Antioxidant activity and total phenolic compounds in extracts of selected grasses (*Poaceae*)

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S u m m a r y

The total content of phenolic compounds and DPPH$^{\bullet}$ radical scavenging were determined in the aerial parts of three selected grass species: *Miscanthus sinensis*, *Dichanthium caucasicum* derived from the collection of grasses at the Plant Breeding and Acclimatization Institute (IHAR), Botanical Garden in Bydgoszcz and *Phragmites australis* derived from natural source. In general, the average amount of polyphenols in selected species: 1.1795±0.1608; 1.4108±0.1086; 0.8103±0.0335 (*M. sinensis*; *D. caucasicum*; *P. australis*, respectively) was low. The DPPH$^{\bullet}$ radical scavenging activity (%) in each investigated sample was also relatively low: 27.00±1.98; 21.22±1.14%; 15.89±0.49 (*M. sinensis*; *D. caucasicum*; *P. australis* respectively).

Key words: *Phragmites australis, Miscanthus sinensis, Dichanthium caucasicum, Poaceae, DPPH$^{\bullet}$, total phenolic compounds contents*

INTRODUCTION

Reactive oxygen species (ROS) are generated in physiological processes to produce energy and metabolites or to generate defences against invasive microorganisms [1]. It has been proved that ROS generated in the human body can cause oxidative damage associated with many degenerative diseases such as cardiovascular disease, cancer and neurodegenerative diseases such as Parkinson's and Alzheimer's diseases [2]. It has been recognized that there is an inverse association between consumption of some fruits, whole cereal grains and vegetables and mortality from degenerative diseases. The link between high intake of fruit, grain products and vegetable (plants) and reduced risk of chronic diseases may be related to antioxidant protection. Antioxidants are substances that delay or prevent the oxidation of cellular substrates. They exert their effects by scavenging ROS or preventing their generation [3-5]. Polyphenols as one of the most diverse and widespread group of natural compounds are probably the most important natural antioxidants [3-6]. The great majority of active phenolic compounds isolated from higher plants are flavonoids and phenolics acids [5]. These compounds show a wide spectrum of chemical and biological activities including radical scavenging properties [3, 6].

The grasses – Poaceae (Gramineae) is the most important group of useful plants. Species from this family contain bioactive components including flavonoids (e.g. *C*-glycosides of apigenin, luteolin, tricin), phenolic acids (e.g. ferulic acid, caffeic acid, *p*-hydroxybenzoic acid) and triterpenes, saponins, sterols. Some of the grass species have been proved to show therapeutic effect (e.g. strong antioxidant properties) and have been effective in the treatment of inflammations and sclerosis [6, 7]. The herbal drug *Arundinis radix* (dried cut roots of common reed, *Phragmites australis* Cav. Trin., syn. *P. communis* Trin.) is used, both in traditional and official medicine, as a mild diuretic [8]. The fresh or dried rhizomes of *P. communis* (*Rhizoma Phragmitis*) are used in diabetic complications in the traditional Chinese medicine (TCM) [9]. The herb is also used to treat breast cancer, leukaemia and diabetes in TCM [6]. To date, only traditional uses of *Miscanthus* (*M. sinensis* Andress.; Eulalie) have been reported: the juice is used to *dissolve blood clots* and disperse poisoning [10]. The herb is used to treat fever and diuretic [10]. To the best of our knowledge, the phenolic and antioxidant activity of *Dichanthium caucasicum* has not been investigated yet. The contents of phenolic compounds and their antioxidant activity in selected grass species have been poorly investigated. Therefore, testing their antiradical properties is of interest, primarily in order to find new sources of natural antioxidants. The aim of this study was to investigate the free radical scavenging capacity of methanol extract from aerial parts selected grass species to 1,1-diphenyl-2-picrylhydrazyl radical ($DPPH^{\bullet}$) assay. The total content of polyphenols in aqueous extract from selected plants was determined according to the Folin-Ciocalteau assay.

The thin-layer chromatography (TLC) and paper chromatography (PC) methods were used for preliminary *approximate determination* of polyphenols in the crude methanol extracts from Poaceae species belonging to *Andropogoneae* tribe: *Andropo-*

gon scoparius; *A. gerardi*; *Hyparrhenia hirta*; *Miscanthus sacchariflorus*; *M. sinensis*; *Saccharum ravennae*; *Sorghum halepense, Dichanthium caucasicum* and *P. australis* (*Arundineae* tribe). TLC and PC confirmed that phenolic acids and flavonoid were the principal constituents of the methanol extracts studied (data not shown). Especially rich in polyphenols were the extracts from *P. australis*; *M. sinensis* and *D. caucasicum*.

The contents of phenolic compounds and their antioxidant activities were analyzed in three selected species of grass belonging to the genera, *Phragmites* (*P. australis*); *Miscanthus* (*M. sinensis*) and *Dichanthium* (*D. caucasicum* (Trin.) S.K. Jain & U.R. Deshpande).

