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TOTAL CONTENT OF PHENOLIC COMPOUNDS IN THE ETHANOL EXTRACTS OF *GALEGA OFFICINALIS* L. AND *G. ORIENTALIS* LAM.

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This study represents results of determination of total phenolic compounds in raw of two species of *Galega* L. It was investigated plant extracts of *Galega officinalis* L. (goat's-rue) and *Galega orientalis* Lam. (fodder galega) which are known as fodder crops with high productivity of green mass and medicine plants (Hasani-Ranjbar et al., 2009; Peiretti, 2009; Shojaee et al., 2015; Teleută et al., 2015). The current study was aimed to evaluate an accumulation in selected plants the total content of phenolics as compounds with antioxidant activity. Raw of investigated plants collected from experimental collections of M.M. Gryshko National Botanical Garden of the NAS of Ukraine (Kyiv) during vegetation. Biochemical analysis conducted in the Slovak University of Agriculture in Nitra. The phenolic content in ethanol extracts was measured using Folin-Chocalteu reagent. The procedure for preparing extracts was done according to Singleton and Rossi, 1965 and measuring conducted using spectrophotometer Jenway (6405 UV/Vis, England). Gallic acid was used as the standard and the results were expressed in mg g^{-1} gallic acid equivalent (GAE). Study of *G. officinalis* extracts showed that accumulation of total phenolic content in different organs was in the range from 9.13 to 32.76 mg g^{-1} GAE. In extracts of *G. orientalis* was identified total phenolics content from 6.73 to 26.77 mg g^{-1} GAE. It was established that less concentration of studied compounds found in the stems for both species.

Keywords: *Galega officinalis*, *Galega orientalis*, phenolics

Introduction

Leguminous plants (Fabaceae Lindl.) are a perspective group of crops, which ecological and economic function is important in agriculture. It is one of the most important plant families in the production of food for humans and livestock, as well as in the production of industrial products. These crops have provided interesting as forage grasses with high productivity and play an important role as N fixators (Peiretti, 2009; Teleută et al., 2015). Plants from Fabaceae family are of interest in relation to biologically active compounds, especially individual, in different organs (Danilčenko et al., 2017).

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Among economically important leguminous plants can be highlight goat's rue (*Galega officinalis* L.) and fodder galega (*Galega orientalis* Lam.). Plants of species of *Galega* L. are valuable perennial and productive crops with the protein-rich chemical composition of plant raw material (Baležentienė, 2008). Results obtained by Peiretti (2009) showed that *G. officinalis* has the potential for large-scale ensiling if plants are harvested at the budding stage or during regrowth. These species cultivated as medicinal plants due to the biochemical composition of plant raw material and as garden plants (Baležentienė and Spruogis, 2011; Kumar et al., 2012). As described in some reports, *G. officinalis* uses in traditional phytotherapy due to hypoglycemic, diuretic properties, and weight-reducing ability (Hasani-Ranjbar et al., 2009; Shojaee et al., 2015). Biochemical composition of *Galega* species is ascorbic acid, carotene, soluble sugars, lipids, protein, ash, alkaloids, macroelements, etc. (Symanowicz and Kalembasa, 2012; Vergun et al., 2012; Shymanska et al., 2017). Also, the phytochemicals screening revealed that in aqueous, methanolic, ethanolic and acetone extracts were found flavonoids, tannins, cardiac glycosides, terpenes and steroids. Methanolic extracts of goat's rue significantly improved the lipid profile in a clinical study (Luka et al., 2017).

The aim of this study was to determine the content of polyphenol compounds in different organs of *G. officinalis* and *G. orientalis* during vegetation in the conditions of M.M. Gryshko National Botanical Garden of the NAS of Ukraine (NBG).

Material and methodology

The plants were grown in 2017 in the experimental fields of the M.M. Gryshko National Botanical Garden of the NAS of Ukraine in the Kyiv city (50° 24' 55" N, 30° 33' 45" E). The total area of the experimental field for each species was 25 m^2. For this study, the average weight of ten plants selected by randomized method was used. In 2017, the active plant growth started on 21th of March for *G. officinalis* and the 5th of April for *G. orientalis*. Plant growth was ended in November for both species of investigated plants. Many years observations showed that the end of vegetation depends on early frosts. Monthly average temperatures in the vegetation period of year of study were following: March – +3.9 °C, April – +10.7 °C, May – +16.2 °C, June – +19.1 °C, July – +21.2 °C, August – +21.2 °C, September – +15.8 °C, October – +6.9 °C, November – +2.6 °C.

