Journal of the Serbian Chemical Society
JSCS-info@shd.org.rs • www.shd.org.rs/JSCS
J. Serb. Chem. Soc. *86* (6) 603–613 (2021)
JSCS–5447
Original scientific paper

Subcritical water extraction of horse chestnut (*Aesculus hippocastanum*) tree parts

TANJA GAGIĆ[1], ŽELJKO KNEZ[1,2] and MOJCA ŠKERGET[1]*

[1]*Laboratory for Separation Processes and Product Design, Faculty of Chemistry and Chemical Engineering, University of Maribor, Smetanova ulica 17, 2000 Maribor, Slovenia and* [2]*Faculty of Medicine, University of Maribor, Taborska ulica 8, 2000 Maribor, Slovenia*

(Received 11 November 2020, revised and accepted 18 February 2021)

Abstract: Subcritical water extraction of horse chestnut (*Aesculus hippocastanum*) parts, such as seeds, seed shell, bark and leaves has been performed in a batch reactor at temperatures of 150, 200 and 250 °C and extraction times of 5, 15 and 30 min. The obtained extracts were analyzed by spectrophotometric methods to determine the total phenols, total carbohydrates and antioxidant activity. Furthermore, the compounds detected in the extracts, such as triterpene saponins (escins), coumarin glycosides (esculin and fraxin), phenolic compounds (chlorogenic, neochlorogenic and gallic acids) and furfurals (5-hydroxymethyfurfural, furfural, and 5-methylfufrual) were quantified using HPLC. High amount of escins was obtained in the seed extracts, while the highest amounts of esculin and fraxin were obtained in bark extracts. The total phenol content was the highest in shell and bark extracts, which implies that these extracts gave the highest antioxidant activity.

Keywords: antioxidant activity; extraction; escins; coumarin glycosides; phenolic compounds.

INTRODUCTION

The horse chestnut (*Aesculus hippocastanum*) tree is widely distributed all over the world due to its excellent resistance to environmental conditions.[1] Horse chestnut seeds contain high amounts of starch, minerals, vitamins, flavonoids, carotenoids, fatty acids, but they are not edible due to presence of saponins.[2] Knowing that horse chestnut seeds are not edible, they are usually treated as biowaste.[3] The most important bioactive component found in horse chestnut seeds is aescin, which represents a mixture of triterpene saponins mainly consisting of escin Ia, escin Ib, isoescin Ia and isoescin Ib.[4] Escin Ia and Ib are β-escins that are dominant in seeds, while isoescin Ia and Ib are α-escins.[4] In order to use horse chestnut seeds in the food industry and as a safe addition to the

* Corresponding author. E-mail: mojca.skerget@um.si
https://doi.org/10.2298/JSC201111013G

food supply, Rafiq *et al.* studied the method for reduction of toxic compounds found in horse chestnut (*Aesculus indica*) seeds.[2] They showed that pre-treatments, such as soaking and especially microwave heating, drastically reduced the anti-nutritional components.[2]

Although these escins are toxic compounds and are the reason why seeds are not edible, they have other positive properties, such as anti-oedematous, anti--inflammatory and venotonic properties.[1] Thus, the extracts of horse chestnut seed could find application in the cosmetic and pharmaceutical industry in the treatment of chronic venous insufficiency, varicose veins, haemorrhoids, post-operative edemas, burns, epidermis abrasion, skin inflammations and frostbites.[5]

Takahashi *et al.* showed that horse chestnut seed, especially the outer theca (seed shell), has strong antibacterial properties toward *Staphylococcus aureus*, *Escherichia coli* and *Streptococcus mutans*.[3] Furthermore, the polyphenol content of the outer theca was around three times higher than that of "sarcocarp and endodermis". The good antioxidant properties are probably the reason why the theca possess high antibacterial properties. The outer theca also showed high deodorizing activity against basic and acidic odors. Moreover, Kimura *et al.* showed that Japanese horse chestnut seed shell contains highly polymerized pro-anthocyanidins that possess strong antiobesity effects.[6]

In this research subcritical water was used for the extraction of different parts of horse chestnut (seeds, bark, seed shells and leaves). No comparable study could be found in the literature. This method was used because generally, compared to conventional extraction techniques, subcritical water shows high extraction efficiency, the process needs shorter extraction time and it is a clean and non-toxic method.

