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Research Article

Chemical Profile of washing water extracts obtained from *Lupinus termis* seeds cultivated under Sudanese Conditions

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Abstract

In the present study, the effect of different washing treatments on the content of isoquiolizidine alkaloidal compounds of Lupinus termis cultivated under Sudanese conditions were investigated. Thin-Layer Chromatography and Gas Chromatography/Mass Spectrometry were used to analyze the composition of alkaloids in washing water extracts (first washing and sum of three sequential washings) of *L. termis*. The lupanine, 1-benzylnaphthalene and 13-OH-lupanine were the principal alkaloids in the washing water extracts of Sum of three sequentials washing of *lupine termis* was better method to leach the high toxic quinolizidine alkaloids from the lupine seed compared with first washing extracts

Keywords: *Lupinus termis*, chemical profile, quinolizidine alkaloid, GC-MS and TLC.

Introduction

Quinolizidine alkaloids (QAs) are toxic secondary metabolites found within the genus Lupinus, some species of which are commercially important grain legume crops. While QAs offer the plants protection against insect pests, the accumulation of QAs in lupine grain complicates its use for food purposes as QA levels must remain below the industry threshold (0.02%), which is often exceeded. (Frick *et al*, 2017) .

Several species of lupine (*Lupinus spp*.) are toxic to livestock, causing death losses in sheep and cattle but more commonly “crooked calf disease” in pregnant range cows. The major toxic alkaloids in lupine are of the quinolizidine alkaloid group and include the teratogen anagyrine, which is primarily responsible for crooked calf disease. Lupines also contain teratogenic piperidine alkaloids including ammodendrine. Previous work in sheep has shown that lupine alkaloid clearance

may be influenced by the animal's physiological status. (Lee *et al*, 2008). Hernández *et al* (2011) reported that *N*-formylangustifoline to be the main QA, at 17%, and some other esters to be minor quinolizidine alkaloid.

The objective of this study was: to determinate the quinolizidine alkaloids through GC-MS analysis of *Lupinus termis* washing water extractions of Sudanese variety (first washing and sum of three sequential washings).

Materials and Methods:

Preparation of Lupine aqueous extracts:

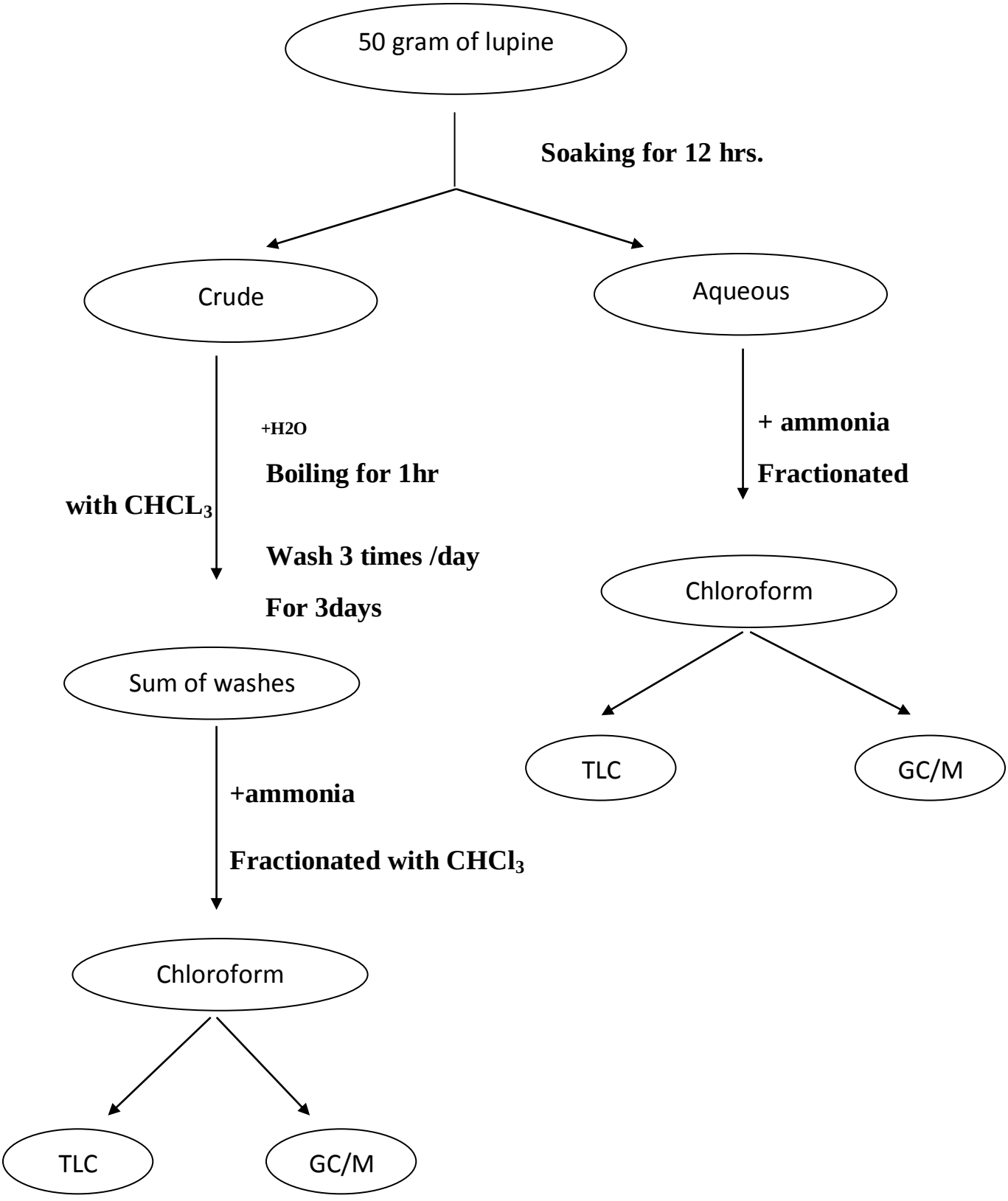
A 50g of Lupine seeds were macerated in distilled water over night (12 hours) at room temperature, and then they were boiled at 100°C for 20 minute until the color of the seed change to yellow and water has been collected. After that seeds were macerated again and the water that used in maceration of the seed has been changed three times per day for three days and water was collected again.

4.2 Extraction of quinolizidine alkaloid

Sample A. 50ml of water that used in maceration of lupine seed overnight**.** Sample B 50ml of water that use for boiling and maceration of lupine seed over the three days after being mixed.

20ml of sample A was made alkaline by addition of ammonia (color of litus paper change to blue). Then it was fractionated three times with 20ml chloroform. After that chloroform layers were combined and placed in a clean vial. These procedures were repeated for sample B.

Figure 1: Extraction and isolation of quinolizidine alkaloids by TLC and GC/MS


50 gram of lupine
Soaking for 12 hrs.
Crude
Aqueous
+H2O
Boiling for 1hr
with CHCL3
Wash 3 times /day
For 3days
+ ammonia
Fractionated
Chloroform
TLC
GC/M
Sum of washes
+ammonia
Fractionated with CHCl3
Chloroform
TLC
GC/M

Chloroform extracts of sample A and B were developed in a silica gel plate with solvent system code 1 and 2, and then the plate was sprayed with Dragendorff's reagent Table 1. Dragendorff's reagent constituents:

Table 1: Solvent systems used for TLC:

Code	Solvent	Ratio
1	$CHCL_3$-MeOH- concNH_4OH	85:15:1
2	MeOH-concNH_4OH	64:1

GC-MS analysis

GC-MS analysis was done for sample A (washing water extract, first washing) and B sum of three sequential washings) with respect to quinolizidine alkaloids. GC/MS analysis was conducted using Ashimadzu QP (2010) GC/MS instrument equipped with reference libraries. Identification of the constituents was based on computer matching against commercial Wiley Library. Conditions for GC Ashimadzu QP (GC-2010) Gas chromatograph apparatus (Japan). Separation of quinolizidine alkaloid mixtures was carried out with a Carlo Erba ICU 600gas chromatograph equipped with FID (flame ionization detector) and spectra physics integrator. Column: PEGA 10%. Column temp: 240°C. Conditions: Carrier gas nitrogen. Detector temperature: 220°C. Injector: 280°C. Sample volume injector: 0.5 ml. Flow rate: 1.5 ml /min. Attenuation: 16x10. Detector chart speed: 1u n /mw.

