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LEAVES EXTRACTS OF SELECTED CROPS AS POTENTIAL SOURCE OF ANTIOXIDANTS

Objective — to evaluate the antioxidant potential of leaves extracts of four selected forage plants in conditions of M.M. Gryshko National Botanical Garden of the NAS of Ukraine.

Material and methods. In this study used dried raw of *Brassica campestris* L. f. *annua* D.C., cv. Chanita (*Brassicaceae* Burnett), *Desmodium canadensis* D.C. (*Fabaceae* Lindl.), *Rhaponticum carthamoides* (Willd.) Iljin. (*Asteraceae* Bercht. & J. Presl.), *Sinapis alba* L., cv. Karolina (*Brassicaceae* Burnett) harvested from collections of the M.M. Gryshko National Botanical Garden of the NAS of Ukraine. 0.2 g of dried plant raw material was extracted with 20 mL of 80 % ethanol for 2 hours. The total polyphenol content of extracts was measured by the method described by Singleton and Rossi (1965) using Folin—Chicalteu reagent and results were expressed in mg of gallic acid equivalent per one gram of dry matter (mg GAE/g). Determination of total flavonoids content was conducted using the modified method described in Shafii et al. (2017) and results expressed in mg quercetin equivalent per one gram of dry matter (mg QE/g). Detection of total phenolic acids content of extracts was carried out using the method described in *Farmakopea Polska* (1999) and results expressed in mg caffeic acid per one gram of dry matter (mg CAE/g). The antioxidant activity of samples was measured using 2,2-diphenyl-1-picrylhydrazyl (DPPH method) according to Sanchez-Moreno et al. (1998). Also, the antioxidant activity of extracts was determined by the phosphomolybdenum method described by Prieto et al. (1999) with slight modifications. Results of these parameters expressed in mg Trolox equivalent per one gram of dry matter (mg TE/g). Experimental data were evaluated by using Excel 2010.

Results. Total content of polyphenol compounds of investigated leaves extracts was from 32.43 to 73.58 mg GAE/g, total content of phenolic acids — from 4.87 to 9.15 mg CAE/g, total content of flavonoids — from 10.16 to 92.61 mg QE/g. Determination of antioxidant activity by DPPH method demonstrated results from 7.15 to 9.00 mg TE/g and by phosphomolybdenum method — from 77.87 to 190.64 mg TE/g.

Conclusions. Investigation of ethanol leaves extracts of four forage plants identified high content of polyphenol compounds and flavonoids what characterizes these plants as a valuable source of biologically active compounds. Obtained results can be of interest for deep pharmacological investigations.

Key words: *Brassica campestris*, *Desmodium canadensis*, *Rhaponticum carthamoides*, *Sinapis alba*, polyphenols, flavonoids, phenolic acids, antioxidant activity.

Biological activity of plants caused by the presence of biologically active compounds that related to different groups. One of the most actual and demanded directions in this area is identification of antioxidant agents and antioxidant activity in plant raw material. Plants are valuable source of natural antioxidants due to content of different groups of phenolic compounds [33]. The founding of

new natural powerful but nontoxic compounds from plants extracts have been searching [27]. Study of forage plants and their antioxidant activity is of interest of last year's investigations [34]. Among forage plants can be highlighted following crops:

- *Brassica campestris* L. is representative of *Brassicaceae* Burnett family. It is the most widely known group of useful plants that includes many of food and forage crops. Numerous studies resulted in the antioxidant potential of raw due to the content

of polyphenol compounds and investigated their antimicrobial activity [2; 11]. In addition, using of Brassicaceae plants has reduced risk of cancer illness [6; 12];

- *Desmodium canadensis* D.C. belongs to Fabaceae Lindl. family. Plants of this species are a rich source of phytochemicals such as phenolic compounds, saponins, triterpenic steroids, alkaloids, complex biologically active compounds in the volatile oil. Plant raw of different *Desmodium* species demonstrated antileishmanial, immunomodulatory, antioxidant, antiulcer, cardio-protective, antidiabetic, antiviral, anticonvulsant, hepatoprotective activities [1; 3; 9; 10];

- *Rhaponticum carthamoides* (Willd.) Iljin. is a perennial plant belonging to Asteraceae Bercht. & J. Presl., roots and leaves of which accumulated biologically active compounds that used in folk and traditional medicine. Plant extracts demonstrated antioxidant activity and no mutagenic effect [4; 25];

- Extracts of *Sinapis alba* L. (Brassicaceae) demonstrated the high antiproliferative, antioxidant and good antimicrobial activities. This plant contains the glucosinolates (sinalbin) that well accepted by the food industry. Finish compounds obtained during sanalbin conversion by endogenous myrosinase are responsible for mustard flavor [6; 23; 34].

