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Comparative study between homemade and commercial hawthorn (*Crataegus* spp.) extracts regarding their phenolic profile and antioxidant activity

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Abstract: *Crataegus* species (hawthorn) have been commonly used in traditional medicine, especially for the treatment of congestive heart failure. Many studies confirmed that they are rich in polyphenols, thus exhibiting strong antioxidant activity, which contribute to the beneficial effects of hawthorn on the cardiovascular system. In the market, there are many herbal medicinal products based on hawthorn, which consumption as adjuvant therapy in heart-related issues is supported by European Medicines Agency. Since there is a global trend of making homemade herbal preparations, this study aimed to compare whether there is a difference in polyphenol profile and antioxidant potential between homemade and commercial ethanol extracts of hawthorn. Polyphenol profile was evaluated by determination of total phenolic and flavonoid contents, and by quantitative analysis of selected polyphenols by liquid chromatography–mass spectrometry/mass spectrometry. Antioxidant potential was examined by 2,2-diphenyl-1-picrylhydrazyl, ferric ion reducing antioxidant power and lipid peroxidation inhibition assays. The results of this study suggest that homemade ethanol extracts of hawthorn flowers, leaves and fruits are just as good source of polyphenols and antioxidants as commercial ones, and their utilization should be supported. Furthermore, hawthorn extracts made of leaves and flowers are better source of bioactive polyphenols and have higher antioxidant activity compared with the same of fruits, regardless of the method of preparation.

Keywords: polyphenols; LC-MS/MS; DPPH; FRAP; lipid peroxidation.

INTRODUCTION

Crataegus spp. are commonly known as hawthorn. It is a large genus of shrubs, belonging to the Rosaceae family, which comprises around 280 species. Hawthorn plants grow in areas with mild climate in Europe, Asia and North

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America.[1] In Europe and North America, the most common species are *Crataegus monogyna* Jacq, *C. laevigata* Poir (syn. *C. oxiacantha* L.) and *C. pentagyna* Waldst, while in China the most frequent species is *C. pinnatifida* Bge.[2]

Thanks to its medicinal properties, hawthorn species, particularly its flowers, leaves and fruits, have a long history of usage in the folk medicine of many countries. Traditionally, hawthorn preparations have been used to treat congestive heart failure, a disease defined by the inability of a weakened heart to pump blood well and to effectively provide oxygen and nutrients to peripheral tissues. Nowadays, numerous *in vivo* and *in vitro* studies confirmed different biological activities of *Crataegus* species, including antioxidant, antimutagenic, antimicrobial, hypolipidemic, hypoglycemic, immunostimulatory, hepatoprotective, cardiotonic, antihypertensive, diuretic, *etc*., which all can be associated with its traditionally recognized heart-protective benefits. The listed beneficial medicinal properties of hawthorn species can be attributed to a variety of bioactive compounds, including polyphenols, organic acids and amines, whose presence in hawthorn is confirmed in previous studies.[1–5]

Numerous herbal medicinal products made from hawthorn, mainly for treating heart failure, are available on the worldwide market. Hawthorn flowers and leaves can be found in various herbal preparations, such are: comminuted or powdered herbal substance, dry or liquid extracts, tinctures or expressed juices from fresh herbal material.[6] Also, different pharmaceutical forms of hawthorn preparations, including herbal teas or solid and liquid dosage forms for oral use are present. They may be sold as authorized prescription pharmaceuticals, over-the-counter (OTC) medications, authorized herbal medicinal items, dietary supplements or unregulated herbal remedies. They are often registered and sold as herbal or traditional herbal medicinal products, herbal dietary supplements or unregulated herbal remedies. Even though the products from hawthorn flowers & leaves have been granted a positive monograph by the European Medicines Agency (EMA), its latest guidelines strongly recommend against employing hawthorn preparations as monotherapy in heart failure. Instead, they should be used as an adjuvant therapy.[7] Specifically, the Committee on Herbal Medicinal Products (HMPC) of the EMA concluded that hawthorn flower and leaf preparations can be used alone to ease symptoms of transitory cardiac complaints associated with anxiousness, such as palpitations, only once serious heart conditions have been excluded by a medical expert. They can also be applied to alleviate moderate symptoms of stress and to improve sleep.[6] In addition, due to the rich occurrence of bioactive polyphenols, phytopreparations made of hawthorn could also be used as antioxidants for alleviating oxidative stress.[8]

