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THE EFFECT OF THE OPERATION CONDITIONS AND THE EXTRACTION TECHNIQUES ON THE YIELD, KINETICS AND COMPOSITION OF METHANOL EXTRACTS OF *Hieracium pilosella* L.

The optimal operational extraction conditions were determined by investigating the influence of the methanol concentration, solvomodule and temperature of the maceration extraction on the yield and kinetics of total extractive matter, chlorogenic acid, umbelliferone and apigenin-7-O-glucoside from Hieracium pilosella L. Based on the results of Soxhlet and Tillepape extraction kinetics investigations of the total extractive matter and the components under the optimal maceration operation conditions it was found that the highest yields of the extractive matter and investigated bioactive components extracted from the dry plant material were obtained by using the Soxhlet extraction method. The contents of chlorogenic acid, umbelliferone and apigenin-7-O--glucoside in the extracts were determined by HPLC method. Chlorogenic acid is the component with the highest share in all the extracts.

The large genus *Hieracium* L. consists of over 1000 species. Some species, such as *H. pilosella*, *H. auranticum* and *H. murorum*, are used in traditional European medicines as they display diuretic and anti-inflammatory effects [1].

Hieracium pilosella L. (Family: Asteraceae) is a perennial herbaceous plant. It is widely spread in mountain and foothill pastures, in the areas of oak woods and underbrush. It is mainly used as a traditional medicine for bronchitis, bronchial asthma, edema, and as an ointment for wound healing. It is especially recommended for intensifying urination and eliminate slime, sand and small stones from the urinary tract and the kidneys [2,3]. Because of its medicinal value, it has been used in traditional Serbian medicine for centuries [3].

In traditional European medicine it is used for its diuretic and anti-inflammatory effects [1]. The components most commonly found in all *Hieracium* species are phenolic acids and flavonoids, in plant material from New Zealand [4-6]. The chemical composition and quantitative content of individual components depend on the species and the locality where the investigated species thrive [7]. A phytochemical screening of diethyl ether extract revealed that *H. pilosella* L. leaves contained coumarins, flavonoids and terpenes [8]. The common phenol components found in all *Hieracium* species methanol extracts are: chlorogenic acid, 3,5-dicaffeoylquinic acid, and, among flavonoids, luteolin 7-*O*-glucoside [9]. The chlorogenic and caffeic acids are found in green leaves and the water extracts from *H. pilosella* L. dry leaves, and the root contains umbelliferone [10]. *H. pilosella* L. flowers contain flavonoids and phenolic acids [11]. Phenolic acids and flavonoids are natural antioxidants [11–13] with anti-mutagenic and anti-carcinogenic [14,15], cardio-protective [16] and antimicrobial properties [12,17,18]. For the extraction of phenolic compounds from the plant material methanol, ethanol and acetone are most often used as extractants [19,20]. Chlorogenic acid is a highly valuable natural polyphenol compound used in medicine and industries. Chlorogenic acid is used for various additives in beverages, cosmetics, tea products, and foods as well as in medical substances. Chlorogenic acid has antibacterial and antiviral properties, and it is a natural antioxidant and anticancer agent. The current commercial sources of chlorogenic acids are from extracts of plants such as *Lonicera japonica Thunb* and *Eucommia ulmoides* Oliver. These sources are generally limited and therefore expensive [21].

In the available reference works there is no data on the composition of the methanol extracts from the leaves and roots of this herb from Southeast Serbia or data on the effects of the operational conditions and extraction techniques on the yield and composition of extractive matter and the kinetics of the extractions.

Based on the comparative investigation of the extracts composition and the kinetics of the macerations extraction, Soxhlet and Tillepape extraction, the aim of this work is to define the optimal operational conditions and extraction techniques for obtaining the maximum yields of the extractive matter, chlorogenic acid, umbelliferone and apigenin-7-*O*-glucoside and to determine the parameters in the extraction kinetics equations.

EXPERIMENTAL

Plant material

A whole plant (leaves and roots) of *Hieracium pilosella* L. (Asteraceae) was collected in Barje, Southeast Serbia, in June 2007. A voucher specimen (16 186 BEOU) is deposited in the herbarium of botany and botanical Garden, Faculty of Biology, University of Belgrade. The plant material was dried in the shade in an airy place and then stored in paperbags and kept at room

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temperature. The plant material was milled by an electrical mill with a fast-rotating knife (15000 rpm; 1 min) immediately before extraction.

