The Pharmaceutical and Chemical Journal, 2021, 8(1):61-66

Available online www.tpcj.org

Research Article

ISSN: 2349-7092
CODEN(USA): PCJHBA

GC-MS Studies and Antioxidant Activity of *Lepidium sativus* L. Grown in Turkey

Abdel Karim, M.*[1], Salwa A. Ateem[1,2], Faiza, I.[3]

[1]Sudan University of Science and Technology, Faculty of Science, Sudan
[2]Alzaiem Alazhari University, Faculty of Education, Department of Chemistry, Sudan
[3]University of Hail, Faculty of Science, Kingdom of Saudi Arabia

Abstract *Lepidium sativum* oil was characterized by GC-MS. A total of 25 constituents were identified. Major constituents are: cis-13-eicosenoic acid methyl ester (16.48%), 9,12-octadecadienoic acid (Z,Z)-, methyl ester (15.90 %), hexadecanoic acid methyl ester (13.11%) and 9,12,15-octadecatrienoic acid methyl ester(Z,Z,Z)-(10.16%). The antioxidant activity of *Lepidium sativeum* oil was conducted by three methods: metal chelating, free radical (DPPH$^{\cdot}$) and superoxide anion scavenging method. The studied oil showed a free radical scavenging capacity in the DPPH assay (IC_{50} 97.36±0.9) close to the positive control: butylated hydroxyl anisole (BHA)–(IC_{50} 80.14±1.02). In the metal chelating assay, the studied oil showed IC_{50}: 118.25±0.31 while the positive control – EDTA gave: IC_{50} 7.05±0.29. In the superoxide anion scavenging assay, the oil sample showed: IC_{50} 11.72±0.17, while the positive controls gave: BHA (IC_{50} 18.22±0.17); Trolox (IC_{50} 54.98±0.17) and BHT (IC_{50}, 60.5±0.22).

Keywords *Lepidium sativum*, Oil, GC-MS Analysis, Antioxidant Activity

Introduction

Lepidium sativum L. is a fast growing annual herb in the family Brassicaceae. This plant is native to west Asia and Egypt [1]. Various parts of *Lepidium sativum* are traditionally used against arthritis, jaundice, gastrointestinal disorders, menstrual problems, liver disorders, spleen disease, fracture, and inflammatory conditions [2-4]. The plant is also used in treatment of bronchitis, asthma and cough. *Lepidium sativum* is diuretic, laxative, stomachic, abortifacient, expectorant, ophrodisiac, antibacterial and gastro protective [5]. It has been reported that *Lepidium sativum* possesses antioxidant effect [6-8]. *Lepidium sativum* seeds contain among others protein, fatty acids, vitamins, flavonids essential oil, fatty acids and carbohydrates [9].

Material and Methods

Plant Material

Lepidium sativum seeds were purchased from a local marketin Cankiri Kartekin city, Turkey. The plant material was taxonomically authenticated by direct comparison with a reference herbarium sample.

Chemicals

2,2-Diphenyl-1-picryldhydrazyl (DPPH); 2,2`-anizobis(3-ethylbenzothiazoline)-6-sulphonic acid (ABTS); ascorbic acid (ASC); butylated hydroxytoluene (BHT); nicotamide adenine dinucleotide (NADH); nitroblue tetrazolium (NBT); butylated hydroxyanisole (BHA) were procured from sigma chemical company (USA).

Extraction of oil

Powdered *Lepidium sativum* seeds (50g) were extracted with n-hexane (300ml) at room temperature and filtrated. The solvent was removed under reduced pressure giving the oil.

GC-MS analysis

Lepidium sativum seed oil was analyzed by gas chromatography –mass spectrometry. Instrumentation and chromatographic conditions are presented in Table 1.

Table 1: Instrumentation and chromatographic conditions

Instrumentation		
Instrument	Shimadzo GC-MS – QP 2020 Ultra (Japan)	
Column	RIX- 5MS; Length (50m); Diameter (0.25mm); Thickness (0.25 μm)	
Carrier gas	Helium (purity 99%)	
Oven temperature program		
Rate	Temperature (^{o}C)	Hold time (min.$^{-1}$)
--	60.0	0.00
10	300.0	0.00
Chromatogrphic conditions		
Column oven temperature	60.0^{o} C	
Injection temperature	300.0^{o}C	
Injection mode	Split	
Flow control mode	Linear velocity	
Pressure	100.0KPa	
Purge flow	3.0 ml/min.	
Split ratio	-1.0	

Antioxidant activity

The antioxidant activity of *Lepidium sativum* oil was conducted by three methods: metal chelating, free radical (DPPH$^{\cdot}$) scavenging and superoxide anion scavenging methods. The metal chelating, free radical and superoxide anion scavenging activity were tested at different concentration to calculate IC_{50} (μg/ml) and r^2 values. All antioxidant assays were carried out in triplicate.

