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GC-MS Analysis and Antimicrobial Activity of Sudanese *Lupinus temis* L.(Fabaceae) Fixed Oil

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Abstract

The present study was carried out to investigate the chemical constituents of the medicinally important : *Lupinus temis* seed oil and to evaluate its antimicrobial activity. Sixteen components were detected by GC-MS analysis. Major constituents are: 9-octadecenoic acid methyl ester (50.99%) , 9,12-octadecadienoic acid methyl ester(15.87%),11-eicosanoic acid methyl ester(7.74%) and and methyl-20-methyl-hencicosanoate(6.54%).
Butylated hydroxytoluene, a potent antioxidant, was detected as a minor constituent(0.03%).The oil was also screened for antimicrobial activity against six standard human pathogens. Against the test organisms , the oil showed different antimicrobial responses. It exhibited excellent antibacterial activity against all test organisms . Also it showed significant antifungal activity against the yeast *Candida albicans* .

Keywords:, *Lupinus temis* , Fixed Oil, GC-MS, Antimicrobial Activity.

Introduction

Lupinus is a genus of flowering plants in the legume (Fabaceae) family. The genus include over 200 species with major center of diversity in the Mediterranean region and south America(Dervas,et.al.,1999 ; Hyghe,1997; Swiccicki et.al.,2000). White lupin is an annual legume cultivated in some Mediterranean countries. The seeds are roasted, mixed with malt grains and infused in boiling water to produce coffee-like beverage(URL-1).Yellow Lupin-*Lupinus temis* –is grown in Sudan for its economic value. It is used in Sudanese system of medicine in the treatment of some diseases. Seeds, being rich in protein, are boiled with water , salted and eaten as food.

Quinolizidine alkaloids usually occur in Lupin species and other plants of the Genisteae tribe. They are biosynthesized in green tissues and stored in all organs including seeds.(Boschin et.al.,2013). A lupin alkaloid - 13–β-hydroxymultiflorine was reported from the seeds of the species : *Lupinus*

varius (Mohamoud and Hashem, 1997).Another eleven known alkaloids were also isolated. Wink et. al.(1995) reported the alkaloid composition of 56 species of the genus Lupinus . The relative abundance of 100 alkaloids in leaves and seeds were recorded. The lupin alkaloid, (−)-Δ^5-dehydroalbine, was isolated from the ethanol extract of the seeds of *Lupinus termis* together with some unusual lupin alkaloids(Mahmoud , 1991).

Hamdy et. al.(2002) claimed that administration of suspensions of *Lupinus termis* seeds to alloxan-induced diabetic models exerted antihyperglycemic effects and consequently may alleviate liver and renal damage caused by alloxan-induced diabetes. Beside the hypoglycemic effect other pharmacological actions were reported for seeds including: antfungal(Antoun and Taha,1981), hypotensive(Medhiu et.al.,1986) and treatment of eczema(Antoun and Taha,1981;Al-Zaid et.al.,1991; El-Dandily,1996). It has been demonstrated that lupinus extract inhibited acetylcholinesterase which is responsible for the hydrolysis of acetylcholine. Loss of acetylcholine plays a vital role in learning and memory in Alzheimer disease patients(Greenblatt et.al.,1999 ; Orhan et.al.,2004 ; Lahiri et.al.,2002 ; Howes and Houghton, 2003 ; Mukherjee et.al.,2007 ; Heinrich and Lee Teoh, 2004).

Materials and Methods

Plant material

The seeds of *Lupinus temis* were purchased from the local market – Omdurman, Sudan.The plant was authenticated by The Institute of Aromatic and Medicinal Plants- Khartoum ,Sudan.

Instruments

A Shimadzo GC-MS-QP2010 Ultra instrument with a RTX-5MS column (30m,length ; 0.25mm diameter ; 0.25 µm, thickness) was used for GC-MS analysis .

Test organisms

Lupinus temis oil was screened for antibacterial and antifungal activities using the standard microorganisms: *Bacillus subtilis* (Gram +ve) , *Staphylococcus aureus* (Gram +ve), *Pseudomonas aeroginosa* (Gram –ve) , *Escherichia coli* (Gram – ve) and the fungal species *Aspergillus niger* and *Candida albicans*.

Methods

Extraction of oil from *Lupinus temis* seeds

Dry-powdered seeds of *Lupinus temis* (400g) were extracted with n-hexane at room temperature for 48h..The solvent was removed under reduced pressure to afford the oil.For GC-MS analysis , the oil was esterified via a methanolic solution of sodium hydroxide and a methanolic sulphuric acid .

GC-MS analysis

Lupinus temis fixed oil was analyzed by gas chromatography – mass spectrometry. Oven temperature program and other chromatographic

conditions are depicted in Tables 1 and 2 respectively.

