

MEXICAN WILD LUPINES AS A SOURCE OF QUINOLIZIDINE ALKALOIDS OF ECONOMIC POTENTIAL**M.A. Ruiz-López, P.M. García-López, R. Rodríguez-Macías, J.F. Zamora Natera***Laboratorio de Biotecnología, Departamento de Botánica y Zoología, CUCBA. Universidad de Guadalajara. Carretera Guadalajara-Nogales Km 15.5 AP 1-139 Zapopan, Jalisco, México. Correo electrónico: mruiz@cucba.udg.mx***M.L. Isaac-Virgen***Instituto de Enfermedades Crónico-Degenerativas, Departamento de Fisiología, CUCS. Universidad de Guadalajara.***M. Múzquiz***SGIT-INIA, Área de Tecnología de Alimentos, Apartado 811, 28080 Madrid, España.***ABSTRACT**

Quinolizidine alkaloids such as lupanine, 13-hydroxylupanine, multiflorine, angustifoline and sparteine, which are present in the species of the genus *Lupinus*, have been reported to have biopesticide and pharmacological activities. The aim of this study was to quantify the content and variation of the individual alkaloids in seeds of *L. mexicanus*, *L. exaltatus*, *L. montanus* and *L. stipulatus* collected in different states of Mexico. Lupanine was the major (5.05 ± 0.37 mg/g) alkaloid found in *L. mexicanus*, whereas sparteine was the main alkaloid present in *L. montanus* (3.97 ± 0.49 mg/g). Conversely, *L. stipulatus* contained only small quantities of lupanine and sparteine (0.1 ± 0.002 and 0.04 ± 0.01 mg/g, respectively). Angustifoline was detected only in *L. montanus*, but in a very low amount (0.048 ± 0.03). The results of this study indicate that *L. mexicanus* and *L. montanus* can be considered as important sources of lupanine

and sparteine for their use as natural pesticide or pharmacological agents.

Key words: *L. mexicanus*, *L. exaltatus*, *L. montanus* and *L. stipulatus*; lupanine, angustifoline, sparteine, 13-hydroxylupanine, angustifoline; biopesticide; pharmaceutical activity.

RESUMEN

Los alcaloides quinolizidinicos lupanina, 13-hidroxilupanina, multiflorina, angustifolina y esparteina, presentes en el género *Lupinus* poseen actividades bioplággida y farmacológica. El objetivo del presente estudio fue cuantificar el contenido y variación de los alcaloides mencionados en semillas de *L. mexicanus*, *L. exaltatus*, *L. montanus* y *L. stipulatus*, colectados en diferentes estados de México. La lupanina fue el principal (5.05 ± 0.37 mg/g) alcaloide encontrado en *L. mexicanus*, mientras que la esparteina fue el mayor alcaloide presente

en *L. montanus* (3.97 ± 0.49 mg/g). Sin embargo, en *L. stipulatus* se encontraron pequeñas cantidades de lupanina y esparteína (0.1 ± 0.002 y 0.04 ± 0.01 mg/g, respectivamente). La angustifolina sólo se detectó en bajos niveles en *L. montanus* (0.048 ± 0.03). Los resultados de este estudio indican que *L. mexicanus* y *L. montanus* pueden ser considerados como una fuente importante de lupanina y esparteína, los cuales pueden ser utilizados como pesticidas o hipoglucémicos naturales.

Palabras clave: *L. mexicanus*, *L. exaltatus*, *L. montanus*, *L. stipulatus*; lupanina, angustifolina, esparteína, 13-hidroxilupanine, angustifolina; bio-plaguicidas; actividad farmacéutica.

INTRODUCTION

The sweet lupin varieties *Lupinus luteus*, *Lupinus angustifolius*, and *Lupinus albus* with low alkaloid content, are recognized throughout the world as a potential source of high quality protein, fiber and fat (Lopez and Fuentes, 1991). However, they have lower adaptation and are preferred by a number of insects, bacteria, fungi, and herbivores (Wink, 1998), as compared to the alkaloid-rich wild type varieties.

