Journal of Medicinal Plants Research Vol. 6(31), pp. 4698-4706, 15 August, 2012
Available online at http://www.academicjournals.org/JMPR
DOI: 10.5897/JMPR12.874
ISSN 1996-0875 ©2012 Academic Journals

Full Length Research Paper

South Siberian fruits: Their selected chemical constituents, biological activity, and traditional use in folk medicine and daily nutrition

Pawel Pasko[1]*, Justyna Makowska-Was[2], Joanna Chlopicka[1], Marek Szlosarczyk[3], Malgorzata Tyszka-Czochara[4], Justyna Dobrowolska-Iwanek[1] and Agnieszka Galanty[2]

[1]Department of Food Chemistry and Nutrition, Medical College, Jagiellonian University, Krakow, Poland.
[2]Department of Pharmacognosy, Medical College, Jagiellonian University, Krakow, Poland.
[3]Department of Inorganic and Analytical Chemistry, Medical College, Jagiellonian University, Krakow, Poland.
[4]Radioligand Laboratory, Department of Pharmacobiology, Medical College, Jagiellonian University, Krakow, Poland.

Accepted 25 July, 2012

The aim of this work was to determine the content of total phenolic and flavonoid compounds; to evaluate the total antioxidant capacity (ferric reducing ability of plasma (FRAP) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) methods); to determine selected trace elements (Zn, Cu) content; to identify the selected organic acid composition; and to determine cytotoxic activity against human prostate cancer cells of six selected South Siberian fruits (Siberian mountain ash, bird cherry, Nanking cherry, Siberian apricot, Siberian elder, and prickly wild rose). These fruits have been used in traditional Siberian medicine before conventional medications were developed. During our scientific expedition, different places as big city, small villages near the lake Baikal and in the mountains were visited. We observed local customs and also interviewed women, responsible for preparing meals, about typical usage, recipes, and practical or medical application of native fruits. Siberian apricots revealed the highest total antioxidant activity, concentration of polyphenols and also the best cytotoxic activity among the examined fruits. Bird cherry and Siberian elder had the highest content of copper and zinc among all the evaluated Siberian fruits. The fruits of some of Siberian species, especially apricots and prickly wild rose, can be a good source of antioxidant compounds. Moreover, Siberian apricot, Siberian mountain ash, and bird cherry, due to their interesting activity against prostate cancer cells, may be considered as a potential anticancer prophylaxis. The findings suggest that ethno-medicinal and ethno-nutritional aspects of Siberian fruits should not be neglected.

Key words: Siberian fruits, antioxidant activity, trace element, organic acids, cytotoxic activity, ethno-nutrition, ethno-medicine.

INTRODUCTION

During the last decades, a global trend for the revival of interest in the traditional use of plants in folk medicine and also in daily menu has been observed. Unfortunately, information about typical use of fruits which are available in South Siberia is scarce. This information is highly required to evaluate the chemical composition and biological activity (antioxidant and cytotoxic) of fruits used in indigenous medical systems and traditional diet. Some of the phytochemical compounds: flavonoids, anthocyanins, phenolic acids, carotenoids, as well as vitamins and trace elements, exhibit proved and high antioxidant capacity (Finley et al., 2011). It is well known that these natural antioxidants inhibit the generation of

*Corresponding author. E-mail: paskopaw@poczta.fm. Tel: +48 12 620 56 70. Fax: +48 12 620 56 93.

free radicals and thus prevent protein, lipids, and also nucleic acids oxidative damage. A lot of information has been published recently, describing anticancer and anti-inflammatory activity of fruits, especially different kinds of berries (Ferretti et al., 2010; Arancibia-Avila et al., 2011). Fruits also exhibited these effects in some *in vivo* models (Parades-Lopez et al., 2010), causing an increase in antioxidant activity of plasma and selected organs and a decrease in lipid peroxidation.

Indigenous Siberian people are recognized as having unique social, cultural, and health needs. Specific economic situation of this region limited the access to natural products, an important part of daily diet, to natural resources (that is, forest, orchards). Siberian people are well adapted to harsh local environmental conditions, so the knowledge about how and why they used natural products in folk medicine and daily nutrition is highly valuable. The connections between the use of traditional food resources and culinary practices with the health of the Siberian habitants are also of great importance. South Siberian fruits remedies have been used for centuries to cure common illnesses and treat various health problems. Without access to hospitals, people could only rely on plants that grew around their houses and yards. Really, interesting evaluation of the indigenous peoples of the North (Chukchi and Siberian Eskimos-Yupik) nutritional habits were carried out by Kozlov and Zdor (2003). However, data available on ethno-nutrition and role of native fruits in ethno-medicine, especially on South Siberia are still not enough in international literature. These fruits have been used for generations, and many were in common medical use before conventional medications were developed. Much of this knowledge has been forgotten; however, some attempts have been made to document the remaining recipes of typical using.

