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Biological activity and stability *in vitro* of polyphenolic extracts as potential dietary supplements*

Aktywność biologiczna i stabilność *in vitro* ekstraktów polifenolowych jako potencjalnych suplementów diety

Authors' Contribution:
A Study Design
B Data Collection
C Statistical Analysis
D Data Interpretation
E Manuscript Preparation
F Literature Search
G Funds Collection

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Summary

Introduction: In times of worsening civilization diseases the interest in natural healing substances is on the increase. To reduce unwanted side effects of many synthetic drugs, it is reasonable to introduce to the daily diet foods rich in natural compounds of plant origin that are beneficial for health. The purpose of the study was to determine the biological activity and stability of selected ethanol extracts of the fruit of chokeberry, blackcurrant, hawthorn, rosehip, quince and Japanese quince as potential nutraceuticals.

Materials and methods: Antioxidant activity of the extracts was determined in relation to model phospholipid membranes (IC_{50}^{PC}). Antiradical activity was determined in a test with the DPPH$^{\bullet}$ radical (IC_{50}^{DPPH}). Also the inhibition of enzymatic (1-LOX) oxidation of linoleic acid was determined at the beginning of the period of storage of the extracts at room temperature and after 12 months.

Results: After 12 months of storage the highest antioxidant stability was shown by blackcurrant extract (1.5% increase in IC_{50}^{PC}), the highest antiradical stability by quince extract (1.0% reduction in IC_{50}^{DPPH}), and the highest stability of 1-LOX enzyme inhibition by chokeberry extract (6.3% reduction in inhibition at a concentration of 8 $\mu g \cdot ml^{-1}$). Japanese quince extract showed the strongest regenerating properties with respect to oxidized phospholipid membranes and the highest ability to eliminate the free radical DPPH$^{\bullet}$.

Conclusion: It can be concluded that the ethanol extracts of the fruits (in particular blackcurrant, chokeberry and Japanese quince) are a potential source of dietary supplements of expected effectiveness in preventive treatment.

Key words: **polyphenolic fruit extracts • antioxidants • antiradical • anti-inflammatory activities • liposome membrane • dietary supplements**

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Introduction

In recent years there has been a large increase in consumer interest in the influence of nutrition on human health. There is increased demand for food and dietary supplements with beneficial effects on the body. Current trends in food technology lean in the direction of placing on the market many new preparations, dietary supplements containing health-supporting components. Their biological properties are well documented [44,45].

Compounds of the polyphenol group are a rich source of natural substances of antioxidant character [20]. The literature reports that natural polyphenols reduce the risk of civilization diseases such as hypertension, coronary artery disease, atherosclerosis, and cancer [8,33,42]. Research conducted on extracts derived from fruits, vegetables and herbs indicates that they may have a significant role in the prevention and treatment of diseases. Their pharmacological effectiveness is often connected with the capture of free radicals, lipid oxidation protection and anti-inflammatory action [47]. There is also growing interest in functional foods and food additives, which may assist the defense mechanisms against diseases caused by oxidative stress.

The fruit of chokeberry, hawthorn, quince, Japanese quince, blackcurrant and rosehip, originating from areas of south-east Poland, which so far are relatively little appreciated by consumers, was selected for the study. Chokeberry and blackcurrant, rich in anthocyanins, are a valuable source of biologically active compounds. Consumption of this fruit causes, among other effects, lowering of blood pressure [16]. Chokeberry shows antimutagenic [14] and anticlotting activity [31]. Also confirmed is the ability of blackcurrant to inhibit development of the type A and B influenza virus in a high temperature environment [21]. Research on the human colorectal cancer cell line Caco-2 showed a significant reduction in proliferation of cancerous cells in the presence of chokeberry and blackcurrant extract [2,3,4]. Scientific reports underline the broader aspects of health beneficial effects of rosehip and hawthorn. Rosehip has been known for years in herbal medicine and used in treating diseases of the liver, gall bladder and kidney [17]. It positively affects the maintenance of healthy and flexible joints, and is effective in the treatment of osteoarthritis and rheumatoid arthritis [24,43]. It is used for the treatment of early stage heart failure, effectively reduces the level of cholesterol in the plasma, and inhibits the growth of breast cancer [26,35]. Quince and Japanese quince are plants derived from the Far East. In Europe they have been grown only recently; hence they are little appreciated by consumers. Research in recent years has shown that the common quince has antibacterial and anti-allergenic properties, and can be used in the prevention and supportive treatment of illnesses such as cardiovascular disease, bronchial asthma and coughing [10,36,46]. It has been used for a long time in Chinese folk medicine for the treatment of depression, migraine and neuralgia. At the same time, pharmacological studies have reported the role of Japanese quince in the prevention of gastric ulcer and tricuspid neuralgia, and it has shown promise in treatment of Parkinson's disease [15,48,49].

