doi: 10.5281/zenodo.3241204
UDC 581.48:581.192

O.M. VERGUN, D.B. RAKHMETOV, O.V. SHYMANSKA, S.O. RAKHMETOVA, V.V. FISHCHENKO
M.M. Gryshko National Botanical Garden, National Academy of Sciences of Ukraine
Ukraine, 01014 Kyiv, Timiryazevska str., 1

ANTIOXIDANT ACTIVITY OF SEED EXTRACTS OF SELECTED FORAGE PLANTS

Objective *— to investigate the antioxidant activity of seed extracts of selected forage plants in conditions of M.M. Gryshko National Botanical Garden of the NAS of Ukraine.*

Material and methods. *Seeds of seven forage plants researched in this study: Baptisia australis (L.) R.Br. (Fabaceae), Bunias erucago L., B. orientalis L. (Brassicaceae), Galega officinalis L., G. orientalis Lam. (Fabaceae), Isatis littoralis Steven, I. tinctoria L. (Brassicaceae). The method described by Brand-Williams et al. (1995) used to determine the antiradical activity of plant extracts. Biochemical analyze included following stages: preparation of methanol, ethanol and water extracts (1 g of dried material mixed with 25 ml of solvent); 12 hours of extraction; spectrophotometric procedure of determination of antiradical activity with a 2,2-diphenyl-2-picrylhydrazyl solution. A solution of radical prepared in methanol and diluted according to method. Data were expressed in ascorbic acid equivalent (AAE) and Trolox equivalent (TE) on a gram of dry weight. Measurements of extracts carried out on spectrophotometer UNICO UV 2800 at 515 nm. Experimental data processed by Excel.*

Results. *Methanol extracts of investigated plants had an antiradical activity of 18.27—80.57 %, ethanol extracts of 11.07—79.73 % and water extracts of 23.31—80.26 %. Antioxidant activity of methanol extracts expressed on ascorbic acid equivalent was 1.75—2.72 mg AAE per gram, ethanol extracts 1.62—2.72 mg AAE per gram and water extracts 1.82—2.66 mg AAE per gram. Results exhibited antioxidant activity expressed on Trolox equivalent of methanol extracts from 1.30 to 7.51 mg TE per gram, ethanol extracts from 0.56 to 7.76 mg TE per gram and water extracts from 1.19 to 7.37 mg TE per gram.*

Conclusions. *Obtained data demonstrated that in conditions of M.M. Gryshko National Botanical Garden of the NAS of Ukraine seed extracts of investigated forage plants have high antioxidant potential as well as leaves or shoots. Antiradical activity of extracts was of 11.07—84.60 %. All seed extracts had the least values of this parameter in the ethanolic extracts. Antioxidant activity expressed in ascorbic acid equivalent was maximal for Bunias orientalis and Galega officinalis and minimal for Isatis tinctoria. Antioxidant activity by Trolox equivalent was high for Bunias orientalis methanol extracts and slow for Bunias erucago ethanol extracts. Investigation of seeds extracts of crops from different plant families is need to evaluate antioxidant potential of it and recommend for use.*

Key words: seeds, alcoholic and water extracts, antiradical activity, antioxidant activity.

The growing demand for natural antioxidants exists in food and cosmetic industries and requires the new sources of these compounds. Antioxidant activity is an important and widely studied parameter of plant raw material investigation. Last time there are numerous reports about the accumulation of different antioxidants in different parts of plant organism [6]. Plant antioxidants are known as agents that reduce the risk of many chronic diseases and play an important role in plants [14]. According to Kumar et al. (2017), antioxidants include two main types such as antioxidants based on solubility (ascorbic acid, glutathione, lipoic acid, uric acid etc.) and based on the occurrence (vitamin A, vitamin C, vitamin E, beta-carotene etc.) [14]. Investigation of seed antioxidant capacity actual concerning to active oxygen species, which, as reported Bailly (2004), may play a role in desiccation-related damage, particularly in dehydration-intolerant recalcitrant seeds [2].

