

ANTIRADICAL ACTIVITY OF PLANT EXTRACTS OF *GALEGA OFFICINALIS* L. AND *G. ORIENTALIS* LAM.

Objective — to estimate the antiradical scavenging ability of extracts of plants of *Galega officinalis* L. and *G. orientalis* Lam., depending on phase of growing in the conditions of M.M. Gryshko National Botanical Garden of the NAS of Ukraine (NBG).

Material and methods. Plant material of this investigation was two species of *Galega* L. (*G. officinalis* and *G. orientalis*) collected from experimental collection of Cultural Flora Department of NBG: GOF-SV (*G. officinalis*, spring vegetation), GOF-B (*G. officinalis*, budding stage), GOF-F (*G. officinalis*, flowering stage), GOF-SR (*G. officinalis*, seed ripening stage), GOR-SV (*G. orientalis*, spring vegetation), GOR-B (*G. orientalis*, budding stage), GOR-F (*G. orientalis*, flowering stage), GOR-SR (*G. orientalis*, seed ripening stage). The antiradical activity of methanol, ethanol and aqueous extracts, based on the discoloration reaction on the solution of DPPH (2,2-diphenyl-1-picrylhydrazyl free radical), was determined by spectrophotometric method according to Brandt-Williams et al. Biochemical preparation and analyze was carried out in the Institute of Biodiversity Conservation and Biosafety, the Slovak University of Agriculture in Nitra (Slovak Republic) and NBG. The total content of tannins was determined by titrimetric method according to Krischenko (water extracts with indigo carmine were titrated by permanganate solution).

Results. We determined that methanol extracts of *Galega officinalis* plants had antiradical activity in range from 19.39 % (GOF-B) to 95.18 % (GOF-SR), ethanol extracts — from 11.24 % (GOF-B) to 92.87 % (GOF-F), and water extracts — from 28.64 % (GOF-SR) to 74.63 % (GOF-F). Methanol extracts of *Galega orientalis* plants had antiradical activity from 20.20 % (GOR-SR) to 91.72 % (GOR-B), ethanol extracts — from 11.74 % (GOR-SR) to 84.74 % (GOR-F), and water extracts — from 22.90 % (GOR-SV) to 77.72 % (GOR-F). The total content of tannins for *G. officinalis* was in range of 1.22 to 4.17 % and for *G. orientalis* — from 1.55 to 4.42 % during vegetation.

Conclusions. Plant raw material of two *Galega* L. species is potential source of antioxidants. During vegetation antiradical activity of plant extracts of *Galega officinalis* exhibited 11.24–95.18 % and *Galega orientalis* — 11.74–91.72 % depending on extract and phase of growing in conditions of NBG. Generative organs such as flowers and fruits had less content of tannins than vegetative.

Key words: *Galega*, antioxidant activity, 2,2-diphenyl-1-picrylhydrazyl (DPPH), tannins.

Plants have played a major role in the introduction of new antioxidant and therapeutic agents [24]. Species of *Galega* L. now are widely recommended plants administered for production of first-line antidiabetic drugs, which is safe and efficient in the treatment of diabetes and usually does not induce hypoglycemia such as Metformin [1, 8, 10, 15, 27, 28]. As reported Shojaee et al. (2015) *G. officinalis* L. extracts possess compounds with hypoglycemia and weight-reducing potential [12, 18]. Karakas et al. (2012) resulted that this plant has been used for treatment of the

plague, malignant fevers, and parasitic infection [17]. According to Leporatti and Ivancheva (2003), aerial parts of *G. officinalis* use in traditional medicine of Bulgaria and Italy for treatment of hypoglycemia and for increasing milk secretion [19].

Also, *G. officinalis* is used as an ornamental plant and occurs as a weed [29]. These plants contain vasicine and a poisonous alkaloid known as galegin. Plant extracts were tested against gram-positive and gram-negative bacteria and the antibacterial effect was shown [17]. Also, an antimicrobial activity of *G. officinalis* was described and found that extracts against bacteria was more effective than against fungus [21].

Kiselova et al. (2006) reported, that *G. officinalis* plant extracts have strong correlation between content of polyphenols and antioxidant activity [11].

Galega orientalis Lam. is very persistent with a high yielding ability that makes focus on study of this plant as plant raw material for energy and fodder production [7, 13].

