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Chemical composition and antimicrobial activity of Sudanese *Lupinus termis* L. root extracts

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Abstract
The root of *Lupinus termis* L., a leguminous plant of great potential growing in northern Sudan and native to the Mediterranean basin was investigated phytochemically and assessed for its antimicrobial activity. Previous phytochemical, pharmacological and clinical investigations based on ethnopharmacologic approach recommended the use of the seed ethanolic extract as a safe herbal remedy in treatment programs of shingles. Herein we report the Lupin root oil extracted with petroleum ether from the sweet variety investigated for its lipid profile as well as the quinolizidine alkaloid profile of the root methanolic extract. The GC-MS analysis showed that the oil was composed mainly of oleamide (43.1%); hydrocarbons (26.2%); fatty alcohols (15.8%) and fatty acids (09.58%). The semi-purified alkaloid methanolic extract was composed mainly of lupanine (72%); 13-hydroxylupanine (6.6%); sparteine (4.6%); ammodendrine (3.8%) and 13- Methoxylupanine (2.5%). The two extracts were assessed for their antimicrobial activity and the microorganisms were resistant to the oil extract and were moderately sensitive to the methanolic extract.

Keywords: *Lupinus termis*, roots, lipid profile, alkaloid profile, antimicrobial activity, phytochemical screening, shingles.

1. Introduction
About 200 characterized species of the *Lupinus* genus are distributed in Europe, Africa and America (1). Lupin is an annual legume, native to the mediterronean basin, with protein content of the seed from 38 to 62%; oil content from 1 to 11%, depending on the genotype and starch content up to 5 % (2). Flour and isolates processed from lupin are used for human consumption and animal feed after reduced alkaloid-content treatment. Endogenous concentrations of quinolizidine alkaloids in the *lupinus* genus are widely known and considered as chemotaxonomic markers and they are toxic for humans, microorganisms and for some plant species (3). Extracts of the seeds were effective in the treatment of chronic eczema with reference to lupin alkaloids (4). A herbal ointment prepared from the seeds of *L. termis* and leaves of *Ambrosia maritima* (Asteraceae), prescribed by a Sudanese herbalist for treatment of Herpes Zoster (shingles) was investigated by phytochemical and pharmacological procedures and clinical trials (5). The study concluded that the ointment could be recommended as a safe herbal remedy in treatment programs of shingles. As a continuation in this field we report in this study the results of our attempts at obtaining a lipid profile of the root petroleum ether 40-60 °C extract and the alkaloid profile of the semi- purified alkaloid methanolic extract of the root. The results of antimicrobial activity assessment of the two extracts, are reported also in the present study.

2. Materials and methods
2.1 Plant material
The plant was collected from the field near the city of Merawi, northern Sudan during the fruiting stage in the year of 2009. Voucher specimens have been deposited and identified in the herbarium of Medicinal and Aromatic Plant Research Institutes, the National Center for Research, Khartoum Sudan.

2.2 Extraction
The plant collected was divided into roots, stems, leaves and seeds. The air-dried powdered material (100g) was defatted with petroleum ether 40-60 °C in a Soxhlet apparatus followed by extraction with methanol three times at room temperature. The petroleum ether extract gave

0.26 g oily extract and the combined methonolic extracts gave 6.7 g residue after removal of methanol under reduced pressure. Half of the petroleum ether extract was used for antimicrobial activity assessments and the other half for TLC and GC- MS analysis. Half of the methoanolic extract was used for antimicrobial tests, and the other half for alkaloid extractions.

2.3 Alkaloid extraction

About 3g of the methanolic extract was dissolved in 150 ml 0.5 N HCl and extracted three times with 300ml diethyl ether. The aqueous layer was adjusted to PH 10 with 25% NH4OH and extracted three times with dichloromethane until it become negative to Dragendorff's reagent (6). The dichloromethane extracts were combined and dried over anhydrous Na2SO4 and evaporated to dryness under reduced pressure. The total alkaloid dry weight was 0.4% (0.12 g).

2.4 Phytochemical Screening

The screening of roots, stems, leaves and seeds for phytochemical constituents was performed using generally accepted laboratory techniques and procedures for qualitative determinations (7). The constituents screened for were alkaloids; anthracene glycosides; flavonoids; sterols and triterpenes; saponins and cardiac glycosides; tannins and carbohydrates.

2.5 Antimicrobial assays

The root petroleum ether extract and the root methanolic and semi-purified alkaloid methanolic extract were resuspended in 20% DMSO to obtain concentrated stocks. The microorganisms were *Staphylococcus aureus* (Sa); *Bacillus subtilis* (Bs); *Escherichia coli* (Ec); *klebsiella pneumonia* (Kp), *Candida albicans* (Ca) and *Aspergilluss flavus* (Asp).

