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Biological activities of polyphenols from *Polygonum lapathifolium*
[Actividades biológicas de polifenoles de *Polygonum lapathifolium*]

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Abstract: *Polygonum lapathifolium* is an invasive plant spread worldwide, which has been used in traditional medicine for its biological activities. We studied chemical profile of the aerial part using HPLC/DAD/ESI-MS. Eight flavonoids, two chalcones and gallic acid were isolated, identified nad tested for their activity in seven experimental models (for antioxidant, α/β-glucosidase inhibitory, antimicrobial, anticholineserase activity). Our results showed that *P. lapathifolium* is a valuable source of compounds with perspective biological activities.

Keywords: *Polygonum lapathifolium*; antioxidant activity; enzyme inhibitory activity; antimicrobial activity; flavonoids; chalcones.

Resumen: *Polygonum lapathifolium* es una planta invasora cuya presencia se extiende por todo el mundo y que ha sido utilizada en la medicina tradicional por sus actividades biológicas. Analizamos el perfil químico de la parte superficial utilizando HPLC/DAD/ESI-MS. Ocho flavonoides, dos chalconas y ácido gálico fueron aislados, identificados y analizados en relación a sus actividades en siete modelos experimentales (de actividad antioxidante, α/β-glucosidasa inhibitoria, antimicrobiana, anticholineserasa). Nuestros resultados demuestran que *P. lapathifolium* es una valiosa fuente de compuestos con actividades biológicas prometedoras.

Palabras clave: *Polygonum lapathifolium*; actividad antioxidante; actividad inhibidora de la enzima; actividad antimicrobiana; flavonoides; chalconas

INTRODUCTION

Polygonum lapathifolium L. (syn. *Persicaria lapathifolia* (L.) S. F. Gray, Polygonaceae) is widely distributed from the tropics in the Northern hemisphere to the temperate zone and grows wildly on green fields, along rivers and on ditch banks. It has spread also to other parts of the world and is distributed also in Chile, Argentina and Uruguay where it can be found especially in lower altitudes (Ciadella & Cocucci, 1997). *P. lapathifolium* is a species which exhibits a wide range of variation and this leads to discrepancies in it´s classification, where some authors use categorization at the level of species (Timson, 1963) and others at the level of subspecies (Smolarz, 2002).

Aerial parts of *P. lapathifolium* have been used in folk medicine for its anti-inflammatory, antibacterial, antiviral, antifungal and hepatoprotective effects and in the treatment of dysentery. Extracts from *P. lapathifolium* have also anthelmintic, anti-emetic properties (Svetaz *et al.,* 2010; Bulbul *et al.,* 2013). *P. lapathifolium* is very common plant and is widely used as a medicinal plant in the Czech Republic. In our preliminary screening of several medicinal plants most often used in the Czech Republic, *P. lapathifolium* showed significant antioxidant activity. Based on this preliminary results, we aimed to prove some of it´s biological activities.

Contained phenylpropanoid esters of sucrose show significant inhibitory effects on induction of Epstein-Barr virus early antigen by 12-*O*-tetradecanoylphorbol-13-acetate (Takasaki *et al.,* 2001), gallic acid is a strong antioxidant compound and exhibits antiapoptotic potential in normal human lymphocytes (Kim *et al.,* 2008), taxifolin acts as a potential chemopreventive agent through activation of the antioxidant response element, pinostrobin gives intensive apoptotic response in stimulating leukemic cells *in vitro* and is a good candidate for a leukemia chemopreventic agent (Matsuo *et al.,* 2005; Smolarz *et al.,* 2006), chalcones are cytotoxic agents (Li *et al.,* 2008) and flavonoid glycosides are known as antioxidants (Rice-Evans *et al.,* 1996).