The present study researched *in vitro* biological activity of polyphenolic ethanol extracts of chokeberry, hawthorn, quince, Japanese quince, blackcurrant and rosehip as a source of healthy substances. The aim was to investigate the stability of the extracts after 12 months of storage at room temperature. Their antioxidant activity was examined with respect to phosphatidylcholine liposomes, a simple model of the biological membrane, antiradical activity in relation to the free radical DPPH$^{\bullet}$, and anti-inflammatory activity in inhibition of enzymatic oxidation of linoleic acid, at the beginning and at the end of the storage period. We also evaluated the content of polyphenols, flavonoids and anthocyanins in the extracts.

Materials and methods

Trolox (6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid), ibuprofen, DPPH$^{\bullet}$ (1,1-diphenyl-2-picrylhydrazyl), linoleic acid, lipoxidase from *Glycine max* (soybean), Folin-Ciocalteu's phenol reagent, gallic acid, rutin hydrate, Tween® 20, trichloroacetic acid (TCA), and 2-thiobarbituric acid (TBA) were purchased from Sigma-Aldrich (Poznań, Poland). Egg yolk phosphatidylcholine (PC) was obtained from Lipid Products, UK. Aluminum chloride hexahydrate ($AlCl_3 \cdot 6H_2O$) and tris(hydroxymethyl)-aminomethane (TRIS) were obtained from "Chempur" (Piekary Śląskie, Poland). Boric acid and sodium tetraborate were purchased from Carl Roth Karlsruhe, Germany.

Preparation of extracts

The raw materials for the study were chokeberry (*Aronia melanocarpa* (Michx.) Elliott) fruit of the Galician variety, blackcurrant (*Ribes nigrum* L.) of the Tisel variety, hawthorn (*Crataegus monogyna* Jacq.), rosehip (*Rosa canina* L.), quince (*Cydonia oblonga* Mill.), and Japanese quince (*Chaenomeles speciosa* (Sweet) Nakai). Fruits of hawthorn and rosehip were collected in Szczytnicki Garden in Wrocław. The fruits of quince and Japanese quince were collected in the Arboretum and Institute of Physiography in Bolestraszyce and in the Botanical Garden of Wrocław, respectively. Blackcurrant was from the Lubin area and chokeberry from the Sady Trzebnickie plantation. The fruits were frozen and then freeze-dried (ChRISTALPhA 1-4 LSC), and just before extraction they were disintegrated with an analytical mill (A11 basic of IKA-Werke, Germany).

Fruit extracts were obtained in the following way: 50 g of fruit lyophilizate was all covered in 200 ml of 70% water-ethanol solution, sonicated (20 kHz, Sonic, Italia) for 15 min, and the alcoholic extract drained. The extraction process by sonication was repeated 2 more times. The extract thus obtained was spun for 15 min in a centrifuge (2500 rev/min) at room temperature and then the ethanol was evaporated to dry weight with a rotary evaporator (IKA RV 05B, Germany) for approx. 50 min. Obtained extracts of blackcurrant and chokeberry were dissolved in distilled water and passed through a column (70 cm x 7.5 cm) filled with Amberlite® resin (XAD4). The column was washed with distilled water (about 2600 ml) until the wash-out of total sugars (the concentration of sugars in the extraction, measured with a Pocket PAL-1 refractometer, came to zero). Anthocyanin colorant was obtained after washing the column with 70% ethanol (about 1300 ml). The collected fraction was evaporated in a vacuum evaporator for about an hour until dry mass. The extracts thus obtained were stored at room temperature without light.

