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Phenolic Content, Antiradical, Antioxidant, and Antibacterial Properties of Hawthorn (*Crataegus elbursensis*) Seed and Pulp Extract

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ABSTRACT

The hawthorn fruits have been used as food and medicine for centuries. In the present study, pulp and seed extract of *Crataegus elbursensis* Rech. F. fruits belonging to the family *Rosaceae* and native of northern part of Iran were evaluated for the polyphenol contents, antiradical, antioxidant, and antibacterial activities. The total phenolic, flavonoid, and anthocyanin contents of methanolic pulp extract were found to be more than those of methanolic seed extract. The DPPH radical scavenging, iron (III) reducing capacity, and total antioxidant activity of the extracts depended on concentration. A 200 µg ml^{-1} of *C. elbursensis* pulp and seed extract and butylated hydroxytoluene (BHT) exhibited 82.13, 83.47, and 85.44% inhibition, respectively. However, effect of the extracts in the total antioxidant activity and reducing power were not significantly as good as BHT. In addition, the results showed that both pulp and seed extract had inhibitory activity against the four bacteria tested, with the pulp extract showing more activity than the seed extract. Also, phenolic acids were identified by RP-HPLC and chlorogenic acid was the predominant phenolics in the samples. In conclusion, our results showed that *C. elbursensis* pulp and seed extract had strong antioxidant and antibacterial activities, which were correlated with its high level of polyphenols.

Keywords: DPPH radical, Flavonoid, HPLC, Polyphenolics, Total Antioxidant Capacity.

INTRODUCTION

For many years, different synthetic and chemical compounds have been used as antioxidant and antimicrobial agents in food to delay the oxidation of food-lipids and inhibit spoilage and pathogenic microorganism growth. However, due to the presence and increase in numerous drug-resistant micro-organisms and some toxic properties of synthetic antioxidants, safe and new natural antioxidant and antimicrobial agents need to be developed (Tajkarimi *et al*., 2010; Ait-Ouazzou *et al*., 2012). Recently, natural plants have received much attention as sources of biologically active substances that have wide range of pharmacological activities including antimicrobial, anti-inflammatory, antioxidant, and anticancer effects (Meot-Duros *et al*., 2008; Sadeghi *et al*., 2009). Also, studies have focused on health functions of phenolic compounds, including flavonoids and anthocyanins (Lin and Tang, 2007). Phenolic compounds, in addition to their antioxidant properties (because of their role as free radical scavengers), are able to inhibit foodborne pathogens and spoilage bacteria. Their antioxidant potential depends on the number and arrangement of the hydroxyl groups and the extent of structure conjugation (Gutiérrez-Larraínzar *et al*., 2012). Crude extracts of fruits, vegetables, and other plants rich in phenolic compounds,

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especially flavonoid-rich, are increasingly of interest in the food and drug industry because they improve the quality and nutritional value of food and, also, help with preservation of good health (Yasoubi *et al*., 2007; Petti and Scully, 2009). Furthermore, the seeds of many fruits are usually considered as waste material in food industry and can be used as a rich source of bioactive compounds. For example, loquat seed is called "good for health" in traditional Japanese lore (Koba *et al*., 2007).

Hawthorn, a common name of all plant species in the genus *Crataegus* of the *Rosaceae* family, has long been used as herbal medicine. The hawthorn fruit has been used as food and medicine in China and Europe for centuries. The proanthocyanidin and flavonoid constituents of hawthorn appear to be responsible for beneficial physiological effects on cardiovascular system. Hawthorn also seems to have antioxidant activity (Liu *et al*., 2010). The main constituents of hawthorn are flavonoids, phenolic acids, proanthocyanidins (especially B-type procyanidins), organic acids and some amines (Urbonaviciute *et al*., 2006; Arslan *et al*., 2010). The leaf, flower, and fruit constituents responsible for free radical scavenging activity are especially epicatechin, hyperoside, and chlorogenic acid. They are also among the best antilipoperoxidants (Kwok *et al*., 2010; Jurikova *et al*., 2012). Among the fruits belonging to the *Rosaceae*, Hawthorn fruit (*C. pinnatifida* Bge.) show the highest rate of neuro-protective phenols (gallic acid, 4-aminobenzoic acid, rutin and quercitrin). To date, more than 50 flavonoids have been isolated from genus *Crateagus* spp. (Jurikova *et al*., 2012). *Crataegus elbursensis* Rech. f. is a native fruit of forested areas of northern Iran. Hawthorn shrubs produce small black-coloured apple-like fruits that ripen during late autumn season. The aims of the present study were to identify and quantify phenolic acids, investigate the antioxidant activities of methanolic pulp and seed extracts of *C. elbursensis* fruit through various *in vitro* models, and determine antimicrobial activity by using broth microdilution method.

