Polyphenol Contents and Antioxidant Activities of Extracts from Flowers of Two *Crataegus azarolus* L. Varieties

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Abstract: Phenolic contents of the ethyl acetate extracts prepared from floral buds and opened flowers harvested on *Crataegus azarolus* trees native in two localities were performed. The antioxidant activity was measured by DPPH (2,2-diphenyl-1-picyrylhydrazyl), ABTS+ (2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) radicals scavenging using spectrophotometric method. The *C. azarolus* var. *aronia* (Willd.) Batt., producing yellow fruits, was richer in total phenols (1638.7±89.9 mg acid gallic/100 g dry weight) according to *C. azarolus* var. *eu-azarolus* Maire (1415.5±23.8 mg acid gallic/100 g dry weight), producing red ones. Ethyl acetate extract from opened flowers has less content in total phenols, proanthocyanidins and flavonoids compared to this from floral buds. Floral buds from the two *C. azarolus* varieties occurring in Siliana-Djebel Serdj showed the highest radical scavenging activities (2431.8±32.7 and 2267.7±22.7 μmol Trolox/100 g dry weight). Hawthorn from Tunisia contains eight antioxidants of phenolic type (chlorogenic acid, hyperoside, rutin, spiraeoside, isorquercitin, quercetin, (-)-epicatechin and the dimer procyanidin B2). These compounds identified especially in floral bud extracts presented a strong radical-scavenging activity.

Key words: *Crataegus azarolus* L., phenolic contents, organic extract, DPPH free radical scavenger

INTRODUCTION

Excessive production of reactive oxygen species (superoxide anion: O$_2^-$), hydrogen peroxide (H$_2$O$_2$) and hypochlorous acid (HOCl), by the organism is thought to be involved in a number of pathological phenomena including anemia, asthma, arthritis, inflammation, neurodegeneration, Parkinson’s and Alzheimer’s diseases, ageing process, atherosclerosis and cancer diseases (Bermeudez-Soto et al., 2007; Boudeg, 2007; Kao et al., 2007; Mandel et al., 2007; Seifried et al., 2007; Stangl et al., 2007). Free radicals can be scavenged through chemoprevention utilizing natural antioxidant compounds present in medicinal plants (Sabu and Kuttim, 2002).

Among plant compounds, a growing number of reports deal with the free radical scavenging and antioxidant properties of polyphenolics (Skerget et al., 2005; Katalinic et al., 2006; Zhou and Yu, 2006; Ruberto et al., 2007). Different classes of these active phenolics are found in hawthorn species (Bahorun et al., 1994, 1996, 2004; Zhang et al., 2001; Cai et al., 2004; Chang et al., 2006; Cui et al., 2006; Svedstrom et al., 2006; Urbonaviciute et al., 2006) and were used in therapy as a cardioselective drugs. In fact, the pharmacological properties of hawthorn is growing in therapeutics, since derived pharmaceuticals and extracts are known and recognized to improve the coronary blood flow and cardiac contraction in moderate heart failure not requiring a major cardiotoxic therapy, as well as in the ageing heart
(Zajie Jun, 2001; Degenring et al., 2003; Schröder et al., 2003; Veveris et al., 2004; Long et al., 2006). In France, Crataegus is also generally prescribed as a sedative to treat nervousness and sleep disorders (Bruneton, 1993; Harus et al., 2004). In China, hawthorn has also been used in herbal medicines (Cui et al., 2006). Studies indicate that Chinese hawthorn extracts have beneficial effects such as antioxidant (Zhang et al., 2001; Chu et al., 2003), anti-inflammatory (Kao et al., 2005) and also hypolipidemic effects (Zhang et al., 2002). Hawthorn extract exhibit other effects such as anticancerogen (Kao et al., 2007) and antimicrobial (Güven et al., 2006; Orhan et al., 2007) ones.

The genus Crataegus, known as Zisor in Tunisia, is represented by two species in the flora of Tunisia: C. oxyacanthus ssp. monogyna (Jacq.) Rouy and Caruso and Crataegus azarolus L. (Pottier-Alapetite, 1979). C. oxyacanthus ssp. monogyna has been often studied and it’s the major hawthorn species utilized in European Pharmacopoeia (Bahroun et al., 1994). Up to date, no analytical study has been performed on the Tunisian Crataegus species mainly Crataegus azarolus, which is represented by two varieties: Crataegus azarolus var. aronia (Willd.) Butt. and C. azarolus var. eu-azarolus Maire, they differ by the color of fruit: yellow fruits for the former and red ones for the later.

