JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

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Isolation, Chemical and Free Radical Scavenging Characterization of Phenolics from *Trifolium scabrum* L. Aerial Parts

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Supporting Information

ABSTRACT: For the first time *Trifolium scabrum* L. was researched for its phenolic profile. Sixteen phenolics (isoflavones and flavonoids) were isolated and identified in the aerial parts of *T. scabrum* L. Their structures were established by electrospray ionization−mass spectrometry (ESI-MS) and nuclear magnetic resonance (NMR) techniques. Quantitative analysis of individual phenolics performed by means of ultraperformance liquid chromatography−mass spectrometry (UPLC-MS) was based on calibration curves obtained for previously isolated standard compounds. Additionally, the free radical scavenging potential of these substances was assessed by means of a simple benchtop thin-layer chromatography−2,2-diphenyl-1-picrylhydrazyl radical (TLC-DPPH$^{\bullet}$) bioassay. Thus, *T. scabrum* L. can be regarded as a potential source of estrogenic and antioxidant compounds, both of significance in the pharmaceutical as well as the food industry. The results show that *T. scabrum* L. can be considered as a natural and very good commercial source of phenolic compounds (mainly isoflavones).

KEYWORDS: *Trifolium scabrum L., isoflavones, TLC-DPPH$^{\bullet}$ test, free radical scavengers*

INTRODUCTION

Isoflavones are a subclass of phenolics and are also described as phytoestrogen compounds, because they exhibit estrogenic activity. Natural isoflavones represent the main class of phytoestrogens of current interest in clinical nutrition. Clinical studies have suggested a positive effect of isoflavonoids in human health and nutrition, such as a decreased risk of heart disease, hormonally dependent cancers,[1] menopausal symptoms, cardiovascular disease, and osteoporosis.[2,3] On the basis of their structural similarity with 17-β-estradiol, isoflavones are able to bind to estrogen receptors (ERs).[4] Among the isoflavones, genistein is the most active estrogen, with the highest binding affinity for the estrogen receptor.[5] Isoflavones have a very limited distribution in the plant kingdom with substantial quantities being found only in leguminous species.[6] In plants, they are found as glucoconjugates, which are biologically inactive. They are hydrolyzed to active forms (aglycones) by the action of intestinal bacteria. In humans, daidzein and genistein are considered to be the most important biologically active forms of isoflavones.

Trifolium pratense L. (red clover) is (besides soybean) one of the plant source of these compounds. Extracts of red clover are used in dietary supplements and medicinal products as tablets (e.g., Promensil), capsules, tea, liquid preparations, and several other forms.[7,8] Red clover is a medicinal herb used to treat whooping cough and gout,[9] and others have used it in the treatment of asthma, bronchitis, coughs, athlete's foot, eczema, and psoriasis.[10] *T. pratense* L. isoflavone supplementation in postmenopausal women significantly decreased menopausal symptoms and had a positive effect on vaginal cytology and triglyceride levels.[3,11−13] Extracts of *T. pratense* L. are commercially available as isoflavone-enriched dietary supplements meant for long-term use as a phytoestrogen source and "natural" form of hormone replacement therapy.[14]

Preliminary analysis of 57 *Trifolium* species for isoflavone concentration showed that *Trifolium scabrum* L. (rough clover) has a higher total isoflavone content than *T. pratense* L., 72.76 versus 39.51 mg/g dry matter (equivalent of daidzin), respectively.[15] Our preliminary results demonstrated also that the phenolic fraction of *T. scabrum* L. aerial parts has antiplatelet properties[16] and is active in reduction of plasma oxidative stress simulated by hyperhomocysteinemia.[17] We may suggest that the composition of chemical profiles of the phenolic fraction may explain these processes. On the basis of these results the main focus of our research was to check whether *T. scabrum* L. can be considered as a natural and very good commercial source of phenolic compounds.