MATERIALS AND METHODS

Materials

Hawthorn fruits were harvested in October 2010 from the Shastkalateh forest, Golestan province (Gorgan), north of Iran (36° 45' N Latitude, 54° 23' E Longitude) with Caspian climate and average annual rainfall of 649 mm, average annual temperature of about 12°C and average humidity of 76.5%; and soil pH was found between 6.5-7.5. Sampling was done manually.

All fruits were harvested at their optimum ripeness and only fruits without damage were selected for this study. All the chemicals and reagents used were of analytical grade and were purchased from Fluka, Merck and Sigma Chemical Co.

Preparation of the Sample and Extract

Fruits were divided into pulp and seed fractions (it was not possible to separate the peel from the pulp). The fractions were dried in an oven at 40°C for 24 hours and pulverized into fine powder using a stainless steel blender, passed through a 40-mesh sieve, and mixed with 80% methanol at the ratio of 1:20 (w/v) in a mechanical shaker (Memmert WB14, Germany) at 50ºC for 18 hours. The extracts were filtered with Whatman No. 1 filter paper. Then, the filtrates were evaporated at 40ºC in a rotary evaporator (IKA RV 05 basic, Germany) for the removal of solvent and finally, freeze-dried (Epron FDV5503, South Korea). The dried sample of each fraction was stored at -18ºC, until use. These hawthorn fruit powders (freeze-dried fruit extracts were in powder forms) were dissolved in solvent and used for the assessment of antioxidant activity (Arabshahi and Urooj, 2007).

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Determination of Total Phenolic Content

Total phenolic content (TPC) in two fraction extracts of *C. elbursensis* was determined with Folin–Ciocalteau reagent according to the method of Slinkard and Singleton (1977) using gallic acid as phenolic compound standard. The results were expressed as mg gallic acid equivalents (GAE) per g extract. Briefly, 20 µl of the extract solution were mixed with 1.16 ml distilled water and 100 µl of Folin–Ciocalteu reagent, followed by addition of 300 µl of Na_2CO_3 solution (20%) within 1-8 minutes. Subsequently, the mixture was incubated in a shaking incubator at 40°C for 30 minutes and its absorbance was measured at 760 nm (PG Instruments T80, UK). The experiment was replicated three times.

Determination of Total Flavonoid Content

The total flavonoid content (TFC) was determined using the aluminum chloride colorimetric method (Chang *et al*., 2002) using quercetin as standard and the results were expressed as mg quercetin equivalents (QE) per g extract. Briefly, 0.5 ml of the extract solution were mixed with 1.5 ml of 95% alcohol, 0.1 ml of 10% aluminum chloride hexahydrate ($AlCl_3$), 0.1 ml of 1 M potassium acetate (CH_3COOK), and 2.8 ml of deionized water. After incubation at room temperature for 30 min, absorbance of the reaction mixture was measured at 415 nm against a deionized water blank. The experiment was replicated three times.

