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REGULAR ARTICLE

CYTOTOXIC AND ANTIOXIDANT ACTIVITY OF BUCKWHEAT HULL EXTRACTS

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ABSTRACT

Buckwheat contains many prophylactic compounds that are concentrated mainly in outer layers of buckwheat grain. The aim of this study was to prepare buckwheat hull extracts. Ten buckwheat cultivars were screened for their antioxidant and anticancer properties. Total polyphenol content was determined using Folin-Ciocalteau's reagent. Antioxidant activity was established by the method of binding free radical DPPH. Cytotoxic properties were measured on human cervical cancer cells HeLa using mitochondrial cytotoxic test (MTT). Total polyphenol content ranged from 166.67 to 635.31 mg GAE/100 g DW. The highest content displayed tartary buckwheat cultivar *Madawaska* (0.64% of hulls weight). Among common buckwheat the richest in polyphenols were cultivars *Bamby* and *KASHO-2*. The best free radical binding antioxidant activity was found for cultivars with highest polyphenol content. This relationship was not observed for cytotoxic action on human cervical cancer cells. The best growth inhibitory activity on HeLa cancer cells displayed common buckwheat cultivars *Bamby* and *KASHO-2* (up to 50%, extract concentration 100 µg/ml). This was not found for tartary buckwheat cultivar *Madawaska*.

Keywords: antioxidant activity, buckwheat hull, cytotoxic effect, polyphenol content

INTRODUCTION

Buckwheat represents a raw material interesting in term of its nutritional and health beneficial suitability. Buckwheat grain is a source of valuable proteins, starch with low glycemic index or high amount of unsaturated fatty acids. It contains compounds with prophylactic value, too. Buckwheat is one of the richest sources of polyphenols and flavonoids. These are concentrated mainly in outer layers of buckwheat seed **(Krkošková *and* Mrázová, 2005)**. Among them the most abundant is rutin with its content 0.02% to 2% **(Jiang *et al.*, 2007)**.

Data obtained from laboratory and epidemiological experiments indicate that buckwheat can play an important role in cancer prevention. Buckwheat extracts effectively inhibited the growth of lung, hepatic, colon, breast, gastric or cervical cancer cells **(Chan, 2003; Kim *et al.*, 2007)**. Buckwheat flavonoids were able to induce apoptosis in human leukemic cells HL-60 **(Ren *et al.*, 2003)**. Common buckwheat extracts exhibited antimutagenic action in Ames testing **(Brindzová *et al.*, 2009)**. Among other mechanisms of action antioxidant and antiproliferative properties are important **(Cao *et al.*, 2008; Guo *et al.*, 2010)**.

In our study nine common buckwheat cultivars and one tartary buckwheat cultivar were screened for their anticancer properties. There was determined total polyphenol content, the ability to scavenge free radicals and cytotoxic effect on human cervical cancer cells HeLa. Correlation between polyphenol content and observed beneficial properties was evaluated.

MATERIAL AND METHODS

Nine common buckwheat cultivars and one tartary buckwheat cultivar were kindly provided from Plant production research center in Piešťany. Overview of tested cultivars is outlined in table 1. Buckwheat grains were mechanically dehulled. Obtained hulls were extracted using methanol (p. a.) for 24 hours at room temperature, filtered and used for polyphenol content determination and antioxidant activity testing. For purposes of cytotoxicity evaluation extracts were evaporated and dissolved in dimethylsulfoxide.

