*The Pharmaceutical and Chemical Journal*, 2021, 8(1):47-52

*Available online www.tpcj.org*

**Research Article**

**ISSN: 2349-7092**
**CODEN(USA): PCJHBA**

# Constituents and Antioxidant Activity of *Raphanus 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** The oil from *Raphanus sativus* L. seeds was characterized by GC-MS. A total of 25 components were identified and quantified. Major constituents are: 13-docosenoic acid, methyl ester, (Z)-(28.02%), cis-13-eicosenoic acid, methyl ester (15.35%), 9,12- octadecadienoic acid -(Z)-, methyl ester (12.75%) and hexadecanoic acid, methyl ester (8.63%). The antioxidant activity of *Raphanus sativeus* essential oil was conducted by different methods: metal chelating, free radical (DPPH˙) and superoxide anion scavenging activity compared to standard antioxidants: BHT (butylated hydroxyl toluene), BHA (butylated hydroxyl anisole) and trolox. In the DPPH assay, the studied oil showed $IC_{50}$: 68.65±1.23 close to that of the standard antioxidant: butylated hydroxyl toluene (BHT)–($IC_{50}$ 61.11±1.78). In the superoxide anion scavenging assay, the oil sample gave: $IC_{50}$ 123.19±7.63 while the positive controls showed: BHT($IC_{50}$, 60.5±0.22) and Trolox ($IC_{50}$ 54.98±0.17). However, in the metal chelating assay the oil showed $IC_{50}$: 132.53±0.80) while the positive control -EDTA gave: $IC_{50}$ 7.05±0.29.

**Keywords** *Raphanus sativus* L., Oil, GC–MS Analysis, Antioxidant Activity

## Introduction

*Raphanus sativus* L. is an annual herb in the family Cruciferous. This herb is consumed as vegetable throughout the world. It has many varieties differing mainly in the size, shape and color of their roots [1]. Though leave and stem have been used in foods as flavoring agents, the root is valued as a natural remedy [2]. Root is used traditionally for stones, gravel, and scorbutic conditions [3,4]. It is also used against disorders of the respiratory, urinary and gastrointestinal systems, female and male infertility and skin infections [1,5]. *Raphanus sativus* contains many essential minerals and vitamins.

The plant also contains carbohydrates and fiber [6]. Many of the interesting pharmacological properties attributed to *Raphanus sativus* are due to a wide range of secondary metabolites, including alkaloids, phenolics, flavonods, coumarins, carotenoids, terpenes and other phytochemicals.

## Material and Method

### Collection of Plant Material

*Raphanus sativus* L. seeds were purchased from a local market - Cankiri Kartekin city, Turkey. The plant material was taxonomically authenticated by 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 from *Raphanus sativus***

Dry powdered of *Raphanus sativus* seeds (150g) were extracted with n-hexane at room temperature for 72h and filtrated. The solvent was removed under reduced pressure to yield the oil.

**Instrumentation and chromatographic conditions**

Instrument: Shimadzu GC-MS QP2010 Ultra (Japan)

Carrier gas: Helium

**Oven temperature program**

| Rate | Temperature (°C) | Hold time (min $^{-1}$) |
|---|---|---|
| - | 60.0 | 0.00 |
| 10 | 300.0 | 0.00 |

**Chromatographic conditions**

| | |
|---|---|
| Colunm Oven Temp.: | 60.0 °C |
| Injection Temp.: | 300.0 °C |
| Injection Mode: | Split |
| Flow Control Mode: | Linear Velocity |
| Pressure: | 100.0 kPa |
| Purge Flow: | 3.0mL/min |
| Split Ratio: | 1.0 |

**GC Program (GC-MS –QP2010 Ultra)**

| | |
|---|---|
| Ion Source Temp.: | 200.00°C |
| Interface Temp.: | 250.00°C |
| Solvent Cut Time: | 2.50min |
| Detector Gain Mode: | Relative |
| Detector Gain: | 0.86 kv +0.00kv |
| Threshold: | 0 |

**Determination of antioxidant activity**

The antioxidant activity of *Raphanus sativeus* oil was conducted by different methods: metal chelating, free radical (DPPH$^{\cdot}$**)** and superoxide anion scavenging activity compared to positive standards. 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**

In this assay, the bleaching rate of a stable free radical (DPPH$^{\cdot}$**)** is monitored at a characteristic wavelength ($\lambda_{max}$ 517 nm) in the presence of sample. The test sample was mixed with DPPH$^{\cdot}$ **(**0.1 mM, 0.5 ml). The absorbance was recorded at $\lambda_{max}$ 517 nm and compared with standards. During this assay there was a change in color from purple to yellow.

