CHEMIJA. 2012. Vol. 23. No. 4. P. 306–311

Distribution of formononetin, daidzein and genistein in *Trifolium* species and their aerial plant parts

Giedrė Dabkevičienė[1],

Bronislava Butkutė[1]*,

Nijolė Lemežienė[1],

Valdas Jakštas[2],

Egidijus Vilčinskas[1],

Valdimaras Janulis[2]

[1] *Institute of Agriculture, Lithuanian Research Centre for Agriculture and Forestry, LT-58344 Akademija, Kėdainiai District, Lithuania*

[2] *Department of Pharmacognosy of Lithuanian University of Health Sciences, A. Mickevičiaus Str. 9, LT-44307 Kaunas, Lithuania*

Isoflavones are phenolic phytochemicals of major interest for health. Clovers are one of their alternative sources. The current study was designed to quantify three isoflavones in various clover species and their aerial plant parts by a high-performance liquid chromatography and to identify the most promising source for medicinal use. Isoflavone contents were quantified in the plants of 11 perennial and 4 annual species of genus *Trifolium*. Subject to aerial part and perennial clover species, the concentrations of formononetin, daidzein and genistein varied in the wide ranges: <Limit of detection (LOD) – 2.19, <LOD – 1.42 and <LOD – 2.898 mg g^{-1}, respectively. According to the sum of three isoflavones quantified in leaves–stems–flowers, perennial clover species were arranged as follows: *T. medium* (6.01–3.50–2.93 mg g^{-1}) > *T. pratense* > *T. fragiferum* > *T. alpestre* > *T. rubens* > *T. ochroleucum* > *T. pannonicum* > *T. hybridum* > *T. ambiguum* > *T. repens* > *T. montanum* (0.092–0.022–0.090 mg g^{-1}). Regarding the total isoflavone content, the annual clover species were ranked in the sequence: *T. incarnatum* (3.27 mg g^{-1}) > *T. campestre* > *T. resupinatum* > *T. alexandrinum* (0.517 mg g^{-1}).

Key words: *Trifolium* spp., isoflavone, formononetin, daidzein, genistein, HPLC

INTRODUCTION

Isoflavones are phenolic compounds, belonging to a widespread group of natural products flavonoids, which are found in a number of plant species, though predominately in legumes. Isoflavones such as genistein [5, 7-dihydroxy-3-(4'-hydroxyphenyl)chromen-4-one] and daidzein [7-hydroxy-3-(4-hydroxyphenyl)chromen-4-one] are common phytoestrogens of soy products. Daidzein, genistein and their 4'-methylated derivatives, formononetin [7-hydroxy-3-(4-methoxyphenyl)chromen-4-one] and biochanin A [5,7-dihydroxy-3-(4'-methoxyphenyl) chromen-4-one], respectively, are present in red clover (*Trifolium pratense* L.) [1, 2, 3] as well as in other *Trifolium* species [4–6]. Epidemiological studies show that due to their phytoestrogenic effect isoflavones exert a positive influence on hormonal functions and can prevent osteoporosis [7, 8], Alzheimer disease [9], cancer [3], vascular pathologies and alleviate menopausal symptoms [2, 10]. Isoflavones are structural mimics of endogenous 17β-estradiol [11] and structures of the three isoflavones discussed in the current work are shown in Fig. 1.

* Corresponding author. E-mail: brone@lzi.lt

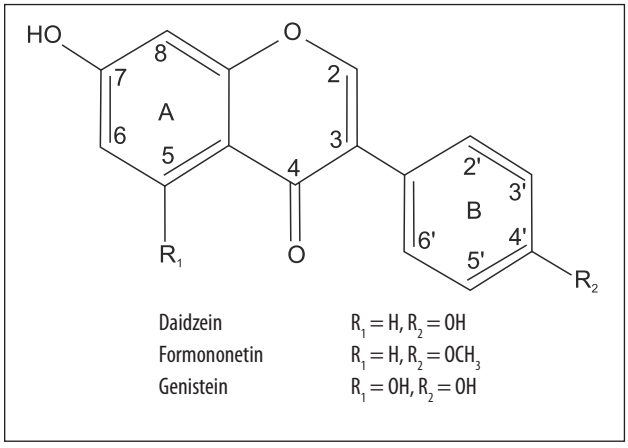

Fig. 1. The molecular structures of isoflavones daidzein, formononetin and genistein