One of the most popular products made of hawthorn use on the market are liquid herbal extracts, obtained from various plant parts (*e.g.* flower, leaf and

fruit) made by hydroalcoholic (methanol or ethanol) or water-based extraction. They are known as hawthorn tinctures or drops. According to the official European Pharmacopoeia, these liquid extracts can be made by extraction with ethanol (30–70 vol. %) and are standardized to a minimum of 0.8–3.0 % of flavonoids, expressed as hyperoside.[9,10] On the other side, in the German Pharmacopoeia, the liquid extract (*Crataegi extractum fluidum*) is officinal preparation, standardized to 0.25–0.5 % of flavonoids, calculated to hyperoside.[11]

Nowadays, trend of consuming herbal extracts, such as liquid herbal extracts, is growing. Namely, the global herbal extract market is forecasted to experience a compound annual growth rate (CAGR) of 6.63 % during the forecast period 2022–2027.[12] In parallel, a trend of making homemade products, including herbal preparations, is rising. Globally, do-it-yourself (DIY) culture is developing in many areas, due to different reasons such as: products costs rising, consumer mistrust in the quality of ingredients used in the industrial production processes as well as in medical approaches and health professionals, and a growing trend among consumers to turn to a more natural and simple way of life. Also, making one's own product can be a type of hobby that causes a feeling of joy upon preparing something beneficial for oneself.[13,14] Bearing all the above mentioned in mind, the aim of this study was to reveal is there a difference in polyphenol quantitative and qualitative composition and antioxidant potential between homemade and commercially available hawthorn (*Crataegus* spp.) herbal ethanol extracts, made from hawthorn fruits or a mixture of flowers and leaves. The end purpose of this study was to conclude whether homemade hawthorn herbal ethanol extracts have sufficient quality and if their production by individuals should be encouraged and promoted.

EXPERIMENTAL

Extract preparation

A commercially available plant material of European origin was purchased in health food stores in Novi Sad, Serbia. The first material was a mixture of dried comminuted flowers and leaves, while the second one was dried hawthorn fruits. Each plant material was purchased from three different stores (different producers) and mixed in order to obtain composite sample, which was used for extracts preparation by maceration. On 30 g of dry flowers and leaves 150 mL of 70 % aqueous EtOH was added and maceration was performed overnight at room temperature. Afterwards, the extract was filtered and maceration was repeated one more time on plant leftovers following the same procedure. This extract is named homemade flowers and leaves extract (HMFL). For the purpose of further analysis, extract was evaporated to dryness *in vacuo* (35 °C), after which dry extract was dissolved in 50 % aqueous EtOH to the final concentrations of 200 mg/mL. The same procedure was repeated for preparation of fruit extract, except that 10 g of dry fruits was mixed with 50 mL of 70 % aqueous EtOH. This extract is named homemade fruit extract (HMF).

The samples of two types of commercial extracts of *Crataegus* spp., produced by Institute for medicinal plants research "Dr Josif Pančić", were purchased in pharmacies in Novi

Sad, Serbia. The first type of extract was 56 % aqueous EtOH extract of flowers and leaves (labelled as CFL) and the second was aqueous-EtOH extract of fruits (CF). The commercial extracts were evaporated to dryness *in vacuo* (35 °C) and dry extracts were dissolved in 50 % aqueous EtOH to the final concentration of 200 mg/mL.

Chemical characterization of homemade and commercial hawthorn extracts

Chemical characterization of extracts was done by determination of total phenolic and flavonoid contents, as well as quantitative liquid chromatography–mass spectrometry/mass spectrometry (LC–MS/MS) analysis of 45 selected compounds. Total phenolic content was determined according to Lesjak *et al*. by the Folin–Ciocalteu method.[15] Results were expressed as mg of gallic acid equivalents per g of dry weight (mg GAE/g dw). Total flavonoid content was determined according to Lesjak *et al*. using the colorimetric method and results were expressed as mg of quercetin equivalents per g of dry weight (mg QE/g dw).[15]

