Original paper

Pharmaceutical availability of hydrogels with extracts of *Arnica montana*, *Aesculus hippocastanum* and *Ruscus aculeatus* and their potential use as antioxidant polyphenol-rich material

Dostępność farmaceutyczna hydrożeli z ekstraktami z Arnica montana, Aesculus hippocastanum i Ruscus aculeatus oraz ich potencjalne zastosowanie jako surowca bogatego w polifenole o działaniu antyoksydacyjnym

Monika Michalak[1], Ewelina Maria Błońska-Sikora[2], Katarzyna Paradowska[3], Agnieszka Zielińska[3]

[1]Department of Dermatology, Cosmetology and Aesthetic Surgery, *Collegium Medicum*, Jan Kochanowski University, Kielce, Poland
[2]Department of Pharmacology, *Collegium Medicum*, Jan Kochanowski University, Kielce, Poland
[3]Department of Organic and Physical Chemistry, Faculty of Pharmacy, Medical University of Warsaw, Warsaw, Poland

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Key words: plant extracts, polyphenols, antioxidant activity, pharmaceutical availability.

Słowa kluczowe: ekstrakty roślinne, polifenole, aktywność antyoksydacyjna, dostępność farmaceutyczna.

Abstract

Introduction: Plant extracts are important sources of natural bioactive compounds, commonly used in the pharmaceutical and cosmeceutical industries. Phytochemical and antioxidant screening of plant extracts provides valuable information about their potential use. In the case of polyphenol-rich plant raw materials for external use, it is extremely important to test the ability of active substances to permeate the skin.
Aim of the research: To determine the polyphenolic content and antioxidant activity of horse chestnut, butcher's broom, and mountain arnica glycerol-water extracts and to assess the diffusion capacity of plant extracts from hydrogel formulations.
Material and methods: The content of polyphenols was determined using a spectrophotometric method. Antioxidant properties were analysed using DPPH-EPR assay and the FRAP method. The pharmaceutical availability of the extracts in the hydrogels was tested using a simplified model of human skin represented by a cellulose dialysis membrane.
Results and conclusions: The total polyphenol content in the extracts was highest in horse chestnut, followed by butcher's broom and mountain arnica, whereas the arnica extract had the highest flavonoid content. The obtained results indicate that the extracts have high antioxidant potential, which can be ranked as follows: *A. montana* > *A. hippocastanum* > *R. aculeatus*. The pharmaceutical availability analysis showed that the extract from *A. hippocastanum* is released most efficiently. Analyses of the kinetics of active substance release from hydrogel formulations have proven to be a useful tool for assessing the efficacy of semi-solid products. Tested plant extracts can be used as polyphenol-rich natural materials with antioxidant properties.

Streszczenie

Wprowadzenie: Ekstrakty roślinne są ważnym źródłem naturalnych związków bioaktywnych, powszechnie stosowanych w przemyśle farmaceutycznym i kosmetycznym. Badania fitochemiczne i antyoksydacyjne ekstraktów roślinnych dostarczają cennych informacji na temat ich potencjalnego zastosowania. W przypadku surowców roślinnych bogatych w polifenole przeznaczonych do stosowania zewnętrznego niezwykle ważne jest badanie zdolności substancji aktywnych do przenikania przez skórę.
Cel pracy: Określenie zawartości polifenoli i aktywności antyoksydacyjnej glicerolowo-wodnych ekstraktów z kasztanowca zwyczajnego, ruszczyka kolczystego i arniki górskiej oraz ocena zdolności dyfuzyjnej tych ekstraktów roślinnych z preparatów hydrożelowych.
Materiał i metody: Zawartość polifenoli oznaczono metodą spektrofotometryczną. Właściwości antyoksydacyjne analizowano przy użyciu metody DPPH-EPR oraz FRAP. Dostępność farmaceutyczną ekstraktów w hydrożelach badano z wykorzystaniem uproszczonego modelu skóry ludzkiej reprezentowanego przez celulozową błonę dializacyjną.
Wyniki i wnioski: Całkowita zawartość polifenoli była najwyższa w ekstrakcie z kasztanowca, następnie z ruszczyka oraz arniki, natomiast ekstrakt z arniki cechował się najwyższą zawartością flawonoidów. Uzyskane wyniki wskazują, że ekstrakty mają wysoki potencjał antyoksydacyjny, który można uszeregować następująco: *A. montana* > *A. hippocastanum* > *R. aculeatus*. Analiza dostępności farmaceutycznej wykazała, że ekstrakt z *A. hippocastanum* uwalnia się najefektywniej. Potwierdzono, że analiza kinetyki uwalniania substancji czynnej z preparatów hydrożelowych może stanowić użyteczne narzędzie do oceny skuteczności produktów półstałych. Badane ekstrakty roślinne mogą być wykorzystane jako bogate w polifenole surowce naturalne o właściwościach antyoksydacyjnych.

