ACTA SCIENTIARUM POLONORUM
Acta Sci. Pol. Hortorum Cultus, 21(6) 2022, 143–154
https://czasopisma.up.lublin.pl/index.php/asphc ISSN 1644-0692 e-ISSN 2545-1405 https://doi.org/10.24326/asphc.2022.6.12
ORIGINAL PAPER
Accepted: 1.12.2022

EXTRACTION AND ANALYSIS OF RUSCOGENINS FROM BUTCHER'S BROOM (*Ruscus aculeatus* L.) RHIZOMES USING HPLC

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ABSTRACT

Butcher's broom (*Ruscus aculeatus* L.) is a plant with valuable chemical composition and many medical applications. The underground rhizomes of the plant contain steroidal saponins, compounds with proven therapeutic effects and used mostly in treating venous insufficiency. The research aimed to optimise the extraction of saponins from butcher's broom rhizomes to obtain the highest possible content of active compounds in the dry extract. The extraction was carried out in five variants using pure water or a mixture of water and ethanol as solvents in different potions. Three samples of butcher's broom rhizomes from Albania, Bulgaria, and Germany were examined for the presence of highest level of sapogenin active ingredient. Results show that Albanian sample has the highest percentage of ruscogenins, and hence Albanian butcher's broom rhizomes were chosen for the extraction of active ingredient by alcoholic solution with different concentration. The sapogenin content in the extracts was determined by the pharmacopoeial method using high performance liquid chromatography (HPLC). A strong, positive correlation was found between ethanol concentration and the content of ruscogenins in the dry extract. The most efficient variant of the extraction turned out to be the use of 50% ethanol as a solvent, where 304 mg of ruscogenins were obtained from 50 g of the raw material.

Key words: saponins, extraction, butcher's broom rhizomes, ruscogenin, neoruscogenin, HPLC

INTRODUCTION

Saponins are secondary plant metabolites found in many wild and cultivated plants, including triterpenoids, steroids, and glycosylated steroid alkaloids. Triterpenoid saponins are predominant in crops such as soybeans, beans, peas, alfalfa, tea, spinach, sugar beet, quinoa, and sunflower. They are also found in horse chestnut and ginseng. At the same time, steroid saponins are common in plants used for their pro-health properties (including oats, peppers, aubergine, tomato, asparagus, yam, and fenugreek). The structural diversity of saponin compounds is reflected in their physicochemical and biological properties. It thus enables their use in pharmacy, medicine, and production of cosmetics or other products [Francis et al. 2002, Güçlü Üstündağ and Mazza 2007, El Aziz et al. 2019, Wang et al. 2021]. One of the plants that accumulate saponins is the butcher's broom (*Ruscus aculeatus* L.), an Eurasian evergreen shrub from the Asparagaceae family, with a rich history and many applications. The healing properties of the underground rhizomes of butcher's broom were known in antiquity and successfully used in the treatment of heavy legs, varicose veins and other problems with venous circulation [European Medicines Agency 2019, Raposo et

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al. 2021, Rodrigues et al. 2021]. The aerial parts of this plant are traditionally used as diuretics, mainly in the Mediterranean countries and the Middle East [Hadžifejzović et al. 2013, Rodrigues et al. 2021]. In the 1950s, scientific research proved the presence of valuable saponin compounds in the butcher's broom, which are responsible for the healing properties of the raw material. The development of science allowed identifying and describing the structure and properties of saponins [Schwarz 2000, Masullo et al. 2016]. It is currently known that these are primarily steroidal saponins. Apart from saponins, butcher's broom extract contains flavonoids, sterols (sitosterol, stigmasterol and campesterol), tyramine, coumarin, triterpenes, lignoceric acid, glycolic acid and benzofurans [Urbanek 2017, Rodrigues et al. 2021].

The content of saponins in the rhizomes of the butcher's broom varies depending on the origin of the raw material, the age of the plant, and the determination method [Ozer et al. 2018]. According to the Polish Pharmacopoeia XI [2017], the herbal raw material of butcher's broom is dry, whole or broken underground parts (*Rusci rhizoma*), with a content of not less than 1.0% of the sum of sapogenins, expressed as ruscogenins (a mixture of neoruscogenin and ruscogenin, Fig. 1). Ivanova et al. [2015], by evaluating the *in vitro* culture of the butcher's broom, suggest that the origin of the clone and the type of culture may influence the saponin biosynthesis. However, later studies [Ivanova et al. 2019] report that major components such as neoruscogenin show opposite biosynthetic tendencies in shoots and roots during their *in vitro* development. Extracts from butcher's broom are currently used in treating chronic venous disease, varicose veins, haemorrhoids, orthostatic hypotension, and their beneficial effect on the damaged venous and lymphatic system microcirculation has been confirmed in experimental studies [Masullo et al. 2016, Chudek and Ziaja 2017]. Balica et al. [2013], when assessing the *in vivo* anti-inflammatory effect of crude steroidal saponin extracted from butcher's broom rhizomes, they showed a dose-dependent anti-inflammatory effect, recommending that the mechanism of action was probably prostaglandin inhibition [Balica et al. 2013]. Extracts of the herb and rhizomes of the butcher's broom and the compounds isolated from them show antibacterial, especially antifungal properties. In some cases, this activity was better than standard drugs (streptomycin, ampicillin, bifonazole, ketoconazole) and more pronounced with some isolated compounds. The results of antioxidant determinations turned out to be strongly correlated with the total content of phenols [Hadžifejzović et al. 2013].

