

COMPOSITION AND ALKALOID PROFILE OF *LUPINUS EXALTATUS* ZUCC. DURING ITS DEVELOPMENT

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ABSTRACT

The content and profiles of quinolizidine alkaloids in different plant organs of *Lupinus exaltatus* were analyzed by Gas Chromatography (GC) and Gas Chromatography -Mass Spectrometry (GC-MS). Plant parts (leaves, stems, flowers, and pods) were collected at different stages of the growing plant. The contents of total alkaloids varied in the different plant parts organs, while the alkaloid profile did not exhibit changes in the organs examined. The profile was characterised by six major alkaloids: epiaphylline, aphylline, α -isolupanine, lupanine, dehydro-oxosparteine, 3 β hydroxylupanine and one that do not appear to correspond to any known alkaloid.

KEY WORDS

Quinolizidine alkaloids, *Lupinus exaltatus*, plant development, alkaloid profile

INTRODUCTION

The genus *Lupinus* comprises above 200 species but from this number only a few species have become domesticated and cultivated on a large scale. The main region of wild lupin species is the South, North and Central America (Planchuelo, 1994). In Mexico, more than 110 wild species have been reported (Bermudez, 1998). In recent years great interest in wild lupin species has been observed due to their high protein content in

their seeds and foliage. *Lupinus exaltatus*, is a native species from the central region of Mexico in America. Similar to others legumes, the foliage of *Lupinus* is attracting worldwide attention as a potential source of forage. In this regard a protein and fibre content of 23.5% and 26.5%, respectively has been reported for the foliage of *Lupinus exaltatus* (Ruiz *et al.* 2007). However, one factor that could limit the utilisation of lupin foliage as feedstuff for herbivores is the presence and type of alkaloids. In this study we analyzed the concentration and alkaloid profile of different plant organs of *Lupinus exaltatus* by GC/MS.

MATERIALS AND METHODS

HARVEST

The plants were grown in open ground from sowing (22-09-2004) to maturity, (20-04-2005), and were harvested for chemical analyses at five different stages of development (Table 1). To avoid confounding treatment effects with diurnal changes in alkaloid content, plants were harvested alternately among treatments between 12:30 and 15:30 hours. During growth six plants selected at random were harvested on the first date and three plants were harvested on the subsequent date. The harvested plants were divided into stems, young (not fully expanded) or mature leaves, flowers, immature pods, and then air dried to constant weight at 50°C.

Table 1. Date of harvest, harvested organs and phenological growth stages of *Lupinus exaltatus*.

Date of harvest	Harvested organs	Stages during growth plant	Morphological plant features
1 = 10-01-2005	leaves, stem	Before flowering	4-6 nodes visible- no laterals
2 = 05-02-2005	leaves, stem	Began flowering	Primary inflorescence
3 = 29-02-2005	leaves, stem, flowers	Flowering	All branches with flowers
4 = 26-03-2005	leaves, stem, flowers, immature pods	Pods growth	All racemes with immature pods
5 = 20-04-2005	leaves, stem, immature pods	Seed ripe	All mature pods and seeds

ALKALOIDS EXTRACTION

The alkaloids extraction method used was as described by Muzquiz *et al.* (1994). Finely grounded lupin organs (0.5 g) was homogenised in 5% trichloroacetic acid (3 x 5 mL) with an Ultra Turrax and centrifuged at 700 g for 5 min. After centrifugation, 1 mL of 10 M NaOH was added to the supernatant. The alkaloids were then extracted with dichloromethane (3 x 15 mL). The dichloromethane extract was evaporated to dryness at 30°C. Methanol (1 mL) was added to dissolve the alkaloids. A 0.5 mL aliquot of the extract was added to 0.5 mL of a solution of codeine, which was the internal standard, in methanol (2 mg/mL).