Biological material

Observation on plants was conducted in the experimental collection of Cultural Flora Department of NBG. Plant raw material of two species *Galega officinalis* and *G. orientalis* were collected in the stages according to Biologische Bundesanstalt, Bundessortenamt and Chemische Industrie (BBCH) coding system. According to BBCH scale, plant samples were taken at the phenological growth stages described for faba bean (*Vicia faba* L.) (Meier, 2001). Four principal growth stages were assigned: leaf development (19 – nine or more leaves infolded), inflorescence emergence (50 – flower buds present, still enclosed by leaves), flowering (65 – full flowering: flowers open on 5 racemes per plant), and ripening (80 – beginning of ripening: seed green, filling pod cavity). For chemical analyses plant raw material was dried at 35 °C for three days. After this, the samples were milled in the powder condition.

Biochemical analysis

The biochemical analysis was done in the Slovak University of Agriculture in Nitra (Slovak Republic). For planned analyses, 0.2 g of milling fraction was extracted with 20 ml of 80% ethanol for 24 hours. After centrifugation at 4000 g with Rotofix 32 A (Hettich, Germany) for 20 min, the supernatant was used for measurement of the total content of polyphenols. The total phenolic content of extracts was measured using Folin-Chocalteu reagent (Singleton, Rossi, 1965). 0.1 ml of each sample was mixed with 0.1 ml of the Folin-Ciocalteu 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 was used as the standard and the results were expressed in mg g^{-1} gallic acid equivalents (GAE) (25–300 mg l^{-1}; R^2 = 0.998).

Statistical analysis

The statistically treated data are given in the table as the arithmetical mean values and their standard errors. Data were submitted ANOVA and differences between means compared through the Tukey-Kramer test (α = 0.05).

Results and discussion

The therapeutic potential of natural medicinal plants as antioxidants in reducing free radicals suggests that most of the plants have a high antioxidant potential, which can be useful therapeutically (Veeru et al., 2009). Plant secondary metabolites such as polyphenol compounds play an important role in the defence against free radicals. Polyphenols exhibit a wide range of biological effects including antibacterial, anti-inflammatory, hepatoprotective, antiviral, anticancerogenic etc. (Soobratee et al., 2005; Piluzza and Bullitta, 2011). Numerous studies described the good or high linear correlation between the concentration of phenolic compounds and antioxidant capacity determined by the DPPH-method (Mareček et al., 2016; Ivanišova et al., 2017). This suggests that phenolic content could be used as an indicator of antioxidant properties of the plants (Piluzza and Bullitta, 2011).

Polyphenols can form several hydrogen bonds and even ionic bonds with most proteins. They modulate the activity of many proteins, involving enzymes, ion channels etc. As a consequence many polyphenols are pharmacologically active, being among antioxidants, anti-inflammatory, antibacterial, antifungal, and antiviral (Wink, 2013). The previous study of *G. officinalis* extracts showed the presence of phenolics as flavonol triglycosides, kaempferol, galegin, medicagol, quercetin etc. (Kahkeshani et al., 2015).

The content of phenolic compounds in different parts of *G. officinalis* parts ranged from 9.13 to 32.76 mg g^{-1} GAE (Table 1). It should be noticed, that most of the results about the content of phenolic compounds concern to the *G. officinalis* plants. According to Tusevski et al. (2014), total phenolic content for plants of *G. officinalis* was 32.53 ±2.80 mg g^{-1} GAE. Pehlivan Karakas et al. (2016) obtained twenty phenolics compounds from methanol leaves extracts of *G. officinalis*. Total phenolic content in them was 36.69 mg g^{-1} of dry extract.

Table 1 The content of total phenolic compounds in plant raw material of *Galega officinalis* L. and *G. orientalis* Lam., mg g^{-1} GAE

Stage of growing	Organ of plan	*Galega officinalis* L.	*Galega orientalis* Lam.
Leaf development	aerial part	17.06 ±0.89 b	19.96 ±0.62 ab
Inflorescence emergence	leaves	19.50 ±1.14 b	25.21 ±0.17 a
	stems	9.61 ±1.45 b	8.09 ±0.44 d
	buds	31.51 ±1.11 a	23.43 ±1.53 a
Flowering	leaves	22.58 ±0.67 b	15.43 ±0.28 c
	stems	9.13 ±0.30 d	6.73 ±0.23 e
	flowers	32.76 ±1.26 a	19.58 ±0.85 ab
Ripening	leaves	18.61 ±0.95 b	26.77 ±0.51 a
	stems	15.28 ±0.67 c	10.87 ±0.99 d
	fruits	19.61 ±1.33 b	13.58 ±0.62 c

Notes: Means in columns followed by different letters are different at p = 0.05; each value represents the mean of three independent experiments (±SD)

