

GC-MS ANALYSIS AND ANTIOXIDANT ACTIVITY OF SAUDI *LINUM USITATISSIMUM* (LINACEAE) OIL

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ABSTRACT

In this study *Linum usitatissimum* seed oil was analyzed by GC-MS. The analysis revealed the presence of 15 components. Major constituents are: 9-octadecenoic acid, hexadecyl ester (27.58%), 9, 12-octadecadienoic acid, methyl ester (25.44%), 9, 12, 15-octadecatrienoic acid, methyl ester (19.73%), hexadecanoic acid, methyl ester (14.12%) and methyl stearate (11.20%). The oil was screened for its free radical scavenging properties using propyl gallate as positive standard. *Linum usitatissimum* seed oil exhibited moderate antioxidant activity.

KEYWORDS: *Linum usitatissimum*, Seed Oil, GC-MS analysis, Antioxidant activity.

INTRODUCTION

Linseed -*Linum usitatissimum* L. is a crop plant in the family Linaceae. The plant has been cultivated for its oil and fiber.^[1,2] The common names flax and linseed are used in North America and Asia, respectively.^[2] The plant is probably native to the Mediterranean region and Southwest Asia^[2]; however the exact location is uncertain.^[3] The initial use of *Linum usitatissimum* has also been debated, but an evidence suggests that the plant was used first for its oil.^[4]

The oil from *Linum usitatissimum* is an excellent source of linolenic acid (an omega-3 fatty acid) with typical levels of 55%^[5] making it ideal for paints, varnishes, ink and many industrial uses.

Large amount of linseed oil which contains significant percentage of omega-3 fatty acids are nowadays consumed in diet. The oil is also added to animal feed to improve animal health.^[6, 7] *Linum usitatissimum* seed oil has various industrial and medicinal uses and various studies have emphasized the need for increased consumption of omega-3 fatty acids such as linolenic acid which is abundant in *Linum usitatissimum* seed oil.^[8]

MATERIALS AND METHODS

Plant material

Linum usitatissimum seeds were collected from, Jeddah, Saudi Arabia.

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.

Methods

Extraction of oil

Powdered seeds of *Linum usitatissimum* (500g) were macerated with n-hexane for 72h. at room temperature. The solvent was removed under reduced pressure giving the oil.

GC-MS analysis

GC-MS analysis of *Linum usitatissimum* oil was conducted and the identification of the constituents was initially accomplished via retention times and further confirmed by the database on MS library.

Testing of antibacterial susceptibility

The paper disc diffusion method was used to screen the antibacterial activity of the studied oil and performed by using Mueller Hinton agar (MHA). The experiment was carried out according to the National Committee for Clinical Laboratory Standards Guidelines (NCCLS, 1999). Bacterial suspension was diluted with sterile physiological solution to 10⁸cfu/ ml (turbidity = McFarland standard 0.5). One hundred micro liters of bacterial suspension were swabbed uniformly on surface of MHA and the inoculum was allowed to dry for 5 minutes. Sterilized filter paper discs (Whatman No.1, 6 mm in diameter) were placed on the surface of the MHA and soaked with the solution of test sample. The

inoculated plates were incubated at 37 °C for 24 h in the inverted position. Tests were carried out in duplicates and the diameters (mm) of the inhibition zones were measured and averaged.

Testing for antifungal activity

The above mentioned method was adopted for antifungal activity, but instead of Muller Hinton agar Sabouraud dextrose agar was used and incubation continued for four days at 25°C. Samples were used here by the same concentrations used above.

Antioxidant activity

DPPH radical scavenging

The DPPH radical scavenging was determined according to the method of Shimada *et. al.*^[9] with some modification. The test sample was allowed to react with

2,2 Di- (4-tert-octylphenyl)-1-picryl-stable free radical (DPPH) for half an hour at 37° C. The concentration of DPPH was kept as (300µM). The test sample was dissolved in DMSO, while DPPH was prepared in ethanol. After incubation, decrease in absorbance was measured at 517nm using multiple reader spectrophotometer. Percentage radical scavenging activity by sample was determined in comparison with a DMSO treated control group. All tests were run in triplicate. Propyl gallate was used as positive control at concentrations 0.5 Mm.^[9]

RESULTS AND DISCUSSION

Constituents of oil

The GC-MS analysis of the studied oil revealed the presence of 15 components see (Table 1).

Table 1: Constituents of *Linum usitatissimum* oil.

