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Research Article

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GC-MS Analysis and Antimicrobial Activity of Sudanese *Lens culinaris* Seed Oil

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Abstract Lens is a small genus in the family Fabaceae consisting of the cultivated variety- *Lens culinaris* and six other wild taxa. *Lens culinaris* legume herb containing several important minerals,vitamins beside some flavonoids. The present study was carried out to characterize the constituents of *Lens culinaris* seed oil and to assess its antimicrobial activity. Twenty six components were detected by GC-MS analysis being dominated by: 9,12-octadecadienoic acid methyl ester (37.49%), hexadecanoic acid methyl ester (20.28%), 9-octadecenoic acid methyl ester (17.19%) and methyl stearate (12.45%). The antimicrobial activity of the oil was evaluated against: Gram positive: *Staphylococcus aureus* and *Bacillus subtilis*; Gram negative : *Escherichia coli* and *Pseud*om*onasa aeruginosa* and the yeast *Candida albicans*. The oil showed good antibacterial activity against *Escherichia coli*. However, it exhibited partial activity against *Bacillus subtilis.*

Keywords *Lens culinaris*, Oil, GC-MS, Antimicrobial activity

Introduction

Lens culinaris L. (Lentil) is an annual edible legume reaching 40cm in height. Lens is a small genus in the family Fabaceae consisting of the cultivated variety- *Lens culinaris* and six other wild taxa [1,2]. In 2018 the global production of Lentil reached 6.3 million tones mainly produced by Canada (33%) and India(25%) [3,4]. Lentil contains proteins beside some amino acids [5]. It also contains starch and insoluble dietary fibers [6,7]. The plant is a good source of prebiotics [8] beside considerable amount of prebiotic carbohydrates thus preventing gut-associated diseases [9,10]. Lentil is rich in potassium and has low fat and sodium content [11]. It is a good source of iron [12]. This legume herb contains several important minerals including boron, copper, zinc, manganese, selenium and molybdenum. It also contains vitamins, niacin, riboflavin, pyridoxine, pantothenic acid, folate, α, β and γ-tocopherols [6,13,14]. Beside these vitamins , the plant contains polyphenolics which act as free radical scavengers [15,16]. It seems that Lantil is a diet with health promoting effects.

Materials and Methods

Materials

Plant material

Seeds of *Lens culinaris* were purchased from the local market, Khartoum, Sudan and authenticated by the Medicinal and Aromatic Plants Research Institute, Sudan.

Instruments

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

Test organisms

Lens culinaris oil was screened for antibacterial and antifungal activity using the standard microorganisms : *Bacillus subtilis, Staphylococcus aureus*, *Pseudomonas aeroginosa*, *Escherichia coli* and the fungal species *Candida albicans*.

Methods

Extraction of oil

Powdered seeds of *Lens culinaris* (400g) were macerated with n-hexane . The solvent was removed *in vauo* to give the oil.

GC-MS analysis

The constituents of *Lens culinaris* oil were investigated by GC-MS using a Shimadzo GC-MS-QP2010 Ultra instrument with a RTX-5MS column (30m, length; 0.25mm diameter; 0.25 µm, thickness). Chromatographic conditions are as follows: column oven temperature: 150.0 ^{o}C; injection temperature: 300.0 ^{o}C; injection mode: split; flow mode: linear velocity; pressure: 139KPa; total flow: 50.0ml/min; column flow:1.54ml/sec.; linear velocity: 47.2 cm/sec.; purge flow: 3.0 ml/min.; split ratio: -1.0. Oven temperature program is presented below:

Table 1: Oven temperature program

Rate	Temperature (^{o}C)	Hold Time (min.$^{-1}$)
-	150.0	1.00
4.00	300.0	0.00

***In vitro* Antimicrobial Assay**

Muller Hinton agar and Sabouraud dextrose agars were used for bacterial and fungal cultures respectively. The disc diffusion method was used to determine the antimicrobial activity of the oil. Fresh cultures of microorganisms grown for 24 h were used and diluted to 10^{-1} with sterile physiological saline solution (0.85% NaCl). 100 µl of test microorganisms containing 2.0×10^{6} colony forming units (CFU/ml) for bacteria were inoculated on the surface of agar plates. Sterile discs with a diameter of 6 mm were placed onto each agar plate containing microorganisms. Then the test solution was dropped onto discs under sterile conditions and incubated at 37 °C for 24 h (for bacteria), for fungi the incubation continued for 3 days at 25^{o}C. After incubation, the diameters of inhibition zones were measured in millimetres. All experiments were repeated two times. Ampicillin, gentamicin and clotrimazole were used as positive controls ,while DMSO was used as negative control. Control discs were tested on the same microorganisms under the same conditions.