The content of quinic acid and other 44 selected phenolic compounds (14 phenolic acids, 25 flavonoids, 2 lignans and 3 coumarins) was determined by LC–MS/MS according to the previously reported method.[16] Standards of the compounds were purchased from Sigma–Aldrich Chem, Fluka Chemie GmbH or from ChromaDex (Santa Ana, CA, USA). Samples and standards were analyzed using Agilent Technologies 1200 Series high-performance liquid chromatograph coupled with Agilent Technologies 6410A Triple Quad tandem mass spectrometer with electrospray ion source, and controlled by Agilent Technologies MassHunter Workstation software – Data Acquisition (ver. B.03.00). All extracts were diluted with 50 % aqueous MeOH to the concentrations of 20 and 0.1 mg/mL. The sample (5 μL) was injected into the system, and compounds were separated on Zorbax Eclipse XDB-C18 (50 mm×4.6 mm, 1.8 μm) rapid resolution column. Data were acquired in dynamic multiple reaction monitoring (MRM) mode, peak areas were determined using Agilent MassHunter Workstation Software – Qualitative Analysis (ver. B.06.00). Calibration curves were plotted by OriginLabs Origin Pro (ver. 2019b) software and used for calculation of investigated compounds concentration in the extracts. Results are expressed as μg per g of dry weight (μg/g dw).

Determination of antioxidant potential of homemade and commercial hawthorn extracts

Antioxidant potential was evaluated by 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, ferric reducing power assay (FRAP) and inhibition of lipid peroxidation (LP) assay. DPPH assay was performed according to Lesjak *et al.* The results were expressed as IC_{50} (mg/mL).[15] FRAP assay was performed according to Lesjak *et al.* and results were expressed as mg of ascorbic acid equivalents per g of dry weight (mg AAE/g dw).[15] The ability of extracts to inhibit LP was determined by TBA assay according to Pintać *et al*.[17] Results were expressed as IC_{50} (mg/mL).

Statistical analysis

The percent of inhibition achieved by different extract concentrations was calculated with the following equation: I (%) = 100(1–A/A_0), where A was the absorbance of the investigated extracts corrected for the absorbance of the blank probe, and A_0 was the absorbance of the control. The IC_{50} values were determined by plotting inhibition–concentration curves in the OriginLabs Origin Pro (ver. 2019b) software. Results were expressed as mean ± standard deviation (*SD*) of three replicates. A comparison of the group means and the significant difference between the groups was verified by one-factor ANOVA followed by Tukey's HSD post-hock test ($p \leq 0.05$). This was performed in Excel using the add-in Xrealstats from Real Statistics.

RESULTS AND DISCUSION

Chemical composition of homemade and commercially available hawthorn extracts

Phenolic profile of hawthorn extracts was determined by measuring the total phenolic and flavonoid contents, as well as by quantitative LC–MS/MS analysis of selected compounds.

The total phenolic content was in the range of 17.0–190 mg GAE/g dw, while the total flavonoid content was in the range of 1.4–48.0 mg QE/g dw (Fig. 1). Flowers and leaves extracts had a significantly higher content of both total phenolic and flavonoid contents compared with fruit extracts, while homemade HMFL extract had the highest amount of both total phenolic and flavonoid contents among all examined samples. There was no significant difference in the contents of total phenolics and flavonoids between HMF and CF. The same conclusions can be made if TPC and TFC are calculated to a daily dose of the extracts (60 drops = 3 mL). If HMFL extract is consumed, TPC in a daily dose is 26.8 mg GAE, while with CFL extract it is 20.1 mg GAE. In the case of TFC, the daily intake through HMFL extract will be 6.77 mg QE, and through CFL extract 3.36 mg QE, which is twice lower. The TPC and TFC in a daily dose of fruit extracts were significantly higher in HMF compared to CF (2.98 mg GAE for HMF and 1.80 mg GAE for CF; 0.25 mg QE for HMF and 0.175 mg QE for CF).

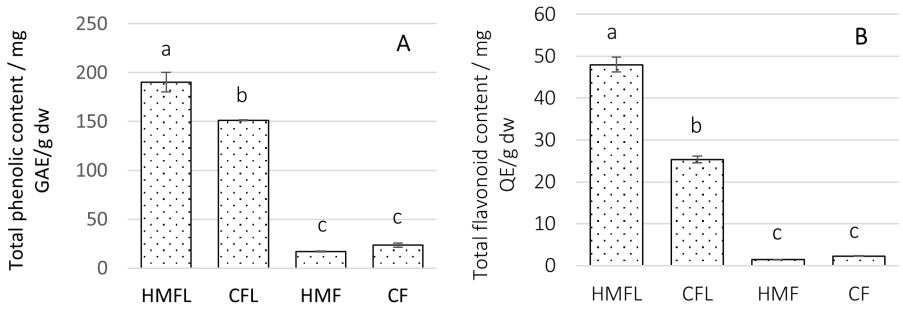

Fig. 1. Total phenolic (A) and flavonoid (B) contents in homemade and commercial hawthorn extracts. HMFL - homemade flowers and leaves extract, CFL – commercial flowers and leaves extract, HMF – homemade fruit extract, CF – commercial fruit extract. Letters a–c denote a significant difference between samples ($p \leq 0.05$).