Determination of Total Anthocyanin Content

Total anthocyanin compounds (TAC) of the samples were estimated using a UV-spectrophotometer by the pH-differential method (Muanda *et al*., 2011). Two buffer systems, potassium chloride buffer, pH 1.0 (0.025 M) and sodium acetate buffer, pH 4.5 (0.4M) were used. Briefly, 400 ml of extract was mixed in 3.6 ml of corresponding buffer solutions and read against a blank at 510 and 700 nm. Absorbance (ΔA) was calculated as:

$$\Delta A = (A510-A700)_{pH1.0} - (A510-A700)_{pH4.0} \quad (1)$$

Monomeric anthocyanin pigment concentration in the extract was calculated and expressed as *Equivalent cyanidin-3-glucoside (mg l^{-1}):*

$$\Delta A \times Mw \times Df \times 1000/(Ma \times c). \quad (2)$$

Where, ΔA: Difference of absorbance, *Mw:* Molecular weight for cyanidin-3-glucoside (449.2); *Df:* The dilution factor of the samples, *Ma:* Molar absorptivity of cyaniding-3-glucoside (26,900); and *c:* Concentration of the buffer in mg ml^{-1}. Results were expressed as mg of cyanidin-3-glucoside equivalents (CgE). The experiment was replicated three times.

Determination of DPPH Radical Scavenging Activity

The free radical scavenging activity of extracts was measured in terms of hydrogen donating or radical scavenging ability using the stable radical DPPH (Shimada *et al*., 1992). Briefly, 1 ml of a 1 mM methanolic solution of DPPH was added to 3 ml of extract solution in methanol at different concentrations (10–200 µg ml^{-1} of dried extract). The mixture was then vortexed vigorously and left for 30 min at room temperature in the dark. The absorbance was measured at 517 nm. Butylated hydroxytoluene (BHT) was used as the reference compound. Radical scavenging activity was expressed as the inhibition percentage of free radical by the sample and was calculated using the following formula:

$$\% \ inhibition = [(A_0 - A_t)/A_0 \times 100] \quad (3)$$

Where, A_0 is the absorbance of the control (blank, without extract) and A_t is the absorbance in the presence of the extract. All the tests were performed in triplicate.

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The EC_{50} value is the concentration of the sample required to scavenge 50% of the DPPH free radical.

Determination of Total Antioxidant Capacity

The total antioxidant capacity of extracts was assayed according to the method of Prieto *et al.* (1999). An aliquot of 0.1 ml of sample solution (containing 100–500 μg ml^{-1} of dried extract in corresponding solvent) was combined with 1 ml of reagent solution (0.6M sulphuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). The reaction mixture was incubated at 95°C for 90 minutes. After the mixture had cooled to room temperature, the absorbance was measured at 695 nm against a blank. A typical blank solution contained 1 ml of reagent solution and the appropriate volume of the same solvent used for the sample, and it was incubated under the same conditions as the rest of the samples. The antioxidant activity of BHT was also assayed for comparison. The experiment was replicated three times.

Determination of Reducing Power

The ability of extracts to reduce iron (III) was assessed by the method of Yildirim *et al.* (2001). Briefly, 1 ml of different concentrations of the samples (200–800 μg ml^{-1} of dried extract in corresponding solvent), 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of potassium ferricyanide (1%) were mixed, and then incubated at 50°C for 30 minutes. At the end of the incubation, trichloroacetic acid (2.5 ml, 10%) was added to the mixtures, followed by centrifuging (Centurion k2042, USA) at 1,650g for 10 minutes. The upper layer (2.5 ml) was mixed with distilled water (2.5 ml) and ferric chloride (0.5 ml, 0.1%), and the absorbance was measured at 700 nm. BHT was used for comparison. The experiment was replicated three times.

Antimicrobial Activity

Microorganisms

The chosen bacteria for evaluating inhibitory activity of the pulp and seed extract included *Escherichia coli* (ATCC 8739) and *Salmonella enterica* (ATCC 19430), which belong to Gram-negative strains, and *Bacillus cereus* (ATCC 11778) *and Staphylococcus aureus* (ATCC 6538), which are Gram-positive strains. The tested bacteria were obtained from Iranian Research Organization for Science and Technology.

