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Inulin as an effectiveness and safe ingredient in cosmetics

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Jerusalem artichoke (*Helianthus tuberosus*) and chicory (*Cichorium intybus*) are valuable pharmaceutical raw materials on account of their high content of inulin, a natural prebiotic. Inulin-rich plants are also increasingly employed in the formulation of cosmetic products. The paper presents the biological properties of aqueous and aqueous-ethanolic extracts of Jerusalem artichoke and chicory. The extracts have been found to have a high free radical scavenging ability, with the most beneficial antioxidant properties being observed for the aqueous-ethanolic extract of Jerusalem artichoke. Inulin isolated from both plant types is a safe and non-toxic raw material. Inulin added to model body wash gel formulations markedly reduces their potential to cause skin irritation and sensitization.

Keywords: Inulin, Jerusalem artichoke, *Helianthus tuberosus*, chicory, *Cichorium intybus*.

INTRODUCTION

Cosmetic industry is currently one of the most rapidly growing sectors both in Poland and across the world. Plant-based cosmetic raw materials play a very important role on account of their content of biologically active ingredients with a broad spectrum of action, safety of use and easy availability. Consequently, they have a wide range of benefits. Plant-based raw materials have rich chemical compositions, which makes them appropriate for a variety of applications. For example, they are suitable for consumers of different age and with various skin types, and for the primary or adjunctive treatment of dermatological diseases[1, 2].

One of the plant-based raw materials with applications in cosmetology is inulin. The ingredient can be obtained, among other sources, from Jerusalem artichoke (also called topinambour, *Helianthus tuberosus* L.) and common chicory (*Cichorium intybus* L.)[3].

Chemically, inulin is an unbranched polysaccharide belonging to the class of fructans. It is composed of 30–35 fructose units linked by β-1,2-glycosidic bonds[4–8].

The benefits of inulin in the cosmetic industry include its antimicrobial protective effect on the skin and mucous membranes due to prebiotic properties, i.e. promotion of healthy bacterial flora[6–9]. Inulin and its surfactant derivatives can be used for the production of antibacterial soaps which are effective in removing gram-positive and gram-negative bacteria and viruses[10–13]. Fructans, including inulin, have also found cosmetic applications in hair shampoo production. An advantage of fructans and their derivatives used as shampoo ingredients is that they make it possible to eliminate ionic surface-active agents from shampoo formulations, which has a beneficial impact on the natural environment[6–8, 14–17].

Furthermore, inulin is used as a stabilizer in cosmetic emulsions and detergents. It is suitable as a base for powders and sprinkles, and as a nutritious ingredient in cosmetics[3–9, 14–16]. According to[18] nanoemulsions produced with inulin-based systems are described. In combination with fatty acids, inulin forms safe surface-active ingredients which do not cause any skin irritation. For the purpose of producing stable O/W emulsions, inulin is usually used at low concentrations, ranging from 0.2 to 1 wt%. In the formulation of nanoemulsions, on the other hand, the concentrations of this surface-active agent should preferably be higher, i.e. 8 wt% of the weight of the oil phase used.

The present paper is an attempt to assess the antioxidant properties of aqueous and aqueous-ethanolic extracts of Jerusalem artichoke and chicory. Inulin isolated from the two plants was used as a body wash gels ingredient. It was applied in the cosmetic formulations at varying concentrations: 1, 2.5, 5, 7.5 and 10 wt%. The skin irritation potential of the formulated body wash gels and the effect of isolated inulin on fibroblasts were evaluated.

MATERIALS AND METHOD

Chemicals

Antioxidant 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), ethyl alcohol (Heneywell), di-potassium hydrogen phosphate pure p.a. (Chempur), potassium dihydrogen phosphate pure p.a. (POCH Gliwice). Resazurin R7017 (Sigma Aldrich),Human skin fibroblasts BJ (ATCC®CRL-2522) and Eagle's Essential Minimum Medium (EMEM) with L-glutamine were purchased from ATCC.Foetal bovine serum (FBS) was purchased from Invitrogen. 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). All reagents were analytical grade.