Introduction

Many plants are essential in human health care. Plant extracts are widely used as active ingredients in the pharmaceutical and cosmetic industries. Plant materials are a source of natural secondary metabolites, which are valuable compounds with a wide range of biological activity. Plants containing polyphenolic compounds play an important role in prevention and treatment. Polyphenols, including flavonoids, are compounds with documented anti-radical, antioxidant, anti-inflammatory, anti-allergic, and antimicrobial activity. Polyphenolic compounds exhibit health-promoting properties, protect the human body against external factors, and also have many potential positive pharmacological effects on the skin. Human skin is exposed to both external factors, such as radiation, smoking, pollutants, and pesticides, as well as internal products of reactive oxygen species (ROS) generated by normal cell metabolism. Free radicals initiate a redox reaction cascade, resulting in changes in proteins, lipids, and DNA or in disturbances of human cell metabolism [1]. Plant extracts containing polyphenols, alongside other bioactive compounds, are an important component of natural products that protect the skin against ROS, promote healing processes, stabilize the capillaries, improve the microcirculation, and exert anti-oedematous, anti-exudative, and anti-inflammatory effects. Examples of such plants include mountain arnica, horse chestnut, and butcher's broom [2–4].

Mountain arnica (*Arnica montana* L.) is a herbaceous, perennial plant of the family Asteraceae, with dark green leaves, hairy stems, and bright yellow flowers. The herbal material is the flowers, *Arnicae flos*, which contain phenolic compounds, including flavonoids (rutin, luteolin, quercetin, myricetin, apigenin) and phenolic acids (gallic, chlorogenic, caffeic, coumaric, ferulic acid), as well as sesquiterpene lactones (helenalin, dihydrohelenalin, and their esters), essential oils, and thymol derivatives [5–7]. The medicinal activity of the flowers includes anti-inflammatory, antibiotic, antibacterial, antioxidant, immunomodulatory, antiplatelet, antirheumatic, and analgesic effects. In addition, there are literature reports of local external application in the form of creams, ointments or gels, used to treat skin bruises, contusions, wounds, osteoarthritis, inflammation, chronic venous insufficiency, and alopecia [4, 8].

Horse chestnut (*Aesculus hippocastanum* L.) is a large, tall tree of the family *Hippocastanaceae*, with large leaves divided into five or seven leaflets, numerous white flowers with tinges of red, and smooth, shiny seeds inside spiny capsules. It is mainly the seeds that have therapeutic applications, but also the flowers and bark [9]. The main constituents of *A. hippocastanum* are triterpene saponins (e.g. aescin, also called escin), flavonoids (e.g. quercetin, kaempferol, epicatechin, and anthocyanins), and coumarins (e.g. esculin and esculetin) [2, 9]. Among phenolic acids, gallic acid, protocatechuic acid, *p*-hydroxybenzoic acid, caffeic acid, and *p*-coumaric acid were detected [10]. Clinical studies support the use of horse chestnut in chronic venous insufficiency (i.e. varicose veins accompanied by pain, oedema, pruritus, a sense of heaviness) and haemorrhoids, and confirm that it has anti-oedematous, anti-inflammatory and venotonic effects. Moreover, free radical scavenging ability, antioxidant properties, decreased vascular permeability, and inhibition of elastase, collagenase and hyaluronidase in the vascular wall have been observed [2, 9, 11, 12].

