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Original article

ALKALOID PROFILES AND BIOLOGICAL ACTIVITIES OF DIFFERENT *SOPHORA JAUBERTII* EXTRACTS

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Abstract

In this study, the aerial parts and seeds of Sophora jaubertii Spach (Leguminosae) growing in Turkey were investigated for their alkaloid compositions and antimicrobial activities. The alkaloid extracts were analyzed by capillary gas chromatography-mass spectrometry (GC-MS). The main components were identified as matrine (34.64 %), sophocarpine (15.32 %), cytisine (10.32 %), anagyrine (9.10 %), and sophoridine (6.35 %) in the aerial parts, while matrine (32.34 %), sophocarpine (14.67 %), cytisine (13.30 %), sophoranol (10.98 %), and sophoridine (8.57 %) in the seeds of the plant. The alkaloid extracts were also evaluated for their antibacterial and antifungal activities. The alkaloid extract of the aerial parts of S. jaubertii presented significant activity against Bacillus subtilis with a minimum inhibitory concentration (MIC) of 31.25 µg/ml. The remaining MIC values were found in the range of 62.5-500 µg/ml.

Key words: *Sophora jaubertii, Leguminosae, Alkaloid, Antimicrobial activity, GC-MS*

Sophora jaubertii'nin Farklı Ekstrelerinin Alkaloit Profilleri ve Biyolojik Aktiviteleri

Bu çalışmada, Türkiye'de yetişen Sophora jaubertii Spach (Leguminosae) bitkisinin topraküstü kısımları ile tohumlarının alkaloit bileşimleri ve antimikrobiyal aktiviteleri incelenmiştir. Alkaloit ekstreleri kapiller gaz kromatografisi-kütle spektrometresi (GC-MS) ile analiz edilmiştir. Bitkinin toprak üstü kısmında matrin (% 34.64), sofokarpin (% 15.32), sitizin (% 10.32), anagirin (% 9.10) ve soforidin (% 6.35), tohumlarında ise matrin (% 32.34), sofokarpin (% 14.67), sitizin (% 13.30), soforanol (% 10.98) ve soforidin (% 8.57) başlıca bileşikler olarak tayin edilmiştir. Alkaloit ekstrelerinin ayrıca antibakteriyel ve antifungal aktiviteleri incelenmiştir. S. jaubertii'nin toprak üstü kısımlarının alkaloit ekstresi Bacillus subtilis'e karşı 31.25 µg/mL minimum inhibitör konsantrasyon (MİK) ile önemli bir aktivite göstermiştir. Diğer MİK değerleri 62.5-500 µg/mL arasında bulunmuştur.

Anahtar kelimeler: *Sophora jaubertii, Leguminosae, Alkaloit, Antimikrobiyal aktivite, GC-MS*

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INTRODUCTION

Sophora jaubertii Spach (Leguminosae) is distributed in North Anatolia and Amanos Mountains in South Anatolia. It also grows naturally in Romania and Crimea. *S. jaubertii* grows amongst scrub and in open communities from 30 m to 730 m. It is Euxine element. *S. jaubertii* is a perennial plant with rhizomatous, oblong and 7-12-paired leaflets, cream flowers and a narrowly cylindrical lomentum fruit (1).

Genus *Sophora* are commonly used in traditional Chinese medicine. Among them, such as 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 gynecological diseases such as eczema, dermatitis and colpitis, as well as fever, sore throat and inflammation. *Sophora* species are known to contain quinolizidine alkaloids (QA) as their principal bioactive constituents, which have been shown to exhibit sedative, analgesic, antipyretic, anti-inflammatory, anti-tumor and notable antiviral activities (2-5). QA are characteristic secondary metabolites of the Leguminosae family and are especially abundant in the tribes Genisteae, Sophoreae and Thermopsideae (5,6). QA also play a chemical defensive role against to herbivores and pathogen microorganisms (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*, *Genista vuralii* and *Genista sandrasica* (9-11). Thus, in the course of our ongoing studies on QA, we aimed to evaluate (i) the alkaloid composition using gas chromatography-mass spectrometry (GC-MS), (ii) the antibacterial and antifungal activities of the aerial parts and seeds of *S. jaubertii* growing in Turkey, respectively.