The total content of phenolics of *G. orientalis* plant raw material ranged from 6.73 to 26.77 mg g^{-1} GAE during vegetation. According to Baležentienė (2009) report, the highest total content of phenols was determined at the budding stage which was characterized as the most intensive growth period of the plant shoot. Moreover, the study of Kahkeshani et al. (2015) demonstrated that mixture of *G. officinalis* and *Nigella sativa* L. (4 : 1 w/w) had shown milk stimulating activity and the total phenol content 77.72 µg mg^{-1} GAE. Also, we detected in the previous study the total antioxidant activity of methanol, ethanol and water extracts (Shymanska et al., 2018). According to obtained data, antiradical activity of *G. officinalis* and *G. orientalis* plant extracts was minimal in stems for both species (11.24 and 11.74% respectively). Likewise, high antiradical activity was identified in methanol and ethanol extracts of generative organs (up to 90%). These data consistent with the obtained results as interrelated. Further research should be focused on the study of more species of the investigated genus and detailed investigation of their polyphenol compounds.

Conclusions

Thus, in conditions of M.M. Gryshko National Botanical Garden of the NAS of Ukraine, plants of *Galega officinalis* and *G. orientalis* accumulated uneven quantity of phenolic compounds during vegetation. Comparing the analysis of two investigated species showed that the most content of total phenolics was determined in the *G. officinalis* flowers extracts in the stage of flowering and the least – in the stems extracts of *G. orientalis*. Both *G. officinalis* and *G. orientalis* accumulated the least content of phenolic compounds in stems. Generative organs of *G. officinalis* had higher total content of phenolics than vegetative.

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References

BALEŽENTIENĖ, L. 2008. Evaluation of galega suitability for cattle feeding. In *Grassland Science in Europe,* vol. 13, p. 777–779.

BALEŽENTIENĖ, L., SPRUOGIS, V. 2011. Experience of fodder galega (*Galega orientalis* Lam.) and traditional fodder grasses use for forage production in organic farm. In *Veterinarija ir Zootechnika,* vol. 56(78), p. 19–26.

DANILČENKO, H., DABKEVIČIUS, Z., JARIENĖ, E., TARASEVICIENE, Ž., TELEVIČIUTĖ, D., TAMOŠIŪNAS, A., JEZNACH, M. 2017. The effect of stinging nettle and field horsetail extracts on the synthesis of biologically active compounds in germinated leguminous and quinoa seed. In *Zemdirbyste-Agriculture,* vol. 104(4), p. 337–344. http://dx.doi.org/10.13080/z-a.2017.104.043

HASANI-RANJBAR, SH., NAYEBI, N., LARIJANI, B., ABDOLLAHI, M. 2009. A systematic review of the efficacy and safety of herbal medicines used in the treatment of obesity. In *World Journal of Gastroenterology,* vol. 15(25), p. 3073–3085. http://dx.doi.org/10.3748/wjg.15.3073

IVANIŠOVA, E., KRAJČOVIČ, T., TOKÁR, M., DRAB, Š., KANTOR, A., KAČÁNIOVÁ, M. 2017. Potential of wild plants as a source of bioactive compounds. In *Scientific Papers: Animal Science and Biotechnologies,* vol. 50(1), p. 109–114.

KAHKESHANI, N., HADJIAKHOONDI, A., MAAFI, N., KHANAVI, M. 2015. Standardization of a galactogogue herbal mixture based on its total phenol and flavonol contents and antioxidant activity. In *Research Journal of Pharmacognosy,* vol. 2(1), p. 35–39.

KUMAR, S., SAINI, M., KUMAR, V., PRAKASH, O., ARYA, R., RANA, M., KUMAR, D. 2012. Traditional medicinal plants curing diabetes: a promise for today and tomorrow. In *Asian Journal of Traditional Medicines*, vol. 7(4), p. 178–188.

LUKA, C.D., ADOGA, G.I., ISTIFANUS, G. 2017. Phytochemical studies of different fractions of *Galega officinalis* extracts and their effects on some biochemical parameters in alloxan-induced diabetic rats. In *European Journal of Medicinal Plants,* vol. 19(1), p. 1–10. http://dx.doi.org/10.9734/EJMP/2017/32145

MAREČEK, J., IVANIŠOVA, E., FRANČAKOVA, H., MUSILOVA, J., KRAJČOVIČ, T., MENDELOVÁ, A. 2016. Evaluation of primary and secondary metabolites in selected varieties of potatoes. In *Potravinarstvo,* vol. 10 (1), p. 145–151. http://dx.doi.org/10.5219/562

MEIER, U. (2001). *Faba Bean. Growth Stages of Mono- and Dicotyledonous Plants,* 2nd edn. Federal Biological Research Centre for Agriculture and Forestry, Braunschweig, Germany, p. 34–36.