Moisture content, determined by drying at 105 °C to constant weight, was 14.87%.

Chemicals and reagents

HPLC grade acetonitrile (Merck, Darmstadt, Germany) and filtered bidistilled water were used for HPLC analysis. Chlorogenic acid was obtained from Sigma-Aldrich (Steinheim, Germany), and apigenin-7-*O*-glucoside and umbelliferone were purchased from Extrasynthese (Genay, France). All other chemicals were of analytical reagent grade.

Extractive matter content in the plant material

The measured quantity (10 g) of crushed and homogenized plant material was placed in a Soxhlet extraction apparatus. 200 ml of solvent (80% v/v methanol) was added into a receiving flask. The extraction was carried out at boiling temperature for 6 h. The solvent was evaporated on a rotary vacuum evaporator at 40 °C. The obtained extract was dried in a vacuum dryer at 40 °C until constant mass and the content of the extractive matter in the plant material was calculated.

HPLC analysis

HPLC analysis was used to determine the quailtative and quantitative composition of chlorogenic acid, umbelliferone and apigenine-7-*O*-glucoside in the extracts based on the calibration curves of the investigated components' standards. The identification and determination of the content of bioactive components in the extracts was carried out by HPLC analysis under the following conditions: Agilent 1100 Series, Waldbronn, Germany; column: Zorbax-Eclipse XDB-CN; 4.6 mm×250 mm, 5 μm. Eluent: acetonitrile:water, 30:70 v/v. Flow rate: 1 ml/min. Injection volume: 20 μl. Detection: 205 nm UV detector. The calibration curves for quantitative determination were constructed with seven different concentrations of standard components' solution under the same conditions of HPLC analysis of extract composition. The concentration ranges were: 1–500 μg/ml, 0.15–15 μg/ml and 4–670 μg/ml of chlorogenic acid, umbelliferone and apigenine-7-O-glucoside, respectively.

Extraction processes

Maceration

Dried, ground plant material (3 g; average diameter of plant-solid material was 0.35 mm) was extracted for 2 h at 25 °C with 1:15 m/v methanol (10–100 % v/v). The extract was separated by filtering under a weak vacuum (water aspirator pump, absolute presure was 9.6 kPa). The content of the extractive matter (dry extract) was determined on a SCALTEC SMO 01 apparatus (Scaltec Instruments, Germany) at 105 °C. The yield of the extractive matter was calculated on the basis of the dry residue content. Based on the extractive matter yield, the yield and content of the chlorogenic acid, umbelliferone and apigenine-7-*O*-glucoside in the dry extracts, the optimal concentration of methanol was determined. With the optimal concentration of methanol, and other operational conditions unchanged, the effect of solvomodule (ratio of plant material:solvent, 1:10, 1:20 and 1:25 (m/v)) on the yield of extractive matter was investigated, as well as the yield and content of the investigated components in the dry extracts. Based on the obtained results the optimal solvomodule was determined. Similarly, by employing the optimal methanol concentration and the optimal solvomodule, the effect of temperature on the extraction kinetics and the investigated bioactive components was investigated to select the optimal extraction temperature.

Soxhlet extraction

Under the optimal maceration conditions (solvent: 80 v/v methanol, solvomodule: 1/20 m/v, temperature: solvent boiling point: –72 °C) the kinetics of Soxhlet extraction, the extractive matter, chlorogenic acid, umbelliferone and apigenine-7-*O*-glucoside were investigated. The dried, ground plant material (10 g) and 80 % v/v methanol (200 ml) were put into the Soxhlet apparatus. The solvent was boiled and refluxed for a period of 240 min. The liquid extract was evaporated under vacuum at 40 °C to constant weight. The extracts were stored in the refrigerator for subsequents analysis.

Tillepape extraction

Under the optimal maceration conditions (solvent: 80 v/v methanol, solvomodule: 1/20 m/v, temperature: solvent boiling point: –72 °C) the kinetics of Tillepape extraction, the extractive matter, chlorogenic acid, umbelliferone and apigenine-7-*O*-glucoside were investigated. Dried, ground plant material (10 g) and 80 %v/v methanol (200 ml) were put into the Tillepape apparatus. The solvent was boiled and refluxed for a period of 240 min. The liquid extract was evaporated under vacuum at 40 °C to constant weight. The extracts were stored in the refrigerator for subsequents analysis.