As part of the Polish Scientific Group interested in Traditional Medicinal System of South Siberia (Bajkal lake area and Sayan mountains region), we conducted studies on dietary and medicine, using six popular indigenous fruits. Our researches are especially important, because the lifestyle of the native people (the Buriat of Southern Siberia) is changing. A lot of negative health outcomes, including an increased prevalence of obesity, hypertension, type 2 diabetes, an elevated risk for various chronic degenerative conditions, and declines in fitness and physiological work capacity are observed (Snodgrass et al., 2007). Snodgrass et al. (2007) suggested that the mechanisms responsible for this health transition remain incompletely understood, although dietary changes, alcohol consumption, tobacco use, chronic psychosocial stress, and physical inactivity have all been implicated.

This work is designed additionally to present typical usage of native Siberian fruits and provide significant data associated with selected chemical constituents and biological activity of these fruits which popular names are: Siberian mountain ash, bird cherry, Nanking cherry, Siberian apricot, Siberian elder, and prickly wild rose. The objectives were to (i) determine the content of total phenolic compounds and total flavonoids, (ii) evaluate the total antioxidant capacity (ferric reducing ability of plasma (FRAP) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) methods), (iii) determine selected trace elements (Zn, Cu) content, (iv) identify selected organic acid composition, and finally (v) determine cytotoxic activity of fruits on human prostate cancer cell line. Our investigation was focused on dry material which is in such state normally stored.

MATERIALS AND METHODS

Plant

For this study, five species were used: *Armeniaca sibirica* (L.) Lam., *Padus avium* Mill. *(syn. Prunus padus* L.), *Prunus tomentosa* Thunb., *Sorbus aucuparia* L. *subsp. sibirica* (Hedl.) Krylov, and *Rosa acicularis* Lindley, and one species from Adoxaceae family *(Sambucus sibirica* Nakai). All the selected species are wild-growing plants, belonging to the *Rosaceae* family (with one exception), and their fruits are edible. The species are also used for cooking in the lake Baikal region.

Fruits of all species were collected in their natural environment in the lake Baikal area and Sayan mountains region in July and August, 2009. *A. sibirica* and *P. tomentosa* were collected near Irkutsk, and *P. avium*, *R. acicularis*, *S. aucuparia subsp. sibirica*, and *S. sibirica* were collected in the Eastern Sayan (Tunka range, Arshan). Voucher specimens are deposited in the Department of Food Chemistry and Nutrition, Faculty of Pharmacy, Medical College, Jagiellonian University with referring number AS/PP/PL 1026 to *A. sibirica*, PA/PP/PL 1027 to *P. avium*, PT/PP/PL 1028 to *P. tomentosa*, SA/PP/PL 1029 to *S. aucuparia subsp. sibirica*, RA/PP/PL 1030 to *R. acicularis*, and SS/PP/PL 1031 to *S. sibirica*. All plants were identified and described by Justyna Makowska-Was from the Department of Pharmacognosy, Faculty of Pharmacy, Medical College, Jagiellonian University, Krakow, Poland.

Samples of fruits used for the experiment were dried at room temperature or in 40°C (*A. sibirica* and *P. tomentosa*). The dried fruits were stored in a freezer until analysis. They were conditioned at room temperature before use. The detailed composition of the seeds was not studied in this work.

Interview

During our scientific expedition, different places as city (Irkutsk), small villages near the Baikal lake (Bolshiye Koty) and in the mountains (Arshan) were visited. We observed local community and we interviewed women responsible for preparing meals about typical usage, recipes, and practical or medical application of native fruits. Moreover, some herbalists from local market were also interviewed to broaden our knowledge and confirmed the obtained information. Finally, 24 people were interviewed, including 21 women and 3 men, aged from 42 to 73 years.

Extracts preparation

Powdered samples of dried fruits (1 g) were extracted for 2 h with 40 ml of solvent consisting of methanol, 0.16 mol/L hydrochloric acid, and water, 8:1:1, respectively. The extracts were decanted and the residues were extracted again with 40 ml of 70 g/100 g acetone for 2 h. Both extracts were then combined, decanted, centrifuged, and stored in darkness in a freezer in temperature of –20°C. These extracts were used for the estimation of total antioxidant activity (FRAP, DPPH) and total phenolic content. Plant material for free flavonoid analysis was extracted with 50% methanol/water and the sample was vortexed for 1 min and was heated at 90°C for 3 h (Gorinstein et al., 2007; Vinson et al., 2001).

Determination of total polyphenols

Total phenols (TP) were determined colorimetrically using Folin–Ciocalteu reagent, as described previously (Paśko et al., 2009). Total phenols assay was conducted by mixing 2.7 ml of de-ionized water, 0.3 ml of extracts, 0.3 ml 7 g/100 g Na_2CO_3, and 0.15 ml Folin–Ciocalteu reagent. Absorbance of mixture was measured at 725 nm using the spectrophotometer Jasco UV-530. A standard curve was prepared with gallic acid. Final results were given as gallic acid equivalents (GAE).

