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Iridoids from *Cornus mas* L. and their potential as innovative ingredients in cosmetics

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Dogwood berries represent a valuable source of a variety of active ingredients. A group that deserves special attention comprises iridoids – compounds with potent antioxidant, antiinflammatory and antibacterial properties. The present study is an attempt to obtain an innovative plant material from dogwood berries. To this end, water and water/ethanol-based extracts (1:1) were prepared and, as the next step, an iridoids-rich fraction was isolated. The total content of iridoids was determined spectrophotometrically, and antioxidant properties of the isolates were concurrently assessed. Additionally, skin whitening activity of isolated fractions was assessed on the basis of tyrosinase inhibition measurement. The testing schedule also involved the formulation of model washing systems based on anionic surfactants. The effect of adding the fractions obtained by the above method on the irritant potential was assessed by determining the zein number

Keywords: dogwood, antioxidant activity, zein test, tyrosinase inhibition, iridoids.

INTRODUCTION

Cosmetic industry is one of the fastest growing industrial sectors both in Poland and across the world. As the market is very competitive, cosmetic manufacturers are required to offer innovative products. One method of obtaining products of this type is enhancing their formulas by adding a novel ingredient which is not commonly used in cosmetics. Recent years have seen a particular increase in consumer interest in cosmetics containing substances of plant origin. A number of scientific studies have shown that plant-based materials exhibit antibacterial, antiviral, antifungal and antiinflammatory properties which counteract adverse changes in the body, and have a positive effect on the condition and functioning of the skin[1, 2]. The group of beneficial plant-based materials also comprises dogwood berries. Biologically active substances contained in dogwood berries are known to have powerful antioxidant, antibacterial, toning and astringent effects. In addition, they effectively prevent inflammations in living organisms[3–6]. Dogwood has been found to contain numerous phenolic compounds, flavonoids, anthocyanins and vitamin C[7–9].

Particular attention should be paid to iridoids, i.e. secondary metabolites in the form of cyclopentane monoterpenes. They may have a simple iridoid structure or occur as glycoside or ester compounds. Based on their chemical structure, iridoids can be divided into four main groups: iridoid glycosides, nonglycosidic (aglycone) iridoids, secoiridoids, and bisiridoids[10]. Dogwood berries have been shown to contain primarily oleanolic acid, ursolic acid, morroniside[11], loganin[12], sweroside, and cornuside. Iridoids have powerful antiinflammatory and antibacterial properties both in internal and external applications[13]. They are potent antioxidants, and may thus display anticancer activity in skin cancer[14, 15], cancer of the colon, lungs, or nose and throat[16, 17].

Since dogwood berries are associated with beneficial effects, extracts or substances isolated from them can be successfully used in cosmetic products. Active substances derived from dogwood and added to cosmetics successfully replace synthetic astringent agents which have a beneficial effect on human skin[18]. Iridoids reacting with amines have also been shown to yield a coloured product which is used as a colourant in the food and cosmetic industries. In addition, iridoids have found applications as hair dyes[19].

The study reported here was an attempt to obtain an innovative cosmetic material. To achieve this goal, water and water/ethanol (1:1) extracts were prepared. Next, an iridoid fraction was isolated from them. The content of iridoids in each obtained fraction was determined, and an analysis of antioxidant properties was conducted. Moreover, each fraction was assessed to determine its effect on the irritant potential of model washing systems by determining the zein number. The test simulates the interaction that takes place between the cosmetics under study and the skin proteins.