In search for plant compounds with chemoprotective activity we isolated 2´,4´-dihydroxy-3´,6´-dimethoxychalcone (**1**) and 5,8-dimethoxy-7-hydroxyflavanone (**2**). This chalcone (**1**) was previously isolated from *P. senegalense* and *P. lapathifolium,* and has molluscidal activity against

Biomphalaria pfeifferi and *B. sudanica* (Maradufu & Ouma, 1978) and antiplasmodial activity (Midiwo *et al.,* 2007). Isolation of different flavanones without substituents on ring B from *Polygonum* genus was published previously (Datta *et al.,* 2002). 5,8-dimethoxy-7-hydroxyflavanone (**2**) was isolated from *P. ferrugineum* (Lopéz *et al.,* 2006) and *P. senegalense* (Midiwo *et al.,* 1992). This is the first report on isolation of this flavanone from *P. lapathifolium.* From methanolic extract of *P. lapathifolium* we isolated also 2´-hydroxy-4´,6´-dimethoxychalcone (**3**), taxifolin (**4**), gallic acid (**5**), kaempferol (**6**), quercitrin (**7**), isoquercitrin (**8**), astragalin (**9**), kaempferol-3-*O*-galactoside (**10**) and hyperoside (**11**). Compounds **4-11** were tested by chemiluminescence methods for antioxidant activity and for enzyme inhibitory activity.

Since specific enzyme reactions are implicated in many diseases, we tested the isolated compounds for enzyme inhibitory activity. Glucosidases can catalyze the cleavage of glycosidic bond in oligosaccharides and thus inhibitors of α/β-glucosidase are promising drugs in the treatment of diabetes, HIV infection, metatastatic cancer etc. Cholinesterase inhibitors can be used in the treatment of Alzeimer´s disease.

2´,4´-dihydroxy-3´,6´-dimethoxychalcone (**1**), 5,8-dimethoxy-7-hydroxyflavanone (**2**) and 2´-hydroxy-4´,6´-dimethoxychalcone (**3**) were tested for antibacterial activity.

MATERIALS AND METHODS
Plant material
The aerial parts of *Polygonum lapathifolium* subsp. *lapathifolium* L. (syn. *Polygonum nodosum* Pers., Polygonaceae) were collected near České Budějovice in August 2008, south Bohemia, Czech Republic and identified by Assoc. Prof. Milan Žemlička, Faculty of Pharmacy, University of Veterinary and Pharmaceutical Sciences Brno, Czech Republic. A voucher specimen (Nº OF01/2007) has been deposited in the Herbarium of the Department of Natural Drugs, Faculty of Pharmacy, Brno.

Material
2,2-diphenyl-1-picrylhydrazyl radical (DPPH), 30% hydrogen peroxide (H_2O_2), Tris buffer, phosphate buffer (KH_2PO_4, Na_2HPO_4), lucigenin, luminol, xanthine oxidase (XO), xanthine, superoxide dismutase (SOD), 2,2'-azobis-(2-amidinopropane)

dihydrochloride (AAPH), Trolox C, quercetin, acarbose, galanthamine, tetracycline, β-galactosidase, trifluoroacetic acid (TFA), 5,5-dithiobis-(2-nitrobenzoic acid) (DTNB), acetylthiocholine iodide (ATCH-I), butyrylthiocholine iodide (BTCH-I), α/β-glucosidase, 4-nitrophenyl α-D-glucopyranoside (α-PNPG), 4-nitrophenyl β-D-glucopyranoside (β-PNPG), acetonitrile (ACN) and HCOOH were purchased from Sigma-Aldrich (CZ). n-buthanol, n-hexane (Hex), CHCl₃, ethylacetate (EtOAc), Na₂CO₃ and DMSO were purchased from Penta (CZ). Silica gel for the column chromatography (silica gel particle size 63 -200 μm) and plates for TLC (DC-Alufolien 60 silica gel F₂₅₄) were purchased from Merck (NJ, USA).

Instrumental

NMR spectra were recorded using a Bruker DRX-400 (400 MHz) (Bruker, USA) in CDCl₃. IR spectra were recorded on FTIR Impact 410 Nicolet (ThermoScientific, USA). For HPLC Agilent technologies 1100 (Agilent Technologies, USA) was used (column Supelcosil ABZ+Plus 150 x 4.6 mm, particle size 3 μm, method of gradient elution - in 0. min 10% of acetonitrile and 90% of 40 mM HCOOH, till 36. min to 100% of acetonitrile, till 40. min 100 acetonitrile, flow-rate 1 mL/min, column temperature 40 °C, injection volume 5 μL) with DAD detector (wavelenghts used: 280, 350 nm) and MS detector (ESI–MS in negative mode). Preparative HPLC was performed on column Supelcosil (250 x 10 mm, particle size 5 μm, with the same gradient elution, flow-rate 5 mL/min, column temperature 40° C, injection volume 100 μL). The system of HPLC-DAD-ELSD was YL9100 (Young Lin Instrument Co., The Republic of Korea) with DAD + ELSD detector Agilent 1200 Series (Agilent Technologies, USA) (column LiChrospher 100 DIOL 250 x 4.1 mm, particle size 5 μm, isocratic elution with mobile phase acetonitrile : water 91:9; flow-rate 2 mL/min, column temperature 40 °C). Antioxidant activity, enzyme inhibitory activities and antibacterial activity were measured on a microplate reader (BioTek Synergy HT, USA).