EXPERIMENTAL

Materials

Seeds, bark, seed shell and leaves of horse chestnut (*Aesculus hippocastanum*) were collected in Serbia in June 2018. All parts of the horse chestnut were air-dried and ground before the extraction. Furfural (purity 99%) and 5-HMF (purity 98 %) were purchased from Acros Organics (Geel, Belgium). Chlorogenic acid (purity 98 %), neochlorogenic acid (purity 98 %), gallic acid, escin, esculin hydrate and fraxin were purchased from Sigma–Aldrich (Steinheim, Germany).

Subcritical water extraction

The subcritical water extraction was performed in a 75 mL Parr batch reactor (series 4740 stainless steel, Parr instruments, Moline, IL, USA) at temperatures of 150, 200 and 250 °C and extraction times of 5, 15 and 30 min using a material–solvent ratio of 1 g in 20 mL. The procedure of the extraction was described in a previous work.[7] Nitrogen was used to remove the oxygen present and to control the pressure. The mixture was stirred at 600 rpm. The reactor was heated by electrical wire. The reaction time was measured when the reactor reached the desired temperature. After the reaction, the reactor was rapidly cooled. The reactor content was filtrated and washed with water.

The extraction yield was calculated by Eq. (1):

$$Y_i = 100\frac{m_e}{m_0} \quad (1)$$

where Y_i – the extraction yield; m_e – the mass of the extract; m_0 – the initial mass of material.

Total phenol content

The total phenol content in the extracts was determined using the Folin–Ciocalteu method, as described in a previous work.[7] Extract (0.5 mL) was mixed with 2.5 mL of Folin–Ciocalteu reagent (diluted 1:10 with water) and 2 mL of Na_2CO_3 solution (75 g L^{-1}). The sample was heated at a temperature of 50 °C for 5 min in a water bath and then cooled to room temperature. after 30 min, the absorbance was measured at 760 nm using a UV-Vis spectrophotometer. Results are expressed as mg of gallic acid per g of material.

Total carbohydrate content

The total carbohydrates in the extracts was determined with the phenol–sulfuric colorimetric method explained in a previous work.[8] Extract (1 mL) was mixed with 0.5 mL of 5 % aqueous solution of phenol and 2.5 mL of concentrated sulfuric acid. The sample was placed in an ultrasonic bath for a 10 min and then kept at room temperature for 20 min for color development. The absorbance was measured at 490 nm using a UV–Vis spectrophotometer. All measurement were performed in triplicate. The results are expressed in mg g^{-1} of material.

Antioxidant activity

The antioxidant activity of the extracts obtained by subcritical water extraction was determined by the DPPH method, as explained in a previous work.[7] The extract (77 μL) (concentration 1 mg mL^{-1}) was mixed with 3 mL of DPPH solution. The mixture was incubated in a dark room for 15 min. The absorbance of the sample at 515 nm was measured using a UV–Vis spectrophotometer. The antioxidant activity is expressed in %.

HPLC methods

The HPLC method for detection of esculin, fraxin, 5-HMF, furfural and 5-MF is described in the literature.[9] The extracts were analyzed using an Agilent 1100 Series HPLC system (Agilent Technologies, Waldbronn, Germany) equipped with a binary pump, an autosampler, a column heater, a variable wavelength detector (VWD) and an Agilent Zorbax Eclipse XDB C18 (4.6 mm×150 mm, 3.5 m) column. The column temperature was 25 °C. The mobile phase consisted of two solvents: methanol (solvent A) and water + 0.1 % trifluorocetic acid (solvent B). The flow rate was 1 mL min^{-1}. The following gradient was set: 0 min 90 % B, 18 min 65 % B, 20 min 90 % B. The injection volume was 10 μL. Detection of the compounds was achieved at 254 nm for esculin and fraxin and 280 nm for 5-HMF, furfural, 5-MF.

The escins were determined by the HPLC method as described in the literature[10] with minor modifications. The same HPLC system was used with a C18 column (150 mm×4.6 mm, 3.5 μm) heated to 30 °C. The method was isocratic and the mobile phase was a mixture of acetonitrile and 0.1 % orthophosphoric acid (39:61 volume ratio). The flow rate was 1 mL min^{-1}, while the injection volume was set to 10 μL. The escins were detected at 210 nm. The quantifications was performed using standard calibration curves.