Table 1 Overview of tested buckwheat cultivars

Buckwheat cultivar	Buckwheat variety	Crop year
Pyra	Common buckwheat	2011
Špačinská 1	Common buckwheat	2011
Siva	Common buckwheat	2011
Emka	Common buckwheat	2011
Bamby	Common buckwheat	2011
Aiva	Common buckwheat	2011
Madawaska	Tartary buckwheat	2011
KASHO-2	Common buckwheat	2011
JANA C1	Common buckwheat	2011
Hrusowska	Common buckwheat	2011

Total polyphenol content

The content of polyphenols was determined using Folin-Ciocalteau's colorimetric method **(Singleton *and* Rossi, 1965)**. 500 µl of Folin-Ciocalteau's reagent was added to 100 µl of sample. After 3 minutes, 1.5 ml of 20% Na_2CO_3 was pipetted to the reaction mixture and allowed to react for 2 hours at room temperature. The absorbance was measured at 765 nm using UV-visible spectrophotometer (UV-1700, Shimadzu, China). Samples were measured in three replicates. Standard curve of gallic acid was prepared using the similar procedure. Results were expressed in gallic acid equivalents (mg GAE/g dry sample).

Free radical scavenging activity

For determination of the ability to scavenge free radicals there was used simple spectrophotometric method according **Yen *and* Chen (1995)**. In this method 12 mg of 2,2'-diphenyl-1-picrylhydrazyl (DPPH) were dissolved in 100 ml of methanol. 50 µl of sample, 100 µl of methanol and 100 µl of DPPH solution were pipetted into microplate well. The reaction of samples with DPPH solution lasted for 10 min at room temperature in dark. After that, the absorbance changes were measured using microplate reader (Multiskan FC, Thermo Scientific, China). Samples were measured in five replicates. Trolox was used as standard antioxidant control. Results were expressed in trolox equivalents (TE/g dry sample).

Cytotoxic activity (MTT test)

Cytotoxic effect of extracts to cancer cells was detected *in vitro* using mitochondrial cytotoxic test according **Theiszová *et al.* (2005)** with modifications. Cell viability was evaluated using thiazolyl blue tetrazolium bromide (MTT) which measures the metabolic activity of cells. Experiment was performed in 96-well microplates. The cells were seeded at a density of $3.5x10^3$ HeLa cells per well. Samples were diluted in medium to the final concentration of 12.5 µg/ml to 100 µg/ml and after 24 h added to cells. Microplates were cultivated for 72 hours in thermostat at 37 °C and 5% CO_2. After incubation 30 µl of thiazolyl blue tetrazolium bromide (3.33 mg/ml phosphate buffered saline, pH = 7.4) was pipetted to each well and left to incubate for another two hours. After that, medium with MTT solution was removed. Formazan crystals in viable cells were dissolved in 150 µl of lysis solution (4 mM HCl and 0.1% Nonidet P40 in ethanol). Microplates were shaken 15 min at 1500 rpm. Absorbance was measured at 540 nm and reference wavelength 740 nm. Samples were measured in six replicates. Inhibition activity was expressed as percentages of control with DMSO.

RESULTS AND DISCUSSION

Total polyphenol content and antioxidant activity determination

Buckwheat is nutritionally valuable food source. Additional value lies in the presence of many prophylactic components. Phytosterols, group B vitamins or minerals have a part in managing of many diseases **(Danihelová *and* Šturdík, 2012)**. Among them the most abundant are polyphenols, especially the group of flavonoids. The main and content-dominant flavonoid is rutin. Except of rutin there are present other flavonoids (quercetin, kaempferol, luteolin, catechin,…) and phenolic acids (caffeic acid, syringic acid, chlorogenic acid,…) **(Alvarez-Jubete *et al.*, 2010)**.

One of the best described properties of polyphenols is their ability to act as antioxidants. Beneficial effects for organisms are attributed to inhibition of lipid peroxidation, binding metals, reactive oxygen and nitrogen species or free radicals. Through their positive action they can lower oxidative damage of DNA, proteins, lipids and other important biomolecules **(Rice-Evans *et al.*, 1997)**.