Results and Discussion

GC-MS analysis

GC-MS analysis of *Lens culinaris* oil was conducted and the identification of the constituents was initially accomplished by comparison of the retention times and consulting the MS library (NIST). Excellent matching was observed when comparing the mass spectra with the database on MS library.

Constituents of oil

The GC-MS spectrum of the studied oil revealed the presence of 26 constituents (Table 2). The typical total ion chromatograms (TIC) is depicted in Figure 1.

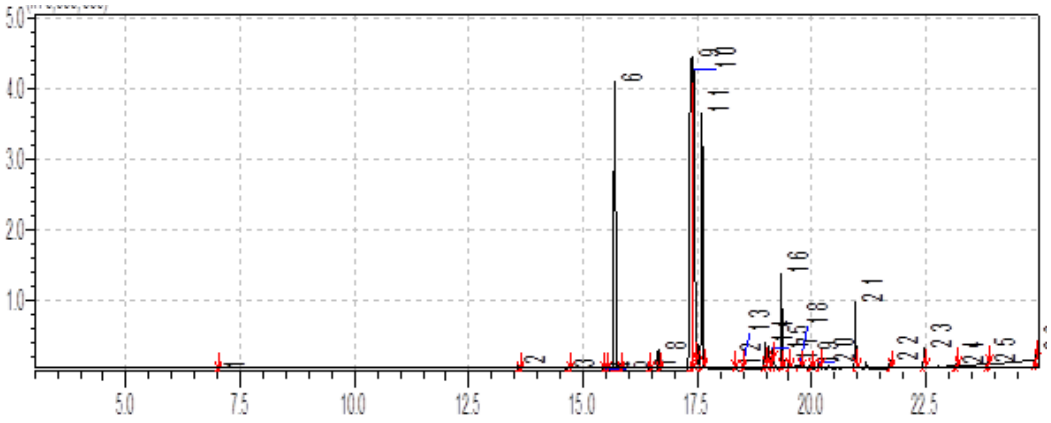


Figure 1: Total ions chromatograms

Table 2: Constituents of *Lens culinaris* oil

No.	Name	Ret. Time	Area%
1.	L-.alpha.-Terpineol	6.981	0.06
2.	Methyl tetradecanoate	13.565	0.25
3.	Pentadecanoic acid, methyl ester	14.639	0.17
4.	6-Octadecenoic acid, methyl ester, (Z)-	15.435	0.07
5.	9-Hexadecenoic acid, methyl ester, (Z)-	15.471	0.07
6.	Hexadecanoic acid, methyl ester	15.698	20.28
7.	7,10-Hexadecadienoic acid, methyl ester	16.433	0.19
8.	Heptadecanoic acid, methyl ester	16.643	0.83
9.	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	17.383	37.49
10.	9-Octadecenoic acid (Z)-, methyl ester	17.415	17.19
11.	Methyl stearate	17.598	12.45
12.	cis-10-Nonadecenoic acid, methyl ester	18.288	0.13
13.	Nonadecanoic acid, methyl ester	18.473	0.07
14.	Z,Z-3,13-Octadecadien-1-ol	18.973	0.85
15.	cis-13-Eicosenoic acid, methyl ester	19.135	0.38
16.	Eicosanoic acid, methyl ester	19.335	3.70
17.	8,11,14-Docosatrienoic acid, methyl ester	19.501	0.42
18.	Methyl 2-octylcyclopropene-1-octanoate	19.738	0.31
19.	2-Furanpentanoic acid, tetrahydro-5-nonyl-, methyl ester	19.976	0.24
20.	Heneicosanoic acid, methyl ester	20.158	0.28
21.	Docosanoic acid, methyl ester	20.954	2.74
22.	Tricosanoic acid, methyl ester	21.717	0.39
23.	Tetracosanoic acid, methyl ester	22.455	0.87
24.	Pentacosanoic acid, methyl ester	23.168	0.12
25.	Hexacosanoic acid, methyl ester	23.858	0.07
26.	Hexatriacontane	24.916	0.38