RESULTS AND DISCUSSION

The components most commonly found in all *Hieracium* species are phenolics and flavonoids [4–6]. Phenolic acids and flavonoids are natural antioxidants [11–13] with anti-mutagenic, anti-carcinogenic [14,15], cardio-protective [16] and antimicrobial properties [12,17,18]. All obtained extracts of *Hieracium pilosella* L. contains three phenolic components: chlorogenic acid, umbelliferone and apigenine-7-*O*-glucoside, according to HPLC analysis.

Maximum contents of extractive matter, chlorogenic acid, umbelliferone and apigenine-7-*O*-glucoside in the initial plant material determined by Soxhlet extraction with 80 %v/v methanol were 42.33, 19.20, 0.58 and 0.068 g/100 g dry plant material, respectively.

Maximum contents of chlorogenic acid, umbelliferone and apigenine-7-*O*-glucoside in the dry extracts, determined by Soxhlet extraction with 80 % v/v methanol, were 45.63, 1.36 and 0.16 g/100 g dry extract, respectively.

Retention times of the components chlorogenic acid, umbelliferone and apigenine-7-*O*-glucoside obtained by HPLC method were 2.07, 4.60, 5.67 min, respectively. The equation obtained from the calibration graph for the determination of the concentrations of the components: the chlorogenic acid, umbelliferone and apigenine-7-*O*--glucoside, in the extracts through the dependence of the peak area upon the concentration of the standards obtained by HPLC method is: $P = q + rc$, where P (mAU) is peak surface, c (mg/ml) standard concentration and q and r are constants.

Investigation results of the influence of methanol on the yield of extractive matter and the bioactive components in dry maceration extracts are shown in Table 1. Based on the HPLC analysis results and the corresponding equations for the determination of the bioactive components concentration in the extracts, the content of bioactive components in total dry extracts was determined.

The highest yields of extractive matter (22.06 g/100 g of dry plant material), chlorogenic acid (10.56 g/100 g of dry plant material), umbelliferone (0.40 g/100 g of dry plant material), and apigenine-7-*O*-glucoside (0.04 g/100 g of dry plant material) were obtained by extraction with 80 % v/v methanol. Further investigations were carried out with extractions with 80 % methanol, as optimal solvent concentration.

Table 2 shows the effect of solvomodule on the yield of extractive matter, the yield and content of the investigated bioactive components in dry extracts obtained by the maceration.

With the increase of solvomodule above 1:20 m/v the content of total extractive matter was decreased (1.12% for solvomodule 1:25 m/v). With the increase of solvomodule (1:25 m/v) the content of chlorogenic acid (13.64%), umbelliferone (28.07%), and apigenine-7-*O*--glucoside (5.55%) was decreased in the dry extracts. The maximum yield of extract and investigated bioactive components were obtained by solvomodule 1:20 m/v. Increasing of solvomodule does not contribute to further increasing of total extractive matter but only extracts dilutions. Therefore, further increasing of solvomodule would not make sense. Solvomodule 1:20 m/v was accepted as the optimal one.

Table 1. The effect of methanol concentration on the yield of the extractive matter and the bioactive components (extraction time: 120 min, solvomodule: 1:15 m/v, temperature: 25 °C)
Tabela 1. Uticaj sadržaja metanola na prinos ekstrakta i bioaktivnih komponenata (vreme ekstrakcije: 120 min, solvomodul: 1:15 m/v, temperatura: 25 °C)

Methanol content, v/v%	Extractive matter content g/100 g d.p.m.[a]	Chlorogenic acid content		Umbelliferone content		Apigenin-7-*O*-glucoside content	
		g/100 g d.e.[b]	g/100 g d.p.m.	g/100 g d.e.	g/100 g d.p.m.	g/100 g d.e.	g/100 g d.p.m.
10	19.24 ± 0.27	28.97 ± 0.30	5.57 ± 0.11	1.53 ± 0.03	0.29 ± 0.03	0.10 ± 0.004	0.02 ± 0.002
20	19.50 ± 0.60	38.53 ± 0.40	7.51 ± 0.08	1.68 ± 0.03	0.33 ± 0.02	0.13 ± 0.003	0.02 ± 0.001
30	20.21 ± 0.46	40.29 ± 0.80	8.14 ± 0.56	1.70 ± 0.04	0.34 ± 0.05	0.13 ± 0.004	0.03 ± 0.001
40	20.82 ± 1.10	39.84 ± 1.06	8.30 ± 0.25	1.77 ± 0.05	0.37 ± 0.06	0.15 ± 0.003	0.03 ± 0.001
60	21.53 ± 1.45	40.36 ± 0.81	8.69 ± 0.38	1.79 ± 0.11	0.39 ± 0.06	0.17 ± 0.005	0.04 ± 0.002
80	22.06 ± 1.48	47.87 ± 0.87	10.56 ± 0.81	1.81 ± 0.03	0.40 ± 0.07	0.17 ± 0.004	0.04 ± 0.001
100	10.59 ± 0.83	40.55 ± 0.91	4.29 ± 0.52	0.68 ± 0.04	0.07 ± 0.0	0.42 ± 0.007	0.04 ± 0.001