**Metal chelating activity**

Ferrozine produces a violet complex with $Fe^{2+}$. In the presence of a chelating agent, complex formation is interrupted and as a result the violet color of the complex is decreased [7]. The $Fe^{2+}$-chelating activity of the sample was recorded using the absorbance of the ferrozine-$Fe^{2+}$complex at $\lambda_{max}$ 562 nm. The sample was added to $FeCl_2$ (2 mM, 0.05 mL). The test was initiated by the addition of ferrozine. The absorbance of the mixture was recorded at $\lambda_{max}$ 562 nm after incubating at room temperature for 10 min.

**Superoxide anion scavenging activity**

The activity of the sample was estimated according to the methods of Nishikimi et al [8] and Zhao et al [9] with minor modification. Superoxide radicals were generated in a PMS-NADH system by oxidation of NADH and assayed by reduction of NBT. Briefly, a (1 ml) sample was thoroughly mixed separately with of (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 $\lambda_{max}$ 532 nm. A decrease in absorbance of the mixture indicates an increase in superoxide anion-scavenging activity.

**Results and Discussion**

Gas chromatography - mass spectrometry has been used for the identification and quantification of the studied oil. The analysis revealed the presence of 25 components – Table 1.

**Table 1:** Constituent of *Raphanus sativus* L. seed oil

| No. | Name | Ret. Time | Area% |
|---|---|---|---|
| 1. | Methyl tetradecanoate | 14.198 | 0.18 |
| 2. | Pentadecanoic acid, methyl ester | 15.326 | 0.05 |
| 3. | 7,10-Hexadecadienoic acid, methyl ester | 16.098 | 0.18 |
| 4. | 7-Hexadecenoic acid, methyl ester, (Z)- | 16.175 | 0.09 |
| 5. | 9-Hexadecenoic acid, methyl ester, (Z)- | 16.204 | 0.54 |
| 6. | Hexadecanoic acid, methyl ester | 16.410 | 8.63 |
| 7. | cis-10-Heptadecenoic acid, methyl ester | 17.221 | 0.10 |
| 8. | Heptadecanoic acid, methyl ester | 17.433 | 0.10 |
| 9. | 9,12-Octadecadienoic acid (Z,Z)-, methyl ester | 18.174 | 12.75 |
| 10. | 9-Octadecenoic acid (Z)-, methyl ester | 18.253 | 7.10 |
| 11. | 9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)- | 18.279 | 4.26 |
| 12. | Methyl stearate | 18.424 | 4.33 |
| 13. | gamma.-Linolenic acid, methyl ester | 19.930 | 1.10 |
| 14. | cis-13-Eicosenoic acid, methyl ester | 20.090 | 15.35 |
| 15. | cis-11-Eicosenoic acid, methyl ester | 20.129 | 1.66 |
| 16. | Eicosanoic acid, methyl ester | 20.267 | 3.33 |
| 17. | 8,11-Eicosadienoic acid, methyl ester | 20.551 | 0.07 |
| 18. | 13-Docosenoic acid, methyl ester, (Z)- | 21.852 | 28.02 |
| 19. | Docosanoic acid, methyl ester | 21.971 | 2.59 |
| 20. | cis-10-Nonadecenoic acid, methyl ester | 22.602 | 0.14 |
| 21. | Tricosanoic acid, methyl ester | 22.769 | 0.08 |
| 22. | 15-Tetracosenoic acid, methyl ester, (Z)- | 23.391 | 5.55 |
| 23. | Tetracosanoic acid, methyl ester | 23.544 | 2.46 |
| 24. | D:B-Friedo-B':A'-neogammacer-5-en-3-ol, (3.beta.)- | 23.875 | 1.17 |
| 25. | Hexacosanoic acid, methyl ester | 25.112 | 0.17 |

The fallowing compounds were detected in the chromatogram as major constituents:

i) 13-Docosenoic acid, methyl ester, (Z)-(28 .02 %)

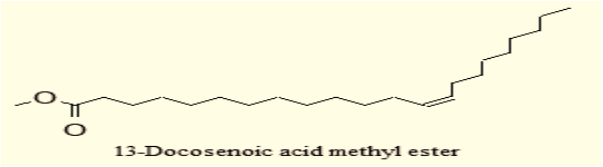
ii) cis-13-Eicosenoic acid, methyl ester (15.35 %)

iii) 9,12-Octadecadienoic acid (Z,Z)-, methyl ester ( 12.75 %).