They were obtained from the stock cultures of the department of microbiology, Medicinal and Aromatic Plants Research Institute, NCR, Khartoum, Sudan. The antimicrobial activity was assayed via the agar diffusion method (6).

2.6 Gass chromatography- Mass spectrometry Analysis

The GC- Ms analysis was performed on Shimadzu QP 2010 system. Column: capillary 30m, 0.25mm ID; conditions: carrier gas He at 1ml/min, and initial temperature set 50°C, temperature ramp 15°C/min to 250°C. spectra were obtained over m/z: 100-800.

The petroleum ether extract sample was esterified with methanol and KOH and the semi- purified alkaloid methanolic extract were used for qualitative and quantitative determination by GC-MS analysis.

3. Results and Discussion

The prepared petroleum ether and methanolic root extracts were calculated as 0.26% and 6.07% respectively and were subjected to phytochemical screening and assessment of antimicrobial activities. Results were displayed in table (1) and table (2). Sterols; triterpenes and fatty acids were detected in the oily petroleum ether extract and the methanolic root extract screening confirmed the presence of alkaloids, flavonoids, saponins, tannins and carbohydrates in addition to sterols and triterpenes.

Table 1: Results of phytochemical screening of petroleum ether and Methanolic extracts of *L. termis* roots

Extract Test	Petroleum ether (40-60 °C)	Methanol
Alkaloids	-	+
Anthraquinones	-	±
Sterols	+	+
Triterpenes	+	+
Saponins	-	+
Cardiac glycosides	-	±
Carbohydrates	-	+
Flavonoids	-	+
Tannins	-	+

(+) positive (-) Negative (±) Traces

The antimicrobial activity of the two extracts was variable. The Gram positive and Gram negative bacteria in addition to the fungi were resistant to the petroleum ether extract, while *B. subtilis, k. pneumonia* and *A.flavus* were sensitive to the methanolic extract.

Table 2: Results of Antimicrobial activity of Petroleum ether and Methanolic extracts of *L. Termis* roots

Extract MDIZ (mm)	Petroleum ether (40-60 °C)	Methanol
Staphylococcus aureus	-	11
Bacillus subtilis	-	28
Escherichia coli	11	-
Klebsiella pneumonia		15
Candida albicans	12	-
Aspergillus flavus	-	14

Concentration: 5% of extract in DMSO. MDIZ: mean Diameter of Inhibition Zone.

MDIZ: >15 mm= sensitive; <15mm= resistant; (-)= no activity.

To spot more light on the chemical composition of the two extracts, and their variable antimicrobial activity, further investigations by GC-MS analysis were performed. It is known that the oil content of lupin seeds may range from 1 to 17%, with a high variation in fatty acid composition (8, 9). The scant information about the root oil content and its chemical composition prompted us to investigate the Sudanese lupin for its root oil content and composition. Results of GC-MS analysis of the methylated petroleum ether root extract were displayed in table (3) and Fig (1).

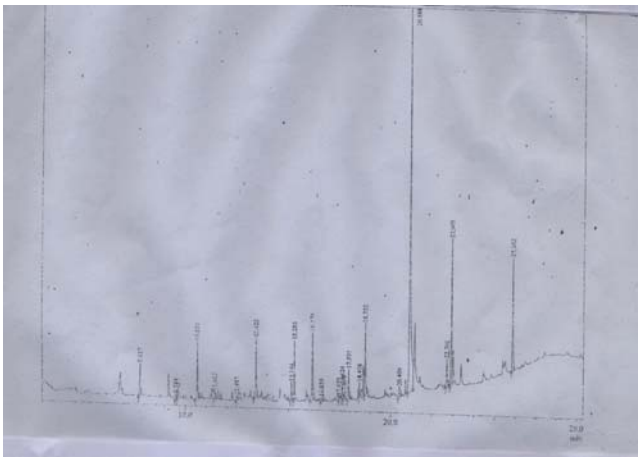


Fig 1: Gas Chromatogram of the Petroleum ether extract of *L. termis* roots

Table 3: Result of GC- MS analysis of petroleum ether extract of *L.termis* root

Peak No.	Retention time RT	Area under the peak AUP%	Compound
1	7.81	2.63	1-Pentadecene
2	9.52	0.30	Unknown*
3	9.63	0.32	Methyl dodecanoate
4	10.57	4.03	1-Heptadecene
5	11.41	0.46	Unknown *
6	12.48	0.35	Methyl tetradecanoate
7	13.42	4.61	1-Nonaclecene
8	15.14	1.30	Unknown *
9	15.28	3.46	Methyl hexadecanoate
10	16.17	6.00	Nonadecane
11	16.63	0.47	Methyl-15-methylhexadecanoate
12	17.45	0.19	Unknown
13	17.62	0.96	(E)-9-Methyl octadecenoate
14	17.69	0.55	(z)-9-Methyl octadecenoate
15	17.93	2.19	Methyl stearate
16	18.46	1.12	Ethyl oleate
17	18.75	5.41	1-Heptacosanol
18	20.40	0.92	Methyl-18-methylnonadecanoate
19	20.88	43.14	(z)-9-Octadecenamide
20	22.70	2.17	Methyl-20-methyl heneicosanoate
21	22.90	10.42	1-Nonadecanol
22	25.86	8.47	Squalene