The purpose of the present study was to evaluate phenol, flavonoid and procyanidin contents of each Ethyl Acetate (E.A.) extract prepared from reproductive organs harvested on native trees of two Crataegus azarolus varieties spreading out in two localities of the Tunisian territory. Estimation of the antioxidant activities of all extracts was performed.

MATERIALS AND METHODS

Chemicals: ABTS (2,2’-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid), DPPH (2,2-diphenyl-picrylhydrazyl), Trolox C (6-hydroxy-2,5,7,8-tetramethylochroma-2carboxylic acid), potassium sulphate (di-potassium peroxdisulfate), HPLC grade of (-)-epicatechin, procyanidin dimer B2, chlorogenic acid, hydroxide, rutin, spiroasido and isoquercetin were obtained from Sigma-Aldrich (Taufen, Germany). HPLC grade of quercetin and cyanidin chloride were obtained from Extrasynthèse (Genay, France).

Plant material: Native trees of two C. azarolus varieties were spreading out at two localities; one in the centre of the country, on a mountainous region, in Siliana Village, called Serdj, the second locality was in the east coast near Hammam-Sousse Town: C. azarolus var. aronia with yellow fruits and C. azarolus var. eu-azarolus with red ones (Pottier-Alapetite, 1979).

Floral buds and opened flowers were collected in March 2004. All voucher specimens are deposited in the Higher Institute of Agronomy of Chott-Meriem, Botanic Laboratory Herbarium and were assigned for each one a corresponding number and code R401 and R404 for C. azarolus var. aronia and C. azarolus var. eu-azarolus respectively, together from Serdj; R412 and R414 for C. azarolus var. aronia and C. azarolus var. eu-azarolus, respectively, together from Hammam-Sousse (Table 1).

Extraction procedure: One hundred gram of fresh plant material was macerated in absolute methanol for 48 h at room temperature (thrice). The pooled methanol filtrates were concentrated using a vacuum rotary evaporator to eliminate the solvent. The methanolic extract was then dissolved in 500 mL of water and extracted subsequently using petroleum ether (150 mL×3), ethyl acetate (150 mL×3) and butanol (150 mL×3) in an order of increasing solvent polarity. The solvents were then evaporated to produce petroleum ether, ethyl acetate and butanol, respectively extracts. In this study, we give results related to the ethyl acetate extract.

<table>
<thead>
<tr>
<th>Abbreviations</th>
<th>Reproductive organs</th>
<th>Crataegus species and varieties</th>
<th>Locality of harvest</th>
<th>Localization, bioclimatic stage, latitude longitude</th>
</tr>
</thead>
<tbody>
<tr>
<td>F.B.a.Ši</td>
<td>Floral Buds</td>
<td>Crataegus azarolus var. aronia</td>
<td>Siliana Serdj mountain</td>
<td>Centre of Tunisia; low arid; 36°4’-9°22’</td>
</tr>
<tr>
<td>F.B.e.Ši</td>
<td>Floral Buds</td>
<td>Crataegus azarolus var. eu-azarolus</td>
<td>Siliana Serdj mountain</td>
<td>Siliana Serdj mountain; 35°51’-10°35’</td>
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<tr>
<td>O.F.a.Ši</td>
<td>Opened Flowers</td>
<td>Crataegus azarolus var. aronia</td>
<td>Siliana Serdj mountain</td>
<td>Siliana Serdj mountain; 35°51’-10°35’</td>
</tr>
<tr>
<td>O.F.e.Ši</td>
<td>Opened Flowers</td>
<td>Crataegus azarolus var. eu-azarolus</td>
<td>Hammam Sousse</td>
<td>East Coast of Tunisia; low suburban; 35°51’-10°35’</td>
</tr>
<tr>
<td>F.B.a.H.S.</td>
<td>Floral Buds</td>
<td>Crataegus azarolus var. aronia</td>
<td>Hammam Sousse</td>
<td>Hammam Sousse</td>
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<td>F.B.e.H.S.</td>
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<td>Hammam Sousse</td>
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</tbody>
</table>

