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POLYPHENOL CONTENT AND BIOACTIVITY OF SASKATOON (*AMELANCHIER ALNIFOLIA NUTT.*) LEAVES AND BERRIES

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Abstract: The studies were designed to determine the polyphenolic composition and biological activity of extracts from fruits (SFE) and leaves (SLE) of Saskatoon *(Amelanchier alnifolia* Nutt.*)* in relation to erythrocyte membranes. A detailed quantitative and qualitative analysis of extracts was conducted, using the chromatographic (UPLC-DAD, UPLC-ESI-MS) and spectrophotometric (Folin-Ciocalteu) methods. The biological activity of the extracts was investigated in relation to erythrocytes and isolated membranes of erythrocytes by using spectrophotometric, fluorimetric and microscopic methods and determined on the basis of hemolytic and antioxidant activity of the extracts and their impact on physical properties of the membrane such as: osmotic resistance, shape of erythrocytes, packing order of the polar head of lipids and fluidity of the membrane. The results showed that the tested extracts are rich sources of polyphenols, primarily from the group of flavonoids; in leaves dominating flavonols and anthocyanins in fruits. The SFE and SLE extracts to varying degree modify the physical properties of the erythrocyte membrane, causing formation of echinocytes, an increase in osmotic resistance and changes in the polar part of the membrane. Furthermore, the substances markedly protect erythrocytes and their membranes against oxidation induced by different physico-chemical factors. The findings indicate that the polyphenolic compounds contained in extracts of Saskatoon do not destroy biological membranes but effectively protect them against oxidation by way of interacting with the membrane surface. The extracts could effectively protect the organism and food products from the harmful effects of free radicals.

Keywords: Saskatoon extracts, antioxidant activity, erythrocyte membrane, UPLC-MS

Abbreviations: A – anisotropy, AA – L(+) ascorbic acid, AAPH – 2,2'-azobis (2-amidinopropane) dihydrochloride, DPH – 1,6-diphenyl-1,3,5-hexatriene, GP – generalized polarization, Laurdan – 6-dodecanoyl-2-dimethylaminonaphthalene, RBC – red blood cells, SFE – Saskatoon fruits extract, SLE – Saskatoon leaves extract, TMA-DPH – 1-(4-trimethylammonium-phenyl)-6-phenyl-1,3,5-hexatriene p-toluenesulfonate

Saskatoon (*Amelanchier alnifolia* Nutt.) is a shrub belonging to Rosaceae family, cultivated in many regions of the world because its fruit has nutritional value and high content of polyphenols (1). Studies conducted by Farah et al. (2) showed that fruits of Saskatoon have high content of anthocyanins and flavonols, compared with other berry fruits. Due to that they possess very strong antioxidant, antifungal, and antitumor abilities. The fruits of Saskatoon is also a rich source of vitamins and minerals, in particular vitamin C, tiamines, riboflavins, vitamins B6, A, E, folic acid, potassium, magnesium and iron (3). Its properties can be used for the production of a wide range of dietary supplements and nutrients to assist the human body in the prevention of many civilizational diseases and deficiencies of minerals. Leaves are also rich in valuable health-promoting substances but they are less used in folk medicine. Their main ingredients are quercetin, (-)-epicatechin and chlorogenic acid. Bioactive components contained in the leaves of Saskatoon can reduce the serum level of glucose. Due to this effect, they can be useful in the treatment and prevention of diabetes and cardiovascular diseases (4).

Both the leaves and fruits of Saskatoon are a rich source of nutrients, antioxidants and other components that can potentially exert protective role in living organisms. This work aimed at studying a protective effect of extracts from Saskatoon fruits and leaves on membranes of red blood cells. We also examined the structural changes in the mem-

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brane of red blood cells under influence of the extracts. Cell membrane is a protective barrier against substances harmful to an organism, in particular against free radicals. In order to understand the relationship between phytochemical profile and studied bioactivities the polyphenol composition of extracts was analyzed using ultra-high performance liquid chromatography coupled to tandem mass spectrometry (UPLC-MS/MS).

EXPERIMENTAL

Plant material

Saskatoon (*Amelanchier alnifolia* Nutt.) fruits and leaves were picked in the Garden of Medicinal Plants of the Medical University in Wrocław, Poland, and directly frozen in liquid nitrogen and freeze-dried (24 h; Alpha 1-4 LSC, Christ, Germany). The homogeneous powders were obtained by crushing the dried tissues using a closed laboratory mill to avoid hydration. Powders were kept in a refrigerator (-80^{o}C) until extract preparation.