No.	Name	Ret.Time	Area	Area%
1	Methyl tetradecanoate	13.317	189936	0.07
2	Pentadecanoic acid, methyl ester	14.373	70754	0.03
3	7-Hexadecenoic acid, methyl ester, (Z)-	15.155	104077	0.04
4	9-Hexadecenoic acid, methyl ester, (Z)-	15.196	476941	0.19
5	Hexadecanoic acid, methyl ester	15.392	35779007	14.12
6	cis-10-Heptadecenoic acid, methyl ester	16.159	270452	0.11
7	Heptadecanoic acid, methyl ester	16.365	508829	0.20
8	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	17.053	64486368	25.44
9	9-Octadecenoic acid (Z)-, hexadecyl ester	17.155	69889815	27.58
10	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	17.164	50002070	19.73
11	Methyl stearate	17.307	28380442	11.20
12	cis-11-Eicosenoic acid, methyl ester	18.887	1343975	0.53
13	Eicosanoic acid, methyl ester	19.092	684609	0.27
14	Docosanoic acid, methyl ester	20.722	818972	0.32
15	Tetracosanoic acid, methyl ester	22.233	436726	0.17

Major constituents of the oil are :

- (i)9-Octadecenoic acid, hexadecyl ester (27.58%)
- (ii)9, 12-Octadecadienoic acid, methyl ester (25.44%)
- (iii)9, 12, 15-Octadecatrienoic acid, methyl ester (19.73%)
- (iv)Hexadecanoic acid, methyl ester (14.12%)
- (v)Methyl stearate (11.20%)

In the mass spectrum of 9-octadecenoic acid, hexadecyl ester (Fig.1), the peak at m/z 296(R.T. 17.155) corresponds $M^+[C_{34}H_{66}O_2]^+$, while the peak at m/z 265 is due to loss of a methoxyl. In Fig. 2(mass spectrum of 9, 12-octadecadienoic acid, methyl ester), the molecular ion $[C_{19}H_{34}O_2]^+$ corresponds m/z 294(RT 17.053). The signal at m/z263 is attributed to loss of a methoxyl.The mass spectrum of 9, 12, 15-octadecatrienoic acid, methyl ester is shown in Fig.3. The peak at m/z 292(R.T.,17.164) corresponds $M^+[C_{19}H_{32}O_2]^+$.The peak

at m/z 261 corresponds to loss of a methoxyl .In Fig. 4- mass spectrum of hexadecanoic acid methyl ester – the molecular ion $M^+[C_{17}H_{34}O_2]^+$ corresponds m/z 270 (RT. 15.392), while the signal at m/z239 accounts for loss of a methoxyl. The mass spectrum of methyl stearate is shown in Fig.5. The peak at m/z 298, which appeared at R.T. 17.307 in total ion chromatogram, corresponds $M^+[C_{19}H_{38}O_2]^+$. The peak at m/z 267corresponds to loss of a methoxyl function.

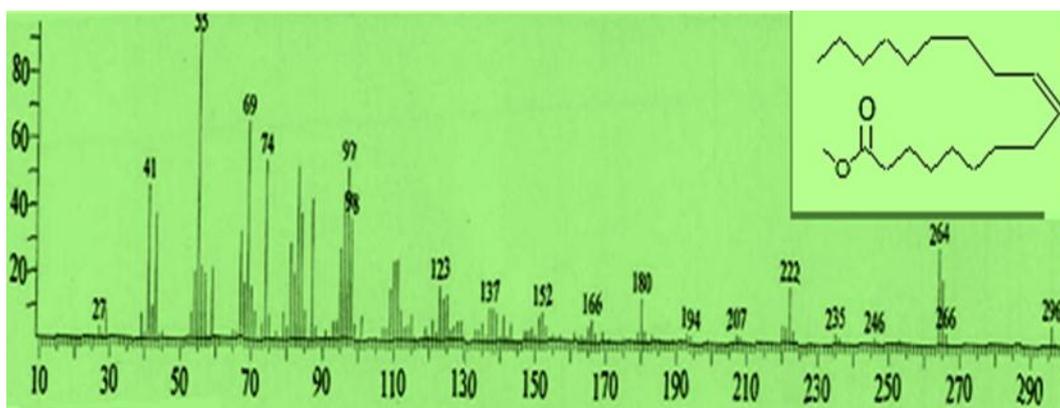


Fig. 1: Mass spectrum of 9-octadecenoic acid, hexadecyl ester.

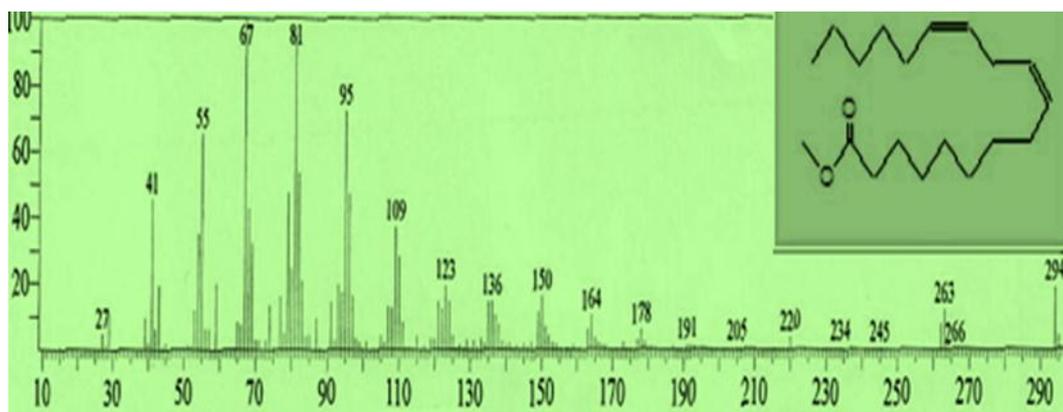


Fig. 2: Mass spectrum of 9,12-octadecadienoic acid methyl ester.