During seed sprouting a multitude of biochemical processes takes place, which caused radical changes in biochemical composition. It can lead to a change in phenolic compounds profile and antioxidant activity [5]. Some results showed that germinated seeds increased the polyphenol content and relating to this antioxidant activity [13;

19]. But the study of some Fabaceae seeds demonstrated that total phenolic compounds decreased during sprouting. Thus, accumulation antioxidants and linear relationship between polyphenol compounds and antioxidant activity of seeds depends on plant species and, also, conditions of germination [9; 16].

Investigation of forage plants still is an actual branch of modern biology because of their use in agriculture and, also, these plants are an important source of biologically active compounds. In addition, these plants can be used as medicinal, plant raw material of which exhibit different biological activities such as microbiological, antioxidant, anticancer effect [8; 11; 12; 17].

Objective — to investigate the antioxidant activity of seed extracts of selected forage plants in conditions of M.M. Gryshko National Botanical Garden of the NAS of Ukraine.

Material and methods

Seeds were collected from the experimental collection of Department of Cultural Flora in M.M. Gryshko National Botanical Garden of the NAS of Ukraine (NBG) at the stage of full seed ripening. In this study following species from a collection of forage plants were tested: *Baptisia australis* (L.) R.Br. (Fabaceae), *Bunias erucago* L., *B. orientalis* L. (Brassicaceae), *Galega officinalis* L., *G. orientalis* Lam. (Fabaceae), *Isatis littoralis* Steven, *I. tinctoria* L. (Brassicaceae).

Biochemical analyses were conducted in the laboratory of Department Cultural Flora of NBG. Determination of the antiradical activity of extracts detected according to Brand-Williams [4].

Extracts preparation

For extraction, 1 g of dried and milled seeds were mixed with 25 ml of solvent (methanol, ethanol, and water). The procedure of extraction continued for 12 hours at the constant shaking. After this conducted filtration of obtained mixtures.

DPPH radical scavenging activity

Obtained extracts were analyzed on antioxidant capacity with DPPH-radical (2,2-diphenyl-2-picrylhydrazyl). A radical solution prepared by the following procedure: 0.025 g of radical mixed with 100 ml of methanol. The obtained solution was diluted for the next analyses (1:10). 3.9 ml of the radical solution measured at the wavelength 515 nm on the spectrophotometer UNICO UV 2800 and added 100 µl of plant extract. After 10 minutes in darkness obtained solution measured again at the same wavelength. Results of measurements were calculated by equation:

$$\% \text{ Inh} = ((A_0 - A_{10}) : A_0) \cdot 100,$$

where % Inh — % of inhibition of radical solution; A_0 — control measurement without plant extract; A_{10} — measurement with plant extract after 10 minutes.

Obtained results also were expressed in ascorbic acid equivalent (AAE) and Trolox equivalent (TE) on a gram of dry weight.

Experimental data were evaluated by using Excel 2010. Mean values of three replicates and standard deviation are given in Figures 1, 2 and Table.

Results and discussions

Investigation of forage plants plays an important role in agricultural science. Plants from Fabaceae such as *Galega orientalis* characterized by high productivity and as N-fixators [18]. *Bunias orientalis* that belongs to Brassiceae, besides value characteristics, demonstrated also antimicrobial activity [21]. It is known, that plants of *Isatis* spp. accumulate two indoxyl-forming substances in leaves, which when exposed on air form indigo [10].

Our previous study of antioxidant activity showed that different plants such as forage, energetic, oil plants, medicinal etc. and it plants raw material have the high antioxidant potential [20].

Various methods are used to identify the antioxidant property of plant raw material. Alam et al. (2012) reviewed two basic groups of methods to evaluate antioxidant properties of samples: *in vitro* and *in vivo* methods. The first group of methods includes DPPH scavenging activity, hydrogen peroxide scavenging assay, nitric oxide scavenging activity, Trolox equivalent antioxidant capacity, total radical-trapping antioxidant parameter (TRAP) method, ferric reducing-antioxidant power, phosphomolybdenum method etc. The second group of methods usually use on animals such as mice, rats

etc. and their tissues can be used for the assay [1]. DPPH method is the most widespread and simple assay based on the reaction of discoloration of the radical solution [15].