Butcher's broom (*Ruscus aculeatus* L.), of the family *Asparagaceae*, is a perennial, evergreen shrub with multiple stems arising from a creeping, thick rhizome to form an oval, pyramidal bush. The herbal material is the rhizomes, *Rusci rhizoma*. The underground parts of *R. aculeatus* have traditionally been used as a remedy for cardiovascular disease, for treatment of venous fragility, varicose veins, haemorrhoids, and atherosclerosis. The biological activity of butcher's broom is linked to its content of steroidal saponins (ruscogenin, ruscogenen, and neoruscogenin) and phenolic compounds (*C*-glycosylated derivatives of apigenin, *O*-glycosylated derivatives of quercetin and kaempferol), phytochemicals that support vein health. Butcher's broom extracts exhibit anti-inflammatory, astringent, and anticoagulatory properties, as well as remarkable anti-elastase activity. *R. aculeatus* is an important phlebotherapeutic agent which can be used to treat chronic venous insufficiency and vasculitis [3, 13–15].

According to current trends in pharmacy and cosmetology, extraction solvents should conform to the ideas of green chemistry. The glycerol-water mixture is an alternative to typical solvents used for extraction of biologically active compounds from plants, such as water, ethyl alcohol, acetone, and hexane [16]. Although the literature contains a great deal of information on the role of mountain arnica, horse chestnut, and butcher's broom in the pharmaceutical and cosmetics industries, there is still a need for more information on their potential use in formulations used for topical application to the skin.

Aim of the research

The objective of the present study was to determine the polyphenolic content and antioxidant properties of glycerol-water extracts from *A. montana*, *A. hippocastanum*, and *R. aculeatus*, as well as to assess the diffusion capacity of plant extracts from self-prepared hydrogels.

Material and methods

Glycerol-water extracts of flowers of horse chestnut, rhizomes of butcher's broom, and flowers of mountain arnica (Greenvit, Poland) were used in the study. De-

mineralized water, ethanol 96% p.a. (Avantor Performance Materials, Poland), gallic acid, Folin-Ciocalteu reagent, catechin, sodium carbonate, sodium nitrite, sodium hydroxide, aluminium chloride, DPPH (1,1-diphenyl-2-picrylhydrazyl), phosphate buffer, $FeCl_3$, TPTZ (2,3,5-triphenyltetrazolium chloride), HCl, and Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) (Sigma Aldrich, Poland) were used. All solvent chemicals were of analytical grade.

The total polyphenol content (TPC) of the extracts was determined using Folin-Ciocalteu reagent (FCR) [17], with gallic acid as the reference standard. The Folin-Ciocalteu reaction is an antioxidant assay based on electron transfer, which measures the reductive capacity of an antioxidant. It has been widely used to determine the total polyphenol content of plant samples. The phenolic compound reacts with FCR in an alkaline medium (sodium carbonate). Dissociation of a phenolic proton produces a phenolate anion, resulting in the reduction of FCR, whereby the molybdate in the testing system is reduced to form molybdenum oxide, yielding a blue colour. The total quantity of phenolic compounds present in the sample is proportional to the intensity of the blue colour [18]. A standard curve of gallic acid was prepared using acid solutions from 50 to 500 mg/l (standard curve equation: Abs = $0.0011x + 0.0028$ ($R^2 = 0.9997$), where Abs is the absorbance at 325 nm, and x is the concentration of gallic acid (mg/100 ml). The results were expressed as the gallic acid equivalent (mg GAE per 100 ml of extract). All determinations were performed in triplicate. TPC was determined spectrophotometrically using the Evolution 60S UV-Vis spectrophotometer (Thermo Scientific, USA).

The total flavonoid content (TFC) of the extracts was measured according to Kim *et al.* [19], using catechin as a standard. A standard catechin curve was prepared using acid solutions from 0.05 to 0.40 mg/ml (standard curve equation: Abs = $0.0016x + 0.0053$ ($R^2 = 0.9981$), where Abs is the absorbance at 510 nm, and x is the concentration of catechin (mg/ml)). The results were expressed as the catechin equivalent (mg CE per 100 ml of extract). Each sample was prepared in triplicate. TFC was determined spectrophotometrically using the Evolution 60S UV-Vis spectrophotometer (Thermo Scientific, USA).

Antioxidant properties were analysed by the ferric reducing antioxidant power (FRAP) method, as well as using a 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical assay by electron paramagnetic resonance (EPR) spectroscopy.