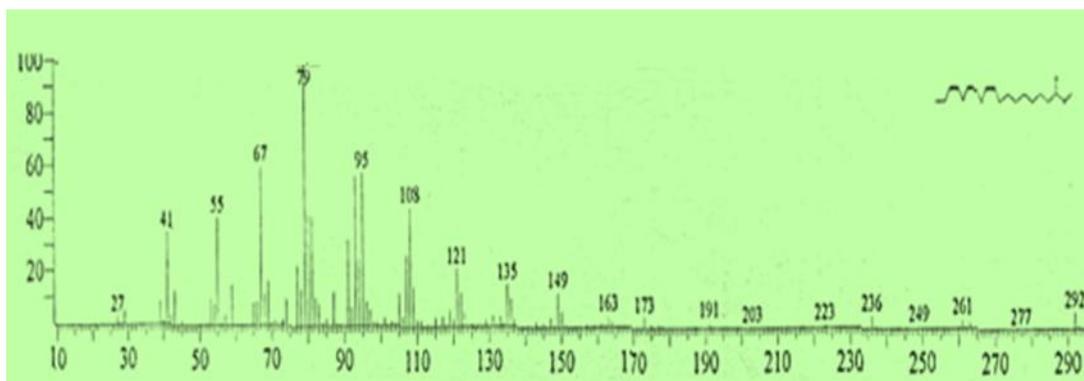


Fig. 3: Mass spectrum of 9, 12, 15-octadecatrienoic acid, methyl ester.

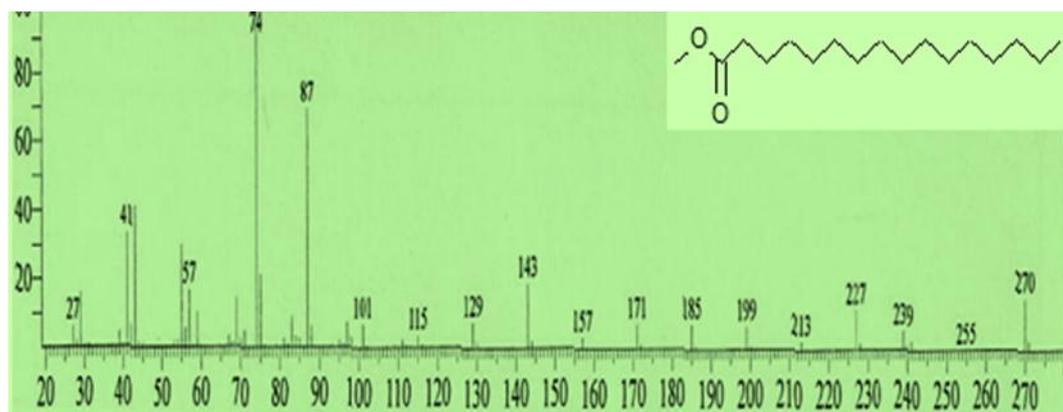


Fig. 4: Mass spectrum of hexadecanoic acid, methyl ester.

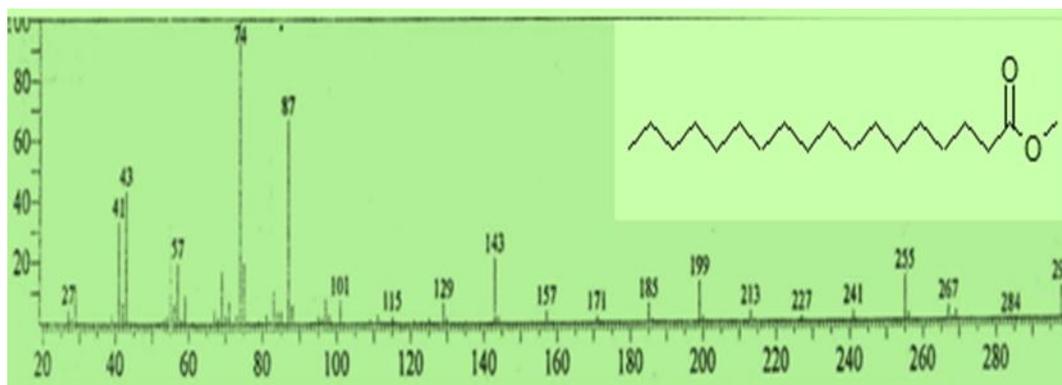


Fig. 5: Mass spectrum of methyl stearate.

Antioxidant and antimicrobial activities

The studied oil failed to give any antimicrobial activity. The antioxidant capacity of *Linum usitatissimum* seed oil was carried out by measuring the capacity of the test compound against stable DPPH radical.

The change in color is measured spectrophotometrically at 517 nm. As depicted in (Table 2) *Linum usitatissimum* seed oil exhibited moderate antioxidant activity. Propyl gallate was used as positive control.

Table 2: Radical scavenging activity of *Linum usitatissimum* seed oil.

Sample	Antioxidant activity
Propyl gallate	92.00%
<i>Linum usitatissimum</i> oil	48.00%

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