RESULTS AND DISCUSSION

Extraction yield

The yields of the extractions performed under different operating conditions are presented in Table I. The highest yields were obtained in the case of seeds, *i.e.*, 82.66 % at 150 °C and 15 min and 82.22 % at 200 °C and 5 min. The extraction yield in the case of seeds was much higher than in the case of the other materials (shell, bark and leaves) because seeds contain much higher amounts of compounds that are soluble in subcritical water (carbohydrates and especially fats). Kapusta *et al.* obtained a significantly lower extraction yield (25.2 %) using 80 % ethanol as extraction solvent at room temperature and extraction time of 72 h.[11]

TABLE I. The extraction yields of seed (Y_{SE}), bark (Y_{BE}), seed shell (Y_{ShE}) and leaf (Y_{LE})

T / °C	t / min	Y_{SE} / %	Y_{BE} / %	Y_{ShE} / %	Y_{LE} / %
150	5	69.01	23.91	26.91	31.64
150	15	82.66	29.37	36.51	32.21
150	30	75.92	30.91	34.00	32.67
200	5	82.22	38.43	24.45	36.96
200	15	69.63	43.59	27.14	31.90
200	30	64.82	26.32	27.02	31.82
250	5	32.13	23.04	11.63	34.41
250	15	25.23	23.54	14.39	34.41
250	30	24.97	16.40	13.74	30.39

Total phenols, total carbohydrates and antioxidant activity

The amounts of total phenols and carbohydrates extracted from different parts of chestnut are given in Table II. The highest amounts of total phenols isolated from seeds and leaves were obtained at 250 °C and 5 min and were 26.01 and 24.48 mg g^{-1}, respectively, while the maximal amount of total phenols from seed shell and bark were obtained at 200 °C and 15 min and were 50.99 mg g^{-1} and 33.29 mg mL^{-1}, respectively.

TABLE II. The amount (mg g^{-1}) of total phenols (Y_{TP}) and total carbohydrates (Y_{TC}) extracted from seeds (S), bark (B), seed shell (Sh) and leaves (L)

T / °C	t / min	Parameter							
		Y_{TPS}	Y_{TPB}	Y_{TPSh}	Y_{TPL}	Y_{TCS}	Y_{TCB}	Y_{TCSh}	Y_{TCL}
150	5	6.46	20.18	36.06	7.81	198.12	49.49	57.76	36.54
150	15	5.63	22.32	39.87	11.87	227.91	60.85	57.78	36.30
150	30	6.47	22.78	35.55	9.47	260.99	76.37	57.75	46.13
200	5	17.32	29.40	33.10	18.98	252.40	77.76	59.39	44.56
200	15	20.98	33.29	50.99	21.28	253.30	85.25	87.22	37.36
200	30	21.58	28.57	22.16	19.95	244.12	81.78	38.65	21.20
250	5	26.01	29.21	25.96	24.48	139.58	48.29	28.17	13.40
250	15	23.73	25.77	24.72	20.86	91.24	37.23	25.72	7.48
250	30	22.70	24.29	25.60	17.63	82.40	30.87	13.16	5.83

The effect of temperature on the amount of carbohydrates obtained from each material was similar. In the case of seeds, seed shell and leaves at extraction times of 5 and 15 min, on increasing the temperature from 150 to 200 °C, the amount of carbohydrate increased and then decreased on further increasing of the temperature, while at 30 min it decreased on increasing the temperature over the whole temperature range. The exception was horse chestnut bark, where the carbohydrate amount increases at temperatures from 150 to 200 °C and then decreases at 250 °C.

The highest amount of total carbohydrates extracted from seed and leaves were 260.99 mg g^{-1} material and 46.13 mg g^{-1} material obtained at 150 °C and 30 min, respectively, while the maximal amount of total carbohydrates from seed shell and bark obtained at 200 °C and 15 min were 87.22 and 85.25 mg g^{-1}, respectively.

The antioxidant activities of the horse chestnut extracts are presented in Table III. The antioxidant activity of seed extracts was the lowest compared to the other materials and it increased with extraction temperature and time, reaching the maximal value of 54.15 % at 250 °C and 30 min.

