SHORT REPORT

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Gas Chromatographic Determination of Quinolizidine Alkaloids in *Genista sandrasica* and Their Antimicrobial Activity

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Abstract: Alkaloid profile of the aerial parts of *Genista sandrasica* Hartvig & Strid. (Fabaceae) growing in Turkey was studied by capillary gas chromatography-mass spectrometry (GC-MS). Ten quinolizidine alkaloids were identified in the alkaloid extract of *G. sandrasica*. The main alkaloids were: sparteine (13.68%), *N*-acetylcytisine (6.48%), 13-methoxylupanine (13.12%), anagyrine (40.49%) and baptifoline (10.76%). In addition, antibacterial and antifungal activities of the alkaloid extract of *G. sandrasica* were tested against standard strains of the bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Staphylococcus aureus*) as well as the fungi (*Candida albicans* and *Candida krusei*). The alkaloid extract of *G. sandrasica* showed significant activity against *B. subtilis* and *S. aureus* with minimum inhibitory concentrations (MIC) of 31.25 and 62.5 µg/mL.

Keywords: *Genista sandrasica*; Fabaceae; alkaloids; GC-MS; antimicrobial activity.

1. Plant Source

Genista sandrasica (Fabaceae), prostrate shrublet with several spreading stems, grows on Sandras Mountain of Southwest Anatolia [1]. The quinolizidine alkaloids (QA) are principal secondary metabolites of Fabaceae family. A number of studies on QA have been concerned with their phytochemical characterization and biological activities [2,3]. In the course of our ongoing studies on *Genista* species growing in Turkey, we investigate the alkaloid composition and antimicrobial activity of the aerial parts of *G. sandrasica*.

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The aerial parts of *Genista sandrasica* Hartvig & Strid. (Fabaceae) were collected at flowering stage from Sandras mountains, the vicinity of Ağla, Muğla, Turkey in June 2003, and identified by one of us (Prof. Dr. Fatma Tosun). A voucher specimen (No. GUE 2613) was kept at the Herbarium of Faculty of Pharmacy, Gazi University, Ankara, Turkey.

2. Previous Studies

In our previous studies on Turkish *Genista* species, we isolated alkaloids from 11 *Genista* species [4-10], as well as flavonoids from *G. aucheri* and *G. involucrata* [11,12]. In other previous studies, the aerial parts of 11 *Genista* species were analyzed for their total and free genistein and daidzein contents by using liquid chromatography/mass spectrometry (LC-MS) method [13,14]. In our recent study, the alkaloid profile and antimicrobial activity of *G. vuralii* were investigated [15].

3. Present Study

Alkaloid extraction was carried out as described by Wink [3]: 2 g plant material was homogenized in 30 ml 0.5 N HCl. After 30 min at room temperature, the homogenate was centrifuged for 10 min 5,000*g*. For quantitative work, the pellet was resuspended in 0.5 N HCl and centrifuged again. Both supernatants were then pooled and adjusted to pH 12-14 with NH_4OH (25%). Alkaloids were extracted by solid-phase extraction using Extrelut column (Merck, Darmstadt). Total alkaloids were eluted with CH_2Cl_2 and the solvent evaporated *in vacuo*.

Gas Chromatography-Mass Spectrometry: The alkaloid extract was dissolved in CH_2Cl_2 and applied into a GC-MS apparatus (Hewlett Packard Model 6890 series) equipped with a mass selective detector. Experimental conditions for capillary GC-MS analysis were developed under the following conditions. Capillary column HP-5 (Crosslinked 5% phenylmethylsiloxane, 50 m x 0.32 mm (i.d.), with 0.17 μm film thickness, model no. HP 19091J-015), detector temperature 280ºC, injector temperature 250ºC, carrier gas helium (1 ml/min), split ratio 1/20, injection volume 0.2 μl, and mass range (*m/z*) 20-440. GC oven temperature was kept at 120ºC for 2 min, programmed to 300ºC at a rate of 6ºC/min, and kept constant at 300ºC for 10 min.

Antimicrobial Activity: Standard strains of four bacteria, namely *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Bacillus subtilis* (ATCC 6633), and *Staphylococcus aureus* (ATCC 25923) were used for determination of antibacterial activity, along with standard strains of *Candida albicans* (ATCC 10231) and *Candida krusei* (ATCC 14243) were used for determination of antifungal activity. The minimum inhibitory concentrations (MIC) of the extracts and references (ciprofloxacin and flucanozole) were determined by broth microdilution techniques according to the National Committee for Clinical Laboratory Standards [16].