Liposome oxidation assay

The procedure was presented in the paper by Gabrielska et al. [12]. Lipid peroxidation was measured as the thiobarbituric acid reactive substance (TBARS) level, based on the method of Buege et al. [6]. TBARS concentrations were estimated using molar extinction coefficient ε = 156 $mM^{-1} \cdot cm^{-1}$. The percentage of phosphatidylcholine (PC) liposome oxidation inhibition was calculated using the formula:

$$\%\ Inhibition = \frac{A_0 - A}{A_0} \cdot 100\%$$

- where: A_0 is the concentration of malondialdehyde (MDA) in a sample without extract added (control) and A is the concentration of MDA in a sample with extract added, measured at λ=535 nm. All measurements were performed for two independent preparations (n=4) using a Cary 300 spectrophotometer (Varian).

The parameter IC_{50}^{PC} was determined on the basis of a plot showing the relation between the percentage of inhibition of lipid oxidation and concentration of plant extracts. This parameter specifies the antioxidant concentration that results in 50% inhibition of lipid oxidation.

Free-radical scavenging assay

The effect of the studied extracts on reduction of DPPH• radical concentration was measured spectrophotometrically, as previously described by Brand-Williams et al. [5]. Briefly, a DPPH• methanol solution with absorption of approx. 0.9 was mixed with an appropriate amount of extract, or Trolox solution, and immediately placed in a spectrophotometer. As a control, the absorption of DPPH• (without addition of extract) was measured at time t=0. Reduction of DPPH• in the sample after 15 min incubation with an antioxidant (of fixed concentration) was determined using the formula:

$$\%\ Reduction = \frac{A_0 - A}{A_0} \cdot 100\%$$

- where: A_0 is the change of absorbance at λ=517 nm, after 15 min in the absence of an antioxidant; and A is the change in absorbance at λ=517 nm, after 15 min in the presence of an antioxidant. All determinations were performed in six replicates (n=6).

Soybean lipoxygenase inhibition

Fruit extracts were tested on inhibition of soybean lipoxygenase (1-LOX, Sigma, Poznań) activity on the basis of the procedure described by Axelrod et al. [1], with modification. Fruit extracts at a concentration of 8 $\mu g \cdot ml^{-1}$ were used in the experiments. For comparative purposes the non-steroidal anti-inflammatory agent ibuprofen (used at 2.0 $\mu g \cdot ml^{-1}$) was also tested. Reactions were carried out in 10 mm path-length quartz cuvettes containing, in a final volume of 2.6 ml: borate buffer pH 9.0, 0.1 $mg \cdot ml^{-1}$ 1-LOX, the extracts tested, and 50 μM linoleic acid (previously prepared in borate buffer pH 9.0 plus 1% Tween 20 and 0.1 M NaOH). The incubation was carried out for 3 min at room temperature. Reference cuvettes (of 2.6 ml volume) contained sodium linoleate borate buffer and an appropriate volume of solvent extract tested (substrate solution). Inhibition of 1-LOX activity was assessed via spectrophotometric monitoring of absorbance increase at 234 nm (at 3 min after linoleic acid addition) due to formation of conjugated diene hydroperoxides (CDH) during enzymatic oxidation processes according to Sudina et al. [37]. All results are presented as the mean ± SD of at least two sets of independent experiments (n=6). Percentage inhibition was calculated using the following formula:

$$\%\ Inhibition = \frac{\Delta A_{control} - \Delta A_{sample}}{\Delta A_{control}} \cdot 100\%$$

- where: $\Delta A_{control}$ and ΔA_{sample} denote the increase of absorbance 3 min after substrate addition to the probe without or with extract tested, respectively. All results are presented as the mean ± SD of at least two sets of independent experiments (n=6).