TABLE III. Antioxidant activity of seed (AA_S), bark (AA_B), seed shell (AA_{Sh}) and leaf (AA_L) extracts

T / °C	t / min	AA_S / %	AA_B / %	AA_{Sh} / %	AA_L / %
150	5	3.34	67.79	89.20	18.35
150	15	2.45	61.01	90.47	25.22
150	30	3.52	57.08	90.06	21.33
200	5	9.30	55.42	89.46	32.89
200	15	15.10	60.05	87.89	42.86
200	30	16.51	83.51	84.90	41.29
250	5	47.34	92.05	89.57	57.87
250	15	53.04	88.98	88.86	47.02
250	30	54.15	91.34	90.54	40.39

The highest antioxidant activity in the case of leaf extracts was 57.87 % obtained at 250 °C and 5 min. The bark and seed shell extracts show the highest antioxidant activities (around 90 %), which could be probably attributed to higher total phenol content in extracts of these materials (in the range from 73.71 to 148.11 mg g^{-1} of extract and from 82.0 to 223.25 mg g^{-1} of extract, respectively) compared to seeds and leaves (in the range from 6.81 to 94.06 mg g^{-1} of extract and from 24.68 to 71.14 mg g^{-1} of extract, respectively). Otajagić *et al.* determined total phenolic content and antioxidant activity of ethanolic extracts of different parts *of Aesculus hippocastanum* (bark of young twigs (BT) and fruits (BF), bark of fruit with pulp (BF+P) and pulp itself (P)).[12] The highest amounts of total phenolics were detected in the BF and BT extracts, 158–202 mg GAE g^{-1} and 178–216.97 mg GAE g^{-1}, respectively, and the antioxidant activities of these

extracts were around 90 %. These results are in agreement with the presented results for seed shell and bark, but in present work, where subcritical water was used as extraction medium, the total phenol content and antioxidant activity of seed extract was 94.06 mg g^{-1} of extract and 53.04 %, respectively, at 250 °C and 15 min, which is much higher than in the case of ethanol extraction (8.80––12.36 mg GAE g^{-1} and 15–25 %, respectively). Probably, at higher temperatures (200 and 250 °C), the hydrolysis of phenolic glycosides occurred and phenolic aglycones, which possess higher antioxidant activity than phenolic glycosides, were formed in the extracts. Kimura *et al.* studied the antioxidant activities and content of flavonol *O*-glycosides of whole seeds, peeled seeds and seed shells from Japanese horse chestnut (*Aesculus turbinata*) extracts, obtained with an acetone–water–acetic acid mixture (70:29.5:0.5 volume ratio).[13] The results indicate that the peeled seeds are a good source of flavonol *O*-glycosides serving as antioxidants to be used as food additives and dietary supplements. They obtained lower content of polyphenols (5.71 mg g^{-1} of seed extract and 68 mg g^{-1} of seed shell extract) compared to results obtained using subcritical water extraction.

Triterpene saponins, coumarin glycosides, phenolic acids and furfurals

The amount of escins, esculin and fraxin extracted from seed, seed shell, bark and leaf of horse chestnut are presented in Table IV. The escins were detected only in seed extracts. From the results, it could be observed that at 150 °C, the amount of escins decreases with increasing extraction time, while at 200 °C after 5 min, they are completely degraded. At 150 °C and 5 min, the amount of escins extracted from seeds was 33.11 mg g^{-1} of material, which shows that the amount of escins in seed extracts obtained by subcritical water extraction was almost the same as that obtained by extraction with methanol (34.9 mg g^{-1} for the year of 2014) published by Abudayeh *et al.*[10]. Chen *et al.* determined four main saponins (escin Ia, escin Ib, isoescin Ia, isoescin Ib) from a different variety of horse chestnut (*Aesculus chinensis*) using accelerated solvent extraction.[14] The solvents used were *n*-butanol, 70 % methanol, methanol, 30 % ethanol, 50 % ethanol and 80 % ethanol with 0.5 % of acetic acid. The quantities of these compounds extracted by 70 % methanol were the highest (average content of escins in different chestnut samples were 42.8 mg g^{-1} of extract), which is similar to the results obtained by subcritical water extraction.