Major constituents are briefly discussed below:

i)-9,12-Octadecadienoic acid methyl ester (37.49%)

The mass spectrum of 9,12-octadecadienoic acid methyl ester is depicted in Figure 2. The signal which was observed at m/z 294 (R.T. 17.383) is due to $M^+[C_{19}H_{34}O_2]^+$, while the signal at m/z 263 corresponds to loss of a methoxyl.

ii) Hexadecanoic acid methyl ester (20.28%)

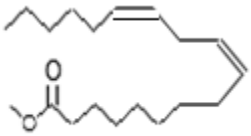
Figure 3 shows the mass spectrum of hexadecanoic acid methyl. The peak m/z 270 (R.T. 15.698) was detected in the spectrum. It corresponds $M^+[C_{17}H_{34}O_2]^+$. The peak at m/z 239 is due to loss of a methoxyl.

iii) 9-Octadecenoic acid methyl ester (17.19%)

The mass spectrum of 9-octadecenoic acid methyl ester is displayed in Figure 4. The peak at m/z 296 (R.T. 17.415) corresponds $M^+[C_{19}H_{36}O_2]^+$, while the signal at m/z 266 is attributed to loss of a methoxyl.

iv)-Methyl stearate(12.45%)

The EI mass spectrum of methyl stearate is displayed in Figure 5. The peak at m/z 298 with R.T. 17.598 is due to $M^+[C_{19}H_{38}O_2]^+$, while the signal at m/z267 corresponds to loss of a methoxyl group.



9,12-Octacedienoic acid methyl ester

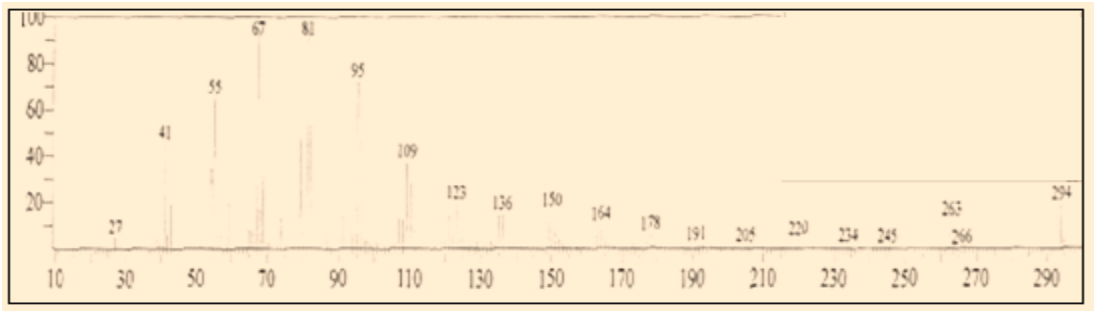
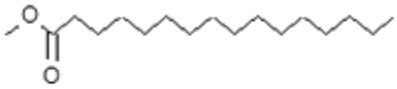


Figure 2: Mass spectrum of 9,12-octadecadienoic acid methyl ester



Hexadecanoic acid methyl ester

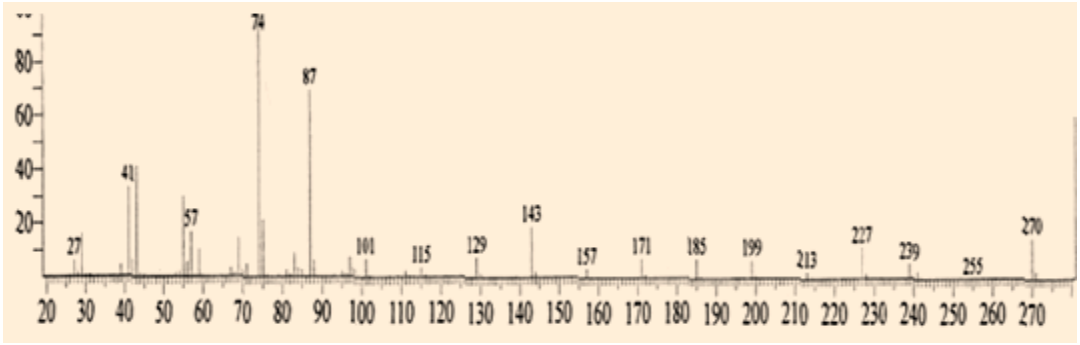
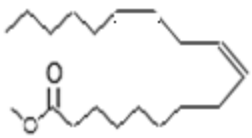