To evaluate whether the isolated compounds possess direct antioxidant properties, a simple TLC-DPPH$^{\bullet}$ test was applied. The TLC-DPPH$^{\bullet}$ assay can be regarded as one of the popular tests performed for assessing free radical scavenging ability of individual components found in plant extracts.[18] It has been used, for example, in the so-called biologically guided fractionation, which led to isolation of antioxidants present in plant extracts.[19] The popularity of this assay stems from a

Received: January 14, 2013
Revised: April 8, 2013
Accepted: April 15, 2013
Published: April 15, 2013

Table 1. MS Parameters and Calibration and Concentration Data for 16 Phenolics in *Trifolium scabrum* L.

no.	compound	time[a] (min)	concentration[b] (mg/g dw)	parent → daughter transition[c] (*m*/*z*)	cone voltage (V)	collision energy (eV)	LOD[d] (μg/g)	LOQ[d] (μg/g)	slope	intercept	R^{2e}
1	3′-hydroxydaidzin	2.17 ± 0.00	0.391 ± 0.007	431 → 269	45	15	0.042	0.140	990.6	−168.8	1.0000
2	daidzin	2.99 ± 0.00	10.930 ± 1.284	*461 → 415, 253	30	20	0.046	0.152	756.8	624.9	0.9992
3	isoquercitrin	3.96 ± 0.01	3.832 ± 0.143	463 → 300	40	25	0.054	0.181	1131.0	−59.7	0.9999
4	calycosin-7-*O*-β-D-glucoside	3.97 ± 0.00	1.199 ± 0.032	*491 → 283	35	15	0.090	0.299	409.0	−5.7	0.9998
5	genistin	4.14 ± 0.00	2.359 ± 0.070	431 → 268	45	25	0.044	0.146	661.9	118.0	0.9996
6	astragalin	4.77 ± 0.01	0.208 ± 0.011	447 → 284	60	25	0.012	0.041	1707.0	10.5	0.9996
7	6″-*O*-acetyl-daidzin	4.78 ± 0.01	6.347 ± 1.375	457 → 253	20	10	0.195	0.651	247.2	36.1	0.9995
8	pratensein-7-*O*-β-D-glucoside	5.21 ± 0.01	2.323 ± 0.084	461 → 299	40	10	0.212	0.708	145.7	11.9	0.9987
9	pseudobaptigenin-7-*O*-β-D-glucoside	5.90 ± 0.00	1.673 ± 0.070	*489 → 281	35	15	0.010	0.034	1633.0	77.6	1.0000
10	daidzein	5.98 ± 0.00	2.301 ± 0.071	253 → 132	65	35	0.438	1.461	1403.0	12.9	0.9995
11	ononin	6.30 ± 0.00	5.901 ± 0.227	*475 → 267	35	15	0.012	0.040	2122.0	609.4	0.9994
12	genistein	7.65 ± 0.01	0.142 ± 0.018	269 → 133	50	30	4.126	13.753	551.3	−111.9	0.9995
13	formononetin-7-*O*-β-D-glucoside-6″-*O*-malonate	7.82 ± 0.00	3.532 ± 0.010	515 → 267	18	10	0.053	0.175	1006.0	253.1	0.9999
14	sissotrin	8.22 ± 0.00	2.281 ± 0.140	445 → 283	40	10	0.180	0.600	467.0	82.3	0.9995
15	formononetin	9.42 ± 0.00	0.286 ± 0.008	267 → 252	45	22	0.434	1.448	6490.0	−275.2	0.9999
16	biochanin A	10.43 ± 0.00	0.060 ± 0.017	283 → 268	45	25	4.566	15.220	2357.0	−405.0	0.9999

[a]Retention times. Values are means ± SD (standard deviation) (n = 27) of intraday assays. [b]Values are means ± SD (n = 3). [c]An asterisk indicates an adduct with formic acid. [d]Limits of detection (3 S/N) and limit of quantitation (10 S/N). Values are based on a dry matter basis. [e]Regression coefficients. Calibration range of 0.1−20 μg/mL was used.

dx.doi.org/10.1021/jf400153c | J. Agric. Food Chem. 2013, 61, 4417−4423

variety of TLC advantages, namely, flexibility, high sample throughput, direct access to separated components, ability to detect the activity of individual compounds present in complex samples, easy accessibility by both sophisticated and poorly equipped laboratories, and still others. As it has been shown recently, the test can be used to obtain quantitative results, as well, giving the possibility to compare compounds with different free radical scavenging activities.[20,21]

T. scabrum L. has never been researched for its phenolic profile. Thus, the aim of the present research was to isolate and identify phenolics in the aerial parts of this plant species and to check their possible free radical scavenging abilities (mainly flavonoids and isoflavones).