FRAP was measured by spectrophotometry according to Benzie and Strain [20]. The assay involved measuring the increase in absorbance during the reduction of Fe^{3+}-TPTZ (iron-2,4,6-tris(2-pyridyl)-s-triazine complex) to Fe^{2+}-TPTZ by the extracts. The FRAP reagent was prepared by mixing acetate buffer (300 mM, pH 3.6), a solution of 10 mM TPTZ in 40 mM HCl, and 20 mM $FeCl_3$ at 10 : 1 : 1 (v/v/v). The reagent and sample solutions were added to each well and mixed thoroughly. Then this solution was mixed with a specified concentration of the plant extract and incubated at 37°C for 4 min. A standard curve of Trolox was prepared using solutions from 0.0625 mol/l to 0.5 mmol/l (standard curve equation: Abs = $1.892x + 0.0044$ ($R^2 = 0.9966$), where Abs is the absorbance at 593 nm, and x is the concentration of Trolox (mmol/l). The results were expressed as mmol Trolox equivalent/l of extract. Each sample was prepared in triplicate. The measurements were made with the Evolution 60S UV-Vis spectrophotometer (Thermo Scientific, USA).

DPPH radical scavenging activity was measured by electron paramagnetic resonance (EPR) spectroscopy with microwaves of 9.3 GHz from an X-band. Most natural antioxidants possess reactive hydrogen atoms which serve as reductants. The DPPH assay is a good measure of the standard antioxidant profile. EPR spectra of DPPH were recorded on a MiniScope MS 200 EPR spectrometer (Magnettech GmbH, Germany), under the following conditions: centre field 330.48 mT, range of sweep 9.92 mT, amplitude of modulation 0.10 mT, microwave power 12 mW, sweep time 20 s, room temperature (25°C). A DPPH solution plus ethanol was used as a negative control, and glycerol-water extracts were used as a positive control. Briefly, 50 µl of extract or ethanol (as a blank) was mixed with 0.5 ml of DPPH solution and left to stand for 20 min in the dark. Then the residual DPPH content was determined by EPR spectroscopy. The results were expressed in mg of DPPH radical neutralized by 1 ml of extract. The EPR spectra were recorded in triplicate for each sample. The mean of three measurements (double integral of the signal) for three samples of each extract was used for calculations.

The pharmaceutical availability of the extracts in the self-prepared hydrogels was tested under *in vitro* conditions using a simplified model of human skin represented by a semipermeable, cellulose dialysis membrane (Membra-Cel, 44 mm, 30 m, Roth, Karlsruhe, Germany).

Three carbomer polymer hydrogels containing the extracts were prepared using Carbopol (1.0 g), triethanolamine (1.5 g), glycerol-water extract of mountain arnica, horse chestnut or butcher's broom (10.0 g), and aqua (ad 100.0 g). The carbomer was slowly dispersed in water, mixed, homogenized, and left to swell for 1 h. Next, the carbomer was neutralized by adding triethanolamine, and the test extract was added. The pH of the hydrogel formulations was determined using a CP-501 pH meter with an EPS-1 electrode (Elmetron).

Pharmaceutical availability was tested using standard procedures described by Szcześniak and Pluta

Table 1. Total polyphenolic and flavonoid content of the glycerol-water extracts

Glycerol-water extracts	TPC (mg GAE/100 ml ± SD)	TFC (mg CE/100 ml ± SD)
Arnica montana	503 ±5[c]	174 ±0.5[a]
Aesculus hippocastanum	761 ±42[a]	104 ±3[b]
Ruscus aculeatus	670 ±25[b]	59 ±2[c]

TPC – total polyphenol content, TFC – total flavonoid content, GAE – gallic acid equivalent, CE – catechin equivalent, SD – standard deviation. Mean values marked with different letters in columns differ statistically significantly ($p < 0.05$), n = 3.