Extraction procedure and content of polyphenols

Saskatoon fruits and leaves extracts were obtained from the Department of Fruit, Vegetable and Cereals Technology, Wroclaw University of Environmental and Life Sciences. Polyphenols were isolated from fruits and leaves by extraction (described previously by Gąsiorowski et al. (5)) with water containing 200 ppm SO_2, the ratio of solvent to fruits or leaves being 3 : 1 (v/v). The extract was adsorbed on Purolite AP 400 resin (UK) for further purification. The polyphenols were then eluted out with 80% ethanol, concentrated and freeze-dried. The content of polyphenols in individual preparations was determined by means of the liquid chromatography (UPLC-DAD-MS) method.

Erythrocyte membrane and fluorescent probes

Pig erythrocyte membranes were obtained from fresh blood using the method described by Dodge et al. (6). The content of erythrocyte membranes in the samples was checked on the basis of protein concentration, using Lowry's method (7), and it was 10 mg/mL. Pig blood was taken each time to a physiological solution of sodium chloride with heparin added.

The fluorescent probes Laurdan (6-dodecanoyl-2-dimethylaminonaphthalene), DPH (1,6-diphenyl-1,3,5-hexatriene) and TMA-DPH (1-(4-trimethylammoniumphenyl)-6-phenyl-1,3,5-hexatriene p-toluenesulfonate) were purchased from Molecular Probes (Eugene, Oregon, USA). The Folin-Ciocalteu phenol reagent, 2,2'-diazobis(2-amidinopropane) dihydrochloride (AAPH), Trolox® and L(+) ascorbic acid (AA) were purchased from Sigma-Aldrich, Inc., Steinheim, Germany.

UPLC-DAD and UPLC–ESI-MS methods

The Saskatoon fruits and leaves preparations were analyzed by UPLC-ESI-MS-MS systems using an ACQUITY Ultra Performance LC system equipped with a photodiode array detector with a binary solvent manager (Waters Corporation, Milford, USA) with a mass detector G2 and G2-Q/Tof Micro mass spectrometer (Waters, Manchester, U.K.) equipped with an electrospray ionization (ESI) source operating in negative and positive modes. Separations of individual polyphenols were carried out using a UPLC BEH C18 column (1.7 m, 2.1 × 100 mm, Waters Corporation, Milford, MA) at 30^{o}C. Qualitative analysis obtained by LC-DAD-MS-MS and quantitative analysis obtained by UPLC-MS (quantified using DAD and MS detection) were described by Oszmiański et al. (8).

Folin-Ciocalteu method

Total phenolic content was determined using the Folin-Ciocalteu (F-C) reagent, adapted from (9). The standard curve was prepared for gallic acid. The results were expressed as mg gallic acid equivalents (GAE) per 1 g of dry sample.

Hemolysis and osmotic resistance of erythrocytes

The hemolytic and osmotic resistance assays were as described earlier by Cyboran et al. (10) with a minor modification. The Saskatoon fruits and leaves extracts were added in the concentrations 0.01, 0.05, 0.1 mg/mL. Hemoglobin concentration in supernatant, expressed as a percentage of hemoglobin concentration in the supernatant of totally hemolyzed cells, was assumed as the measure of the extent of hemolysis.

In the osmotic resistance assay, a red blood cell suspension (at 2% hematocrit) containing SFE and SLE extracts at 0.05 mg/mL concentration was used. On the basis of the results obtained, the relation between percentage of hemolysis and NaCl concentration in the solution was determined. Next, using obtained plots, the NaCl percent concentrations (C_{50}) that caused 50% hemolysis were found. The C_{50} values were taken as a measure of osmotic resistance. If a determined sodium chloride concentration is higher than that of control cells, the osmotic resistance of the erythrocytes is regarded as lower, and vice versa.

Spectrophotometric assay for antihemolytic activity

To test the effect of an extract on hemolysis induced by free radicals, RBCs were pre-incubated with varying concentrations of extract (2 to 30 µg/mL) at 37°C for 1 h. Hemolysis of RBCs was carried out by mixing a 3% suspension of RBCs (unmodified or modified by extract) in phosphate buffer of pH 7.4 with AAPH solution (final concentration 40 mM). This reaction mixture was incubated for 3 h at 37°C. After incubation, samples were centrifuged for 15 min (2000 × g) at 23°C. The

Table 1. The content and characterization of phenolic compounds of the Saskatoon preparations obtained using their spectral characteristics by UPLC-DAD (retention time, λmax) and negative ions by UPLC-ESI-MS.