The results of the LC–MS/MS analysis revealed that 36, out of 45 analyzed compounds, were present in examined hawthorn extracts (Table I). Quinic acid, an intermediate in biosynthesis of chlorogenic acids, was the most abundant, especially in flowers and leaves extracts. Most phenolic acids and flavonoids were present in significantly higher amounts in flowers and leaves extracts compared with extracts of fruits. Analyzed but not detected compounds were: umbel-

liferon, *o*-coumaric acid, 3,4-dimethoxicinnamic acid, daidzein, genistein, matairesinol, secoisolariciresinol, baicalin, epigallocatechin gallate.

TABLE I. The content of selected phenolic compounds and quinic acid (μg/g dw) in homemade and commercially available hawthorn extracts analyzed by LC–MS/MS; abbreviations: HMFL – homemade flowers and leaves extract, CFL – commercial flowers and leaves extract, HMF – homemade fruit extract, CF – commercial fruit extract. Letters a–d denote a significant difference between samples ($p \leq 0.05$)

Compound	HMFL	CFL	HMF	CF
Quinic acid	60680±6068^{b}	75730±7573^{a}	22450±2245^{c}	23700±2370^{c}
Hydroxybenzoic acids				
p-Hydroxybenzoic acid	132.4±7.945^{a}	97.28±5.837^{b}	19.26±1.156^{d}	58.92±3.535^{c}
Gentisic acid	3.679±0.294^{a}	/^{e}	2.460±0.197^{b}	/
Protocatechuic acid	46.21±3.697^{c}	85.99±6.879^{b}	63.65±5.092^{c}	169.6±13.57^{a}
Gallic acid	2.811±0.253^{c}	7.338±0.660^{a}	<2.450^{f}	6.126±0.551^{b}
Vanillic acid	61.23±18.37^{a}	22.62±6.785^{bc}	43.79±13.14^{ab}	11.22±3.366^{c}
Syringic acid	4.557±0.911^{c}	4.935±0.987^{c}	14.89±2.978^{b}	23.75±4.750^{a}
Hydroxycinnamic acids				
Cinnamic acid	40.46±8.091^{a}	50.59±10.119^{a}	5.836±1.167^{b}	5.937±1.187^{b}
p-Coumaric acid	16.28±1.465^{bc}	75.52±6.796^{a}	11.64±1.048^{c}	23.34±2.100^{b}
Caffeic acid	20.65±1.446^{b}	57.45±4.021^{a}	4.949±0.346^{d}	11.27±0.789^{c}
Ferulic acid	7.629±0.763^{b}	12.94±1.294^{a}	4.899±0.490^{c}	11.08±1.108^{a}
Sinapic acid	/	0.852±0.085^{c}	1.481±0.148^{b}	2.363±0.236^{a}
5-*O*-Caffeoylquinic acid	14020±701^{b}	23500±1175^{a}	159.3±7.966^{c}	185.7±9.284^{c}
Coumarins				
Esculetin	2.303±0.138^{c}	4.087±0.245^{a}	0.495±0.030^{d}	3.599±0.216^{b}
Scopoletin	<0.600	<0.600	/	/
Flavonols				
Quercetin	104.6±31.38^{b}	245.2±73.56^{a}	39.26±11.78^{d}	47.62±14.29^{d}
Rutin	2309±69^{b}	3529±106^{a}	100.8±3.023^{c}	140.8±4.224^{c}
Quercetin 3-*O*-glucoside + quercetin 3-*O*-galactoside	13640±818^{a}	11040±663^{b}	1901±114^{c}	2931±176^{c}
Quercitrin	8.940±0.536^{a}	4.086±0.245^{b}	0.525±0.031^{c}	0.464±0.028^{c}
Isorhamnetin	3.534±0.212^{b}	6.385±0.383^{a}	2.039±0.122^{c}	3.545±0.213^{d}
Kaempferol	13.15±0.920^{a}	15.00±1.050^{a}	3.418±0.239^{c}	8.538±0.598^{b}
Kaempferol 3-*O*-glucoside	1227±086^{a}	951.7±66.62^{b}	66.23±4.636^{c}	109.5±7.665^{c}
Flavones				
Baicalein	2.068±0.621^{a}	2.616±0.785^{a}	/	/
Amentoflavone	<0.600	/	/	<0.600
Luteolin	8.268±0.413^{b}	21.31±1.066^{a}	1.213±0.061^{c}	2.356±0.118^{c}
Luteolin 7-*O*-glucoside	190.1±5.702^{a}	140.0±4.199^{b}	1.332±0.040^{c}	2.527±0.076^{c}
Apigenin	1.672±0.117^{b}	8.090±0.566^{a}	<0.075	0.146±0.010^{c}
Apigenin 7-*O*-glucoside	31.94±1.597^{a}	9.866±0.493^{b}	<0.075	0.198±0.010^{c}
Vitexin	88.77±4.438^{a}	93.93±4.696^{a}	5.978±0.299^{b}	13.02±0.651^{b}
Apiin	<0.075	0.698±0.035^{a}	<0.075	<0.075
Chrysoeriol	0.949±0.028^{b}	8.533±0.256^{a}	0.267±0.008^{c}	0.926±0.028^{b}