Extraction and isolation

Air-dried aerial parts of *P. lapathifolium* (1.5 kg) were extracted with MeOH (15 L, 48 h, 25 °C). The MeOH extract was concentrated *in vacuo* to a dark gum, suspended in H₂O and partitioned successively with *n*-hexane, EtOAc and *n*-buthanol using liquid-liquid extractor to give EtOAc (80 g), *n*-buthanol (25

g) and *n*-hexane (Hex; 5,1 g) extracts. EtOAc and *n*-buthanol extracts were column chromatographed on silica gel using elution with CHCl₃/MeOH (9:1 – 1:1), *n*-hexane extract with Hex/CHCl₃ (9:1 – 1:1). Preparative HPLC with gradient elution was used to isolate the constituents from the obtained fractions. The CHCl₃/MeOH (8:2) fraction of EtOAc extract afforded compounds **4** (2 mg), **5** (5 mg), **6** (10 mg), the CHCl₃/MeOH (6:4) fraction of EtOAc extract afforded compounds **7** (6 mg), **8** (5 mg), **9** (15 mg), **10** (2 mg) and the CHCl₃/MeOH (1:1) fraction of *n*-buthanol extract afforded compound **11** (8 mg). Compounds **1** (10 mg), **2** (15 mg) and **3** (60 mg) were obtained from the Hex/CHCl₃ (1:1) fraction of *n*-hexane extract. Compouds **1-3** were identified by spectroscopic methods (UV, IR, ESI-MS, NMR) and compounds **4-9** and **11** were identified by co-HPLC-DAD with standards and by ESI-MS. For identification of compound **10** a new HPLC method in HILIC mode was used (Špačková & Pazourek, 2013), where compound **10** was measured after acid hydrolysis (4M TFA diluted with acetonitrile, 60 min at 90 °C) and enzymatic hydrolysis (galactosidase, pH 7.3, 30 min, 37 °C).

Antioxidant activity

Trolox C was used as a reference compound in these methods and the results were expressed in terms of Trolox equivalent antioxidant capacity (TEAC) (Choi *et al.,* 2007). Area under the curve (AUC) was recorded in each experiment and scavenging effect (%) was calculated according to the formula: [(1 - AUC_{sample}/ $AUC_{control}$)] x 100. The dose-response curve for Trolox was constructed in each experiment and the mean TEAC values of compounds were derived from the Trolox dose-response curve. Activity of compounds was analysed in triplicate with quercetin used as the standard. SOD was also used as positive reference substance for the method of scavenging of superoxide anion radical and the results in this method were expressed as % inhibition of superoxide anion radical.

Scavenging of H₂O₂

The H₂O₂ scavenging activity was measured by monitoring of the H₂O₂ induced oxidation of luminol according to previously described procedure (Guo *et al.,* 2007). The reaction mixture contained (at final concentrations): 1% H₂O₂, 50 mM Tris buffer, pH 7.4 and the tested sample. The reaction was started by injection of 1 mM luminol. The resulting chemiluminescence was monitored for 5 minutes.

Scavenging of hydroxyl radical

The hydroxyl radical scavenging activity was measured by monitoring of the hydroxyl radical induced oxidation of luminol according to previously described procedure with slight modifications (Parejo *et al.,* 2000). Hydroxyl radical was generated by Fenton system. The reaction mixture contained: 0.1 M phosphate buffer (pH 7.4), 1 mM luminol in 0.1 M NaOH and the tested sample. Initiation of reaction was achieved by adding 25 μM $FeSO_4$ and then 0.5 mM H_2O_2 into the mixture. The dynamic curves were recorded for 30 minutes.