Currently, the main source of isoflavones used in human nutrition is soy [10, 12]. However, some people are allergic to soy products. As a result, researchers are looking for alternative sources of isoflavones. Most research has focused on the quantification of isoflavones in red clover and its separate parts [4, 13–15]. Increasingly more countries are joining the search for clover species that could be applied to meet specific needs, including their use as a valuable source of phytoestrogens.

Our study was intended to quantify the content of formononetin, daidzein, genistein in various clover species and aerial plant parts by a high-performance liquid chromatography and to identify the most promising sources of isoflavones.

EXPERIMENTAL

Plant material. Isoflavone studies were done on the plants of the 11 perennial and 4 annual clover species: *T. alpestre* L., *T. ambiguum* Bieb., *T. fragiferum* L., *T. hybridum* L., *T. medium* L., *T. montanum* L., *T. ochroleucum* Huds., *T. pannonicum* Jacq., *T. pratense* L., *T. repens* L., *T. rubens* L., *T. alexandrinum* L., *T. campestre* Schreb., *T. incarnatum* L., *T. resupinatum* L. In 2007 and 2008, genetic collections of clover species were established in a trial field using seedlings grown in a greenhouse. Thirty plants per accession (2 replications, 15 plants per replication) were planted at 50 × 50 cm distances. The plants were cut at full flowering stage. Fresh samples were chopped, fixed at 105 °C for 20 min, dried at (65 ± 5) °C, ground in a cyclonic mill with a 1 mm sieve and assayed for the following isoflavones: daidzein, formononetin and genistein. Isoflavones were quantified by a reversed-phase high-performance liquid chromatography (RP-HPLC).

Isoflavone quantification. An HPLC grade acetonitrile and trifluoracetic acid were purchased from Sigma-Aldrich (Steinheim, Germany), ethanol from Stumbras AB (Kaunas, Lithuania). Ultra pure water (>18 MΩ cm) was used throughout the HPLC experiment. Standards of daidzein (purity 99.9%) and genistein (purity 99.3%) were obtained from ChromaDex (Irvine, CA, USA) and formononetin (purity ≥99%) from Fluka-Sigma-Aldrich (Steinheim, Germany). The standard solutions were prepared by dissolving standard compounds in methanol.

Sample preparation procedure included acid hydrolysis of glycoside forms and was performed under conditions described in the USP monograph [16]. The analyses were carried out with the Waters 2695 Alliance HPLC system (Milford, MA, USA), equipped with a Waters 2487 UV/Vis detector and a Waters XTerra RP18 150 × 3.9 mm column with a guard column. The dual λ absorbance detector allowed monitoring of two wavelengths, 258 nm (for genistein analyte) and 301 nm (for daidzein and formononetin analytes). A Waters 996 PDA detector was used for peak spectral identification. Chromatographic and PDA data were recorded and peak area responses were integrated by the software Waters Empower2 (Milford, MA, USA). The gradient chromatographic elution was carried out according to Raudonis et al. [17]. The linear through zero type calibration curves were generated using external standard solutions, prepared by dissolving standard compounds in methanol (0.2–30 μg mL^{-1}). Value of the coefficient of determination for the curves was ≥0.9998 and the equation was y = 160329691 · x for genistein, y = 55761134 · x for daidzein and y = 55059861 · x for formononetin. Isoflavone concentrations were expressed as mg g^{-1} of sample dry matter (DM). Limit of detection (LOD), i. e. the lowest quantity of isoflavone that can be reliably detected by the method used, was determined with the standard solution at a signal-to-noise ratio of three and was <0.100 μg mL^{-1}. That corresponds to the isoflavone content <0.004 mg in 1 g DM of the tested material.