The content and type of alkaloids found in different *Lupinus* species depend on the variety, habitat, phenology, plant organs (leaves, stems, flowers, pods, roots, and seeds) and weather conditions (Carey and Wink 1994; Gremigni *et al.*, 2000; Christiansen *et al.*, 1997; Muzquiz *et al.*, 1994a). The total content of quinolizidine alkaloids (QA) in seeds of lupin species ranges from 1.5% to 4.0% (Hatzold *et al.*, 1983; Ruiz and Sotelo, 2001). In addition, more than

150 different QA's have been reported within this genus, of these, lupanine, multiflorine and sparteine have been found in both old and new world lupins (Wink *et al.*, 1995). Sparteine was used in the treatment of cardiac arrhythmias and to induce uterine contractions. It has also been shown to depress the central nervous system and to have hypotensive, diuretic and anti-inflammatory activities (Schmeller and Wink, 1998; Szczawinska *et al.*, 1994). The QA's lupanine, 13-hydroxylupanine and multiflorine have been reported to have pharmacological activities such as anticonvulsant, antipyretic and hypoglycemic (Hatzold *et al.*, 1983; Kubo *et al.*, 2006; García-López *et al.*, 2004). Lupanine, 13-hydroxylupanine, angustifoline and sparteine were shown to have bactericide-like activity against *Staphylococcus aureus*, *Bacillus subtilis*, and *Bacillus thuringiensis* (De la Vega *et al.*, 1996). Lupanine and lupin alkaloid extracts have shown to have herbicidal activity, and the capacity to inhibit the growth of *Fusarium avenaceum*, *Fusarium solani*, *Pythium aphanidermatum*, *Botrytis cinerea*, *Sclerotium rolfsii*, *Rhizoctonia solani*, and *Fusarium oxysporum* (De la Cuadra *et al.*, 1994; Muzquiz *et al.*, 1994b; Zamora *et al.*, 2005; Zamora *et al.*, 2008). Lupin alkaloids also have feeding deterrence effects on the red-legged earth mite *Halotydeus destructor* (Wang *et al.*, 2000).

There are approximately 100 wild lupin species throughout Mexico, with the highest concentration being found in the "Sierra Madre Occidental" and "Eje Neovolcánico Transversal" (Bermúdez *et al.*, 2000). However, the alkaloid content or profile of some of these species is unknown and can be potentially use as natural products in agriculture and /or medicine.

The aim of this study was to determine the content and variability of lupanine, 13-hydroxylupanine, multiflorine, angustifoline and sparteine in seeds of *L. exaltatus*, *L. montanus*, *L. stipulatus* and *L. mexicanus* collected from several states and regions of Mexico.

MATERIAL AND METHODS

Seeds of several wild lupins were collected during the winter and spring of 2006 and 2007 at several locations in the Mexican states of Jalisco, Zacatecas, Mexico, and Guanajuato (table 1). The voucher specimens of *L. exaltatus* (No. 165321, 165435, 165641), *L. montanus* (No. 164989, 164575, 172672), *L. stipulatus* (No. 178432) and *L. mexicanus* (No. 167885, 167890, 177884), were deposited in "Herbario del Instituto de Botánica, Universidad de Guadalajara (IBUG), Mexico.

Seeds were separated, dried and ground to pass through a 150 µm sieve (Tecator, Cyclotec 1093). The alkaloid extraction was performed as described elsewhere (Muzquiz *et al.*, 1993). One half gram of seed flour was homogenized in 5% trichloroacetic acid (3 x 5 mL) with a homogenizer and centrifuged at 3 000 r.p.m. for 5 min. After centrifugation, 1 mL of 10 M NaOH was added to the supernatant and the alkaloids were then extracted with dichloromethane (3 x 5 mL). The dichloromethane was evaporated and the alkaloids were dissolved in 1 mL of methanol. A 0.5 mL aliquot of the extract was added to 0.5 mL of a solution of codeine (internal standard) in methanol (2 mg/mL), each sample was diluted ten-fold.

The samples were analyzed using a Perkin-Elmer Capillary Gas-Chromatograph, equi-

pped with a phosphorous-nitrogen detector (PND) and Turbochrom for instrument control and data analysis. The samples were separated in an SPB-1 column (30 m x 0.25 mm i.d., 0.25-µm film thickness), using helium as the carrier gas (1.38 bar). The temperatures of the injector and detector were kept at 240°C and 300°C, respectively. The initial oven temperature was 150°C, with a temperature ramp of 5°C/min to 235°C, and finally held at 235°C for 15 minutes.