MATERIALS AND METHODS

Plant Material. Seeds of *T. scabrum* L. were obtained from Genebank, Zentralinstitute fűr Pflanzengenetik and Kulturpflanzenforchung (Gatersleben, Germany). The origin of this annual species of the genus *Trifolium* is Portugal, and its herbarium voucher number is TRIF 120/79. The plants were cultivated (1 m × 1 m plot) in an experimental field of the Institute of Soil Science and Plant Cultivation−State Research Institute in Pulawy, Poland. Plants were harvested at the beginning of flowering in August 2011. The plant material directly after harvest was frozen in the laboratory freezer (−18 °C) and then lyophilized using a Gamma 2-16 LSC freeze-dryer from Martin Christ Gefriertrocknungsanlagen GmbH, Germany (time, 3 days; pressure, 1 mbar). The *T. scabrum* L. aerial parts were powdered using a ZM 200 ultra centrifugal mill (Retsch); this is a modern grinder, which does not cause sample warm-up (12 tooth rotor, sieve size = 0.50 mm). The plant material was then used for successive extraction.

Chemical Reagents. 2,2-Diphenyl-1-picrylhydrazyl radical (DPPH•) was purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). The solvents used in the study (methanol, acetonitrile, formic acid, toluene, chloroform, ethyl acetate, 85% orthophosphoric acid, acetic acid) were of the analytical purity grade, purchased from J. T. Baker (Deventer, The Netherlands). Fractions were analyzed by TLC (25DC − Alufilien 20 cm × 20 cm cellulose; Merck, Darmstadt, Germany). The TLC-DPPH• test was performed on the surface of aluminum-backed silica gel 60 F254 plates (Merck). The standard solution of phenolics was prepared in a concentration of 1 mg/mL.

Extraction and Isolation of Phenolic Standards. Powdered aerial parts of *T. scabrum* L. (350 g) were extracted with 80% aqueous MeOH at room temperature. After 24 h, the extract was filtered and the solvent was removed under reduced pressure (35 °C). The crude extract was suspended in water, and the solution was applied to a 6 cm × 10 cm, 40−63 μm LiChroprep RP-18 (Merck) preparative column previously preconditioned with water. The column was washed first with water to remove sugars and then with 40% MeOH to elute phenolics. Using analytical high-performance liquid chromatography (HPLC) as a check, the fraction washed out with 40% MeOH was used for isolation of phenolics according to a previously used protocol.[22] In short, the 40% MeOH fraction was condensed nearly to dryness in vacuo, redissolved in distilled water, and loaded onto a preparative column (4 cm × 50 cm, 40−63 μm LiChroprep RP-18; Merck). The column was washed with water and then with increasing concentrations of MeOH in water (5% increments from 0 to 100% MeOH). Ten milliliter fractions were collected, checked by TLC (DC − Alufilien cellulose; Merck), developed in 15% acetic acid, and observed under long-wavelength UV (366 nm) illumination. Fractions showing similar TLC patterns (40 fractions) were further analyzed by HPLC. The HPLC analyses were performed on a Waters system (Waters Corp., Milford, MA, USA) equipped with a model 616 pump, a model 600s controller, and a model 996 PAD detector. Millenium Chromatography Manager software (Waters Corp.) was used to monitor chromatographic parameters and to process the data. Twenty microliters of each fraction was injected for analysis. Fractions were applied to a Eurospher-100 C18 column (Knauer, Germany) (0.4 cm × 25 cm i.d., 5 μm) and eluted at 1 mL/min with a linear gradient of 1% H_3PO_4 in water/60% MeOH in 1% aqueous H_3PO_4 (80:20%), increasing to 0:100% over 60 min. The column was maintained at 50 °C. Fractions possessing one compound were combined and evaporated to dryness. Fractions containing more than one compound were further purified on a C18 glass column (2 cm × 50 cm, 25−40 μm; Merck) using an isocratic system (MeOH/1% H_3PO_4). Sixteen individual compounds were obtained.