No.	Compound	Content fruits [mg/g]	Content leaves [mg/g]	Rt (min)	λ_{max} (nm)	[MS-]	[MS-MS-]
1	Cyanidin-3,5-*O*-diglucoside	1.28	0	3.15	516	609.0849	447.0887/285.0313/229.0230
2	Neochlorogenic acid	34.66	3.98	3.19	320	353.0866	235.9249/190.9269/146.9378
3	Caffeoylglucose	7.65	0.78	4.38	320	341.0840	179.0331/135.0464
4	Chlorogenic acid	190.64	18.16	4.50	320	353.0866	235.9249/190.9269/146.9378
5	Cryptochlorogenic acid	2.78	3.45	4.75	320	353.0866	235.9249/190.9269/146.9378
6	Procyanidin B2	16.12	1.85	4.81	280	577.1356	407.0765/289.0709
7	p-Coumaroylglucoside	0.88	0.82	5.07	314	325.0986	163.0380/146.9399
8	Cyanidin-3-*O*-galactoside	134.67	0	5.16	520	447.0887	285.0313/229.0230
9	Unknown hydroxycinnamic acid derivative	0	2.78	5.17	320	357.1183	177.0549
10	Cyanidin-3-*O*-glucoside	33.97	0	5.56	520	447.0887	285.0313/229.0230
11	(-)-Epicatechin	23.28	4.76	5.66	280	289.0713	245.0814/203.0706
12	Cyanidin-3-*O*-arabinoside	14.12	0	5.91	520	417.0840	285.0313/229.0230
13	B-type PA-trimer	9.49	7.35	6.10	280	865,1974	577.1334/407.0765/289.0709
14	Cyanidin-3-*O*-xyloside	11.53	0	6.87	520	417.0840	285.0313/229.0230
15	Quercetin-3-*O*-vicianoside	7.71	23.52	7.56	354	595.1328	433.1406/301.0277/151.0034
16	Quercetin-3-*O*-robinobioside	5.95	18.82	7.93	354	609.1427	447.0397/301.0277/151.0034
17	Quercetin-3-*O*-galactoside	13.26	25.95	8,16	355	463.0843	301.0277/151.0034
18	Quercetin-3-*O*-glucoside	4.37	11.60	8.41	352	463.0843	300.0277/151.0034
19	Kaempferol-*O*-hexoside-*O*-pentoside	0.23	7.10	8.67	350	579.1349	417.0802/285.0187
20	Kaempferol-*O*-hexoside-*O*-rhamnoside	0.27	2.44	8.80	350	593.1559	431.0968/285.0187
21	Quercetin-3-*O*-arabinoside	0.67	12.80	9.05	355	433.0778	300.0277/151.0034
22	Di-caffeoyl quinic acid	8.73	2.65	9.25	320	515.1189	353.0866/235.9249/ 190.9269/146.9378
23	Kaempferol-3-*O*-rhamnoside-7-*O*-glucoside	3.29	9.42	9.32	350	593.1559	447. 007/285.0187
24	Quercetin-3-*O*-rhamnoside	0.25	16.35	9.59	355	447.0918	300.0277/151.0034
25	Izorhamnoside-3-*O*-rutinoside	0.11	1.19	9.79	350	623.1640	447.1003/315.0549
26	Kaempferol-3-*O*-arabinoside	0.30	1.98	10.01	350	417.0802	285.0187
27	Di-caffeoyl quinic acid	0	2.77	10.78	320	515.1189	353.0866/235.9249/ 190.9269/146.9378
28	Kaempferol-3-*O*-rhamnoside	0.28	4.71	10.98	350	431.0968	285.0187
	Total	526.49	185.23				

extent of hemolysis was determined spectrophotometrically by measuring the absorbance of supernatant at 540 nm. For reference, RBCs were treated with redistilled water and the absorbance of the hemolysate was used as 100% hemolysis. The IC_{50} concentration of extract responsible for 50% inhibition of hemolysis induced by AAPH was determined and was compared with IC_{50} determined for the standard antioxidants AA and Trolox®.