Statistical data analysis was done using the software ANOVA from the package SELEKCIJA [18].

RESULTS AND DISCUSSION

Isoflavone amounts in the aerial parts of perennial *Trifolium* species

Isoflavones were quantified separately in leaves, stems and flowers. The clover species and their aerial plant parts differed in the concentrations of the isoflavones tested.

The highest concentration of formononetin was determined in aerial parts of *T. fragiferum, T. medium* and *T. pratense* (Table): in leaves 1.83, 1.69 and 1.51 mg g^{-1}, in stems 1.98, 1.86 and 2.19 mg g^{-1}, in flowers 0.763, 1.13 and 1.66 mg g^{-1}, respectively. For other species tested, the levels of formononetin in various plant parts were considerably lower. The contents of this isoflavone in aerial parts of *T. rubens* ranged from 0.395 to 0.644 mg g^{-1}. *T. repens* plants accumulated even less formononetin – from 0.129 to 0.155 mg g^{-1}. In leaves and stems of *T. pannonicum*, formononetin was found in traceable concentration only, i. e. below the limit of detection (<LOD); however, in flowers

Table. **The concentration of formononetin, daidzein and genistein in different aerial parts of perennial *Trifolium* species**

	Formononetin			Daidzein			Genistein		
	Leaves	Stems	Flowers	Leaves	Stems	Flowers	Leaves	Stems	Flowers
T. rubens	0.395	0.644	0.401	0.308	0.253	0.065	0.058	0.143	0.129
T. repens	0.135	0.155	0.129	0.054	0.037	0.041	<LOD	<LOD	<LOD
T. pratense	1.51	2.19	1.66	0.786	0.947	0.324	0.452	0.181	0.231
T. pannonicum	<LOD	<LOD	0.752	0.092	0.040	0.267	0.526	0.277	0.129
T. ochroleucum	<LOD	<LOD	<LOD	0.044	0.011	0.026	0.970	0.682	0.498
T. montanum	0.028	<LOD	0.040	0.064	0.022	0.050	<LOD	<LOD	<LOD
T. medium	1.69	1.86	1.13	1.42	0.917	0.961	2.90	0.719	0.841
T. hybridum	0.126	0.257	0.031	0.205	0.326	0.116	0.011	0.015	<LOD
T. fragiferum	1.83	1.98	0.763	0.636	0.422	0.268	0.290	0.203	0.302
T. ambiguum	0.297	0.095	0.096	<LOD	0.018	0.038	<LOD	<LOD	<LOD
T. alpestre	0.076	0.504	0.197	0.052	0.444	0.097	1.17	0.388	0.543
Average	0.553	0.699	0.473	0.333	0.312	0.205	0.580	0.237	0.243
MSE*	0.246	0.322	0.167	0.143	0.107	0.084	0.328	0.092	0.099

* Mean squared error.

its content amounted to 0.752 mg g^{-1}. In *T. montanum* and *T. ochroleucum* plants, the level of formononetin concentration was particularly low.

In terms of average formononetin concentration and its differences among aerial plant parts, stems of most species contained the largest amount of this isoflavone (Table). Such pattern of compound distribution among plant parts was less specific to the other isoflavones – daidzein and genistein. The species *T. medium, T. fragiferum* and *T. rubens* had the highest daidzein concentrations in leaves (1.42, 0.636 and 0.308 mg g^{-1}), compared with stems (0.917, 0.422 and 0.253 mg g^{-1}) and flowers (0.961, 0.268 and 0.065 mg g^{-1}), whereas stems of *T. pratense*, *T. hybridum* and *T. alpestre* contained more daidzein (0.947, 0.326 and 0.444 mg g^{-1}) than other plant parts. *T. hybridum* accumulated only modest amounts of daidzein in all plant parts (0.205, 0.326 and 0.116 mg g^{-1}). Similar concentrations of this phytoestrogen were determined in the plants of *T. rubens* and *T. alpestre* species. In the rest of the clover species assayed daidzein was found in low concentrations.