Free radical scavenging activity

The activity was evaluated by bleaching of the purple-colored solution of DPPH. In this assay, the bleaching rate of a stable free radical (DPPH$^{\cdot}$) is monitored at a characteristic wavelength (λ_{max} 517 nm) in the presence of different concentrations of the test sample to calculate IC_{50}. The test sample was mixed with DPPH$^{\cdot}$ (0.1 mM, 0.5 ml) and the absorbance was recorded at λ_{max} 517 nm and compared with standards.

Metal chelating activity

The ferrous ion chelating activity was evaluated by a standard method [10] with minor changes. The reaction was carried out in HEPES buffer (20 mM, pH 7.2). Briefly, various concentrations (0–120 μg/ml) of test sample were added to 12.5 μM ferrous sulfate solution and the reaction was initiated by the addition of ferrozine (75 μM). The mixture was shaked vigorously and incubated for 20 min at room temperature, then the absorbance was measured at λ_{max} 562 nm. All tests were performed in triplicate and compared to controls.

Superoxide anion scavenging activity

The activity of the sample was estimated according to the methods of Nishikimi et al [11] and Zhao et al [12] with minor modification. Superoxide radicals were generated in a PMS-NADH system and assayed by reduction of NBT. Briefly, a (1 ml) sample was thoroughly mixed separately with (156 μM) NBT and (468 μM) NADH, respectively.

The reaction started by adding (60 μM) PMS. After incubation, the absorbance of the mixture was measured at λ_{max} 532 nm.

Results and Discussion

GC-MS analysis of *Raphanus sativus* oil

Gas chromatography - mass spectrometry has been used for the identification and quantification of the studied essential oil. The analysis revealed the presence of (25) components - Table 2.

Table 2: Constituent of *Lepidium sativum* L. seed oil

No.	Name	Ret. Time	Area%
1.	Methyl tetradecanoate	14.189	0.26
2.	cis-5-Dodecenoic acid, methyl ester	15.047	0.03
3.	Pentadecanoic acid, methyl ester	15.319	0.06
4.	7,10-Hexadecadienoic acid, methyl ester	16.095	0.05
5.	7-Hexadecenoic acid, methyl ester, (Z)-	16.157	0.16
6.	9-Hexadecenoic acid, methyl ester, (Z)-	16.200	0.42
7.	Hexadecanoic acid, methyl ester	16.413	13.11
8.	cis-10-Heptadecenoic acid, methyl ester	17.217	0.14
9.	Heptadecanoic acid, methyl ester	17.430	0.21
10.	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	18.172	15.90
11.	9-Octadecenoic acid (Z)-, methyl ester	18.264	8.73
12.	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	18.293	10.16
13.	Methyl stearate	18.427	7.16
14.	Nonadecanoic acid, methyl ester	19.359	0.08
15.	.gamma.-Linolenic acid, methyl ester	19.929	2.18
16.	cis-13-Eicosenoic acid, methyl ester	20.085	16.48
17.	11,14,17-Eicosatrienoic acid, methyl ester	20.125	1.92
18.	Eicosanoic acid, methyl ester	20.270	7.09
19.	cis-10-Nonadecenoic acid, methyl ester	21.795	8.34
20.	Docosanoic acid, methyl ester	21.966	2.69
21.	13-Docosenoic acid, methyl ester, (Z)-	22.600	0.23
22.	Tricosanoic acid, methyl ester	22.767	0.19
23.	15-Tetracosenoic acid, methyl ester, (Z)-	23.389	2.72
24.	Tetracosanoic acid, methyl ester	23.542	1.61
25.	.gamma.-Tocopherol	26.552	0.08

Major constituents of the oil are:

i) cis 13 -Eicosenoic acid methyl ester(16.48%)

ii) 9,12-Octadecadienoic acid (Z,Z)-, methyl ester (15.90 %)

iii) Hexadecanoic acid, methyl ester (13.11%)

iv) 9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)- (10.16%)