Esculin and fraxin were the dominant compounds in bark extracts, but they were also found in other extracts. The amounts of both esculin and fraxin isolated from bark decrease with extraction time and temperature, except at 150 °C when the esculin amount increased from 5 min to 15 min. In the case of bark extracts, esculin was not detected anymore at the conditions of 250 °C and 15 min, while fraxin was no longer present already at 200 °C and 15 min. Similar amounts of esculin and fraxin in horse chestnut bark were obtained by Stanić *et al.*,[15] where

methanol was used as the extraction solvent and the highest amounts obtained in bark samples (branch diameter of 5 cm) were in the range from 36 to 59.6 mg g^{-1} of material for esculin and from 15.3 to 26.2 mg g^{-1} of material for fraxin. Thus, subcritical water as non-toxic and environmentally friendly medium could be good replacement for organic extraction solvents for the extraction of these compounds. In the case of other materials (seeds, seed shell and leaves), esculin and fraxin were detected in the extracts obtained at 200 °C, while above 250 °C at an extraction of 5 min, they were no longer present in the extracts.

TABLE IV. The amount (mg g^{-1}) of escins (Y_{Escin}), esculin (Y_{Escul}) and fraxin (Y_{Frax}) extracted from seed (S), bark (B), seed shell (Sh) and leaves (L)

T / °C	t / min	Parameter								
		Y_{EscinS}	Y_{EsculS}	Y_{EsculB}	$Y_{EsculSh}$	Y_{EsculL}	Y_{FraxS}	Y_{FraxB}	Y_{FraxSh}	Y_{FraxL}
150	5	33.11	n.d.	41.63	n.d.	2.26	n.d.	20.38	n.d.	1.79
150	15	29.85	n.d.	46.18	n.d.	2.33	n.d.	18.01	n.d.	1.67
150	30	26.02	n.d.	44.19	n.d.	2.23	n.d.	n.d.	n.d.	1.79
200	5	17.08	n.d.	32.21	1.87	2.33	2.69	4.06	2.08	n.d.
200	15	n.d.	2.12	21.76	1.88	n.d.	1.74	n.d.	2.09	n.d.
200	30	n.d.	3.09	9.98	1.87	n.d.	1.74	n.d.	2.02	n.d.
250	5	n.d.	2.95	1.71	n.d.	1.92	2.75	n.d.	n.d.	1.67
250	15	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
250	30	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

Typical chromatograms are shown in Fig. 1 of the escin mixture standard (A) and chestnut seed extract treated with water at a temperature of 150 °C and an extraction time of 5 min (B). As can be seen from the chromatograms, the detected peaks represent escins, such as escin Ia (1), escin Ib (2), isoescin Ia (3) and isoescin Ib (4). The peaks were identified based on the data published by Abudayeh *et al.*[10]

The amounts of chlorogenic acid, neochlorogenic acid and galic acid extracted from different parts of horse chestnut are presented in Table V. Chlorogenic acid was detected in higher amounts only in extracts from bark, while in extracts from other chestnut parts, it was present only in trace amounts. The results obtained for bark at 150 and 200 °C show that the amount of chlorogenic acid decreases with increasing temperature and time, while at 250 °C it was no longer present in the extract. Furthermore, the highest amount of neochlorogenic acid was detected in seed shell extracts. In the cases of seed shell, bark and leaf, the amount of neochlorogenic acid increases with increasing extraction time at 150 °C and reaches the highest value at 200 °C and 5 min. However, in the case of seeds, the amount of neochlorogenic acid increases with increasing time and temperature and reaches the highest value at 200 °C and 30 min, while at 250 °C, it was no longer present in the extracts. Oszmaiański *et al.* extracted leaves of horse chestnut with methanol acidified with 1 % acetic acid for 20 min under

sonication.[16] They found different phenolic compounds (neochlorogenic acid, epicatechin, proanthocyanidins and quercetin and kaempferol derivatives) in the extracts of white and red horse chestnut leaves. The content of neochlorogenic acid found in the literature was 1.26 mg g^{-1} of leaf extracts, while in the present work, the highest content of neochlorogenic acid was 2.41 mg g^{-1} of leaf extract at 200 °C and 15 min. Gallic acid was detected only in leaf extracts and at 150 °C, the amount decreased with increasing extraction time, while after 200 °C and 5 min, it was no longer present in the extracts. Postoyuk *et al.* identified various components in leaf extracts: 1) different phenolic compounds among which the dominant were gallic acid, hyperoside, epigallocatechin-gallate, luteolin-7-glucoside and cichoric acid; 2) organic acids such as citric, tartaric, malic, ascorbic, oxalic and lactic acid; 3) sugars such as glucose and fructose; 4) escin.[17] They obtained an amount of gallic acid of 146 mg g^{-1} of leaf extract, while in the case of subcritical water extraction, this value was 4.96 mg g^{-1} of leaf extract at 150 °C and 5 min, probably because hydrolysis of gallic acid occurred.