According to the results obtained from the GC-MS analysis, it was found that the petroleum ether oily extract was composed of saturated and unsaturated aliphatic hydrocarbons (26. 24%); saturated long chain aliphatic alcohols (15.83%); saturated and unsaturated fatty acids (09.51%) and the main constituent was (z)-9- octadecenamide (43.14%) known as oleamide. The unknowns were about 02.25%. oleamide was first isolated from the cerebrospinal fluid of sleep- deprived cats, and has been characterized and identified as the signaling molecule responsible for causing sleep(10).

Oleamide

In addition to its sleep-including properties; oleamide has other neurological activities including regulation of memory processes, decreasing body temperature and locomotive activity; stimulating Ca++ release; modulation or activation of receptors, and effect on perception of pain (10). The high content of oleamide in the lipid fraction of the lupin root, may spot more light on its chemical composition as a natural source of this important fatty amide, bearing in mind its synthetic production in industry.

It is known that bitter and sweet lupin differ in the alkaloidal content which could exceed 2%, but by boiling and steeping the seeds in water, the alkaloidal content in sweet lupin could be successfully removed (8). Along with the lipid profile, the present work attempts at obtaining an alkaloid profile of the root to spot more light on the quinolizidine alkaloid profile as chemotaxonomic markers and their biological functions. The GC-MS analysis showed the presence of previously reported quinolozidine alkaloids as well as additional alkaloids (6, 11). Results are displayed in Fig (2) and table (4).

The results revealed that the main quinolizidine alkaloid was lupanine (72.03%), followed by 13- hydroxy lupanine (6.66%), sparteine (4.62%); ammodendrine (3.84%) and 13-methoxylupanine (6.66%). The results were not compatible with data published in the literature about the lupine seed alkaloids and this could be due to different geographic locations and seasonal variations.

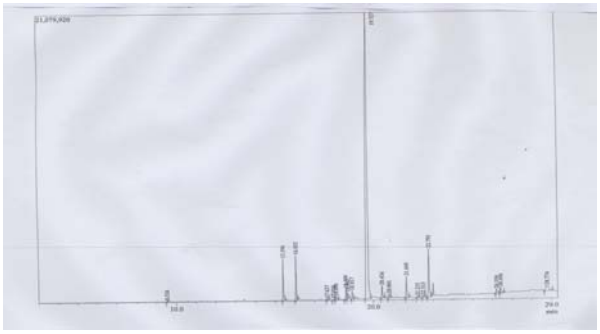
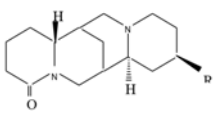
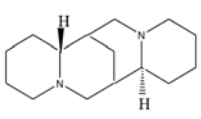


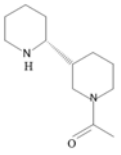
Fig 2: Gas chromatogram of the semi-purified alkaloid methanolic extract of *L.termis* roots

Table 4: Results of GC-MS analysis of the semi-purified alkaloid extract of *L.termis* root

Peak No	Retention time Rt	Area under the peak AUP%	Compound
1	9.52	0.10	Lupinine
2	15.39	3.84	Ammodendrine
3	16.05	4.62	Sparteine
4	17.63	0.16	Isoangustifoline
5	17.95	0.17	Tetrahydro Rhombifolia
6	18.09	0.37	Dehydroangustifoline
7	18.59	1.30	Angustifoline
8	18.68	0.70	Matrine
9	18.91	0.90	α-isolupanine
10	19.72	72.03	Lupanine
11	20.43	1.62	Aphylline
12	20.80	0.59	Dehydrolupanine
13	21.66	2.56	13-methoxylupanine
14	22.23	0.20	Genisteine
15	22.51	0.25	Multiflorine
16	22.79	6.66	13-OH-Lupanine
17	26.19	0.66	N-oxide-isosophordine
18	26.40	0.98	Sophocarpine
19	28.77	2.29	Baptifoline







(1) Lupanine; R=H
(2) 13-Hydroxylupanine, R = OH
(3) 13- Methoxylupanine, R = OCH3

(4) Sparteine

(5) Ammodendrine (Spherocarpine)

4. Conclusion

Based on the present studies conducted to characterize the lupin seed alkaloids and oil of different varieties, it can be concluded that the oily extract of the root is a rich source of oleamide and other antioxidant markers. It is known that, lupin contains endogenous concentrations of quinolizidine alkaloids as chemotoxonomic markers and the high content of lupanine in the roots could be considered, based on the fact that, alkaloid biosynthesis starts in the roots.

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