MATERIALS AND METHODS

Plant material

The aerial parts of *A. scoparius, A. gerardi, H. hirta, M. sacchariflorus, M. sinensis, S. ravennae, S. halepense,* and *D. caucasicum* at flowering stage were harvested from the collection of grasses at the IHAR Botanical Garden in Bydgoszcz in September and October 2008. The herb of *P. australis* at flowering stage was harvested in July 2008 near Biechowo (kujawsko-pomorskie, Poland). The herbs were dried at room temperature in dark.

Determination of total contents of phenolic compounds

Total contents of phenolic compounds in the three selected grass species (*P. australis*; *M. sinensis* and *D. caucasicum*) extracts were determined by the Folin-Ciocalteau colorimetric method described in Pharmacopoeia Polonica VI [11].

Extraction

Five hundred milligrams of freshly crushed dry plant material were weighed accurately on an analytical balance (WAS 100/X, Radwag, Poland) and extracted with 150 ml distilled water. The extraction solvent was heated in boiling water bath (AJL electronic MLL 147, Poland) for 30 minutes. The resulting aqueous extract was allowed to cool under tap water and was transferred into 250 ml volumetric flasks and diluted with distilled water. The infusion was filtered through filter paper (Whatman No. 1). Five millilitres of extraction solvent were transferred into 25 ml calibrated flasks and diluted with distilled water.

Extract solution (2 ml) was mixed with 1 ml of the Folin-Ciocalteau reagent and 10 ml distilled water and supplemented with sodium carbonate Na_2CO_3 (290 g/L) to the volume of 25 ml. After 30 min of incubation at room temperature, the absorbance at 760 nm was measured in 1 cm cuvettes in triplicate, using a UV/VIS spectrophotometer (U 1800 Hitachi, Japan). All determinations were performed in triplicate. Total content of phenolic compounds (%) was expressed as pyrogallol (PG) equivalents.

Solution of reference substance

Pyrogallol in quantity of 50 mg was dissolved in distilled water and filled up to 100 mL in calibrated flask. 5.0 mL of obtained solution were diluted in another 100 mL calibrated flask. Absorbance of 2.0 mL of pyrogallol solution (with the adequate reagents) was measured as described above. Pyrogalol and Folin–Ciocalteau's reagent were obtained from Sigma-Aldrich Chemie, (Germany).

Results were expressed as the mean ± standard deviation (SD). Total content of phenolic compounds was expressed as equivalent to g PG/g dry weight of plant material.

The total content of phenolic compounds in each plant extract was calculated as follows:

$$X = \frac{62{,}5 \cdot A_1 \cdot m_2}{A_2 \cdot m_1}$$

where
X – total phenolic compounds content expressed as equivalent of pyrogallol [%];
A_1 – absorbance of extract investigated;
A_2– absorbance of pyrogallol solution;
m_1 – mass of sample investigated [g];
m_2 – mass of pyrogallol (g).

Free radical-scavenging activity of plant extracts using DPPH•

Free radical scavenging activity was evaluated with the DPPH• (1,1-diphenyl-2-picrylhydrazyl radical) assay. The antiradical capacity of the sample extracts was estimated according to the procedure reported by Miliauskas, Venskutonis & van Beek [12].

Extraction

The plant sample was ground to powder using a coffee-mill. A precisely weighed amount (2.5 g) of the powder was extracted with 25 ml of methanol (99.5% POCh, Poland) at room temperature for 24 h. The extracts were filtered and evaporated to dryness under reduced pressure at approximately 40°C by a Rotavapor R-200 Büchi (Switzerland).

Extract solutions were prepared by dissolving 25 mg of dry extract in 10 ml of methanol. The solution of DPPH• in methanol (6×10^{-5} M) was freshly prepared in methanol every day and was kept protected from light, before UV measurements. Three ml of DPPH• solution were mixed with 77 μL plant extract. The reaction mixtures were shaken and then kept in the dark for 15 min. The absorbance of the resulting solutions was measured in 1 cm cuvettes at 515 nm, against blank without DPPH•. Decrease in the DPPH•

solution absorbance indicates an increase in the DPPH• radical scavenging activity. This activity is given as % DPPH• radical scavenging calculated from the equation:

$$\%\ \text{Inhibition} = \frac{A_B - A_A}{A_B} \times 100$$

where
A_A – absorbance in the presence of the plant extract in DPPH• solution;
A_B – absorbance of the control solution (containing only DPPH•)