Objective — to evaluate the antioxidant potential of leaves extracts of selected forage plants in conditions of M.M. Gryshko National Botanical Garden of the NAS of Ukraine.

Material and methods

Plant materials

In this work used dried leaves raw of selected crops: *Brassica campestris* L. f. annua D.C., cv. Chanita, *Desmodium canadensis* D.C., *Rhaponticum carthamoides* (Willd.) Iljin., *Sinapis alba* L. from collections of the M.M. Gryshko National Botanical Garden of the NAS of Ukraine.

Chemicals

All chemicals were analytical grade and were purchased from Rechem (Slovakia) and Sigma Aldrich (USA).

Sample preparation

0.2 g of dried plant raw material was extracted with 20 ml of 80 % ethanol for 2 hours. After centrifugation at 4000 g (Rotofix 32 A, Hettich, Germany) for 10 min, the supernatant was used for the next measurements: antioxidant activity, polyphenols, and flavonoids.

Total polyphenol content

Total polyphenol content (TPC) of extracts was measured by the method of Singleton and Rossi (1965) using Folin—Chiocalteu reagent [24]. 0.1 ml of each sample extract was mixed with 0.1 ml of the Folin—Chiocalteu reagent, 1 ml of 20 % (w/v) sodium carbonate and 8.8 ml of distilled water. After 30 min in darkness, the absorbance at 700 nm was measured using the spectrophotometer Jenway (6405 UV/Vis, England). Gallic acid (25—250 mg/L; $R^2=0.996$) was used as the standard and the results were expressed in mg/g gallic acid equivalents.

Total flavonoid content

Determination of total flavonoids content (TFC) was conducted using the modified method described in Shafii et al. (2017) [21]. 0.5 ml of sample extract was mixed with 0.1 ml of 10 % (w/v) ethanolic solution of aluminum chloride, 0.1 ml of 1 M sodium acetate and 4.3 ml of distilled water. After 30 min in darkness the absorbance at 415 nm was measured using the spectrophotometer Jenway (6405 UV/Vis, England). Quercetin (0.01—0.5 mg/L; $R^2=0.997$) was used as the standard and the results were expressed in mg/g quercetin equivalents.

Total phenolic acid content

Determination total phenolic acids content (TPA) of extracts was carried out using the method described in Farmakopea Polska (1999) [8]. 0.5 ml of sample extract was mixed with 0.5 ml of 0.5 M hydrochloric acid, 0.5 ml Arnova reagent, 0.5 ml of 1 M sodium hydroxide (w/v) and 0.5 ml of distilled water. Absorbance at 490 nm was measured using the spectrophotometer Jenway (6405 UV/Vis, England). Caffeic acid (1—200 mg/L, $R^2=0.999$) was used as a standard and the results were expressed in mg/g caffeic acid equivalents.

Antioxidant activity by DPPH method

The radical scavenging activity of samples was measured using 2,2-diphenyl-1-picrylhydrazyl (DPPH) according to Sanchez-Moreno et al., 1998 [20]. The extracts (0.5 ml) were mixed with 3.6 ml of radical solution (0.025 g of DPPH in 100 ml ethanol). The absorbance of the sample extract was determined using the spectrophotometer Jenway (6405 UV/Vis, England) at 515 nm. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) (10–100 mg/L; $R^2=0.988$) was used as the standard and the results were expressed in mg/g Trolox equivalents.