Scavenging of peroxyl radical

The luminescence of luminol was induced by thermolysis of AAPH which produces the alkylperoxyl radicals (Ritov *et al.,* 1995). The reaction mixture contained: 0.1 M phosphate buffer (pH 7.4), 0.1 M AAPH and the tested sample. Initiation of reaction was achieved by adding 1 mM luminol in 0.1 M NaOH into the mixture after thermal decomposition of AAPH to carbon-centred radical at 37 °C. The dynamic curves were recorded for 30 minutes.

Scavenging of superoxide anion radical

The superoxide anion radical scavenging activity was measured by monitoring of the superoxide anion radical induced oxidation of lucigenin according to previously described procedure with slight modifications (Yeung *et al.,* 2002). Superoxide radicals were generated by the xanthine-xanthine oxidase system. The reaction mixture contained: 50 mM Tris buffer (pH 7.4), 1 mM lucigenin and the tested sample. Then, xanthine oxidase (0,06 U/well) was added. The reaction was started by the injection of 30 μM xanthine in 0.1 M NaOH. The dynamic curves were recorded for 5 minutes.

Inhibition of α/β-glucosidase

The inhibition of α/β-glucosidase was assayed on a microplate-reader according to the standard method (Fan *et al.,* 2010) with slight modification: 180 μL of 0.1 M phosphate buffer (pH 7.0), 20 μL of enzyme solution (0.2 U/mL α-glucosidase in 0.1 M phosphate buffer) and 10 μL of tested sample were mixed. After an incubation time (15 min, 37 °C) the reaction was initiated by adding 20 μL of substrate solution (2.5 mM α-PNPG in 0.1 M phosphate buffer). After an additional 15 min at 37 °C the reaction was stopped by adding 0.2 M Na_2CO_3 (80 μL). For β-glucosidase inhibition 10 μL of enzyme solution (0.5 U/mL β-

glucosidase in 0.1 M phosphate buffer) and 10 μL of substrate solution (10 mM β-PNPG in 0.1 M phosphate buffer) were used. The amount of released PNP was quantified at 405 nm. One set of mixtures prepared with an equivalent volume of MeOH instead of tested samples was used as a control. Another set of mixtures prepared with an equivalent volume of phosphate buffer instead of enzyme was used as a blank. The inhibitory rates (%) were calculated according to the formula $[1 - (Abs_{sample} - Abs_{blank}) / Abs_{control}] \times 100$. All samples were tested in triplicates. Quercetin and acarbose were used as the positive reference substances.

Inhibition of cholinesterase

The inhibition of acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) was assayed on a microplate-reader according to the Ellman method (Fan *et al.,* 2010) with slight modification: 120 μL of 0.1 M phosphate buffer (pH 7.0), 20 μL of enzyme solution (0.5 U/mL), 20 μL of 10 mM DTNB and 20 μL of tested compound were mixed. After an incubation time (15 min, 25 °C) the reaction was initiated by adding 20 μL of 1.5 mM ATCH-I or BTCH-I in 0.1 M phosphate buffer. After 2 min at 25 °C absorbance at 405 nm was measured. One set of mixtures prepared with an equivalent volume of MeOH instead of tested samples was used as a control. Another set of mixtures prepared with an equivalent volume of phosphate buffer instead of enzyme was used as a blank. The inhibitory rates (%) were calculated according to the formula $[1 - (Abs_{sample} - Abs_{blank}) / Abs_{control}] \times 100$. All samples were tested in triplicates. Quercetin and galanthamine were used as the positive reference substances.

Antimicrobial activity assay

Tested compounds were dissolved in DMSO to a concentration of 128 μg/mL (stock solution) and subjected to an *in vitro* antimicrobial activity assay using the broth microdilution method (Jorgensen *et al.,* 1997; Schwalbe *et al.,* 2007). Nine 2-fold dilutions of each compound were mixed with appropriate Mueller-Hinton broth to final concentrations of 0.25 - 64 μg/mL in 96-well microtiter plates and were further inoculated with 3 μL of suspension of standard bacteria strain of *Klebsiella pneumoniae* ATCC 700603, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 29213 (deposited at Department of Infectious Diseases and Microbiology, VFU Brno) and several MRSA strains at a density of 0.5 McF. Used resistant