Figure 3: Mass spectrum of hexadecanoic acid methyl ester



9-Octadecenoic acid methyl ester

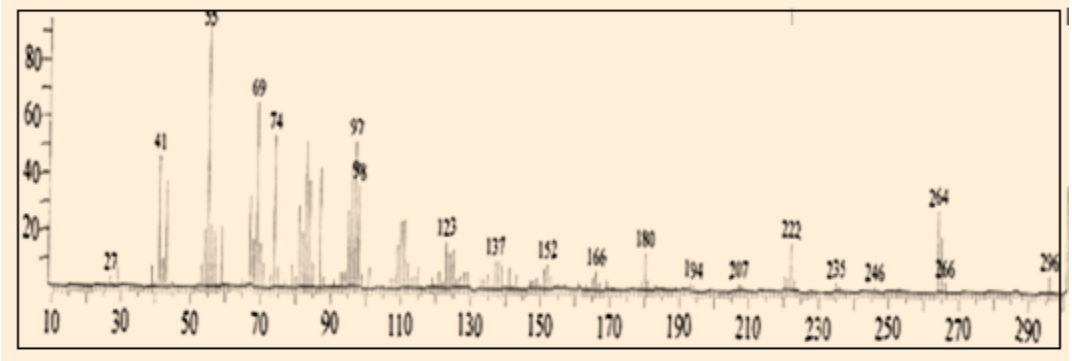


Figure 4: Mass spectrum of 9-octadecenoic acid methyl ester

Methyl stearate

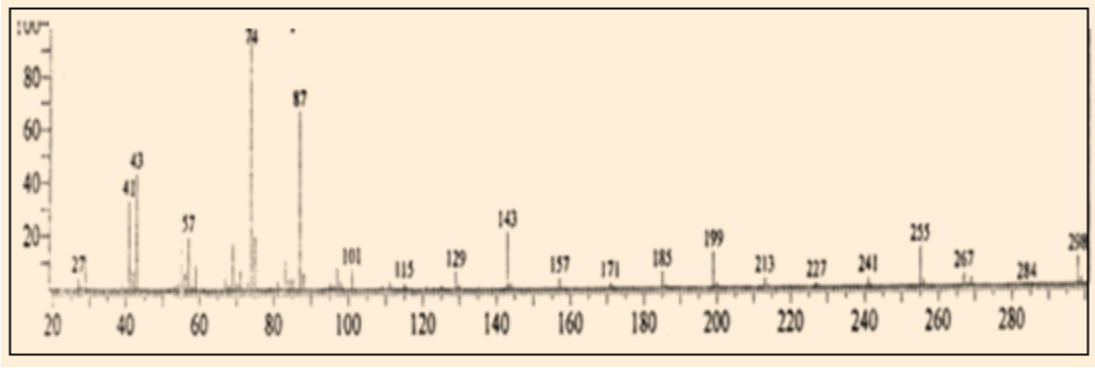


Figure 5: Mass spectrum of methyl stearate

Antimicrobial activity

Lens culinaris seed oil was screened for antimicrobial activity against five standard human pathogens. The average of the diameters of the growth of inhibition zones are depicted in Table 3.The results were interpreted in commonly used terms (<9mm: inative;9-12mm:partially active; 13-18mm: active. >18mm:very active). The oil showed good antibacterial activity against *Escherichia coli*. However, it exhibited partial activity against *Bacillus subtilis.*

Table 3: Inhibition zones (mm/mg sample)

Sample	Sa	Bs	Ec	Ps	Ca
Oil (100mg/ml)	--	10	14	--	--
Ampicilin (40mg/ml)	30	15	--	--	--
Gentamicin (40mg/ml)	19	25	22	21	--
Clotrimazole (30mg/ml)	--	--	--	--	38

Sa.: *Staphylococcus aureus*, Bs.: *Bacillus subtilis*, Ec.: *Escherichia coli*, Pa.: *Pseudomonas aeroginosa*, Ca.: *Candida albicans*.