[21]. This test can be used to assess the ability of the active substance to diffuse from the substrate through a membrane into an appropriate solution. The acceptor fluid was a phosphate buffer pH of 5.8 (with acidity closer to the natural pH of the skin than the more commonly used PBS buffer pH of 7.2). The membrane with 5 g of hydrogel was immersed vertically in a double-walled beaker containing 150 ml of phosphate buffer, thermostated with a water jacket, and mixed using a magnetic stirrer. The release tests were carried out at 32°C, as recommended by Polish Pharmacopoeia X [22] for testing transdermal therapeutic systems. The speed of the release process was determined by measuring the quantity of the substances released at defined time intervals, using gallic acid as a reference. Samples of 1.5 ml were taken after 15, 30, 60, 90, 120, 150, 180, 240, and 300 min and replaced with an equal volume of phosphate buffer to a volume of 150 ml. Each sample was prepared in triplicate. The speed and quantity of passage of active substances through the semipermeable membrane into the acceptor fluid at each time interval were determined by spectrophotometry using the Evolution 60S UV-Vis spectrophotometer (Thermo Scientific, USA) and expressed as the gallic acid equivalent in the sample according to the standard curve equation Abs = $0.0011x$ – 0.0028 (R^2 = 0.9997), where Abs is the absorbance at 325 nm, and x is the concentration of gallic acid [mg/ml]. The amount of substance released after time t [min] was calculated according to Hudemowicz *et al.* [23], using the following equation:

$$M_t = \frac{V}{v} m_t + \sum m_i$$

where: M_t is the amount of substance released over time t, V is the volume of fluid (ml) inside the apparatus, v is the volume of fluid (ml) taken once from the apparatus for dialysis, m_t is the amount of substance found in the sample, m_i is the amount of substance found in samples of volume v previously taken, and $\sum m_i$ is the amount of active substance (sum) determined in the samples previously taken.

Statistical analysis

Statistical analysis was performed with Statistica 12 software (StatSoft, Poland), using one-way ANOVA (significance level $p < 0.05$). Intergroup differences were assessed by the Tukey test ($n = 3$). In addition, Pearson correlation coefficients (r) between the results obtained for extracts from individual plant materials were calculated.

Results and Discussion

The results of TPC and TFC determination in glycerol-water extracts are compared in Table 1. The horse chestnut extract had a higher level of total polyphenols (761 mg GAE/100 ml) than the butcher's broom and arnica extract. In a study by Dudek-Makuch *et al.* [24], the total phenolic content in a hydroalcoholic extract of *Hippocastani flos* was 88.84 mg of chlorogenic acid equivalent/g of dry matter. In the present study, arnica extract was found to have the highest content of flavonoids, which accounted for 35.6% of total polyphenols, while in the horse chestnut extract and butcher's broom extract the contribution of flavonoids to the total polyphenol pool was 13.6% and 8.8%, respectively. These findings are in agreement with literature data indicating that apart from flavonoid compounds, horse chestnut extract is a rich source of triterpene saponins (e.g. escin) [25], and butcher's broom extract is rich in steroidal saponins (e.g. ruscogenin) [13]. In our study, TFC in the glycerol-water extract of mountain arnica flowers expressed as catechin equivalent was found to be 174 mg/100 ml of extract (Table 1). Another study of methanol extract from *A. montana* flower heads found that the amount of total flavonoids expressed as the hyperoside equivalent was 25.48 mg/g of dry extract [5]. Craciunescu *et al.* [6] reported TFC of 38.62 mg CE/g dry extract in *A. montana* ethanolic extract. The differences in the cited and present data are probably due to differences in quantification and in the type of extract.

The content of such bioactive compounds as phenols, flavonols, and flavonoids in plant extracts is generally strongly associated with antioxidant activity [26]. The present study evaluated the correlation between the content of phenolic compounds and the antioxidant activity of extracts from mountain arnica, horse chestnut, and butcher's broom. Table 2 presents the results obtained for the 1,1-diphenyl-2-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) assays of the extracts. The DPPH and FRAP assays, based on the ability to scavenge synthetic free radicals, are common spectrophotometric methods

Table 2. Antioxidant activity of glycerol-water extracts

Glycerol-water extracts	DPPH [mg/ml]	FRAP [mmol/l]
Arnica montana	3.61±0.24[a]	1.48 ±0.03[a]
Aesculus hippocastanum	2.17 ±0.05[b]	1.22 ±0.04[b]
Ruscus aculeatus	1.38 ±0.06[c]	1.08 ±0.06[c]

DPPH – 1,1-diphenyl-2-picrylhydrazyl, FRAP – ferric reducing antioxidant power. Mean values marked with different letters in columns differ statistically significantly ($p < 0.05$), n = 3.

for determining the antioxidant capacity of plant extracts [5].