MATERIAL AND METHODS

Chemicals

Biological activity tests were conducted using: DPPH (2,2-diphenyl-1-picrylhydrazyl, Sigma Aldrich), ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid), Sigma Aldrich), 4-(dimetyloamino)benzaldehyde (Sigma Aldrich), aucubin (Sigma Aldrich), hydrochloric acid (36%, Chempur), ethyl alcohol (Heneywell), aluminium oxide (Sigma Aldrich), tyrosinase from mushroom (Sigma Aldrich), L-DOPA (Sigma Aldrich), hydrochinone (POCH Gliwice), di-potassium hydrogen phosphate pure p.a. (Chempur), potassium dihydrogen phosphate pure p.a. (POCH Gliwice). Measurements of the irritant potential were carried out with: zein from corn (Sigma Aldrich), sulfuric acid (98%, Chempur), copper sulphate penthahydrate (Chempur), potassium sulphate (Chempur), sodium hydroxide (Chempur), Tashiro indicator (Chempur). Model washing

systems were prepared from: sodium dodecyl sulphate (SLS, Sigma Aldrich) and sodium dodecyl ether sulphate (SLES, Brensurf, Brenntag). For a colorimetric analysis Aquamate Helios spectrophotometer were used.

Plant material

Dogwood (*Cornus mas L.* cultivar Bolestraszycki) fruits, collected in August 2016 in Bolestraszyce, were kindly supplied by Arboretum Bolesztraszyce.

Methods

Iridoid fraction derivation method

Isolation of the iridoid fraction, and determination of the total content of iridoids in the final product were performed using the method proposed by Baj et al.[20] with certain modifications.

To obtain appropriate product, freshly squeezed juice from pitted dogwood berries were used. The study was conducted using 25 g of dogwood berry juice which was extracted with 75 g of water (water extract, WE+IR). For the water/ethanol extract (WE+E+IR), extraction was performed in a water : ethanol mixture (1:1 w/w), with 75 g of extraction solvent in total. Both mixtures also contained 15 g of calcium carbonate each. The mixtures were boiled in a heating mantle under a reflux condenser for 30 minutes. The extracts thus prepared were decanted and filtered under reduced pressure through filter paper (Whatman no. 1). The resulting extracts were separated on a chromatography column filled with activated aluminium oxide (6 g). The first 5 mL portion of the filtrate was discarded.

Iridoid analysis

Analysis of total iridoid concentration

A 0.5 mL portion of the product was transferred to a 10 mL graduated tube, combined with 1 mL of methanol and 2 mL of 4-dimethylamino benzoic aldehyde solution with an addition of hydrochloric acid and 1.5 mL of distilled water. The mixture was heated for 3 minutes in boiling water bath, after which the test tube contents were immediately cooled down and filled with distilled water to 10 mL. After incubating the mixture at room temperature for 15 minutes absorbance was measured at the wavelength λ = 540 nm.

To calculate an amount of total iridoid concentration in final product, calibration curve of aucubin [0 – 100 mg/L] have been prepared with analogous procedure.

$DPPH^{\bullet}$ radical scavenging activity

$DPPH^{\bullet}$ radical scavenging by iridoid isolate was performed according to[21]. 1 mL of iridoid isolate or appropriate solvent was mixed with 1 mL 25 mM $DPPH^{\bullet}$ solution in 96% ethanol. Following 40 min incubation at room temperature the absorbance of the sample was measured at λ = 515 nm using AquaMate spectrophotometer (Thermo Scientific). 96% ethanol was used as a blank sample. All samples were analyzed in triplicates. The percentage of $DPPH^{\bullet}$ scavenging was calculated for each sample based on the equation:

% of $DPPH^{\bullet}$ scavenging = [1 – (As/Ac)] x 100%

where: As – absorbance of the sample; Ac – absorbance of the control sample ($DPPH^{\bullet}$ solution).

$ABTS^{\bullet+}$ radical scavenging activity

Scavenging of $ABTS^{\bullet+}$ free radical was evaluated according to[22]. The scavenging reaction is based on decolourisation of the green ABTS radical cation ($ABTS^{\bullet+}$). To prepare the $ABTS^{\bullet+}$ solution 19.5 mg ABTS and 3.3 mg potassium persulphate was mixed with 7 mL of phosphate buffer pH = 7.4 and dissolved for 16 hours in darkness. The solution was diluted to reach the absorbance at λ = 414 nm around 1.0. 20 μL of iridoids fraction or appropriate solvent was mixed with 980 μL diluted $ABTS^{\bullet+}$ solution and incubated for 10 min. The decrease in $ABTS^{\bullet+}$ absorbance was measured at λ = 414 nm using AquaMate spectrophotometer (Thermo Scientific), using distilled water as a blank. All samples were analyzed in triplicates The percentage of $ABTS^{\bullet+}$ scavenging was calculated based on the equation:

% of $ABTS^{\bullet+}$ scavenging = [(1 – (As/Ac)] x 100

where: As – absorbance of the sample; Ac – absorbance of the control sample ($ABTS^{\bullet+}$ solution).