strains were defined as MRSA based on their mecA gen. MRSA 287, MRSA 4211, MRSA 6975, MRSA 630 are deposited at National Reference Laboratory for Antibiotics, National Institute of Public Health, MRSA 62059 (clinical isolate) is stored at Department of Infectious Diseases and Microbiology, VFU Brno. After inoculation, plates were incubated for 24 h at 37 °C. The growth of microorganisms was observed using UV-Vis spectrophotometer monitoring absorbance at 600 nm. Minimum inhibitory concentrations (MICs) were established based on the ratio of absorbance of the growth of negative control and absorbance of sample and were calculated as the lowest compound concentrations that resulted in an 80% reduction in growth of microorganism. The solution of DMSO was measured simultaneously as a negative control. Antibiotic tetracycline was used as a positive control.

Statistical analysis
Difference between the controls and compounds were analyzed by Student's *t*-test and referred to as significant at $p < 0.05$.

RESULTS AND DISCUSSION
Isolation and identification of compounds
Compounds **1** (2´,4´-dihydroxy-3´,6´-dimethoxychal-cone) and **3** (2´-hydroxy-4´,6´-dimethoxychalcone) are dominant compounds in crude extracts from *P. lapathifolium* and revealed a strong UV absorption maximum at 345 nm characteristic for chalcones. Fragmentation of **1** and **3** in the mass spectrometer follows fragmentation patterns similar to those described for chalcones (Porter *et al.,* 2008). The chemical structure of isolated compounds was determined by means of spectroscopic methods (^{1}H and ^{13}C NMR, [1]H–[13]C gs-HMQC and ^{1}H–^{13}C gs-HMBC, [1]H–[1]H NOESY, [1]H–[1]H COESY) and by comparison of the spectroscopic data with those in literature (Maradufu & Ouma, 1978; Jhoo *et al.,* 2006). Chalcone **1**, 2´,4´-dihydroxy-3´,6´-dimethoxychal-cone, was isolated earlier from *P. lapathifolium* (Ahmed *et al.,* 1990) and *P. senegalense* (Bratoeff & Pérez-Amador, 1994). Chalcone **3**, 2´-hydroxy-4´,6´-dimethoxychalcone (flavokawain B), was isolated earlier from *P. lapathifolium* (Ahmed *et al.,* 1990) and *P. ferrugineum* (López *et al.,* 2006).

5,8-dimethoxy-7-hydroxyflavanone (**2**) was found in the same fraction from which the chalcone **1** was isolated and this chalcone is the precursor of flavanone **2**. Coumpound **2** was obtained as a pale yellow powder. UV data (with peaks around 280 nm and 335 nm) are consistent with flavanone structures. Small amount of flavanone **2** was isolated by preparative HPLC from MeOH solution of chalcone **1** after isomerization with acid treatment. The UV and mass spectra of the two flavanones obtained from different fractions were identical and we suppose, that flavanone **2** had arisen from chalcone **1** by cyclization (Figure 1). The flavanone structure was determined by comparison of the spectral data with those in literature (Bratoeff & Pérez-Amador, 1994).

Figura 1
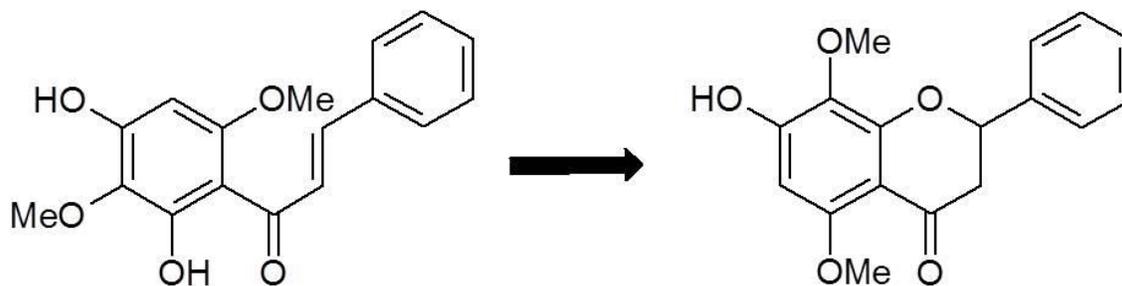
Scheme of the conversion of the chalcone (1) to the flavanone (2)