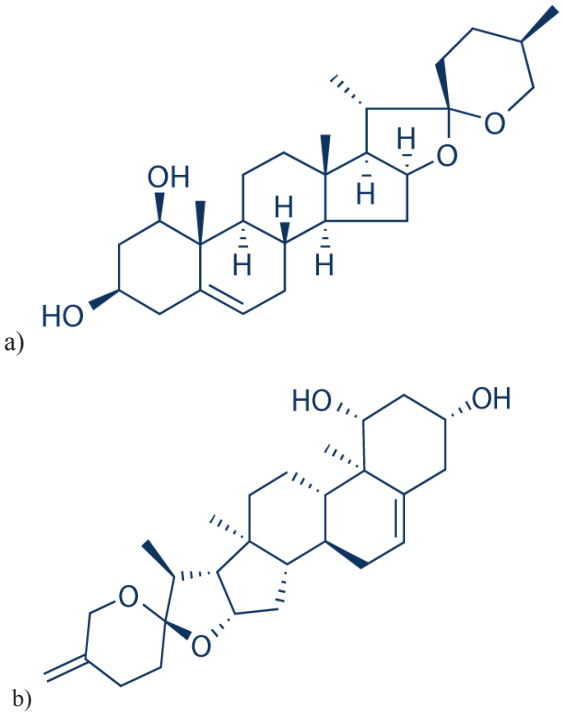

Fig. 1. Chemical structure of: a) ruscogenin $C_{27}H_{42}O_4$ and b) neoruskogenin $C_{27}H_{40}O_4$

There are various methods of saponin extraction. Akbarizare et al. (2019) extracted saponins from *Spirulina platensis* using distilled water and n-butanol. The result showed that saponins from *Spirulina* decrease cancer cellular viability, and these compounds can be a candidate for anticancer agents. In turn, Akbari et al. [2019] obtained saponins from fenugreek seeds using microwave-assisted extraction under different extraction parameters such as irradiation time, microwave power, ethanol concentration (40–80%), solid-to-solvent ratio (1:8–1:12 g/mL), and a constant microwave temperature of 70°C. Literature data show that saponins are often extracted using methanol or butanol as solvents [Kite et al. 2007, De Marino et al. 2012, Hadžifejzović et al. 2013, Ivanova et al. 2015, El Aziz et al. 2019]. Also the latest research shows

that chloroform and ethanol extracts from the rhizome of butcher's broom show beneficial pharmacological effects and can be used in the pharmaceutical industry [Taşkın et al. 2020, Rodrigues et al. 2021]. The use of an appropriate solvent is a key element from the point of view of the dietary supplement industry, which is regulated, among others, by control of residual solvents. Presents research aimed to optimise the extraction process of ruscogenins from the rhizome of the butcher's broom by analysing the influence of the process conditions on the accumulation of the tested compounds in the extract. The selected factor was the concentration of the extraction solvent – water and ethyl alcohol. Research also sought to find a lower concentration of ethanol to reduce the cost of ruscogenins extraction.

MATERIAL AND METHODS

Plant material and extract preparation. The plant material consisted of butcher's broom rhizomes obtained in 2018 from Albanian, Bulgarian, and German suppliers. The tests were performed in the Greenvit development laboratory in Zambrów, Poland. After preliminary analysis using high-performance liquid chromatography (HPLC), the raw material with the highest content of ruscogenins was selected, and further investigations studies were carried out with this material. Butcher's broom rhizomes were extracted in five variants (A, B, C, D, E), using water or a mixture of water and ethanol as solvents (Tab. 1). One-stage extractions were carried out using a heating mantle under a reflux condenser connected with 1 L round bottomed flask at the solvent boiling temperature (78°C). The ratio of the raw material to the solvent was 1:10 (sample mass – 50 g). The extraction time was 60 minutes.

In variant B, additionally, a change of pH was used. For this purpose, after 10 min of heating, the extract was acidified with phosphoric acid (85%). From each obtained liquid diluted extract (LDE), a sample of approximately 50 g was taken to determine the dry weight and the pH value. The residue was concentrated in a vacuum evaporator (Heidolph, Germany) at 70°C until a thick extract (more concentrated extract – MCE) was obtained with approximately 50% a dry matter content. The extracts were then dried in a vacuum oven (Binder, Germany) at 60°C to obtain dry weight of liquid extract (solid dry extract – SDE). Samples were taken from the prepared MCE and SDE extracts, in which the content of ruscogenins was determined using the HPLC method.