EXPERIMENTAL

Plant material

The aerial parts and seeds of *Sophora jaubertii* Spach (Leguminosae) were collected at flowering and fruiting stages from the vicinity of Azdavay, Kastamonu, Turkey in June and October 2002, respectively. The plant material was obtained from clearing forest at the altitude of 450 meters. An authenticated voucher specimen (U. Ozbek 1175) was kept in the Herbarium of GAZI.

Extraction of alkaloids

Alkaloid extraction was carried out as described by Wink (6). The aerial parts and seeds of *Sophora jaubertii* were air-dried until dryness at room temperature and under shade, and then powdered to a fine grade by using a laboratory scale mill. 2 g plant material was homogenized in 30 ml 0.5 N HCl and was left for 30 min at room temperature. The homogenate was centrifuged for 10 min 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 x 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.

Microbiological studies

Microorganisms

Standard strains of *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.

Antibacterial and antifungal tests

The minimum inhibitory concentrations (MICs) of the extracts and references (ciprofloxacin and flucanozole) were determined by broth microdilution techniques according to the Clinical Laboratory Standards Institute (12,13). 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]-propansulfonic acid (MOPS). The extracts were dissolved in dimethylsulfoxide (DMSO). Extracts concentrations ranging from 1.000 to 3.75 μg/mL were prepared. Microorganism inoculums were standardized 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 × 10^3 cells/mL for yeasts and 1-5 x 10^4 for bacteria. Microtiter 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 microorganisms and pure media (positive and negative controls) were placed in the wells of a microtiter plate. The MIC was defined as the lowest concentration of extracts that produced an 80% reduction in visible growth compared with 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 as 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 to investigate the alkaloid profile of the aerial parts and seeds of *S. jaubertii* by capillary GC-MS analysis and the antimicrobial activity of their alkaloid extracts. Two alkaloid extracts were obtained from the aerial parts and seeds of *S. jaubertii* by solid-phase extraction using Extrelut column and both samples were analyzed by capilllary GC-MS. Relative alkaloid contents 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-three compounds, representing 96.70 % of the total, were detected in the aerial parts of *S. jaubertii*, and twenty-one compounds, representing 97.96 % of the total, in the seeds of the plant. 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 anagyrine and cytisine. Matrine (34.64 %), sophocarpine (15.32 %), cytisine (10.32 %), anagyrine (9.10

%), and sophoridine (6.35 %) were identified as the main components in the aerial parts of *S. jaubertii* alkaloid extract. In addition, *N*-methylcytisine, lupanine, oxymatrine, 14*β*-hydroxymatrine, lamprolobine, isosophoramine, sophocarpine-*N*-oxide, sophoranol-*N*-oxide, and adenocarpine were detected in only trace amounts by GC-MS. In the seeds of the alkaloid extract of *S. jaubertii*, matrine (32.34 %), sophocarpine (14.67 %), cytisine (13.30 %), sophoranol (10.98 %), and sophoridine (8.57 %) were determined as the main alkaloids. *N*-methylcytisine, 5,6-dehydrolupanine, lupanine, 14*β*-hydroxymatrine, lamprolobine, and isosophoramine were detected as only in trace amounts in the seeds of the alkaloid extract of *S. jaubertii*. In addition, sophocarpine-*N*-oxide and adenocarpine were not determined in the seeds alkaloid extract of the plant.

Table 1. Alkaloid composition and alkaloid content of the aerial parts and seeds of *Sophora jaubertii*.