Antioxidant activity by phosphomolybdenum method

Antioxidant activity of extracts was determined by the phosphomolybdenum method of Prieto et al. (1999) with slight modifications [16]. The mixture of sample (1 ml), monopotassium phosphate (2.8 ml, 0.1 M), sulfuric acid (6 ml, 1 M), ammonium heptamolybdate (0.4 ml, 0.1 M) and distilled water (0.8 ml) incubated at 90 °C for 120 min, then rapidly cooled and detected by monitoring ab-

sorbance at 700 nm using the spectrophotometer Jenway (6405 UV/Vis, England). Trolox (10–1000 mg/L; $R^2=0.998$) was used as the standard and the results were expressed in mg/g Trolox equivalents.

Experimental data were evaluated by using Excel 2010. Data were analyzed with ANOVA test and differences between means were compared through the Tukey-Kramer test ($\alpha = 0.05$); significance level at $p < 0.05$. Mean values of three replicates and standard deviation are given in Tables 1, 2.

Results and discussions

Last time study of antioxidant potential of plant raw due to the content of polyphenol compounds is a very discussed branch of modern biology [13]. In M.M. Gryshko National Botanical Garden has carried out complex investigations including the biochemical study of different groups of plants, among which forage, medicinal, energetic, aromatic, wild, etc. [17; 18; 28–30; 32]. Our previous data showed that plants from different families characterized by different values of DPPH scav-

Table 1. The total content of polyphenol compounds, phenolic acids, and flavonoids in leaves extracts of selected crops

Samples	Polyphenol compounds, mg GAE/g (DW)	Phenolic acids, mg CAE/g (DW)	Flavonoids, mg QE/g (DW)
<i>Brassica campestris</i> , cv. Chanita	41.69 ± 0.62 ^b	4.87 ± 0.27 ^c	28.37 ± 1.10 ^b
<i>Desmodium canadensis</i>	71.43 ± 1.50 ^a	8.70 ± 0.07 ^{ab}	61.05 ± 1.80 ^a
<i>Rhaponticum carthamoides</i>	32.43 ± 0.28 ^c	6.75 ± 0.50 ^c	10.16 ± 0.19 ^c
<i>Sinapis alba</i>	73.58 ± 5.83 ^a	9.15 ± 0.19 ^a	62.91 ± 0.44 ^a

Note: GAE — gallic acid equivalent; CAE — caffeic acid equivalent; QE — quercetin equivalent; DW — dry weight; means in columns followed by different letters are different at $p = 0.05$.

Table 2. Antioxidant activity of leaves extracts of selected crops

Samples	AA by phosphomolybdenum method, mg TE/g (DW)	AA by DPPH-method, mg TE/g (DW)
<i>Brassica campestris</i> , cv. Chanita	91.94 ± 3.12 ^b	8.17 ± 0.06 ^a
<i>Desmodium canadensis</i>	190.64 ± 4.03 ^a	8.29 ± 0.16 ^a
<i>Rhaponticum carthamoides</i>	77.87 ± 6.43 ^c	9.00 ± 0.05 ^a
<i>Sinapis alba</i>	148.43 ± 2.03 ^{ab}	7.15 ± 0.10 ^{ab}

Note: AA — antioxidant activity; TE — Trolox equivalent; DW — dry weight; means in columns followed by different letters are different at $p = 0.05$.

enging activity depending on raw (leaves, stems, flowers, inflorescences, seeds, roots, etc.) [31]. Leaves, as well as flowers, had the most antioxidant capacity among other organs of plants as showed some studies [22].

Phenolic compounds it's a very large group of phytochemicals that correlated often with antioxidant activity of plant extracts [7]. As demonstrated in Table 1, the content of polyphenol compounds was of 32.43–73.58 mg GAE/g. According to Ayoola et al. (2018), the content of polyphenols in stems and leaves of *Desmodium abscondens* was 10 and 24 mg GAE/g, respectively [1]. Study of another species *D. gyrans* showed that phenolic compounds determined in the acetone, methanol and water extracts whereas in petroleum ether, toluene, chloroform, ethyl acetate extracts didn't determine it [33].

In this study, the highest content of polyphenol compounds found for *Sinapis alba* ethanol extracts. Investigations of Thangi et al. (2016) resulted that seed extracts of *S. alba* demonstrated content of polyphenols of 8 mg GAE/g [26]. Content of polyphenols in *Rhaponticum carthamoides* extracts obtained by Miliuskas et al. (2004) was 2.4 times less (13.3 mg GAE/g) comparing with our results. Content of flavonoids in this study was 10.16 mg Rutin equivalent/g [15].