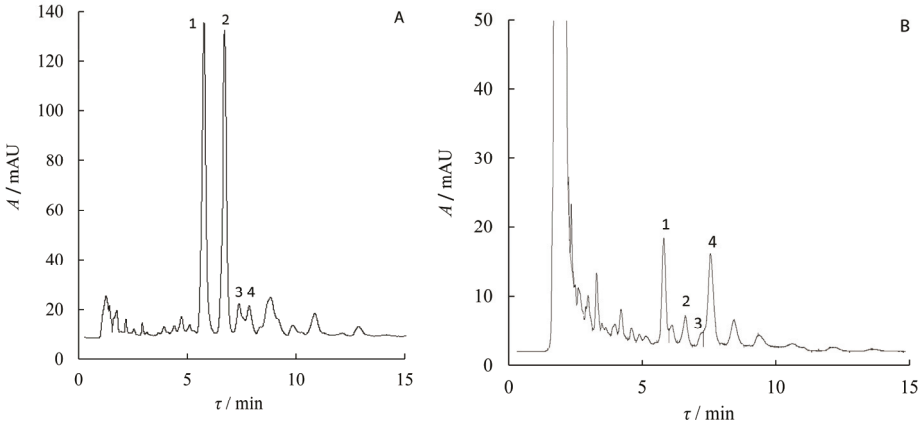

Fig. 1. Typical chromatogram of :A) escin mixture standard and B) chestnut seed extract treated with water at a temperature of 150 °C and an extraction time of 5 min. Identified peaks: 1 – escin Ia, 2 – escin Ib, 3 – isoescin Ia, 4 – isoescin Ib.

The extracted amounts of 5-HMF, furfural and 5-MF are presented in Table VI. Furfurals are the main sugar degradation products. Baraldi *et al.* studied chemical composition of seed samples (pure and hybrid) of *Aesculus hippocastanum* found in Italy and they obtained a glucose concentration of 6.8 % and a fructose concentration of 8.4 % in pure seed sample.[18] As can be seen from Table VI, the highest amounts of 5-HMF and furfural were obtained in seed extracts, which is a consequence of higher content of total carbohydrates in seeds than in the other materials. The furfurals are in negligible low content or even not found in the extracts obtained at 150 °C. The amounts started to increase by inc-

reasing the temperature to 200 °C and by increasing the extraction time. At 250 °C, the amounts of 5-HMF and furfural started to decrease with increasing extraction time. For all materials, the amount of 5-HMF reached the maximal value at 250 °C and 5 min (42.60 mg g^{-1} for seed, 4.52 mg g^{-1} for bark and 2.90 mg g^{-1} for seed shell and 0.143 mg g^{-1} for leaf). In the case of seed, the maximal amount of furfural was 6.34 mg g^{-1} at 250 °C and 5 min, while in the cases of bark, seed shell and leaf, the amount of furfural reached the maximum at 200 °C and 30 min (7.42, 3.22 and 0.496 mg g^{-1}, respectively). The leaf extracts contained the lowest amount of total carbohydrates and thus they contained significantly lower amounts of 5-HMF and furfural than the other parts of chestnut. 5-Methylfurfural was detected in seed, bark and seed shell extracts. In the case of seed, the amount of 5-methyfurfural increased with extraction temperature and time, while in the case of bark and seed shell, it reached the maximal values at 200 °C and 30 min and then started to decrease at 250 °C.

TABLE V. The amount (mg g^{-1}) of chlorogenic (Y_{Ch}), neochlorogenic (Y_{NCh}) and gallic (Y_{GA}) acids extracted from seed (S), bark (B), seed shell (Sh) and leaves (L)

T / °C	t / min	Parameter					
		Y_{ChB}	Y_{NChS}	Y_{NChB}	Y_{NChSh}	Y_{NChL}	Y_{GAL}
150	5	0.69	0.27	0.34	0.91	0.48	1.57
150	15	0.68	0.30	0.45	1.21	0.55	1.56
150	30	0.61	0.38	0.52	1.36	0.65	1.34
200	5	0.49	0.45	0.88	2.19	0.79	1.10
200	15	0.48	0.53	0.85	2.10	0.77	n.d.
200	30	n.d.	0.56	0.62	1.51	0.54	n.d.
250	5	n.d.	n.d.	0.37	0.58	0.31	n.d.
250	15	n.d.	n.d.	n.d.	0.40	n.d.	n.d.
250	30	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

