Quantitative and qualitative detection of metabolic compounds in the seeds of cress plant (*Lepidium sativum*.L) using chromatography technique

Inas A. Ismael[1]* and Ayad C. Khorsheed[1]

[1]Biology Department, College of Educating for Girls, Mosul University, Mosul, Iraq
***Corresponding author e-mail: enasahmadismail@gmail.com**

Received:
July 01, 2021

Accepted:
Aug. 22, 2021

Published:
Oct. 01, 2021

Abstract

The natural compounds are considered one of the important natural source for giving effective and medicinal compounds, especially in the varions aspects due to the nature of their composition, safety of use and of access to their sources in nature. One of these plants is *Lepidium sativum* that grown in Iraq. In this research, volatile oils were separated from the seeds and calculating their concentration in ratio and also identification with GLC- technique which included. (alpha-pinene, Linalool, comphour, camphere, limonene, terpene and Myrcene). Also chromatographic analysis showed that the seeds contain of many of many of fatty acid compounds which included: (palmitic acid, oleic acid, Linoleic acid, stearic acid, Eicosenoic acid, linolinic acid, butyric acid and undeconaic acid).

Keywords: Lepidium sativum, Fatty acids, Volatile oils, Saponificaiton

Introduction

Lepidium sativum L. (Family: Brassicaceae) Commonly known as “Garden Cress”, a native of the Mediterranean region is a small, smooth, erect, annual herb. It is widely cultivated in temperate and to some extent in sub- tropical climates throughout the world as a culinary and medicinal herb [1] in India, *Lepidium sativum* is mainly cultivated in the states of Gujarat, Madhya Pradesh, Maharashtra, Rajasthan and Uttar Pradesh [2]. It is a fast- growing, edible plant botanically related to watercress and mustard with a similar peppery, sharp flavor and aroma. Nowadays, due to the availability of commercial antioxidants which are unsafe and toxic there is increased interest in the therapeutic potential of plants for the development of healthy fats and nutraceutical foods. The seed has diuretic, tonic, aphrodisiac, carminative, galactagogue and emmenagogue properties [3]. In addition, health drink and food products incorporated with seed and its fractions are traditionally known and acceptable. Seed oil is reported to modulate the functions of immune competent cells [4].

Lepidium sativum (garden cress) seed oil was examined for its antimicrobial, antioxidant and anti-inflammatory [5]. *Lepidium sativum* has been widely used to treat a number of diseases in traditional medicine throughout Iraq. It is useful in case of lumbago or any other pains about the loins through rheumatism. Its external application

with lime juice for relief of internal inflammation and rheumatic pain is useful. Garden cress is genetically related to water stress and mustard, sharing their peppery, tangy flavor and aroma. In some regions, garden cress is known as mustard and cress, garden pepper cress, pepperwort, pepper grass, or poor man's pepper [6].

The seeds of *lepidium sativum* contain 27% of protein, 14-26% of lipids, 35, 54% of carbohydrate and 8% of fiber [7]. Garden cress (*Lepidium stivum*) is an annual edible herb [8]. This species belongs to Brassicaceaefamily which includes 338 genera and 3709 species [9].

Garden cress is native to Iran, and is widely distributed as a cultivated plant eastward to Tibet [10]. Genetic diversity study is used for efficient utilization and for development of improved cultivar/ varieties [11]. The cultivated cress has several medical benefits. It is taken in cases of vitamin C deficiency, cases of constipation, a tendency towards infection (weak immune system) and fluid retention. The cress plant and its seeds were also used in the past to increase the speed of healing of bone fractures by increasing the deposition of collagen in areas of broken bones and increasing the tensile strength in these areas. Cress seeds also have analgesic and anti-inflammatory properties. Which helps relieve joint pain and reduce stiffness and swelling corresponding to this pain [1].

Material and methods

Plant seeds collection

The seeds of *Lepidium sativum* were collected from the Dam district/Mosul governorate and classified in the Directorate of the Medicinal plants development project in the Mosul Dam of the Iraq. The seeds were cleaned, grinded, and put in a paper batch, and kept in conditions away from moisture.

Preparation of some plant Extracts by using continuous soxhlet Apparatus.

After the seeds were dried and also crushed by and electrical mill, where 25g of the well- ground powder was placed in the soxhlet batch system using 400µl of pet. Ether (60- 80) °C was adeded to the flax seeds extracted oil (L_1). The extraction process continued at a rate of 6 hrs. per day until the solvent in the device became colorless. Mreover, the extract was concentrated by a rotary vacuum evaporator (RVE) [13]. Moreover, we used other solvents chloroform (L_2) ethyl acetale (L_3), IMS (95% Etolt: 5% Medolt; L_4 and also Hot equeous water (L_5).