As reported earlier, flavanones can be formed from related chalcones during drying of plant or during preparation of extracts. Occurrence of similar flavanones (5-hydroxy-7,8-dimethoxyflavanone and 5-hydroxy-6,7-dimethoxyflavanone) was previously described in farinose exudate of the fern *Onychium siliculosum* (Ramakrishnan *et al.,* 1974) but later this was revised and it was assumed that those flavanones

are artefacts and not naturally occurring flavonoids. The workers prepared those two flavanones by heating at 180 °C from pashanon, a constituent of yellow farina on fertile pinnules of *O. siliculosum* (Wollenweber, 1982). According to our experience and previous reports, for cyclization of chalcones to related flavanones the temperature over 100 °C or acid treatment is necessary; under these conditions flavanones and related homoisoflavonoids can arise

from the chalcone precursors (López *et al.,* 2006). We demonstrated by HPLC that the isolated flavanone **2** is present in small amount also in the extract from fresh plant and so we can state that it is a natural flavonoid in *P. lapathifolium*. The quantity of flavanone **2** in the extract is increasing in the course of time; after two years, chalcone **1** almost completely disappeared, whereas flavanone **2** became the dominant compound of the extract.

Table 1
Antioxidant activity of compounds. Data are expressed as the TEAC value = the concentration of standard Trolox solution with equivalent antioxidant potential to a 1 μM concentration of the antioxidant compounds in the present study

Compounds	TEAC (μM)		
	Scavenging of H_2O_2	Scavenging of hydroxyl radical	Scavenging of peroxyl radical
Taxifolin	0.30 ± 0.06	-	1.16 ± 0.01
Gallic acid	0.68 ± 0.01	0.59 ± 0.02	0.47 ± 0.05
Kaempferol	0.90 ± 0.01	0.50 ± 0.06	0.78 ± 0.07
Quercitrin	0.65 ± 0.01	0.46 ± 0.04	1.16 ± 0.01
Isoquercitrin	0.46 ± 0.15	0.47 ± 0.06	1.17 ± 0.01
Astragalin	0.37 ± 0.01	0.15 ± 0.01	0.98 ± 0.05
Kaempferol-3-O-galactoside	0.62 ± 0.01	0.30 ± 0.02	0.86 ± 0.04
Hyperoside	0.54 ± 0.08	0.27 ± 0.02	1.16 ± 0.01
Quercetin	1.48 ± 0.05	0.80 ± 0.02	1.10 ± 0.01

Antioxidant activity
Aerial parts of *P. lapathifolium* contain flavonoids and phenolic acids with strong antioxidant activity. We isolated from the MeOH extract taxifolin (**4**), gallic acid (**5**), kaempferol (**6**), quercitrin (**7**), isoquercitrin (**8**), astragalin (**9**), kaempferol-3-*O*-galactoside (**10**) and hyperoside (**11**). We evaluated radical scavenging capacities of these compounds in four experimental models. The scavenging activities against H_2O_2, superoxide anion radical, peroxyl radical and hydroxyl radical were determined using chemiluminescence methods. The results of TEAC of the scavenging activities against H_2O_2, peroxyl radical and hydroxyl radical are given in Table 1. According to TEAC method, compounds with TEAC values higher than 1.0 indicate higher radical-scavenging effect than Trolox C. Glycosides of quercetin were strong scavengers of peroxyl radicals.

Their scavenging activity against peroxyl radicals was even higher than the activity of the standard Trolox C. Gallic acid and kaempferol were the most potent scavengers of hydroxyl radical and H_2O_2, their free 3-OH group is very important for the scavenging process. Substances with a 2,3-double bond showed stronger activity, taxifolin was weaker than flavon-3-ols. Glycosides of kaempferol had lower activity than glycosides of quercetin; this result supports the importance of the *ortho*-diphenolic arrangement in the B ring (Husain *et al.,* 1987). The scavenging activity of compounds against hydroxyl radical was lower than the activity of the standard Trolox C.