T. medium, *T. pratense* and *T. fragiferum* were characterized by the apparently largest concentration of daidzein (like that of formononetin) among perennial *Trifolium* species, whereas the highest genistein concentrations were typical of *T. medium*, *T. alpestre* and *T. ochroleucum*. With regard to genistein distribution among aerial plant parts, leaves of most species contained the highest concentration of this isoflavone (Table). Significantly higher concentration (2.90 mg g^{-1}) was detected in leaves of *T. medium* compared with other plant parts and other species. Fairly large amount of this isoflavone was found in leaves of *T. alpestre* and *T. ochroleucum* (1.17–0.970 mg g^{-1}). Although compared with leaves, stems and flowers of *T. medium*, *T. ochroleucum* and *T. alpestre* accumulated markedly less genistein, its concentration was the highest (in stems – 0.719, 0.682 and 0.388 mg g^{-1}, in flowers – 0.841, 0.498 and 0.543 mg g^{-1}) paralleled with the same plant parts of other species tested. In plant parts of *T. pratense* and *T. fragiferum*, the genistein concentrations were the lowest in comparison with those of daidzein and, especially, of formononetin. Only trace amounts of genistein were detected in aerial parts of *T. repens, T. montanum* and *T. ambiguum* species.

From some literature sources it follows that the formononetin content in a whole plant and / or individual plant parts is from twice to many more times higher than that of daidzein and genistein [6, 15, 19–21]. In our study, the concentration of formononetin was in many cases higher in stems and flowers than that of the other two isoflavones. The concentrations of formononetin, daidzein and genistein of a similar order of magnitude have been reported [6, 15, 22], moreover, according to Zgórka [6], like in our study, *T. medium* and *T. pratense* were characterised by relatively high isoflavone amounts, while *T. montanum* exhibited the lowest concentrations. Screening of 57 *Trifolium* species for total isoflavone concentration, done by Oleszek et al. [5], has suggested that there are a number of species with extremely high amounts of these compounds, and the regularity of isoflavone content variation between species significantly differed from that established in our study as well as in Zgórka [6] research: according to Oleszek et al. [5], isoflavone concentration in *T. montanum* was very high and in *T. medium* it was quite low. Thus comparison of our findings with those obtained by other researchers indicates that they do not always agree both in terms of isoflavone contents within species and between species. A number of factors may be responsible for this. The varied isoflavones extraction methodologies are investigated and used for isoflavones quantification: they differ in sample preparation and storage, origin of extractive solutions, temperature of extraction, hydrolysis (with or without) of glycosides to free aglycones etc. [6, 20, 22]. Grynkievicz et al. [23] have reported over 40 methods for determination of isoflavones in plants and food samples differing in instrumentation, detection systems and other factors. Year, environment, maturity, number of cuts could be also the sources of variance

in isoflavone content [15, 20, 24–26]. Moreover, a great variation of isoflavones within clover species was identified [5, 15, 19, 21, 26]. As Oleszek et al. [5] confirmed, these compounds could differ about 3–4 times within one species *T. pratense*.

The computed sum of quantified isoflavones in each aerial part revealed that the largest concentration of the phytoestrogens in plant DM of most species: *T. medium, T. fragiferum, T. ochroleucum, T. ambiguum, T. repens* and *T. montanum* came from leaves (Fig. 2). *T. medium* leaves were characterized by an especially high total concentration of formononetin, daidzein and genistein (6.01 mg g^{-1}). In fact, stems and flowers of this species contained more isoflavones tested than those plant parts of other species; however, the gap was not so sharp. Among aerial parts, stems of *T. pratense*, *T. alpestre, T. rubens* and *T. hybridum* were the richest in the sum of quantified isoflavones. Commonly, among structural components of plants, flowers contained the lowest total amount of daidzein, formononetin and genistein, with one exception: *T. pannonicum* exhibited the highest total isoflavone concentration in flowers.