RESULTS AND DISCUSSION

The total contents of phenolic compounds in the three investigated Poaceae species (*P. australis*; *M. sinensis* and *D. caucasicum*) are reported in table 1. As the phenolic substances make one of the major groups of compounds acting as primary antioxidants or free radical scavengers, it was reasonable to determine their total amount in the selected plant extracts. The total content of phenolic compounds in the extracts was determined by the Folin-Ciocalteau method. The total contents of phenolic compounds were calculated as equivalents of pyrogallol. The aerial parts of the species studied contained phenolic compounds in small concentrations. The highest amounts were found in the extracts from *D. caucasicum* (1.410±0.108%), while the other grass species contained lower amounts of phenolic compounds. The lowest average amount of total phenolic compounds was found in *P. australis* (0.810±0.033%). Li, Wong, Cheng & Chen [13] reported that 80% methanolic extract from *P. australis* yielded 5.78±0.07 phenolic content expressed as milligram gallic acid equivalent (mg GAE/g) dry weight of plant material. To date, the total content of phenolic compounds in *M. sinensis* and *D. caucasicum* has not been reported in literature.

Table 1.

Total amount of phenolic compounds in percent

sample number	plant material	total amount of phenolic compounds [% dry weight] in PG eq.
I	*P. australis*	0.8428
II		0.7758
III		0.8124
mean±SD		**0.8103±0.0335**
I	*M. sinensis*	1.2182
II		1.3174
III		1.0028
mean±SD		**1.1795±0.1608**
I	*D. caucasicum*	1.4505
II		1.4940
III		1.2878
mean±SD		**1.4108±0.1086**

The DPPH• radical scavenging activity (%) in each investigated sample are shown in table 2. The results demonstrate that the most active radical scavenger was the extract from *M. sinensis* (27.00±1.98%). The lowest scavenging activity was observed for the extract from *P. australis* (15.89±0.49%). To our knowledge, there was no prior report on the DPPH• radical scavenging activity of these plants. The present study provided valuable preliminary data on the antioxidant activity of phenolic compounds from selected grass species. Isolation and characterization of individual active components awaits further comprehensive studies.

Table 2.

DPPH absorption inhibition (%) of plant extracts

sample series	sample number	plant material	DPPH inhibition (%)
I	1	*P. australis*	16.5949
	2		16.0483
	3		16.0721
	mean±SD		16.2384±0.3089
II	1		15.0147
	2		16.2028
	3		15.4186
	mean±SD		15.5454±0.6041
mean±SD from two series			**15.8919±0.4901**
I	1	*M. sinensis*	22.3691
	2		25.8502
	3		28.5829
	mean±SD		25.6007±3.1144
II	1		28.3096
	2		26.8007
	3		30.1036
	mean±SD		28.4047±1.6535
mean±SD from two series			**27.0027±1.9827**
I	1	*D. caucasicum*	20.1354
	2		20.8839
	3		20.2067
	mean±SD		20.4087±0.4131
II	1		22.0245
	2		22.1314
	3		21.9176
	mean±SD		22.0245±0.1069
mean±SD from two series			**21.2166±1.1425**

CONCLUSIONS

It can be concluded that the aerial parts of all the selected grass species contained phenolic compounds in small concentrations. For complete evaluation of the extracts quality more phytochemical analyses have to be carried out.

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OZNACZANIE AKTYWNOŚCI PRZECIWUTLENIAJĄCEJ ORAZ ZAWARTOŚCI ZWIĄZKÓW POLIFENOLOWYCH W WYCIĄGACH Z WYBRANYCH GATUNKÓW TRAW (*POACEAE*)

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S t r e s z c z e n i e

W częściach nadziemnych wytypowanych gatunków traw pochodzących z kolekcji Ogrodu Botanicznego IHAR w Bydgoszczy: *Miscanthus sinensis* i *Dichanthium caucasicum* oraz pochodzących ze stanowiska naturalnego (*Phragmites australis*) oznaczono ogólną zawartość polifenoli oraz aktywność przeciwutleniającą. Do określenia ogólnej zawartości związków polifenolowych wykorzystano metodę spektrofotometryczną z zastosowaniem odczynnika Folin-Ciocalteau. Najwyższą zawartością polifenoli charakteryzował się wyciąg z *D. caucasicum* (1,41±0,10%). Niższe wartości uzyskano w przypadku wyciągu z ziela *M. sinensis* (1,18±0,16%), a najniższą zawartość związków polifenolowych odnotowano dla wyciągu z *P. australis* (0,81±0,03%). Aktywność przeciwutleniającą metanolowych wyciągów z badanych roślin określono, wykorzystując roztwór rodnika DPPH$^{\bullet}$. Najwyższą aktywnością przeciwutleniającą charakteryzował się wyciąg z *M. sinensis* (27,00±1,98%), nieco niższą wyciąg z *D. caucasicum* (21,22±1,14%), a najniższą zdolność do zmiatania wolnych rodników wykazywał wyciąg z *P. australis* (15,89±0,49%).

Słowa kluczowe: *Phragmites australis, Miscanthus sinensis, Dichanthium caucasicum, Poaceae, DPPH$^{\bullet}$, ogólna zawartość związków fenolowych*