Determination of total flavonoids

Briefly, a 0.25 ml of extract was diluted with 1.25 ml of distilled water. Then, 75 ul of 5% $NaNO_2$ solution was added to the mixture. After 6 min. 150 ul of 10% $AlCl_3 \cdot 6H_2O$ solution was added and the mixture was allowed to stand for another 5 min. Then, 0.5 ml of 1 M NaOH was added and, after mixing, the absorbance was measured immediately at 510 nm (Gorinstein et al., 2007). The final concentration of flavonoids was expressed as an equivalent of rutin (mg rutin/g dry weight).

Determination of FRAP activity

FRAP assay was carried out according to Benzie and Strain (1996), and modified to 48-well plates and automatic reader (Synergy-2, BioTek/USA) with syringe rapid dispensers. Briefly, the oxidant in the FRAP assay (reagent mixture) consisted of ferric chloride solution (20 mmol/L), 2,4,6-Tripyridyl-s-Triazine (TPTZ) solution (10 mmol/L TPTZ in 40 mmol/L HCl), and acetate buffer (pH = 3.6) in a proportion of 5:5:10, respectively, and was freshly prepared. To each plate, 0.4 ml of acetate buffer (pH 3.6) was dispensed, followed by 50 µl of sample, standard or blank. The plate was conditioned at the temperature of 37°C for 2 min, and then 0.2 ml of reagent mixture was added and shaken for 30 s; afterwards, absorbance at 593 nm was measured with kinetic mode for 15 min. The final results were expressed as mmol Fe^{2+}/kg dry weight.

Determination of DPPH radical scavenging activity

DPPH radical-scavenging activity was measured according to the method of Yen and Chen (1995) with modification (Paśko et al., 2009). For the measurement of sample scavenging activity, 0.4 ml of methanolic acetate buffer was added to the cuvettes containing the increasing volumes of sample (e.g. 0, 0.1, 0.2, 0.3, 0.45, and 0.6 ml) with adequate volumes of methanol to make total volume of 1 ml. Acetate buffer was made from 0.2 mol/L solutions of sodium acetate and acetic acid in methanol mixed at the volume ratio 7.9:2.1. The pH of the buffer was 5.2. One millilitre of DPPH stock solution (12 mg DPPH was dissolved in 100 ml of methanol; absorbance 1.3) was added to each cuvette, and then absorbance was measured after 24 h. The absorbance of the resultant solution was determined using Jasco UV-530 spectrometer (Japan) at 514 nm. The total antioxidant capacities (TAA) were estimated as Trolox equivalents (TEAA) by interpolation to 50% inhibition (TEAA50).

Determination of selected organic acids

The extracts for organic acids analysis were prepared according to standard procedure: boiling water was poured over 1.8 g of dried fruits and then it was left to steep for at least 5 min. Isotachophoretic separation was performed using the Electrophoretic Analyser EA 202M (Villa Labeco, Spišská Nová Ves, Slovakia) with conductivity detection. The system was equipped with sample valve of 30 µm fixed volume and two capillary: the preseparation capillary (90 × 0.9 mm inner diameter (ID)) and analytical capillary (160 × 0.3 mm ID). The leading electrolyte was hydrochloric acid (10 mM) including 0.2% methylhydroxyethylcellulose (M-HEC) adjusted with beta-alanine to pH 3.5. The terminating electrolyte contained 5 mM caproic acid and 5 mM histidine. The current in the preseparation column was 250 µA and in the analytical column 60 µA. During detection, it was reduced to 50 µA. Samples (infusions of examined fruits) were diluted with distilled water to give final organic acid concentration range of 25 to 125 mg/L. The analysis of each sample was performed in triplicates.

Cytotoxic assay

The cytotoxic activity was tested against human prostate cancer cell line Du-145. The cells were grown in Dulbecco's Modified Eagle's Medium Nutrient Mixture F-12, supplemented with 10% calf serum and antibiotics. Cells were seeded on 24-well plates (density 1.5×10^4 $well^{-1}$) and incubated at 37°C and 5% CO_2. After 24 h, the cultured medium was replaced with fresh medium, containing different concentration of the tested extracts from 0 (control) to 100 µg/ml. The controls were incubated in the culture medium with 0.05% methanol. As a positive control, a standard cytostatic drug mitoxanthrone was used. The cells were incubated for 48 h. Cell viability was determined using trypan blue exclusion dye test. Cytotoxic activity was measured by microscopic examination as a percentage of living cells.

Determination of selected trace elements

Zinc and copper presence were determined using a differential pulse anodic stripping voltammetry (DP-ASV) with a controlled growth mercury drop electrode (CGMDE) (Szlósarczyk et al., 2011) with a differential pulse stripping step. Samples were powdered in agate mortar and then dried at over 70°C for 4 h. Approximately, 250 to 500 mg of sample material was weighed and inserted in a high pressure Teflon container and was treated with 5 to 6 ml of nitric acid and 1 ml of perhydrol. Next, the vessel was placed in a microwave oven (Multiwave 3000, Anton Paar). The digestion of the sample was carried out according to the following on the ramp program: 30 min under microwave irradiation (600 W, 60 bar), 20 min under microwave irradiation (400 W, 60 bar), and 15 min cooling time. The digested sample was placed at the heated plate to let it evaporate and to remove the nitrate for copper and zinc analysis. The sampled solutions were cooled to room temperature and transferred quantitatively into volumetric flasks (10 ml), and was filled up to the mark with double distilled water. All the procedures were repeated three times for each sample.