Zein test

Irritant potential of the products was measured using zein test. In the surfactants solution zein protein is denatured and then is solubilized in the solution. This process simulates the behavior of surfactants in relation to the skin proteins. To 40 mL of the samples solution (10 wt%) was added 2 ±0.05 g of zein from corn. The solutions with zein were shaken on a shaker with water bath (60 min at 35°C). The solutions were filtered on Whatman No. 1 filters and then centrifuged at 5000 rpm for 10 min. The nitrogen content in the solutions was determined by Kjeldahl method. 1 mL of the filtrate was mineralized in sulphuric acid (98%) containing copper sulphate pentahydrate and potassium sulphate. After mineralization the solution was transferred (with 50 mL of MiliQ water) into the flask of the Wagner–Parnas apparatus. 20 mL of sodium hydroxide (25 wt%) was added. The released ammonia was distilled with steam. Ammonia was bound by sulfuric acid (5 mL of 0.05 M H_2SO_4) in the receiver of the Wagner–Parnas apparatus. The unbound sulfuric acid was titrated with 0.1 M sodium hydroxide. Tashiro solution was used as an indicator. The zein number (ZN) was calculated from the equation:

$ZN = (10 - V1) \cdot 100 \cdot 0.7$ (mg N/100 mL)

where V1 is the volume (mL) of sodium hydroxide used for titration of the sample.

The final result was the arithmetic mean of five independent measurements.

Patch test

The study was performed in a group of 20 healthy individuals (10 women and 10 men) aged between 20 and 40 years. None of the study participants had taken any medications for at least two weeks prior to the epidermal patch tests.

The epidermal patch tests were conducted using IQ Ultra test chambers from Chemotechniqe Diagnostics. The aim of the patch test was to assess the irritant potential of water (WE+IR) and water/ethanol (WE+E+IR) isolates. An open test was also performed for an extract

containing no iridoids. 30 μl portions of the test material were transferred into each chamber in accordance to the manufacturer's recommendations.

The epidermal patch tests were conducted in compliance with guidelines issued by the International Contact Dermatitis Research Group. Three identical patches containing the test emulsions were applied onto the skin of the test participants, in the region between the scapulas. The first patch was removed after 24 hours and the second (if no allergic reaction was observed) – after another 24 hours. Subsequent patches were removed 72, 96 and 168 hours after the application of the first patch test. The results were interpreted in accordance to the recognised international system for recording results of epidermal patch tests[23].

Tyrosinase inhibition activity

Tyrosinase inhibitory activity was determined by a spectrophotometric method described by Lim et. al[24] and modified by Studzińska-Sroka et. al[25]. Briefly, 0.6 mL of isolate was mixed with 1.35 mL of 0.1 M of phosphate buffer (pH 6.8) and 0.75 mL of tyrosinase water solution (192 U/mL). Samples were incubated for 10 minutes in room temperature and after incubation 0.1 mL water solution of L-DOPA (4mM) was added. Absorbance of samples was measured after 20 minutes at $\lambda = 475$ nm. Each sample was accompanied by a reference that contains all components except L-DOPA, which was substituted with distilled water. The mixture of ingredients with distilled water in place of isolates was used as a control for reference. Hydrochinone was used as a standard as substance with well-known tyrosinase inhibitory properties. The percentage of tyrosinase inhibition was calculated as:

$$\frac{A_{control}-A_{sample}}{A_{control}} *100\%$$

Presented values are the mean value from five independent measurements.

Statistical analysis

Each analysis was performed in triplicates. Obtained values were presented as mean ± SD. Significant differences between obtained values were analyzed using GraphPad Prism 5.0 software using One-way ANOVA and Tukey's test. The differences were considered significant when $p < 0.05$.