The mass spectrum of cis-13-eicosenoic acid, methyl ester is presented in Fig. 1. The peak at m/z 324 which appeared at (RT.20.085) is due to M^+ [$C_{21}H_{40}O_2$], while the peak at m/z 292 corresponds to loss of a methoxyl. Fig. 2 illustrates the mass spectrum of 9,12-octadecadienoic acid (Z,Z)-, methyl ester. The signal at m/z 294 which appeared at RT. 18.172 accounts for the molecular ion: M^+ [$C_{19}H_{34}O_2$]. The peak at m/z 263 is attributed to loss of a methoxyl. Fig. 3 shows the mass spectrum of hexadecanoic acid methyl ester. The signal at m/z 270 (RT.16.413) is due to M^+ [$C_{17}H_{34}O_2$], while the peak at m/z 239 is attributed to loss of a methoxyl function. Fig. 4 illustrates the mass spectrum of 9,12,15-octadecatrienoic acid methyl ester. The signal at m/z 292 which appeared at RT. 18.293 accounts for the molecular ion: M^+ [$C_{19}H_{32}O_2$].

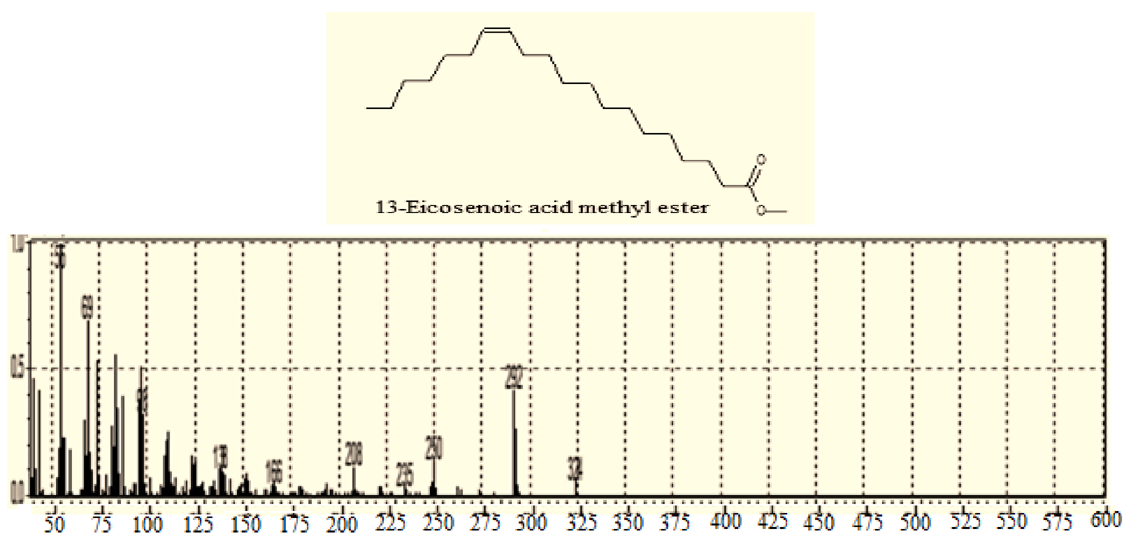

Figure 1: Mass spectrum of cis-13-eicosenoic acid, methyl ester

