

Characterisation and antimicrobial activity of *Sophora alopecuroides* L. var. *alopecuroides* alkaloid extracts

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Abstract: The aerial parts and seeds of *Sophora alopecuroides* L. var. *alopecuroides* growing in Turkey were investigated for their alkaloid compositions and antimicrobial activities. The alkaloid extracts were analysed by capillary gas chromatography-mass spectrometry (GC-MS). The main components were identified as sophoridine (33.07%), matrine (24.03%), and sophocarpine (8.01%) in the aerial parts, and matrine (36.67%) and sophoridine (34.53%) in the seeds of the plant. The alkaloid extracts were also evaluated for their in vitro antibacterial and antifungal activities using microdilution techniques. The alkaloid extract of the aerial parts of *S. alopecuroides* var. *alopecuroides* presented significant activity against *Staphylococcus aureus* and *Bacillus subtilis* while the alkaloid extract of the seeds displayed good activity against *Pseudomonas aeruginosa* and *Bacillus subtilis* with minimum inhibitory concentrations (MICs) of 62.5 µg/mL. In the antifungal assay, the alkaloid extract of the seeds of the plant showed significant activity against *Candida krusei* (MIC = 62.5 µg/mL). To the best of our knowledge, the current work is the first to report the alkaloid profile and antimicrobial activity of *S. alopecuroides* var. *alopecuroides* growing in Turkey.

Key words: *Sophora*, *Sophora alopecuroides* var. *alopecuroides*, Leguminosae, alkaloid, antimicrobial activity, GC-MS

Sophora alopecuroides L. var. *alopecuroides* alkaloit ekstrlerinin kimyasal bileşimi ve antimikrobiyal aktivitesi

Özet: Bu çalışmada, Türkiye’de yetişen *Sophora alopecuroides* L. var. *alopecuroides* bitkisinin toprak üstü kısımları ile tohumlarının alkaloit bileşimleri ve antimikrobiyal aktiviteleri incelenmiştir. Alkaloit ekstrleri kapiller gaz kromatografisi-kütle spektrometresi (GC-MS) ile analiz edilmiştir. Bitkinin toprak üstü kısmında sofridin (% 33,07), matrin (% 24,03) ve sofokarpin (% 8,01), tohumlarında ise matrin (% 36,67) ve sofridin (% 34,53) başlıca bileşikler olarak tayin edilmiştir. Alkaloit ekstrlerinin ayrıca in vitro mikrodilüsyon teknikleri kullanılarak antibakteriyel ve antifungal aktiviteleri incelenmiştir. *S. alopecuroides* var. *alopecuroides*’in toprak üstü kısımlarının alkaloit ekstresi *Staphylococcus aureus* ve *Bacillus subtilis*’e karşı, tohumları ise *Pseudomonas aeruginosa* ve *Bacillus subtilis*’e karşı 62,5 µg/mL minimum inhibitör konsantrasyonlar (MİK) ile belirgin bir aktiviteye sahip bulunmuştur. Bitkinin tohumlarının alkaloit ekstresi *Candida krusei*’ye karşı önemli bir antifungal aktivite (MİK = 62,5 µg/mL) göstermiştir. Bilgimize göre, bu çalışma Türkiye’de yetişen *S. alopecuroides* var. *alopecuroides*’in alkaloit içeriği ve antimikrobiyal aktivitesinin incelendiği ilk çalışmadır.

Anahtar sözcükler: *Sophora*, *Sophora alopecuroides* var. *alopecuroides*, Leguminosae, alkaloit, antimikrobiyal aktivite, GC-MS

Introduction

The plant *Sophora alopecuroides* L. var. *alopecuroides* (Leguminosae) is widely distributed in south-west and east Asia, Greece, and south Russia. It grows at the edges of fields, banks and less frequently on sand dunes, from sea level to 1750 m. *Sophora alopecuroides* has 2 varieties in the *Flora of Turkey and the East Aegean Islands*, namely var. *alopecuroides* and var. *tomentosa* (Boiss.) Chamberlain, and among them var. *alopecuroides* is more common than the other in Turkey. *S. alopecuroides* var. *alopecuroides* (SA) is a perennial plant with rhizomatous, oblong and 7-12-paired leaflets, cream flowers, and a narrowly cylindrical lomentum fruit (1).

Some *Sophora* species are commonly used in traditional Chinese medicine. Among them, the roots of *S. flavescens*, the roots of *S. tonkinensis*, and the seeds of *S. alopecuroides* are widely used for the treatment of some skin and gynaecological diseases such as eczema, dermatitis, and colpitis, as well as fever, sore throat, and inflammation. *Sophora* species are known to contain quinolizidine alkaloids (QAs) as their principal bioactive constituents, which have been shown to exhibit sedative, analgesic, antipyretic, anti-inflammatory, anti-tumour and notable antiviral activities (2-5). QAs are characteristic secondary metabolites of the family Leguminosae and are especially abundant in the tribes Genisteae, Sophoreae, and Thermopsidae (5,6). QAs also play a chemical defensive role against herbivores and pathogen micro-organisms (7,8).