Preparation of samples for HPLC analysis. To determine the content of sapogenins in both the dry plant material and the prepared extracts (MCE and SDE), the HPLC method given in the Polish Pharmacopoeia XI [2017] was used. For this purpose, approximately 2.0 g of finely ground raw material and approx. 1.0 g of extracts were weighed, and then 60 ml of anhydrous ethanol, 15 ml of water and 0.2 g of potassium hydroxide were added. The mixtures were extracted for 4 hours in an oil bath under reflux, keeping the mixture at reflux temperature (95°C). After heating the mixture, it was allowed to cool and filtered into a 100 mL volumetric flask, then made up to 100 mL with anhydrous ethanol. 25 mL of the resulting solution was evaporated to dryness under reduced pressure in a vacuum evaporator. The residue was dissolved in 10 mL of 1-butanol, 3 mL of hydrochloric acid (250 g/L; 7 mol/L) and 8 mL of water were added, keeping the mixture at reflux temperature (95°C). The mixtures were heated for 1 hour in an oil bath under reflux. It was allowed to cool and transferred to

Table 1. Variants of extraction

Variant	A	B	C	D	E
Solvents	Aqueous solution		Alcoholic solution		
	Water, pH 7	Water, pH 3	Water – ethanol	Water – ethanol	Water – ethanol
Ethanol concentration (% v/v)	0	0	35	50	70

Table 2. Gradient parameters of the used mobile phases

Time (min.)	Mobile phase – water (1) (% *v/v*)	Mobile phase – acetonitrile (2) (% *v/v*)
0–25	40	60
25–27	40 → 0	60 → 100
27–37	0	100

a 100 mL volumetric flask and made up to 100 mL with methanol. The samples were filtered through a syringe filter into the vials and placed in an HPLC autosampler tray.

Determination of ruscogenins by HPLC. The determinations were made by high-performance liquid chromatography (HPLC) using a Prominence Shimadzu chromatograph (Japan). The analysis was performed against a reference solution prepared by dissolving 5.0 mg of the standard CSP ruscogenin (EDQM, France) in methanol. Raw material analyses were performed in triplicate, while EG and ES analyses were duplicated. HPLC analysis was performed in reverse phase using a stepwise gradient of the mobile phase consisting of: 1) water and 2) acetonitrile (Tab. 2). A Kinetex 5 µm C18 100 Å chromatographic column (Phenomenex, Germany) with dimensions: length 0.25 m, internal diameter 4.6 mm, packed with silica gel with octadecylsilyl groups, diameter 5 µm, was used for the analysis. The flow rate of the mobile phase through the column was 1.2 mL/min. In each case, 20 µL of the test sample was dosed into the distribution system. Compounds were detected at a wavelength of 203 nm with a diode array detector (photo diode array – PDA). Each extract was analysed for 37 minutes in 3 stages. The identification of the peaks of neoruscogenin and ruscogenin was carried out based on the chromatogram obtained for the CSP ruscogenin standard and the literature data.

To determine the content of sapogenins in raw materials and extracts, the formula included in the monograph in Polish Pharmacopoeia XI [2017] was used, according to which the percentage content of sapogenins was calculated in terms of ruscogenins (neoruscogenin and ruscogenin) (percentage):

$$X = \frac{A_1 \times m_2 \times 4 \times p_1}{A_2 \times m_1} + \frac{A_3 \times m_2 \times 4 \times p_2}{A_4 \times m_1}$$

where: X – percentage of ruscogenin;
A_1 – ruscogenin peak area in the chromatogram of the test solution;
A_2 – ruscogenin peak area in the chromatogram of the reference solution;
A_3 – neoruscogenin peak area in the chromatogram of the test solution;
A_4 – neoruscogenin peak area in the chromatogram of the reference solution;
m_1 – mass of test extract used to prepare the test solution, in grams;
m_2 – mass of CSP ruscogenins used to prepare the reference solution, in grams;
p_1 – percentage of ruscogenin in CSP ruscogenins;
p_2 – percentage of neoruscogenin in CSP ruscogenins.

Determination of dry matter of extracts. The dry matter was determined at approx. 1.5 g LDE and approx. 0.5 g EG. The extracts were dried at 105°C using a moisture analyser (Radwag, Poland). The assay was performed in triplicate and expressed as a percentage by weight, noting the weight loss by evaporation of the water.

Determination of pH. The pH of the extracts was determined with a pH meter (Mettler Toledo, Germany).

Statistical analysis. A statistical analysis of the extraction efficiency results, dry matter content, and the sapogenin content in raw materials and extracts were analysed. The analysis was performed in the Statistica 13 program, using one-way ANOVA, applying the Fisher's NIR test and correlation analysis.

