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Determination of genistein in the Turkish *Genista* L. species by LC-MS

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In this study, *Genista* species growing in Turkey were analysed for their total and free genistein content by using LC-MS method. The highest amount of total and free genistein was found in *Genista tinctoria* as 1.05% and 0.27% respectively and trace amount in *G. libanotica*. Total and free genistein content of the aerial parts of other *Genista* species varied from 0.01 to 0.18% and from 0.01 to 0.13% respectively.

1. Introduction

The phytoestrogen genistein (4',5,7-trihydroxyisoflavone) has estrogenic, antiestrogenic, antioxidant, anticarcinogenic, antiaetherogenic, antiosteoporotic and antiandrogenic activities [1–6]. Genistein and its glycosides are mainly found in the family Leguminosae [7–9].

The genus *Genista* L. (Fabaceae) is represented by eleven species in the Turkish flora [10]. Among these species *G. aucheri*, *G. burdurensis* and *G. involucrata* are endemic. We previously reported alkaloids from eleven species, flavonoids from *G. aucheri* and *G. involucrata* [11–13]. In this research, the genistein content of Turkish *Genista* species which has not been reported before was investigated.

2. Investigations, results and discussion

In the current research, *Genista* species growing in Turkey were analysed for their total and free genistein content by LC-MS. The amounts of genistein were calculated from the regression equation obtained from a standard curve which was linear over the concentration range 5×10^{-5} – 2×10^{-4} mg \times ml $^{-1}$ ($y = 744330589.1x + 104126.764$, $r^2 = 0.9814$). The assay was rapid and simple to perform. As

shown in the Table, the highest amount of total and free genistein was found in *Genista tinctoria* as 1.05% and 0.27% respectively and trace amounts in *G. libanotica*. Total and free genistein content of the aerial parts of other *Genista* species varied from 0.01 to 0.18% and from 0.01 to 0.13% respectively.

3. Experimental

3.1. Plant material

Voucher specimens of research material used in this investigation were collected in flowering periods and deposited in "Ankara Üniversitesi Eczacılık Fakültesi Herbaryumu", Ankara, Turkey. Their collecting locations are given below. *G. acanthoclada* DC.: Zeytinalan (zmir); *G. anatolica* Boiss.: Bornova (İzmir); *G. sessilifolia* DC.: Lalahan (Ankara); *G. aucheri* Boiss.: Seyitgazi (Eskişehir); *G. carinalis* Gris.: Kemalpaşa-Ovacık (İzmir); *G. involucrata* Spach : Akdağmadeni (Yozgat); *G. albida* Willd.: Beynam (Ankara); *G. tinctoria* L.: Ünye (Ordu); *G. burdurensis* P. Gibbs: Yeşilova (Burdur); *G. lydia* Boiss. var. *lydia*: Sipyl Mt. (Manisa); var. *antiochia* (Boiss) P. Gibbs: Bülke-Dörtlöy (Hatay); *G. libanotica* Boiss.: Sarıgöl-İskenderun(Hatay).

3.2. LC-MS analysis

LC-MS analyses were carried out on a ThermoQuest Finnigan AQA Mass Spectrometer linked to a ThermoQuest Spectra System Liquid Chromatograph equipped with a C₁₈ Phenomenex column (50 \times 4.6 mm, 3 μ m). The mobile phase was methanol (HPLC grade) : water (60 : 40) at a flow rate 0.5 ml \cdot min $^{-1}$. The column and probe temperatures were at 25 and 300 °C respectively. Analyses were performed on Single-Ion-Monitoring (SIM) mode. Positive and negative ion mass spectra of genistein were recorded in the Electrospray ion mode. As the molecular [M + H] $^{+}$ ion was not observed in the positive ion mode, [M – H] $^{-}$ ion was the most abundant ion in the negative ion mode and the analyses were carried in the negative ion mode.

3.3. Sample preparation

Air-dried and powdered aerial parts of plant material (1 g) were extracted with methanol under ultrasonic vibration. This extract was analyzed by LC-MS for free isoflavones.

Air-dried and powdered aerial parts of plant material (1 g) were hydrolysed by heating for 1 h under reflux with a mixture of equal volume methanol and 2 N HCl. After filtering the mixture, 1 ml of the filtrate was diluted with 9 ml water and loaded on to a Sep-Pak C₁₈ cartridge (Waters). Isoflavones were retained on the Sep-Pak C₁₈ cartridge which was then washed with 10 ml of water twice and eluted with 70% methanol. This extract was used for the analysis of total isoflavones.

Table: Genistein contents of studied *Genista* species

Plants	Total genistein (%)	Free genistein (%)
<i>G. tinctoria</i>	1.05	0.27
<i>G. acanthoclada</i>	0.18	0.06
<i>G. lydia</i> var. <i>lydia</i>	0.14	0.13
<i>G. carinalis</i>	0.1	0.03
<i>G. sessilifolia</i>	0.08	0.05
<i>G. lydia</i> var. <i>antiochia</i>	0.06	0.06
<i>G. burdurensis</i>	0.05	0.05
<i>G. anatolica</i>	0.05	0.04
<i>G. involucrata</i>	0.02	0.01
<i>G. albida</i>	0.02	0.02
<i>G. aucheri</i>	0.01	0.01
<i>G. libanotica</i>	Trace	Trace

3.4. Preparation of standards

A set of six standard solutions were prepared containing 5×10^{-5} , 1×10^{-4} , 4×10^{-4} , 8×10^{-4} , 1.6×10^{-4} , 2×10^{-4} mg ml⁻¹ of genistein (Sigma).

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