The scavenging mechanism of hydrogen peroxide by phenolic acids is not known but in relevant literature it is stressed that no radical reaction is involved. Cinnamate derivatives are weaker scavengers of hydrogen peroxide than their

benzoate analogues. *p*-hydroxybenzoic acid is not able to act as antioxidant but gallic acid is the most potent antioxidant among phenolic acids (Sroka *et al.,* 2005; Zhao *et al.,* 2008).

Cinnamate derivatives, flavonoids and hydroxylated coumarins scavenge superoxide anion radical (Zhao *et al.,* 2008). Our results indicate that gallic acid can scavenge especially superoxide anion radical; it showed an activity similar to SOD (Table 2). Strong antioxidant activities possess in this experimental model mainly flavonoids and alkaloid boldin (Sichel *et al.,* 1991; O´Brien *et al.,* 2006); 3´,4´-dihydroxyl on the B ring of flavonoids is the key functional group for scavenging of superoxide anion radical.

Table 2
Scavenging of superoxide radicals of compounds in the dose of 5 µM. Values mean ± SD, *n* = 3

Compounds	Scavenging of hydroxyl radical	Inhibition (%)
Taxifolin		51.9 ± 2.8
Gallic acid		70.8 ± 0.3
Kaempferol		33.7 ± 2.1
Quercitrin		59.9 ± 2.7
Isoquercitrin		53.1 ± 5.3
Astragalin		38.4 ± 3.1
Kaempferol-3-O-galactoside	40.8 ± 2.3	
Hyperoside	59.8 ± 3.3	
SOD (18U/ml)	77.2 ± 0.8	
Quercetin	66.2 ± 1.5	

Inhibition of enzymes

In AChE and BChE assay (Table 3) the aglycone kaempferol showed strong inhibitory activity. The isolated flavonoid glycosides are stronger inhibitors of BuChE than of AChE. The effect of gallic acid on AChE (51.2%) is similar to that of the kaemferol (60.4%). The rate of AChE inhibition by gallic acid (**5**) is very high as was demonstrated in previous study (Kulišić-Bilušić *et al.,* 2008). Kaempferol was better inhibitor of BuChE than standard galanthamine.

In the α-glucosidase inhibitory assay (Table 3) flavonoids showed the most potent enzyme inhibitory activity. Compared to the activity of acarbose (11.4%), the isolated flavonoid glycosides showed 8-fold higher activity. There is no difference between the glycosides. The sugar moiety is important for the inhibition, however, inhibitory activity of flavonoids is not dependent on the type of sugar.

All compounds had low β-glucosidase inhibitory activity (Table 3). Flavonoids were stronger inhibitors than phenylpropanoids (Fan *et al.,* 2010); quercetin glycosides (**7**, **8** and **11**) showed β-glucosidase inhibitory activity from 23 to 28%.

Antibacterial activity
Chalcones and flavanones are compounds exerting a broad range of pharmacological activities (Rahman, 2011). They are responsible for the cytotoxicity and antibacterial activity of extracts, in dihydroxy-derivatives also for antioxidant activity (De Cunha *et al.,* 2003; Shindo *et al.,* 2006; Li *et al.,* 2008). An *in vitro* study demostrated that hydroxychalcones and chalcones with methoxyl groups were cytotoxic towards isolated rat hepatocytes which results from the depletion of hepatic GSH. Flavokawain B is cytotoxic to human hepatoma cells *in vitro* and can disrupt the mitochondrial membrane potential of hepatocytes (Jhoo *et al.,* 2006). On the other hand, chalcones are compounds with potent apoptotic activity and might be novel promising compounds for treatment of some types of cancer (Kuo *et al.,* 2010; Tang *et al.,* 2010).

Flavanones and chalcones isolated from Leguminosae plants possess antibacterial activity

against methicillin-resistant *Staphylococcus aureus* (MRSA). The structure-activity relationship indicated that 2′,4′- or 2′,6′-dihydroxylation of the B ring and 5,7-dihydroxylation of the A ring in the flavanone structure are important for significant anti-MRSA

activity and the substitution with an aliphatic group at the 6- or 8-position also enhances the activity (Tsuchiya *et al.,* 1996).

Table 3
Enzyme inhibitory activity of compounds in the dose of 100 µM for cholinesterase activity and 30 µM for α/β-glucosidase activity. Values mean ± SD, *n* = 5.