Model washing systems were prepared from: sodium dodecyl sulphate (SLS, Sigma Aldrich), cocamidopropylbetaine (Dehyton PK, Basf), lauryl glucoside (Plantacare 1200, Basf), sodiumchloride (Chempur), citric acid (Chempur), sodium benzoate and potassium sorbate (Chempur).

Extract production

Jerusalem artichoke and common chicory extracts were obtained using the method of continuous solvent extraction in a Soxhlet extractor. The extract was prepared from 10 g of ground common chicory root and 10 g of ground Jerusalem artichoke tuber. As the extraction solvents, istilled water with ethanol and pure distilled water were used. In the aqueous-ethanolic extract the weight ratio of ethanol to water was 70:30. The process of extraction was conducted for 2 hours from the start of boiling of the solvent contained in a flask. Next, the extract thus obtained was passed through filters made of Whatman filter paper No. 1. The finished extract was stored in the refrigerator at a temperature of 4°C.

Isolation of inulin

Inulin extraction was performed using 50 g of Jerusalem artichoke tuber and 50 g of commonchicory root. The raw materials were blended and extracted with use of 300 mL of distilled water and 0.45 g of salt at 80°C for 45 minutes. Following filtration, the filtrates were extracted with ethyl alcohol overnight and centrifuged for about 30 minutes (5000 rpm). The precipitate was washed with ethyl alcohol and dried at a temperature of 50°C.

DPPH$^{\bullet}$ radical scavenging activity

DPPH$^{\bullet}$ radical scavenging by extracts was performed according to[19] with[23] modification. 1 mL of extract or appropriate solvent was mixed with1 mL 25 mM DPPH$^{\bullet}$ solution in 96 wt% 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 wt% 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[20] with[21] modification. 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 extracts 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).

Formulations of the model body cleaning gels

On the basis of literature reports and our own experiments prototype formulation of the body cleaning gels were developed. The model formula are listed in Table 1.

Table 1. Model body wash gel formulation

Ingredients (INCI)	Sample					
	Base	I1	I2	I3	I4	I5
Aqua	81.4	80.4	78.9	76.4	73.9	71.4
Sodium Lauryl Sulfate	8.0					
Cocamidopropyl Betaine	1.8					
Lauryl Glucoside	1.5					
Sodium Chloride	1.0					
Citric Acid	0.2					
Potassium Sorbate	0.4					
Inulin	-	1.0	2.5	5.0	7.5	10.0

The formulations of model body wash cosmetics contained a total of 11.3 wt% of surfactants. Three surfactants, Sodium Lauryl Sulfate (8.0 wt%), Lauryl Glucoside (1.5 wt%) and Cocamidopropyl Betaine (1.8 wt%), were selected on the basis of their most widespread use in body wash cosmetics. In addition, the formulation also contained citric acid (pH regulator), potassium sorbate (preservative, 0.4 wt%) and sodium chloride (NaCl, viscosity regulator). The variable parameter in the composition of analyzed samples was the type of isolated inulin. An additive-free sample was also used in the study as a reference (baseline) sample. The technology of formulating model cosmetics involved dissolution in water ingredients in the sequence specified in the formulation, and mixed using the mechanical stirrer (mechanical stirrer ChemLand O20).

Zein test

Irritant potential of the model washing gels 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 wt%) 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 solution (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:

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

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.

Resazurin assay

Cell proliferation/metabolism was assessed by resazurin assay. The assay was performed using a model of BJ human skin fibroblasts (ATCC CRL-2522). The cells were cultured in EMEM (Eagle's Minimum Essential Medium) with an addition of 10 wt% FBS (Foetal Bovine Serum). Resazurin R7017 – 1 g (Sigma Aldrich) was used in the assay. The cells were seeded into 96-well plates. Isolated inulin were diluted in the range of 1 mg · mL^{-1} to 5 mg · mL^{-1}. Next, the culture medium was substituted for the isolated inulin at appropriate dilutions. The control cells were cultured in EMEM with 1 wt% FBS. Absorbance was measured after 24 hours at the wavelength of λ = 570 nm, using the microplate reader FilterMax F5 (Molecular Devices).