GC AND GC-MS ANALYSIS

Capillary gas chromatography was carried out using a Perkin-Elmer Autosystem equipped with a phosphorus-nitrogen detector (NDP) and Turbochrom for instrument control and data analysis. The column used was a SPB-1 (30 m x 0.25 mm i. d., 0.25 µ film thickness). Helium was the carrier gas (1.3 bar). The temperature of the injector and detector were 240°C and 300°C respectively. The injection volume was 0.1 µL for each sample. GC-MS analyses were carried out on Hewlett Packard capillary gas chromatograph quadrupole mass spectrometry system fitted with a DB5 fused silica capillary column (50 m x 0.2 mm, 0.25 µ film thickness). Chromatographic conditions were the same as mentioned above, and the mass spectrometer was operated at 70 eV. Alkaloids identification was carried out by comparison with authentic reference compounds, spectrometric electronic libraries and published mass spectra. Quantitative analysis of each alkaloid (expressed in percent) was carried out by peak area normalisation measurements.

RESULTS AND DISCUSSION

Six quinolizidine alkaloids were quantified and determined by Gas Chromatography and Gas Chromatography-Mass Spectrometry (GC-MS) analysis in stems, leaves, flowers, pods and seeds of *Lupinus exaltatus* collected at different phenological growth

stages. The alkaloids identified according to retention time and mass spectrometric data registered in the Software for MS Chem-Station data system correspond to epiaphylline aphylline, α-isolupanine, lupanine, dehydro-oxosparteine, 3βhydroxylupanine. The analysis also shows the presence of one compound that does not appear to correspond to any known alkaloid. The corresponding mass spectral dates are presented in Table 2. It is important to indicate that alkaloids such as anagyrene and ammodendrine with mutagenic properties, were not found in *Lupinus exaltatus*.

The alkaloids profile was similar in all organs collected during the different stages of plant growth. These results are similar to those reported by Kinghorn *et al.* (1980) when different parts of the same plants collected simultaneously, were examined. However, considerable variation in the alkaloid profile of *Lupinus campestris* was observed in samples of various organs collected in different months (Martinez *et al.* 2001). In general, concentration of total alkaloids varied in the different parts of the same plant and between different phenological growth stages (Table 3).

Alkaloid concentration was greater in the immature pods than in the leaves, stems and flowers. Alkaloid concentration was similar in flowers and leaves, but lower in stem at all phenological growth stages. Total alkaloids percentages in stem decreased with plant maturity, while the concentrations in leaves decreased significantly only after pod growth. This suggests that the main loss of alkaloids from all vegetative parts is due to transport to fruits and seeds. Similar trends were observed in *L. angustifolius*, *L. albus* and *L. mutabilis* at different phenological growth stages (Williams and Harrison, 1983). However, are different to those observations reported by Lee *et al.* (2007) in stems and leaves samples of *L. leucophyllus* collected in different years. In summary, these results suggest that quinolizidine alkaloids are not waste products or end products of metabolism, but that they are metabolically dynamic compounds involved in defense against predation from herbivores.

Table 2. Identification of quinolizidine alkaloids in plant parts from *Lupinus exaltatus*.

Alkaloids	M ⁺	Characteristic ions (abundance %) ^a
Lupanine	248	248(32) 149(52) 136(100) 98(30) 150(34) 110(12)
epiaphylline	248	248(28) 136(100) 137(51) 96(38) 220(34) 247(29)
α-isolupanine	248	248(40) 149 (52) 150(31) 136(100) 110 (19) 84(25)
aphylline	248	248 (21) 136(100) 96(44) 37(48) 97(48) 220(22)
3β- hydroxylupanine	264	136(100) 264(41) 134(48) 150(41) 98(38) 110(22)
Dehydro-oxosparteine	246	136(100) 96(60) 122(18) 246(93) 98(40) 134(40)

^a Six significant fragments and their relative abundance (%).

M⁺ = Molecular ion.

Table 3. Alkaloid concentration in plant parts at different phenological growth stages.

Phenological growth stages	Stems	Leaves	Flowers	Immature pods
Percent dry weight (%)				
Before flowering	0.91 a Δ	0.93 b		
Began flowering	0.87 a	1.5 a		
Flowering	0.56 b	1.4 a	0.92 a	
Pods growth	0.48 b	1.2 a	1.20 a	1.8 a
Seed ripe	0.31 c	0.61 c		2.1 a
Mean	0.63	1.12	1.06	1.95

Δ Within columns, means followed by the same letter are not significantly different at the 0.05 probability level according to Duncan's multiple range test.

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