Because present antioxidant polyphenols are mainly concentrated in outer layers of buckwheat seed and buckwheat hulls represent waste material that has no important commercial utilization, buckwheat hulls from ten cultivars were used for their screening of beneficial compounds and activities. In prepared methanolic hull extracts there was assessed total polyphenol content using simple spectrophotometric assay. Results are expressed in mg GAE/100 g of dry sample. Along with this the antioxidant capacity was evaluated through monitoring the ability of samples to scavenge free radical DPPH. Inhibition activities were compared to standard antioxidant trolox. Obtained data are presented in table 2.

Table 2 Total polyphenol content in ten buckwheat cultivars as determined using Folin-Ciocalteau's reagent and their antioxidant activity assessed via binding free radical DPPH

Buckwheat cultivar	TP (mg GAE/100 g DW)	DPPH (µM TE/ g DW)
Pyra	168.75 ± 8.38	20.24 ± 0.67
Špačinská 1	217.39 ± 10.09	25.34 ± 0.91
Siva	187.64 ± 2.45	19.32 ± 0.28
Emka	193.69 ± 9.03	22.94 ± 1.11
Bamby	406.03 ± 18.64	471.35 ± 16.12
Aiva	177.47 ± 8.28	20.69 ± 0.44
Madawaska	635.31 ± 23.13	1038.67 ± 4.23
KASHO-2	361.39 ± 18.26	452.38 ± 5.79
JANA C1	166.67 ± 4.36	21.08 ± 0.75
Hrusowska	177.44 ± 8.78	21.22 ± 0.78

Detected polyphenol content in buckwheat hull samples was in the range from 166.67 to 635.31 mg GAE/100 g of dry hulls weight. The highest content of polyphenols was found in the only tested cultivar of tartary buckwheat *Madawaska* (about 0.64% of hulls weight). Among common buckwheat the richest in polyphenols were cultivars *Bamby* (0.41%) and *KASHO-2* (0.36%). Most of common buckwheat samples achieved polyphenol content values about 0.2% of hulls weight, what represents one third of polyphenols present in examined tartary buckwheat sample.

Our findings are in accordance with informations from the literature. **Sharma *et al.* (2012)** published that tartary buckwheat samples expressed higher total phenolic content compared to the common buckwheat. Tartary buckwheat groats have been shown to contain two times higher amount of total phenolics than common buckwheat **(Cao *et al.*, 2008)**. Differences in polyphenol content between buckwheat varieties can be much higher because of varietal and environmental influences.

Buckwheat samples with high content of polyphenols achieved the highest ability to scavenge free radicals. We have found a good correlation between polyphenol content and

antioxidant activity in tested buckwheat cultivars (R^2 = 0.9889). Although tartary buckwheat was three times higher in phenolics than common buckwheat, its antioxidant activity as determined by binding free radical DPPH exhibited even fifty times better value. The best antioxidant effect in common buckwheat was determined for cultivars *Bamby* and *KASHO-2*. Their activity was about twenty-five times higher in comparison with other common buckwheat cultivars.

Positive relationship between the present polyphenolic compounds and their antioxidant activity has been described by many authors **(Zieliński *and* Kozłowska, 2000; Gorinstein et al., 2007; Kishore *et al.*, 2010)**. Rutin appears to be the major antioxidant in buckwheat **(Morishita *et al.*, 2007)**. Tartary buckwheat cultivars display several times higher antioxidant activity than the common one **(Morishita *et al.*, 2007; Cao *et al.*, 2008)**.

Cytotoxicity evaluation

Performed studies indicate that buckwheat possess anticancer properties. However this literature is quite scarce. Available informations document that common and tartary buckwheat extracts induce apoptosis **(Ren *et al.*, 2003)** and have antimutagenic action **(Brindzová *et al.*, 2009)**. They are effective in growth inhibition of various cancer cells **(Chan, 2003; Zheng *et al.*, 2012)**.

Ten buckwheat cultivars were tested for their ability to inhibit growth of human cervical cancer cell line HeLa. Cell viability was detected *in vitro* using mitochondrial cytotoxic test. Viable cells convert yellow tetrazolium salt to purple formazan. Colour changes are measured spectrophotometrically. Samples were tested at the concentration of 100 µg extract/ml. Results are shown in figure 1.