Total phenols, flavonoids and anthocyanins content

Total polyphenols were determined by the Folin-Ciocalteu method according to Gao et al. [13]. The results were calculated as mg of gallic acid equivalent in 1 g dry mass of extract (mg GAE/g d.m.). Measurements were made using a Cary 300 UV-VIS spectrophotometer (Varian). Each extract was analyzed for total flavonoid content according to the previously reported colorimetric method with modifications by Lamaison et al. [23]. The absorbance of the solution was measured at 430 nm. The results were expressed as the rutin equivalent (mg RE/g d.m.) and compared with

the rutin standard curve, which was made under the same conditions. Total anthocyanin amount, calculated as cyanidin-3-*O*-glucoside, was determined by means of the pH differential method of Trappey et al. [38]. Absorption was measured at 510 and 700 nm, using the formula:

$$Total\ anthocyanins = \frac{A \cdot MW \cdot DF \cdot 1000}{\varepsilon \cdot 1}$$

• where: A = (A_{510}-A_{700})pH 1.0 – (A_{510}- A_{700})pH 4.5.
MW – molecular weight, DF – dilution factor, ε – molar absorbance.

Results were expressed as mg equivalent of cyanidin-3-*O*--glucoside in 1 g dry mass of extract (mg C3GE/g d.m.).

Statistical analysis

All results are expressed as mean ± SD. The correlation coefficient between total phenolics content and antioxidant activity was calculated, and variance analysis was done with the Duncan test. P values < 0.05 were considered statistically significant. The program Statistica 10.0 was used for all of the statistical calculations.

Results

The work presents the results of a study of polyphenolic extracts from selected fruits. The following have been characterized: the antioxidant properties of the extracts in the process of auto-oxidation of phosphatidylcholine membranes, the antiradical properties with respect to the free radical DPPH•, and the anti-inflammatory effect in enzymatic oxidation of linoleic acid. We also determined the total content of polyphenols, flavonoids, and anthocyanins (in the case of chokeberry and blackcurrant).

Antioxidant activities of extracts from the fruit of the chokeberry, hawthorn, rosehip, quince, Japanese quince, and blackcurrant were expressed as IC_{50}^{PC}. This parameter is listed in Figure 1 for the start of the storage period, and after 12 months. A low IC_{50}^{PC} value means a high antioxidant activity of the extract. The IC_{50}^{PC} parameters for the start of the storage period of the extracts follow the sequence [μg·ml^{-1}]: Japanese quince (19.38 ± 0.43) < chokeberry (27.69 ± 0.92) < blackcurrant (32.65 ± 0.16) < rosehip (0.68 ± 39.46) < hawthorn (47.05 ± 2.42) ≈ quince (45.71 ± 1.03, p < 0.05).

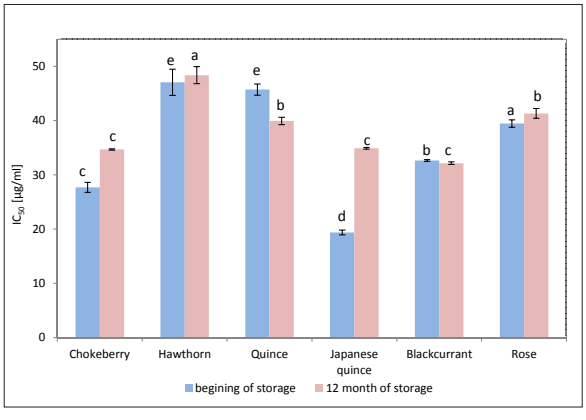

Fig. 1. The antioxidant activity (IC_{50}^{PC}) of extracts from fruits of chokeberry, hawthorn, quince, Japanese quince, blackcurrant and rosehip in the process of autoxidation PC liposomes for the initial time and after 12 months of storage at room temperature. IC_{50}^{PC} values are the averages of two independent measurements ± SD (n= 4). Different letters (a-e) in the same row indicate significant differences (p < 0.05)