RESULTS

Ruscogenins from different sources. The tested plant material turned out to be heterogeneous in terms of the content of sapogenins. The highest content of ruscogenins (1.40%) was obtained in a sample of butcher's

broom rhizomes obtained from Albania (Fig. 2, Tab. 3). Lowest contents were recorded for the samples from Bulgaria and Germany – 1.18% and 1.13% of the sum of ruscogenins, respectively (Tab. 3). Peaks of neoruscogenin (retention time 10.17 min.) and ruscogenin (retention time 12.08 min.) were identified from the standard chromatogram.

Assessment of dry matter content and pH value in tested liquid extracts for Albanian butcher's broom rhizomes. The dry matter content in the extracts, pH values and the initial extraction efficiency are presented in Table 4. During the extraction, differences in the colour of the solutions depending on the solvent used were observed. The water extracts were dark brown, while the increasing concentration of ethanol in the mixture resulted in a lighter colour and greater clarity of the extracts. The increase in ethanol concentration also reduced the foaming of the solution in the extraction flask. The highest dry matter was obtained in the case of water extractions – in extract B, it was

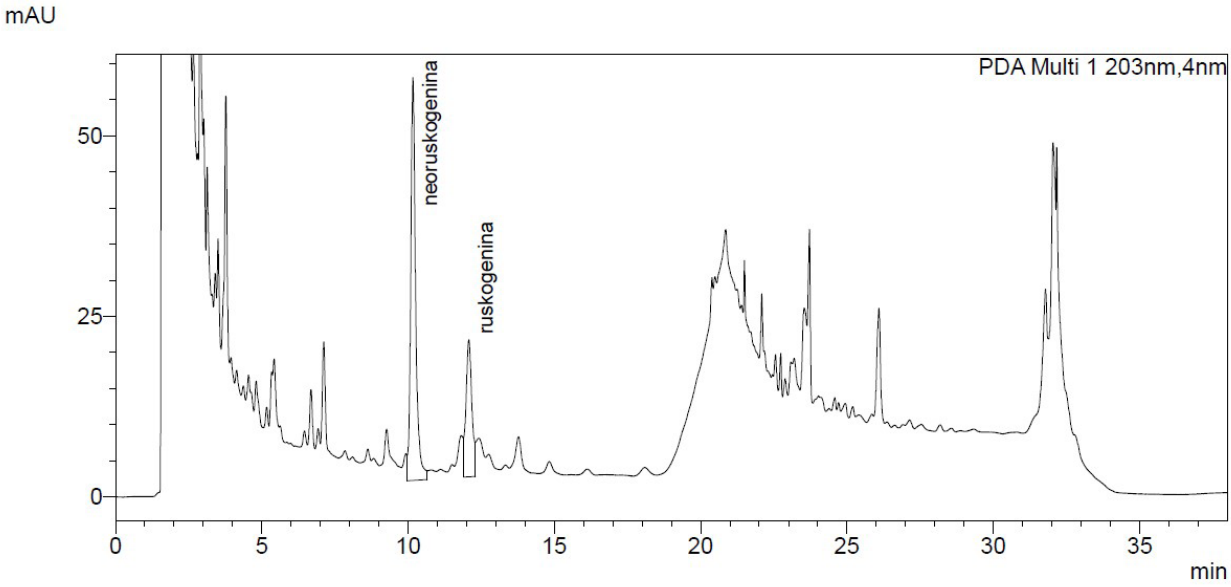

Fig. 2. Chromatogram of the rhizome of butcher's broom from Albania, neoruscogenin (retention time 10.17 min.), ruscogenin (retention time 12.08 min.)

Table 3. The content of the sum of ruscogenins (ruscogenin and neoruscogenin) in the tested samples of butcher's broom rhizomes

Active compounds in raw material (%)	The origin of the raw material		
	Albania	Bulgaria	Germany
Ruscogenin	0.38 a	0.31 b	0.30 b
Neoruscogenin	1.02 a	0.86 b	0.83 b
Sum of ruscogenins	1.40 a	1.18 b	1.13 b

The results marked with the same letters do not differ significantly, assuming the significance level of $p < 0.05$.

3.07%, while in extract A – 2.97%. In variants C and D, the differences were minor – 2.86% and 2.71%, respectively, similar to the case of A and B, where they amounted to 2.97% and 3.07%, respectively. The lowest dry matter content was found in extract E, where 2.22% of dry matter was obtained (Tab. 4).

The amounts of extracts formed after one hour of extraction varied depending on the variant used (Tab. 4). The highest amount of LDE (400 g) was obtained using 70% ethanol solution (variant E). Smallest amounts were extracted in variants C and D (respectively 376 g and 370 g of the extract with 35 and 50% ethanol). The values obtained in the water extractions were very similar and amounted to 358 g in variant B and 356 g in variant A.

Analysing the initial extraction efficiency of butcher's broom rhizomes, a highest efficiency was found in water variants with a predominance of water in the extraction mixture. The yield expressed as the theoretical amount of dry matter in the obtained extract for variants A, B, and C ranged from 10.58–10.99 g. There were no significant differences in the yield of the described variants. Significantly lower yields was obtained in extract extract E (8.88 g).