iv) Hexadecanoic acid, methyl ester (8.63 %)

v) 9-Octadecenoic acid methyl ester (7.10%)

Fig. 1 shows the mass spectrum of 13-docosenoic acid, methyl ester. The peak at m/z 352 which appeared at RT. 21.852 in total ion chromatogram corresponds $M^+$ [$C_{23}H_{44}O_2$], while the peak at m/z 320 corresponds to loss of a methoxy. The mass spectrum of cis-13-eicosenoic acid, methyl ester is presented in Fig. 2. The peak at m/z 324 which appeared at (RT.20.090) is due to $M^+$ [$C_{21}H_{40}O_2$], while the peak at m/z 292 corresponds to loss of a methoxy. Fig. 3 illustrates the mass spectrum of 9,12-octadecadienoic acid (Z,Z)-, methyl ester. The signal at m/z 294 which appeared at RT 18.174 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. 4 shows the mass spectrum of hexadecanoic acid methyl ester. The signal at m/z 270 (RT.16.410) 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. 5 shows the mass spectrum of 9- octadecenoic acid methyl ester. The molecular ion: $M^+$ [$C_{19}H_{36}O_2$] appeared at m/z 296.



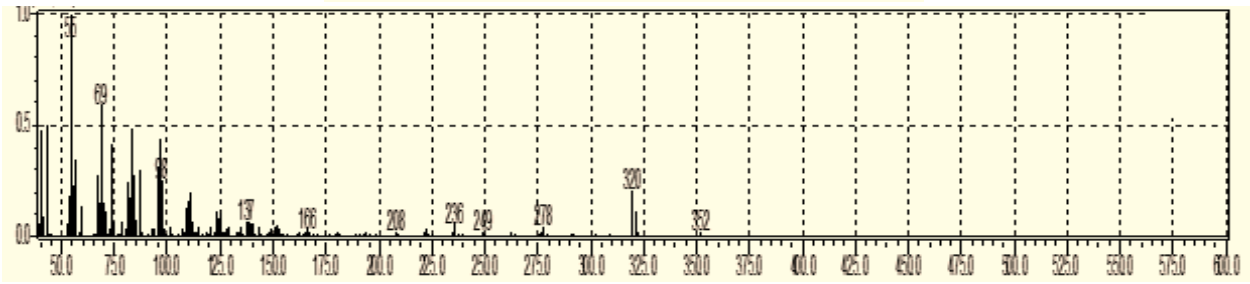
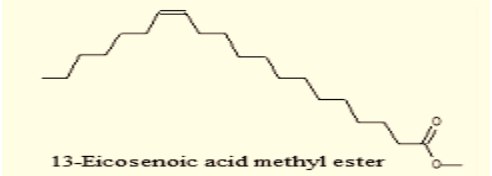




*Figure 1: Mass spectrum of 13-docosenoic acid, methyl ester(Z)-*



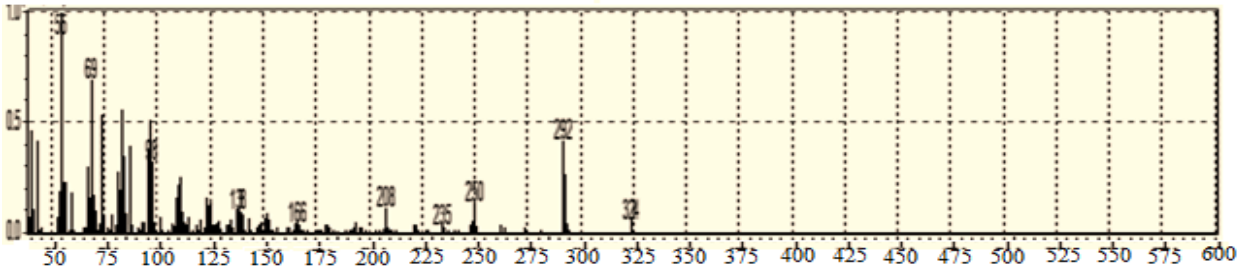
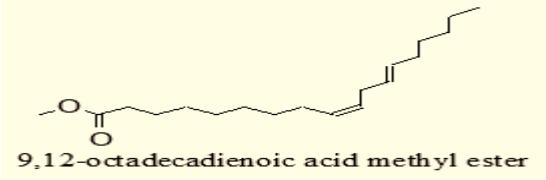




