Chemistry of Natural Compounds, Vol. 42, No. 5, 2006

LC-MS ANALYSIS OF DAIDZEIN IN THE TURKISH *Genista* SPECIES

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UDC 547.972

In this research, total and free daidzein content in Genista *species growing in Turkey were investigated using the LC-MS method. The highest amount of total and free daidzein in these species was found in* Genista sessilifolia *and* G. lydia *var. antiochia as 0.0056 and 0.0009%, respectively. Total and free daidzein content of the aerial parts of other* Genista *species varied from 0.0003 to 0.0044%, and from 0.0001 to 0.0008%, respectively.*

Key words: *Genista* L., Fabaceae, isoflavones, daidzein, LC-MS.

Daidzein (7,4′-dihydroxyisoflavone) is known as a phytoestrogen because of its estrogenic activity in humans. Phytoestrogens are polyphenolic nonsteroidal plant compounds with estrogen-like biological activity. Phytoestrogens can be classified into four main groups: isoflavonoids, flavonoids, stilbenes, and lignans. Isoflavones, the largest group of natural isoflavonoids, have up to now received the most attention, especially genistein, daidzein, and their respective 4-methyl ether derivatives, biochanin A and formononetin [1–4]. Phytoestrogens may have protective effects on estrogen-related conditions such as menopausal symptoms [5, 6], and estrogen-related diseases such as prostate [7], breast cancer [8], osteoporosis [9], and cardiovascular diseases [10].

Isoflavones are widely distributed in the Fabaceae family. Especially, soy (*Glycine max* (L.) Merr.) is a very rich source of isoflavones, mainly daidzein and genistein [11–13]. Isoflavone phytoestrogens based on structural resemblance to 17β-estradiol have been found to exert potential health benefits in age-related and hormone dependent diseases. Therefore, various commercial preparations of isoflavone-rich soy extracts as nutritional supplements or phytopharmaceuticals are sold in increasing demand in the market worldwide [1, 4, 14].

There are thirteen *Genista* L. (Fabaceae) species in the Flora of Turkey and the East Aegean Islands; among these species *G. aucheri, G. burdurensis, G. sandrasica,* and *G. involucrata* are endemic [15, 16]. Recently, *G. vuralii* from Central and North Anatolia in Turkey has been described [17]. Adding the species described here raised the number of Turkish *Genista* species to 14. In our works on *Genista* species, we previously isolated alkaloids from eleven *Genista* species, as well as flavonoids from *G. aucheri* and *G. involucrata* [18–20].

Several methods to characterize the content of isoflavones, including genistein and daidzein, using high-performance liquid chromatography (HPLC), liquid chromatography combined with ultraviolet and/or electrospray ionization-mass spectrometry (LC-MS), and gas chromatography-mass spectrometry (GC-MS) have been reported [21–25]. In our previous study, the aerial parts of eleven *Genista* species growing in Turkey were analyzed for their total and free genistein content using the LC-MS method. The highest amount of total and free genistein found in *G. tinctoria* was 1.05 and 0.27%, respectively [21]. The purpose of this research was to study the total and free daidzein content in eleven *Genista* species growing in Turkey (Table 1) using the LC-MS method.

The total and free daidzein amount of eleven *Genista* species growing in Turkey are given in Table 1. The amounts of daidzein were calculated from the linear regression equation obtained from the daidzein standard curve, which was linear over the concentration range 0.2–5 μg/mL. The regression equation and correlation coefficient determined for daidzein were $y = 275488.17\ x + 31333.78$ and $r^2 = 0.999$. The limit of detection was 0.02 μg/mL. The assay was rapid and simple to perform.

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0009-3130/06/4205-0517

TABLE 1. Collection Sites and Daidzein Contents of *Genista* Species

Plant	Collected Area	Total daidzein, %	Free daidzein, %
G. acanthoclada DC	Zeytinalan, Izmir	0.0044	0.0002
C. anatolica Boiss.	Bornova, Izmir	0.0004	0.0001
G. sessilifolia DC.	Lalahan, Ankara	**0.0056**	0.0008
G. aucheri Boiss.	Seyitgazi, Eskisehir	0.0003	0.0001
G. carinalis Gris.	Kemalpassa-Ovacik, Izmir	0.0044	0.0002
G. involucrata Spach.	Akdagmadeni, Yozgat	0.0027	0.0004
G. albida Willd.	Beynam, Ankara	0.0038	0.0005
G. tinctoria L.	Unye, Ordu	0.0007	0.0002
G. burdurensis P. Gibss	Yesilova, Burdur	0.0015	0.0006
G. lydia Boiss. var. lydia	Sipyl Mt., Manisa	0.0028	0.0007
G. lydia var. antiochia (Boiss) P. Gibbs	Bulke-Dortyol, Hatay	0.0037	**0.0009**
G. libanotica Boiss.	Sarigol-Iskenderun, Hatay	0.0003	0.0001

As shown in Table 1, the highest amount of total and free daidzein was found in *Genista sessilifolia* and *G. lydia* var. *antiochia* as 0.0056 and 0.0009%, respectively. The total and free daidzein content of the aerial parts of other *Genista* species varied from 0.0003 to 0.0044% and from 0.0001 to 0.0008%, respectively. To the best of our knowledge, this is the first report of the content of daidzein in Turkish *Genista* species by LC-MS analysis.

EXPERIMENTAL

Plant materials were collected during flowering periods from different localities. Authenticated voucher specimens were deposited in the Pharmaceutical Botany Department Herbarium at Ankara University (AEF) Ankara, Turkey. Collection sites for each plant material are given in Table 1.

An authentic compound, daidzein, was obtained from Sigma Chem. (St. Louis, MO, USA). HPLC grade methanol (Merck, Darmstadt, Germany) and bidistilled water were used for chromatographic studies.

Extraction. Air-dried aerial parts of each plant were milled homogenously and 1 g was precisely weighed. The powdered materials were extracted with methanol under ultrasonic vibration [21]. This extracts were analyzed for free isoflavones.

One gram of precisely weighed aerial parts of plant materials was hydrolyzed by heating with a mixture of an equal volume of methanol and 2 N HCl for 1 h under reflux. After filtering the mixture, 1 mL filtrate was diluted with 9 mL water and loaded on to a Sep-Pak C_{18} cartridge (Waters). Isoflavones were retained on the Sep-Pak C_{18} cartridge, which was then washed with 10 mL of water twice and eluted with 70% methanol [21]. This extracts were analyzed for total isoflavones.

LC-MS Analysis. LC-MS analysis was performed on a ThermoQuest Finnigan AQA Mass Spectrometer linked to a ThermoQuest Spectra System Liquid Chromatograph. Separation was achieved on a C_{18} Phenomenex column, 50×4.6 mm, particle size 3 μm. The mobil phase was methanol–water (60:40, v/v) and the flow rate was 0.5 mL/min. The probe temperature was at 300°C. The system was operated at room temperature (25°C) [21]. Analyses were performed in the Single-Ion-Monitoring (SIM) mode. Positive and negative ion mass spectra of daidzein were recorded in the Electrospray ion mode. Although the molecular $[M+H]^+$ ion was observed in the positive ion mode, $[M-H]^-$ ion was the most abundant ion in the negative ion mode. Therefore, the analyses were carried out in the negative ion mode.

Preparation of Standards. A set of eight standard solutions was prepared containing 0.2, 0.25, 0.5, 1, 1.25, 1.66, 2.5, and 5 μg/mL of daidzein.

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