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Original Article

Antioxidant Properties of Some Traditional Iranian Edible Fruits Growing Wild in Lorestan Province in the Western Iran

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Abstract

Background and Aim: This study aimed to determine the antioxidant properties of *Ziziphus nummularia (ZN), Crataegus spp (CS)* (red), *Crataegus spp (CS)* (yellow), and *Berberis integerrima (BI)* which grow in the Western Iran.

Materials and Methods: The free radical scavenging ability, total antioxidant capacity, and phenol as well as flavonoid contents were measured using the 2, 2 diphenyl-1-picrylhdrazyl (DPPH), phosphomolybdate, Folin-Ciocalteu, and Zishen methods, respectively.

Results: The total antioxidant capacities of *ZN, CS (red), CS (yellow),* and *BI* were 0.93±1.15, 1.01±1.14, 1.42±3, and 3.36±4.84 (µmol ascorbic acid/gram of dry extract), respectively. The total flavonoid contents of *ZN, CS (red), CS (yellow),* and *BI* were 0.46±0.14, 0.98±0.17, 1.25±0.12, and 3.55±1.80 (mg quercetin/gram of dry extract), respectively. The total phenol content of the extracts ranged from 255.96±78.17 (*ZN*) to 5128.04±501.61 (*CS* yellow) mg of gallic acid/gram of dry extract. The lowest IC_{50} value (3892.25±1298.51 µg/ml of extract) was observed in *CS* (yellow) and the highest case (5571.35±31.06 µg/ ml) belonged to *CS* (red).

Conclusion: The results of the present study indicated that all the samples were rich in phenolic and flavonoid contents.

Keywords: Antioxidants, Flavonoids, Wild plants, Total antioxidant capacity

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Introduction

Plants and fruits are highly significant because of their medicinal and nutritional properties. They are the major sources of bioactive compounds. Historically, plants and their products have been consumed as herbal supplements such as botanicals, nutraceuticals, and drugs (1). Wild and traditional edible species are indeed significant in family food security, even though the attraction of food and non-economic crops has influenced their consumption. Hence, they are culturally accepted and used in

various ways (2). One hundred and fifty-one edible plants have been identified in Lorestan province, Western Iran, which are used not only for nutritional purposes but also as traditional treatments for different diseases (3).

Nowadays, antioxidants have gained high significance due to their potential to serve as preventive and therapeutic agents in different diseases caused by free radicals. The excessive production of damaging free radicals above the capacity of the body's antioxidant defenses to detoxify them results in the production of oxidative stress (4). Antioxidants are able to act as catalysts in biochemical reactions. That is, they react to oxygen-free radicals, though they are not damaged or altered by them. Such a reaction leads to the formation of a stable molecule, and the unaltered antioxidant pursues its helpful work in converting oxygen free radicals into harmless molecules. Antioxidants could be found in food or taken as supplements and could remarkably reduce the adverse impacts of oxygen free radicals. They exist in vitamins, minerals, and phytochemicals, and beneficial nutrients found in foods such as fresh fruits and vegetables. Phenols, falavonoids and flavons are among the most significant plant-derived compounds that act as antioxidant agents (5).

Several studies have indicated that reactive oxygen species (ROS) and free radical-mediated reactions have their roles in degenerative or pathological events such as aging, cancer, coronary heart ailments, and Alzheimer's disease (5).

Several epidemiological researches have revealed the clear significant positive association between the consumption of fruits and vegetables and a decreased rate of heart disease, mortality, common cancers and other degenerative diseases as well as aging. This is due to the fact that these foods might provide an optimal blend of phytochemicals such as natural antioxidants, fibers and other biotic compounds (6).

Vegetables and fruits are potential sources of natural antioxidant compounds. Among different edible and medicinal plants, some indigenous variants are more appealing in terms of raw herbal materials or certain phytochemical properties, and have a high antioxidant capacity to maintain human health. Polyphenols and flavonoids have the potential to inhibit the activity of free radicals and delay lipid peroxidation (7, 8).