Estimating dry matter content and quantitatively assessing sapogenin content in concentrated extracts. Concentrated liquid extract (MCE) was concentrated to a dry weight of approx. 50–60%. Like during extraction, increased foaming of solutions with lower ethanol concentrations was observed. The summary of dry weights, amounts of MCE obtained and theoretical amount of native extract in the obtained amount is summarised in Table 4. The highest theoretical content of the native extract 8.70 g, was obtained in variant D. Much less native extract was obtained in variants A, B and E (7.30 g, 7.10 g and 6.90 g, respectively).

The content of the sum of ruscogenins determined by the HPLC method in the dense extracts is presented in Table 5. The highest mean content of the sum of ruscogenins (neoruscogenin and ruscogenin) was

Table 4. The results obtained during the analyzes carried out using the HPLC method

Type of extract	Parameter	Variants				
		A	B	C	D	E
Liquid diluted extract (LDE)	average dry weight after extraction (%)	2.97 ±0.17	3.07 ±0.30	2.86 ±0.17	2.71 ±0.06	2.22 ±0.14
	amount of liquid extract (LDE) after extraction (g)	356	358	376	370	400
	yield expressed as the theoretical amount of dry matter in the obtained amount of extract (g)	10.58 a ±0.63	10.99 a ±1.10	10.74 a ±0.64	10.03 b ±0.22	8.88 b ±0.59
	pH after extraction	4.92	3.55	5.20	5.33	5.45
More concentrated extract (MCE)	the amount of extract used for concentration (g)	300	300	320	320	350
	average dry matter after concentration (%)	59.88 ±0.71	47.17 ±1.21	53.37 ±0.24	51.09 ±0.48	59.32 ±1.13
	the amount of extract after concentration (g)	12.20	15.00	15.60	17.10	11.70
	theoretical amount of native extract in the obtained amount of concentrated extract (g)	7.30 c ±0.09	7.10 d ±0.18	8.30 b ±0.04	8.70 a ±0.08	6.90 d ±0.13
Solid dry extract (SDE)	amount of extract used for drying (g)	6.80	no	10.10	11.40	6.80
	theoretical amount of extract after drying (g)	4.07	no	5.39	5.82	4.03
	the amount of extract after drying (g)	3.90 c	no	5.00 b	5.60 a	3.70 c
	process efficiency (%)	57.35	no	49.50	49.12	54.41

no – not marked
The results marked with the same letters do not differ significantly, assuming the significance level of $p < 0.05$.

Table 5. Percent content of ruscogenins (neoruscogenin and ruscogenin) in MCE extracts determined by HPLC

Type of extract	Parameter		Variants				
			A	B	C	D	E
More concentrated extract (MCE)	average content of HPLC sapogenins in the concentrated extract (%)	neoruscogenin	0.44 ±0.12	0.32 ±0.01	0.71 ±0.01	0.81 ±0.15	0.80 ±0.07
		ruscogenin	1.90 ±0.22	1.33 ±0.10	2.93 ±0.08	2.87 ±0.11	3.02 ±0.33
		sum of ruscogenins	2.34 ±0.35	1.65 ±0.11	3.64 ±0.10	3.68 ±0.26	3.82 ±0.40
	average content of HPLC sapogenins calculated on dry weight (%)	neoruscogenin	0.73 ±0.21	0.68 ±0.03	1.33 ±0.03	1.59 ±0.30	1.35 ±0.12
		ruscogenin	3.17 ±0.38	2.82 ±0.21	5.48 ±0.16	5.62 ±0.21	5.10 ±0.55
		sum of ruscogenins	3.90 b ±0.59	3.50 b ±0.24	6.81 a ±0.19	7.21 a ±0.52	6.44 a ±0.68
Solid dry extract (SDE)	average content of HPLC sapogenins in the dry extract (%)	neoruscogenin	0.58 c ±0.06	no	1.11 b ±0.11	1.16 b ±0.16	1.19 a ±0.01
		ruscogenin	2.57 d ±0.10	no	3.86 c ±0.11	4.27 b ±0.14	4.76 a ±0.05
		sum of ruscogenins	3.15 d ±0.03	no	4.96 c ±0.22	5.43 b ±0.01	5.95 a ±0.04
	amount of ruscogenins in the obtained amount of dry extract (mg)		123 d	123 d	no	248 b	304 a

no – not marked
The results marked with the same letters do not differ significantly, assuming the significance level of $p < 0.05$.