Research sources provide contradictory findings on which aerial plant parts accumulate maximal isoflavone contents [4, 14, 27]. Although isoflavones present in some commercial products were extracted from red clover flowers [25], our results suggest that leaves and stems of most clover species are more valuable parts for this purpose, and flowers would be the last plant part to be used as a nutraceuticals source. This observation is in agreement with some literature data related to red clover [15, 20, 26]. In contrast, J. Vetter [4] found that the stem fractions of *T. pratense* have in general the lowest total isoflavone concentration and are rich in daidzein.

Characteristics of isoflavone distribution in four annual *Trifolium* species

Formononetin, daidzein and genistein concentrations in annual clover plants were quantified on a whole aerial part of the plant sample without division into leaves, flowers and stems. In the plants of *T. incarnatum,* formononetin made up the largest share of the three isoflavones: its concentration (2.49 mg g^{-1}) was found to be more than 3-fold higher than that of genistein and more than 60-fold higher than that of daidzein (Fig. 3).

The content of formononetin in *T. resupinatum* plants amounted to 0.508 mg g^{-1}, and in *T. alexandrinum* plants to 0.335 mg g^{-1}, while *T. campestre* plants did not contain formononetin as well as daidzein. Overall, annual clover species were poor in daidzein: its concentration varied from <LOD (*T. campestre*) to 0.138 mg g^{-1} (*T. alexandrinum* and *T. resupinatum*). The highest genistein content (3.04 mg g^{-1}), which is practically equal to the summarized isoflavone amount, was established in *T. campestre* plants. Four-fold lower genistein concentration (0.742 mg g^{-1}) was established in the aerial part of *T. incarnatum* plants, while *T. resupinatum* contained even less (0.485 mg g^{-1}), and *T. alexandrinum* was characterized by the significantly lowest genistein concentration (0.044 mg g^{-1}) among the four annual clover species tested. Having summed the content of all quantified isoflavones, it is seen that the highest content of these bioactive compounds was accumulated by *T. incarnatum* plants (3.27 mg g^{-1}), slightly less by *T. campestre* (3.04 mg g^{-1}). Considerably lower isoflavone amount (1.13 mg g^{-1} and 0.517 mg g^{-1}) was established in *T. resupinatum* and *T. alexandrinum* plants DM.