Consumption of medicinal herbs requires scientific information and the identification of their chemical compounds, for they are responsible for the therapeutic and bioactive effects of herbal medicines. Traditionally, medicinal herbs have been widely used in Iran. Hence, discovering the chemical composition (secondary metabolites) of herbal plants and their bioactive effects such as antioxidant properties is of great significance. The present study aimed to determine the total phenols, flavonoids, radical scavenging activity (by IC_{50} assay) and total antioxidant capacity of the extracts of *Ziziphus Nummularia, Crataegus spp (red), Crataegus spp (yellow),* and *Berberis Integerrima* that are found in Lorestan province.

Materials and Methods

Materials and Reagents

The Folin-Ciocalteu (FC) reagent, 2, 2-diphenyl -1-picrylhydrazyl (DPPH), gallic acid, and disodium hydrogen phosphate used in this study were obtained from Merck Company. Quercetin, ascorbic acid and other solvents and chemicals were purchased from Sigma.

Fruits and Sampling Methods

Fruit samples were collected from nature in various regions of Lorestan province in April and October, 2016. The information pertaining to these plants has been exhibited in Table 1. Samples were allowed to stand in room temperature away from sunlight, and were air-dried for several days. Dry samples were powdered using a clean grinder. Finally, powdered samples were kept at 4°C in a dark and dry environment.

Extraction

To prepare the extracts by maceration, 50 ml of 80% methanol was added to 5 g of dried powdered fruit material. The solution was kept at room temperature for 72 hours and stirred every 4 or 5 hours. After being filtered through filter papers, the solution was concentrated using a rotary device, and chlorophyll was removed. Subsequently, 50 ml distilled water was added, and the solution was filtered again. The resultant extract was kept at 4°C for further phytochemical analysis.

Determination of Total Phenolic Content

The contents of total phenolic compounds in the fruit extracts were measured using the Folin-Ciocalteu method (9). One ml of methanol was added to 10 mg of dried powdered extract. Then, 200 µl of Folin-Ciocalteu reagent was added to 40 µl of the extract, and the solution was diluted with 1000 µl distilled water. Afterwards, 600 µl of sodium carbonate was added, and the samples were allowed to stand for 2 hours at room temperature in darkness. Finally, the amount of light absorbance by the solution was measured by spectrophotometry at 765 nm (Lightwave II UV/VIS Spectrophotometer). The standard curve was drawn based on different amounts of gallic acid, and the concentration of total phenolic compounds was expressed as gallic acid equivalents (GAE) in milligrams per gram of dry fruit extract.

Determination of Total Flavonoids

Total flavonoid contents were measured using ammonium chloride colorimetric analysis with quercetin as the standard. Initially, standard solutions with concentrations of 0-100 mg/l were prepared from quercetin in absolute methanol. Subsequently, 0.2 ml of aluminum chloride and 0.1 ml of 33% acetic acid were added to 0.2 ml of fruit extract or standard solution. The solution was vortexed, and then the volume was brought to 5 ml using 90% ethanol. After 30 minutes, absorbance was recorded at 420 nm, and the total flavonoid content was obtained by a standard curve based on mg quercetin per gram of the dry fruit extract (10).

Determination of Total Antioxidant Capacity and IC_{50}

To analyze the antioxidant activity of the alcoholic extract of fruits, the inhibition of the production of free radicals was measured using the DPPH method (11). Initially, 3 test tubes were prepared for each concentration. Then, 200 µl of the samples with various concentrations were mixed with 1 ml of 90 µmol DPPH, and the volume was increased to 4 liters with 95% methanol. The solution was shaken in darkness for 1 h. Subsequently, absorbance was measured at 517 nm. Inhibition percentage was calculated based on the following equation comparing extracts and controls:

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

I%= inhibition percentage, A_{blank} = absorption in controls at 517 nm and A_{sample}= absorption in samples at 517 nm. To compare the antioxidant activity of the extracts, IC_{50} was used. The IC_{50} value is the concentration of each extract required to scavenge the free radicals to 50% of the controls. Using the Excel software program, a diagram was drawn based on the sample absorption and the concentration of the extract. The resultant equation was used to calculate IC_{50} in µg/ml fruit extract.

