

Alkaloid profile and antimicrobial activity of *Lupinus angustifolius* L. alkaloid extract

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Abstract Purpose of the present study was to evaluate alkaloid profile of the aerial parts of *Lupinus angustifolius* growing in Turkey by capillary gas chromatography-mass spectrometry (GC-MS). Fifteen alkaloids were identified by capillary GC-MS. 13 α -Hydroxylupanine (50.78%) and lupanine (23.55%) were determined as the main alkaloids in the aerial parts of *L. angustifolius*. Ammodendrine, isoangustifoline, tetra-hydrorhombifoline, angustifoline, α -isolupanine, 5,6-dehydrolupanine, 11,12-dehydrolupanine, 13 α -acetoxylupanine, 13 α -isovaleroyloxylupanine, 13 α -valeroyloxylupanine, 13 α -tigloyloxylupanine, 13 α -*cis*-cinnamoyloxylupanine and 13 α -*cis*-cinnamoyloxy-17-oxolupanine were identified as the minor alkaloids of the plant. Furthermore, antibacterial and antifungal activities of *L. angustifolius* alkaloid extract were tested against standard strains of the following bacteria; *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Staphylococcus aureus* as well as the fungi; *Candida albicans* and *C. krusei*. The alkaloid extract showed significant activity on *B. subtilis*,

S. aureus and *P. aeruginosa* while it was weakly active on *E. coli*. On the other hand, the extract possessed moderate activity against *C. albicans* and *C. krusei*.

Keywords *Lupinus angustifolius* · Fabaceae · Alkaloids · GC-MS · Antimicrobial activity

Introduction

The genus *Lupinus* L. (Fabaceae), a large and diverse genus, comprises more than 200 species. There are five *Lupinus* species in flora of Turkey, namely *Lupinus albus* L., *L. angustifolius* L., *L. micranthus* Guss., *L. varius* L., *L. hispanicus* Boiss. & Reuter. Among them, *Lupinus angustifolius* L. is an annual herbaceous plant characterized by narrow palmate-compound leaves, and regular blue “papilionaceous” flowers allocated in terminal raceme (Chamberlain 1970). *Lupinus* species are named as “termiye, aci bakla, delicebakla, gavurbaklasi, lupen, kurtbaklasi, misirbaklasi, tirmis and yahudibaklasi” in Turkey. Lupine seeds obtained from *L. albus* are used as diuretic, antihelmintic and tonic in folk medicine. *L. albus* is known as indigenous lupine and is cultivated mainly Inner Anatolia. In addition, Lupine seeds are used in the treatment of liver disorders, diabetes, hemorrhoids and eczema (Başer et al. 1986; Baytop 1999).

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More than 200 naturally occurring lupine alkaloids, most of which have been isolated from leguminous plants, especially the subfamily Papilionaceae are well-known. Plants of the genus *Lupinus* are known to contain a variety of structural types of lupine alkaloids (Ohmiya et al. 1995). The quinolizidine alkaloids (QA), the main lupine alkaloids, play a chemical defensive role against herbivores and pathogen microorganisms (Wink 1988, 1992). Lupine seeds, especially rich in QA (2–8%), contain up to 40–50% protein and up to 20% lipids. Seeds are a good source for animal feeding due to their high amount of protein content. The knowledge of the alkaloid patterns of lupines is of importance, since many lupines might be of potential use for animal feeding besides toxicity potential to domestic wild ranging animals (Wink 1993; Wink et al. 1995).

In this study, we report on the alkaloid profile of the aerial parts of *L. angustifolius* growing in Turkey by capillary GC-MS. The structure of alkaloids was identified according to their mass fragmentation patterns that combined with library search (Wiley GC-MS library databank).

Materials and methods

Plant material

The aerial parts of *Lupinus angustifolius* L. (Fabaceae) were collected at flowering stage from the vicinity of Avclar, Aydın, Turkey in May 2005. An authenticated voucher specimen (GUE 2460) was kept in the Herbarium of Faculty of Pharmacy, Gazi University.