DISCUSSION

Plant substances are an abundant source of primary and secondary metabolites. A large proportion of these compounds have antioxidant properties and are used both as carriers and active ingredients in cosmetic formulations. In addition, these compounds play a very important role in preventing cell damage induced by free radicals[22–25]. Reactive oxygen species may contribute to the development of oxidative stress which ultimately leads to cell metabolism disorders and peroxidation of cell membrane lipids[23, 26]. Free radicals also afect amino acids and proteins by changing their chemical structure, leading to mitochondrial DNA damage, elastin degradation or changes in collagen structure. As a result, modified proteins become inactivated and accumulate in cells, accelerating their ageing. The effects of free radicals in carbohydrates include, among others, depolymerisation of hyaluronic acid which is responsible for proper skin hydration[23–28].

Oxidative stress, and an increased number of free radicals which is associated with it, play a part in accelerating the ageing process, but they may also contribute significantly to the development of diseases including atopic dermatitis[28], acne[29] or psoriasis[30].

Cosmetics enriched with antioxidant substances are more readily absorbed by the human skin and less liable to cause skin allergy and sensitization than products based on synthetic ingredients[31]. In addition, they restrict the processes of oxidation of substances contained in cosmetics, e.g. fragrances. In this way, they may potentially extend the stability of cosmetic products[33].

The group of plants that are rich in active substances and have potential applications in the cosmetic industry includes, among others, Jerusalem artichoke (topinambour) (*Helianthus tuberosus* L.) or common chicory (*Cichorium intybus* L.). The plants are rich in phenolic compounds including phenolic acids or flavonoids. Common chicory root contains chicoric acid, and chlorogenic or isocholorogenic acid[33–36]. Jerusalem artichoke tubers contain primarily derivatives of hydroxybenzoic and hydroxycinnamic acids, which constitute approximately 16% of their dry matter content[8]. Moreover, the two plants are valuable sources of vitamins, among others C and E and B-group vitamins including thiamine and riboflavin. According to[36], the average content of vitamin C in Jerusalem artichoke tubers is 7.6 mg 100 g^{-1}, and in chicory root it is 5.2 mg 100 g^{-1}.

However, the most abundant dry matter components of the two plants are carbohydrates, particularly polysaccharide fructans, chiefly inulin[3–6]. As[37] claim, the antioxidant activity can also be attributed to polysaccharides which scavenge the superoxide radical anion, hydroxyl radical or hydrogen peroxide.

The present study has assessed the antioxidant activity of ethanol-aqueous and aqueous extracts obtained from common chicory and Jerusalem artichoke. The antioxidant activity of the extracts under study was assessed using the DPPH• and ABTS•$^+$ methods. The analyses were carried out within the concentration range of 0.3–10 mg · mL^{-1} and showed all the extracts under study to have an ability to neutralize reactive oxygen species depending on their concentration. According on the DPPH$^•$ method, the highest antioxidant activity was determined for the aqueous-ethanolic extract of Jerusalem artichoke. At the concentration of 10 mg · mL^{-1}, the free radical scavenging ability was equal to 80%. The values noted for the aqueous-ethanolic extract of common chicory were lower at all measurement points. At the concentration of 10 mg · mL^{-1}, the free radical neutralizing ability was 71%. The values determined for the aqueous extracts were lower than those obtained for the aqueous-ethanolic extracts – both in the case of common chicory and Jerusalem artichoke. The lowest values were observed for the aqueous solution of Jerusalem artichoke at the concentrations of 5 and 10 mg · mL^{-1}. At the remaining concentrations, the lowest ability to scavenge reactive oxygen species was found for the aqueous extract of common chicory (Fig. 1).