Determination and Quantification of Phenolics Composition (NMR, UPLC, and MS analyses). NMR experiments were performed on a Bruker Avance-500 spectrometer (Bruker BioSpin GmbH, Rheinstetten, Germany) equipped with a 5 mm probe. The ^{1}H NMR spectra (at 500 MHz) were measured at 300 K. Samples were dissolved in 500 μL of CD_3OD. Chemical shifts are given on the δ scale and referenced to tetramethylsilane (TMS) at 0.00 ppm. NMR data of all isolated compounds were collected in tables and are available as Supporting Information.

A Waters ACQUITY UPLC system equipped with a binary pump system, sample manager, column manager, and PDA detector (Waters Corp.) and coupled to a Waters ACQUITY TQD (tandem quadrupole mass detector) with an electrospray ionization (ESI) source was used for quantitative analyses. All data were acquired and processed using Waters MassLynx 4.1 and QuanLynx software. A Waters BEH C18 column (100 mm × 1.0 mm i.d., 1.7 μm particles) was used to separate the analytes. A linear gradient elution program was conducted for chromatographic separation with mobile phase A (Milli-Q water containing 0.1% HCOOH) and mobile phase B (MeCN containing 0.1% HCOOH) as follows: 0.0−0.5 min (7% B); 8.0 min (25% B); 11.5 min (60% B); 12.0 min (80% B); 13.0 min (80% B). The flow rate was 0.19 mL/min, and the column temperature was 50 °C. Injection wash solvents were MeCN/H_2O (5:95, v/v) and MeOH/H_2O (95:5, v/v) for weak (900 μL) and strong washes (300 μL), respectively. One microliter of sample was injected for analysis. For MS detection, negative ESI was used as the ionization mode. Nitrogen was used as the desolvation and cone gas with flow rates of 800 and 80 L/h, respectively. Argon was used as the collision gas at a flow rate of 0.2 mL/min. The optimal MS parameters were as follows: capillary, 2.8 kV; extractor and radiofrequency voltage were fixed to 3.0 and 0.1 V, respectively; source temperature, 140 °C; desolvation temperature, 350 °C. Cone voltages (varied from 18 to 65 V) and collision energies (varied from 10 to 35 eV) are presented in Table 1. Dwell time was from 0.02 to 0.04 s. Quantification was performed using the selected reaction monitoring (SRM) mode (parent and daughter ions are presented in Table 1).

TLC-DPPH• Test. Both standard compounds as the analyzed fraction (Ex) were applied to aluminum-backed silica gel chromatographic plates by means of a micropipet with a scale. All of the samples were applied band-wise (5 mm wide), with a distance of 10 mm between them and a 10 mm distance from both the left and low edge of the plate. The plates were developed in vertical chambers presaturated for 15 min with proper mobile phase. The plates were developed to the distance of 90 mm. Then 5 μL aliquots of standard solutions and 10 μL of the analyzed fraction were applied onto the plates. As the analyzed extract contained compounds characterized with a wide range of polarity, different chromatographic systems were used to screen the free radical scavenging potential of individual compounds. The following mobile phases were used in the study: toluene/ethyl acetate/formic acid (60:30:5, v/v/v), for the least polar compounds; ethyl acetate/formic acid (95:5, v/v), for the medium polar constituents; and chloroform/methanol/ethyl acetate/water (20:40:22:10, v/v/v/v) for the most polar ones. The plates were dried in a hood for 10 min before derivatization. TLC plates were immersed for 5 s in freshly prepared 0.2% (w/v) methanolic DPPH• solution. After removal of DPPH• excess, plates were kept in the dark for 30 min and then scanned by means of a flatbed scanner. A comparison of the results was performed by means of an image processing program, ImageJ, according to an already published protocol with slight modifications.[21]