Content of phenolic acids of investigated plants determined from 4.87 to 9.15 mg CAE/g. Phenolic acids are widespread phenolic compounds in plant organism that perform different important functions (as defense molecules, chemo-attractants, concentration-dependent root growth inhibition, a stimulator of indole acetic acid, alternative source of carbon for some diazotrophs, etc.) depending on plant species [14].

Also, we found that the content of flavonoids was from 28.37 to 62.91 mg QE/g among investigated leaves extracts.

We determined the antioxidant capacity of ethanol leaves extracts by both phosphomolybdenum and DPPH methods (Table 2). We found that antioxidant activity by phosphomolybdenum method was from 77.87 to 190.64 mg TE/g and by DPPH method from — 7.15 to 9.00 mg TE/g of DW.

Antioxidant activity determination by different methods in extracts of *Rh. carthamoides*, as reported Biskup and Lojkowska (2009), using of phosphomolybdenum method identified more activity than others but the most interesting were chloroform extracts [4]. Also, the antioxidant activity of investigated extracts depended on plants origin [5].

Thangi et al. (2016) found a positive correlation between the content of phenolic compounds and antioxidant activity in *Sinapis alba* extracts [26]. In addition, leaves of representatives of Brassicaceae characterized not antioxidant potential only but have anticancer activity also [19].

Conclusions

Summarizing obtained data, it should be noticed that leaves extracts of forage plants *Brassica campestris* L. f. annua D.C., cv. Chanita, *Desmodium canadensis* D.C., *Rhaponticum carthamoides* (Willd.) Iljin., *Sinapis alba* L. from the M.M. Gryshko National Botanical Garden of the NAS of Ukraine had high values of antioxidant activity by two different methods. The highest content of phenolic compounds had extracts of plants *D. canadensis* and *S. alba*. Evidently, that leaves of investigated plants can be a valuable source of antioxidants and obtained data can be used in further pharmacological investigations.

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ЕКСТРАКТИ ЛИСТКІВ ДЕЯКИХ КУЛЬТУРНИХ РОСЛИН ЯК ПОТЕНЦІЙНЕ ДЖЕРЕЛО АНТИОКСИДАНТІВ

Мета — оцінити антиоксидантний потенціал листових екстрактів чотирьох кормових рослин в умовах Національного ботанічного саду імені М.М. Гришка НАН України.

Матеріал та методи. Використовували суху рослинну сировину *Brassica campestris* L. f. annua D.C., cv. Chanita (Brassicaceae Burnett), *Desmodium canadensis* D.C. (Fabaceae Lindl.), *Rhaponiticum carthamoides* (Willd.) Iljin. (Asteraceae Bercht. & J. Presl.), *Sinapis alba* L., cv. Karolina (Brassicaceae Burnett), зібрану в колекціях Національного ботанічного саду імені М.М. Гришка НАН України. 0,2 г сухої рослинної сировини екстрагували в 20 мл 80 % етанолу протягом 2 год. Загальний вміст поліфенолів в екстрактах визначали методом, описаним у Singleton та Rossi (1965), використовуючи реагент Фоліна—Чокалтеу. Результати представлено в міліграмах на 1 г еквівалента галової кислоти (мг ГКЕ/г). Визначення вмісту флавоноїдів проводили методом, описаним у Shafii et al. (2017). Результати представлено в міліграмах на 1 г кверцетин-еквівалента сухої маси (мг КЕ/г). Визначення загального вмісту фенольних кислот проводили за методом, описаним у Farmakorea Polska (1999). Результати представлено у міліграмах еквівалента кофейної кислоти на 1 г сухої маси (мг ККЕ /г). Антиоксидантну активність зразків вимірювали з 2,2-дифеніл-1-пікрилгідразилом (ДФПГ-метод) згідно із Sanchez-Moreno et al. (1998). Також визначали антиоксидантну активність екстрактів фосфомолібденовим методом, описаним у Prieto et al. (1999) з незначною модифікацією. Результати цих досліджень представлено в міліграмах тролокс-еквівалента на 1 г сухої речовини (мг ТЕ/г). Експериментальні дані опрацьовано в Excel 2010.