Antioxidant activity of the extracts

The antioxidant activity of SFE and SLE extracts towards erythrocyte membranes was determined with spectrophotometric and fluorimetric methods using three oxidation inducers: UVC and UVB radiation and AAPH, as described earlier by Cyboran et al. (11). The fluorimetric experiment was carried out with the fluorescence probe TMA-DPH.

In both methods the antioxidant activity was determined on the basis of the half maximal inhibitory concentration (IC_{50}) – the concentration at which lipid peroxidation is inhibited by 50%. Antioxidant activity of extracts was compared with activity of the standard antioxidant L(+) ascorbic acid (AA) and with activity of its major constituents.

Microscopic studies of erythrocyte shapes

The impact of extracts on the shape of erythrocytes was determined by using the optical microscope and scanning electron microscope (SEM) according to the methods described earlier by Bonarska-Kujawa et al. (12), with minor modifications. For investigation with an optical microscope the SFE and SLE were added at concentrations of 0.01 and 0.05 mg/mL. For investigation with an electron microscope the Saskatoon fruits and leaves extracts were added at a concentration of 0.1 mg/mL.

Packing order and fluidity of the membrane

The effect of polyphenols on the packing arrangement of lipids and fluidity of erythrocyte membrane was investigated using the fluorimetric method described earlier by Bonarska-Kujawa et al. (13), with minor modification. On the basis of changes in fluorescence intensity of the Laurdan and DPH probes, the two parameters: generalized polarization (GP) and anisotropy (A) for unmodified and extract's modified erythrocyte membranes were determined and compared.

Increased values of GP and A signify increased packing density of the membrane lipid polar heads or decrease in membrane fluidity. Decreased values of GP and A indicate decreased polar group packing arrangement of the erythrocyte membrane lipid bilayer or increased membrane fluidity.

Statistical analysis

Statistical analysis of results was performed using the STATISTICA 12.0 (StatSoft PL) software. For results obtained from fluorimetric and spectrophotometric tests the statistical analysis was performed using the Dunnett test (*post-hoc* test – ANOVA) at significance level $\alpha = 0.05$. All the experiments were done in at least three replicates, the results being presented as the mean ± standard deviation.

Table 2. IC_{50} values for Saskatoon fruits (SFE) and leaves (SLE) extracts and standard antioxidants AA and Trolox®, obtained by spectrophotometric method. Hemolysis was induced by AAPH compound.

Extract/ Inducer	IC_{50} (µg/mL) ± SD AAPH
SFE	14.81 ± 0.98
SLE	15.87 ± 0.94
Trolox®	4.57 ± 0.20
AA	32.54 ± 4.24

RESULTS

Qualitative analysis performed with LC-DAD-MS-MS methods and quantitative results obtained using UPLC-MS (quantified using DAD and MS

Table 3. IC_{50} values for Saskatoon fruits (SFE) and leaves (SLE) extracts and standard antioxidants AA. Erythrocyte membranes oxidation was induced with UVC and UVB radiation for SEF, SLE and AAPH compound.

Extract/ Inducer	IC_{50} (µg/mL) ± SD		
	AAPH	UVC	UVB
SFE	6.3 ± 0.8	10.5 ± 1.0	6.7 ± 0.9
SLE	12.3 ± 0.3	56.4 ± 0.9	31.6 ± 1.0
AA	20.5 ± 1.8		

detection) are summarized in Table 1. A total of 28 polyphenolic compounds found in Saskatoon fruits and leaves preparations were identified. Cyanidin, quercetin, kaempferol and caffeic acid derivatives were the most abundant phenolic groups found in Saskatoon fruits and leaves preparations, they constituted 526.49 mg/g and 185.23 mg/g of fruits and leaves preparation, respectively.

Folin-Ciocalteu method

The calculated content of polyphenols in the extracts, expressed as mg gallic acid equivalents