TABLE VI. The amount (mg g^{-1}) of extracted 5-HMF (Y_{HMF}), furfural (Y_F) and 5-methyfurfural (Y_{MF}) from seed (S), bark (B), seed shell (Sh) and leaves (L)

T	t	Parameter										
°C	min	$Y_{5\text{-HMFS}}$	$Y_{5\text{-HMFB}}$	$Y_{5\text{-HMFSh}}$	$Y_{5\text{-HMFL}}$	Y_{FS}	Y_{FB}	Y_{FSh}	Y_{FL}	Y_{MFS}	Y_{MFB}	Y_{MFSh}
150	5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
150	15	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
150	30	0.16	0.11	n.d.	n.d.	0.41	0.41	n.d.	n.d.	n.d.	n.d.	n.d.
200	5	3.10	0.62	0.24	0.11	0.79	1.03	0.94	0.42	0.42	0.56	0.52
200	15	13.82	1.90	0.69	0.12	2.85	3.48	2.11	0.47	0.72	0.61	0.78
200	30	33.45	4.05	1.95	0.13	5.09	7.42	3.22	0.50	0.83	1.66	0.92
250	5	42.60	4.52	2.90	0.14	6.34	4.31	2.07	0.43	1.11	1.09	0.80
250	15	31.32	3.88	1.96	0.14	4.64	3.36	1.33	0.43	1.30	1.03	0.68
250	30	18.79	2.47	0.99	0.14	3.25	1.95	0.76	0.43	1.38	0.82	0.49

CONCLUSIONS

In the present work it was shown that subcritical water could be a suitable replacement for organic solvents and could be efficiently used as the extraction medium to extract important compounds from horse chestnut parts. Based on the results, it could be concluded that by adjusting the extraction conditions, the extraction of certain compounds could be favored. Namely, the results showed, that higher content of escins, esculin, fraxin, phenolics and carbohydrates were obtained at a lower extraction temperature (150 °C) and time, due to their degradation under rigorous conditions. On the other hand, if the desired compounds are furfurals, subcritical water extraction has to be performed at higher temperatures (200 and 250 °C) and longer times.

Acknowledgement. The authors would like to acknowledge the Slovenian Research Agency (ARRS) for financing this research within the frame of Program P2-0046 (Separation processes and production design).

ИЗВОД

ЕКСТРАКЦИЈА САСТОЈАКА ИЗ ДЕЛОВА ДИВЉЕГ КЕСТЕНА (*Aesculus hippocastanum*) СУБКРИТИЧНОМ ВОДОМ

TANJA GAGIĆ[1], ŽELJKO KNEZ[1,2] и MOJCA ŠKERGET[1]

[1]*Laboratory for Separation Processes and Product Design, Faculty of Chemistry and Chemical Engineering, University of Maribor, Smetanova ulica 17, 2000 Maribor, Slovenia и* [2]*Faculty of Medicine, University of Maribor, Taborska ulica 8, 2000 Maribor, Slovenia*

Екстракција састојака из делова дивљег кестена (*Aesculus hippocastanum*), као што су плодови, омотач плода, кора и листови, субкритичном водом је изведена у шаржном реактору на температурама од 150, 200 и 250 °C и екстракцијским временима од 5, 15 и 30 min. Добијени екстракти су анализирани методом спектрофотометрије ради одређивања тоталних фенола, угљених хидрата и антиоксидативне активности. Осим тога, детектована једињења у екстрактима, као што су тритерпеноидни сапонини (ескини), кумаринski гликозиди (ескулин и фраксин), фенолна једињења (хлорогенска, неохлорогенска и гална киселина) и фурфурали (5-хидроксиметилфуфурал, фурфурал, 5-метилфурфурал) су квантификовани применом HPLC. Велика количина ескина је добијена у екстрактима плода, док су највеће количине ескулина и фраксина добијене у екстрактима коре. Садржај тоталних фенола је био највећи у екстрактима омотача и коре, што имплицира да ти екстракти дају највећу антиоксидативну активност.