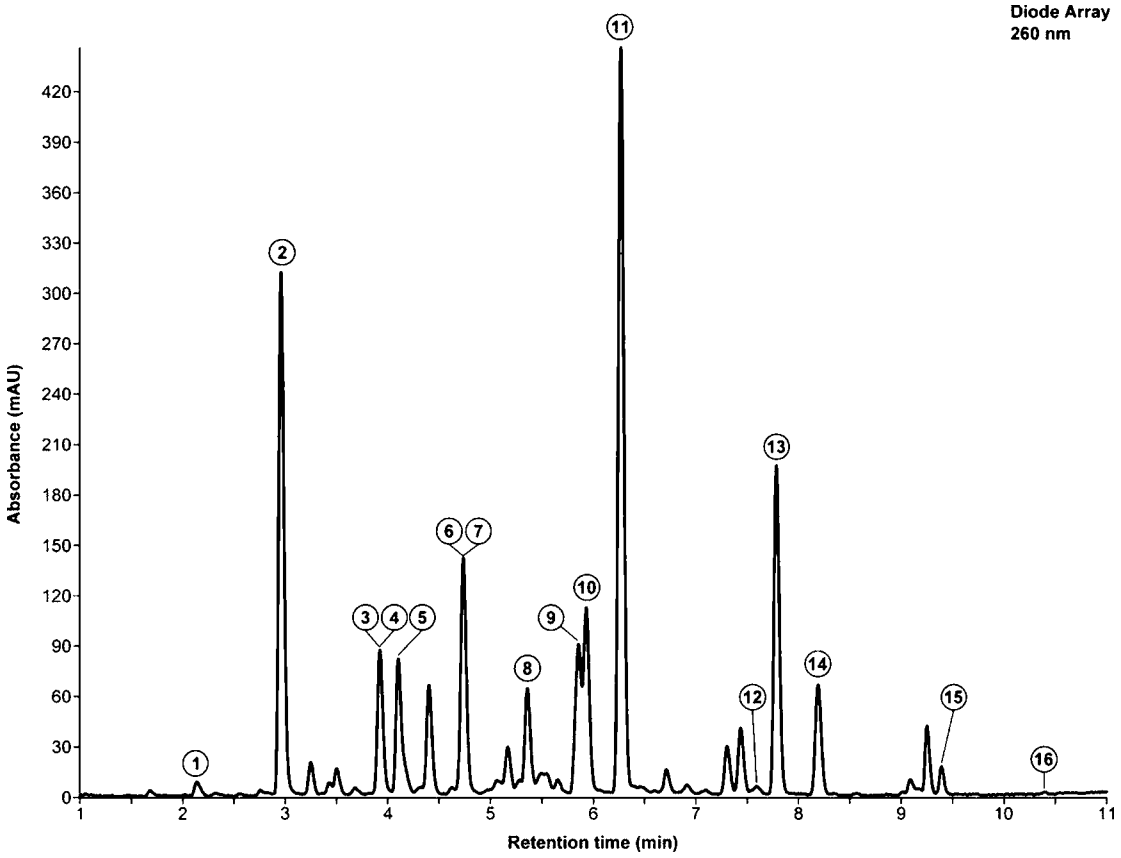
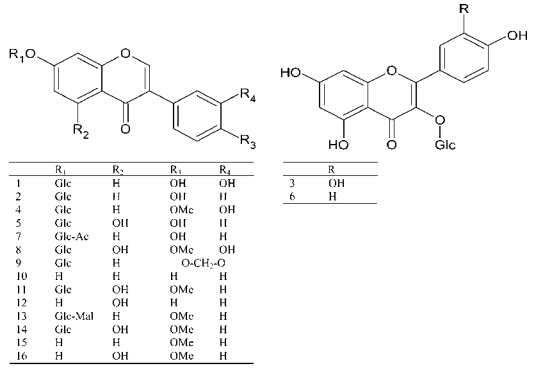


Figure 1. UPLC profile of *T. scabrum* extract (peak numbers correspond to the numbering of phenolics in Table 1).

Statistical Analysis. Each calibration curve was constructed by running standards of six different concentrations in triplicate. All samples were prepared in triplicate, and chromatographic analyses were performed in triplicate.