TABLE I. Continued

Compound	HMFL	CFL	HMF	CF
Flavones				
Myricetin	<9.750	<9.750	<9.750	<9.750
Flavanones				
Naringenin	46.42±3.249[b]	72.42±5.070[a]	1.756±0.123[c]	3.784±0.265[c]
Flavan-3-ols				
Catechin	18.10±1.810[a]	/	8.686±0.869[b]	8.818±0.882[b]
Epicatechin	3426±343[a]	1720±172[b]	398.8±39.88[c]	240.9±24.09[c]

[e]Concentration is below the instrument's limit of detection (*LOD*); [f]concentration is below the limit of quantification (*LOQ*)

The amounts of selected polyphenols quantified by LC–MS/MS calculated to a daily dose of examined hawthorn extracts are available from authors upon request. The amount of examined phenolic acids and flavonoids in a daily dose of HMFL and CFL were significantly higher than the same in a daily dose of HMF and CF.

Antioxidant activity of homemade and commercially available hawthorn extracts

The antioxidant potential of hawthorn extracts was evaluated by DPPH, FRAP and LP assays, and the results are shown in Fig. 2.

From the results it can be seen that all samples displayed some level of antioxidant activity. Flowers and leaves extracts had significantly higher antioxidant potential compared to fruit extracts. In the FRAP assay, antioxidant activity was in the range of 10.1–100.8 mg AAE/g dw, with the highest activity shown by HMFL. In the DPPH assay, IC_{50} was in the range of 0.03–0.41 mg/mL, with the highest activity expressed by HMFL and CFL. Compared to the standard antioxidant butylated hydroxytoluene (BHT), with IC_{50} of 0.009 mg/mL[17], hawthorn extracts were less potent quencher of DPPH radicals. All hawthorn samples inhibited LP, and IC_{50} was in the range of 0.14–1.56 mg/mL. The highest inhibitory activity towards LP was expressed by HMFL and CFL, while fruit extracts were significantly less potent inhibitors. Compared to BHT, with IC_{50} of 0.001 mg/mL,[17] hawthorn extracts were significantly less potent inhibitors of LP. However, HMFL and CFL, but not HMF and CF, were more potent compared to propyl gallate (PG), with IC_{50} of 0.05 mg/mL.[17]

According to the results obtained in this study, homemade extract of flowers and leaves of hawthorn had the highest TPC and TFC, as well as content of individual polyphenols determined by LC–MS/MS, among all examined samples. Furthermore, both HMFL and CFL contained higher amounts of polyphenols than fruit extracts, which is in accordance with the findings of other researchers mentioned below. Also, even though extracts examined in this study can be regarded as rich sources of polyphenols, some other researchers reported higher con-

tents of total phenolics and flavonoids, as well as individual polyphenols. These differences could be a consequence of different geographical origin of the samples, different *Crataegus* species and chemotypes, or different extraction procedures. In addition, to our knowledge, there was no literature data regarding evaluation of polyphenol composition and antioxidant activity of commercial preparations made of hawthorn, just ones prepared in the laboratory.