Nr.	Alkaloid	% of total alkaloid content in the aerial parts	% of total alkaloid content in the seeds
1	*N*-Methylcytisine	tr.	tr.
2	Cytisine	10.32	13.30
3	5,6-Dehydrolupanine	0.43	tr.
4	Lupanine	tr.	tr.
5	7,11-Dehydromatrine	4.43	1.22
6	Lehmannine	1.34	2.07
7	Sophocarpine	15.32	14.67
8	Matrine	**34.64**	**32.34**
9	Sophoridine	6.35	8.57
10	Sophoridine-*N*-oxide	2.86	1.75
11	Sophoramine	2.14	2.90
12	5,17-Dehydromatrine	4.41	2.76
13	Oxymatrine	tr.	0.65
14	14*β*-Hydroxymatrine	tr.	tr.
15	Lamprolobine	tr.	tr.
16	Isosophoramine	tr.	tr.
17	Anagyrine	9.10	2.91
18	Sophocarpine-*N*-oxide	tr.	nd.
19	12*β*-Hydroxysophocarpine	3.32	2.20
20	Sophoranol	1.17	10.98
21	Sophoranol-*N*-oxide	tr.	1.10
22	Baptifoline	0.87	0.54
23	Adenocarpine	tr.	nd.
	Number of identified compounds	**23**	**21**
	Total	**96.70**	**97.96**

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

There have been several studies on the alkaloid profiles of *Sophora* species (2, 3, 15-17, 19-23). However, we found two studies on the alkaloid profile of *S. jaubertii*. In Gürkan and Hırlak's study (17), matrine, allomatrine, sophocarpine, sophocarpine-*N*-oxide, sophoranol, sophoridine, and anagyrine were isolated through chromatographic techniques from the aerial parts of *S. jaubertii* collected from Turkey. Comparing the present results with the above-mentioned literature findings, the alkaloid compositions have been found to be similar. In agreement with previous findings in *S. jaubertii* (17), except for the presence of allomatrine, matrine, sophocarpine, sophocarpine-*N*-oxide, sophoranol, sophoridine, and anagyrine were found in the alkaloid profile of the aerial parts of the plant in the present study. In addition, matrine have been also isolated in the seeds of *S. jaubertii* by Kavalalı (21), which is consistent with our data.

Furthermore, the antibacterial and antifungal activities of *S. jaubertii* alkaloid extracts against standard strains of bacteria (*Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis*, and *Staphylococcus aureus*) as well as fungi (*Candida albicans* and *Candida krusei*) were also investigated in the present work. Results of the antibacterial and antifungal tests are given in Table 2. The alkaloid extract of the aerial parts of *S. jaubertii* presented significant activity against *B. subtilis* (MIC=31.25 µg/mL) and *P. aeruginosa* (MIC=62.5 µg/mL), moderate activity against *S. aureus* (MIC=125 µg/mL), and weak activity against *E. coli* (MIC=500 µg/mL). The alkaloid extract of the seeds of the plant possessed significant activity on *B. subtilis* (MIC=62.5 µg/mL) and moderate activity on both *P. aeruginosa* and *S. aureus* with MICs of 125 and 250 µg/mL, respectively, as well as weak activity against on *E. coli* (MIC=500 µg/mL). In the anti-yeast assay, the alkaloid extract of the aerial parts of the plant displayed moderate activity against *C. albicans* with a MIC value of 250 µg/mL and a weak activity (MIC=500 µg/mL) against *C. krusei*. The alkaloid extract of the seeds showed moderate activity on both *Candida* species at MIC of 250 µg/mL.

Table 2. Antibacterial and antifungal activities of alkaloid extracts of *Sophora jaubertii*.

Microorganisms	MIC (µg / ml)		
	Aerial parts	Seeds	Standards
Bacteria			Ciprofloxacine
Staphylococcus aureus	125	250	0.08
Bacillus subtilis	**31.25**	62.5	0.02
Escherichia coli	500	500	0.02
Pseudomonas aeruginosa	62.50	125	0.04
Fungi			Flucanozole
Candida albicans	250	250	1.75
Candida krusei	500	250	1.75

MIC: Minimum inhibitory concentration.

In conclusion, in our study, the alkaloid profile of the aerial parts and seeds of *S. jaubertii* exhibited a higher diversity. Matrine was determined as the major component in both extracts of the plant. Both of the alkaloid extracts also displayed varied antimicrobial activity profile. The most significant microbiological activity was found against *B. subtilis*. To the best of our knowledge, for the first time, we herein report the alkaloid profile of *S. jaubertii* by capillary GC-MS as well as the antimicrobial activity.

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