The results obtained in the present study indicate that the extracts have high antioxidant potential, which can be ranked as follows: *A. montana* > *A. hippocastanum* > *R. aculeatus* (Table 2). The arnica extract manifests higher DPPH and FRAP activity than the horse chestnut and butcher's broom extracts. The antioxidant capacity of the arnica extract was 1.7 times as high in the DPPH assay and 1.2 times as high in the FRAP assay as that of horse chestnut, as well as 2.6 times as high in the DPPH assay and 1.6 times as high in the FRAP assay as that of butcher's broom extract. This was correlated with the amount of flavonoids, which was found to be 1.7 times as high in arnica as in horse chestnut extract and 3 times as high as in butcher's broom extract (Tables 1 and 2). Literature reports confirm that *A. montana* shows strong scavenging potential, which is mainly attributed to the presence of flavonoids [5, 6].

The analysis of the correlations between total flavonoid content (TFC) and antioxidant properties – 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay ($r = 0.9982$, $p < 0.05$) and ferric reducing antioxidant potential (FRAP) assays ($r = 0.9769$, $p < 0.05$) – showed significant correlations. In general, samples with high TFC levels exhibited higher antioxidant activity. In addition, the DPPH assay results were in good agreement with the FRAP results (Table 2).

Phytochemical and antioxidant screening of plant extracts provides valuable information about plants and plant extracts that have been known and used for centuries, especially pharmaceuticals. In the case of polyphenol-rich plant raw materials for external use, it is also extremely important to test the ability of active substances to permeate the skin. The release test is of great importance for both researchers and manufacturers [27]. A significant element of the action of bioactive substances is their ability to penetrate into deeper layers of the skin. A dialysis membrane was used to confirm the ability of the extracts of *A. montana*, *A. hippocastanum*, and *R. aculeatus* to pass through the first skin barrier. The kinetics of the release of extracts from the hydrogels into the acceptor fluid was presented as the relationship between the amount of active substance released, expressed as the reference substance gallic acid, and

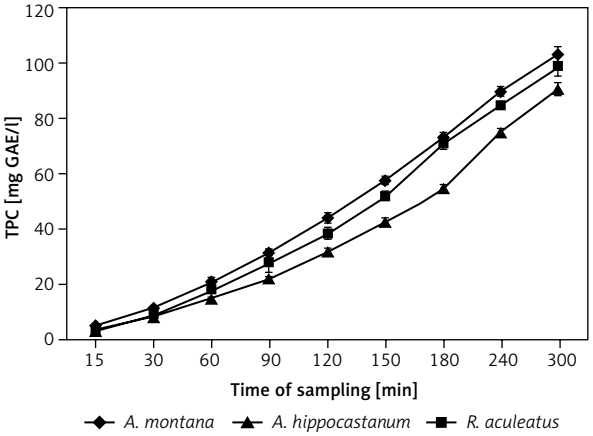

Figure 1. Zero-order kinetics of release of the active substance from hydrogels with tested extracts: *A. montana* ($y = 12.622x - 14.453$; $R^2 = 0.9857$), *A. hippocastanum* ($y = 10.813x - 15.844$; $R^2 = 0.9562$), *R. aculeatus* ($y = 12.252x - 16.325$; $R^2 = 0.9784$)

TPC – total polyphenol content, GAE – gallic acid equivalent.

the exposure time. The tests of the diffusion capacity of plant extracts showed that the release of active compounds was ranked as follows: *A. hippocastanum* > *R. aculeatus* > *A. montana* (Figure 1). The *in vitro* release tests of the active substance confirmed the usefulness of this procedure for assessing the effectiveness of semisolid formulations. The experiment makes it possible to evaluate the release rate of gallic acid from the hydrogel formulations as a carrier for the extract. Based on the available literature, it can be concluded that the studies of pharmaceutical availability of plant extracts and their components are rather limited. Zillich *et al.* [28] examined the skin permeation kinetics of polyphenols (mixtures of catechin, epigallocatechin gallate, resveratrol, quercetin, rutin, and protocatechuic acid), incorporated into O/W emulsions, and the release of polyphenols from semisolid emulsions. Another study focused on estimating the pharmaceutical availability of arbutin from a hydrogel formulation produced on Carbopol base, as well as assessing the effect of glycol plant extract components on the process of arbutin diffusion from the model formulations. The results of these studies showed that the introduction of glycol extracts of ginkgo leaf and rosemary into arbutin formulations increased the release of arbutin

through the semipermeable membrane into the acceptor fluid [29].