Seed is a potential source of antioxidants [7]. In our study, we used to investigate methanol, ethanol and water extracts of seeds to determine DPPH scavenging effect. On Fig. 1 demonstrated that antiradical activity of seed extracts was the least for ethanol extracts of all investigated plants. Antiradical activity of methanol extracts of investigated plants decreased in the following the order: *Galega officinalis* > *Bunias orientalis* > *Galega orientalis* > *Bunias erucago* > *Baptisia australis* > *Isatis littoralis* > *Isatis tinctoria*. This parameter in ethanol extracts decreased in following order: *Bunias orientalis* > *Galega orientalis* > *Galega officinalis* > *Bunias erucago* > *Isatis littoralis* > *Baptisia australis* > *Isatis tinctoria*. Scavenging effect of seed extracts against DPPH radical decreased in the following the order: *Bunias orientalis* > *Galega officinalis* > *Galega orientalis* > *Baptisia australis* > *Bunias erucago* > *Isatis littoralis* > *Isatis tinctoria*.

Generally, methanol extracts of investigated plants had an antiradical activity of 18.27—80.57 %, ethanol extracts of 11.07—79.73 % and water extracts of 23.31—80.26 %. Our previous investigation of antioxidant activity of Brassicaceae species demonstrated that plant raw material of above-ground part of the plant has an antiradical activity of different extracts from 25.67 to 84.60 %, wherein methanol extracts had higher results [22].

As positive control during DPPH assay can be used ascorbic acid, gallic acid, quercetin, rutin, catechin [14]. In our experiment chosen control compound the ascorbic acid equivalent (AAE). Generally, methanol extracts showed antioxidant activity from 1.75 to 2.72 mg AAE per gram, ethanol extracts from 1.62 to 2.72 mg AAE per gram, water extracts from 1.82 to 2.66 mg AAE per gram (Fig. 2).

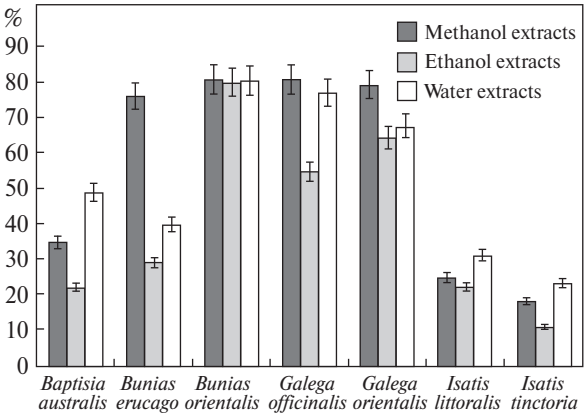

Fig. 1. Scavenging effect of seed extracts on DPPH radical, %

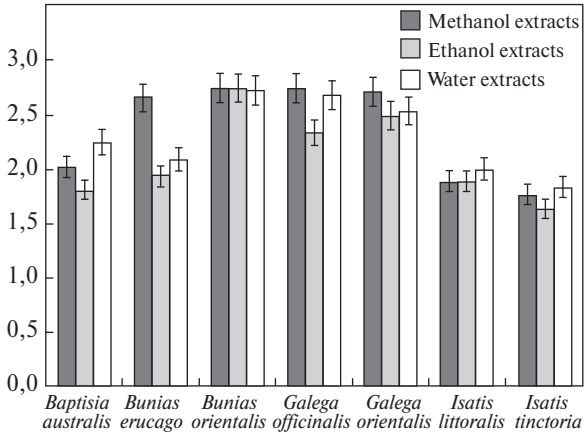

Fig. 2. Antioxidant activity of seed extracts of selected plant species, mg AAE/g

Antioxidant activity of selected plant extracts, mg TE/g

Species	Methanol extracts	Ethanol extracts	Water extracts
Baptisia australis	1.30 ± 0.13	—	3.27 ± 0.11
Bunias erucaga	6.74 ± 0.17	0.56 ± 0.04	1.96 ± 0.16
Bunias orientalis	7.51 ± 0.12	7.46 ± 0.05	7.37 ± 0.14
Galega officinalis	7.50 ± 0.39	4.00 ± 0.23	6.98 ± 0.21
Galega orientalis	7.28 ± 0.26	5.34 ± 0.31	5.68 ± 0.30
Isatis littoralis	—	—	1.19 ± 0.17
Isatis tinctoria	—	—	—