Determinations of the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

A broth microdilution method was employed for the determination of *in vitro* antimicrobial activities of *C. elbursensis* extracts according to the National Committee for Clinical Laboratory Standards (NCCLS, 2001). All tests were performed in Mueller Hinton broth (MHB). A serial doubling dilution of the extract was prepared in a 96-well microtiter plate over the range of 0.15–40 mg ml^{-1} and the final concentration of microbial suspensions was 10^6 CFU ml^{-1} (colony forming units). After a 24-h-incubation at 37°C, the MIC was read. The MIC is defined as the lowest concentration of the extract at which the microorganism does not demonstrate visible growth. To determine MBC, broth was taken from each well and inoculated in Mueller Hinton agar (MHA) for 24 hours at 37°C. The MBC is defined as the lowest concentration of the extract at which inoculated microorganisms are completely

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killed. All determinations were performed in duplicate.

RP-HPLC-DAD Analysis

Phenolic acids were determined following the method of Kwok *et al*. (2010) and slightly modified. Two grams of dried powder were extracted with 20 ml 80% methanol and shaken at ambient temperature, 200 rpm for 12 hours. Then, the solution was centrifuged at 4,000 rpm for 10 minutes. The supernatant was collected and the residue was re-extracted once again with the same volume of 80% methanol. Finally, all of the supernatant was filtered using filter paper (Whatman No. 4). The combined supernatant was evaporated under vacuum at 40°C to about 10 ml. The phenolic acids identification was carried out using Hitachi C18, 5µ, 4.6×250 mm column (reversed-phase liquid chromatography coupled with an UV-vis multi-wavelength detector). A solvent system consisting of water/acetic acid/methanol (Isocratic, 80:2:18, v/v/v) was used as mobile phase at a flow rate of 1 ml min^{-1} at room temperature. The extracted samples (pulp and seed extract) were filtered through a 0.45 µm membrane filter before injection. The injection volume was 20 µl, and peaks were monitored at 280 and 330 nm. The experiment was replicated three times.

Statistical Analysis

Data were recorded as means±standard deviation of triplicate measurements. Analyses of variance were performed by ANOVA test and significance differences between the means were determined by Duncan's New Multiple Range Test ($P< 0.05$) by the SAS software.

RESULTS AND DISCUSSION

Total Phenolic, Flavonoid, and Anthocyanin Contents

The content of phytochemical compounds (phenolics, flavonoids, and anthocyanins) of *C. elbursensis* fruit (the methanolic extracts of pulp and seed) is shown in Table 1. The results obtained showed that the total phenolic content (TPC), total flavonoid content (TFC), and total anthocyanin content (TAC) of the pulp extract were higher than those of the seed extract. The phenolic composition in hawthorn fruit varies among species and cultivars. Geographic locations also should be considered. The time of harvest (the stage of maturity) is also a very important factor. Besides, the polyphenolic content of hawthorn fruit is dependent on the conditions of extract preparation and the method of chemical determination of polyphenols (Jurikova *et al*., 2012). Total content of polyphenols in Chinese hawthorn fruit *C. pinnatifida* Bge. was 96.9±4.3 mg gallic acid equivalents per gram weight, measured by the Folin-Ciocalteu reagent method (Liu *et al*., 2010 b). The highest level TPC, TFC, and TAC in fresh and dry fruit extracts of *C. monogyna* have been reported as 12.82 mg equivalents gallic acid g^{-1} DW, 1.5 mg equivalents rutin g^{-1} DW, and 0.58 mg equivalents cyanidin-3-O-glucoside g^{-1} DW, respectively (Froehlicher

Table 1. Phenolic compounds of pulp and seed extracts.[a]

Sample	TPC (mg GAE g^{-1} extract)	TFC (mg QE g^{-1} extract)	TAC (mg CE g^{-1} extract)
Pulp extract	163.32 ± 6.32	12.06 ± 0.33	1.94 ± 0.08
Seed extract	58.60 ± 2.21	1.73 ± 0.01	0.26 ± 0.1

[a] TPC: Total Phenolic Compounds, TFC: Total Flavonoid Compounds, TAC: Total Anthocyanin Compounds.