Statistical Analysis

Data analysis was conducted using SPSS, version 18. For each sample, experiments were repeated three times and the mean of the results was calculated. The data have been exhibited as mean ± standard deviation.

Results and Discussion

It has been reported that the antioxidant potential of plant-derived phenolic compounds, including flavonoids, might decrease the risk of developing some diseases such as heart failure (12).

Total Phenol Contents

The phenolic contents of the extracts that were measured using the Folin Sykaltv method ranged from 255.96±78.17 to 5128.04±501.61 gallic acid equivalents in milligrams per gram of dry extract. The highest phenolic content was observed in *Crataegus SP* (Yellow), and the lowest was observed in *Ziziphus nummularia* (Table 2). Previous studies have indicated that oligomeric procyanidins and their glycosides are the major phenolic compounds in *Berberis integerrima* (13). *Berberis integerrima* also had a high total phenolic content (5036.375±1465.275 mg/g plant extract in gallic acid equivalent). The total phenolic contents of *Berberis integerrima* fruits were reported as 48000 /100g extract in a study carried out in Turkey (14). Berenji Ardestani *et al.* reported the total phenol content as 8530 mg/100 g fresh fruit for *Berberis integerrima* (15). The total amount of phenol obtained in their study was less than the amount obtained in our research, which might be because of the ripening steps of fruits or certain environmental factors (16).

Total Flavonoids

As it has been indicated in Table 2, the flavonoid contents of the extracts measured with ammonium chloride colorimetric analysis ranged from 0.46±0.14

Table 1: The list of some wild fruits consumed by residents of Lorestan province in Western Iran.

Scientific name	Family	Common name	Local name	Plant part used	Time of collection
Cratagus SP(Yellow)	Rosaceae	Hawthorns	Zalzalak zard	Fruit	October
Berberis integrrima	Berberidaceae	Seedless Barberry	Zereshk koohi	Fruit	September
Cratagus SP(RED)	Rosaceae	Hawthorns	Zalzalak ghermez	Fruit	October
Zizphus numularia	Rhamnaceae	Jharber	Konar parsi	Fruit	September

Table 2: Mean and standard deviation of total phenol, flavonoids and total antioxidant capacity of 4 wild plants consumed by residents of Lorestan province in Western Iran[a].

	Plant Name	Total flavonoid*	Total phenol content **	IC50***	Total antioxidant capacity****
1	*Cratagus SP(Yellow)*	1.25±0.12	5128.04±501.61	3892.25±1298.51	1.42±3
2	*Berberis integrrima*	3.55±1.80	5036.38±1465.28	4576.46±2698.52	3.36±4.84
3	*Cratagus SP(RED)*	0.98±0.17	401.38±131.77	5571.35±31.06	1.01±1.14
4	*Zizphus numularia*	0.46±0.14	255.96±78.17	4239.35±2490.93	0.93±1.15

[a] For each studied extract, experiments were repeated 3 times
* mg/g plants extract based on quercetin
** mg/g plant extract in galic acid equivalent
*** µg/ml plant extract
**** Micromole ascorbic acid/gr plant extract

to 3.55±1.80 mg per gram of dry plant extract based on quercetin. *Berberis integerrima* had the highest flavonoid content (3.549±1.802 mg/g plants extract based on quercetin), whereas the lowest was observed in *Ziziphus nummularia* (0.46±0.14 mg/g plants extract based on quercetin).