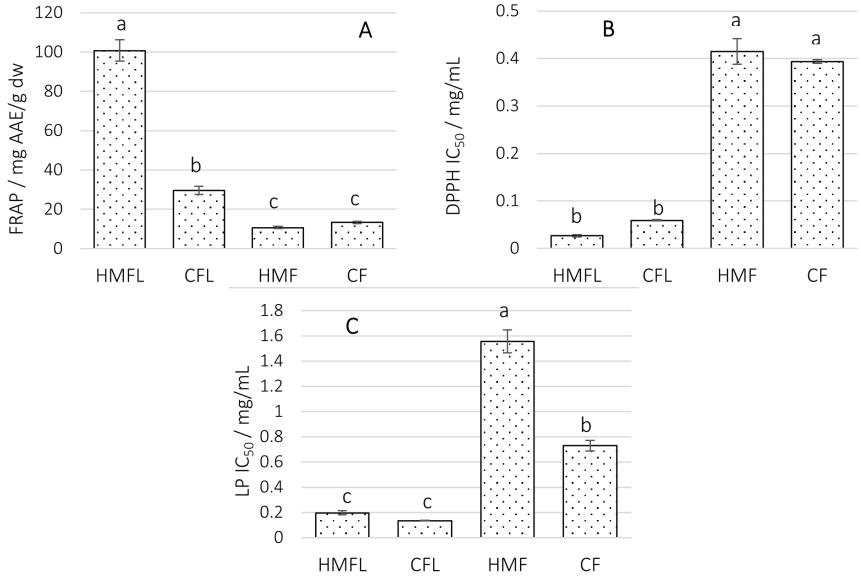

Fig. 2. Antioxidant activity of homemade and commercial hawthorn extracts; A – FRAP results, B – DPPH assay results and C – LP inhibition assay results. HMFL – homemade flowers and leaves extract, CFL – commercial flowers and leaves extract, HMF – homemade fruit extract and CF – commercial fruit extract. Letters a–c denote a significant difference between samples ($p \leq 0.05$).

Namely, in the available literature, there is abundance of data regarding the total phenolic and flavonoid contents of EtOH hawthorn extracts. Specifically, it was reported that leaf EtOH extract of *C. monogyna* contains 473.4 mg GAE/g dw of total phenols and 80.9 mg QE/g dw of total flavonoids.[18] Also, it was determined that total phenolic content in fruit extracts of different *Crataegus* spp. grown in Turkey was in the range of 360–380 mg GAE/g of dried fruit, while total flavonoid content was in the range of 13–20 mg QE/g dw.[3] Furthermore, the total phenolic content in EtOH extract made of mixture of *C. monogyna* and *C. oxycantha* fruits grown in Serbia, was 35.4 mg GAE/g dw as published by Tadić *et al.*[19] Zhang and collaborators determined that the total phenolic and flavonoids contents in ethanol extract of fruits of *C. pinnatifida* grown in China was 65 and 63 mg GAE/g dw, respectively,[20] while total phenolic content in methanol ext-

ract of dry aerial parts (flowering tops or flowers with young leaves) was 562.9 mg GAE/g dw and in dry fruit methanol extract 128.2 mg GAE/g dw, both from *C. monogyna* grown in France, as publishes by Froechlicher *et al*.[21]

LC–MS/MS analysis in the present study has shown that ethanolic flowers and leaves extracts, especially homemade extract, contain high amounts of chlorogenic acid, rutin, quercetin 3-*O*-glucoside and hyperoside, kaempferol 3-*O*-glucoside and epicatechin. Amounts of these compounds were significantly lower in fruit extracts. Also, different HPLC methods were performed for analysis of polyphenols in hawthorn extracts in previous studies. Specifically, Bardakci *et al.* detected epicatechin, vitexin, isoorientin, hyperoside, chlorogenic acid and quercetin in fruit extracts of different *Crataegus* species.[3] Tadic *et al.* analyzed chemical composition of fruit extracts using HPLC-DAD method and determined chlorogenic acid, isoquercitrin, hyperosid and quercetin in higher concentrations, as well as rutin and vitexin in traces.[19] Zhang and collaborators detected epicatechin and procyanidin B2 as the main components of methanolic extract of *C. pinnatifida* fruits in the concentrations of 2.816 and 2.435 mg/g dw, respectively,[20] which is lower than epicatechin determined in HMFL, and higher then epicatechin determined in CFL and fruit extracts from this study. Froechlicher *et al.* analyzed the content of chlorogenic acid, caffeic acid, hyperoside and epicatechin in extracts of fresh and dried fruits and dried aerial parts. Dry flowering tops (flowers with young leaves) extract contained the highest amounts of these compounds, followed by extracts of fresh and dried fruits,[21] which is also in accordance with the results reported here.