(Примљено 11. новембра 2020, ревидирано и прихваћено 18. фебруара 2021)

REFERENCES

1. C. R. Sirtori, *Pharmacol. Res.* **44** (2001) 183 (http://dx.doi.org/10.1006/phrs.2001.0847)
2. S. I. Rafiq, S. Singh, D. C. Saxena, *J. Food Meas. Charact.* **10** (2016) 302 (http://dx.doi.org/10.1007/s11694-016-9307-2)
3. T. Takahashi, Y. Tsurunaga, W. F. Schmidt, K. Yoshino, *J. Wood Sci.* **63** (2017) 484 (http://dx.doi.org/10.1007/s10086-017-1649-9)
4. P. A. De Almeida, M. C. Alves, H. C. Polonini, L. S. Dutra, M. N. Leite, N. R. B. Raposo, A. D. O. Ferreira, M. A. F. BrandÃo, *Lat. Am. J. Pharm.* **32** (2013) 1082 (http://www.latamjpharm.org/resumenes/32/7/LAJOP_32_7_1_19.pdf)

5. M. Dudek-Makuch, I. Matławska, *Acta Pol. Pharm. - Drug Res.* **70** (2013) 517 (https://pubmed.ncbi.nlm.nih.gov/23757942/)
6. H. Kimura, S. Ogawa, A. Sugiyama, M. Jisaka, T. Takeuchi, K. Yokota, *Food Res. Int.* **44** (2011) 121 (http://dx.doi.org/10.1016/j.foodres.2010.10.052)
7. S. Jokić, T. Gagić, E. Knez, D. Ubarić, M. Kerget, *Molecules* **23** (2018) (http://dx.doi.org/10.3390/molecules23061408)
8. T. Gagić, A. Perva-Uzunalić, Ž. Knez, M. Škerget, *Ind. Eng. Chem. Res.* **57** (2018) (http://dx.doi.org/10.1021/acs.iecr.8b00332)
9. S. Jokić, T. Gagić, Ž. Knez, M. Banožić, M. Škerget, *J. Supercrit. Fluids* **153** (2019) 104593 (http://dx.doi.org/10.1016/j.supflu.2019.104593)
10. Z. H. M. Abudayeh, K. M. Al Azzam, A. Naddaf, U. V. Karpiuk, V. S. Kislichenko, *Adv. Pharm. Bull.* **5** (2015) 587 (http://dx.doi.org/10.15171/apb.2015.079)
11. I. Kapusta, B. Janda, B. Szajwaj, A. Stochmal, S. Piacente, C. Pizza, F. Franceschi, C. Franz, W. Oleszek, *J. Agric. Food Chem.* **55** (2007) 8485 (http://dx.doi.org/10.1021/jf071709t)
12. M. Otajagić, S., Pinjić, Dž., Ćavar, S. Vidic, D. Maksimović, *Bull. Chem. Technol. Bosnia Herzegovina* (2012) 35 (https://www.semanticscholar.org/paper/Total-phenolic-content-and-antioxidant-activity-of-Otajagi%C4%87-Pinji%C4%87/71221e61f9b6c99ae23b0d91b3719d32d650f6c8)
13. H. Kimura, S. Ogawa, T. Ishihara, M. Maruoka, S. Tokuyama-Nakai, M. Jisaka, K. Yokota, *Food Chem.* **228** (2017) 348 (http://dx.doi.org/10.1016/j.foodchem.2017.01.084).
14. J. Chen, W. Li, B. Yang, X. Guo, F. S. C. Lee, X. Wang, *Anal. Chim. Acta* **596** (2007) 273 (http://dx.doi.org/10.1016/j.aca.2007.06.011)
15. G. Stanić, B. Jurišić, D. Brkić, *Croat. Chem. Acta* **72** (1999) 827 (https://hrcak.srce.hr/132302)
16. J. Oszmiański, S. Kalisz, W. Aneta, *Molecules* **19** (2014) 14625 (http://dx.doi.org/10.3390/molecules190914625)
17. N. A. Postoyuk, A. A. Markaryan, T. D. Dargaeva, *Glob. J. Pharmacol.* **7** (2013) 321 (http://dx.doi.org/10.5829/idosi.gjp.2013.7.3.1109)
18. C. Baraldi, L. M. Bodecchi, M. Cocchi, C. Durante, G. Ferrari, G. Foca, M. Grandi, A. Marchetti, L. Tassi, A. Ulrici, *Food Chem.* **104** (2007) 229 (http://dx.doi.org/10.1016/j.foodchem.2006.11.032).