Statistical analysis

Results of analyses are given as means ± standard deviation (SD) based on three measurements for each sample of Siberian fruit extracts. Where appropriate, the data were tested by one-way analysis of variance (ANOVA), followed by Tukey post hoc test. Differences with $P < 0.05$ were considered to be statistically significant.

RESULTS AND DISCUSSION

Interview

Basic information about traditional usage of the examined fruits in medicine and nutrition, and also, some data on the chemical compounds which probably can be responsible for biological activity of these natural products are presented in Table 1. During the interviews with South Siberian herbalists, we gathered much more detailed information about traditional processing of the fruits. Smashed fresh bird cherry fruits, mixed with butter and sugar are recommended as a remedy for common cold, while dried and ground fruits of the species can be used in baking breads or cakes as an additive to flour. Sometimes, dried roasted fruits of bird cherry are used as a substitute of coffee. Bird cherry fruits are also often eaten by the native children directly from the trees. Fresh Siberian mountain ash fruits are used for dumplings stuffing and from fresh ripe fruits of fruits are used for dumplings stuffing and from fresh ripe fruits of Siberian elder soups can be prepared.

Table 1 was worked up according to personal interview and scientific information (Komarov, 1964; Shreter, 1975; Odonmažig et al., 1985; Kawecki et al., 2002; Kumarasamya et al., 2004; Uusitalo, 2004; Jordheim, 2007; Kim et al., 2008).

Polyphenol compounds and total antioxidant capacity of Siberian fruits

The total phenolic compounds and flavonoid contents and also antioxidant activity of the evaluated Siberian wild fruits are shown in Table 2. Total phenolic content varied greatly among the native fruits harvested in south Siberia, ranging from 155 to 4375 mg GAE/100 g of dry weight. The highest content of polyphenols were observed in Siberian apricot, while the lowest content was measured in Nanking cherry and Siberian mountain ash, 155 and 190 mg GAE/100 g dry weight, respectively (significant differences are indicated in Table 2). Data obtained for Siberian apricots are similar to results presented by Akin et al. (2008) who evaluated dry Malatya apricot fruits harvested in Turkey. Jabłońska-Ryś et al. (2009) examined total polyphenols content in similar wild fruits (dog rose, elderberry) which are grown in East Poland and obtained comparable results. In our evaluation, total polyphenols content in Siberian mountain ash was 10 fold lower than in rowan berry grown in the northern climate (Finland) (Kahkonen et al., 2001). The reason for such differences in total phenolic content could be associated with the ripeness of the fruit; concentration of these compounds is usually higher in young fruits than in mature rowan fruits used in our analysis. It is also probable that fruits harvested in the cool northern climate have higher phenolic content as compared to fruits grown in a warm climate (South Siberia) (Kahkonen et al., 2001). The most prominent levels of free flavonoids were also observed in the extracts of Siberian apricot: 206.1 mg rutin/100 g dry weight. The extracts of 3 species (Siberian mountain ash, bird cherry, and Nanking cherry), exhibited similar low content of flavonoids, 68.1, 59.4, and 48.65 mg rutin/100 g dry weight, respectively.

It is obvious and also proved in our study that all examined extracts of dried fruits contain several different chemical compounds that contribute to the overall antioxidant activity. Two methods (FRAP and DPPH) were used to test the antioxidant activity of Siberian fruits. FRAP method is based on the determination of ferric-tripyridyltriazine complex reducing capacity of the evaluated extracts. Among polyphenols, the greatest antioxidant efficacies in this test were shown for quercetin, tannic acid, caffeic acid, and gallic acid, while catechin had the lowest ones (Pulido et al., 2000). On the other hand, DPPH method is based on the evaluation of the reducing ability of antioxidants toward DPPH, which is stable nitrogen radical, possessing an odd electron. In this case, steric accessibility is a major determinant of the analytical reaction. Thus, this assay is adequate mainly for reactive small molecules that have good access to the radical site. The most effective antioxidants scavenging DPPH are gallic acid, tannic acid, ascorbic acid, and quercetin (Chlopicka et al., 2012) which are widely represented in evaluated fruits. Siberian apricots showed the highest antioxidant activity, as indicated by FRAP and DPPH methods. The FRAP value ranged from 17.52 to 326.37 mmol Fe^{2}+/kg dry fruits and the DPPH radical scavenging activity from 26.5 to 309 mmol Trolox/kg dry fruits. With both methods, Nanking cherry expressed the lowest antioxidant capacity. There is a significant correlation between total phenolic or flavonoids content and antioxidant activity measured by FRAP and DPPH methods. It is especially observed in the case of Siberian apricot and prickly wild rose, which were the two most active species with the highest content of polyphenols and flavonoids. Moreover, correlation coefficients (r) were calculated for the obtained results. A strong correlation was observed in all examined fruits between the results of DPPH versus FRAP ($r = 0.98$; $P < 0.05$). The highest rate of correlation coefficients was observed in the case of Nanking cherry: TP versus TF ($r = 0.97$; $P < 0.05$), TP versus DPPH (0.98; $P < 0.05$), DPPH versus TF ($r = 0.99$; $P < 0.05$). Some interesting results were also obtained for Siberian elder, with TP versus DPPH (0.95, $P < 0.05$); TF versus FRAP ($r = 0.93$; $P < 0.05$).