Results and Discussion

Thin layer analysis results

The results of TLC of chloroform extracts are presented in Tables 2 and 3. Rf values obtained by TLC patterns are useful to establish their identity and purity of the plant species. It is evident that solvent system chloroform: methanol: conc. Ammonium hydroxide (85:15:1) achieved good separation, showing 6 spots in water extract 1 in lupine, which gave orange-brown color with Dragendorffs reagent that indicates the probability of being alkaloids. From Table 4, it is evident that solvent system Methanol: conc Ammonium Hydroxide (64:1), showing 10 spots in water extract 2. Dragendorff's reagent and solvent system chloroform: methanol: conc. Ammonium hydroxide (85:15:1) have given better separation of the components of explosive mixtures. The RF values of Dragendorff's reagent and chloroform: methanol: conc. Ammonium hydroxide (85:15:1) in different solvent systems is mainly attributed to the C-methylgroup, which reduces the adsorption capability and thereby increases the RF value gradually. The RF value as is evident in (Table 2 and 3) in which RF values extract 1 and 2. The TLC analysis of two lupinus extractions (yellow and brown

color) indicated that alkaloids are existed. Furthermore, present of brown color in all samples tested normally correlated with the existence of alkaloids in thin layer analysis.

Table 2: The results of TLC examination of alkaloids in *L. termis* water extract 1:

Material	No. of spots	Day light		UV 254nm		Dragendorff's reagent	
		Color	R_F values	Color	R_F value	Color	R_F values
	1	Yellow	0.36	Brown	0.38	Brown	0.38
	2			Brown	o.55	Broun	0.45
	3			Brown	o.84	Brown	0.55
	4					Brown	0.66
	5					Brown	0.78
	6					Brown	0.84

Table 3: The results of TLC examination of alkaloids in *L. termis* water extract 2:

Material	No of spot	Day light		UV 254nm		Dragendorff's reagent	
		Color	R_f values	Color	R_f values	Color	R_f values
	1	Yellow	0.07	Brown	0.07	Brown	0.07
	2	Yellow	0.14	Brown	0.16	Brown	0.16
	3	Yellow	0.85	Brown	0.25	Brown	0.25
	4			Brown	0.30	Brown	0.30
	5			Brown	0.39	Brown	0.39
	6			Brown	0.47	Brown	0.46
	7			Brown	0.56	Brown	0.56
	8			Brown	o.59	Brown	0.59
	9			Brown	o.79	Brown	0.79
	10			Brown	0.85	Brown	0.85

Analysis of Alkaloids:

GC/MS analyses of *L.termis* from washing water extracts detected differences in the quantities of three alkaloidal compounds identified as 1-benzylnaphthalene, lupanine and 13-OH-lupanine Tables 4 and 5. Some minority alkaloids were also identified: C17H14 in extract 1 and 2-methylhexanoic acid, 1-(1-cycloheptenyl) pyrodine and C18H32N2O2Si in extract 2. At first washing a new alkaloid (RT 27.059) which has not been reported before was identified tentatively according to its mass fragmentation pattern RT 27.059 shows a molecular ion at mol/wt 218 and base peak mol/wt 218. Alkaloids in the water extracts (sum of three sequential washings) were identified (2-methyl hexanoic acid, 2-cyclohexen-1-one, 2, 4, 4- trim ethyl -3-(3-oxobutyl)-, 1-benzylnaphthalene, 1-(1-cycloheptenyl) pyriodine, 1,5-methano-8-H-pyrido[1,2-a]- [1,5] d—azocin-8-one, decahydro-4-(2-propen-1-yl)-(1S,4S,5S,11aR)-, Lupanine, and 13-OH-Lupanine) table 5.