RESULTS AND DISCUSSION

Analysis of Phenolics. Analysis of the flavonoid profile obtained from analytical liquid chromatography of the methanol extract of the aerial green parts of *T. scabrum* L. using photodiode array detection showed the presence of a number of phenolic compounds (Figure 1). Separation of the compounds by low-pressure liquid chromatography followed by purification of fractions on a semipreparative C18 column afforded 16 single compounds for which structures (Figure 2) have been determined by spectrometric and spectroscopic (ESI/MS, NMR) techniques. Among 16 isolated compounds from *T. scabrum* L. extract, 14 were characterized as isoflavones (aglycones or their glucosylated derivatives) and another 2 were flavonol glucosides. These 16 single compounds have not been previously reported in *T. scabrum* L. Glucosides have been reported as the major forms of isoflavones in *T. scabrum* L. All of the isolated phenolics were identified by comparing UV spectra, ESI-MS data, and ^{1}H NMR data with those found in the literature. The absolute configuration of glucose was assumed to be D, consistent with the stereochemistry of the naturally occurring isomer. The identities, retention times, and spectral information for individual isolates are presented in Table 1 and were in good agreement with reported data for similar structures isolated from different plant sources.[23−25] ^{1}H NMR analysis confirmed the structures of obtained standards, and for all isolated compounds the literature data were fully accessible.[26−28]



	R_1	R_2	R_3	R_4
1	Glc	H	OH	OH
2	Glc	H	OH	H
4	Glc	H	OMe	OH
5	Glc	OH	OH	H
7	Glc-Ac	H	OH	H
8	Glc	OH	OMe	OH
9	Glc	H	O-CH$_2$-O	
10	H	H	H	H
11	Glc	OH	OMe	H
12	H	OH	H	H
13	Glc-Mal	H	OMe	H
14	Glc	OH	OMe	H
15	H	H	OMe	H
16	H	OH	OMe	H

	R
3	OH
6	H

Figure 2. Structures of isolated isoflavones, isoflavone glucosides, and flavonol glucosides. For symbols see Table 1.

Various plants, specifically those belonging to the Leguminosae and Graminae families, are the major sources of various dietary phytoestrogens. Three of the major isoflavones in soybeans are daidzein, genistein, and glycitein and their respective acetyl, malonyl, and aglycone forms.[29] The bioavailable isoflavones (aglycones) are formed by the hydrolysis of glycosides through β-glucosidase present in soybean.[4,30] Other isoflavonoids reported in red clover are formononetin and biochanin A, which are methoxylated derivatives of genistein and daidzein, respectively. The beneficial effects of red clover isoflavones are very similar to those found with soy isoflavones. Although the isoflavone profile of red clover is known to vary from that observed in