Extraction of alkaloids

Alkaloid extraction was carried out as described in Wink (1993): 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 5,000g. For quantitative work, the pellet is resuspended in 0.5 N HCl and centrifuged again. Both supernatants were then pooled and adjusted to pH 12–14 with NH₄OH (25%). Alkaloids were extracted by solid phase extraction using Extrelut (Merck, Darmstadt) column. Total alkaloids were

eluted with CH₂Cl₂ and the solvent evaporated in vacuo.

Analysis of alkaloids

The alkaloid extract was dissolved in CH₂Cl₂ 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

Microorganisms

The standard strains of the following bacteria, namely *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Bacillus subtilis* ATCC 6633 and *Staphylococcus aureus* ATCC 25923 for determination of antibacterial activity, along with standard strains of *Candida albicans* ATCC 10231 and *Candida krusei* ATCC 14243 used were 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 National Committee for Clinical Laboratory Standards (NCCLS 1997, 2000). Mueller–Hinton Broth (Merck, Germany) and Mueller–Hinton agar (Oxoid Ltd., UK) were applied for growing and diluting of the bacteria. Sabouraud liquid medium (Oxoid Ltd., UK) and Sabouraud dextrose agar (Oxoid Ltd., UK) were applied for growing and diluting of the fungi. The medium RPMI-1640

(Sigma Chemical Co.) with L-glutamine was buffered pH: 7 with 3-[N-morpholino]-propan-sulfonic acid (MOPS). The extracts were dissolved in dimethylsulfoxide (DMSO). Extracts concentrations ranging from 1,000 $\mu\text{g/ml}$ to 3.75 $\mu\text{g/ml}$ 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 $1\text{--}5 \times 10^3$ cells/ml for yeasts and $1\text{--}5 \times 10^4$ 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 put 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 $\mu\text{g/ml}$ or less; moderate, when the MIC was 100–500 $\mu\text{g/ml}$; weak, when the MIC was 500–1,000 $\mu\text{g/ml}$; and inactive, when the MIC was over 1,000 $\mu\text{g/ml}$.

Results and discussion

Capillary GC-MS analysis of *L. angustifolius* revealed the presence of 19 alkaloids. Fifteen alkaloids identified unequivocally by comparing mass spectral data to those of reference data in the literature (Wink 1988; Wink et al. 1995) and with library search (Wiley library databank). Relative contents of % alkaloids were determined with area under peaks from total ion chromatography using Hewlett Packard software. The quantitative pattern of the minor alkaloids is given in Table 1. 13 α -Hydroxylupanine (50.78%) and lupanine (23.55%) were determined as the main alkaloids in the aerial parts of *L. angustifolius*. Ammodendrine, isoangustifoline, tetrahydrorhombifoline,

Table 1 Alkaloid composition and alkaloid content of *Lupinus angustifolius*

Nr.	Alkaloid	% of total alkaloid content
1	Ammodendrine	1.10
2	Isoangustifoline	0.78
3	Tetrahydrorhombifoline	1.14
4	Angustifoline	2.94
5	α -Isolupanine	1.67
6	5,6-Dehydrolupanine	*
7	Lupanine	23.55
8	11,12-Dehydrolupanine	*
9	n.i.	0.50
10	13 α -Hydroxylupanine	50.78
11	13 α -Acetoxylupanine	1.54
12	13 α -Isovaleroyloxylupanine	1.13
13	13 α -Valeroyloxylupanine	1.65
14	n.i.	0.71
15	13 α -Tigloyloxylupanine	6.77
16	13 α -cis-Cinnamoyloxylupanine	2.60
17	n.i.	0.74
18	13 α -cis-Cinnamoyloxy-17-oxolupanine	*
19	n.i.	1.79

n.i.: not identified; *: trace (<0.50)

angustifoline, α -isolupanine, 13 α -acetoxylupanine, 13 α -isovaleroyloxylupanine, 13 α -valeroyloxylupanine, 13 α -tigloyloxylupanine and 13 α -cis-cinnamoyloxylupanine were identified as the minor alkaloids. In the alkaloid extract, 5,6-dehydrolupanine, 11,12-dehydrolupanine and 13 α -cis-cinnamoyloxy-17-oxolupanine were detected only in trace amounts by GC-MS. Furthermore, minor alkaloids which could not be identified (% 3.74) were present.