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et al., 2009). The levels of TPC, TFC, and TAC in pulp extract of *C. elbursensis* were higher than other species reported above. Also, the mean TPC value of pulp extract of *C. elbursensis* was higher than that reported for Mayhaws (*Crataegus aestivalis and C. rufula*) (Pande and Akoh, 2010). In addition, TPC of methanolic and ultrasonic extract of *Crataegus pentagyna* subsp. *elburensis* fruits have been reported as 85.15 mg equivalents gallic acid g^{-1} extract powder and 90.83 mg equivalents gallic acid ml^{-1}, respectively (Ebrahimzadeh and Bahramian, 2009; Rabiei *et al*., 2012). According to the mentioned results, ultrasound-assisted extraction was considered as an efficient method for extracting bioactive compounds from plants. Phenolic compounds are very important plant constituents because of their scavenging ability, which is attributed to their hydroxyl (-OH) groups and the methoxy ($-OCH_3$) substituent in the molecules (Cai *et al*., 2006).

DPPH Radical Scavenging Activity

DPPH radicals are extensively used to investigate the scavenging activities of natural compounds. The percent inhibition of DPPH radicals by the pulp and seed extract and BHT as standard are shown in Figure 1. The extracts and BHT had significant scavenging activities with increasing concentration in the range of 10-200 μg ml^{-1}. The DPPH activity of pulp and seed extract was found in the range of 4.86-82.13% and 2.82-83.47%, respectively, as compared to 2.82-85.44% for BHT. The extracts had the highest antioxidant activity in the concentration of 200 μg ml^{-1}, and there were no significant differences among the antiradical activity of the extracts and BHT ($P< 0.05$) in this concentration. In the DPPH assay, antioxidant activities of pulp and seed extracts were approximately the same. Thus, the extracts of pulp and seed at 200 μg ml^{-1} concentration can be used as a natural antioxidant agent instead of BHT in food. DPPH radical scavenging activities based on EC_{50}, were 89.68 for pulp and 92.88 μg ml^{-1} for seed extract. Values of EC_{50} in the extracts of *C. elbursensis* are lower in comparison with *Crataegus pentagyna* subsp. *elburensis* fruit (349.29 μg ml^{-1}) (Ebrahimzadeh and Bahramian, 2009). In conclusion, antiradical activities of *C. elbursensis* extracts were higher than that reported for *Crataegus pentagyna* subsp. *elburensis* fruit.

Reducing Power Activity

In this assay, the reducing abilities of pulp and seed extract of *C. elbursensis* were

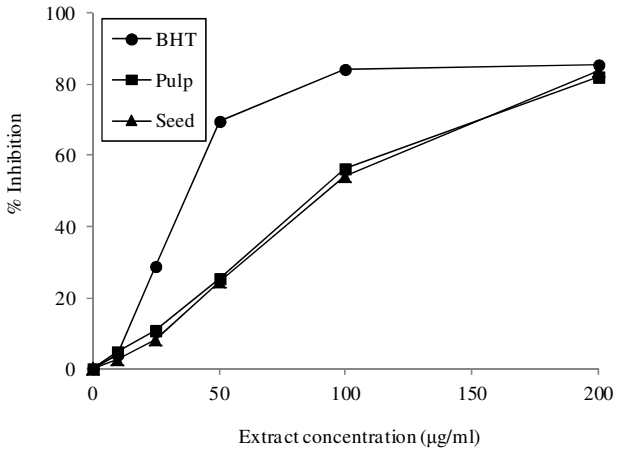

Figure 1. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activities of pulp and seed extract of *C. elbursensis*. butylated hydroxytoluene (BHT) was used as positive control.