[a]d.p.m. – dry plant material; [b]d.e.– dry extract; each value is mean ±*SD* of three measurements. Values are statistically different at the probability level of $P < 0.05$ according to Student's *t*-test

Table 2. The effect of solvomodule on the yield of the extractive matter and the bioactive components (extraction time: 120 min, solvent: 80 % v/v methanol, temperature: 25 °C)
Tabela 2. Uticaj solvomodula na prinos ekstrakta i bioaktivnih komponenata (vreme ekstrakcije: 120 min, rastvarač: 80% v/v metanol, temperatura: 25 °C)

Solvomodule, m/v	Extractive matter content g/100 g d.p.m.	Chlorogenic acid content		Umbelliferone content		Apigenin-7-*O*-glucoside content	
		g/100 g d.e.	g/100 g d.p.m.	g/100 g d.e.	g/100 g d.p.m.	g/100 g d.e.	g/100 g d.p.m.
1:10	21.10 ± 0.93	42.78 ± 0.72	9.06 ± 0.56	1.03 ± 0.03	0.22 ± 0.01	0.17 ± 0.007	0.04 ± 0.002
1:15	22.06 ± 1.48	47.87 ± 0.87	10.56 ± 0.81	1.81 ± 0.03	0.40 ± 0.07	0.17 ± 0.004	0.04 ± 0.001
1:20	23.30 ± 1.70	48.16 ± 0.50	11.22 ± 0.62	1.71 ± 0.05	0.40 ± 0.03	0.18 ± 0.005	0.04 ± 0.001
1:25	23.04 ± 0.21	41.59 ± 0.85	9.57 ± 0.45	1.23 ± 0.02	0.28 ± 0.02	0.17 ± 0.004	0.04 ± 0.003

The influence of temperature (25 °C – solvent boiling point) on the extractive matter yield, the yield and content of the bioactive components in the macerated dry extracts with 80 % v/v methanol and solvomodule 1:20 m/v, was monitored by investigating the kinetics of the extraction of extractive matter, chlorogenic acid, umbelliferone and apigenine-7-*O*-glucoside (Figs. 1a–1d, respectively) at different extraction temperatures.

The yield of extractive matter, chlorogenic acid, umbelliferone and apigenine-7-*O*-glucoside is increased with the increase in temperature. The content of chlorogenic acid, umbelliferone and apigenine-7-*O*-glucoside in the dry extracts was not significantly changed; therefore, the solvent boiling point (72 °C) was accepted as the optimal extraction temperature.

The figure shows that, in the maceration extraction process, there are two characteristic periods, the period of fast extraction and the period of slow extraction, in accordance with Ponomarjev's empirical equation [22]. Independent of the recovery temperature, the extraction occurred in two main stages: first, the dissolution of the material near the surface, characterized by a rapid increase in the extractive matter yield at the beginning of the process (washing or fast extraction), and second, the diffusion of the solute from the porous plant residue into the solution (slow extraction). Based on the results obtained the optimal extraction conditions were defined: 80 %v/v methanol, solvomodule 1:20 m/v and the temperature: 72 °C.

Under the optimal maceration extraction conditions the kinetics of the extractive matter and bioactive components were monitored by the circulation extraction techniques. A comparative survey of the kinetics of the maceration extraction with reflux, Tillepape and Soxhlet extraction of the extractive matter, chlorogenic acid, umbelliferone and apigenine-7-*O*-glucoside (Figs. 2a–2d, respectively) under the optimal maceration extraction conditions is shown in Fig. 2.