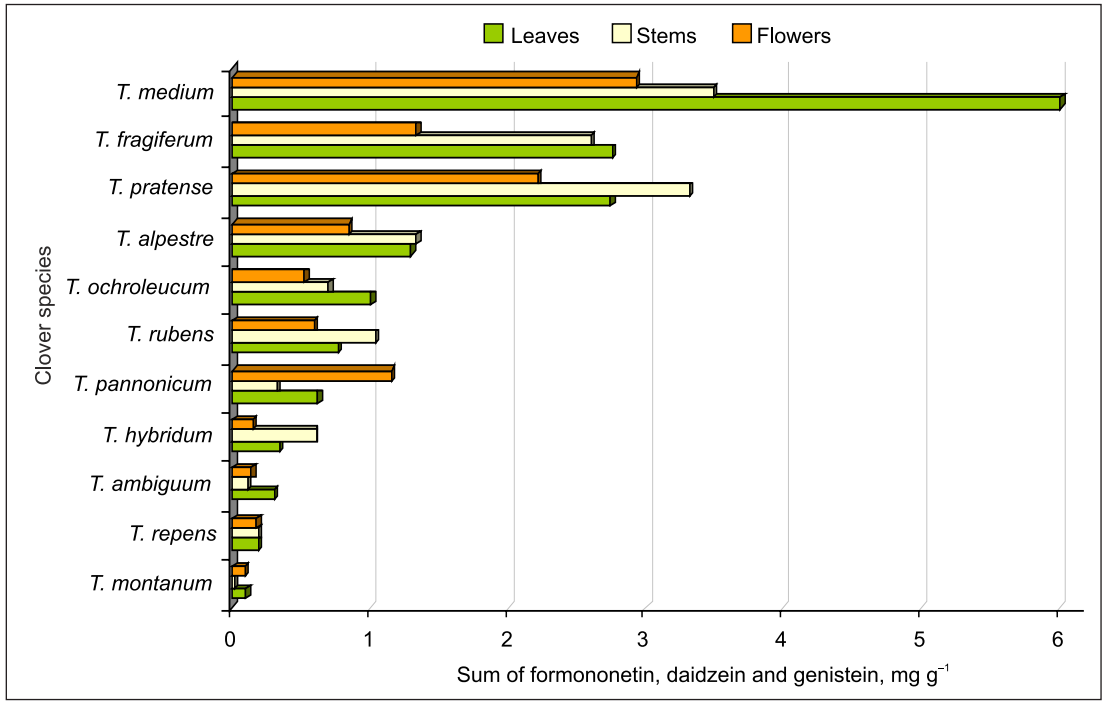

Fig. 2. The summarized concentration of formononetin, daidzein and genistein in aerial parts of perennial *Trifolium* spp.

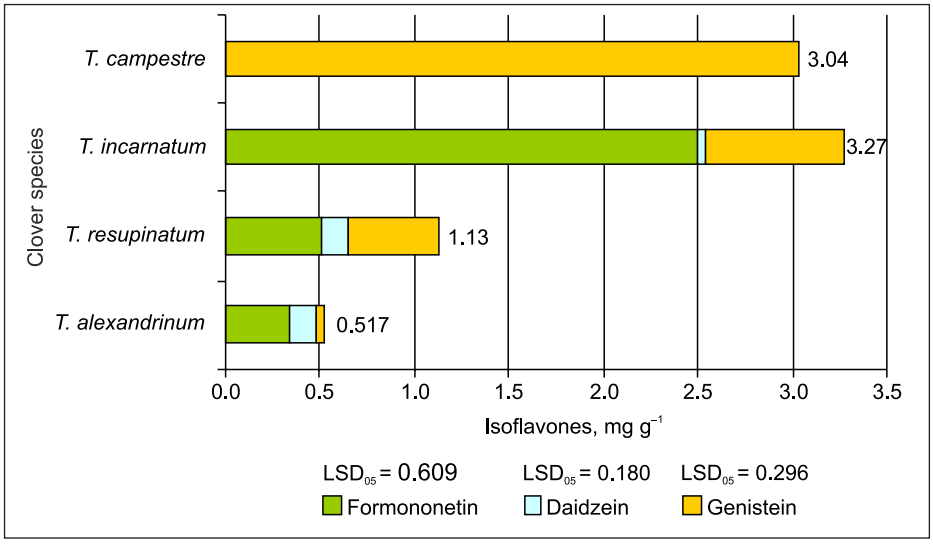

Fig. 3. The concentration of isoflavones in annual *Trifolium* spp.

In terms of species practical applications, a very important aspect is high yield and good reproductive plant characteristics. Our previous research evidenced that the DM yield of clover species varied from 87 to 226 g per plant [28]. Perennial species *T. pratense*, *T. medium* containing high concentrations of isoflavones in aerial plant parts showed high and moderately high productivity and could be considered as a potential medicinal plant in the countries of temperate climate. Although short-growing *T. fragiferum* plants exhibit high seed set and rather high content of isoflavones, the species does not stand out by the DM yield, therefore it can hardly be recommended for isoflavone extraction, like *T. pannonicum* populations topping by DM yield per plant or also high-yielding *T. hybridum, T. ambiguum,* but not distinguishing by isoflavone abundance. Among annual clover species *T. incarnatum, T. alexandrinum* and *T. resupinatum* are considered as valuable and promising agricultural plants [29]. However, only *T. incarnatum* exhibited significant contents of isoflavones and can be considered as a potential source of phytoestrogens for nutraceuticals and functional foods. In our study, *T. campestre* plants, exhibiting high genistein content, performed poorly in terms of herbage yield.

CONCLUSIONS

1. The highest concentration of formononetin was determined in the aerial parts of *T. fragiferum, T. medium* and *T. pratense*: in leaves – 1.83, 1.69 and 1.51 mg g^{-1}, in stems – 1.98, 1.86 and 2.19 mg g^{-1}, in flowers – 0.763, 1.13 and 1.66 mg g^{-1}, respectively.

