 mL of 7.5% sodium carbonate solution (from Sigma St. Louis, MO, USA) were added and then the reaction was allowed to stand for 30 min at room temperature. The absorbance was recorded at 760 nm using a Pye unicam spectrophotometer (UV/VIS Spectrophotometer, Shimadzu, Japan). The total phenolic compounds were determined according to a standard curve prepared from gallic acid (from Sigma St. Louis, MO, USA) (Fig. 1).

Determination of the flavonoids: The total flavonoids in the aqueous and ethanolic extracts were determined according to Zhisben *et al.*[27]. One milliliter of extract solution (1 mg mL^{-1}) was placed in a 10 mL volumetric flask and 5 mL of distilled water and 0.3 mL of 5% $NaNO_2$ (from Sigma St. Louis, MO, USA) solution were added to the extract. After 5 min, 0.6 mL of 10% $AlCl_3$ (Fisher, Springfield, NJ, USA) was added. After another 5 min, 2 mL of 1 M NaOH solution (Fisher, Springfield, NJ, USA) was added and the volume was brought up to 10 mL with distilled water.

The mixture was mixed thoroughly and the absorbance was measured at 510 nm. The flavonoid compounds were determined by comparison to a catechin (from Sigma St. Louis, MO, USA) standard curve (Fig. 2).

Antioxidant activity assay

Reducing power: The reducing power was estimated as described by Chou *et al.*[28]. A 1 mL sample of extract (2-10 mg mL^{-1}) was mixed with 2.5 mL of 1% potassium ferric cyanide (from Sigma St. Louis, MO, USA) and 2.5 mL of 0.2 M (pH 6.6) sodium phosphate buffer (from Sigma, St. Louis, MO, USA) and the solution was incubated at 50°C for 20 min. To stop the reaction, 2.5 mL of 1% trichloroacetic acid (TCA) (from Sigma St. Louis, MO, USA) was added to the mixture, which was then centrifuged for 10 min at 3000 rpm (DuPont, model Sorvall RC-5C). A 0.5 mL aliquot of the supernatant was mixed with 1 mL of 1% ferric chloride (from Sigma St. Louis, MO, USA) and the mixture was allowed to stand for 10 min. The absorbance was measured at 700 nm. BHT (from Sigma St. Louis, MO, USA) (0.02%) was used as a reference.

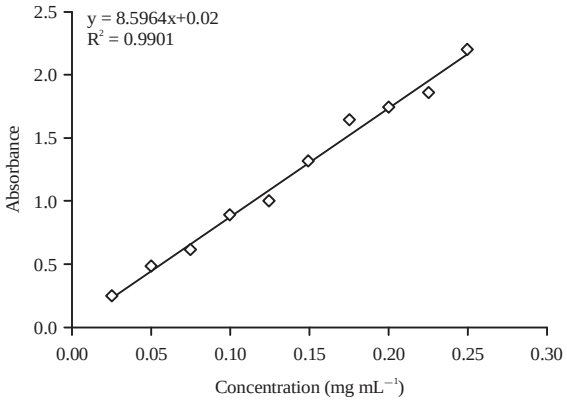

Fig. 1: Concentration-response curve for gallic acid at 760 nm

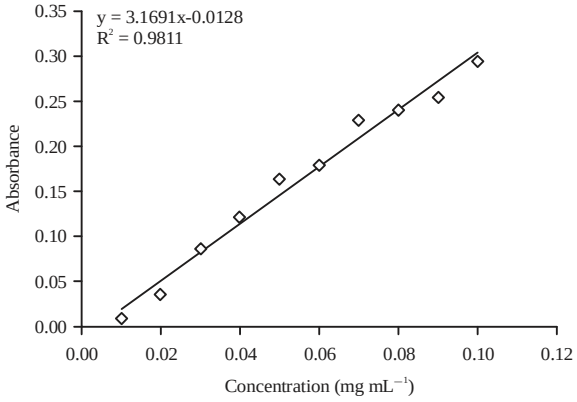

Fig. 2: Concentration-response curve for catechin at 510 nm

Chelating capacity: The chelating capacity was determined according to Su *et al.*[29], with some modifications. One milliliter (2-10 mg mL^{-1}) of extract was mixed with 0.2 mL of 2 mM ferric chloride (from Sigma St. Louis, MO, USA) and 0.2 mL of 5 mM 8-hydroxyquinoline (from Sigma St. Louis, MO, USA). After reacting for 10 min at room temperature, the absorbance was measured at 562 nm. Ethylenediaminetetraacetic acid (EDTA-Na_2) was obtained from Wako Chemicals (Richmond, VA, USA) and was used as a reference.