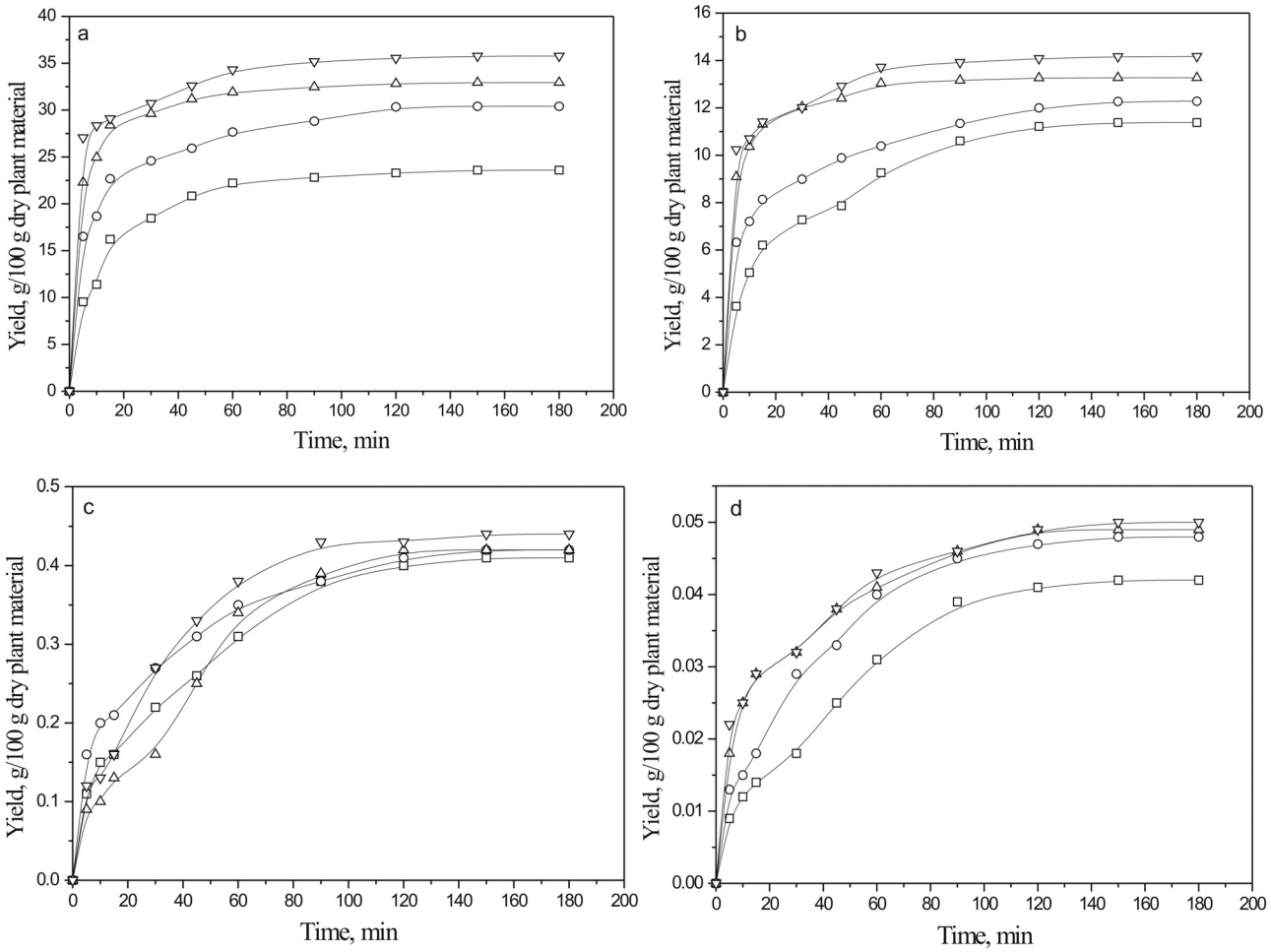

Figure 1. Extraction kinetics of the extractive matter, a) chlorogenic acid; b) umbelliferone; c) apigenine-7-O-glucoside and d) maceration at various temperatures (□-25, ○-40, Δ-50 °C and ∇-solvent boiling point). Solvent: 80 % v/v methanol, solvomodule: 1:20 m/v.
Slika 1. Kinetika ekstrakcije a) hlorogene kiseline; b) umbeliferona; c)apigenin-7-O-glukozida i d)maceracija na različitim temperaturama (□-25, ○-40, Δ-50 °C i ∇-tačka ključanja rastvarača). Rastvarač: 80 % v/v metanol, solvomodul: 1:20 m/v.