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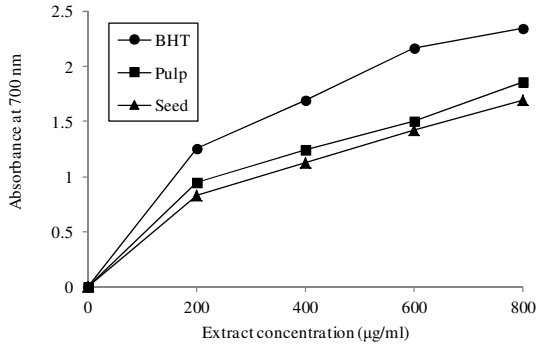

Figure 2. Reducing powers of pulp and seed extract of *C. elbursensis*. butylated hydroxytoluene (BHT) was used as positive control.

measured using the potassium ferricyanide method. The reducing properties of the extracts and BHT as standard were increased with an increase in concentrations (Figure 2). The extracts had significant reducing powers with increasing concentrations in the range of 200– 800 µg ml^{-1}. However, the reducing power of the extracts was significantly lower than that of BHT. Reducing power of the extracts of *C. elbursensis* and standard compound were in the following order: BHT> pulp extract> seed extract

Pulp extract contained higher amount of total phenolics than seed extract and was a more potent reducing agent. The results indicated that the marked reducing power of *C. elbursensis* extracts seemed to be the result of their antioxidant activity. It was reported previously that the reducing power of plant extracts generally increased with increasing sample concentration (Kil *et al*., 2009; Shukla *et al*., 2009; Sun *et al*., 2011). Biological studies showed that the extract from hawthorn (*C. pinnatifida* Bge.) possessed a strong inhibitory effect against DPPH, hydroxyl radicals, as well as strong reducing power (Liu *et al*., 2010 b). Also, in the studies reported by Ebrahimzadeh and Bahramian (2009) and Rabiei *et al*. (2012), reducing powers of the extracts increased with the increase in their concentrations, which is in good agreement with our results.

Total Antioxidant Capacity Assay

In the phosphomolybdenum assay, molybdenum VI (Mo^{6+}) is reduced to form a

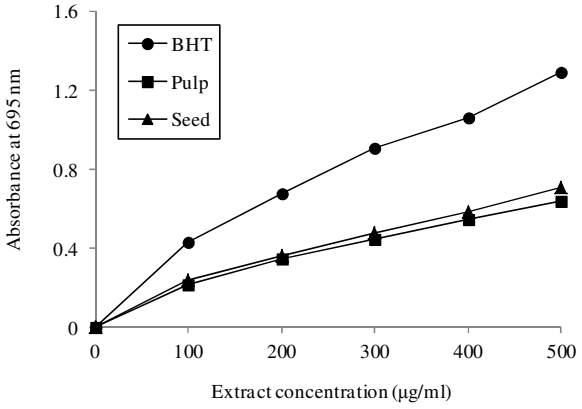

Figure 3. Total antioxidant activity of pulp and seed extract of *C. elbursensis*. butylated hydroxytoluene (BHT) was used as positive control.

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green phosphate/Mo^{5+} complex at acidic pH. This assay is a quantitative method to evaluate water-soluble and fat-soluble antioxidant capacity (total antioxidant capacity) (Arabshahi and Urooj, 2007). As shown in Figure 3, total antioxidant capacity of the extracts and BHT increased with increasing amount of sample, and all of the amounts showed high activities. However, the antioxidant activity of the extracts were found to be low ($P< 0.05$) when compared to BHT as standard. In this assay, pulp and seed extracts were found to be rather similar in their action and the sequence for antioxidant activity samples were: BHT> pulp extract≈ seed extract. According to the results of Pande and Akoh (2010), other natural compounds like tocopherols (vitamin E) along with polyphenols have shown high antioxidant activity in seeds of *Crataegus* aestivalis and *C. rufula*. The high phenolic and flavonoid contents in *C. elbursensis* extracts could be responsible for their antioxidant activity. Highly positive linear relationship exists between antioxidant activity and TPC in many spices and herbs (Arabshahi and Urooj, 2007).