Antioxidant activity of similar fresh fruits, described by other authors, is in opposite to our results. The following order of antioxidant activity by FRAP (mmol $Fe^{2+/}$100 g fresh weight) method was obtained by Halvorsen et al. (2002): dog rose < sour cherry (*Prunus cerasus*) < elderberry < rowan < sweet cherry (*Prunus avium*) < apricot. The study of Jabłońska-Ryś et al. (2009) showed

Table 1. Information about botanical and native name of the examined fruits, their traditional usage in medicine and nutrition and also chemical content.

Botanical name	Family	English name (Russian name)	Parts used	Dietary usage	Traditional medicinal usage	Active constituents
A. sibirica (L.) Lam.	Rosaceae	Siberian apricot, (Abrikos sibirsky)	Fruit	Fruit: raw or cooked, cakes, jam, jelly, liqueur; Seeds: as bitter almond substitute	Seeds: analgesic, antiasthmatic, antitussive, anti-furuncle and emollient; used in the treatment of coughs, asthma, acute or chronic bronchitis and constipation.	Oil , cyanogenic glycosides, (seeds); sugars (free sugars – sorbitol and glucose, carbohydrates), carotenoids, polyphenols.
P.avium Mill. (syn. *Prunus padus* L.)	Rosaceae	Bird cherry, (Ceremucha obyknovennaja)	Fresh and dried fruit, bark, leaf	Raw fruit, dumplings, wine, jam, ground dried fruit used as flour	Fruit for strengthening stomach, in gastritis, colic, diarrhoea, anaesthetic and disinfectants, also to treat coughs, improve complexion and eyesight.	Sugars, organic acids, cyanogenic glycosides, anthocyanins, flavonoids, tannins and lignanxylosides.
P. tomentosa Thunb.	Rosaceae	Nanking cherry, (Vishnya vojlochnaya)	Fruit	Raw fruit, juices, alcohol beverages, compotes, jams, jellies	For cleaning and nourishing blood, increase appetite, regulate alimentary system.	Cyanogenic glycosides (seeds); sugars, organic acids, vitamins, anthocyanins, flavonoids.
S.s aucuparia subsp. *sibirica* (Hedl.) Krylov	Rosaceae	Siberian mountain ash, (Ryabina sibirskaya)	Fruit (frozen or blanched)	Alcohol beverages, jams, jellies, honey, floured dried fruit (minced dried fruits used as flour)	In avitaminosis, arteriosclerosis, as antipyretic or diuretic agent.	Sugars, phenolic acids, carotenoids, flavonoids, vitamins (C, provitamin A) tannins, sorbitol
R. acicularis Lindl.	Rosaceae	Prickly wild rose, (Acicular Rose, Arctic Rose), (Shipovnik iglisty)	Fruit, petals, leaf, root	Teas, jams wine, liquor, marmalade	Fruit - in cold, weakness, anaemia, tuberculosis as vitamin source; also in hepatic failure, stomach ulcer and neurasthenia.	Sugars, organic acids, vitamins (mainly vit. C), carotenoids, pectins, flavonoids, tannins, oil (seeds).
S. sibirica Nakai.	Adoxaceae	Siberian Elder, (Buzina sibirskaya)	Fruit, flowers	Beverages, juices marmalade, jam, jelly, wine, vinegar	Fruit and seeds oil – mild purgative; bark and wood - externally on wounds, furuncles; Flowers – antipyretic, antiphlogistic, diuretic.	Fruit: anthocyanins, tannins, organic acids, sugars, vitamins, oil (seeds); Flower: essentials oil, flavonoids, tannins, sugars, organic acids, glycoside.

the highest antioxidant capacity for dog rose, than for elderberry and rowan. The most significant differences between our results and other results are associated with apricot. Halvorsen et al. (2002) also evaluated dried fruits. Data obtained by these authors about antioxidant activity of dry apricots was 10 fold lower than our results (Halvorsen et al., 2002). These differences between some of the chemical compositions of apricot varieties have been observed previously by Munzuroglu et al. (2003) and explained mainly by genetic and environmental variations. In addition, the time of fruit harvesting and also their ripening stage could also affect these results (Akin et al., 2008). Total antioxidant capacity of prune

Table 2. Content of total polyphenols, flavonoids, and total antioxidant capacity (mean ± SD, n = 3) of examined native Siberian fruits.