RESULTS AND DISCUSSION

Multiple studies have shown that the content of iridoid compounds present in plant-based materials or extracts is directly correlated with their antioxidant potential[26–28]. Consequently, iridoids are a valuable material for a number of industries including the cosmetic industry. Dogwood berries, both fresh and processed, have been identified as a source of a broad spectrum of iridoid compounds at relatively high concentrations amounting to an average of 21.1 mg/g of fresh berry weight (assuming that berries contain 20% of fresh weight)[26–29]. The amount, however, is strictly related to a number of factors including plant variety, harvest time or degree of processing.

The process of isolation of iridoid compounds from the plant material is described in many procedures. In the majority of them, though, methanol is used as the elution solvent[19, 30]. However, the content of this alcohol markedly reduces the scope of potential applications of the extract as a material in the production of most cosmetics, and contributes to an increase in production costs due to the need to eliminate methanol. Consequently, using ethyl alcohol in the process of isolating the fraction rich in iridoid compounds seem to be a purposeful and justified strategy. Such an extract can then be added to cosmetic formulations as is, with ethanol used for isolation – ethanol is commonly present in cosmetics as a solvent, antibacterial agent or rheology modifier reducing the high viscosity of cosmetic products.

The results obtained in the study reveal that the use of water: ethanol (1:1) mixture to extract the iridoids allow to achieve a 4.5-fold increase in the concentration of iridoids in the final product relative to the product extracted with water only. The concentrations (expressed in miligramequivalents of aucubin per liter (mg(Aq)/L) are 69.9 mg/L for the water/ethanol isolate and 19.3 mg/L for the water fraction (Fig. 1).

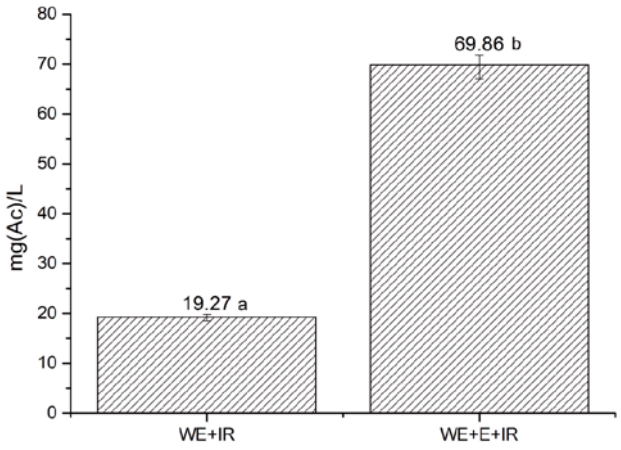

Figure 1. Total concentration of iridoids expressed as miligramequivalents of aucubin [mg(Ac) / L]

Both tests investigating the antioxidant potential corroborated the thesis that the antioxidant capacity of solutions is directly proportional to their iridoid content[26–29, 31]. Within the studied range, the DPPH free-radical scavenging ability determined for the WE+E+IR was on average over 8.6% higher than for the WE+IR and for the ABTS radical it was nearly 1.5% higher. Importantly, for DPPH radicals the potential of the (WE+E+IR) was higher across the entire range of test concentrations. For the ABTS radical, a similar tendency was noted for the extract at concentrations exceeding approx. 1.5 mg/mL. What is interesting, at lower concentrations (between approx. 0.2 and 1.5 mg/mL) the ABTS radical scavenging ability was higher for the water isolate. The scavenging ability of both free radicals is shown in the charts below (Figure 2, 3).