In our previous studies on the analysis of QA containing Leguminosae plants growing in Turkey, we investigated the alkaloid profile of the aerial parts of *Lupinus angustifolius* and *Genista vuralii* (9,10). No phytochemical and biological data on *S. alopecuroides* var. *alopecuroides* growing in Turkey were found in a literature survey. Thus, in the course of our ongoing studies on QAs, we aimed at evaluating (i) alkaloid composition using gas chromatography-mass spectrometry (GC-MS), (ii) antibacterial and antifungal activities of the aerial parts and seeds of *S. alopecuroides* var. *alopecuroides* growing in Turkey, respectively.

Materials and methods

Plant material

The aerial parts and seeds of *Sophora alopecuroides* L. var. *alopecuroides* (Leguminosae) were collected at flowering and fruiting stages from the vicinity of Aralık, Iğdır, Turkey, in June and October 2001, respectively. Plant material was obtained from sandy places at the altitude of 800 m and identified by one of the authors (Prof. Dr. Nezaket Adıgüzel) of the present study. An authenticated voucher specimen (code as N. Adıgüzel 4264) was kept in the Herbarium of Gazi University (GAZI).

Extraction of alkaloids

Alkaloid extraction was carried out as described by Wink (6): 2 g of plant material was homogenised in 30 mL of 0.5 N HCl. After 30 min at room temperature, the homogenate was centrifuged for 10 min at 5000 × g. For quantitative work, the pellet was re-suspended 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 an Extrelut column (Merck, Darmstadt, Germany). Total alkaloids were eluted with CH_2Cl_2 and the solvent evaporated in vacuo.

Analysis of alkaloids

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 × 0.32 mm (i.d.), with 0.17 µm film thickness, model no. HP 19091J-015), detector temperature 280 °C, injector temperature 250 °C, 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 120 °C for 2 min, programmed to 300 °C at a rate of 6 °C/min, and kept constant at 300 °C for 10 min.

Antimicrobial activity

Micro-organisms: Standard strains of 4 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) that were used for determination of antifungal activity.

Antibacterial and antifungal tests: The minimum inhibitory concentrations (MICs) of the extracts and references (ciprofloxacin and flucanazole) were determined by broth microdilution techniques according to the Clinical Laboratory Standards Institute (11,12). Mueller-Hinton Broth (Merck) and Mueller-Hinton agar (Oxoid Ltd, Basingstoke, UK) were applied for growing and diluting of the bacteria. Sabouraud liquid medium (Oxoid Ltd) and Sabouraud dextrose agar (Oxoid Ltd) were applied for growing and diluting of the fungi. The medium RPMI-1640 (Sigma Chemical Co., St. Louis, MO, USA) with L-glutamine was buffered pH 7 with 3-[N-morpholino]-propanesulfonic acid (MOPS). The extracts were dissolved in dimethylsulfoxide (DMSO). Extracts concentrations ranging from 1.00 to 3.75 µg/mL were prepared. Micro-organism inoculums were standardised to a turbidity equivalent to that of a 0.5 McFarland standard (10^6 yeasts or 10^8 bacterial cells), and diluted for the broth microdilution procedure. Final concentrations were approximately $1-5 \times 10^3$ cells/mL for yeasts and $1-5 \times 10^4$ for bacteria. Microtitre plates were incubated under normal atmospheric conditions at 37 °C for 24 h for bacteria and at 30 °C for 48 h for the yeasts. The micro-organisms and pure media (positive and negative controls) were placed in the wells of a microtitre plate. The MIC was defined as the lowest concentration of extracts that produced an 80% reduction in visible growth compared with the control. The bacterial growth was indicated by the presence of a white "pellet" on the well bottom. Each extract was tested in triplicate.

The in vitro antimicrobial results of the extracts were classified as follows: the antibacterial activity was considered significant when the MIC was 100 µg/mL or less, moderate when the MIC was 100-500 µg/mL, weak when the MIC was 500-1000 µg/mL, and inactive when the MIC was above 1000 µg/mL.