Antimicrobial Activities

The results of bacteriostatic and bactericidal activity of pulp and seed extract of *C. elbursensis* fruit against four food borne and food spoilage bacteria are summarized in Table 2. The results indicated that both pulp and seed extracts displayed relatively high antibacterial activity against the four bacteria tested. Meanwhile, it was observed that antibacterial activity of pulp extract was more effective and stronger than seed extract due to high phytochemical contents of pulp extract. MIC and MBC values in this study revealed that pulp and seed extract of *C. elbursensis* tested have significant antibacterial activity. The pulp extract showed bacteriostatic and bactericidal activity against all bacteria, with MIC values ranging from 2.5 to 10 mg ml^{-1} and MBC values ranged from 5 to 20 mg ml^{-1}. As can be seen in Table 2, *C. elbursensis* seed extract showed bacteriostatic activity against all bacteria tested at concentrations ranging from 10 to 40 mg ml^{-1}. Evaluation of MBC revealed that *C. elbursensis* seed extract showed bactericidal effect against all bacteria assayed (with the exception of *Escherichia coli*). Also, for seed extract, there was no significant difference ($P< 0.05$) in MIC and MBC values for *Bacillus cereus* and *Staphylococcus aureus*. As can be seen in Table 2, *Bacillus cereus* was the most sensitive bacterium tested in pulp extract.

Our results demonstrated that phenolic contents were responsible for *C. elbursensis* antibacterial activity. Several authors have concluded that flavonoids and phenolic acids have antioxidant capacity and inhibit growth of a wide range of Gram-negative and Gram- positive bacteria and plant extracts rich in phenolic compounds have high levels of antimicrobial activity (Silici *et al*., 2010; Negi, 2012). Most studies have reported that plant extracts generally are more active against the Gram-positive bacteria than the Gram-negative bacteria (Bajpai *et al*., 2009; Weerakkody *et al*., 2010; Gutiérrez-Larraínzar *et al*., 2012). These reports are in

Table 2. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of pulp and seed extract of *C. elbursensis* against foodborne pathogens and spoilage bacteria.

Bacterial	Pulp extract		Seed extract	
	MIC (mg ml^{-1})	MBC (mg ml^{-1})	MIC (mg ml^{-1})	MBC (mg ml^{-1})
Gram-positive				
Bacillus cereus	2.5	5	10	20
Staphylococcus aureus	5	10	10	20
Gram-negative				
Escherichia coli	10	20	40	>40
Salmonella enterica	10	10	20	40

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agreement with the results of the present study. Although the antibacterial activity of many plant extracts has been studied, their antimicrobial mechanism has not been reported in great detail. This is due to the difference in cell wall structure of bacteria and type of antibacterial compounds in extracts (Kalemba and Kunicka, 2003). The main mechanism reported for antimicrobial activity of plant extracts has been membrane disruption by phenolics and metal chelating by flavonoids. In the case of phenolic compounds cell wall lysis, cytoplasmic and bacterial protein membrane damage may cause leakage of cellular ultrastructure and, as a result, cell death (Negi, 2012).

Identification of Phenolic Acids by RP-HPLC Assay

Phenolic acids of the fruit fractions are reported in Table 3. The amount of gallic acid, chlorogenic acid and caffeic acid of *C. elbursensis* fruit fractions were determined for the first time, in our study. The predominant compound detected was chlorogenic acid in both pulp and seed extract. Caffeic acid was only identified in the pulp extract. The seed extract showed higher proportion of gallic acid (0.050 mg DW^{-1}) compared to the pulp extract (0.022 mg/g DW). In general, the pulp extract showed higher phenolic acids than the seed extract. Pande and Akoh (2010) studied some phenolics of mayhaw fruit fractions including gallic and caffeic acids. These authors also found that the seed fraction was richer in gallic acid than the pulp fraction. Chlorogenic acid has been found in fruits and leaves of all hawthorn species and the most frequent phenolic acid in Chinese hawthorn fruits and caffeic acid was only seen in fruit of European hawthorn (*C. monogyna*) (Jurikova *et al*., 2012). The average contents of chlorogenic acid in mature fruits of Chinese hawthorn (*C. pinnatifida*) by a HPLC-UV method has been reported to be 0.234 mg g^{-1} FW (Cui *et al*., 2006); thus, pulp extract of *C. elbursensis* had higher amount of chlorogenic acid in comparison with *C. pinnatifida*.