Analysis of total phenols in ethyl acetate extracts: The concentration of total phenols in EA extracts was measured by UV spectrophotometry (Denway 6300), based on a calorimetric oxidation/reduction reaction. The oxidizing agent used was Folin-Ciocalteu reagent (Merck) (Singleton and Rossi, 1965; AOAC, 1984). To 50 μL of diluted extract (1 mg/1 mL of methanol) was added, in screw-capped test tubes, 750 μL of distilled water/Folin-Ciocalteu solution (28/2; v/v). After 3 min, 200 μL of
sodium carbonate (Na₂CO₃) (200 g L⁻¹) was added and the test tubes were properly shaken before incubating in boiling water bath for 1 min. The tubes were then allowed to cool in the dark. The absorbance was measured at 765 nm and results were expressed in mg of gallic acid/100 g dry weight (DW) using appropriate standard curve. For a control sample, 50 µL of methanol was used.

Analysis of proanthocyanidins in ethyl acetate extracts: The proanthocyanidins were determined by UV spectrophotometry method based on acid hydrolysis and colour formation. The HCl/butan-1-ol assay was used to quantify the total proanthocyanidins (Porter et al., 1986).

One milligram of each EA extract was dissolved in 1 mL of methanol. 0.25 mL of this solution was added 3 mL of a 95% solution of n-butanol/HCl (95/5; v/v) in stoppered test tubes followed by addition of 0.1 mL of a solution of NH₄Fe(SO₄)₂·12 H₂O in 2 M HCl (0.2%; w/v). The tubes were incubated for 40 min at 95°C. For a control sample, 0.25 mL of methanol was used. After incubation, the samples were cooled and analyzed by measuring absorbance at 540 nm. The results were expressed as mg of cyanidin chloride/100 g DW.

Analysis of total flavonoids in ethyl acetate extracts: The AlCl₃ method (Lamaison and Carnat, 1991) was adopted for the purpose of determining the total flavonoid content of the EA extracts. A quantity of each extract (0.5 mg) was dissolved in 1 mL of methanol. 0.5 mL of this solution was added equal volumes of a solution of 2% AlCl₃·6H₂O (2 g in 100 mL methanol). The mixture was thoroughly mixed and incubated for 10 min. After incubation, the samples were cooled and analyzed by measuring absorbance at 367.5 nm. The results were expressed in mg rutin equivalents/100 g DW.

High performance liquid chromatography analysis: HPLC analysis of the EA extracts was carried out using a Hewlett Packard 1500 series (Waldbronn, Germany) liquid chromatography system equipped with a vacuum degasser, quaternary pump, auto-sampler, thermostated column compartment and diode array detector. After filtration on millipore filter paper (0.22 µm) (Whatman), 20 µL of each diluted ethyl acetate extract (1 mg dry weight/1 mL of absolute methanol) were injected on a Spherisorb ODS2 RP18 (5 µm) reversed phase, C18 column (4.6 mm i.d.×150 mm) (Sigma-Aldrich, Taufkirchen, Germany) eluted by an acidified acetonitrile-water gradient. Elution with a flow rate of 0.7 mL min⁻¹ at 25°C was as follow: 0-5 min, 0-7.5% B in A; 5-13 min, 20% B in A; 13-20 min, 80% B in A; 20-25 min, 100% B in A (solvent A:

- acetonitrile/water, 1/9 v/v, pH 2.5; solvent B: acetonitrile/water, 9/1 v/v, pH 2.5). Chlorogenic acid, (-)-epicatechin and procyanidin dimer B2 were identified and quantified by comparison with authentic standards at 280 nm. Hyperoside, rutin, spiraeoside, isouqueritrin and quercetin were identified and quantified by comparison with authentic standards at 360 nm.

DPPH radical scavenging activity: Measurement of antiradical activity was adapted from Soler-Rivas et al. (2000). The extracts were diluted (1 mg dry weight mL⁻¹) using absolute methanol. Twenty microliter of ethyl acetate extract was added to 980 µL of DPPH radical (90 µM in methanolic solution) in a test tube. Methanol was used in the place of antioxidant solution as a control. The solution was immediately mixed vigorously for 10 sec by a vortex mixer and transferred to the cuvette holder of the spectrophotometer against the blank, which did not contain the extract. After a 30 min incubation period at room temperature, the absorbance was read against a blank at 515 nm. All experiments were performed in triplicate.