Results and discussion

In the present study, we aimed at investigating the alkaloid profile of *S. alopecuroides* var. *alopecuroides* by capillary GC-MS analysis and the antimicrobial activity of its alkaloid extracts. Two alkaloid extracts were obtained from the aerial parts and seeds of SA by solid-phase extraction using an Extrelut column. Both samples were analysed by capillary GC-MS and the relative contents of % alkaloids were determined via areas under the peaks from total ion chromatography using Hewlett Packard software. The quantitative pattern of the alkaloids is given in Table 1. Twenty-seven compounds, representing 97.15% of the total, were detected in the aerial parts of SA, and 21 compounds, representing 99.30% of the total, in the seeds of plant. The EI mass spectra of QA have been well characterised and fragment ions have been identified that are indicative of certain features of the carbon skeleton (13). In this study, the alkaloids were identified according to their mass fragmentation patterns with those of reference data in the literature (6,14-18) as well as by a library search (Wiley GC-MS library databank) and comparison with authentic alkaloids such as anagryne and cytisine. Sophoridine (33.07%) and matrine (24.03%), along with sophocarpine (8.01%), 12β-hydroxysophocarpine (6.57%) and sophoranol (5.42%), were identified as the main components in the aerial parts of SA alkaloid extract. In addition, lupanine, aphylline, 14β-hydroxysophoridine, N-formylcytisine, isosophoramine, and adenocarpine were detected in only trace amounts by GC-MS. In the seeds of the alkaloid extract of SA, matrine (36.67%) and sophoridine (34.53%), along with cytisine (8.53%) and 5,17-dehydromatrine (4.36%), were determined as the main alkaloids. Isosophoramine and anagryne were detected as only in trace amounts in the seed of alkaloid extract of SA. In addition, α-isosparteine, 11, 12-dehydrosparteine, aphylline, sophocarpine, sophoranol-N-oxide, and adenocarpine were not determined in the seed alkaloid extract of the plant.

There are a number of reports on the alkaloid profiles of *Sophora* species (2,3,15-17,19-23). In the study by ur-Rahman et al. (2), 7α-hydroxysophoramine, 12β-hydroxysophocarpine, sophoramine, 14β-hydroxymatrine, adenocarpine, matrine, sophoridine, sophocarpine, and baptifoline

Table 1. Alkaloid composition of *Sophora alopecuroides* var. *alopecuroides*.

No.	Alkaloid	% of total alkaloid content in the aerial parts	% of total alkaloid content in the seeds
1	α -Isosparteine	0.54	n.d.
2	11,12-Dehydrosparteine	3.12	n.d.
3	<i>N</i> -Methylcytisine	1.06	1.24
4	Cytisine	2.21	8.53
5	5,6-Dehydrolupanine	0.51	0.83
6	Lupanine	tr.	1.04
7	Aphylline	tr.	n.d.
8	7,11-Dehydromatrine	1.73	2.12
9	Lehmannine	1.30	0.72
10	Sophocarpine	8.01	n.d.
11	Matrine	24.03	36.67
12	Sophoridine	33.07	34.53
13	Sophoridine- <i>N</i> -oxide	1.02	1.70
14	Sophoramine	2.24	1.92
15	5,17-Dehydromatrine	2.38	4.36
16	14 β -Hydroxysophoridine	tr.	0.52
17	Oxymatrine	0.58	0.83
18	14 β -Hydroxymatrine	0.52	0.62
19	<i>N</i> -Formylcytisine	tr.	0.54
20	Isosophoramine	tr.	tr.
21	Anagyrene	0.96	tr.
22	Sophocarpine- <i>N</i> -oxide	0.86	0.51
23	12 β -Hydroxysophocarpine	6.57	1.42
24	Sophoranol	5.42	0.62
25	Sophoranol- <i>N</i> -oxide	0.50	n.d.
26	Baptifoline	0.52	0.58
27	Adenocarpine	tr.	n.d.
Number of identified compounds		27	21
Total		97.15	99.30

n.d.: Not detected; tr.: Trace (<0.50)

were isolated through chromatographic techniques from the aerial parts of *S. alopecuroides* collected from Pakistan. To the best of our knowledge, this is the first report on the alkaloid pattern of *S. alopecuroides* var. *alopecuroides* growing in Turkey by GC-MS.

Furthermore, the antibacterial and antifungal activities of *S. alopecuroides* var. *alopecuroides* alkaloid extracts against standard strains of bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, and *Staphylococcus aureus*) as well as fungi (*Candida albicans* and *C. krusei*) were also investigated in this study. Results of the antibacterial and antifungal tests are given in Table 2. The alkaloid extract of the aerial parts of SA presented significant activity against *S. aureus* and *B. subtilis* (MIC = 62.5 µg/mL), moderate activity against *P. aeruginosa* (MIC = 250 µg/mL), and weak activity against *E. coli* (MIC = 500 µg/mL). The alkaloid extract of the seeds of SA possessed significant activity on both *P. aeruginosa* and *B. subtilis* (MIC = 62.5 µg/mL) and moderate activity on both *S. aureus* and *E. coli* with MICs of 125 and 250 µg/mL, respectively. In the anti-yeast assay, the alkaloid extract of the aerial parts of SA displayed significant activity against *C. krusei* with the tested concentration of 62.5 µg/mL. Both the alkaloid extracts of SA showed weak activity on *C. albicans* while aerial parts alkaloid extract of SA displayed moderate activity against *C. krusei* with MIC of 250 µg/mL.