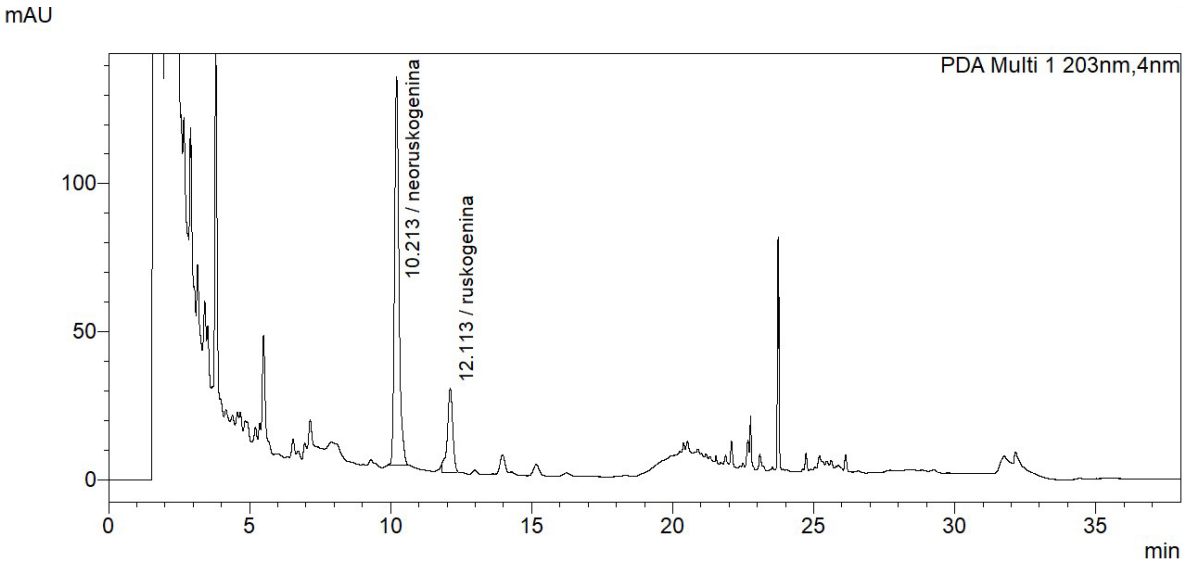

Fig. 3. Chromatogram of the analyzed sample of dry extract E (70% ethanol)

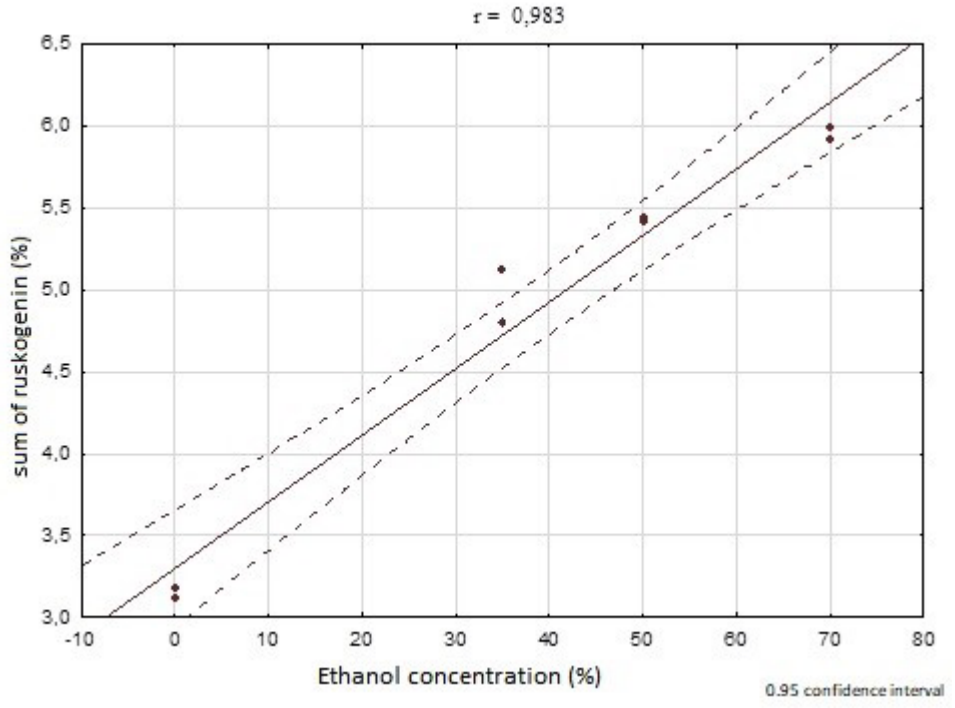

Fig. 4. Graph of the dependence of the content of the sum of ruscogenins determined by HPLC in the dry extract on the concentration of ethanol used for extraction (Statistica 13)

obtained in variant E (3.82%, with a dry matter content of 59.32%). Lower values of the sum of components were obtained in variants D and C (respectively, 3.68% for dry matter, 51.09% and 3.64% for dry matter, 53.37%). The content of ruscogenins in water extracts was much lower, and the lowest value was obtained in variant B (1.65% with a dry matter content of 47.18%).