ABTS radical scavenging activity: ABTS radical scavenging activity was measured using a modified Re et al. (1999) method. ABTS radical cation (ABTS⁺) was produced by reacting 7 mM aqueous solution of ABTS with 2.45 mM potassium persulfate (final concentration). The reaction mixture was allowed to stand in the dark at room temperature for 12-16 h prior to use. ABTS solution was diluted with methanol to an absorbance of 0.70 (±0.02) at 734 nm. To a diluted ABTS solution (980 µL) was added 20 µL of the extract solution (1 mg dry weight/mL). The solution was immediately mixed vigorously for 10 sec by a vortex mixer and transferred to a cuvette. The absorbance was monitored at 734 nm after 6 min.

Radical-scavenging expression: For the two test (DPPH' and ABTS⁺), Trolox, the water-soluble tocopherol (Vitamin E) analog, served as standard. A concentration-response curves, for ABTS⁺ (734 nm) and for DPPH' (515 nm), as a function of different Trolox concentrations were prepared. The decrease in absorption of tested samples was used for calculating the TEAC (Trolox equivalent antioxidant capacity for the two radicals).

Statistical analysis: Simple regression analysis was performed to calculate the dose-response relationship of standard solutions used for calibration as well as test samples. Linear regression analysis was performed,
quoting the correlation coefficient \( r_p \) between antioxidant activities and concentration in phenolic classes. All results are expressed as Mean±SD of three parallel measurements. The results were processed using Microsoft Excel 2003 and the data were subjected to one way analysis of variance (ANOVA) and the significance of differences between sample means were calculated by Duncan multiple range test using SPSS for Windows (Standard Version 12.0 SPSS Inc., Chicago, IL.), p-value ≤0.05 were regarded as significant.

RESULTS

Polyphenolic contents of *Crataegus* ethyl acetate extracts prepared from floral buds and opened flowers: The total phenol, flavonoid and proanthocyanidin contents of the two varieties of *Crataegus azarolus* reproductive organ ethyl acetate extracts are shown in Table 2. The total phenol contents ranged from 931.31 to 1638.7 mg/100 g DW. The highest level was measured in floral buds (F.B.) ethyl acetate extract from *Crataegus azarolus* var. *aronia* developed in Serdj locality. Comparable and significant amounts were obtained in F.B.a.H.S. (1033.3±7.9 mg/100 g DW), F.B.e.H.S. (1023.7±32.1 mg/100 g DW), O.F.a.H.S. (1014.2±11.8 mg/100 g DW), O.F.e.H.S. (991.3±0.8 mg/100 g DW), O.F.e.Sj. (945.6±33.8 mg/100 g DW) and O.F.a.Sj. (931.1±26.6 mg/100 g DW).