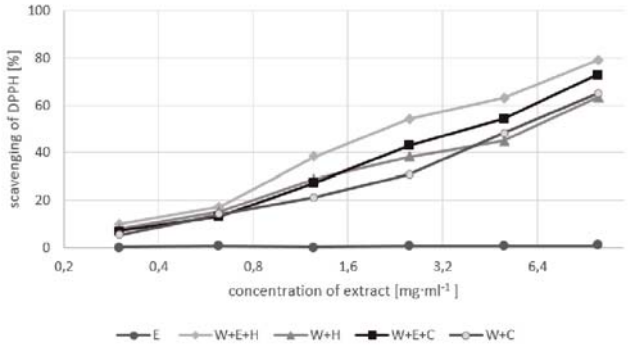

Figure 1. % of DPPH scavenging (E-ethanol, W+E+H – water+ethanol+*Helianthus tuberosus*, W+H – water+*Helianthus tuberosus*, W+E+C – water+ethanol+*Cichorium intybus*, W+C – water+*Cichorium intybus*

The extracts obtained from common chicory and Jerusalem artichoke were characterized by a lower ability to neutralize the ABTS$^{•+}$ radical than the DPPH$^•$ radical. Similarly to DPPH scavenging, the highest antioxidant activity was shown for the aqueous-ethanolic extract derived from Jerusalem artichoke. At the highest concentration studied (10 mg · mL^{-1}), the free radical scavenging ability was 64% and decreased gradually along with increasing dilutions. The aqueous-ethanolic solution obtained from common chicory had a free radical scavenging ability that was lower comparing to the aqueous-ethanolic extract derived from Jerusalem artichoke. The lowest values

were noted for the aqueous extract of common chicory at all measurement points (Fig. 2).

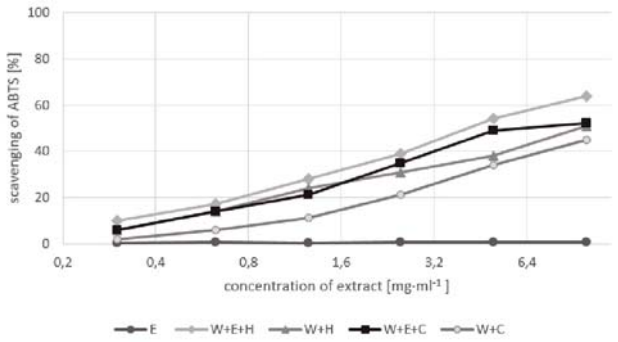

Figure 2. % of ABTS scavenging (E-ethanol, W+E+H – water+ethanol+*Helianthus tuberosus*, W+H – water+*Helianthus tuberosus*, W+E+C – water+ethanol+*Cichorium intybus*, W+C -water+*Cichorium intybus*

The tests evaluating the ability of aqueous-ethanolic extracts of Jerusalem artichoke and common chicory to scavenge the $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ radicals also involved inulin isolation from the extracts. The compound was then added to a cosmetic formulation. The content of inulin in plants usually ranges from 5 to 12%, which remains in agreement to the literature. The content of inulin isolated from *Helianthus tuberosus*. has been found to vary from 3 to 15%[38]. According to[3] Jerusalem artichoke contains about 52% of inulin in its tubers, and from common chicory (*Cichorium intybus*) containing approximately 44% of inulin. The present study found the percentage content of inulin in *Cichorium intybus* to be 12%, and in *Helianthus tuberosus* – 18% (Fig. 3).

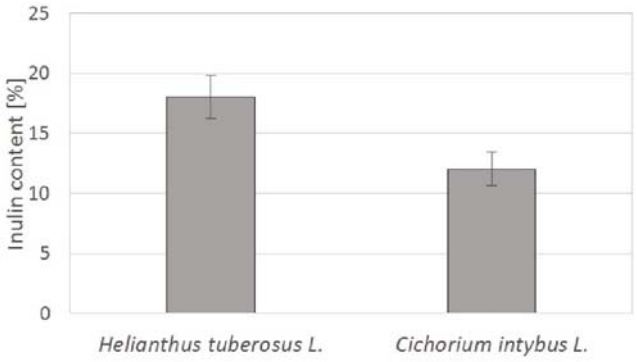

Figure 3. Inulin content isolated from *Cichorium intybus* L. and *Helianthus tuberosus* L