Compounds	Inhibition (%)			
	AChE	BuChE	α-glucosidase	β-glucosidase
Taxifolin	17.6 ± 0.3	17.9 ± 9.5	89.3 ± 2.8a	20.1 ± 7.4
Gallic acid	51.2 ± 8.9	33.1 ± 0.4	16.6 ± 5.1	2.8 ± 1.1
Kaempferol	60.4 ± 5.2	74.5 ± 7.5a	72.6 ± 2.6a	12.8 ± 1.9
Quercitrin	24.9 ± 3.5	42.7 ± 7.0	85.0 ± 3.6a	23.6 ± 3.0b
Isoquercitrin	20.9 ± 2.0	41.5 ± 2.9	93.6 ± 3.0a	23.4 ± 7.2b
Astragalin	21.7 ± 5.1	45.9 ± 2.6	90.1 ± 0.3a	13.1 ± 3.3
Kaempferol-3-O-galactoside	23.1 ± 9.3	41.0 ± 4.6	87.6 ± 4.5a	10.5 ± 1.0
Hyperoside	27.2 ± 6.3	40.5 ± 5.0	97.2 ± 2.5a	28.8 ± 1.3b
Galanthamine	95.7 ± 3.1 (IC50 1.1 µM)	47.9 ± 4.8 (IC50 168 µM)	-	-
Quercetin	-	-	-	16.7 ± 2.7 (IC50 207 µM)
Acarbose	-	-	11.4 ± 1.1 (IC50 131 µM)	-

[a] p<0.001; [b] p<0.05.

Table 4
Antibacterial activity of isolated compounds (2´,4´-dihydroxy-3´,6´-dimethoxychalcone, 1; 5,8-dimethoxy-7-hydroxyflavanone, 2; 2´-hydroxy-4´,6´-dimethoxychalcone, 3)

Bacteria strain	MIC (µg/ml)			
	1	2	3	Tetracycline
Klebsiela pneumoniae	>64	>64	>64	32
Pseudomonas aeruginosa	>64	>64	>64	>64
Staphylococcus aureus	>64	64	>64	1
MRSA SA 287	>64	>64	>64	16
MRSA SA 4211	>64	>64	>64	0.25
MRSA SA 6975	>64	32	>64	8
MRSA 62059	>64	64	8	16
MRSA SA 630	>64	>64	>64	0.5

In our study the antibacterial activity of isolated methoxylated chalcones and flavanone was tested (Table 4). 2´-hydroxy-4´,6´-dimethoxychal-

cone (**3**) showed antimicrobial activity against methicillin resistant *Staphylococcus aureus* strain (MRSA 62059) higher than the standard tetracycline,

nevertheless, tested chalcones possess low antibacterial activity against bacteria strain of *Klebsiella pneumonie*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and several MRSA.

5,8-dimethoxy-7-hydroxyflavanone (**2**) is stronger antibacterial compound than the isolated chalcones (**1** and **3**). However, this compound lacks hydroxylation on B ring and compounds without this hydroxylation or lipophilic group (isopropyl, dimethylallyl, farnesyl) have not significant activity (Sohn *et al.,* 2001).

CONCLUSIONS
In our research on plants from the genus *Polygonum*, which is widely distributed throughout the world and used in traditional medicine, we investigated *P. lapathifolium* for its constituents and activities. From the aerial parts of *P. lapathifolium* we isolated eleven compounds (chalcones, flavonoids, gallic acid) and proved that the flavone 5,8-dimethoxy-7-hydroxyflavanone is not only artefact arising from its precursor during isolation of 2´,4´-dihydroxy-3´,6´-dimethoxychalcone, but that both compounds are natural constituents of the plant.

In order to support the use of *P. lapathifolium* in the traditional medicine, we tested antioxidant, antibacterial, α/β-glucosidase and choliesterase inhibitory activities of the isolated compounds. From these results, we can point out that polar components of *P. lapathifolium* have notable antioxidant activity and these data support the use of decoction as hepatoprotective and anti-inflammatory drugs. Strong antioxidant activity of flavonoids along with their significant activities against AChE and α-glucosidase indicated that *P. lapathifolium* can be developed into a potent drug to treat diabetes mellitus or/and for memory-enhancing purposes in folk medicine.

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