Significantly higher antioxidant activity was found at the start of storage for Japanese quince and chokeberry extracts compared to the activity of hawthorn and quince (p<0.05). After 12-month storage the antioxidant activity followed the sequence [μg·ml^{-1}]: blackcurrant (32.15 ± 0.25) > chokeberry (34.68 ± 0.14) ≈ Japanese quince (34.89 ± 0.16) > quince (39.93 ± 0.67) > rosehip (41.32 ± 0.90) > hawthorn (49.36 ± 1.57) (p<0.05). Thus, there are differences in activity between the start and end of storage. Blackcurrant, hawthorn, rosehip, and quince proved to be stable extracts (differences in the parameter varied from 1.5% to 12.6%). A large decrease in activity was found for Japanese quince and chokeberry: 80 and 25%, respectively. Comparison of the antioxidant activity of the extracts was made in relation to Trolox (IC_{50}^{PC} = 3.27 μg·ml^{-1}) as the positive control (Table 1) with the TEAA parameter, expressing it in mM Trolox per g dry weight of raw material [mM·g^{-1}]. The higher the value of the parameter the stronger the antioxidant property of an extract. One can tell by these values that all extracts showed a lower antioxidant activity than Trolox, from 6-fold (Japanese quince) to 15-fold lower (hawthorn) at the beginning of the storage period, and from 10 to 24 times lower after 12 months of storage.

The total content of polyphenols in the extracts at the beginning of storage ranged from 133.3 to 640.3 [mg GAE/g d.m.] for hawthorn and Japanese quince, respectively. After 12 months of storage the contents of polyphenols had not changed sharply, with the exception of Japanese quince (approx. 53% decline). Analysis of the results obtained confirmed the existence of a strong correlation between antioxidant activity (IC_{50}^{PC}) and the total polyphenols content at the beginning of storage of the plant extracts (r = - 0.8335) and a relatively strong correlation after 12 months (r = - 0.6208), at significance level p < 0.05.

The content of flavonoids in the extracts showed that the richest are extracts of chokeberry (128.18 ± 0.31 mgRE/g d. m) and blackcurrant (79.03 ± 0.19 mgRE/g d.m.). The remaining 4 extracts contained much smaller amounts of flavonoids; they ranged from 9.10 ± 0.02 to 4.71 ± 0.19 mgRE/g d.m. For extracts of chokeberry and blackcurrant the anthocyanins content was assayed to be 1.29 ± 0.01 to 0.92 ± 0.03 mg C3GE/g d.m.

The results on antiradical activity of the extracts are shown in Figure 2, where the values of the parameter IC_{50}^{DPPH} at the beginning of the storage period and after 12 months are compiled. It should be noted that of all the studied plant extracts the highest activity to eliminate the free radical DPPH• was shown by: Japanese quince, rosehip, chokeberry and quince. In fact, there was a sli-

Table 1. The antioxidant and antiradical activity of studied fruits extracts expressed as $TEAA^{PC}$ [mM TE/g d.m.] and $TEAC^{DPPH}$ [mM TE/g d.m.], respectively.

Plants	$TEAA^{PC}$ [mM TE/g d.m.]		$TEAC^{DPPH}$ [mM TE/g d.m.]	
	begining of storage	12 month of storage	begining of storage	12 month of storage
Chokeberry	0.47 ± 0.09	0.38 ± 0.01	3.33 ± 0.01	3.42 ± 0.02
Hawthorn	0.27 ± 0.10	0.27 ± 0.09	1.44 ± 0.07	2.17 ± 0.13
Quince	0.29 ± 0.09	0.33 ± 0.07	3.25 ± 0.02	3.23 ± 0.05
Japanese quince	0.67 ± 0.04	0.37 ± 0.02	4.70 ± 0.01	4.91 ± 0.01
Blackcurrant	0.40 ± 0.02	0.40 ± 0.03	2.94 ± 0.01	2.79 ± 0.03
Rosehip	0.33 ± 0.07	0.32 ± 0.09	4.63 ± 0.02	4.53 ± 0.06

ght change in the IC_{50}^{DPPH} parameter with the exception of hawthorn extract, for which its value significantly decreased by 33% after 12 months of storage. The results of antiradical activity at the beginning of storage and after 12 months, expressed in Trolox equivalent as the $TEAC^{DPPH}$ parameter (Table 1), indicate weaker activity of all the extracts examined in comparison with Trolox (IC_{50}^{DPPH} = 4.87 μg·ml^{-1}). It should be noted that the results of antiradical activity correlate well with the total polyphenol content in the test extracts. Correlation coefficients are r = - 0.7493 at the beginning of storage and r = - 0.7056 after 12 months ($p < 0.05$).