The next stage of the study involved evaluating the effect of inulin isolated from Jerusalem artichoke and common chicory on the metabolism of human dermal fibroblasts. The analysis was based on the resazurin assay which is a quick and sensitive method for assessing proliferation and cytotoxicity in vitro. In response to the reduction of culture medium by living cells, the dye was observed to change colour from blue to red[39–40]. The analyses were carried out within the concentration range of 1–5 mg · mL^{-1} (Fig. 4). The study demonstrated that inulin isolated from *Cichorium intybus* L. and *Helianthus tuberosus* L. at the highest test concentration (5 mg · mL^{-1}) had a beneficial effect on increasing cell proliferation compared to the control sample. Out of all samples tested, the highest increase in cell metabolism was found for inulin isolated from *Helianthus tuberosus* L. at a concentration of 5 mg · mL^{-1}. The highest decrease in relation to the control sample was shown after the addition of inulin derived from common chicory at a concentration of 2.5 mg · mL^{-1}. Based on the studies it can be concluded that inulin added to a cosmetic formulation at a concentration of 5 mg · mL^{-1} should not exhibit any skin irritation activity, and should beneficially affect the proliferation of fibroblasts. Similar conclusions were drawn by[34–35], who demonstrated that an addition of fructans had a beneficial effect on fibroblast stimulation and keratinocyte proliferation.

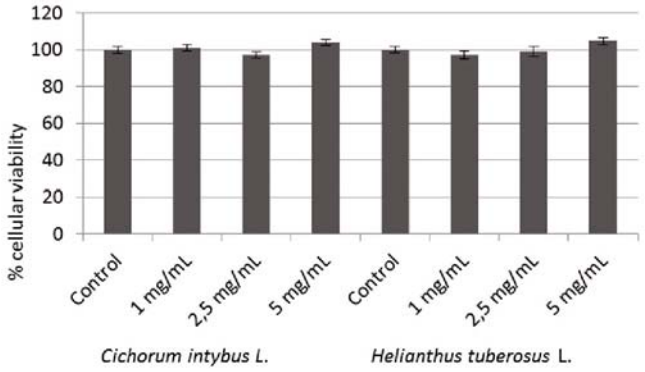

Figure 4. Influence of inulin isolated from *Cichorium intybus* L. and *Helianthus tuberosus* L. on cell viability

As the next stage of the reported study, an attempt was made to apply isolated inulin in the formulations of model body wash gels. The gels thus obtained, enriched with inulin, were subjected to skin interaction tests aimed at evaluating their potential to cause skin irritation (zein value), allergy and sensitization (patch test). The figure presents results of skin irritation potential measurements performed for the gels containing inulin derived from Jerusalem artichoke and chicory.

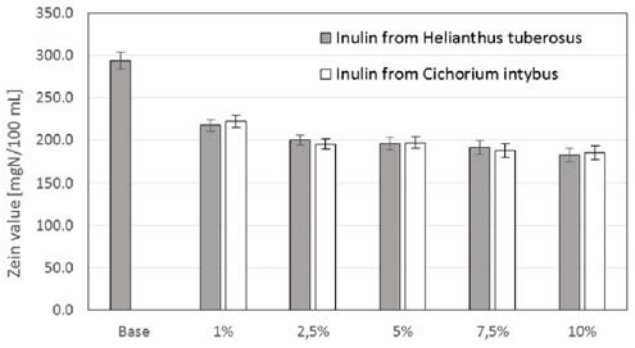

Figure 5. Irritant potential of model body wash gels containing inulin isolated from *Cichorium intybus* L. and *Helianthus tuberosus* L.

The risk of skin irritations is one of the greatest disadvantages associated with using body wash cosmetics. Their skin irritation potential is due to the presence of surfactants in the formulation. Surfactants may interact with the skin surface proteins, cause their denaturation and ultimately wash them away from the skin. The skin irritation potential of body wash gels depends primarily on the type of washing agents used in the formulation. The most severe skin irritation effect is attributable to