The alkaloid standards used were sparteine (Sigma, St Louis, MO, USA), lupanine perchlorate (Koch-Light, LTD, Conbrock, UK), 13-hydroxylupanine, multiflorine and angustifoline (isolated and kindly provided by Prof. Wysocka, University Adam Mickiewicz). Calibration curves were prepared for alkaloid standards; response was linear over a range of 0-1.250 mg/ml. The determination coefficient of alkaloid content was > 0.99. Alkaloids were identified by comparing retention times of standards with sample peaks.

RESULTS AND DISCUSSION

Table 2 shows the alkaloid concentration in seeds of the wild lupin species collected in different locations and times. As shown in this table, lupanine, sparteine, 13-hydroxylupanine, and multiflorine were present in all the samples collected. However, angustifoline was found only in *L. montanus*. Similar to the majority of American lupin species, angustifoline was not detected in *L. exaltatus*, *L. stipulatus* and *L. mexicanus*. The presence of angustifoline in *L. montanus* indicates a possible chemotaxonomic relationship of this species with the European lupins *L. angustifolius*, *L. albus* and *L. polyphyllus* that contain this

alkaloid (Wink *et al.*, 1995; Kinghorn *et al.*, 1980). However, further analysis of other phytoconstituents would help to establish this relationship.

The lupanine was the major alkaloid in all lupin species with exception of *L. montanus* where sparteine was the principal alkaloid follow by lupanine. The average highest lupanine concentration was found in *L. mexicanus* followed by *L. montanus*, *L. exaltatus*, and *L. stipulatus* (5.05 ± 0.37 , 1.65 ± 0.09 , 1.47 ± 0.27 , and 0.10 ± 0.002 mg/g, respectively). Although sparteine was detected in all lupins species the concentration was only significant in *L. montanus* (3.97 ± 0.49 mg/g). This level of sparteine in *L. montanus* is higher than that reported for *L. reflexus* (2.66 mg/g) and slightly lower than that of *L. articus* (4.31 mg/g) (Ruiz and Sotelo, 2001; Majak *et al.*, 1994).

The minor alkaloids 13-hydroxylupanine and multiflorine were found in all lupins species with the highest content found in *L. stipulatus*, 0.12 and 0.2 mg/g, respectively.

A large variation in the average lupanine content among the lupin samples collected was observed, this could be due to differences among the species, locality, year of collection, and/or environmental conditions (Jansen, *et al.*, 2009; Gremigni, *et al.*, 2000; Christiansen, *et al.*, 1997).

L. stipulatus was found to contain only small quantities of sparteine (0.04 ± 0.01 mg/g), lupanine (0.1 ± 0.002 mg/g), 13-hydroxylupanine (0.12 ± 0.004 mg/g) and multiflorine (0.2 ± 0.004 mg/g). Therefore, *L. stipulatus* is a poor source of these four alkaloids. Other peaks were observed in

the chromatogram, however, additional gas chromatographic-mass spectrometry (GC-MS) and nuclear magnetic resonance analysis must be performed in order to determine if these are other alkaloids.

The average highest concentration of 13-hydroxylupanine was found in *L. stipulatus* (0.12 ± 0.004 mg/g), followed by *L. montanus* (0.10 ± 0.09 mg/g), *L. mexicanus* (0.015 ± 0.018 mg/g) and *L. exaltatus* (0.015 ± 0.013 mg/g). The multiflorine content in *L. mexicanus*, *L. exaltatus*, *L. montanus*, and *L. stipulatus* was 0.096 ± 0.09 , 0.004 ± 0.000 , 0.07 ± 0.004 , and 0.2 ± 0.004 mg/g, respectively. Even though these minor alkaloids possess biological activity their isolation and purification could be an expensive and time consuming process to pursue.

CONCLUSIONS

The variability in the alkaloids content among species was larger than within individual species. *L. mexicanus* and *L. montanus* contain a high amount of lupanine and sparteine and can be a potential source of these alkaloids for agricultural and medical applications. Although, multiflorine and 13-hydroxylupanine have pharmacological activities, the isolation from these lupin species would be result impractical due to their low concentration.

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Table 1. Date and place of collection, and typical habitat of each lupin species.