2. *T. medium, T. fragiferum* and *T. rubens* had the highest daidzein concentrations in leaves (1.42, 0.636 and 0.308 mg g^{-1}), compared with stems (0.917, 0.422 and 0.253 mg g^{-1}) and flowers (0.961, 0.268 and 0.065 mg g^{-1}).

3. Significantly higher genistein concentration (2.90 mg g^{-1}) was detected in leaves of *T. medium* compared with other plant parts and other perennial species tested.

4. The highest content of isoflavones among annual clover species was accumulated by *T. incarnatum* plants (3.27 mg g^{-1}), and *T. campestre* was characterised by the highest content of genistein (3.04 mg g^{-1}), which is practically equal to the total isoflavone amount in the plant aerial part.

5. High levels of isoflavones were found not only in *T. pratense*, but also in other species. A favourable combination of isoflavone concentration and plant yield makes *T. medium, T. incarnatum* a novel promising source of these bioactive compounds. Leaves and stems showed a higher total amount of the three isoflavones tested compared with flowers.

ACKNOWLEDGEMENTS

This work was partly supported by the long-term research programs "Genetics and Purposeful Change of Genotypes of Agricultural and Forest Plants" and "Biopotential and Quality of Plants for Multifunctional Use" implemented by the Lithuanian Research Centre for Agriculture and Forestry.

Received 8 May 2012
Accepted 13 June 2012

References

1. J. K. Prasain, C.-C. Wang, S. Barnes, *Free Radical Biol. Med.*, **37**, 326 (2004).
2. J. Liu, J. E. Burdette, H. Xu, C. Gu, R. B. van Breemen, K. P. Bhat, *J. Agric. Food Chem.*, **49**, 2472 (2001).
3. M. J. Messina, *Am. J. Clin. Nutr.,* **70(3)**, 439 (1999).

4. J. Vetter, *J. Agric. Food Chem.*, **43**, 106 (1995).
5. W. Oleszek, A. Stochmal, B. Janda, *J. Agric. Food Chem.*, **5(20)**, 8095 (2007).
6. G. Zgórka, *J. Sep. Sci.*, **32(7)**, 965 (2009).
7. C. Atkinson, J. E. Compston, N. E. Day, M. Dowasett, S. A. Bingham, *Am. J. Clin. Nutr.*, **79**, 326 (2004).
8. F. Occhiuto, R. De Pasquale, G. Guglielmo, et al., *Phytotherapy Res.*, **21**, 130 (2007).
9. M. S. Kurzer, X. Xu, *Annu. Rev. Nutr.*, **17**, 353 (1997).
10. A. Mortensen, S. E. Kulling, H. Schwartz, *Mol. Nutr. Food Res.*, **53**, 266 (2009).
11. D. A. Seielstad, K. E. Carlson, P. J. Kushner, G. L. Greene, J. A. Katzenellenbogen, *Biochemistry*, **34**, 12605 (1995).
12. J. O. Bennett, O. Yu, L. G. Heatherly, H. B. Krishnan, *J. Agric. Food Chem.*, **52**, 7574 (2004).
13. C. A. Williams, J. C. Onyilagha, J. B. Harborne, *Biochem. Syst. Ecol.*, **23**, 655 (1995).
14. L. Z. Lin, X. G. He, M. Lindenmaier, J. Yang, *J. Agric. Food Chem.*, **48**, 354 (2000).
15. Q. Wu, M. Wang, J. E. Simon, *J. Chromatogr., A*, **1016**, 195 (2003).
16. M. H. Sharaf, in: R. L. Williams (ed.), *US Pharmacopoeia USP 32-NF 27*, Rockville, Maryland, USA (2009).
17. R. Raudonis, V. Jakstas, D. Burdulis, R. Beneis, V. Janulis, *Medicina*, **45(5)**, 382 (2009).
18. P. Tarakanovas, S. Raudonius, *The Statistical Analysis of Data of Agricultural Researches Applying Computer Programs ANOVA, STAT, SPLIT-PLOT from a Package SELECTION and IRRISTAT,* LUA, Akademija (2003).
19. N. M. M. Saviranta, M. J. Anttonen, A. Von Wright, R. O. Karjalainen, *J. Sci. Food Agric.*, **88**, 125 (2008).
20. R. Tsao, Y. Papadopoulos, R. Yang, J. C. Young, K. McRae, *J. Agric. Food Chem.*, **54**, 5797 (2006).
21. G. P. Ramos, P. M. B. Dias, C. B. Morais, P. E. Froehlich, M. Dall'Agnol, J. A. S. Zuanazzi, *Chromatographia*, **67**, 125 (2008).
22. L. Krenn, I. Unterrieder, R. Ruprechter, *J. Chromatogr., B*, **777**, 123 (2002).
23. G. Grynkievicz, H. Ksycinska, J. Ramza, J. Zagrodska, *Acta Chromatographica,* **15**, 35 (2005).
24. N. L. Booth, C. R. Overk, P. Yao, S. Totura, Y. Deng, A. S. Hedayat, *J. Agric. Food Chem.*, **54**, 1277 (2006).
25. E. Gikas, A. Alesta, G. Economou, A. Karamanos, A. Tsarbopoulo, *J. Liq. Chromatogr. R. T.,* **31**, 1181 (2008).
26. E. Sivesind, P. Seguin, *J. Agric. Food Chem.*, **53**, 6397 (2005).
27. P. Seguin, W. Zheng, A. Souleimanov, *J. Agron. Crop Sci.,* **190**, 211 (2004).
28. E. Vilčinskas, G. Dabkevičienė, *Žemdirbystė-Agriculture*, **96(4)**, 170 (2009).
29. J. Fraser, D. McCartney, H. Najda, Z. Mir, *Can. J. Plant Sci.*, **84**, 143 (2004).