Результати. Загальний вміст поліфенольних речовин досліджуваних листових екстрактів становив від 32,43 до 73,58 мг ГКЕ/г, загальний вміст фенольних кислот — від 4,87 до 9,15 мг ККЕ/г, флавоноїдів — від 10,16 до 92,61 мг КЕ/г. Антиоксидантна активність, визначена ДФПГ-методом, становила від 7,15 до 9,00 мг ТЕ/г, а визначена фосфомолібденовим методом — від 77,87 до 190,64 мг ТЕ/г.

Висновки. Дослідження етанольних екстрактів листків чотирьох кормових рослин показало високий вміст поліфенольних речовин та флавоноїдів, що характеризує ці рослини як цінне джерело біологічно активних речовин. Отримані результати можуть становити інтерес для поглиблених фармакологічних досліджень.

Ключові слова: *Brassica campestris*, *Desmodium canadensis*, *Rhaponticum carthamoides*, *Sinapis alba*, поліфеноли, флавоноїди, фенольні кислоти, антиоксидантна активність.

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ЭКСТРАКТЫ ЛИСТЬЕВ НЕКОТОРЫХ КУЛЬТУРНЫХ РАСТЕНИЙ КАК ПОТЕНЦИАЛЬНЫЙ ИСТОЧНИК АНТИОКСИДАНТОВ

Цель — оценить антиоксидантный потенциал листовых экстрактов четырех кормовых растений в условиях Национального ботанического сада имени Н.Н. Гришко НАН Украины.

Материал и методы. Использовали сухое растительное сырье *Brassica campestris* L. f. annua D.C., cv. Chanita (Brassicaceae Burnett), *Desmodium canadensis* D.C. (Fabaceae Lindl.), *Rhaponticum carthamoides* (Willd.) Pjin. (Asteraceae Bercht. & J. Presl.), *Sinapis alba* L., cv. Karolina (Brassicaceae Burnett), собранное в коллекциях Национального ботанического сада имени Н.Н. Гришко НАН Украины. 0,2 г сухого растительного сырья экстрагировали в 20 мл 80 % этанола в течение 2 ч. Общее содержание полифенолов в экстрактах определяли методом, описанным у Singleton и Rossi (1965), с реа-

гентом Фолина—Чокалтеу. Результаты представлены в миллиграммах на 1 г эквивалента галловой кислоты (мг ГКЭ/г). Определение содержания флавоноидов проводили методом, описанным у Shafii et al. (2017). Результаты представлены в миллиграммах на 1 г кверцетин-эквивалента сухой массы (мг КЭ/г). Определение общего содержания фенольных кислот проводили по методу, описанному в Farmakopea Polska (1999). Результаты представлены в миллиграммах эквивалента кофейной кислоты на 1 г сухой массы (мг ККЭ/г). Антиоксидантную активность образцов измеряли с 2,2-дифенил-1-пикрилгидразилом (ДФПГ-метод) по Sanchez-Moreno et al. (1998). Также определяли антиоксидантную активность экстрактов фосфолибденовым методом, описанным Prieto et al. (1999) с незначительной модификацией. Результаты этих исследований представлены в миллиграммах тролокс-эквивалента на 1 г сухого вещества (мг ТЭ/г). Экспериментальные данные обработаны в Excel 2010.

Результаты. Общее содержание полифенольных веществ исследованных листовых экстрактов составляло от 32,43 до 73,58 мг ГКЭ/г, общее содержание фенольных кислот — от 4,87 до 9,15 мг ККЭ/г, общее содержание флавоноидов — от 10,16 до 92,61 мг КЭ/г. Антиоксидантная активность, определенная ДФПГ-методом, составляла от 7,15 до 9,00 мг ТЭ/г, а определенная фосфолибденовым методом — от 77,87 до 190,64 мг ТЭ/г.

Выводы. Исследования этанольных экстрактов листьев четырех кормовых растений показало высокое содержание полифенольных веществ и флавоноидов, что характеризует данные растения как ценный источник биологически активных веществ. Полученные результаты могут представлять интерес для углубленных фармакологических исследований.

Ключевые слова: *Brassica campestris*, *Desmodium canadensis*, *Rhaponticum carthamoides*, *Sinapis alba*, полифенолы, флавоноиды, фенольные кислоты, антиоксидантная активность.