*Figure 2: Mass spectrum of cis-13-eicosenoic acid, methyl ester*



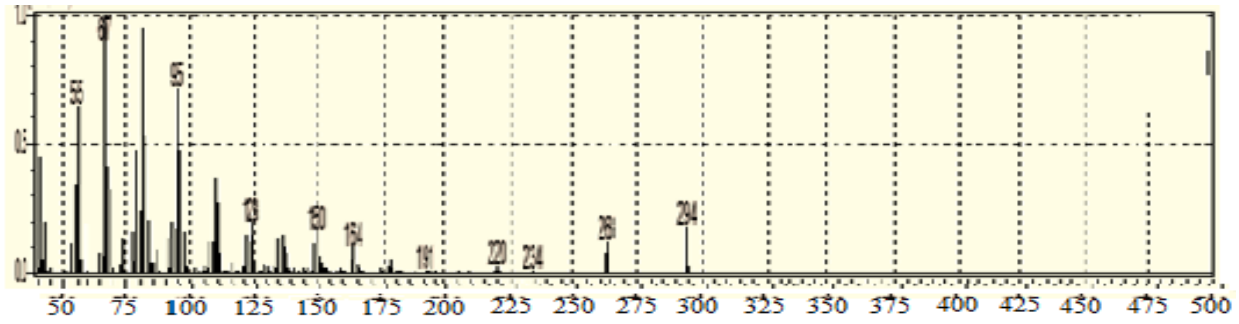
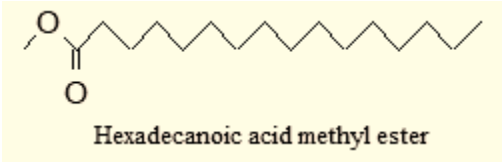


*Figure 3: Mass spectrum of 9,12-octadecadienoic acid methyl ester*



Hexadecanoic acid methyl ester

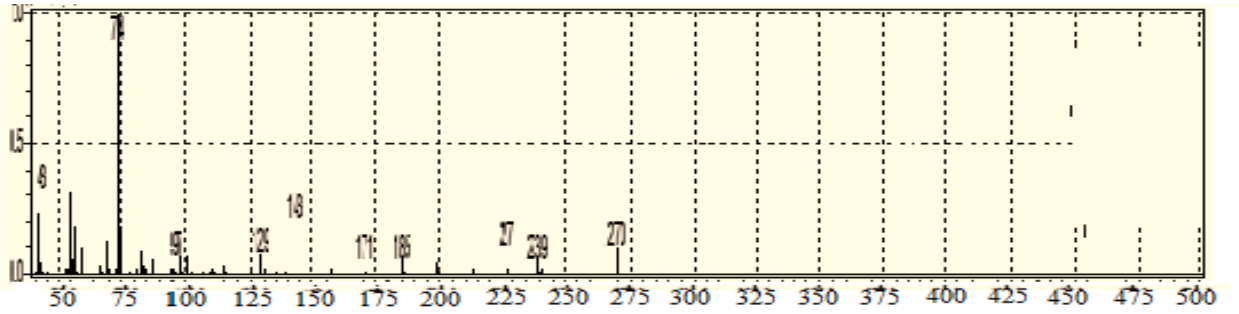
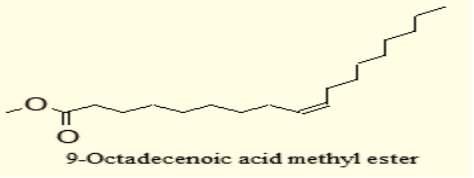