References

[1]. Shyam S., David, M. and Philip, C. (Editors); Lentil :An Ancient crop for Modern Times, 2007, Springer Science and Business Media, Berlin.
[2]. Erskine and William ; The Lentil: Botany, Production and Uses, 2009, CABI, Walling, UK.
[3]. Lentil; Lintilis. Org, Saskatchewan Pulse Growers, 2020, retrieved, 28 March 2020.
[4]. Pulses : Growing Regions , Pulse Canada, 2020, Retrieved 28 March 2020.
[5]. US Dept. of Agriculture, national Nutrient Database for Standard Reference Release, 28(2016), Full Report, 16069, Lentil raw, retrieved 15 Dec. 2015.
[6]. Lombardi-Boccia G., Ruggeri S., Aguzzi A., Cappelloni M. Globulins enhance in vitro iron but not zinc dialysability: A study on six legume species. J. Trace Elem. Med. Biol. 2013;17:1–5. doi: 10.1016/S0946-672X(03)80037-86.
[7]. United States Department of Agriculture (USDA) Agricultural Research Service, National Nutrient Database for Standard Reference Release 28. Nutrient Database Laboratory Home Page. [(accessed on 14 August 2016)]; Available online: https://ndb.nal.usda.gov/ndb/search/list.
[8]. Bednar G.E., Patil A.R., Murray S.M., Grieshop C.M., Merchen N.R., Fahey G.C. Starch and fiber fractions in selected food and feed ingredients affect their small intestinal digestibility and fermentability and their large bowel fermentability in vitro in a canine model. J. Nutr. 2001;131:276–286
[9]. Dwivedi S., Sahrawat K., Puppala N., Ortiz R. Plant prebiotics, and human health: Biotechnology to breed prebiotic-rich nutritious food crops. Electr. J. Biotechnol. 2014;17:238–245. doi: 10.1016/j.ejbt.2014.07.004
[10]. Fooks L.J., Fuller R., Gibson G.R. Prebiotics, probiotics and human gut microbiology. Int. Dairy J. 1999;9:53–61. doi: 10.1016/S0958-6946(99)00044-8.
[11]. Johnson C.R., Combs G.F., Thavarajah P. Lentil (*Lens culinaris* L.): A prebiotic-rich whole food legume. Food Res. Int. 2013;51:107–113. doi: 10.1016/j.foodres.2012.11.025.
[12]. Padovani R.M., Lima D.M., Colugnati F.A., Rodriguez-Amaya D.B. Comparison of proximate, mineral and vitamin composition of common Brazilian and US foods. J. Food Compos. Anal. 2007;20:733–738. doi: 10.1016/j.jfca.2007.03.006
[13]. Soltan S.S.A. The protective effect of soybean, sesame, lentils, pumpkin seeds and molasses on iron deficiency anemia in rats. World Appl. Sci. J. 2013;23:795–807.
[14]. Ryan E., Galvin K., O'Connor T.P., Maguire A.R., O'Brien N.M. Phytosterol, squalene, tocopherol content and fatty acid profile of selected seeds, grains, and legumes. Plant Foods Hum. Nutr. 2007;62:85–91. doi: 10.1007/s11130-007
[15]. Rodriguez C., Frias J., Vidal-Valverde C., Hernandez A. Correlations between some nitrogen fractions, lysine, histidine, tyrosine, and ornithine contents during the germination of peas, beans, and lentils. Food Chem. 2008;108:245–252. doi: 10.1016/j.foodchem.2007.10.073.
[16]. Taylor W.G., Fields P.G., Sutherland D.H. Fractionation of lentil seeds (*Lens culinaris* Medik.) for insecticidal and flavonol tetraglycoside components. J. Agric. Food Chem. 2007;55:5491–5498. doi: 10.1021/jf0705062.