According to Borchardt et al. (2008), antioxidant activity from DPPH scavenging activity for *Baptisia australis* was 12.99 μM Trolox per 100 g, *B. bracteata* of 20.08 μM Trolox per 100 g, *Lepidium virginicum* 25.95 μM Trolox per 100 g, *Capsella bursa-pastoris* of 14.71 μM Trolox per 100 g [3]. In our experiment methanol extracts exhibited antioxidant activity from 1.30 to 7.51 mg Trolox equivalent (TE) per gram, ethanol extracts from 0.56 to 7.46 mg TE per gram, water extracts from 1.19 to 7.37 mg TE per gram (Table). Investigated concentrations of plant extracts of *Isatis littoralis* (methanol, ethanol extracts), *Isatis tinctoria* (all extracts) and *Baptisia australis* (ethanol extract) couldn't be expressed in Trolox equivalent.

Conclusions

This study indicates that seeds of investigated forage plants have high antioxidant potential as well as leaves or shoots. In conditions of M.M. Gryshko National Botanical Garden of the NAS of Ukraine selected seeds of forage plants had antiradical activity from 11.07 to 84.60 % depending on extract. All investigated seeds had the least values of this parameter in the ethanolic extracts. Antioxidant activity expressed in ascorbic acid equivalent (mg AAE/g) and Trolox equivalent (mg TE/g) was of 1.62—2.72 and 0.56—7.76 respectively. Investigation of seeds extracts of crops from different plant families is need to evaluate antioxidant potential of it and recommend for use.

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Recommended by O.V. Grygorieva
Received 31.01.2019

О.М. Вергун, Д.Б. Рахметов, О.В. Шиманська, С.О. Рахметова, В.В. Фіщенко

Національний ботанічний сад
імені М.М. Гришка НАН України,
Україна, м. Київ

АНТИОКСИДАНТНА АКТИВНІСТЬ ЕКСТРАКТІВ НАСІННЯ ДЕЯКИХ КОРМОВИХ РОСЛИН

Мета — дослідити антиоксидантну активність екстрактів насіння деяких кормових рослин в умовах Національного ботанічного саду імені М.М. Гришка НАН України.

Матеріал та методи. Досліджено насіння семи кормових рослин: *Baptisia australis* (L.) R.Br. (Fabaceae), *Bunias erucago* L., *B. orientalis* L. (Brassicaceae), *Galega officinalis* L., *G. orientalis* Lam. (Fabaceae), *Isatis littoralis* Steven, *I. tinctoria* L. (Brassicaceae). Для визначення антирадикальної активності рослинних екстрактів використовували метод, описаний Brand-Williams та ін. (1995). Біохімічний аналіз передбачав такі етапи: підготовка метанольних, етанольних та водних екстрактів (1 г сухого матеріалу змішували з 25 мл розчинника); 12 год екстракції; спектрофотометричне визначення антирадикальної активності з розчином 2,2-дифеніл-пікрилгідразилу. Розчин радикалу готували в метанолі та розводили згідно з методикою. Дані перераховано як еквівалент аскорбінової кислоти (АКЕ) і тролоксу (ТЕ) на 1 г сухої маси. Вимірювання екстрактів проводили на спектрофотометрі UNICO UV 2800 за довжини хвилі 515 нм. Експериментальні дані опрацьовано в програмі Excel.

Результати. Метанольні екстракти досліджуваних рослин мали антирадикальну активність 18,27—80,57 %, етанольні — 11,07—79,73 %, водні — 23,31—80,26 %. Антиоксидантна активність метанольних екстрактів становила 1,75—2,72 мг АКЕ на 1 г, етанольних екстрактів — 1,62—2,72 мг АКЕ на 1 г, водних екстрактів — 1,82—2,66 мг АКЕ на 1 г, або відповідно 1,30—7,51, 0,56—7,76 та 1,19—7,37 мг ТЕ на 1 г.