anionic surfactants (e.g. Sodium Lauryl Sulfate, Sodium Laureth Sulfate) which can interact with proteins via strong ionic bonds. Consequently, anionic surfactants have a relatively strong ability to elute and denaturate the skin surface proteins, which may result in skin irritation and impairment of skin function as a barrier preventing water loss (increase of transepidermal water loss, TEWL) or penetration of pathogens. A markedly lower skin irritation potential is found for nonionic surfactants which are linked to proteins by weak hydrogen bonds. Another factor impacting on the skin irritation potential of body wash gels is the concentration of surfactants which determines the form in which surfactants are found in solutions. Before reaching the critical micelle concentration (CMC) surfactants in the form of individual molecules (monomers) demonstrate the most pronounced skin irritation ability, which is due to the small size of individual molecules, their high mobility and markedly higher capacity to penetrate through the epidermal barrier into the skin. Lower skin irritation ability is associated with micelles arising in solutions after the CMC is exceeded. This is caused by the fact that they are larger in size, which prevents them from permeating deeply into the skin. Surfactant concentrations used in body wash gels exceed the CMC, however on account of the possibility of releasing monomeric molecules due to ongoing disintegration of thermodynamically unstable micelles, the presence of micelles does not completely eliminate the possibility of skin irritations. The literature data show that the skin irritation potential can be reduced for example by introducing into the system substances having an ability to reduce the CMC, increase the number of aggregations (amount of micelle-building monomers) or enhance the size and stability of micelles. Such compounds include polymers, hydrolyzed proteins, proteins, some plant extracts and electrolytes. A reduction in the skin irritation potential can also be achieved by using mixtures of different types of surfactants in the formulations of body wash cosmetics[41–48]. As the results of zein value measurements (Fig. 5) indicate, the addition of inulin to the formulations of body wash gels (based on a mixture of anionic and nonionic surfactants) contributes to a significant decrease in their skin irritation potential. Compared to the inulin-free baseline sample (zein value approximately 300 mgN/100 mL), inulin--containing gels are characterized by an approximately 40% lower skin irritation potential. However, the studies did not demonstrate a significant influence of inulin concentration on the zein value (which is equal to approximately 180 mgN/100 mL within the concentration range of 1–10%) or any impact of the plant type from which inulin was isolated. A review of the literature shows that surfactant systems with an addition of sugar substances have not been thoroughly studied to date. What follows from scanty literature reports[44–47] is that an addition of carbohydrates, such as glucose, fructose, saccharose or maltose, has an effect on increasing the number of aggregations in the micelles of both ionic (Sodium Lauryl Sulphate) and nonionic surfactants (oxyethylated derivatives), and lowers their CMC, which can also be the cause of the drop in zein value associated with inulin, which is a polysaccharide.

CONCLUSION

The aqueous and aqueous-ethanolic extracts of *Helianthus tuberosus* and *Cichoriumintybus* show a high free radical scavenging ability. More beneficial antioxidant properties, both with respect to the ABTS and DPPH radicals, were shown in both cases for the aqueous-ethanolic extracts. A comparison of both plants revealed that a more potent antioxidant capacity was associated with the Jerusalem artichoke extract. At the highest concentration studied (10 mg/mL), the DPPH radical scavenging ability determined for the aqueous-ethanolic extract of Jerusalem artichoke was about 80%, and the ABTS radical scavenging ability was approximately 60%. For the corresponding chicory extract the values were about 75 and 50%, respectively. In aqueous extracts the values were approximately 20–30% lower. The plants under analysis are characterized by a high content of inulin. Using extraction processes, 18 and 12%aof inulin was obtained from *Helianthus tuberosus* and *Cichorium intybus*, respectively. Cytotoxicity tests showed that both inulin isolated from *Cichorium intybus* and *Helianthus tuberosus*, at a concentration of 5 mg/mL, had a beneficial effect on increasing cell proliferation compared to the control sample. Inulin isolated from both plants under analysis can be applied in body wash formulations without any problems, as it becomes completely dissolved, producing clear and stable solutions. Tests determining the skin irritation potential of model body wash gels showed inulin to contribute to a marked decrease in that parameter. Following the addition of inulin, the zein value decreases by approximately 40% compared to the baseline sample, however the concentration of inulin was not found to have a significant effect on the findings. What is more, there was no significant difference with respect to the skin irritation potential between the gels containing inulin derived from *Cichorium intybus* and *Helianthus tuberosus.* The findings of the study show that both extracts of *Cichorium intybus* and *Helianthus tuberosus*, and inulin isolated from them, can be used as a valuable multifunctional ingredient of body wash cosmetics.

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