Siberian fruits	Total polyphenols (mg GAE/100 g dry fruits)	Total flavonoids (mg rutin/100 g dry fruits)	FRAP (mmol Fe^{2}+/ kg dry fruits)	DPPH (mmol Trolox/kg dry fruits)
Siberian apricot	4375 ± 258^{a}	206.1 ± 6.3^{a}	326.37 ± 12.6^{a}	309 ± 10.5^{a}
Bird cherry	367 ± 7.8^{ab}	59.4 ± 2.9^{ab}	45.8 ± 4.5^{ab}	35.8 ± 7.9^{ab}
Nanking cherry	155 ± 6.2^{abc}	48.65 ± 3.5^{ac}	17.52 ± 0.7^{abc}	26.5 ± 4.7^{ac}
Siberian mountain ash	190 ± 14^{abcd}	68.1 ± 6.8^{ad}	315.5 ± 14^{bcd}	357 ± 11.8^{bcd}
Prickly wild rose	2120 ± 23^{abcde}	133.2 ± 5.7^{abcd}	208.6 ± 10.9^{abcde}	222 ± 9.3^{abcde}
Siberian elder	436.8 ± 10.2^{abcde}	113.8 ± 10.2^{abcd}	70.1 ± 4^{abcde}	43 ± 7.2^{abcde}

Means with different letters in the each column for each Siberian fruits are significantly different (P < 0.05).

Table 3. Concentration of organic acids in evaluated water extracts (mg/L fruit tea).

Organic acid	Siberian apricot	Bird cherry	Nanking cherry	Siberian mountain ash	Prickly wild rose	Siberian elder
Oxalic acid	nd	nd	3.47 ± 0.31	nd	nd	4.22 ± 0.17
Citric acid	901.4 ± 7^{a}	567.6 ± 0.9^{ab}	nd	9.4 ± 0.8^{ab}	410.5 ± 1.23^{a}	524.6 ± 0.9^{a}
Malic acid	270.7 ± 1.3^{a}	17.6 ± 1.8^{ab}	54.2 ± 0.7^{abc}	237.3 ± 2.1^{bcd}	95.3 ± 1.8^{abcd}	86.1 ± 3.4^{abd}
Lactic acid	271.02 ± 6.3^{a}	nd	nd	143.8 ± 3.7^{ab}	106.7 ± 0.7^{ab}	nd
Succinic acid	44.6 ± 2.5^{a}	nd	nd	142.1 ± 3.2^{a}	nd	nd
Sorbic acid	nd	nd	nd	146.5 ± 2.9	nd	nd

Means with different letters in the each row for each organic acids in evaluated Siberian fruits are significantly different (P < 0.05). nd: not detected.

(*Prunus nigra*) is similar to our findings about bird cherry and Nanking cherry, but raisins and figs have much more lower antioxidant activity than dried fruits harvested in South Siberia (Halvorsen et al., 2002).

Organic acids profile in Siberian fruits

Samples of water extracts prepared from dried fruits were differed significantly in terms of profile and concentration of organic acids. The amount of organic acids measured in the particular samples is presented in Table 3. Organic acids are responsible for the flavour and taste of fruits. Moreover, they have influence on human health, revealing protective role against various disease due to their antioxidant activity (Silva et al., 2004). Extract of Siberian mountain ash was the richest as to the content of various organic acids. In this material, the dominant compound compared to other organic acids was malic acid. Concentration of lactic, succinic, and sorbic acids were comparable and at the same time about 40% lower than the results obtained for malic acid. The less abundant from among all identified organic acids in Siberian mountain ash extract was citric acid. In Siberian apricots and Siberian mountain ash extracts, the same organic acids were identified except for sorbic acid. In contrast to Siberian mountain ash, Siberian apricot extract revealed the highest concentration of citric acid which was about three times higher than the concentration of malic and lactic acid. Malic and citric acid were found also in Turkish apricot by Akin et al. (2008). The following acids were identified in prickly wild rose extract and Siberian elder extract: malic and citric acid. In addition, lactic acid was found in prickly wild rose extract and oxalic acid was found in Siberian elder extract. Citric acid revealed the highest concentration in both extract samples. Lactic acid concentration found in prickly wild rose extract appeared to be four times lower than the concentration of citric acid in the same samples. Furthermore, oxalic acid concentration in Siberian elder extract was very low.

Bird cherry and Nanking cherry extracts were the poorest in terms of variety of organic acids. In bird cherry, only two organic acids were observed: citric acid and malic acid. The content of citric acid in the sample was the highest as compared to other investigated fruit extracts, except Siberian apricot. Concentration of lactic acid was at the same time about 30 times lower. In Nanking cherry extract, oxalic and malic acid were determined. The level of oxalic acid was similar to that obtained for Siberian elder. Malic acid concentration in the extract was over 15 times higher as compared to oxalic acid content, but lower than the result obtained for most other fruit extracts. With respect to organic acid contents in sweet cherry harvested in Spain, the dominant organic acid was also malic acid