Висновки. Отримані дані демонструють, що в умовах Національного ботанічного саду НАН України екстракти насіння досліджуваних кормових рослин характеризуються високим антиоксидантним потенціалом, як і листки чи пагони. Антирадикальна активність екстрактів становила 11,07—84,60 %. Найменші значення цього параметра зафіксовано в усіх екстрактів. Антиоксидантна активність, виражена як АКЕ, була максимальною для *Bunias orientalis* і *Galega officinalis* та мінімальною — для *Isatis tinctoria*, а виражена як ТЕ була найвищою у метанольних екстрактів *Bunias orientalis* та найнижчою — в етанольних екстрактів *Bunias erucago*. Дослідження екстрактів насіння культур з різних родин є необхідним для оцінки їх антиоксидантного потенціалу та рекомендацій щодо їх використання.

Ключові слова: насіння, спиртові та водні екстракти, антирадикальна активність, антиоксидантна активність.

О.Н. Вергун, Д.Б. Рахметов, О.В. Шиманская, С.А. Рахметова, В.В. Фищенко

Национальный ботанический сад
имени Н.Н. Гришко НАН Украины,
Украина, г. Киев

АНТИОКСИДАНТНАЯ АКТИВНОСТЬ ЭКСТРАКТОВ СЕМЯН НЕКОТОРЫХ КОРМОВЫХ РАСТЕНИЙ

Цель — исследовать антиоксидантную активность экстрактов семян некоторых кормовых растений в условиях Национального ботанического сада имени Н.Н. Гришко НАН Украины.

Материал и методы. Исследованы семена семи кормовых растений: *Baptisia australis* (L.) R.Br. (Fabaceae), *Bunias erucago* L., *B. orientalis* L. (Brassicaceae), *Galega officinalis* L., *G. orientalis* Lam. (Fabaceae), *Isatis littoralis* Steven, *I. tinctoria* L. (Brassicaceae). Для определения антирадикальной активности растительных экстрактов использовали метод, описанный Brand-Williams и др. (1995). Биохимический анализ предусматривал

следующие этапы: подготовка метанольных, этанольных и водных экстрактов (1 г сухого материала смешивали с 25 мл растворителя); 12 ч экстракции; спектрофотометрическое определение антирадикальной активности с раствором 2,2-дифенил-пикрилгидразила. Раствор радикала готовили в метаноле и разводили соответственно методике. Данные пересчитаны как эквивалент аскорбиновой кислоты (АКЭ) и тролокса (ТЭ) на 1 г сухой массы. Измерение экстрактов проводили на спектрофотометре UNICO UV 2800 при длине волны 515 нм. Экспериментальные данные обработаны в программе Excel.

Результаты. Метанольные экстракты исследованных растений имели антирадикальную активность 18,27—80,57 %, этанольные — 11,07—79,73 %, водные — 23,31—80,26 %. Антиоксидантная активность метанольных экстрактов 1,75——2,72 мг АКЭ на 1 г, этанольных экстрактов — 1,62—2,72 мг АКЭ на 1 г, водных экстрактов — 1,82—2,66 мг АКЭ на 1 г, или соответственно 1,30—7,51, 0,56—7,76 и 1,19—7,37 мг ТЭ на 1 г.

Выводы. Полученные данные демонстрируют, что в условиях Национального ботанического сада НАН Украины экстракты семян исследованных кормовых растений характеризуются высоким антиоксидантным потенциалом, как и листья или побеги. Антирадикальная активность экстрактов составила 11,07—84,60 %. Наименьшие значения этого параметра зафиксированы у всех этанольных экстрактов. Антиоксидантная активность, выраженная как АКЭ, была максимальной для *Bunias orientalis* и *Galega officinalis* и минимальной — для *Isatis tinctoria*, а выраженная как ТЭ была наивысшей у метанольных экстрактов *Bunias orientalis* и самой низкой — у этанольных экстрактов *Bunias erucago*. Исследование экстрактов семян культур из разных семейств является необходимым для оценки их антиоксидантного потенциала и рекомендаций относительно их использования.

Ключевые слова: семена, спиртовые и водные экстракты, антирадикальная активность, антиоксидантная активность.