The obtained data indicate that a greater amount of dry extracts was achieved by the circulation extraction techniques for a period of 240 min (41.07 and 40.20 g/100g dry plant material by Soxhlet and Tillepape extraction, respectively), compared to the maceration technique with reflux (35.76 g/100g dry plant material), under the same extraction operation conditions. The amount of dry extracts obtained by Soxhlet and Tillepape extraction increased for 13.0 and 11.0 %, respecttively in comparison with the amount of extract obtained by maceration with reflux. The highest level of matter extracted was achieved with Soxhlet extraction for a period of 240 min (95.0%).

The extraction kinetics curves (Fig. 2) are the typical curves for the extraction of the cellular material [22–24] with two extraction periods. In the first period, the fast extraction occurs where the matter extracted is washed out from the surface of the grounding plant material by solvent. In the second period, a slow molecule diffusion of the matter extracted from the internal part of the porous plant material occurs (the slow extraction). The fast extraction (the curvilinear part of the extraction curve) is characterized by the washing coefficient b, and the slow extraction by the slow extraction coefficient, k, in the extraction curve equation (Eq. (2)) [22]:

$$\frac{q_0 - q_i}{q_0} = b + kt \tag{1}$$

where q_0 is the extractive matter content in the initial plant material; q_i – the content of the extractive matter in the plant material after the period t; b – coefficient of the fast extraction period; k (min^{-1}) – coefficient of the slow extraction period.

The values of coefficients b and k in the kinetics equations for the extraction of matter extracted, chlorogenic acid, umbelliferone and apigenine-7-*O*-glucoside, by using various techniques under the optimal conditions, are given in Table 3. The duration times of the