Giedrė Dabkevičienė, Bronislava Butkutė, Nijolė Lemežienė, Valdas Jakštas, Egidijus Vilčinskas, Valdimaras Janulis

FORMONONETINO, DAIDZEINO IR GENISTEINO PAPLITIMAS TARP *TRIFOLIUM* RŪŠIŲ IR PASISKIRSTYMAS ANTŽEMINĖSE AUGALO DALYSE

S a n t r a u k a

Izoflavonai yra sveikatai svarbūs fenoliniai fitojunginiai. Dobilai yra vienas iš alternatyvių jų šaltinių. Mūsų tyrimai buvo skirti nustatyti trijų izoflavonų kiekį įvairių dobilų rūšių augalų antžeminėse dalyse (efektyviosios skysčių chromatografijos metodu) bei šių fitoestrogenų perspektyviausių šaltinių paieškai. Izoflavonų kiekis ištirtas 11 daugiamečių ir 4 vienametėse dobilo (*Trifolium*) genties rūšyse. Priklausomai nuo antžeminės augalo dalies ir daugiamečių dobilų rūšies, formononetino, daidzeino ir genisteino koncentracijos kito atitinkamai šiose ribose: <Aptikimo riba (LOD) – 2,19, <LOD – 1,42 ir <LOD – 2,90 mg g^{-1}. Pagal bendrą trijų tirtų izoflavonų kiekį lapuose–stiebuose–žieduose daugiametės rūšys išsidėstė taip: *T. medium* (6,01–3,50–2,93 mg g^{-1}) > *T. pratense* > *T. fragiferum* > *T. alpestre* > *T. rubens* > *T. ochroleucum* > *T. pannonicum* > *T. hybridum* > *T. ambiguum* > *T. repens* > *T. montanum* (0,092–0,022–0,090 mg g^{-1}). Pagal bendrą izoflavonų kiekį visoje antžeminėje augalo dalyje vienametės rūšys išsirikiavo: *T. incarnatum* (3,27 mg g^{-1}) > *T. campestre* > *T. resupinatum* > *T. alexandrinum* (0,517 mg g^{-1}).