Ten alkaloids were detected in the alkaloid extract of the aerial parts of *G. sandrasica* by GC-MS, a powerful method for the analysis of the quinolizidine alkaloids. Sparteine, *N*-acetylcytisine, 13-methoxylupanine, anagyrine and baptifoline were identified as the major alkaloids in the alkaloid extract. Besides these main alkaloids, cytisine, 5,6-dehydrolupanine, lupanine, *N*-formylcytisine and 13*α*-benzoyloxylupanine were detected as the minor compounds in the plant. The quantitative pattern of the minor alkaloids is given in Table 1. The structures of alkaloids were identified based on comparison of their Kovats retention indices (KI) and mass spectral fragmentation with those of reference data in the literature [3,17] as well as by a library search (Wiley GC-MS library databank) and comparison with authentic alkaloids namely, anagyrine and cytisine. Relative contents of % alkaloids were determined via areas under the peaks from total ion chromatography using Hewlett Packard software.

Table 1. Alkaloid composition and alkaloid content of *G. sandrasica*

Nr.	Alkaloid	KI	M^+	% of total alkaloid content
1	Sparteine	1785	234	13.68
2	Cytisine	1990	190	3.05
3	5,6-Dehydrolupanine	2132	246	3.41
4	Lupanine	2165	248	2.95
5	*N*-Formylcytisine	2315	218	3.65
6	*N*-Acetylcytisine	2325	232	6.48
7	13-Methoxylupanine	2370	278	13.12
8	Anagyrine	2390	244	40.49
9	Baptifoline	2650	260	10.76
10	13*α*-Benzoyloxylupanine	3100	368	2.41

KI: Kovats retention index; M^+: Molecular ion

A number of studies on the alkaloid patterns of *Genista* species have been determined by capillary GC-MS [15,18-20]. Our results compared with literature findings, the alkaloid compositions were similar, but the relative amount of the alkaloids showed a higher diversity. In agreement with our previous findings in Turkish *Genista* species [4-10,15], sparteine, cytisine, 5,6-dehydrolupanine, lupanine, *N*-formylcytisine, *N*-acetylcytisine, 13-methoxylupanine, anagyrine and baptifoline were determined in the alkaloid extract of the aerial parts of *G. sandrasica*. In this study, 13α-benzoyloxylupanine, previously detected in *G. cinerea* ssp. *ausetana*, *G. cinerascens* and *G. majorica* [19], was identified for the first time from Turkish *Genista* genus.

In addition, the antibacterial and antifungal activities of *G. sandrasica* alkaloid extract were also tested against standard strains of the bacteria and fungi (Table 2). According to our findings, the alkaloid extract of *G. sandrasica* showed significant activity against the Gram-positive bacteria *B. subtilis* and *S. aureus* with MICs of 31.25 and 62.5 µg/ml respectively, moderate activity against the Gram-negative bacteria *P. aeruginosa* and *E. coli* (MIC=125 µg/ml). In the yeast assay, the alkaloid extract exhibited moderate activity against *C. albicans* and *C. krusei* (MIC=125 µg/ml). QA have been shown to have antimicrobial activity by several researchers [15,21,22]. Our antimicrobial findings in this research support that QA may be involved in the antimicrobial defense system of *Genista sandrasica* [21].

In this study, we aimed to investigate the alkaloid profile of *G. sandrasica* by GC-MS and antimicrobial activity of this alkaloid extract of the plant. From the GC-MS analysis, it appeared that the alkaloid extract of *G. sandrasica* consisted of anagyrine, sparteine, 13-methoxylupanine, baptifoline and *N*-acetylcytisine as the main alkaloids. The alkaloid extract of *G. sandrasica* exhibited a marked inhibition towards Gram-positive bacteria, whereas the antimicrobial activity against Gram-negative bacteria and fungi are considered to be moderate. To best of our knowledge, the alkaloid profile and antimicrobial activity of *Genista sandrasica* growing in Turkey are reported for the first time.

Table 2. Antimicrobial activity of *G. sandrasica* alkaloid extract

Microorganisms	MIC (µg/ml)	
	Alkaloid extract	**Standards**
Bacteria		Ciprofloxacin
Staphylococcus aureus ATCC 25923	62.5	0.08
Bacillus subtilis ATCC 6633	31.25	0.02
Escherichia coli ATCC 25922	125	0.02
Pseudomonas aeruginosa ATCC 27853	125	0.04
Fungi		Flucanozole
Candida albicans ATCC 10231	125	1.75
Candida krusei ATCC 14243	125	1.75

MIC: Minimum inhibitory concentration

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