RESULTS AND DISCUSSION

The aqueous and ethanolic extracts of *Beta vulgaris* L. were analyzed for their phytoconstituents. Quantitatively, the total phenolic contents accounted for 16.88 and 7.81% of the ethanolic and aqueous extracts, respectively (Table 1). The phenolic content was determined to be much higher in the ethanolic extract than in the aqueous extract, which might be due to the solvent used for extraction. These results showed that *Beta vulgaris* L. is rich in phenols[14,20].

Quantitatively, the total flavonoid contents were 10.80 and 4.77% for the ethanolic and aqueous extracts, respectively (Table 2).

The reducing power is a measure of the concentration of compounds that are electron donors and can act as primary and secondary antioxidants[30]. The reducing powers of the aqueous extract of beetroot were 52.09, 56.29, 61.88, 70.01 and 80.89% at concentrations of 2-10 mg mL^{-1}, respectively (Fig. 3.), while the reducing powers of the ethanolic extract were 55.09, 57.29, 63.88, 75.01 and 88.89% over the same concentrations range (Fig. 4). From these results, we found that the reducing power increased as the sample concentration increased[14]. Higher reducing powers can be attributed to higher amounts of total phenolic compounds and flavonoids and the reducing power of a compound may reflect its antioxidant potential[31].

Phenolic compounds have been identified as antioxidant agents that can act as free radical oxidation terminators[32] and the reducing properties of these compounds are generally associated with the occurrence of reductions in solution[33]. The

Table 1: Phenolic contents in the beetroot extracts

Plant	Extraction	Phenolic contents (%)
Beetroot	Ethanolic	16.88
	Aqueous	7.81

Table 2: Flavonoid contents in the beetroot extracts

Plant	Extraction	Flavonoid contents (%)
Beetroot	Ethanolic	10.80
	Aqueous	4.77

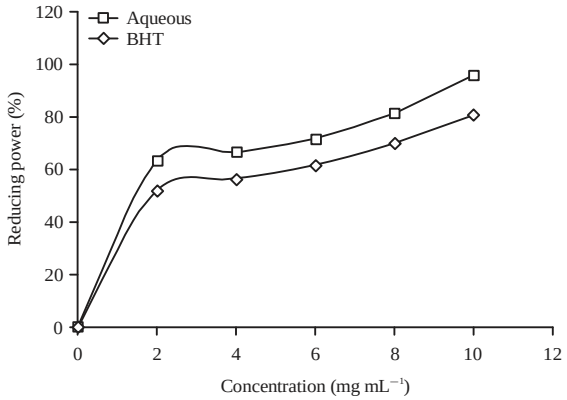

Fig. 3: Reducing power of the aqueous extract of beetroot compared with BHT at the same concentration

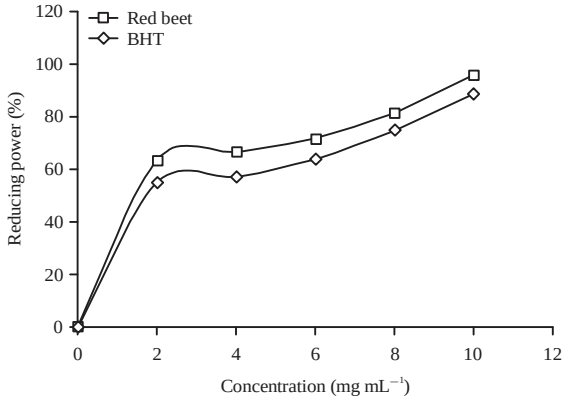

Fig. 4: Reducing power of the ethanolic extract of beetroot compared with BHT at the same concentration