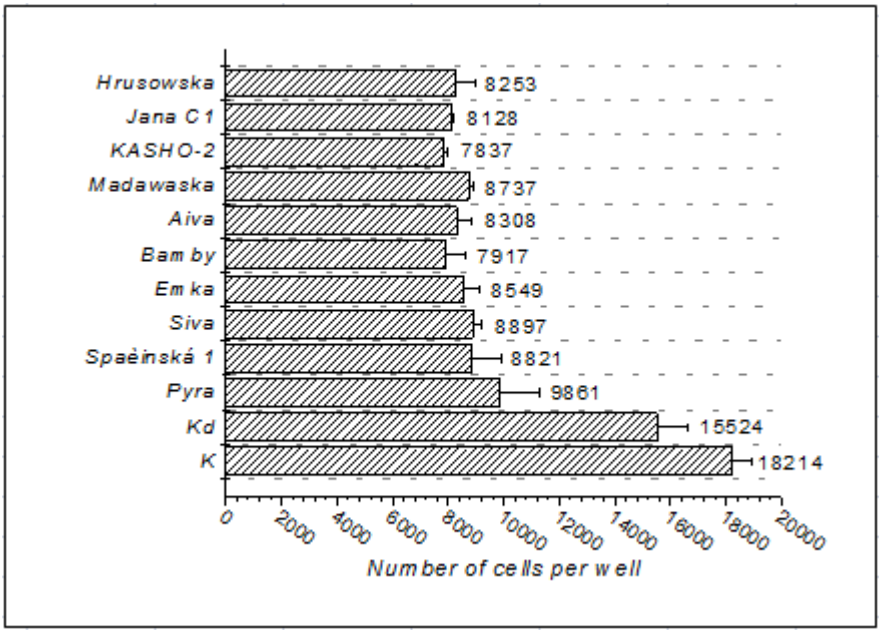

Figure 1 Antiproliferative activity of ten buckwheat cultivars on HeLa cancer cell line as measured by MTT test (100 µg extract/ml, 72 h)

Obtained results indicate that buckwheat hull extracts at the concentration of 100 µg/ml are able to inhibit the growth of human cervical cancer cells. After 72 hours of exposure there were observed inhibitory activities in the range from 36.5% to 49.5%. Majority of samples displayed similar inhibitory effect. The best results have reached common buckwheat cultivars *Bamby* and *KASHO-2*. Among common buckwheat these two have shown to contain the highest polyphenol content. In contrary tartary buckwheat cultivar *Madawaska* containing from tested samples the most polyphenols didn't exhibit high antiproliferative effect.

Data from the literature describe for buckwheat extracts potent cytotoxic effect against various cancer cells. **Kim *et al.* (2007)** published that buckwheat hulls effectively inhibited the growth of breast, hepatic, lung, gastric and cervical cancer cells. They observed inhibitory action up to 93%, but they used higher extract concentrations (250 µg/ml to 1000 µg/ml). Tartary buckwheat flavonoids induced apoptosis in human leukemic cells **(Ren *et al.*, 2003)**. Tartary buckwheat was active against skin cancer cells **(Park *and* Park, 2004)**. Authors observed slight cytotoxic activity.

CONCLUSION

Buckwheat material provides nutritional and health benefits. Many prophylactic compounds such as polyphenols and flavonoids are concentrated in outer layers of buckwheat

grain. In our study there were screened 9 common buckwheat cultivars and 1 tartary buckwheat cultivar. Tartary buckwheat hulls were more abundant in polyphenols than common buckwheat. Cultivars rich in polyphenols displayed higher antioxidant activity. The highest cytotoxic action on human cervical cancer cell line HeLa have shown hulls of common buckwheat cultivars *Bamby* and *KASHO-2*. Obtained results indicate that buckwheat has potential in prevention of cancer diseases.

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