Recent years have seen growing interest in the use of hydrogels in pharmaceutical and cosmetic formulations [30]. Hydrogels, which are based on a hydrophilic polymer in water, can be applied to the skin, mucous membranes, or eye, as well as rectally or in the form of hydrogel dressings. Hydrogels are created using organic gelling agents, which may be natural (e.g. agar, tragacanth or alginate), semisynthetic (cellulose derivatives: methylcellulose or hydroxyethylcellulose) or synthetic (a cross-linked poly(acrylic acid) polymer (carbomer)), or using inorganic gelling agents (bentonite or colloidal silica) [31]. Carbopol (polyacrylic acid), used to prepare the hydrogels for the present study, is a rheological modifier ensuring that cosmetic and pharmaceutical products based on hydrogels are of suitable viscosity. Glycerol or propylene glycol is often added to hydrogels to prevent them from rapidly drying out, as well as ethanol or isopropanol to facilitate dissolution and improve absorption of the active substance. In addition, auxiliary substances should be chosen so as to obtain a hydrogel pH close to the physiological pH of the skin [31]. The pH of skin is usually acidic, from 4 to 6, which protects against the development of microorganisms. The tolerance of the skin is much broader, and some formulations may have a higher pH due to the technology used to obtain them, as in the case of Carbopol, with a pH slightly higher than the physiological level [31, 32]. In our study, the pH values of the hydrogel formulations were within the limits for human skin: 7.06 for the hydrogel with *A. montana*, 6.71 for the hydrogel with *A. hippocastanum* extract, and 7.07 for the formulation with *R. aculeatus*.

An important advantage of hydrogels used for topical treatment of diseases and for skin care, apart from their favourable toxicology profile and good organoleptic and aesthetic properties, is that they improve the pharmaceutical availability of medicinal and skin care substances. This means that hydrogels can very successfully be used to treat many skin disorders and to enhance skin regeneration processes [30]. The use of hydrogels is also recommended for skin in cases of intolerance to lipophilic substrates, in diseases affecting skin with hair, in cases of acute inflammation, acne vulgaris, fungal infections, or psoriasis, and also for treating indolent wounds. Hydrogels containing anti-inflammatory, astringent, and capillary-stabilizing substances can also be used for the care of vascular skin and as support in the treatment of varicose veins [30, 31]. Plant materials that exhibit vasoconstriction and anti-inflammatory properties, strengthen blood vessels, reduce capillary fragility, inhibit the activity of elastase, which damages elastin fibres, and support healthy circulation include the extracts of *A. hippocastanum*, *R. aculeatus*, and *A. montana* analysed in the present study [2–4, 26]. An important element of the action of the active substances is their ability to pass through the epidermal barrier into the deeper layers of the skin, where they can act not only on skin cells (keratinocytes and fibroblasts), but also on the blood vessels. In our preliminary experiments, the use of a dialysis membrane as a model of human skin confirmed the ability of the plant extracts to pass through the first barrier of the skin. The question of the ability of active substances from plant extracts to penetrate the deeper layers of the skin requires further scientific investigation, including *in vitro* and *in vivo* studies.

Conclusions

Based on our reported values for TPC, TFC, and antioxidant activity (DPPH and FRAP), extracts of mountain arnica, horse chestnut, and butcher's broom may be considered valuable polyphenol-rich material with antioxidant properties. The results of the pharmaceutical availability tests showed that model hydrogel formulations with the tested extracts may serve as a basis for developing new preparations for use in skin care or the treatment of skin diseases, including chronic venous disorders.

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Conflict of interest

The authors declare no conflict of interest.

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Address for correspondence

Dr **Monika Michalak**
Department of Dermatology, Cosmetology and Aesthetic Surgery
Collegium Medicum
Jan Kochanowski University
19 a IX Wieków Kielc St, 25-317 Kielce, Poland
E-mail: monika.michalak@ujk.edu.pl