When converting the content of the sum of ruscogenins to the dry mass of extracts, it was found that the highest content of active compounds was found in extract D (7.21%). However, the differences between the water-ethanol extracts were not statistically significant. After conversion to dry matter, much lower values occurred in A and B aqueous extracts, which were 3.90% and 3.50%, respectively. Based on the obtained results, the content of the tested compounds was abandoned, and further research was not carried out on the water extract B.

Quantitative evaluation of sapogenin content in dry extracts. The highest amount of solid dry extract (SDE) was obtained in variant D – 5.60 g. The theoretical and actual amounts of extracts after drying were very similar, and the observed differences resulted from the losses incurred during the drying and grinding of the extracts. Based on the obtained data, the theoretical drying efficiency was calculated and compared to the obtained amounts of dry native extracts (Tab. 5).

The content of ruscogenins in the analysed dry extracts increased with the increase in the concentration of ethanol used for extraction. Based on the statistical analysis, significant differences were shown between all variants (Tab. 5). The extract had the highest content of active compounds – the sum of ruscogenins was 5.95%. Lower values were obtained for extracts D and C (5.43% and 4.96%, respectively), while the lowest content of ruscogenins was obtained in the aqueous extract A (3.15%). The research confirmed the high content of the tested compounds in the extract obtained with 70% ethanol (E), which was suggested by the results of the thick extract analysis. The chro-

matogram of the analysed sample of dry extract E is presented in Figure 3.

During the research, a strong positive correlation (r = 0.983) was found between the concentration of ethanol used for extraction and the content of the sum of sapogenins determined by HPLC in ES (Fig. 4). When calculating the content of ruscogenins in the obtained amounts of ES, extract E turned out to be the most optimal variant of the extraction, where, in the amount of 3.70 g, 304 mg of ruscogenins was obtained. Extract C (35%) is second in terms of the amount of compounds obtained, with 248 mg of ruscogenins. Quantitatively, fewer ruscogenins were obtained in variant E (70%) – 220 mg despite the high percentage. The most negligible amounts of desired substances were contained in extract A (0% of ethanol) – 123 mg of ruscogenins. The 3.82% of the sum of ruscogenins was obtained in the variant E of the MCE extract, which in terms of dry native extract should theoretically give 6.44% of the sum of ruscogenins. However, 5.95% of sapogenins were obtained in the SDE which was 92% of the theoretical sum of sapogenins from the thick extract. In this variant, it was the highest reproducibility because, for variant C, the reproducibility of the sapogenin result the obtained was 4.96%.

DISCUSSION

Sapogenins, as secondary metabolites of plants, exhibit numerous medicinal properties, which is why they are gaining more comprehensive application. The amount of pharmacologically active steroidal sapogenins in butcher's broom plants appears variable but is usually highest in the rhizome and root [Thomas and Mukassabi 2014]. The content of sapogenins in rhizomes varies depending on the origin of the raw material, the age of the plant, and the method of determination. Tansi et al. [2007] when analysing extract petroleum ether/ethanol (95:5) all parts of *R. aculeatus*, found the highest content of ruscogenin (0.12%) in the underground parts by HPLC method. Ozer et al. [2018], by examining the content of the sum of sapogenins from butcher's broom rhizomes obtained from 18 different sites in the Marmara region (Anatolia, Turkey), showed that 7 of the tested samples met the minimum pharmacopoeial requirements for the content of sapogenins in terms of ruscogenins. As determined by HPLC, the total content of these compounds was from 0.51% to 1.50%. Similar values were obtained in the presented study. The sum of ruscogenins in the raw materials ranged from 1.13% to 1.40%. Cited by Ozer et al. [2018] research using other methods, including thin layer chromatography with densitometry (TLC-DM), showed the content of ruscogenin in the underground parts of *R. aculeatus* L. at the level of 0.08%. On the other hand, studies of various parts of the plant using liquid chromatography coupled with mass spectrometry (HPLC-MS) showed the highest content of sapogenins in rhizomes, which was 0.28% of the sum of ruscogenins [Ozer et al. 2018]. Research by Ghorbani et al. [2020] by the HPLC-PDA method using *Ruscus hyrcanus* Woron. confirmed the highest content of ruscogenin and neoruscogenin in rhizomes (1.96 and 0.76 mg/g dry weight, respectively). The above data indicate that our choice of underground parts of the butcher's broom to isolate sapogenins was justified.