Opened flowers from the two varieties from Serdj locality contained the lowest amounts. The total flavonoid contents of the eight E.A. extracts ranged from 317.8±19.2 to 753.6±54.0 mg/100 g DW. F.B.e.Sj. contained the highest total flavonoid contents. Comparable and lowest amounts were obtained in O.F.a.Sj. (317.8±19.2 mg/100 g DW), O.F.e.Sj. (346.4±29.8 mg/100 g DW), F.B.e.H.S. (358.9±0.4 mg/100 g DW) and O.F.e.H.S. (345.6±0.1 mg/100 g DW). Similar moderate levels were obtained in F.B.a.H.S. (472.7±1.4 mg/100 g DW) and O.F.a.H.S. (535.8±1.8 mg/100 g DW). Values obtained for the total proanthocyanidin contents of the eight ethyl acetate extracts varied from 520.9±0.6 to 925.3±8.1 mg/100 g DW. F.B.e.Sj. had also the highest level of total proanthocyanidin while the lowest was measured in O.F.e.H.S. Comparable levels were measured in ethyl acetate extracts from O.F.a.Sj. (673.5±10.5 mg/100 g DW), O.F.e.Sj. (640.1±19.5 mg/100 g DW) and O.F.a.H.S. (659.9±10.3 mg/100 g DW). Table 3 shows the two flavan-3-ol ((-)-epicatechin and the procyanidin B2 dimer), the chlorogenic acid and the five flavonoids (hyperoside, rutin, spiraeoside, isoquercitrin and quercetin) contents. Here (-)-epicatechin and the dimeric procyanidin B2, which were the catechins characterized in the eight ethyl acetate extracts of *Crataegus azarolus* were detected at the upper concentrations of 198.7±0.8 mg/100 g DW in F.B.a.Sj. and 55.2±0.2 mg/100 g DW in F.B.a.Sj. The summation of the amounts of (-)-epicatechin and procyanidin B2 dimer gave an indication of the catechin richness of E.A extracts from the two varieties of *Crataegus azarolus* in the following order F.B.a.Sj.>F.B.e.H.S. >F.B.e.Sj.>O.F.a.Sj.>O.F.e.H.S.>O.F.e.Sj. Chlorogenic acid was the main phenolic acid in ethyl acetate extract found at the upper concentration of 244.1±0.6 mg/100 g DW in F.B.a.Sj. The lowest level is 16.6±0.1 mg/100 g DW (O.F.e.H.S.). The concentrations of hyperoside, rutin, spiraeoside, quercetin and isoquercitrin ranged from 24.7±0.0 to 412.2±0.1 mg/100 g DW; 9.7±0.1 to 198.3±0.6 mg/100 g DW; 65.0±0.2 to 245.9±0.6 mg/100 g DW; 1.8±0.0 to 72.5±0.3 mg/100 g DW and 6.2±0.0 to 101.9±0.2 mg/100 g DW, respectively (Table 3). F.B.a.Sj. present the highest amount of the different individual polyphenols except for hyperoside.

<table>
<thead>
<tr>
<th>Reproductive organ and variety</th>
<th>Total phenol (mg gallic acid/100 g DW)</th>
<th>Total flavonoids (mg rutin/100 g DW)</th>
<th>Total proanthocyanidins (mg chlorogenic acid/100 g DW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F.B.a.Sj.</td>
<td>1638.7±89.9g</td>
<td>640.3±42.4g</td>
<td>922.1±10.9g</td>
</tr>
<tr>
<td>F.B.e.Sj.</td>
<td>1415.5±23.8g</td>
<td>753.6±54.0g</td>
<td>925.3±38.1g</td>
</tr>
<tr>
<td>O.F.a.Sj.</td>
<td>931.1±26.6g</td>
<td>317.8±19.2g</td>
<td>673.5±10.5g</td>
</tr>
<tr>
<td>O.F.e.Sj.</td>
<td>45.6±33.8g</td>
<td>346.4±29.8g</td>
<td>640.1±19.5g</td>
</tr>
<tr>
<td>F.B.a.H.S.</td>
<td>1033.3±7.9g</td>
<td>472.7±1.4g</td>
<td>897.3±6.8g</td>
</tr>
<tr>
<td>F.B.e.H.S.</td>
<td>1023.7±32.1g</td>
<td>358.9±0.4g</td>
<td>850.2±15.1g</td>
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<tr>
<td>O.F.a.H.S.</td>
<td>1014.2±11.8g</td>
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<tr>
<td>O.F.e.H.S.</td>
<td>991.3±0.8g</td>
<td>345.6±0.1g</td>
<td>520.9±6.6g</td>
</tr>
</tbody>
</table>

DW: Dry weight; Data are expressed as Mean±SD of three measurements. In the same column, contents with same superscript letter(s) are not significantly different at p<0.05.

663
Antioxidant capacities of *Craconteus* ethyl acetate extracts from floral buds and opened flowers: The TEAC<sub>eff</sub> values ranged from 317.6±12.2 to 1681.4±58.6 μmol/100 g DW and TEAC<sub>ARTS</sub> values from 966.2±10.4 to 2431.8±32.7 μmol/100 g DW. The F.B.a.Sj. have the highest TEAC<sub>eff</sub> and TEAC<sub>ARTS</sub> values while O.F. from *C. azarolus* var. *azarolus* developed in Sj. show weak free radical scavenging potentials (Table 4). Both TEAC<sub>eff</sub> and TEAC<sub>ARTS</sub> assays show similar trend in antioxidant potentials (r = 0.99) (Table 5). There was a strong correlation between antioxidant activities and total phenol contents (TEAC<sub>eff</sub>: r = 0.91; TEAC<sub>ARTS</sub>: r = 0.89) and with total flavonoids contents (TEAC<sub>eff</sub>: r = 0.88; TEAC<sub>ARTS</sub>: r = 0.88).