Results of this study showed that flowers and leaves extracts had higher DPPH scavenging activity compared to fruit extract, whit the HMFL extract being the most potent among the examined extracts. Determined values in this study are in accordance with values reported by other researchers. Namely, DPPH radical scavenging activity of fruit hawthorn extracts of different *Crataegus* species expressed as IC_{50} published by other authors was in the range of 1.2–2.4 mg/mL,[3] 1.4[19] and 0.013 mg/mL.[22] Froechlicher *et al.* evaluated the DPPH scavenging activity of dry aerial parts and dry fruit extracts of *C. monogyna.* The aerial parts extract was 5 times more active than fruit extract. However, these results were published in Trolox equivalents which cannot be compared with findings from this study.[21] Furthermore, DPPH neutralization activity of mixed flower and leaf and fruit ethanol extract of *C. monogyna* collected in Italy, expressed as IC_{50} were 0.003 and 0.013 mg/mL, respectively,[22] which is in accordance with results reported in this paper for flowers and leaves extracts. Furthermore, it was published that ethanol extract of *C. azarolus* leaves collected from Lebanon expressed IC_{50} value for DPPH 0.050 mg/mL, which is in accordance with results presented herein.[23]

FRAP assay also showed that the flowers & leaves extracts had higher antioxidant capacity compared to the extracts of fruits, with the HMFL being the most potent among all examined extracts herein. Other authors also investigated hawthorn antioxidant potential using this assay. Namely, antioxidant activity determined by FRAP assay was 531.4 and 955.8 mg AAE/L for fruits and aerial parts extracts, respectively, prepared by traditional maceration, and 105.3 and 1630.9 mg AAE/L for fruits and aerial parts extracts, respectively, prepared by sonicated extraction, both with ethanol, for *C. monogyna* collected in Ireland,[24] which is in correlation with our results. Others evaluated the antioxidant activity of fruit extracts of different *Crataegus* spp. and they were in the range of 0.50––2.83 mM $FeSO_4$ eq per 1 g sample, showing good antioxidant activity but less potent than BHT which they used as a reference (4.24 mM $FeSO_4$ eq per 1 g of sample).[3]

Shortle *et al.* have examined the effects of hawthorn extracts on LP and found good inhibitory activity. The most active sample was the extract of aerial parts prepared by sonicated maceration, followed by aerial parts and fruit extracts obtained by traditional maceration, while the least active sample was fruit extract obtained by sonicated maceration.[24] These results are not fully comparable with the results of this study because of different methodologies applied, but in both studies aerial part extracts were more active than fruit extracts.

Hawthorn has been traditionally used for the treatment of heart conditions. Leaf and flower extracts possess inotropic and chronotropic effects on the heart and can enhance coronary blood flow. It is assumed that flavonoids present in examined extracts, such as epicatechin, catechin, rutin, quercetin, vitexin and hyperoside are responsible for these effects.[25] Namely, flavanols, including epicatechin and catechin, can reduce ROS and increase the bioavailability of NO, an important messenger for vasodilation which reduces hypertension.[26] Furthermore, rutin has the ability to improve ejection fraction, left ventricular systolic function and fractional shortening.[27] Rutin also exhibits strong antioxidant activity, like other flavonoids, which can greatly contribute to its cardioprotective activity.[28] Rutin and quercetin protect against cardiac hypertrophy but through different mechanisms of action. Both flavonoids modulate oxidative stress through the inhibition of Ang II-induced NADPH oxidase, a major source of superoxide radicals. They also act on ROS/NO axis, but only quercetin seems to act on MAPK pathways. Quercetin inhibits ERK1/2, JNK1/2 and p38, while rutin showed no significant inhibition toward ERK1/2 and p38.[29] Likewise, vitexin exhibits antioxidant, antitumor, antimetastatic, antimicrobial, neuroprotective, anti-inflammatory, hypotensive and cardioprotective activities.[30,31] Attenuation of cardiac hypertrophy is achieved through the modulation of intracellular calcium concentration.[32] Hyperoside also exhibits cardioprotective activity. It can increase left ventricular ejection fraction and reduce heart size. Additionally, it

has anti-hypertensive activity, which is achieved by influencing blood vessels.[33] Generally, the anti-hypertension activity of flavonoids can be exhibited through the inhibition of ET-1, a vasoconstriction factor produced by endothelium. It has been shown that an increase in flavonoid hydrophilicity and glycosylation can impair their anti-hypertensive activity. Another key element for this activity is the presence of the 4-keto group and flavonoids that do not possess it show low activity.[34]