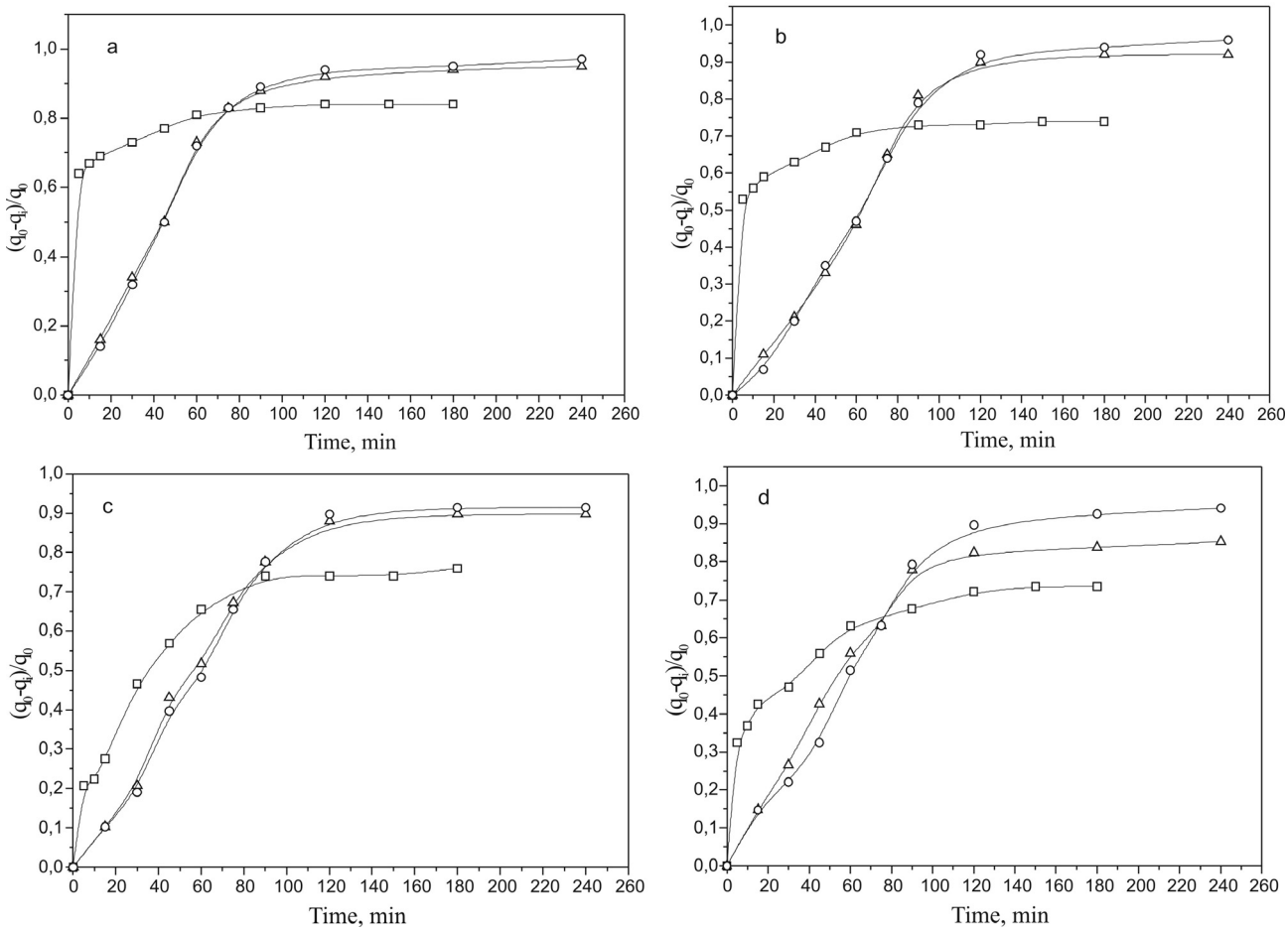

Figure 2. Tillepape and Soxhlet extraction kinetics and reflux maceration of the extractive matter a) chlorogenic acid; b) umbelliferone; c) and apigenine-7-O-glucoside; d) under the optimal maceration conditions (□-reflux, Δ-Tillepape and ○-Soxhlet).
Slika 2. Kinetika Tillepape i Soxhlet ekstrakcije i refluksna maceracija, a) hlorogene kiselina; b) umbeliferon; c) apigenin-7-O-glukozid; d) optimalni uslovi maceracije (□-refluks, Δ-Tillepape i ○-Soxhlet).

fast extraction and extraction levels for total matter extracted and the investigated components are given in Table 4. Based on this data it is clear that, in the period of fast extraction, 81−94% of extractive matter was extracted by elution and dissolution of the matter extracted from the surface of destructed cells of the plant material, 71−92% of chlorogenic acid, 74−90% of umbelliferone, and 72−90% of apigenine-7-*O*-glucoside, depending on the extraction method used (Table 4). This shows that the crushing of the plant material used for the investigation has been relatively high (average diameter of drug particles is 0.35 mm). The high level of destruction of the cells increases the surface whereof the matter extracted is eluted in the fast period and the matters extracted are dissolved very fast, thus providing a high level of their extraction during that period.

In all the extracts a high content of chlorogenic acid was detected. In dry extracts obtained by maceration with reflux, Soxhlet and Tillepape extractions under the optimal conditions it was 39.61, 44.39 and 45.63%, respectively. The preparative isolation of chlorogenic acid confirmed that the yield of chlorogenic acid in the isolated preparation corresponds with this component content established in liquid extracts by HPLC analysis. Considering a high content of this component in the extracts *H. pilosella* L., this plant can represent a potential natural resource.

CONCLUSION

Operation conditions and the extraction methods (maceration with and withouth solvent reflux, Soxlet and Tillepape extraction) used have significant influence on the yield of the extractive matter, extraction kinetics and the composition of the extracts obtained from *Hieracium pilosella* L. The optimal extraction conditions are: solvent: 80 % v/v methanol; solvomodule 1:20 m/v; extraction temperature: solvent boiling point (*i.e.* Soxhlet and Tillepape method compared to maceration with and without reflux extraction). The highest yield of dry extracts and yield of some important bioactive components was achieved by using Soxhlet extraction. Several components present in extract were identified using HPLC: chlorogenic acid, umbelliferone and apigenine-7-*O*-glucoside. Chlorogenic acid is the component with the highest percentage in all the extracts. A relatively high content of chlorogenic acid in the investigated plant material (19.20%) and the high level of its extraction from the plant material (71−92%) under the defined optimal maceration conditions, Soxhlet and Tillepape extraction, facilitate its high level of extraction as a pure substance that has an important application in the pharmaceutical and food industries.