*Figure 4: Mass spectrum of hexadecanoic acid, methyl ester*



9-Octadecenoic acid methyl ester

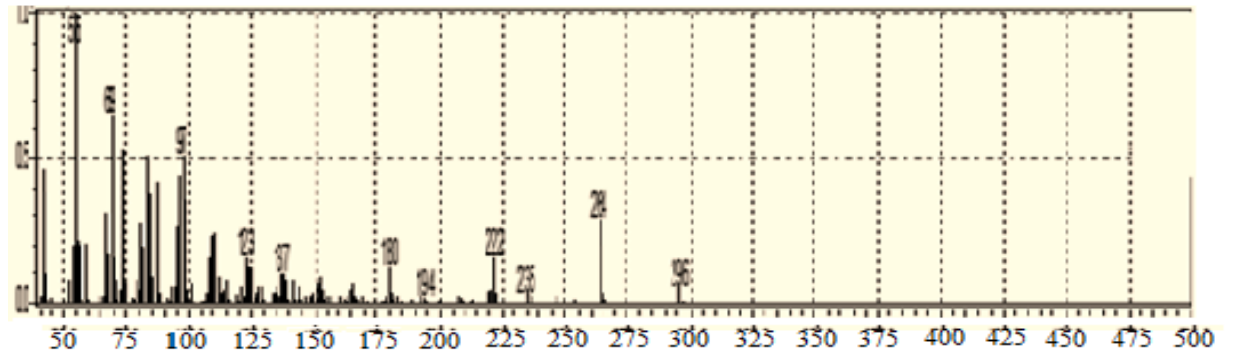


*Figure 5: Mass spectrum of 9-octadecenoic acid methyl ester*

**Antioxidant Assay**

The antioxidant activity of *Raphanus sativeus* essential oil was conducted by different methods: metal chelating, free radical (DPPH$^{\cdot}$**)** and superoxide anion scavenging activity compared to standard antioxidants: BHT (butylated hydroxyl toluene), BHA (butylated hydroxyl anisole) and trolox. In the DPPH assay, the studied oil showed $IC_{50}$: 68.65±1.23 close to that of the standard antioxidant: butylated hydroxyl toluene (BHT) – ($IC_{50}$ 61.11±1.78). In the superoxide anion scavenging assay, the oil sample gave: $IC_{50}$ 123.19±7.63 while the positive controls showed: BHT

($IC_{50}$, 60.5±0.22) and Trolox ($IC_{50}$ 54.98±0.17). However, in the metal chelating assay the oil showed $IC_{50}$: 132.53±0.80) while the positive control-EDTA gave: $IC_{50}$ 7.05±0.29.

**Table 3:** $IC_{50}$ and $r^2$ values of for antioxidant activity

| Sample | Free radical (DPPH·) scavenging activity | | Metal chelating activity | | Superoxide anion scavenging activity | |
|---|---|---|---|---|---|---|
| | **$IC_{50}$, µg/mL** | **$r^2$** | **$IC_{50}$, µg/mL** | **$r^2$** | **$IC_{50}$, µg/mL** | **$r^2$** |
| **Oil** | 68.65±1.23 | 0.87 | 132.53±0.80 | 0.66 | 123.19±7.63 | 0.98 |
| **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]. Aruna, G., Yerragunt, V.G. and Raju, A.B., "Phytochemistry and Pharmacogy of Raphanus sativus, Intrenational Journal of drug Farmudation and Research,2012, l3(1): 43-53.

[2]. Beevi, S.S., Mangamoori, L.N. and Aabrolu, N., Compartive activity against pathogenic bacteria of the root, steem and leaves of Raphanus sativus grown in India, World Journal of Microbiology and Biotechnology, 2009, 25(3): 465-473.

[3]. Kadhim, M.J., Sasa, S.S. and Hamid, I.H., Evaluation of antibacteral activity and bioactive chemical analysisof Ocimum basilicum using fourier trans from infra red (FT-IR) and gas chomatography mass spectrometry (GC-MS) Techniques. International Journal of pharmacognosy and Phytochemical Research, 2016 , 8(6): 127-146.

[4]. Hussen, I.H., Ibraheem, O.A., Antimicrobial Activity and spectral chemical analysis of methenolic leaves extract of Adiantum Capillus Veneris using GC-MS AND FT-IR spectroscopy, International Journal of Pharmacgonsy and Phytochecmical Rrseach, 2016, (2): 10-30.

[5]. Said, O., Khalil, K., Fulder, S. and H. Azaieh, H., Ethnopharmacological survey of medicinal herbs in Israel, the Golan Heights and the West Bank region, Journal of Ethnopharmacology, 2002, 83(3): 251-265.

[6]. Shukla, S., Chidambara, K.N. and Patil, B.S., Crucial facts about health benefits of popular cruciferous vegetables, Journal of Functional Foods 2012, 4(1): 94-106.

[7]. Haro-Vicente, J.F., Martinez-Gracia, C., Ros, G. Optimization of *in vitro* measurement of available iron from different fortificants in citric fruit juices. Food Chem. 2006;98:639–64.

[8]. 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.

[9]. 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.