Total proanthocyanidins contents influenced the antioxidant potency of the *Craconteus* ethyl acetate extract (TEAC<sub>eff</sub>: r = 0.84; TEAC<sub>ARTS</sub>: r = 0.83). Regression correlation coefficients also show important contribution in the antioxidant activity of the contents of each phenolic compound identified of the E.A. extract (i.e., for procyanidin B2 dimer, TEAC<sub>eff</sub>: r = 0.85 and TEAC<sub>ARTS</sub>: r = 0.86), for isocoumarin, TEAC<sub>eff</sub>: r = 0.78; TEAC<sub>ARTS</sub>: r = 0.99). Within the antioxidant capacities (according to TEAC<sub>ARTS</sub>) of ethyl acetate extracts, we can classify the reproductive organs in the following order F.B.a.Sj. > F.B.e.Sj. > F.B.a.H.S. > O.F.a.H.S. > O.F.e.Sj. > O.F.e.H.S. > O.F.e.H.S.

**DISCUSSION**

The medicinal use of *Craconteus* has a long tradition with written records dating back to ancient Roman times (Verderis et al., 2004). The constituents of *Craconteus* have been the subject of intensive investigations for a long time (Shah et al., 1995; Bahour, et al., 1996; Cui et al., 2006; Sokol-Lewotska et al., 2007) but intention has not been directed at *Craconteus azarolus*. In fact, a few studies were interested for the polyphenolic composition of *Craconteus azarolus* and its antioxidant activity (Kerly et al., 1987; Twaij et al., 1987; Ljubuncic et al., 2005). Present results show that ethyl acetate extracts obtained from *Craconteus azarolus* var. *azarolus* (with yellow fruits) are richer in polyphenols than those from *Craconteus azarolus* var. *eu-azarolus* (with red fruits). Whatever was the variety and the region of harvest, the floral buds are richer in polyphenols than opened flowers.
this is in accordance with results reported by Bahorun et al. (1994) on Crataegus monogyna. Nevertheless, Crataegus azarolus ethyl acetate extract present lower contents in total phenols, total flavonoids and total proanthocyanidins in regard to the same extract prepared from Crataegus monogyna (Bahorun et al., 1994). This difference could be attributed to genotypic (different species), locality and/or to extraction protocol.

HPLC analysis of ethyl acetate extracts of Crataegus azarolus, demonstrate the presence of chlorogenic acid as the main phenolic acid. It’s in accordance with studies of Bahorun et al. (1994) and of Urbanaviciute et al. (2006) realized on Crataegus monogyna and with those done by Zhang et al. (2001), Cai et al. (2004, 2006) on Crataegus pinnatifida. Ethyl acetate Crataegus azarolus extract present lower amount in this phenolic acid compared with other species, such as C. monogyna which present 322 mg/100 g DW chlorogenic acid in floral buds and 69 mg/100 g DW in opened flowers (Bahorun et al., 1994). Contrast results in amount of this phenolic acid in C. pinnatifida fruits were identified. In fact, Zhang et al. (2001) demonstrate the presence of 64.9 mg/100 g DW, when Cai et al. (2006) suggest the presence of 1210 mg/100 g DW.

Hyperoside is the main flavonoid detected in ethyl acetate reproductive organ extracts of Crataegus azarolus for the two varieties. This is in accordance with all studies in hawthorn species (Bahorun et al., 1994; Zhang et al., 2001; Cai et al., 2004; Cui et al., 2006; Urbanaviciute et al., 2006). According to Bahorun et al. (1994), ethyl acetate extract of Crataegus monogyna floral buds present 547 mg/100 g DW hyperoside. This amount is higher than this found in this study (maximum of 410.7±0.6 mg/100 g DW). On the other hand, ethyl acetate extract of Crataegus pinnatifida fruits contains 24.6 mg/100 g DW (Zhang et al., 2001) and 280 mg/100 g DW hyperoside (Cui et al., 2006). Rutin and spiranoside were detected with higher amounts than in other species of Crataegus such as C. monogyna and C. pinnatifida. In fact, Zhang et al. (2001), demonstrate the presence of 2.6 mg/100 g DW rutin in ethyl acetate fruit extract.