CONCLUSIONS

In the present study, the antibacterial and antioxidant effect of *C. elbursensis* pulp and seed extracts were evaluated. Overall, the results supported the view that hawthorn fruit contained active antioxidants of the phenolic type with antibacterial properties. The findings clearly demonstrated that there were at least three antioxidants (namely, chlorogenic acid, gallic acid, and caffeic acid) present in hawthorn fruits. These compounds were effective in inhibiting bacterial growth and responsible for free radical scavenging activity. The results of this study showed that *C. elbursensis* extracts were rich in polyphenols with strong antioxidant and antibacterial properties, thus, suggesting the possibility of using the extracts as natural antibacterial and antioxidant agents. These properties may contribute to the reduction of the risk of chronic diseases such as cancer and cardiovascular disease and could be useful for the food and drug industry. Also, these extracts have potential to improve the shelf-life and safety of food. Further research is needed for in vivo confirmation of antimicrobial and antioxidant activities of *C. elbursensis*.

Table 3. The amounts of phenolic compounds in fruit parts (milligrams per gram dried plant).

Fruit parts	Name of compound		
	Gallic acid	Chlorogenic acid	Caffeic acid
Pulp+peel	0.022±0. 1	0.509±1.6	0.012±0.3
Seed	0.050±0.2	0.137±0. 9	nd[a]

[a] Not detected

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محتوای فنولی، ویژگی‌های ضدرادیکالی، آنتی‌اکسیدانی و ضدباکتریایی عصاره هسته و پالپ ولیک (*Crataegus elbursensis*)

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چکیده

میوه‌های ولیک به عنوان غذا و دارو برای قرن‌ها مورد استفاده قرار گرفته است. در این پژوهش محتوای ترکیبات پلی‌فنولی، فعالیت‌های ضدرادیکالی، آنتی‌اکسیدانی و ضدباکتریایی عصاره پالپ و هسته میوه‌های ولیک بومی مناطق شمال ایران (*Crataegus elbursensis*) متعلق به خانواده رزاسه مورد ارزیابی قرار گرفت. محتوای کل ترکیبات فنولی، فلاونوئیدی و آنتوسیانینی عصاره متانولی پالپ بیشتر از عصاره هسته بود. فعالیت‌های مهارکنندگی رادیکال DPPH، احیاءکنندگی آهن (III) و آنتی‌اکسیدانی کل عصاره‌ها وابسته به غلظت بود. در غلظت ۲۰۰ میکروگرم در میلی‌لیتر عصاره پالپ، هسته و BHT به ترتیب ۸۲/۱۳، ۸۳/۴۷ و ۸۵/۴۴ درصد مهارکنندگی رادیکال DPPH را نشان دادند. با این وجود، تاثیر عصاره‌ها در فعالیت آنتی‌اکسیدانی کل و قدرت احیاءکنندگی بخوبی BHT نبود. بعلاوه هر دو عصاره فعالیت ضدباکتریایی علیه چهار باکتری مورد آزمایش نشان دادند و فعالیت ضدباکتریایی عصاره پالپ بیشتر از عصاره هسته بود. همچنین اسیدهای فنولی عصاره‌ها توسط دستگاه RP-HPLC اندازه‌گیری و کلروژنیک اسید غالب‌ترین اسیدفنولی موجود در نمونه‌ها شناسایی شد. در نتیجه، نتایج نشان داد که فعالیت‌های قوی آنتی‌اکسیدانی و ضدباکتریایی عصاره پالپ و هسته *C. elbursensis* ناشی از محتوای بالای ترکیبات پلی‌فنولی آن‌ها می‌باشد.

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