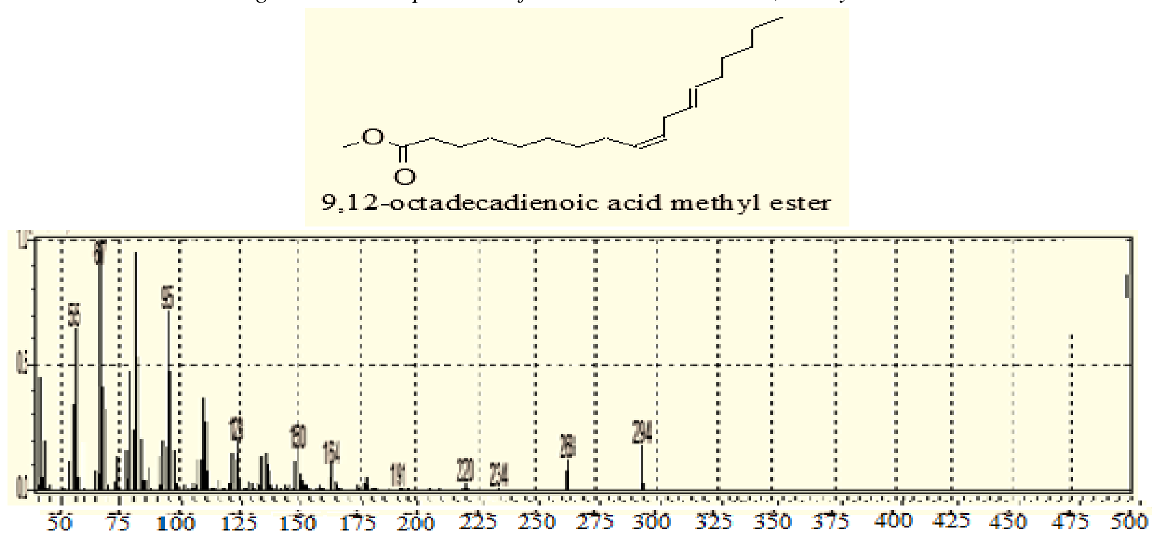

Figure 2: Mass spectrum of 9,12-octadecadienoic acid methyl ester

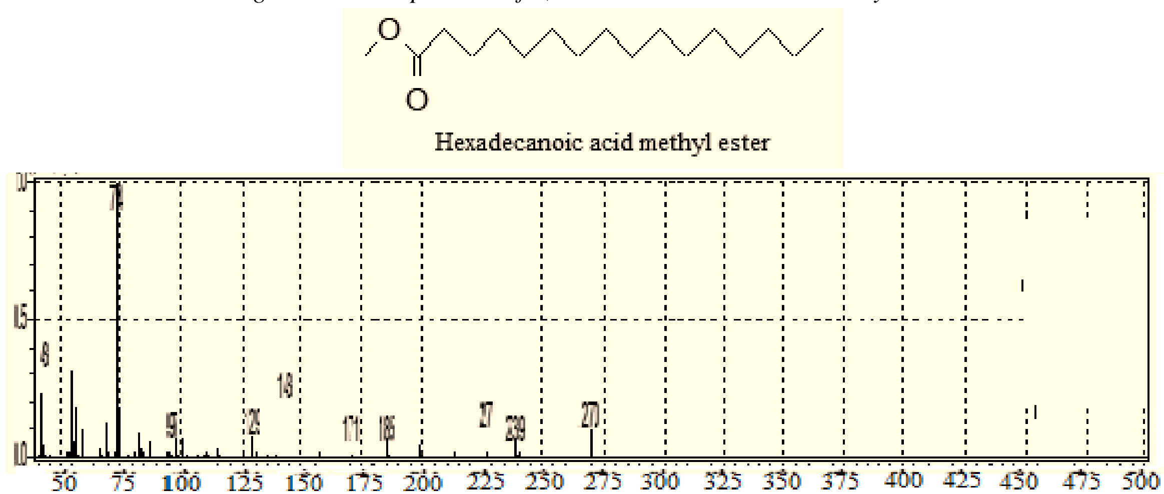

Figure 3: Mass spectrum of hexadecanoic acid, methyl ester

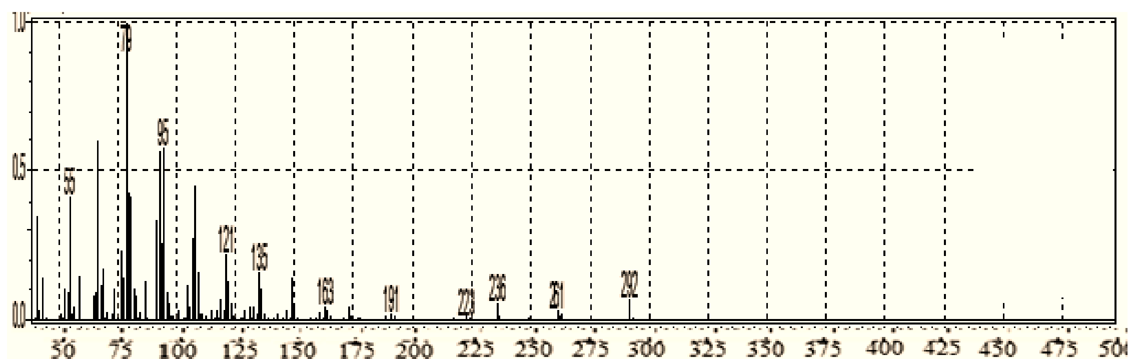

Figure 4: Mass spectrum of 9,12,15-octadecatrienoic acid, methyl ester, (Z,Z,Z)-

Antioxidant Activity

The DPPH –scavenging model is a widely used method of evaluating the antioxidant activity within a relatively short time compared with other methods. The effect of antioxidant potential in the DPPH bioassay is explained in terms of the hydrogen donating ability of the sample. DPPH · is a stable free radical and accepts one electron or hydrogen radical to form a stable diamagnetic molecule. The studied oil showed a free radical scavenging capacity in the DPPH assay (IC_{50} 97.36±0.9) close to the positive control: butylated hydroxyl anisole (BHA)– (IC_{50} 80.14±1.02).