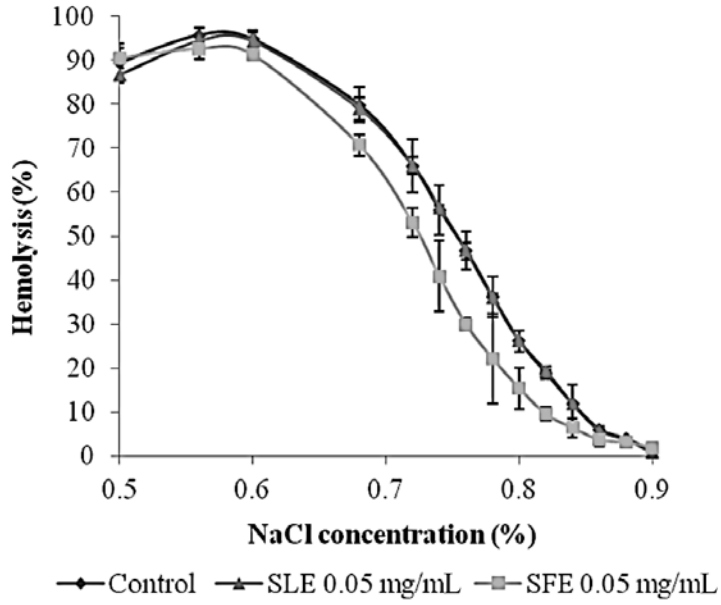

Figure 1 Percent of hemolysis of cells modified with Saskatoon extract: leaves (SLE), fruits (SFE), at 0.05 mg/mL concentration *vs*. sodium chloride concentration

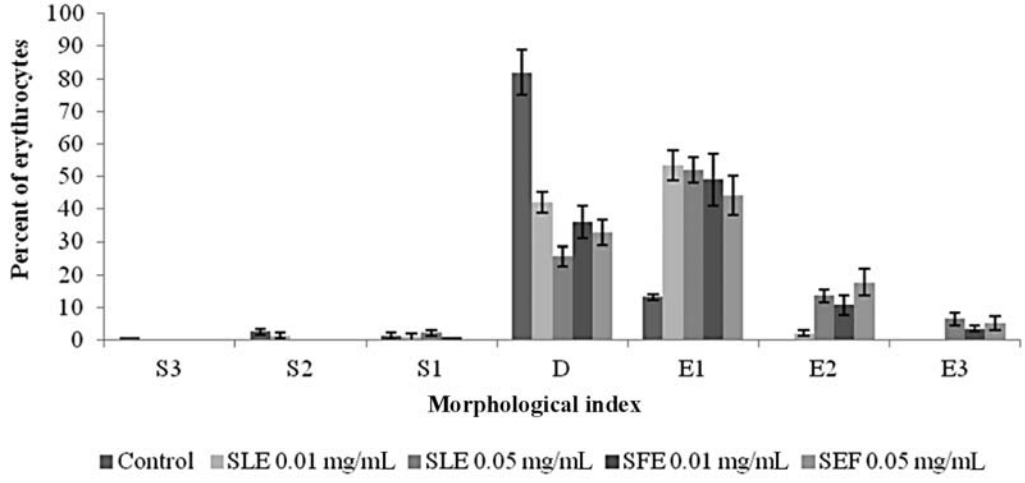

Figure 2. Percent share of different shapes of erythrocytes induced by SLE and SFE at 0.01 and 0.05 mg/mL concentration. On the abscissa there are morphological indices for the respective shapes of cells: stomatocytes II (S3), stomatocytes I (S2), discostomatocytes (S1), discocytes (D), discoechinocytes (E1), echinocytes (E2), spheroechinocytes (E3)

(GAE) per 1 g of dry sample, was as follows: for SFE extract TPC = 406 ± 1.0 mg GAE/g, for SLE extract TPC = 253 ± 4 mg GAE/g.

Hemolysis and osmotic resistance of erythrocytes

In the presence of SFE and SLE, at concentration ranging from 0.01 to 0.1 mg/mL, there was no increased hemolysis of erythrocytes compared to the control.

In the study of the impact of the extracts on erythrocyte osmotic resistance, no significant differences were found between the degree of hemolysis in control cells and those modified with extracts, at different concentrations of sodium chloride (Fig.1). The C_{50} values obtained for blood cells unmodified and treated with extracts at 0.05 mg/mL concentration were as follows: control C_{50} = 0.753 ± 0.03%, SFE C_{50} = 0.752 ± 0.05%, C_{50} = SLE 0.723 ± 0.026%. They point to a slight increase in osmotic resistance of erythrocytes in the case of SFE extract. These results indicate that SFE extracts causes red blood cells to be more resistant to changes in osmotic pressure.