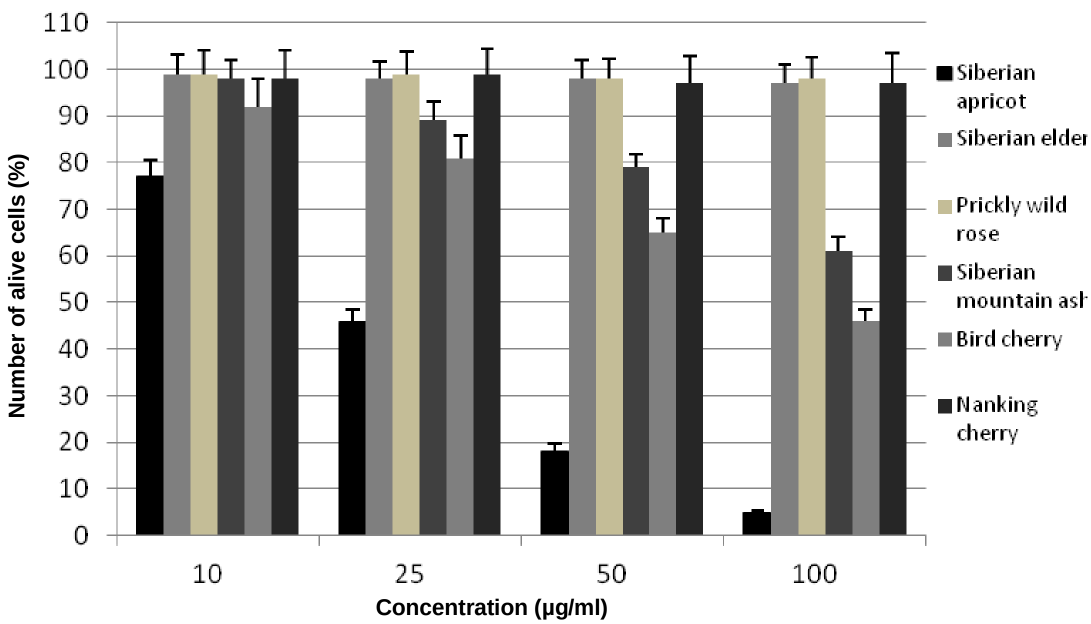

Figure 1. Cytotoxic activity of Siberian fruits on human prostate cancer cell line Du-145 (mean ± SD, n = 3).

(Serrano et al., 2005). Serrano et al. (2005) found citric and succinic acids, which are not present in fruits obtained from South Siberia. Malic acid has been found to be the major organic acid contributing to the acidity in the *Prunus* species, such as plum, peach, apricot, and nectarine (Serrano et al., 2005).

Cytotoxic activity of Siberian fruits

Cell death of human prostate cancer cell line Du-145 was analysed after 48 h of incubation with media containing extracts of six native Siberian fruits, in the concentration range of 0 to 100 mcg/ml (Figure 1). Siberian apricot, Siberian mountain ash, and bird cherry extracts exhibited moderate cytotoxic activity in comparison to mitoxanthrone (IC_{50} 5 mcg/ml), in dose-dependent manner. The best results were obtained for Siberian apricot extract, causing more than 50% cell death at the concentration of 25 mcg/ml, and almost 100% of dead cells were observed at the concentration of 100 mcg/ml. For Siberian mountain ash and bird cherry fruits, at the concentration of 100 mcg/ml, the results were still significant, 40 and 55%, respectively. Nanking cherry, prickly wild rose, and Siberian elder extracts did not exhibit cytotoxic activity against human prostate cancer cells at the tested concentration range (10 to 100 mcg/ml). To our best knowledge, cytotoxic activity of the tested species has been reported for the first time. Data on the cytotoxic activity of fruits from Rosaceae family are scarce. Some cytotoxic effects were observed for prune (*Prunus domestica*) juice (Fujii et al., 2006). The examined substance revealed moderate cytotoxic activity against human colon (Caco-2) and stomach carcinoma (KATO-III) cells, at concentration of 125 mcg/ml causing 60 and 10 % of dead cells, respectively (Fujii et al., 2006). Yamai et al. (2009) examined the influence of extract from Japanese apricot (*Prunus mume*), combined with known cytostatic drugs, on human esophageal squamous carcinoma cells. The tested extract showed synergistic cytotoxic effects on cancer cells (YES-2) together with 5-fluorouracil at the concentration of 10 mcg/ml. Moreover, the extract combined with 5-fluorouracil induced cell cycle arrest at G2/M phase and caused apoptosis in YES-2 cells (Yamai et al., 2009).