<i>Lupinus</i> species	Date of collection	Locality	Mexican state	Typical habitat
<i>L. mexicanus</i>	03/2006	Lagos de Moreno	Jalisco	Dry and disturbed roadsides
<i>L. mexicanus</i>	03/2006	Manalisco	Jalisco	Grassland, arid and disturbed soils
<i>L. mexicanus</i>	04/2006	Teoloyucan	Mexico	Disturbed, roadsides and cultivated grounds
<i>L. mexicanus</i>	04/2006	Guanajuato	Guanajuato	Roadsides and cultivated grounds
<i>L. exaltatus</i>	09/2006	Cd. Guzmán	Jalisco	Grassy openings in pine, roadsides and cultivated grounds
<i>L. exaltatus</i>	10/2006	Zapopan	Jalisco	Spreading to pasture, roadsides
<i>L. exaltatus</i>	09/2007	Cd. Guzmán	Jalisco	Grassy openings in pine, roadsides and cultivated grounds
<i>L. montanus</i>	10/2006	Tonila	Jalisco	Montane meadows, sandy soil in open forest
<i>L. montanus</i>	10/2007	Tonila	Jalisco	Montane meadows, sandy soil in open forest
<i>L. montanus</i>	10/2007	Bolaños	Jalisco	Disturbed and sandy soils
<i>L. stipulatus</i>	12/2007	Monte Escobedo	Zacatecas	Dry and disturbed roadsides

Table 2. Quinolizidine alkaloids in seeds of Mexican wild lupins (mg/g dry matter).

Species	Sparteine	Lupanine	13-hydroxylupanine	Multiflorine	Angustifoline
<i>L. mexicanus</i> ¹	0.0080 ± 0.0001	5.56 ± 0.060	0.006 ± 0.002	0.140 ± 0.001	---
<i>L. mexicanus</i> ²	0.0070 ± 0.001	4.70 ± 0.930	0.042 ± 0.030	0.200 ± 0.004	---
<i>L. mexicanus</i> ³	0.0030 ± 0.001	4.90 ± 0.007	0.004 ± 0.000	0.030 ± 0.000	---
<i>L. mexicanus</i> ⁴	0.0120 ± 0.001	5.06 ± 0.520	0.007 ± 0.007	0.016 ± 0.001	---
Mean ± S.D.	0.0075 ± 0.004	5.05 ± 0.370	0.015 ± 0.018	0.096 ± 0.090	
<i>L. exaltatus</i> ⁵	0.0030 ± 0.000	1.75 ± 0.10	0.006 ± 0.000	0.005 ± 0.000	---
<i>L. exaltatus</i> ⁶	0.0040 ± 0.000	1.47 ± 0.00	0.010 ± 0.003	0.004 ± 0.000	---
<i>L. exaltatus</i> ⁷	0.0030 ± 0.000	1.20 ± 0.01	0.030 ± 0.010	0.002 ± 0.000	---
Mean ± S.D.	0.0033 ± 0.000	1.47 ± 0.27	0.015 ± 0.013	0.004 ± 0.000	
<i>L. montanus</i> ⁸	3.50 ± 0.21	1.75 ± 0.32	0.110 ± 0.007	0.090 ± 0.002	0.013 ± 0.003
<i>L. montanus</i> ⁹	4.47 ± 0.13	1.60 ± 0.051	0.190 ± 0.024	0.100 ± 0.003	0.050 ± 0.004
<i>L. montanus</i> ¹⁰	3.93 ± 0.05	1.60 ± 0.400	0.006 ± 0.000	0.027 ± 0.002	0.081 ± 0.006
Mean ± S.D.	3.97 ± 0.49	1.65 ± 0.090	0.102 ± 0.090	0.07 ± 0.004	0.048 ± 0.034
<i>L. stipulatus</i> ¹¹	0.04 ± 0.01	0.10 ± 0.002	0.120 ± 0.004	0.20 ± 0.004	---

Values are given as mean ± S.D. of three replicates

1= Lagos de Moreno, Jalisco 2006, 2= Manalisco, Jalisco, 2006, 3= Teoloyucan, Edo. de Mexico, 2006, 4= Guanajuato, Guanajuato, 2006, 5= Cd. Guzman, Jalisco, 2006, 6= Cd. Guzman, Jalisco, 2007, 7= Zapopan, Jalisco, 2006, 8= Tonila, Jalisco, 2006, 9= Tonila, Jalisco 2007, 10= Bolaños, Jalisco, 2007, 11= Monte Escobedo, Zacatecas, 2007.