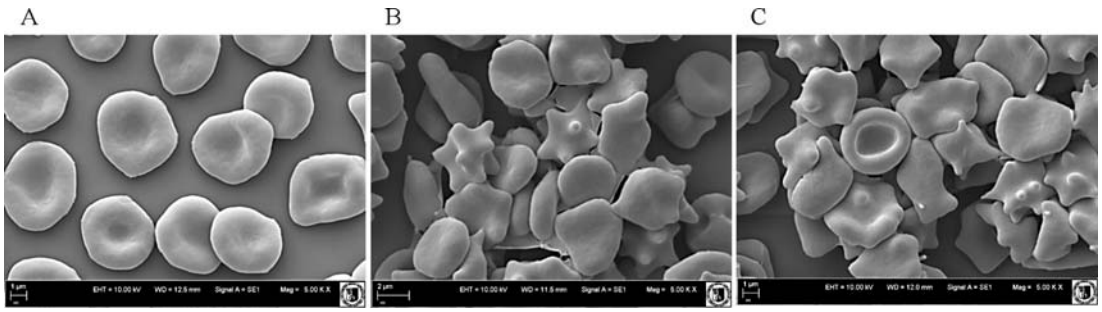

Figure 3. Shapes of unmodified erythrocytes (A), modified with SLE (B) and SFE (C) observed with electron microscope at 0.1 mg/mL concentration

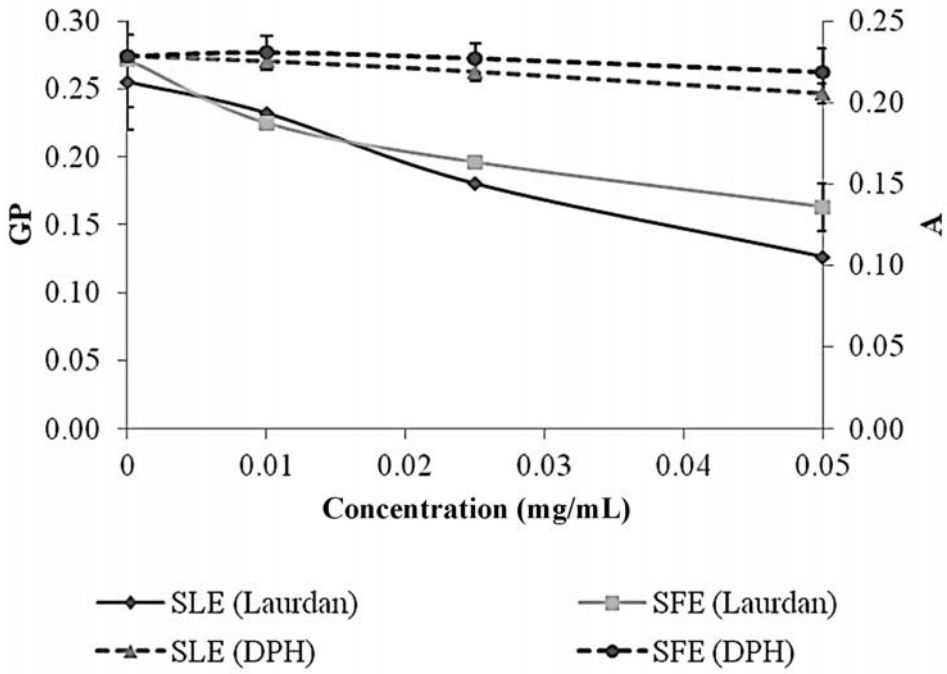

Figure 4. Values of generalized anisotropy (GP) for Laurdan probe and erythrocyte membranes modified with SFE (■) and SLE (♦) extracts at 37°C. Values of fluorescence anisotropy (A) of DPH probe for t erythrocyte membrane modified by SFE (●) and SLE (▲) at 37°C

Spectrophotometric assay for antihemolytic activity

The antihemolytic activity of SFE and SLE extracts was examined by spectrophotometric methods with hemolysis induced by free radicals released in the process of AAPH decomposition. The IC_{50} concentration of extract, responsible for 50% inhibition of free radical-induced hemolysis, was determined and compared with IC_{50} values determined for the standard antioxidant: AA and Trolox® (Table 2).

Antioxidant activity of the extracts

The antioxidant activity of SFE and SLE extracts was examined using fluorimetric and spectrophotometric methods, with three factors inducing oxidation of erythrocyte membranes: UVB and UVC radiation and AAPH inducer.

Free radicals induced by AAPH decomposition caused quenching of TMA-DPH fluorescence probes. The relative decrease in fluorescence intensity (F/F_0) of TMA-DPH was assumed as a measure of the extent of lipid oxidation. Based on the kinetics of oxidation process, the concentrations reducing oxidation by 50% (IC_{50}) for the extracts and standard antioxidant AA were determined and shown in Table 3.