Hawthorn extracts made from flowers and leaves analyzed in this study, especially homemade extract, are rich in flavonoids, such as epicatechin and quercetin glycosides, and they also contain apigenin-*C*-glucoside (vitexin), which altogether affirm their use in the treatment of heart conditions. The examined extracts are also rich in other flavonoids and phenolic acids that can have other health benefits such as antioxidant, cardioprotective, anti-atherosclerotic, anti--inflammatory, anti-diabetic, anticancer and others.[35–39] Considering that fruit extract contained lower amounts of these compounds, flowers and leaves extract usage should be favored. Furthermore, the preparation of flower and leaf extract in home conditions, using a simple extraction method with 70 % ethanol for 24 h at room temperature, in a ratio of 1:5 (dry plant material:70 % ethanol), could be considered as a suitable method, which may yield an extract of equal or even higher quality in terms of the quantity of biologically active phenolic compounds compared to commercial extracts.

CONCLUSION

Hawthorn flowers and leaves are a good source of phenolic compounds that have cardioprotective and other beneficial effects which supports its usage in herbal medicinal products. Flowers and leaves extract is a richer source of beneficial polyphenols than fruit extract. Furthermore, homemade flowers and leaves extract is richer in phenolic compounds responsible for its health-promoting effects, compared to commercial ones, which supports preparation of hawthorn flowers and leaves extract at home by individuals. However, safety issues (contraindications, special warnings and precautions for use and possible interactions with other medicinal products and other forms of interactions) and duration of use should be addressed when supporting homemade herbal preparations especially when used in therapeutic doses!

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ИЗВОД

УПОРЕДНА АНАЛИЗА ФЕНОЛНОГ ПРОФИЛА И АНТИОКСИДАНТНЕ АКТИВНОСТИ ДОМАЋИХ И КОМЕРЦИЈАЛНИХ ЕКСТРАКАТА ГЛОГА (*Crataegus* SPP.)

НЕМАЊА ЖИВАНОВИЋ, НАТАША СИМИН, МАРИЈА ЛЕСЈАК, ДЕЈАН ОРЧИЋ, НЕДА МИМИЦА-ДУКИЋ и ЕМИЛИЈА СВИРЧЕВ

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Врсте рода *Crataegus* (глог) се од давнина примењују у традиционалној медицини, нарочито за лечење конгестивне срчане инсуфицијенције. Резултати бројних истраживања потврдили су да су ове врсте богате биолошки активним молекулима, посебно из класе полифенола, који поседују снажну антиоксидантну активност, што доприноси кардиопротективним особинама глога. На светском тржишту могу се наћи бројни фитопрепарати на бази глога, чију употребу као допунске терапије срчаних обољења подржава Европска агенција за лекове. Терапеутски ефекат препарата глога условљен је његовим хемијским саставом на који утичу бројни генетски и еколошки фактори као и начин припреме препарата. С обзиром на све већу популарност припреме фитопрепарата у кућним условима, циљ овог истраживања био је да се упореди полифенолни профил и антиоксидативна активност комерцијалних препарата глога и препарата добијених једноставним методама екстракције прилагођених кућним условима. Хемијски профил полифенола је одређен мерењем садржаја укупних фенола и флавоноида, као и квантитативном анализом одабраних полифенола помоћу LC–MS/MS технике. Антиоксидантни потенцијал је испитан помоћу DPPH и FRAP теста и мерењем способности инхибиције липидне пероксидације. Добијени резултати показали су да не постоје значајније разлике у саставу полифенолних једињења и у антиоксидативној активности између етанолних екстрактата цветова, листова и плодова глога који су припремљени једноставним методама екстракције (*home-made*) и комерцијалних фитопрепарата. Надаље, етанолни екстракти листова и цветова глога били су богатији полифенолима и показали јачу антиоксидативну активност у поређењу са екстрактима плодова, без обзира на начин припреме.

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