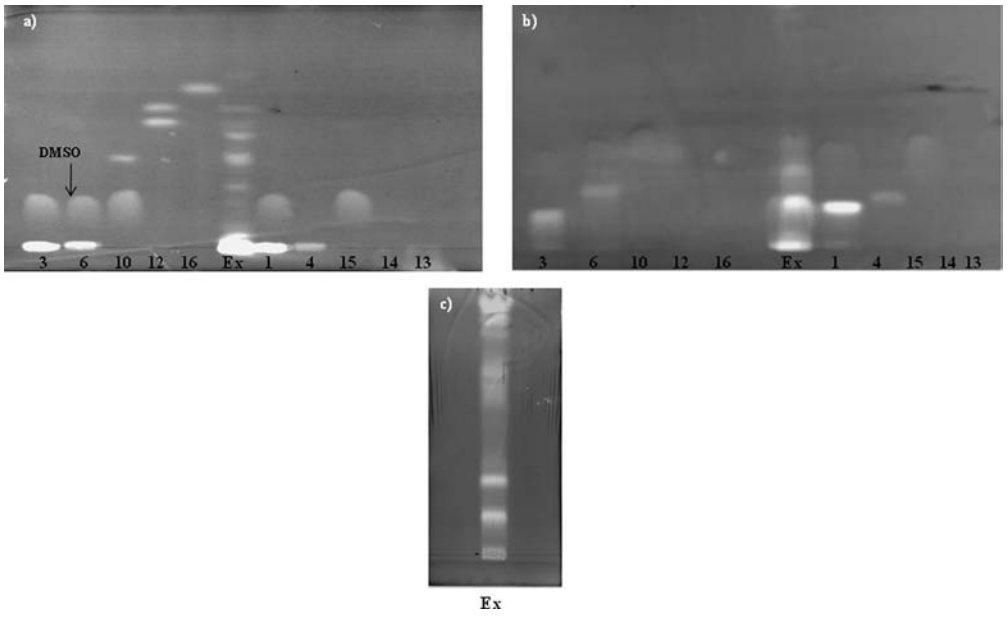


Figure 3. Results of TLC-DPPH• test performed for the investigated phenolic compounds. TLC plates were developed by means of the following mobile phases: (a) toluene/ethyl acetate/formic acid (60:30:5, v/v/v); (b) ethyl acetate/formic acid (95:5, v/v); (c) chloroform/methanol/ethyl acetate/water (20:40:22:10, v/v/v/v) and further dipped into 0.2% (w/v) DPPH• methanolic solution. For symbols see Table 1.

soybeans, their final metabolites, showing phytoestrogenic properties in humans, are similar.[31]

Phytochemical Analysis. Qualitative study revealed that *T. scabrum* L. contained a large amount of isoflavones, which are mainly available as isoflavone glucoside conjugates. The individual phenolics, isolated by preparative column chromatography, were used as a standards for quantitative determination in *T. scabrum* extracts. These determinations were carried out using the external standard method and external calibration curves. Some validation data are given in Table 1. The application of UPLC for determination of phenolics allowed us to separate these compounds in quite a short time of 11 min (Figure 1). The triple-quadrupole mass detector and the SIR mode were used for determination.

The total phenolics concentration in *T. scabrum* determined with UPLC was 45.64 ± 3.57 mg/g of aerial part dry matter (isoflavones, 41.60 ± 3.41 mg/g dry weight (DW)). Daidzein and its derivatives represented 44% of total phenolics content in *T. scabrum* aerial parts. Predominant isoflavones were daidzin, 6″-*O*-acetyl-daidzin, and ononin. Concentrations of these compounds were 10.93, 6.35, and 5.90 mg/g DW, respectively (Table 1). In comparison with soybean and red clover, *T. scabrum* L. aerial parts are a particularly abundant source of isoflavones. Soybeans and soy products contain approximately 0.2−1.6 mg of isoflavones/g DW.[32] Wang and Murphy[29] reported that soybeans contain 1.2−4.2 mg of total isoflavones/g of sample. In another paper it has been reported that the total isoflavone concentration in 10 cultivars (whole plants) ranged between 8.92 and 12.75 mg/g of dry matter (DM) averaged across sites, harvests, and years.[33] Eldridge and Kwolek[34] reported that total isoflavone content ranged from 1.16 to 3.90 mg/g DW in four soybean cultivars. Zgorka[35] quantified the amount of four isoflavones in *T. pratense*, and their total concentration was approximately 2.29 mg/g DW. According to Oleszek et al.,[15] total isoflavone concentration in red clover may range from 11.80 to 39.51 mg/g DW. Wu et al.[24] reported the total isoflavone content in different plant parts of red clover ranged from insignificant amounts to approximately 2.90 mg/g DW. Concentrations and profiles of these compounds may be affected by many factors, such as the effects of environment, cultivar, plant maturity, plant part, preservation method, soil conditions, crop year, and growth location.[33,36] The results of this study indicate that *T. scabrum* L. is an alternative new source of isoflavones other than soybean and red clover.