Bermúdez Torres *et al* (2009) reported sparteine as main QA in leaves of *L. aschenbornii* and *L. montanus*. Lupanine, another tetracyclic QA, was present in stem (19%), flower (9%) and seed (7%) of *L. aschenbornii.* Multiflorine is one of the characteristic QA of seed (11%), stem (6%) and flower (3%) of *L. aschenbornii* Schauer. Whereas sparteine, lupanine and their derivatives are very common in lupin species, multiflorine and their derivatives have a restricted distribution (Wink *et al*., 1995). They were found in Old World species such as *L. albus, L. cosentinii, L. micranthus* and *L. varius* and in South American species such as *L. albescens* and *L. aureonitens* (Planchuelo-Ravelo and Wink, (1993), Planchuelo-Ravelo *et al*, 1993). Traces of this alkaloid were also found in some North American species (Wink *et al*, 1995).

Table4. Composition of alkaloids in the water extract (first washing) obtained from the seeds of *L. termis*:

No.	Alkaloids	RT	Mol/Formula (Mol/Wt)	Base Peak	Area (%)
1	1-benzylnaphthalene	26.951	$C_{17}H_{14}$ (218)	218.05	17.18
2	Unidentified	27.051	$C_{17}H_{14}$ (218)	218.00	2.65
3	Lupanine	30.369	$C_{15}H_{24}N_2O$ (248)	136.10	65.96
4	13-OH-Lupanine	35.227	$H_{15}H_{24}N_2O_2$ (264)	152.10	14.20

The GC and MS analysis of alkaloids at first washing and water extract (sum of three sequential washings) showed that 1-benzyl naphthalene; Lupanine and 13-OH-Lupanine were detected in both first and second lupines extracts, and considered the main alkaloids. Whereas, new alkaloids which have not been identified RT 27.051, 31.296, 31.652 and 33.341, respectively, shows a molecular ion at 218, 336, 153 and 221, respectively, and base peak at 218.00, 246.10, 58.05, and 221, respectively.

Lupanine,1, 5-methano-8-H-pyrido [1,2-a] -[1,5] d — azocin – 8 - one, decahydro - 4-(2 propen-1-yl)-(1S,4S,5S,11aR)-,-cyclohexen-1-one,2,4,4-trimethyl-3-(3-oxobutyl)-, and 13-OH-Lupanine alkaloids were detected as the main component in lupine seed collected from Sudan. The occurrence of alpha pyridine alkaloids in genus lupines is quite unusual and has been detected in a few species only, which are genetically closely related (Kass and Wink, 1997).

Table5. Composition of alkaloids in water extract (sum of three sequential washings) obtained from the seeds of *L. termis*:

No.	Alkaloids	RT	Mol/Formula (Mol/Wt)	Base Peak	Area (%)
1	2-methyl hexanoic acid	21.994	$C_7H_{14}O2$ (130)	74	0.17
2	2-cyclohexen-1-one,2,4,4-trimethyl-3-(3-oxobutyl)-	25.717	C13H20O2 (208)	165	1.94
3	1-benzylnaphthalene	26.941	C17H14 (218)	218.05	4.90
4	Unidentified	27.051	C17H14 (218)	218.00	0.89
5	1-(1-cycloheptenyl) pyridine	28.989	C11H19N (165)	134.05	0.49
6	1,5-methano-8-H-pyrido[1,2-a]-[1,5] d—azocin-8-one, decahydro-4-(2-propen-1-yl)-(1S,4S,5S,11aR)-	29.538	C14H22N2O (234)	193.05	1.78

7	Lupanine	30.366	C15H24N2O (248)	136.10.	72.03
8	Unidentified	31.296	C18H32N2O2Si (336)	246.10	0.89
9	Unidentified	31.652	C10H19N (153)	58.05	1.30
10	Unidentified	33.341	C12H15NO3 (221)	221	4.02
11	13-OH-Lupanine	35.185	C15H24N2O2 (264)	152.05	11.60

Conclusion:

Lupines extracts (first and sum of three sequential washing) detect 1-benzylnaphthalene, Lupanine, 13-OH-Lupanine, and unidentified ones alkaloids. But, the sum of sequential washing helps to detect more available alkaloids through GC-MS and TLC analysis. The sum of three sequential washing is better than the first washing, but takes more time to extract these alkaloids from the lupines.

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