The antioxidant properties of cultivated plants are usually well recognized. There is, however, little data about antioxidant activity of *Galega* species. Therefore further studies must be carried out.

Material and methods

Plant material was collected from experimental collection of Cultural Flora Department of M.M. Gryshko National Botanical Garden of the NAS of Ukraine (NBG). It was selected plants of two species of *Galega* — *G. officinalis* and *G. orientalis*. In this report has used abbreviation: GOF-SV (*G. officinalis*, spring vegetation), GOF-B (*G. officinalis*, budding stage), GOF-F (*G. officinalis*, flowering stage), GOF-SR (*G. officinalis*, seed ripening stage), GOR-SV (*G. orientalis*, spring vegetation), GOR-B (*G. orientalis*, budding stage), GOR-F (*G. orientalis*, flowering stage), GOR-SR (*G. orientalis*, seed ripening stage).

Preparation of plant raw material and determination of tannins content was done in the laboratory of Cultural Flora Department of NBG. Determination of antiradical activity of plant extracts were conducted in the Institute of Biodiversity Conservation and Biosafety, the Slovak University of Agriculture in Nitra (Slovak Republic). To determine antioxidant activity of extracts was investigated dried above-ground part of plants. Antiradical activity of the methanolic, ethanolic and aqueous extracts was carried out according to Brand-Williams et al. (1995) against DPPH radical (2,2-diphenyl-1-picrylhydrazyl) [9]. This method based on the reaction of radical discolouration (colour of the radical solution is purple). The procedure of determination of optical density measured with spectrophotometer Genesis-20 at wavelength 515 nm. Dry mass (1 g) of investigated plants mixed with 25 ml of solvent. Extraction was carried out with methanol and water during 12 hours with constant stir-

ring on a shaker. 0.1 ml of antioxidant solution was added to 3.9 ml of methanol DPPH· solution (25 mg of radical per 100 ml of methanol with further dilution). The optical density of the solution was measured after adding sample immediately and after 10 min of incubation in the dark. Obtained results were calculated in percentage by using the formula

$$(A_0 - A_{10}) : A_0 \cdot 100$$

(A_0 — absorbance of the control solution (containing only DPPH·); A_{10} — absorbance in the presence of the plant extract in DPPH· solution).

The total content of tannins was determined by titrimetric method according to Krischenko (1983) [3]. The samples (5 g of fresh plant raw material) were mixed with distilled water (volume of flask 100 ml) and boiled 2 hours at the 70 °C. The supernatant was analyzed by adding 10 ml of the sample and 25 ml of indigo carmine to a 1 liter flask and then adding 750 ml of water with following titration against the permanganate solution (0.1 N).

Data presented as the mean ± standard deviation for triplicate determinations and given in Table 1—3. Experimental data were evaluated using Excel 2010.

Results and discussions

Medicinal plants synthesize antioxidant compounds as secondary products [1, 4, 6, 22]. Plant raw material of investigated plants is rich source of biologically active compounds such as ascorbic acid, lipids, carotene, tannins that makes above-ground part of *Galega* species potentially rich plant raw material with high level of antioxidant activity. Our previous biochemical investigations showed high content of ascorbic acid in the above-ground part of *G. officinalis* (595.12 mg%), *G. orientalis* (436.70 mg%). Carotene content was 2.07 mg% and 1.49 mg% respectively [2]. Also, Shymanska et al. (2017) found that content of carotenoids for *G. officinalis* was from 0.57 to 0.88 mg·g⁻¹ and for *G. orientalis* — from 0.31 to 1.05 mg·g⁻¹ (per fresh mass) [26].

There is great number of methods for determination of antioxidant capacity of plant raw material based on different principles. One of the most

widely-used procedures for measurements of antioxidant capacity is DPPH. This method is rapid, simple, accurate and inexpensive assay for measuring the ability of different compounds to act as free radical scavengers or hydrogen donor [20].