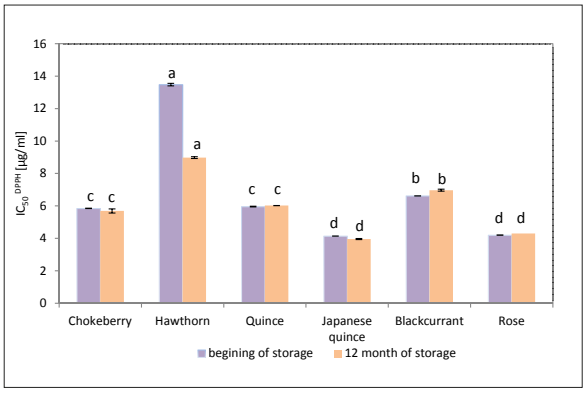

Fig. 2. Comparison of IC_{50}^{DPPH} parameters in the neutralization process of the DPPH• free radicals caused by extracts of chokeberry, hawthorn, quince, Japanese quince, blackcurrant and rosehip. The values are given as the averages of two independent measurements ± SD (n= 6). Different letters (a-d) in the same row indicate significant differences ($p < 0.05$)

Figure 3 shows the results of anti-inflammatory activity of the plant extracts when applied at a concentration of 8 μg·ml^{-1}. The highest activity of 1-LOX inhibition at the beginning of the storage period and after 12 months showed rosehip extracts (up to 46% and 33% inhibition, respectively) and hawthorn (respectively 39 and 31% inhibition). Inhibition of the extracts after 12 months of storage dropped from 6.3% to 77.3%, with the highest decline in Japanese quince activity. Comparison of the inhibition of extracts in relation to ibuprofen as a positive control showed that it

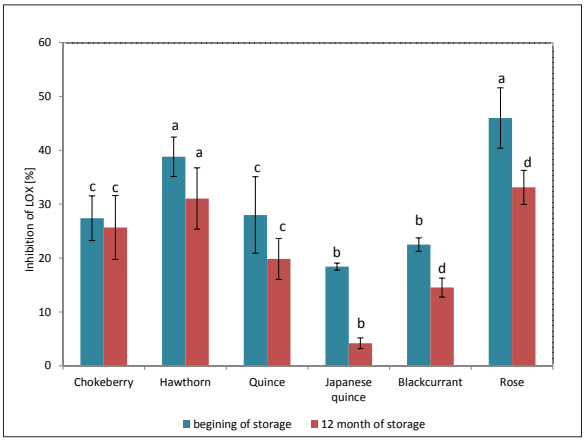

Fig. 3. The percentage of inhibition of 1-LOX activity (anti-inflammatory activity), cause by extracts of chokeberry, hawthorn, quince, Japanese quince, blackcurrant and rosehip at 8 μg·ml^{-1} concentration. The percentage of inhibition are given as average of two independent measurements ±SD (n= 6) Different letters (a-d) in the same row indicate significant differences ($p < 0.05$)

causes 56% inhibition at a concentration of 4-fold less than the test extracts (2 μg·ml^{-1}).

Discussion

The use of substances derived from plants in prevention and treatment of health problems should be preceded by detailed studies. Therefore, polyphenolic compounds extracted from the fruit of blackcurrant, chokeberry, hawthorn, rosehip, Japanese quince and quince were tested *in vitro* during 12 months of storage at room temperature. The extracts were tested for their antioxidant capacity in relation to phospholipid model membranes and also for inhibition of soybean lipoxygenase enzyme activity, which is a good indicator of inflammatory reactions in the body. We also examined the ability of the extracts to eliminate the free radical DPPH•, which showed the extracts' antioxidant action as scavengers of free radicals in the redox process.