Table 1: Oven temperature program

Rate	Temperature(C)	Hold time (mim.$^{-1}$)
-	60.0	0.00
10.00	300.0	0.00

Table 2: Chromatographic conditions

Column oven temperature	1300.0 °C
Injection temperature	280.0 °C
Injection mode	Split
Flow control mode	Linear velocity
Pressure	93.1KPa
Total flow	50.0ml/ min
Column flow	1.50ml/sec
Linear velocity.	44.7cm/sec
Purge flow	3.0ml/min.
Spilt ratio	- 1.0

Antimicrobial screening

In cup plate agar diffusion bioassay, *Lupinus termis* seed oil was assessed for antimicrobial activity against six standard pathogenic microbes.(0.5g) of the oil was weighed and dissolved in 10ml of DMSO to obtain a concentration of 50mg/ml. This was the initial concentration of the oil used to check the antimicrobial activities. Diffusion method was the method used for screening the oil. Mueller Hinton and Sabouraud dextrose agars were the media used as the growth media for the bacteria and the fungus respectively. The media were prepared according to the manufacturer's instructions, sterilized at 121°C for 15 minutes, poured into sterile Petri dishes and were allowed to cool and solidify. The sterilized media were sealed with 0.1ml of the standard inoculums of the test microbe (Mueller Hinton agar was sealed with the bacteria and Sabouraud dextrose agar sealed with the fungus). The inoculums were spread over the surface of the medium by the use of a sterile swab. By the use of a standard cork borer (6mm in diameters), a well was cut at the centre of each inoculated medium. (0.1ml) of the oil (concentration of 100mg/ml) was then introduced into the well on the inoculated medium. Incubation of the inoculated medium was made at 37°C for 24 hours for the bacteria and for 4 days at 30°C for the fungus. After incubation each plate of the medium was observed for the growth inhibition zone. The zone was measured and the results were recorded in millimeters.

Results and Discussion

Constituents of *Lupinus temis* oil were identified and quantified via GC-MS analysis . Identification was accomplished by comparison with the MS library (NIST) and confirmed by interpreting the fragmentation pattern where a 90-95% match was observed. The GC-MS analysis of the oil revealed

the presence of 16 components(Table 3).The typical total ion chromatograms (TIC) is depicted in Fig.1.