In the spectrophotometric method, antioxidant activity of the extracts was determined on the basis of concentration of malondialdehyde (MDA) released in the process of lipid peroxidation. Based on the kinetics of the erythrocyte membrane oxidation induced by UVB or UVC radiation, the concentration reducing MDA formation by 50% (IC_{50}) for the extracts was determined (Table 3).

The microscopic studies of erythrocyte shapes

Figure 3 shows the erythrocyte shapes observed in a scanning electron microscope after modification with extracts at 0.1 mg/mL concentration. Figure 2 shows the proportions of the various forms of cells in a population of erythrocytes modified with SFE and SLE at 0.05 and 0.01 mg/mL. As seen in the Figures, the extracts induce various forms of echinocytes, mainly discoechinocytes.

Packing order and fluidity of the membrane

The DPH steady-state anisotropy is primarily related to the rotational motion restriction due to the hydrocarbon chain packing order. Therefore, the observed decrease of this parameter can be explained by a structural perturbation of the bilayer hydrophobic region caused by incorporation of investigated compounds. The effect of SFE and SLE on fluidity of the lipid phase of erythrocyte membranes was studied on the basis of fluorescence anisotropy (A) measured with the fluorescence probe DPH. Negligible changes in A were recorded for erythrocyte membranes affected by SLE extracts, in particular for the highest concentrations used (Fig. 4).

By using the Laurdan probe, the degree of order in the hydrophilic part of the erythrocyte membrane was investigated. The calculated values of general polarization (GP) decreased with increasing extract concentration (Fig. 4), which is indicative of increasing disorder in the hydrophilic part of the lipid layer and the presence of the compounds in that area.

DISCUSSION AND CONCLUSION

Chromatographic studies have shown that Saskatoon leaves and fruits are rich source of polyphenols. A total of 28 phenolic compounds found in Saskatoon leaves and fruits extracts were identified and are presented in Table 1. The same compounds were identified in Saskatoon berries by Bakowska-Barczak et al. (14). The main polyphenolic components of SFE are derivatives of caffeic acid and cyanidin – approximately 84% of all polyphenols, in particular cyanidin-3-galactoside 26% and chlorogenic acid 36%, whose content in the extract is approximately 62% of all the polyphenols. A different polyphenol composition is observed in SLE extract, where phenolic acid derivatives and quercetin dominate, together accounting for approx. 76% of total polyphenol content of the extract. Similar results for SFE and SLE extracts were obtained by other authors (15-17).

More polyphenolic compounds were identified and assayed in SFE than SLE, both in chromatographic (almost 3 times) and the Folin-Ciocalteu (nearly 2 times) methods. Polyphenols contained in the extracts, in particular, cyanidin and their glycosides and chlorogenic acid were reported to have a number of beneficial properties for human health which are related to their ability to scavenge free radicals, including reactive oxygen species (18-21). Especially, very good antioxidant properties of chlorogenic acid, cyanidin-3-O-galactoside, quercetin-3-O-galactoside and quercetin-3-O-glucoside in relation to biological membranes were observed in our earlier studies (22-24).

In the present study, erythrocytes and erythrocyte membranes were used and treated as a model

of cell and biological membrane. The biological activity of SFE and SLE in relation to erythrocytes and their membranes was determined by fluorimetric, microscopic and spectrophotometric methods. The choice of pig erythrocytes was dictated by the fact that percentage of lipids is closest to that of human erythrocytes, and the blood was easily available.

Hemolytic tests showed that polyphenols contained in the extracts do not induce hemolysis and therefore do not exert lytic action on red blood cells in the range of concentrations studied. Hemolysis caused by various lytic compounds, in particular those of amphiphilic character and in the free radical form, occurs when such substances penetrate into the alkyl chains region of the membrane, weakening the interaction between membrane components, and thus facilitating water transport into the cell interior with resulting structural damage. Studies by Deuticke et al. (25) and Iglic et al. (26) showed that formation of echinocytes occurs when amphiphilic molecules are incorporated in the outer monolayer of the erythrocyte membrane. It can thus be assumed that the Saskatoon extracts concentrate mainly in the outer monolayer of the erythrocyte membrane when inducing echinocytes and practically do not permeate into the inner monolayer of the membrane. The lack of hemolysis with plant extracts was also confirmed by Bors et al. (27) and Cyboran et al. (11).