To date, a number of studies have been reported on antimicrobial activities of pure QA and QA containing plant extracts. In a study by Wink (24), sparteine was reported to possess antimicrobial activity against bacteria and phytopathogenic fungi. In another study, Wippich and Wink (25) reported that sparteine, lupanine, and 13-tigloyloxylupanine inhibited the germination of conidia *Erysiphe graminis* f. sp. *hordei*. Tyski et al. (26) reported that pure QA isolated from *Lupinus angustifolius* var. Mirela, namely, lupanine, 13α-hydroxylupanine, sparteine, and angustifoline as well as the ethanolic extract of the seed of the plant showed bacteriostatic effects on *S. aureus*, *B. subtilis*, *E. coli*, *P. aeruginosa*, and *Bacillus thuringiensis*. In a previous study, the *n*-hexane, chloroform, ethyl acetate, methanol, and water extracts obtained from *Sophora flavescens* were assayed in the antimicrobial potency, and the methanol extract was found to possess strong growth inhibition activity against gram-positive bacteria *B. subtilis* and *S. aureus* (MIC = 25-50 µg/mL), and gram-negative bacteria *P. aeruginosa* (MIC = 25 µg/mL) (27). In a study by Yan et al. (28), the antimicrobial activities of total alkaloids from *Sophora flavescens*, and its monomer matrine, oxymatrine, sophoridine, sophocarpine, and cytosine, were tested against 11 plant pathogenic fungi and 3 bacteria using in vitro methods. They found that only the total alkaloids to *Phytophthora drechsleri* and *Colletotrichum*

Table 2. Antimicrobial activity of the alkaloid extracts of *Sophora alopecuroides* var. *alopecuroides*.

Micro-organisms	MIC (mg/mL)		
	Aerial parts	Seeds	Standards
Bacteria			Ciprofloxacin
<i>Staphylococcus aureus</i>	62.5	125	0.08
<i>Bacillus subtilis</i>	62.5	62.5	0.02
<i>Escherichia coli</i>	500	250	0.02
<i>Pseudomonas aeruginosa</i>	250	62.5	0.04
Fungi			Flucanazole
<i>Candida albicans</i>	500	500	1.75
<i>Candida krusei</i>	250	62.5	1.75

MIC: Minimum inhibitory concentration.

gloeosporioides, and sophoridine to *P. drechsleri* had relatively high activities; the percentage of inhibition on mycelial growth with the tested concentration of 500 mg/L were 96.18%, 54.90%, and 93.62%, respectively. Furthermore, these samples were found to have little effect on the growth of the 3 bacteria: the percentage of growth inhibition were all less than 20% (28). On the other hand, the *n*-hexane, ethyl acetate, *n*-butanol, and water fractions obtained from the methanolic extract of *Sophora japonica* flowers were tested for antimicrobial activity. Among them, the ethyl acetate fraction was effective on bacterial inhibition against *E. coli* and *Klebsiella pneumonia*, with MIC of 125 µg/mL (29).

We previously investigated antimicrobial activity of the alkaloid extracts obtained from *Lupinus angustifolius* and *Genista vuralii*. In our previous study, the alkaloid extract of the aerial parts of *Lupinus angustifolius* showed significant activity on *B. subtilis*, *S. aureus*, and *P. aeruginosa* at MICs of 62.5 µg/mL, while it was weakly active on *E. coli*. On the other hand, the extract possessed moderate activity against *C. albicans* and *C. krusei* at MICs of 250 µg/mL (9). In one of our previous studies, the alkaloid extract of *G. vuralii* presented significant activity against *S. aureus*, *B. subtilis*, and *C. krusei* at MICs of 62.5 µg/mL (10).

Our antimicrobial results support the idea that QAs may be involved in the antimicrobial defence system of plants (24,25). In addition, our GC-MS analysis demonstrated that the alkaloid extract of SA contained matrine and sophoridine as the main components. These compounds might be responsible for antimicrobial activity of the alkaloid extract of SA, which has been herein found to have significant antimicrobial activity against *S. aureus*, *B. subtilis*, *P. aeruginosa*, and *C. krusei* with MICs of 62.5 µg/mL.

To the best of our knowledge, we herein report for the first time the alkaloid profile and antimicrobial activity by the above-mentioned methods of *S. alopecuroides* var. *alopecuroides* growing in Turkey.

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