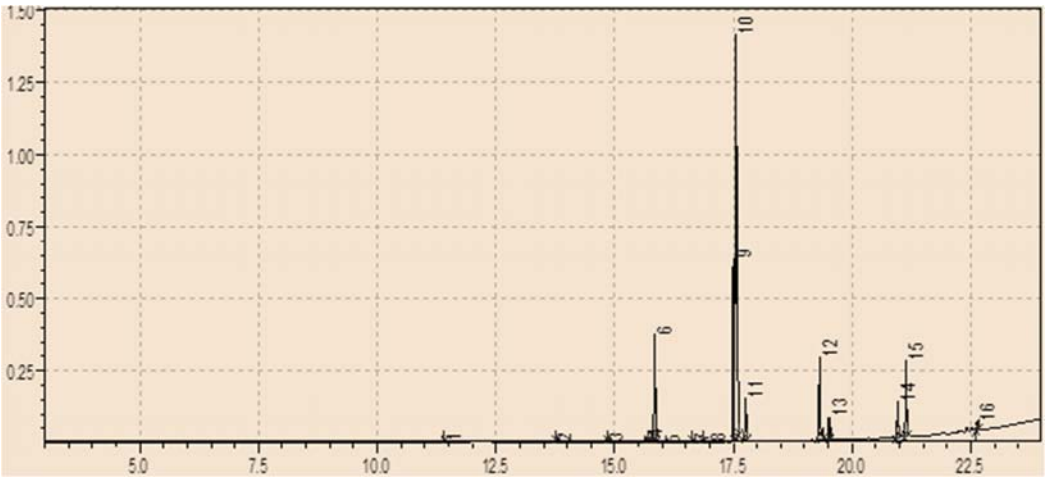

Fig.1: Total ion chromatograms

Table 3: Contituents of *Lupinus temis* oil

Peak#	R.Time	Area	Area%	Name
1	11.389	20401	0.03	Butylated Hydroxytoluene
2	13.740	58355	0.08	Methyl tetradecanoate
3	14.816	52339	0.07	Pentadecanoic acid, methyl ester
4	15.651	274893	0.39	9-Hexadecenoic acid, methyl ester, (Z)-
5	15.745	14688	0.02	Cyclopropaneoctanoic acid, 2-hexyl-, meth
6	15.842	6091082	8.72	Hexadecanoic acid, methyl ester
7	16.615	29059	0.04	cis-10-Heptadecenoic acid, methyl ester
8	16.821	27885	0.04	Heptadecanoic acid, methyl ester
9	17.500	11086000	15.87	9,12-Octadecadienoic acid (Z,Z)-, methyl e
10	17.554	35614548	50.99	9-Octadecenoic acid (Z)-, methyl ester
11	17.757	2371726	3.40	Methyl stearate
12	19.317	5403967	7.74	11-Eicosenoic acid, methyl ester
13	19.513	1295212	1.85	Methyl 18-methylnonadecanoate
14	20.959	2376856	3.40	13-Docosenoic acid, methyl ester, (Z)-
15	21.134	4568566	6.54	Methyl 20-methyl-heneicosanoate
16	22.636	562233	0.80	Tetracosanoic acid, methyl ester
		69847810	100.00	

Major constituents include:

9-Octadecenoic acid methyl ester(50.99%)

Fig. 2 shows the EI mass spectrum of 9-octadecenoic acid methyl ester.The peak at m/z 296, which appeared at R.T. 17.554 in total ion chromatogram, corresponds to $M^+[C_{19}H_{36}O_2]^+$,

while the peak at m/z266 accounts for loss of a methoxyl function.

9,12-Octadecadienoic acid methyl ester (15.87%)

Fig. 3 shows the mass spectrum of 9,12-octadecadienoic acid methyl ester.The peak at m/z294, which appeared at R.T. 17.500 in total ion chromatogram, corresponds $M^+[C_{19}H_{34}O_2]^+$, while the peak at m/z263 corresponds to loss of a methoxyl group.

11-Eicosanoic acid methyl ester(7.74%)

The mass spectrum of Cis-11-eicosenoic acid methyl ester is shown in Fig.4 .The peak at m/z324(R.T. 19.328) corresponds to M+[C21H40O2]+ .The peak at m/z293 corresponds to loss of a methoxyl function.

Methyl 20-methy-heneicosanoate(6.54%)

The molecular ion(C23H46O2)+ for methyl 20-methy-hexeicosanoate appeared at m/z354(Fig.5) with retention time 21.134. The loss of a methoxyl is demonstrated by m/z323.