PEHLIVAN KARAKAS, F., SAHIN, G., TÜRKER, A. 2016 (a). Enhancement of direct shoot regeneration and determination of bioactive secondary metabolites in leaves of *Galega officinalis* L. In *Turkey Journal of Biology,* vol. 40, p. 1311–1319. http://dx.doi.org/0.3906/biy-1603-70

PEIRETTI, P.G. 2009. Ensilability characteristics and silage fermentation of *Galega* (*Galega officinalis* L.). In *Agricultural Journal,* vol. 4(1), p. 41–45.

PILUZZA, G., BULLITTA, S. 2011. Correlations between phenolic content and antioxidant properties in twenty-four plant species of traditional ethnoveterenary use in the Mediterranean area. In *Pharmaceutical Biology,* vol. 49(3), p. 240–247. http://dx.doi.org/10.3109/13880209.2010.501083

SHOJAEE, S.S., VAHDATI, A., ASSAEI, R., SAPEHRIMANESH, M. 2015. Effect of *Galega officinalis* leaf powder and *Trigonella foenum-graecum* seed powder on blood glucose levels and weight gain in a diabetes mellitus rat models. In *Comparative Clinical Pathology,* vol. 24(1), p. 145–148. https://doi.org/10.1007/s00580-013-1873-7.

SHYMANSKA, O., VERGUN, O., RAKHMETOV, D., BRINDZA, J. 2018. Antiradical activity of plant extracts of *Galega officinalis* L. and *Galega orientalis* L. In *Introdukciia Roslyn* [Plant Introduction], vol. 78(2), p. 12–19.

SHYMANSKA, O., VERGUN, O., RAKHMETOV, D., FISHCHENKO, V. 2017. The content of photosynthetic pigments in the leaves of *Galega officinalis* L. and *Galega orientalis* Lam. cultivars. In *Agrobiodiversity for improving nutrition, health and life quality*, vol. 1, p. 398–403. http://dx.doi.org/10.15414/agrobiodiversity.2017.2585-8246.398-403

SINGLETON, V.L., ROSSI, J.A. 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagent. In *American Journal of Enology and Agricultural,* vol. 6, p. 144–158.

SOOBRATTEE, M., NEERGHEEN, V.S., LUXIMON-RAMMA, A., ARUOMA, O.I., BAHORUM, T. 2005. Phenolics aspotential antioxidant therapeutic agents: mechanism and actions. In *Mutation Research,* vol. 579, p. 200–213.

SYMANOWICZ, B., KALEMBASA, S. 2012. Changes of calcium and magnesium content in biomass of goat's-rue (*Galega orientalis* Lam.) during vegetation. In *Ecological Chemistry and Engineering, A,* vol. 19(7), p. 689–698. http://dx.doi.org/10.2428/ecea.2012.19(07)068

TELEUTĂ, A., TÎTEI, V., COŞMAN, S., LUPAN, A. 2015. Forage value of the species *Galega orientalis* Lam. under the conditions of the republic of Moldova. In *Research Journal of Agricultural Science,* vol. 47(2), p. 226–231.

TUSEVSKI, O., KOSTOVSKA, A., ILOSKA, A., TRAJKOVSKA, L., GADZOVSKA SIMIC, S. 2014. Phenolic production and antioxidant properties of some Macedonian medicinal plants. In *Central European Journal of Biology,* vol. 9(9), p. 888–900. https://doi.org/10.2478/s11535-014-0322-1

VEERU, P., KISHOR PANKAJ, M., MEENAKSHI, M. 2009. Screening of medicinal plant extracts for antioxidant activity. In *Journal of Medicinal Plants Research*, vol. 3(8), p. 608–612.

VERGUN, O.M., SHYMANSKA, O.V., RAKHMETOV, D.B. 2012. Biohimichna harakterystyka vydiv rodu *Galega* L. v Pravoberegnomu Lisostepu Ukrajiny [Biochemical characteristic of species of the genus of *Galega* L. in the Right Bank of Forest-Steppe of Ukraine]. In *Visnyk Odeskoho Nacionalnoho Universitetu* [Odesa National University Herald], vol. 17(28), p. 43–50 [In Ukrainian].

WINK, M. 2013. Evolution of secondary metabolites in legumes (Fabaceae). In *South African Journal of Botany,* vol. 89, p. 164–175. http://dx.doi.org/10.1016/j.sajb.2013.06.006