Antiradical activity of extracts exhibit the accumulation of a group of compounds that react with DPPH radical and change the color of radical solution (from purple to yellow or green depending on investigated extracts). Results of the antiradical

Table 1. Antiradical activity of plant extracts of *Galega officinalis* L. depending on phase of growing and parts of plants, %

Sample	Part of plant	Methanol extract	Ethanol extract	Water extract
GOF-SV	All above-ground part	52.04 ± 2.19	23.62 ± 1.44	38.57 ± 1.65
GOF-B	Buds	85.50 ± 0.52	40.05 ± 1.18	71.06 ± 3.46
GOF-B	Leaves	70.43 ± 3.36	42.55 ± 0.98	66.79 ± 0.72
GOF-B	Stems	19.39 ± 1.13	11.24 ± 0.80	29.31 ± 2.94
GOF-F	Flowers	92.26 ± 0.68	92.87 ± 1.03	74.63 ± 0.83
GOF-F	Leaves	88.25 ± 2.83	77.29 ± 1.36	55.33 ± 3.48
GOF-F	Stems	19.54 ± 1.12	20.83 ± 0.50	30.76 ± 0.73
GOF-SR	Fruits	95.18 ± 1.54	53.08 ± 2.82	43.80 ± 2.26
GOF-SR	Leaves	79.72 ± 3.59	62.03 ± 3.01	54.63 ± 0.67
GOF-SR	Stems	26.03 ± 1.05	18.80 ± 1.33	28.61 ± 1.30

Table 2. Antiradical activity of plant extracts of *Galega orientalis* Lam. depending on phase of growing and parts of plants, %

Sample	Part of plant	Methanol extract	Ethanol extract	Water extract
GOR-SV	All above-ground part	53.96 ± 1.66	19.07 ± 2.14	22.90 ± 2.44
GOR-B	Buds	91.72 ± 0.21	58.44 ± 7.26	50.38 ± 1.37
GOR-B	Leaves	85.41 ± 2.06	43.33 ± 1.55	50.22 ± 2.87
GOR-B	Stems	23.69 ± 1.92	12.59 ± 1.25	31.00 ± 1.52
GOR-F	Flowers	90.88 ± 0.56	84.78 ± 2.66	77.72 ± 4.58
GOR-F	Leaves	87.72 ± 2.08	37.74 ± 3.15	48.33 ± 3.06
GOR-F	Stems	23.77 ± 1.81	15.59 ± 0.82	28.40 ± 1.81
GOR-SR	Fruits	90.60 ± 2.63	20.66 ± 1.43	59.72 ± 2.43
GOR-SR	Leaves	41.78 ± 2.95	22.93 ± 2.42	44.65 ± 0.86
GOR-SR	Stems	20.20 ± 0.90	11.74 ± 0.62	24.33 ± 1.56

Table 3. Total content of tannins in water extracts of *Galega* L. species depending on phase of growing and parts of plants, %

Sample	Part of plant	Tannins	Sample	Part of plant	Tannins
GOF-SV	All above-ground part	2.56 ± 0.29	GOR-SV	All above-ground part	1.77 ± 0.21
GOF-B	Buds	2.89 ± 0.04	GOR-B	Buds	2.44 ± 0.13
GOF-B	Leaves	3.23 ± 0.15	GOR-B	Leaves	3.94 ± 0.22
GOF-B	Stems	1.54 ± 0.14	GOR-B	Stems	1.87 ± 0.11
GOF-F	Flowers	2.06 ± 0.11	GOR-F	Flowers	1.55 ± 0.06
GOF-F	Leaves	4.17 ± 0.23	GOR-F	Leaves	3.56 ± 0.18
GOF-F	Stems	1.78 ± 0.13	GOR-F	Stems	2.23 ± 0.16
GOF-SR	Fruits	1.22 ± 0.09	GOR-SR	Fruits	1.94 ± 0.07
GOF-SR	Leaves	4.89 ± 0.32	GOR-SR	Leaves	4.42 ± 0.15
GOF-SR	Stems	2.23 ± 0.11	GOR-SR	Stems	1.73 ± 0.05

activity of plant extracts of two species of *Galega* are reported in Table 1 and 2.

Previous data, obtained by Tusevski et al. (2014), showed a high antioxidant potential of *G. officinale* plant extracts due to content of phenolic compounds [22].