DPPH• Scavenging Activity of Isolated Compounds. From among the isolated and identified compounds, 3′-hydroxydaidzein-7-*O*-glc (**1**), isoquercitrin (**3**), calycosin-7-*O*-glc (**4**), kaempferol-3-*O*-glc (astragalin) (**6**), daidzein (**10**), genistein (**12**), and biochanin A (**16**) showed antiradical activity, as seen in Figure 3a,b. Contradictory reports exist on the free radical scavenging ability of isoflavones in the literature. The results obtained in the present TLC-DPPH• assay indicate that the following isoflavone aglycones do possess antiradical activity: daidzein, genistein, and biochanin. Formononetin was found to be inactive in the performed experiments. This compound possesses only one hydroxyl group, which is apparently insufficient to scavenge DPPH• molecules, in the performed test. An interesting thing can be noted when one compares the free radical scavenging abilities of daidzein and 3′-hydroxydaidzein-7-*O*-glc. The latter is characterized with very potent antiradical activity when compared to other isoflavones. Such enhanced antiradical properties can be ascribed probably to the presence of the catechol moiety (3′,4′-dihydroxy group in the B ring), which was found to favor the free radical scavenging activity of some polyphenols (flavonoids and phenolic acids).[37,38] It is an interesting observation, as isoflavones have been mostly considered to be relatively poor hydrogen donors.[39,40] Although this compound is present in relatively small quantities, it is characterized with potent free radical scavenging activity, as seen in Figure 3a. Antiradical properties were also observed for calycosin-7-*O*-glc, a compound with neighboring hydroxyl and methoxy groups in the B ring (Figure 3b). Although the other isoflavones with a sugar moiety attached to the 7-hydroxy group were inactive in the test (biochanin A 7-*O*-glc and formononetin-7-*O*-glc), the

aforementioned compound scavenged free radical molecules. Such enhanced antiradical activity can be explained in this case by analogy with phenolic acids possessing neighboring hydroxyl and methoxy groups, for example, ferulic acid.[38] In the majority of papers found in the literature authors indicate a poor correlation between the amount of isoflavones and free radical scavenging activity of different *Trifolium* species extracts.[41] As can be seen in Figure 3a, apart from isoflavones some identified flavonoids were also potent free radical scavengers. It is apparent that the analyzed extract contains also polar constituents (probably flavonoid glycosides) with DPPH$^{\bullet}$ scavenging properties (Figure 3c). Apart from the yellow spots of active ingredients, blue bands were also observed on some tracks (Figure 3a). They are due to the dimethyl sulfoxide (DMSO) that was used for preparing stock solutions of some of the compounds, as also reported by Ciesla et al.[42] The DMSO bands do not coelute with the identified free radical scavengers; thus, the addition of it as cosolvent does not influence the results of TLC-DPPH$^{\bullet}$ assay. In the case of genistein (**12**) another band can also be observed in Figure 3a. HPLC analyses performed after the isolation procedure show the presence of a small peak of impurity. In the TLC-DPPH$^{\bullet}$ test the impurity (unknown compound) also gave positive results; it also possesses free radical scavenging activity. However, to compare the quantitative results obtained with TLC-DPPH$^{\bullet}$ and the image processing procedure, the activity was calculated according to the genistein content. Taking into account the above-mentioned results, it may be concluded that non-isoflavone polyphenolic compounds can be mostly responsible for free radical scavenging ability of clover extracts, which confirms the assumptions of Kroyer.[41] It explains also the lack of correlation between total isoflavone content and antioxidant activity, as reported in the majority of scientific papers.[43] *T. scabrum* L. extract can be proposed to be used as a functional food ingredient or dietary food supplement due to the presence of a variety of phenolic compounds with proven antiradical activity. The obtained results indicate also that modification of aglycone structures may influence the observed free radical scavenging properties of isoflavones. This can constitute the basis for synthesis of new isoflavone-derived molecules with chain-breaking antioxidant properties.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Tables 1S and 2S. This material is available free of charge via the Internet at http://pubs.acs.org.

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Funding

The work was supported from the seventh Framework Program of European Community, PROFICIENCY (Contract 245751) and by statutory activity of the Institute of Soil Science and Plant Cultivation − State Research Institute (1.06, 2012−2014).

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank Prof. Dr. A. Graner of Genebank, Zentralinstitut für Pflanzengenetik und Kulturpflanzenforchung (Gatersleben, Germany), for providing authenticated *Trifolium scabrum* L. seeds.

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