The dimer procyanidin B2 and (−)-epicatechin are the main catechins detected in Crataegus azarolus ethyl acetate extracts. It’s in accordance with earlier studies on ethyl acetate extract of Crataegus monogyna (Bahorun et al., 1994; Urbanaviciute et al., 2006), Crataegus pinnatifida (Zhang et al., 2001; Cai et al., 2004; Cui et al., 2006), Crataegus laevigata (Svedstrom et al., 2006) and Crataegus oxyacantha (Svedstrom et al., 2006; Sokoł-Łetowska et al., 2007). Amounts of (−)-epicatechin and procyanidin B2 dimer in ethylacetate extract of C. monogyna floral buds were 884 mg/100 g DW and 135 mg/100 g DW respectively, whereas opened flowers contains 157 mg/100 g DW and 44 mg/100 g DW (Bahorun et al., 1994). Ethyl acetate extract of Crataegus pinnatifida fruits contains 178.3 mg/100 g DW according to Zhang et al. (2001) but according to Cui et al. (2006) this extract contains 9860 mg/100 g DW of (−)-epicatechin and 5900 mg/100 g DW of procyanidin B2 dimer.

We have observed rapid and strong inhibition of both DPPH and ABTS radicals after the addition of ethyl acetate extracts from floral buds and opened flowers of Crataegus azarolus showing a high antioxidant activity of those extracts. Floral buds present the higher antioxidant activity. This is in accordance with Bahorun et al. (1994) study, where authors demonstrate that floral buds present the higher percent of inhibition of malondialdehyde formation compared to opened flowers. Ljubuncic et al. (2005) demonstrated that a decoction of leaf and green fruit of Crataegus azarolus var. aronia inhibit β-carotene and plasmatic oxidation, lipidic peroxidation and scavenged the radical O•−. Zhang et al. (2001) demonstrated the inhibition of Low Density Lipoprotein (LDL) peroxidation with ethyl acetate fraction of Crataegus pinnatifida fruits. Cui et al. (2006) demonstrated the oxygen radical scavenging capacity and enzyme inhibition of ethyl acetate fraction from Crataegus pinnatifida fruits. We demonstrate that ethyl acetate extract from Crataegus azarolus var. aronia floral buds (F.B.a.) present a high antioxidant capacity (1681.4±58.6 μmol Trolox/100 g DW) compared to tomato (from 5.4 to 20.9 μmol Trolox/g DW), to potato (from 2.3 to 9.9 mol Trolox/g DW) (Zhou and Yu, 2006) and to lettuce (1.14 μmol Trolox/g DW) extracts (Luximon-Ramma et al., 2005). According to those researchers, fresh tea leaves extract present an antioxidant activity equivalent to 1637 μmol Trolox/g DW. Roberto et al. (2007) studies suggest similar antioxidant activity (1.58 mmol Trolox/g DW) for Nero d’Avola grape pomace extract.

Strong antioxidant capacity of Crataegus azarolus EA extracts has been related mainly to chlorogenic acid and hyperoside (Bahorun et al., 1994; Zhang et al., 2001), to (−)-epicatechin and procyanidin B2 dimer (Bahorun et al., 1994; Zhang et al., 2001; Cui et al., 2006) and to rutin, quercetin and isoquercitrin (Zhang et al., 2001). Ethyl acetate extract of Tunisian
*Craeagus azarolus* presents all this compounds and we demonstrate a strong correlation with all these compounds and the antioxidant capacity of this extract.

In conclusion, the present study supported the view that hawthorn contains antioxidants of phenolic type. The HPLC analysis led to identify eight antioxidants (chlorogenic acid, hyperoside, rutin, spiracoside, isoquercitrin, queretin, (-)-epicatechin and the dimer procyanidin B2). These compounds presented a strong radical-scavenging activity. Floral bud extracts could be used in clinical trials to study the modulation of risk factors of cardiovascular diseases, diabetes, cancer and neurodegenerative diseases.

**REFERENCES**