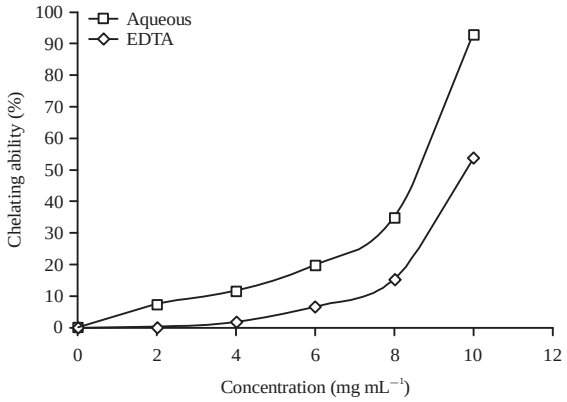

Fig. 5: Chelating capacity of an aqueous extract of beetroot compared with EDTA at the same concentration

results demonstrated that the differences between the two extraction methods might be due to the extract solvent and the compounds that can be generated in the reducing reaction.

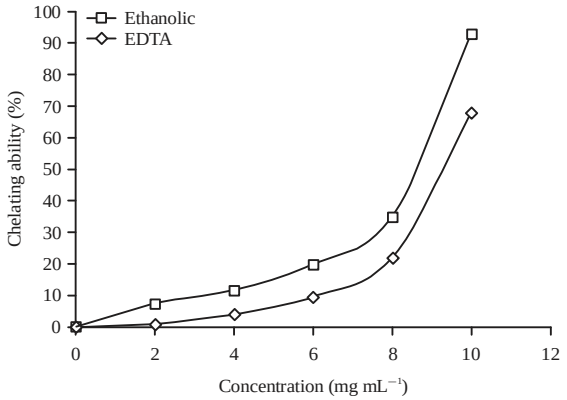

Fig. 6: Chelating capacity of an ethanolic extract of beetroot compared with EDTA at the same concentration

Water was chosen as an extraction solvent since it is often utilized in the food industry in a variety of ways. The aqueous and ethanolic extracts were screened for their possible antioxidant activities and the decrease in absorption was used as a measure of the chelating capacity of the extract. Flavonoids have been demonstrated to display a wide range of pharmacological and biochemical actions, such as antimicrobial, antithrombotic, antimutagenic and anticarcinogenic activities[7,34].

The aqueous extract was found to have a lower chelating capacity than EDTA (absorbances of 53.98 and 93.00, respectively, at 10 mg mL^{-1} (Fig. 5) and it was lower than that of the ethanolic extract, which had an absorbance of 68.11 (Fig. 6).

These outcomes were consistent with many previous studies which reported that the chelating capacities of the aqueous extracts of dates ranged from 62.87-81.30% at concentrations of 5 and 10 mg mL^{-1} [35] or 3.15% at concentrations of 0.25-2 mg mL^{-1}, while others found that the chelating capacity of the ethanolic extracts of dates was 1.06% at 0.25-2 mg mL^{-1} concentration[36].

In contrast, the chelating capacities for alcoholic extracts were 47.19% for pomegranates, 57.23% for fig and 48.58% for black grape at concentrations of 5 mg mL^{-1} [37]. Extracts of dates (Deglet noor) contained antioxidants that included a wide range of phenolic compounds[38]. It was found that chelating compounds acted as antioxidants by forming bonds with metals[39].

CONCLUSION

Consumption of the phenolic compounds present in natural foods may lower the risk of serious health disorders. *Beta vulgaris* L. is rich in total phenols and flavonoids.

Phenolic compounds have potent antioxidant activities as they can effectively scavenge free radicals and chelate transition metals among other properties. Natural antioxidants from edible sources are considered safer alternatives to synthetic antioxidants for food preservation since they avoid the adverse effects on human health caused by synthetic food additives.

SIGNIFICANCE STATEMENT

This study evaluated the phenolic compounds in beetroot (*Beta vulgaris* L.), which is one of the most commonly produced vegetables around the world and it can be a beneficial source of new natural food ingredients. This study will help the researcher to uncover the critical area of the antioxidant activities of phenolic compounds may be mainly due to their redox properties, which can be difficult for researchers to explore. Thus, a new theory on the combination of these micronutrients or other possible combinations may be developed.

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