In the metal chelating method [10] the measurement of the rate of red color reduction allows evaluation of the level of chelating capacity of the test sample [10]. In the metal chelating assay, the studied oil showed IC_{50}: 118.25±0.31 while the positive control – EDTA gave: IC_{50} 7.05±0.29.

In the superoxide anion scavenging assay, the oil sample showed: IC_{50} 11.72±0.17, while the positive controls gave: BHA (IC_{50} 18.22±0.17); Trolox (IC_{50} 54.98±0.17) and BHT (IC_{50}, 60.5±0.22). All assays showed excellent reproducibility as shown in Table 3.

Table 3: IC_{50} and r^2 values of oil and standards

Sample	Free radical (DPPH·) scavenging activity		Metal chelating activity		Superoxide anion scavenging activity	
	IC_{50}, mg/ml	r^2	IC_{50}, mg/ml	r^2	IC_{50}, mg/ml	r^2
Oil	97.36±0.96	0.87	118.25±0.31	0.75	11.72±0.17	0.89
BHA	80.14±1.01	0.79	-	-	18.22±0.17	0.99
BHT	61.11±1.78	0.96	-	-	60.5±0.22	0.94
Trolox	31.57±2.07	0.95	-	-	54.98±0.17	0.89
EDTA	-	-	7.05±0.29	0.95	-	-

References

[1]. Gokavi, N. Malleshi, G. and Guo, M., Chemical composition of garden cress (*Lepidium sativums* L.) seeds and its fractions and use of barn as a functional ingredient, J. plant Food Hum. Nutr., 2004, 59: 105-111.

[2]. Al-Asmari AK, AM. Al–Elaiwi, MT Athar, M Tariq, AEID, SM Al–Asmary, A review of hepatoprotective plants used in Saudi traditional medicine, J. Evid. Based Complement. Altern. Med. 2014, 5(2); 45-53.

[3]. Al–Marzoqi, A.H., Hadi, M.Y., Hameed, I.H., Determination of metabolites products by Cassia angustifalia and evaluate antimicrobial activity .Journal of Pharmacognosy and Phytothorepy, 2016, 8(2) 25-48.

[4]. Sosa, A., Bagi, A., Hameed, S.A., Analysis of bioactive chemical compounds of Euphorbia Iathyrus using gas chromatography–mass spectrometry and fourier–transform infrared spectroscopy, International Journal of Pharmacynosy and phytochemical Research, 2016, 8(5): 109-26.

[5]. Kadhim, M.J., Mohammed, G.J., Hameed, I.H., *In vitro* antibacterial, antifungal and phytochemical analysis of methanolic fruit extract of *Cassia fistula*, Oriental Journal of Chemistry, 2016, 32(2): 10-30.

[6]. Agarwal, J., Verma, D.L., Antioxidant activity-guided fractionation of aqueous extracts from Lepidium sativum and identification of active flavonol glycasides, Acad Arena, 2011, 3: 14 -18.

[7]. Zia-Ui–Haq, M., Ahmad, S., Calani, L., Mazzeo, T., Det Rio, D., Pellegrini, N., Defeo, V., Compositional study and antioxidant potential of I pomoea hederacea Jacq. and Lepidium sativum L. seeds, Molecules, 2012, 17: 103 06 -10321.

[8]. Al-Jasscl, M.J., Mohammed, G.J., Hammed, I.H., Secondary metabolites Analysis of Sacharomyces cerivisiae and Evaluation of Antibacterial Activity, International Journal of Pharmaceutical and Clinical Research, 2016, 8 (5) 304 -315.

[9]. Yadav, Y.C., Srivastav, D.N., Seth, A.K., Saini, V., Balaraman, R., Ghelani, T.K., *In vivo* antioxidant potential of Lepidium sativum seed in abinorats using cisplatin induced nephrotoxicity , lnt . J. Phytomed., 2010, 2: 292- 298.

[10]. Dinis, T. C., Madeira, V. M. and Almeida, L. M. (1994). Action of phenolic derivatives (acetaminophen, salicylate, and 5-aminosalicylate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. Archives of biochemistry and biophysics, 1994, 315(1), 161-169.

[11]. Nishikimi, M., Rao, N. A., and Yagi, K., The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. Biochemical and biophysical research communications, 1972, *46*(2), 849-854.

[12]. Zhao, G.R., Xiang, Z.J., Ye, T.X., Yuan, Y.J., and Guo, Z.X., Antioxidant activities of Salvia miltiorrhiza and Panax notoginseng. Food Chemistry, 2006, 99(4), 767-774.