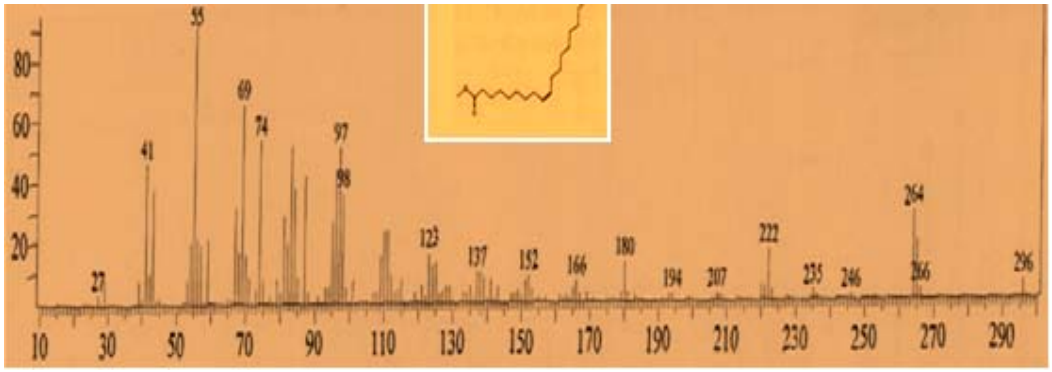

Fig. 2: Mass spectrum of 9-octadecenoic acid methyl ester

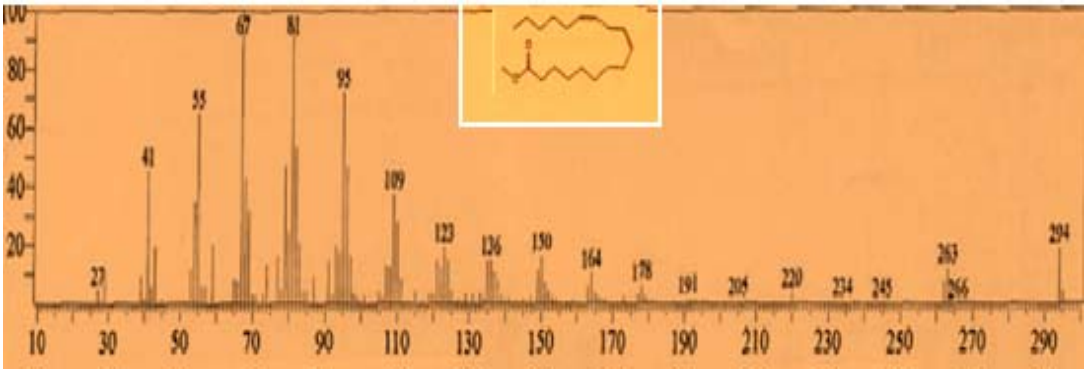

Fig. 3: Mass spectrum of 9,12-octadecadienoic acid methyl ester

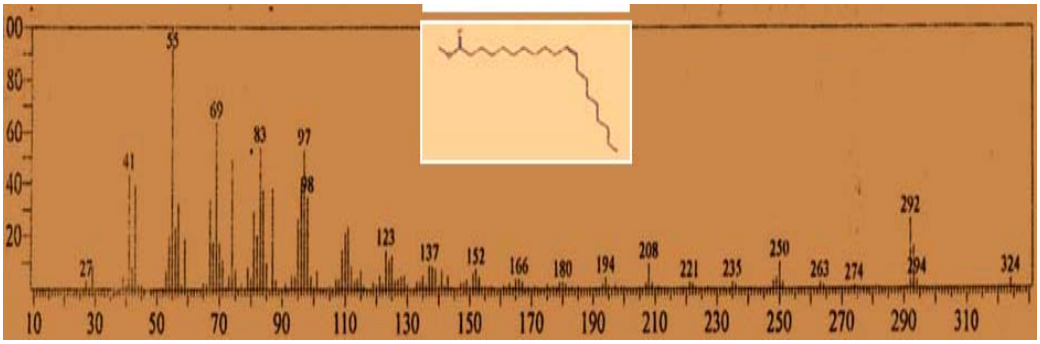

Fig. 4: Mass spectrum of 11-eicosanoic acid acid methyl ester

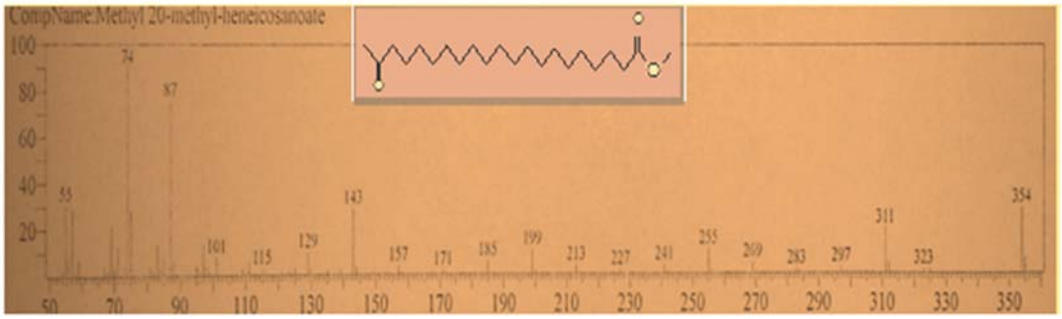

Fig. 5: Mass spectrum of methyl 20-methy-heneicosanoate

Antimicrobial activity

Type	Conc. mg/ml	**Sa**	Bs	Ec	Ps	Ca	An
Oil	100	18	18	18	17	17	13

In cup plate agar diffusion bioassay ,the oil was screened for antimicrobial activity against six standard pathogenic bacteria. The average of the diameters of the growth of inhibition zones are depicted in Table (4) .The results were interpreted in conventional terms : (<9mm: inative;9-12mm: partially active; 13-18mm: active;>18mm:very active) .Tables (5) and (6) represent the antimicrobial activity of standard antibacterial and antifungal chemotherapeutic agents against standard bacteria and fungi respectively.

Table 4 : Antibacterial activity of *Lupinus temis* oil

Table 5 : Antibacterial activity of standard chemotherapeutic agents

Drug	Conc.(mg/ml)	Bs	Sa	Ec	Ps
Ampicilin	40	15	30	-	-
	20	14	25	-	-
	10	11	15	-	-

Gentamycin	40	25	19	22	21
	20	22	18	18	15
	10	17	14	15	12

Table 6 : Antifungal activity of standard chemotherapeutic agent

Drug	Conc.(mg/ml)	An	Ca
Clotrimazole	30	22	38
	15	17	31
	7.5	16	29

Sa.: *Staphylococcus aureus*
Ec.: *Escherichia coli*
Pa.: *Pseudomonas aeruginosa*
An.: *Aspergillus niger*
Ca.: *Candida albicans*
Bs.: *Bacillus subtilis*

The oil exhibited excellent antibacterial activity against all test organisms (Table 4) . Also it showed significant antifungal activity against the yeast *Candida albicans* .

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