There are a number of reports on the alkaloid patterns of *Lupinus* species (Kinghorn et al. 1980; Planchuelo-Ravelo and Wink 1993; Planchuelo-Ravelo et al. 1993; Wink and Carey, 1994; Wink et al. 1995; Torres et al. 2002; Przybylak et al. 2005; Sánchez et al. 2005). In our study, alkaloid profiles of aerial parts of *L. angustifolius* exhibited a higher diversity. *Lupinus* plants usually contain ester lupine alkaloids. Most of the detected alkaloids in our study were the esters of hydroxylupanine, the most common was 13 α -tigloyloxylupanine. Our results support that a possible biosynthetic pathway in that hydroxylated alkaloids in *Lupinus* plants, like 13 α -hydroxylupanine, during germination specific

Table 2 Antibacterial and antifungal activities of alkaloid extract of *L. angustifolius*

Microorganisms	MIC ($\mu\text{g/ml}$)	
	<i>L. angustifolius</i> alkaloid extract	Standards
Bacteria		Ciprofloxacin
<i>Staphylococcus aureus</i>	62.5	0.08
<i>Bacillus subtilis</i>	62.5	0.02
<i>Escherichia coli</i>	500	0.02
<i>Pseudomonas aeruginosa</i>	62.5	0.04
Fungi		Flucanazole
<i>Candida albicans</i>	250	1.75
<i>Candida krusei</i>	250	1.75

alkaloid acyltransferases produces a variety of hydroxylupanine esters (Wink and Witte 1983; Ohmiya et al. 1995).

Furthermore, antibacterial and antifungal activities of *L. angustifolius* alkaloid extract 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 *C. krusei*. Results of the antibacterial and antifungal tests are given in Table 2. According to data, the alkaloid extract showed significant activity on *B. subtilis*, *S. aureus* and *P. aeruginosa* at MICs of 62.5 $\mu\text{g/ml}$, 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 $\mu\text{g/ml}$.

There are a number of reports on the biological activities of lupine alkaloids such as antipyretic activity, hypoglycemic activity, cardiogenic activity, antiulcerogenic activity, inhibition of edema, inhibition of natural killer cell growth and inhibition of acetylcholinesterase (Ohmiya et al. 1995). QA as the main lupine alkaloids have been shown to have antimicrobial activity by several researchers (Wink 1984; Wippich and Wink 1985; Tyski et al. 1988). In Wink's study (1984), sparteine was reported to possess antimicrobial activity against bacteria and phytopathogenic fungi. Moreover, Wippich and Wink (1985) declared sparteine, lupanine and 13-tigloylupanine were also inhibited the germination of conidia *Erysiphe graminis* f. sp. *hordei*.

Tyski et al. (1988) reported that pure QA isolated from *Lupinus angustifolius* var. Mirela, lupanine, 13 α -hydroxylupanine and angustifoline and the ethanolic extract of the seed of the plant and compound sparteine were showed to have bacteriostatic effects against *S. aureus*, *B. subtilis*, *E. coli*, *P. aeruginosa* and *B. thuringiensis*. Besides, these researchers were declared that bacteriostatic effects of QA were supported the allelopathic function of alkaloids (Tyski et al. 1988). All data obtained these study support that QA may be involved in the antimicrobial defense system of lupins (Wink 1984; Wippich and Wink 1985).

In this study, we aimed to investigate the alkaloid profile of the *L. angustifolius* by GC-MS and antimicrobial activity of the alkaloid extract of the plant. Our GC-MS analysis demonstrated that *L. angustifolius* alkaloid extract consisted of 13 α -hydroxylupanine and lupanine as the main alkaloids. These findings may explain the antibacterial activity of the *L. angustifolius* alkaloid extract as Tyski et al.'s (1988) declared that lupanine and 13 α -hydroxylupanine were displayed antibacterial activity.

To the best of our knowledge, for the first time, we herein report alkaloid profile and antimicrobial activity of *Lupinus angustifolius* L. growing in Turkey.

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