This study of antioxidant activity in relation to the process of autooxidation of phosphatidylcholine liposomes showed

significantly higher activity of blackcurrant, chokeberry and Japanese quince extracts in comparison to the remaining quince, rosehip and hawthorn extracts, after 12 months of storage. It should be added that although extracts of chokeberry and Japanese quince showed the largest degradation after that period of storage, they still possessed a relatively high antioxidative potential. The effectiveness of polyphenolic extracts as antioxidants in the process of oxidation of lipid structures has been the subject of relatively numerous studies, including with the use of model lipid membranes [11], that confirmed the high activity of polyphenolic compounds as scavengers of free radicals. It has been suggested that the hydrophilic components of the extracts localize on the surface of the lipid bilayer, neutralizing free radicals that arrive there from the external environment [41]. Other more hydrophobic components of the extracts are capable of penetrating deep into the bilayer [34] and regenerating lipid oxides and peroxides that arise in propagation of the oxidation process.

The use of natural antioxidants to inhibit lipid peroxidation has unquestionable advantages over synthetic antioxidants even if the former should be used in much higher concentrations compared to the concentrations of synthetic antioxidants such as Trolox or BHT. Studies on application of BHT have in fact shown toxic action of this synthetic antioxidant [18,22]. The extracts, being a mixture of several ingredients including the flavonols fraction, can to a varying degree penetrate the lipid membrane and affect repair of its interior. Therefore, the results of research of antioxidant activity in relation to model liposome membranes, which are a simple model of the cell membrane, can be helpful in predicting the beneficial action of these substances *in vivo*.

The results of the antiradical activity of the extracts indicate that the highest ability to scavenge the radical DPPH$^{\bullet}$ is shown by extracts of Japanese quince and rosehip. The high correlation coefficients between total polyphenol content and DPPH$^{\bullet}$ test results, at the beginning of the storage period ($r = -0.7493$) and after 12 months ($r = -0.7056$), show the highest activity for extracts which contained the most polyphenolic compounds, which is in line with the reports of other authors [9]. The health-supporting activity of compounds of the polyphenol group is due to their ability to inhibit the 1-LOX enzyme that catalyses oxidation of fatty acids. Reaction of this enzyme with linolenic acid produces substances that participate in the formation of many diseases including arteriosclerosis, inflammatory bowel disease, psoriasis and asthma [30]. Inhibition of 1-LOX enzyme activity may therefore indicate the potentially beneficial effects of natural polyphenolic compounds, including at the level of the body. The highest activity to inhibit 1-LOX was observed for the rosehip extract (46% inhibition) and hawthorn (38% inhibition). Rosehip extract showed about 10% lower activity than ibuprofen (approx. 56%) as a positive control at 2 $\mu g \cdot ml^{-1}$ concentration. It is reported by various authors that rosehip is rich in quercetin derivatives, kaempferol as well as gallic acid and ellagic acid [39,40]. The presence of these substances in rosehip extract is also confirmed by our research (data not published). Anti-inflammatory effects of quercetin and gallic acid have also been confirmed by independent researchers [7,32]. The anti-inflammatory effect of rosehip extract is confirmed by *in vivo* studies performed on rats. They showed that ethanol-water extract from that fruit inhibits the development of carrageenan-induced edema in rats [25]. The main substances contained in fruits of hawthorn are quercetin-3-*O*-galactoside, quercetin-3-*O*-glucoside and chlorogenic acid [27,28]. It is believed that the anti-inflammatory properties of hawthorn extract are largely due to one of the components, i.e. quercetin-3-*O*-galactoside. Its anti-inflammatory properties *in vivo*, in an experiment on inflammation induced in the liver of rats, has been confirmed in the literature [19].

The literature reports that quercetin and 3-, 4- or 7-*O*-glycosides of quercetin inhibit LDL oxidation speed in the presence of 15-lipoxygenase, while glucosylation of the hydroxyl group in position 4' causes a decrease in the inhibitory activity [29]. The presented relations between the structure and inhibitory activity of lipoxygenase are helpful in predicting the desired *in vitro* activity of polyphenolic extracts as potential nutraceuticals.

In summary, it can be concluded that the polyphenolic extracts of rosehip, hawthorn and chokeberry, which possess the highest stability during 12-month storage, and Japanese quince extract, which has the highest antioxidant and antiradical activity, present a potential source for the production of dietary supplements. Studies confirm that there is a need for nutraceuticals of appropriate composition, which, when available on the market as dietary supplements, can be used both as para-medicines and for enriching the diet.

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