The data given above demonstrate that the isolated iridoid fraction retains its high antioxidant potential despite performing the process of extraction at high temperature, which can lead to the decomposition of some antioxidants contained in fresh juice, e.g. vitamin C[32–37]. Whereas, as some authors report, the DPPH

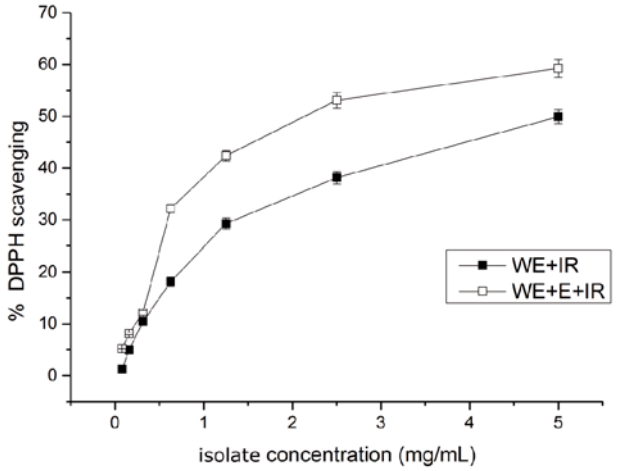

Figure 2. Antioxidant properties of *Cornus mas* L. iridoid fractions against DPPH free radical

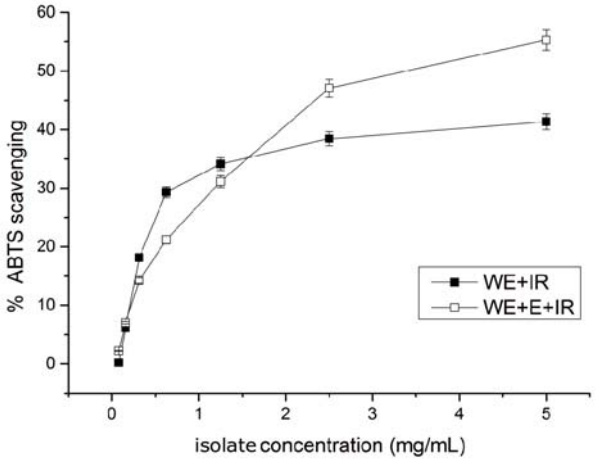

Figure 3. Antioxidant properties of *Cornus mas* L. iridoid fractions against ABTS free radical

free-radical scavenging ability for mashed dogwood berry is 73.30 ±4.07%, and for juice derived from *Cornus officinalis* it is 82.32 ±3.13%[26], the extracts obtained in the present study removed, respectively, 49.92% ±3.21 (for WE+IR) and 59.25% ±3.82 (for WE+E+IR) of the radicals at the concentration of 5 mg/mL. The relatively high antioxidant capacity of the obtained fraction, and its improved stability in relation to fresh juice, justify the use of the isolate as a material for the production of cosmetics.

The study involved an assessment of the capacity of iridoids isolated from dogwood berries to reduce the irritant potential of washing cosmetics. To this end, the irritant potential was determined for model systems based on washing compounds that are commonly used in the formulation of cosmetics, including sodium lauryl sulphate (SLS) and sodium laureth sulphate (SLES).

The literature data indicate that the irritant effect triggered by surfactants stems primarily from their interactions with the protein on the skin surface. The most potent irritant activity is ascribed to anionic surfactants which interact with proteins via ionic bonds. Nonionic and amphoteric washing agents exhibit no irritant effects because of weak hydrogen interactions with the surface protein[38–42].

The results indicate that iridoids have no significant impact on reducing the irritant activity of SLS and SLES. For pure solutions of these compounds, the results were approx. 250 mg N/100 mL (SLS) and approx. 80 mg N/100 mL (SLES). The addition of the iridoid fraction isolated using water and water/ethanol causes a slight, falling within the margin of error, decrease in irritant activity both in SLS and SLES solutions. The results of tests determining the irritant potential support the classification of iridoids as active ingredients of body care cosmetics, in which they contribute to enhancing the antioxidant and nourishing effects (Fig. 4).