Selected trace elements in Siberian fruits

Zinc and copper were chosen to complement the results of the experiments on the antioxidant activity of the tested plant material. The two essential trace elements play an important role in the regulation of anti-oxidative processes. They are especially important as an element of superoxide dismutase (SOD) (Klotz et al., 2003). The concentrations of selected trace element (Figure 2) in Siberian fruits were observed in the range of 6.36 to 28.53 mg/kg of dry fruits and 0.58 to 6.62 mg/kg of dry fruits for zinc and copper, respectively. The maximum amount of copper was observed in the Siberian elder (6.62 ± 0.4 mg/kg dry weight) and bird cherry (6.16 mg/kg dry weight), and the minimum in the Siberian apricots (0.58 ± 0.02 mg/kg dry weight). Concentration of copper in apricots from Turkey was significantly higher (Saracoglu et al., 2009) than in case of Siberian apricots. The highest level of zinc was observed in bird cherry fruits

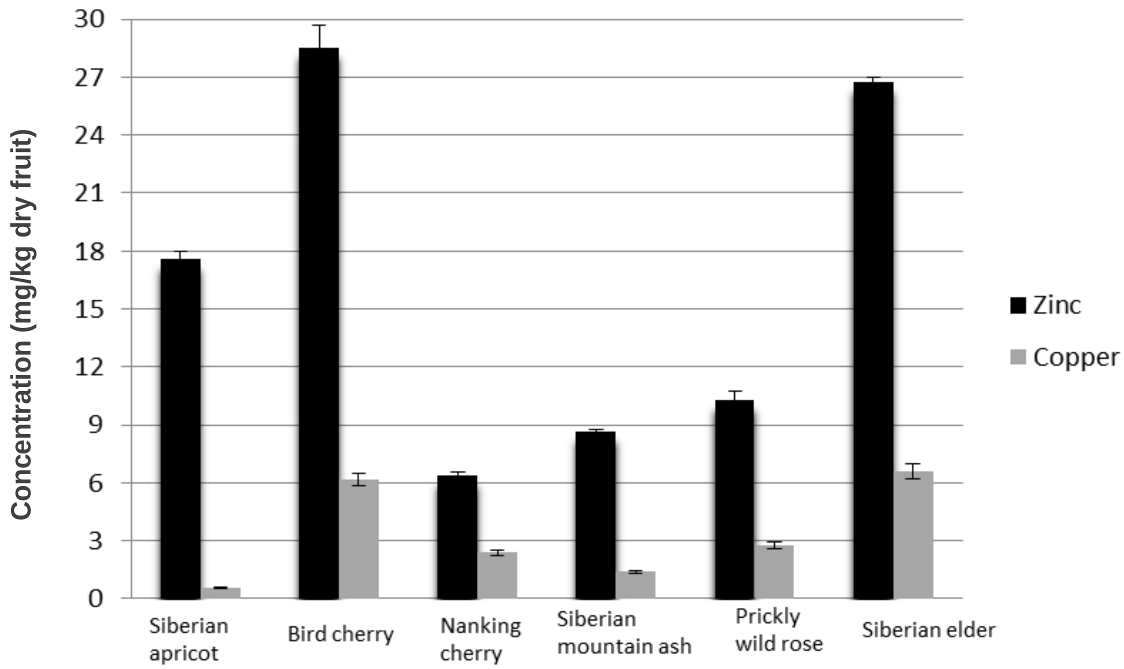

Figure 2. Content of copper and zinc in evaluated Siberian fruits (mean ± SD, n = 3).

(28.53 ± 1.1 mg/kg dry weight) and in Siberian elder fruits (26.75 ± 0.25 mg/kg dry weight). Significantly lower concentration ($P < 0.05$) of this element was presented in prickly wild rose (10.29 ± 0.46 mg/kg dry weight) and Siberian mountain ash (8.67 ± 0.2 mg/kg dry weight). Nanking cherry fruits were the poorest source of zinc (6.36 ± 0.2 mg/kg dry weight) in the group of examined fruits from South Siberia. Our research showed that Siberian apricots were moderate source of zinc (17.57 ± 0.45 mg/kg dry weight) and similar results were obtained by Saracoglu et al. (2009), but in comparison with apricots from Peshawar, Pakistan, the level of this trace element was significantly higher (Sattar et al., 1998). Edible dry fruits (date, raisin or fig) harvested in Pakistan provided much more lower zinc than fruits harvested in South Siberia (Sattar et al., 1998).

Conclusion

A number of fruits included in this study are known to have different medicinal properties (Table 1), some of which may be attributed to their significant free radical scavenging capacity. Some of the examined fruits, especially apricot, bird cherry or Siberian elder, exhibited very interesting properties, which might be of great importance in the prevention of some health problems. The obtained results do not indicate the direct relationship between the antioxidant and cytotoxic activity of the tested species and their use in traditional medicine; however, further investigation of these fruits may be useful in perspective and should be continued. It would be interesting to carry out further investigations on these native natural products in order to provide knowledge of the health benefits, antioxidant, and cytotoxic activities of different cultivars of popular fruits, which had not been well- known until now. Results of ethno-medicinal use and dietary habits could indicate which cultivars should be harvested and included in food products and daily nutrition.

ACKNOWLEDGEMENTS

Polish Scientific Expedition to South Siberia (Baikal lake), Russian Federation – VII – VIII 2009 was supported by Professor Jan Krzek, the Dean of Faculty of Pharmacy, Medical College, Jagiellonian University, Krakow, Poland and by the Bratniak Students' and Graduates' of Jagiellonian University Foundation.

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