The study of osmotic resistance showed that 50% hemolysis of erythrocytes modified with lower concentrations of NaCl in hypotonic solutions with SFE extracts seems to be connected with decreased permeability to water molecules and lesser sensibility to changes of osmotic pressure.

Results of the antihemolytic test done in the presence of SFE and SLE have shown that polyphenolic compounds are effective in protecting erythrocytes against the destructive action of free radicals that arise in the process of AAPH decomposition. The antihemolytic activity of both extracts was higher than of AA (IC_{50} = 32.54 µg/mL), and weaker than that of Trolox® (IC_{50} = 4.57 µg/mL). These results confirm the presence of polyphenols in the hydrophilic area of the erythrocyte membrane, where they form a kind of protective barrier against the hemolytic activity of free radicals in the membrane.

The extracts also exhibited high antioxidant activity toward ROS developed as a result of membrane irradiation with UVC and UVB and AAPH hydrolysis. The antioxidant activity towards free radicals induced by AAPH was much higher than that of the standard antioxidant AA. SFE and SLE effectively protect the RBC membrane from oxidation induced by UV radiation. The antioxidant activity of extracts was higher in UVB induced oxidation, which is associated with fewer radicals emerging under the influence of that radiation. Moreover, SFE was a better antioxidant than SLE and it protects the RBC membrane irradiated by UVC or UVB more efficiently. The greater antioxidant activity of SFE results from a greater content of polyphenols and also from high antioxidant potency of its main constituents. Very good antioxidant properties of chlorogenic acid and cyanidin-3-O-galactoside in relation to biological membranes were observed in our earlier studies (12, 13). High antioxidant activity of Saskatoon berries extract was also reported (15, 28)

As evidenced by the microscopic investigation, both extracts induce changes in erythrocyte shape from the normal discoid to echinocytic form. It can thus be assumed, according to the bilayer couple hypothesis (29), that the extracts concentrate mainly in the outer monolayer of the erythrocyte membrane when inducing various forms of echinocytes and practically do not permeate into the inner monolayer of the membrane. The presence of the extract compounds in the outer monolayer of the erythrocyte membrane may cause its sealing, which also prevents hemolysis. Such results, obtained for erythrocytes, are confirmed on isolated erythrocyte membranes by our fluorimetric experiments, performed with the DPH fluorescent probe that anchors in the hydrophobic part of the membrane and causes practically no changes in membrane anisotropy. The results indicate that the extracts do not alter fluidity of the erythrocyte membrane in the region occupied by acyl chains of fatty acids of lipid molecules. (20, 30, 31). The lack of essential changes in membrane fluidity in this hydrophobic region inspired the authors to conduct studies using the Laurdan probe, which can monitor changes in the hydrophilic region of the membrane, as it completely partitions there. The fluorophore of Laurdan takes position in the phospholipid glycerol backbone and is closely related to the dynamic free movement of water molecules that surround it (32). Decreased GP values in the tested membranes indicated decreased packing arrangement within membrane and interface and causes hydration. A significant decrease in generalized polarization of Laurdan probe was observed with SFE and SLE at a concentration of 0.05 mg/mL. Such changes in GP of the probe may indicate that polyphenol molecules bind to the membrane surface and indicated

changed packing arrangement in the polar head region.

In the presence of the extracts, a concentration-dependent decrease in GP of Laurdan probe occurred. Though the changes induced by SFE in the hydrophilic part of the erythrocyte membrane are smaller than those induced by SLE, the conviction remains that the compounds of the latter extract become incorporated into the membrane, inducing greater disorder in the hydrophilic part of it. Earlier studies (24, 33) of the interaction between the main components of extracts and erythrocyte membrane showed changes in the hydrophilic part of the membrane without essential changes in the hydrophobic part.

In conclusion, the polyphenolic compounds contained in SFE and SLE extracts do not disrupt the structure of the biological membrane and practically do not penetrate deep into the hydrophobic region of RBC membrane, taking position in the hydrophilic part of it. The SFE and SLE extracts effectively protect the cell membrane against free radicals. The protective action of the extracts components with respect to biological membranes depends on the number of polyphenol molecules adsorbed on the membrane surface. The polyphenols contained in the extracts reduce the concentration of free radicals there, constituting a specific protective barrier which hinders infiltration of free radicals into the membrane. The overall effect of the compounds from SFE and SLE on the biological membrane supports the view that they can be safely used as effective natural antioxidants against external oxidizing agents.

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