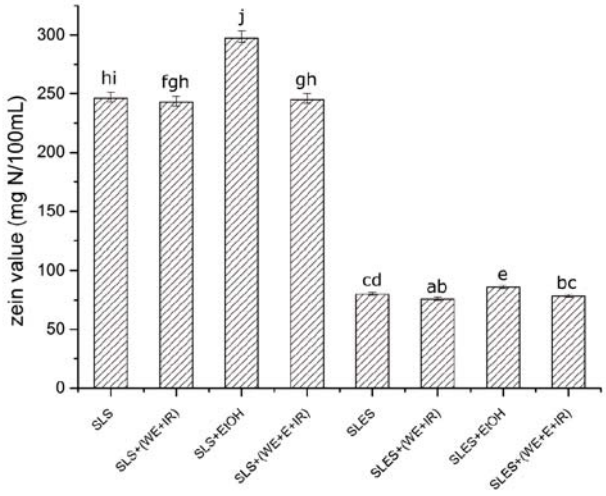

Figure 4. Zein value expressed in mgN/100 ml for 10% (w/w) surfactant solutions (SLS- sodium lauryl sulphate, SLES- sodium laureth sulphate), solutions with an addition of 5% (w/w) of ethanol EtOH instead of water and solutions with an 10% (w/w) addition of isolates

Patch tests are relatively cheap and uncomplicated to perform, which makes them one of the most widely used methods for the diagnosis of contact allergies. They are widely used for the evaluation of safety of cosmetic products. An assessment of the irritant reaction (IR) is usually performed using a 4-grade scale developed by Draelos and Dover[43].

The study reported above proves that the addition of WE+IR and WE+E+IR triggers no allergic responses in adults. Based on the study, it can thus be concluded that the extracts under investigation can be successfully used as an active ingredient in cosmetics.

Tyrosinase is a copper-containing enzyme which is a key enzyme in melanin biosynthesis and plays an important role in melanin pigmentation distribution[44]. An accumulation of an abnormal amount of melanin in different specific parts of skin might result in more pigmented patches, which might be considered as an aesthetic problem. Bioactive compounds with strong tyrosinase inhibitory properties are valuable material for cosmetic industry – especially in field of anti-aging cosmetics. Our research proved that both isolates (WE+IR and WE+E+IR) are very effective tyrosinase inhibitors – the percentage of inhibition was up to two fold higher than for hydrochinone (44.2%) and were equal 83.4% for WE+IR and 88.9% for WE+E+IR (Fig. 5). Obtained values of inhibition are also significantly higher from values obtained by the other authors for water extracts from *Sambucus nigra* L. flower (62.5%), *Calendula* L flower (55.3%) and *Euphrasia* L. herb (48.2%)[25]. Results

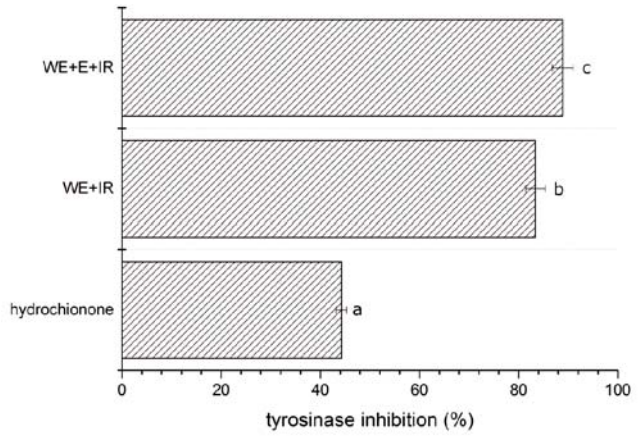

Figure 5. Tyrosinase inhibition [%] for *Cornus mas* L. iridoid fractions in comparison with hydrochinone as well described tyrosinase inhibitor

shows that tyrosinase inhibitory properties of obtained isolates are relatively high, which make them effective cosmetic ingredients against skin hyperpigmentation.

CONCLUSION

The iridoid-containing extracts investigated in the study had a high antioxidant activity. The study demonstrated the water/ethanol isolate have a considerably higher antioxidant potential. Another finding was that the antioxidant capacity of solutions is directly proportional to the content of iridoids. The results of tests determining the safety of cosmetic use support the classification of iridoids as active ingredients of body care cosmetics, in which they contribute to enhancing the antioxidant and nourishing effects.

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