Methanol extracts of *G. officinale* plants exhibited antiradical scavenging from 19.39 % (GOF-B, stems) to 95.18 % (GOF-SR, fruits) during vegetation (Table 1). Ethanol extracts showed antiradical activity from 11.24 % (GOF-B, stems) to 92.87 % (GOF-F, flowers). Antiradical activity of water extracts was of 28.61 % (GOF-SR, stems) to 74.63 % (GOF-F, flowers).

Methanol extracts of *G. orientalis* plants exhibited antiradical scavenging from 20.20 % (GOR-SR, stems) to 91.72 % (GOR-B, buds) during vegetation (Table 2). Ethanol extracts showed antiradical activity from 11.74 % (GOR-SR, stems) to 84.78 % (GOR-F, flowers). Antiradical activity of water extracts was from 22.90 % (GOR-SV, all plant) to 77.72 % (GOR-F, flowers).

Also, we determined the content of total tannins in the water extracts of investigated plants (Table 3). Tannins are naturally occurring polyphenols produced by plants via secondary metabolic processes. Their ability to bind proteins, pigments, and complex metallic ions, together with their flavouring effect are the basis for their extensive use as additives in the food industry [25]. Tannins don't function solely as primary antioxidants but as secondary antioxidants [5]. According to last study in this branch, it was found correlation between antioxidant activity and compounds of phenolic nature [23].

As presented in Table 3, the total content of tannins noticed in the leaves depending on phase of plant growing. Generative organs such as flowers and fruits had less content of tannins than vegetative: *G. officinalis* — from 1.22 to 2.89 % (generative), from 1.54 to 4.89 (vegetative); *G. orientalis* — from 1.55 to 2.44 % (generative), from 1.73 to 4.42 (vegetative).

Conclusions

Thus, this study was shown that plant raw material of the genus *Galega* is potential source of antioxi-

dants. The antiradical activity of plant extracts of *G. officinalis* was minimal in stems in the period of budding (11.24 %) and maximal — in fruits in the fruitage period (95.18 %) depending on extract. The same sign for *G. orientalis* plants was minimal in stem in the seed ripening period (11.74 %) and maximal — in buds in the budding period (91.72 %) depending on extract. The high antiradical activity identified in methanol and ethanol extracts (up to 90 %). The content of tannins for *G. officinalis* was in range of 1.22 to 4.17 % and for *G. orientalis* — from 1.55 to 4.42 % during vegetation.

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АНТИРАДИКАЛЬНА АКТИВНІСТЬ РОСЛИННИХ ЕКСТРАКТІВ *GALEGA OFFICINALIS* L. ТА *G. ORIENTALIS* LAM.

Мета — дослідити антирадикальну дію екстрактів рослин *Galega officinalis* L. та *G. orientalis* Lam. залежно від фази розвитку в умовах Національного ботанічного саду імені М.М. Гришка НАН України (НБС).

Матеріал та методи. Рослинний матеріал дослідження — два види роду *Galega* L. (*G. officinalis* та *G. orientalis*), зібрані з експериментальної колекції відділу культурної флори НБС: GOF-SV (*G. officinalis*, весняне відростання), GOF-B (*G. officinalis*, бутонізація), GOF-F (*G. officinalis*,

цвітіння), GOF-SR (*G. officinalis*, дозрівання насіння), GOR-SV (*G. orientalis*, весняне відростання), GOR-B (*G. orientalis*, бутонізація), GOR-F (*G. orientalis*, цвітіння), GOR-SR (*G. orientalis*, дозрівання насіння). Антирадикальну активність метанольних, етанольних та водних екстрактів, яка ґрунтується на реакції знебарвлення розчину ДФПГ (2,2-дифеніл-1-пікрілгідразил вільний радикал), визначали спектрофотометричним методом за W. Brandt-Williams et al. Біохімічний аналіз проведено в Інституті збереження біорізноманіття та біологічної безпеки Словацького аграрного університету в Нітрі (Словацька Республіка) та НБС. Загальний вміст дубильних речовин визначено методом титрування за В.П. Крищенком (водні екстракти з індигокарміном титрували розчином перманганату).