The fruits of these plants are also widely used as a home remedy (17). Fallah *et al.* recently reported that *Berberis integerrima* could ameliorate insulin resistance (18). Moreover, the hypolipidemic, hypotensive, and anti-inflammatory features of this plant have already been reported (19,20). The results of a study that investigated the physicochemical properties of Iranian native barberry fruits showed that *Berberis integerrima* has high total phenolics and total anthocyanin contents. Hence, it could be a significant dietary source of phenolic compounds (15). In another study, Sharifi and Poorakbar reported that flavonoid contents of *Berberis integerrima* were 1.93±0.03 mg of quercetin /10g (21). Compared to previous studies, *Berberis integerrima* had a higher flavonoid content in our research, which might be related to environmental factors affecting plants growth such as weather conditions, sunlight, soil richness, etc.

Dureja and Dhiman carried out a research in India in which the total flavonoid content of *Ziziphus nummularia* was reported as 54±5.97 mg of quercetin/100 g of fruit (22), which was a little higher than the total flavonoid content of *Ziziphus*

nummularia in our study.

Flavonoids are able to prevent injuries caused by free radicals in different ways. Through *in vitro* experiments, it has been found that flavonoids have anti-inflammatory, antiallergic, antiviral and anticarcinogenic properties (23). Flavonoids are prominent inhibitors of cyclooxygenase, and lipoxygenase flavonoids prevent the synthesis of prostaglandins that suppress T-cells. Immune cells are able to communicate with chemical signals called cytokines, which are controlled by flavonoids (24). Flavonoids are strong bioactive molecules capable of having anticarcinogenic effects, for they are able to interfere with the initiation, development and progression of cancer through the modulation of cellular proliferation, differentiation, apoptosis, angiogenesis and metastasis (25).

Total Antioxidant Capacity and IC_{50}

Total antioxidant capacity ranged from 0.93±1.15 to 3.36±4.84 µmol per gram of dry matter. *Berberis integerrima* had the highest content (3.36±4.84 µmol per gram of the extract), and the lowest activity was observed in *Ziziphus nummularia* (0.93±1.15 µmol per gram of the extract) (Table 2).

The stable free radical 2, 2′-diphenyl-1-picrylhydrazyl (DPPH) has been extennsively used in the evaluation of radical scavenging activity of plant extracts, natural compounds, and foods. The IC_{50} of the extracts ranged from 3892.25±1298.51 to 5571.35±31.06 µg/ml.

The results of total antioxidant capacity and IC_{50} did not match. For instance, the sample with the highest amount of total antioxidant capacity (*Berberis integerrima*) was different from the sample with the lowest IC_{50} value (*Crataegus SP (Yellow)*). *Berberis integerrima* extract contained the highest flavonoid content, but there was no significant association between its phenolics and flavonoids and the total antioxidant capacity. Few studies have reported the lack of correlation between total phenolic content and radical scavenging activity in certain edible plants and wheat (26, 27). However, a strong association between antioxidant activity (DPPH method) and phenolic contents in 113 Indian medical plant extracts was indicated by Surveswaran *et al.* (28). Similarly, Kaur and Kapoor (2002) indicated a correlation between total phenolic content and antioxidant activity (β-carotene bleaching method) in certain Asian vegetables (29). Several factors could be central in the antioxidant activities of plant materials such as the structural features of the antioxidants and the complex composition of extracts (30), which could justify the lack of correlation between phenols and flavonoids and antioxidant activity in the present study. Hence, it is necessary to carry out several types of antioxidant capacity measurement in order to examine various mechanisms of antioxidant action (31).

Conclusion

In conclusion, the results of the present study indicated that all the samples, including *Ziziphus Nummularia, Crataegus spp (red), Crataegus spp (yellow),* and *Berberis Integerrima* were rich in phenolic and flavonoid contents. *Berberis integerrima* had the highest amount of flavonoid contents and *Crataegus spp (yellow)* had the highest amount of total phenolic content. Hence, the species examined in this research might be valuable natural antioxidant sources, and could be used in both health medicine and the food industry.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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