Due to the growing public awareness of preventive health care, there is a significant increase in the literature on the review of recent advances in the extraction of bioactive compounds from medicinal plants. Three techniques for extracting sapogenins are described: simple extraction and Soxhlet extraction (well-known extraction methods) and modern, continuously refined techniques: ultrasound-assisted extraction, microwave-assisted extraction and solvent accelerated extraction methods [Le et al. 2018, El Aziz et al. 2019]. The use of the pharmacopoeial method for determining ruscogenins (HPLC) in this study made it possible to obtain reproducible results. However, the isolation of these compounds is often somewhat problematic, so the choice of solvent and its concentration are crucial in planning the entire process. Sapogenins are often extracted using methanol or butanol as solvents [Kite et al. 2007, De Marino et al. 2012, Hadžifejzović et al. 2013, Ivanova et al. 2015, El Aziz et al. 2019]. which is not beneficial from the point of view of the dietary supplement industry or regulated medicinal products, including control of residual solvents. Recent research [Taşkın et al. 2020] proves that ethanol and chloroform extracts from the rhizome of butcher's broom can be recommended for the pharmaceutical and food industry due to their beneficial pharmacological effects (antioxidant, anticholinesterase and antiurease). Ethanol is one of the basic solvents used in the ex-

traction of active compounds; however, in terms of chemistry, it is classified as a medium-polar solvent. It is a relatively weak solvent for most highly polar bioactive ingredients, such as sapogenins, phenols and flavonoids. For the above reasons, the addition of water is often used. Water, as a polar solvent in combination with ethanol, will improve the process of extracting active compounds, including saponins. However, studies indicate that the addition of water of over 45% may have a negative impact on the extraction of saponin compounds from plant material [Amid and Mirhosseini 2012, Akbari et al. 2019]. To reduce production costs while maintaining a high content of active compounds, extraction methods are sought that could support this process. The European Medicines Agency [2019] recommends extracting the rhizome of the butcher's broom using ethanol at a concentration of 80% or 96% and water. Akbari et al. [2019], to optimise the extraction of the total content of saponins from *Trigonella foenum-graecum* L. seeds, ethanol was used with a concentration in the range of 40–80%. Microwave-assisted extraction (MAE) and the optimal conditions for saponin isolation were achieved using ethanol with a concentration of 63.68%. In the case of our analysis of dense extracts determined by HPLC, the extract obtained with the use of 70% ethanol was characterised by the highest content of the sum of ruscogenins.

Similarly, in the case of the isolation of saponins from *Chenopodium quinoa* Wild. the use of ethanol at a concentration of 70% allowed to develop of optimal conditions for the isolation of these compounds [Espinoza et al. 2021]. In our research, 70% ethanol was not confirmed in the case of isolation of compounds from liquid extracts, where the use of water extractions (A and B) and ethanol with a concentration of 35% (variant C) turned out to be more efficient. Similarly, in the studies of Deng et al. [2019], the optimal conditions for the isolation of saponins from *Sapindus mukorossi* by the MAE method were achieved using 40% ethanol. Espinoza et al. [2021] report that 70% ethanol allows obtaining an extract with good foaming properties confirmed in this study. In our study, the content of saponins in extracts with a lower concentration of ethanol was slightly lower, but at the same time, more extract was obtained. It gives rise to the conclusion that it is beneficial to reduce the ethanol concentration to increase the extraction yield.

Supplementation with preparations containing extracts of butcher's broom rhizomes is successfully used in the European and American markets, mainly due to the beneficial effect on microcirculation [Raposo et al. 2021]. In the available literature, there is little information on the level of ruscogenins in rhizome extracts, which is crucial for standardising the raw material and its use in producing a finished medicinal product. A daily dose of 7–11 mg of ruscogenins is recommended in treating chronic venous disease. If the extract contains about 5% ruscogenins, the daily therapeutic dose is 140–220 mg of the dry extract [Tomkowski 2014, European Medicines Agency 2019]. The dry extracts obtained by us, especially those obtained with 50% ethanol, contain the desired amounts of ruscogenins that meet the criteria of a therapeutic dose. We have shown that the solvent's most effective type and concentration for the extraction of sapogenins from butcher's broom rhizomes is 50% ethanol (variant D), which allows for the preparation of 304 mg of ruscogenins from 50 g of rhizomes. No reports in the literature indicate the use of such a concentration of ethyl alcohol for extracting sapogenins from the rhizome of the butcher's broom; therefore, our results should be considered promising.

CONCLUSION

The content of ruscogenins in the butcher's broom varies depending on the place of obtaining the raw material. The pharmacopoeial method of determination of ruscogenins by HPLC allowed to obtain repeatable results when examining the crushed raw material. However, based on the analysis of the results and the large dispersion in repetitions, it can be concluded that this method of ruscogenins determination is not suitable for testing the content in dense extracts. The most effective solvent concentration for the extraction of ruscogenins from the rhizome of the butcher's broom in the experiment was 50% ethanol (variant D), where 304 mg of ruscogenins was obtained from 50 g of rhizomes. The weakest variant was water extraction (A), because only 123 mg of ruscogenin was obtained from the same amount of raw material. The future use of 50% ethanol as a solvent with a good effect in extracting ruscogenin may reduce the cost of extract production while increasing the efficiency of

the process. Further research is recommended in this direction, analysing the conditions of extraction to obtain sapogenins from butcher's broom rhizomes, using a more considerable amount of raw material, and modifying the time, temperature, amount and concentration of the extractant.

SOURCE OF FUNDING

This research received no external funding.

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