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Table 3. The values of b and k coefficients in the equations of the extraction kinetics of the extractive matter under the optimal maceration conditions (Ponomarjev's empirical equation [22])
Tabela 3. Vrednosti koeficijenata b i k u kinetičkim jednačinama ekstrakcije pod optimalnim uslovima maceracije (empirijska jednačina Ponomarjeva [22])

Extracts	Coefficients					
	Reflux maceration		Tillepape extraction		Soxhlet extraction	
	b	$k\times10^4$, min^{-1}	*b*	$k\times10^4$, min^{-1}	*b*	$k\times10^4$, min^{-1}
Extractive matter	0.804	2.33	0.982	2.50	0.908	2.50
Chlorogenic acid	0.702	2.33	0.883	1.67	0.880	3.33
Umbelliferone	0.757	6.61	0.864	1.50	0.882	1.41
Apigenin-7-*O*-glucoside	0.695	2.33	0.794	2.41	0.855	3.67

Table 4. The fast extraction time and the extraction level in the equations of extraction kinetics for various techniques used in this study under the optimal extraction conditions [22]
Tabela 4. Vreme brze ekstrakcije i nivo ekstrakcije u kinetičkim jednačinama ekstrakcije različitim tehnikama pod optialnim uslovima korišćenim u ovom istraživanju [22]

Extracts	Reflux maceration		Tillepape extraction		Soxhlet extraction	
	PFE, min	*SE*, %	*PFE*, min	*SE*, %	*PFE*, min	*SE*, %
Extractive matter	60	81	120	92	120	94
Chlorogenic acid	60	71	120	90	120	92
Umbelliferone	90	74	120	88	120	90
Apigenin-7-*O*-glucoside	120	72	120	82	120	90

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IZVOD

UTICAJ OPERATIVNIH USLOVA I TEHNIKE EKSTRAKCIJE NA PRINOS, KINETIKU I SASTAV METANOLNIH EKSTRAKATA *Hieracium pilosella* L.

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(Naučni rad)

Hieracium pilosella Linne (Family: *Asteraceae*) je višegodišnja zeljasta biljka. Upotrebljava se u tradicionalnoj evropskoj medicini zbog svojih diuretskih i antiinflametornih efekata. Posebno se preporučuje za pojačano mokrenje i izbacivanje mulja, peska i sitnijih kamenčića iz mokraćnih puteva. Kod svih vrsta roda *Hieracium* najzastupljenije komponente su fenolne kiseline i flavonoidi. Ispitivanjem uticaja koncentracije metanola (10––100% v/v), solvomodula (1:10–1:25 m/v) i temperature (25 °C–temperatura ključanja rastvarača) ekstrakcije maceracijom na prinos i kinetiku ukupnih ekstraktivnih materija, hlorogene kiseline, umbeliferona i apigenin-7-*O*-glukozida iz *Hieracium pilosella* L. određeni su optimalni operativni uslovi ekstrakcije (80% v/v metanol, solvomodul: 1:20 m/v, temperatura ključanja rastvarača). Na osnovu rezultata ispitivanja kinetike Soxhlet i Tillepape ekstrakcije ukupnih ekstraktivnih materija i ispitivanih komponenti pod optimalnim operativnim uslovima maceracije utvrđeno je da se najveći prinos ekstraktivnih materija iz suvog biljnog materijala (41,07 g/100 g suvog biljnog materijala, tj. 97,0% u odnosu na sadržaj ekstraktivnih materija u biljnom materijalu), hlorogene kiseline (18,34 g/100 g suvog biljnog materijala tj. 95,5% u odnosu na sadržaj hlorogene kiseline u biljnom materijalu), umbeliferona (0,53 g/100 g suvog biljnog materijala, tj. 91,4% u sadržaj umbeliferona u biljnom materijalu) i apigenin-7-*O*-glukozida (0,064 g/100 g suvog biljnog materijala, tj. 94,1% u odnosu na odnosu na sadržaj apigenin-7-*O*-glukozida u biljnom materijalu) ostvaruju primenom Soxhlet ekstrakcije, za vreme od 240 min. Sadržaj hlorogene kiseline, umbeliferona i apigenin-7-*O*-glukozida u ekstraktima određivan je HPLC metodom.

Ključne reči: *Hieracium pilosella* L. • Tehnike ekstrakcije • HPLC analiza • Hlorogena kiselina • Umebeliferon • Apigenin-7-*O*-glukozid
Key words: *Hieracium pilosella* L. • Extraction techniques • HPLC analysis • Chlorogenic acid • Umbelliferone • Apigenin-7-*O*-glucoside