Результати. Визначено, що метанольні екстракти рослин *Galega officinalis* виявляли антирадикальну активність від 19,39 % (GOF-B) до 95,18 % (GOF-SR), етанольні екстракти — від 11,24 % (GOF-B) до 92,87 % (GOF-F), водні екстракти — від 28,64 % (GOF-SR) до 74,63 % (GOF-F), метанольні екстракти рослин *Galega orientalis* — від 20,20 % (GOR-SR) до 91,72 % (GOR-B), етанольні екстракти — від 11,74 % (GOR-SR) до 84,74 % (GOR-F), водні екстракти — від 22,90 % (GOR-SV) до 77,72 % (GOR-F). Загальний вміст дубильних речовин для *G. officinalis* становив від 1,22 до 4,17 %, для *G. orientalis* — від 1,55 до 4,42 % протягом вегетації.

Висновки. Рослинна сировина двох видів роду *Galega* — потенційне джерело антиоксидантів. Протягом вегетації рослинні екстракти *Galega officinalis* виявляли антирадикальну активність від 11,24 до 95,18 %, *Galega orientalis* — від 11,74 до 91,72 % залежно від екстракту та фази розвитку в умовах НБС. Генеративні органи характеризувалися нижчим вмістом дубильних речовин, ніж вегетативні.

Ключові слова: *Galega*, антиоксидантна активність, 2,2-дифеніл-1-пікрілгідразил (ДФПГ), дубильні речовини.

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АНТИРАДИКАЛЬНАЯ АКТИВНОСТЬ РАСТИТЕЛЬНЫХ ЭКСТРАКТОВ *GALEGA OFFICINALIS* L. И *G. ORIENTALIS* LAM.

Цель — исследовать антирадикальное действие экстрактов растений *Galega officinalis* L. и *G. orientalis* Lam. в зависимости от фазы развития в условиях Нацио-

нального ботанического сада имени Н.Н. Гришко НАН Украины (НБС).

Материал и методы. Растительный материал исследования — два вида рода *Galega* L. (*G. officinalis* и *G. orientalis*), собранные из экспериментальной коллекции отдела культурной флоры НБС: GOF-SV (*G. officinalis*, весеннее отрастание), GOF-B (*G. officinalis*, бутонизация), GOF-F (*G. officinalis*, цветение), GOF-SR (*G. officinalis*, созревание семян), GOR-SV (*G. orientalis*, весеннее отрастание), GOR-B (*G. orientalis*, бутонизация), GOR-F (*G. orientalis*, цветение), GOR-SR (*G. orientalis*, созревание семян). Антирадикальную активность метанольных, этанольных и водных экстрактов, основанную на реакции обесцвечивания раствора ДФПГ (2,2-дифенил-1-пикрилгидразил свободный радикал), определяли спектрофотометрическим методом по W. Brandt-Williams et al. Биохимический анализ проведен в Институте сохранения биоразнообразия и биологической безопасности Словацкого аграрного университета в Нитре (Словацкая Республика) и НБС. Общее содержание дубильных веществ определяли методом титрования по В.П. Крищенко (водные экстракты с индигокармином титровали раствором перманганата).

Результаты. Установлено, что метанольные экстракты растений *Galega officinalis* проявляли антирадикальную активность от 19,39 % (GOF-B) до 95,18 % (GOF-SR), этанольные экстракты — от 11,24 % (GOF-B) до 92,87 % (GOF-F), водные экстракты — от 28,64 % (GOF-SR) до 74,63 % (GOF-F), метанольные экстракты растений *Galega orientalis* — от 20,20 % (GOR-SR) до 91,72 % (GOR-B), этанольные экстракты — от 11,74 % (GOR-SR) до 84,74 % (GOR-F), водные экстракты — от 22,90 % (GOR-SV) до 77,72 % (GOR-F). Общее содержание дубильных веществ для *G. officinalis* составляло от 1,22 до 4,17 %, для *G. orientalis* — от 1,55 до 4,42 % в течение вегетации.

Выводы. Растительное сырье двух видов рода *Galega* — потенциальный источник антиоксидантов. В период вегетации растительные экстракты *Galega officinalis* проявляли антирадикальную активность от 11,24 до 95,18 %, *Galega orientalis* — от 11,74 до 91,72 % в зависимости от экстракта и фазы развития в условиях НБС. Генеративные органы характеризовались более низким содержанием дубильных веществ, чем вегетативные.

Ключевые слова: *Galega*, антиоксидантная активность, 2,2-дифенил-1-пикрилгидразил (ДФПГ), дубильные вещества.