Volatile Oils extracted using Clevenger Apparatus

Volatile oil was extracted from the seeds of the plant under study, using a specialized and converted Clevenger device to extract the light oil and connected with a volumetric flask with a capacity of 500 µl as 15 gm of powdered seeds was mixed with 200 µl of distilled water, and then the distillation process was carried out by using the powdered plant at a boiling point 100°C and the process of distillation lasted between 1-2 hrs. Distilled water that containing the volatile oil was collected and put in the separating funnel 100 µl of it and 50 µl of diethyl ether was added to it and for two stages. Shaking the mixture well and left to settle, so two lagers were collected an upper layer containing the ether with oil, the aqueous layer was negleded. After collecting the samples

anhydrous mgso4 was added at 2 gm to dry the sample from remaining water in the ether layer. Also, the samples were concentrated using the rotary vacuum evaporate at a temperature of 25-30°C.

The crude oil was placed in sealed bottles and kept in the freezer until identified of their compounds. Was distilled water also zamzam water.

Saponification

By taking 5 µl of the each crude extracts (pet-ether (60-80)°C through defatted process chloroform (L_2), Ethyl acetate (L_3), IMS (L_4) and Hot aqeous ucle (L_5) and added 100 µl of 7.5 m Kolt in methanol: water, (3:2) lteating the mixture for 90 min. at 100°C, then added 100 µl of distilled water and 50 µl ether solvent and put in the separating funnel and tooked the aqueous layer and added of concentrated H_2SO_4 until pH(Acid function)= 2. In the end adel 50 µl of ether and put again in the separting funned and take the organic layer [14].

Identification of fatty acids and volatile oils using GLC-technique.

The free pole of separated fatty acids and volatile oil were identified in the laboratories of ministry of science and technology Dept. of Environment and water by GLC model (Shimanezo) Japanse (2010) by using ionized flame detector and using poetic colum type (SE-30) wavelengths (0.25 µm, 0.5 µm, 30 m).The temperature was in the injection area and the detector (330 and 280) °C, while the column temperature gradually starts from (120-----180) °C at a vate of 8 °C/ min using, passive nitrogen gas as a carrier gas at rate of 100 Kp.

Results and discussion

Qualitative and Quantitative identification of volatile oils of lepidium sativum using GLC- analysis method

Chromatographic diagrams charts were obtained in which the retention time of each compound was determined under study samples compaired the standard sample retention time of each volatiled oil compounds scince ∝- pinene was presented in the distilled water, Zam Zam water, (L_1), (L_2), and also (L_3); 17.0%, 19.8%, 18.2%, 15.7% and 13-2 % respectively.Also, Linalool was presented in the same mentioned extracts (18.6%, 22.6%, 20.1 %, 17-1 % and 15.4% respectively moreover, camphor was investigal (14.2%, 17.8%, 16.4%, 12.5% and 10.3% respectively). Using the above same extracts. Camphere compound was identified in the same above extracts (2.4%, 3.9%, 3%, 2.0% and 1.4% respectively). Limonene was also presented in the same extracts (1.7%, 2.4%, 2.1%, 1.1% and 0.6% respectively. Also, terpinene presented in the various same using extracts (11.6%, 14.8%, 13.0%, and 8.3% respectively). The last one of volatile oil was myrcene that presented in the same above extracts (3.2%, 4.9%, 4.0%, 2.7%, and 1.8% respectively).

The important point and we abtaind from the table (1) refer to the high ratio of volatile oils for compained the other extracts (Table (1), Figure (1), Figure (2), Figure (3), Figure (4), Figure (5), Figure (6), Figure (7), Figure (8), Figure (9), Figure (10), Figure (11), Figure (12). Table (1) volatile oil identified using GLC. Technique of various extracts.

The identification of fatty acid compounds of lepidium sativum extracts (L1, L2, L3, L4, and L5).

The identification of these extracts (L_1, L_2, L_3, L_4 and L_5) after saponification process and liberated fatty acid compounds which showed the presence of these extracts using GLC. Technique as following Table (2).

Fatty acids

Table (2) The ratio of fatty acids identified using GLC- technique of various extracts (L_1, L_2, L_3, L_4, and L_5). → Figure. Figure (13), Figure (14), Figure (15), Figure (16), Figure (17), Figure (18). Palmatic acid was presented in all saponified extracts (L_1, L_2, L_3, L_4 and L_5) whit ratio of (29.2%, 27.4%, 22.1%, 25.0% and 19.6 % respectively). Also, oleic acid was showed in the same extracts with ration of (10.3%, 8.9%, 7.0%, 7.6% and 6.2% respectively). Moreover linoleic acid was also investigated in the same above saponified extracts with ration of (18.4%, 14.5%, 10.3%, 12.0% and 9.7% respectively). Stearic acid was identified in the above saponified extracts with ratio of (1-7%, 1-3%, 0.8%, 1.1% and 0.6% respectively). The eicosenoic acid was showed in thse saponified extracts with ratio of (12.9%, 10.9%, 7.6%, 8.7% and 7.0% respectively). Lenolinic acid identified in these mentioned saponified extracts with ratio of (7.6%, 6.1%, 4.3%, 5.7% and 3.1% respectively). Butyric acid investigated in these saponified extracts with ratio of (2.5%, 2.0%, 1.3%m 1.6% and respectively). Finally, undecanoic acid was also showed in these saponified extracts with ratio of (1.5%, 1.2%, 0.8%, 1.0% and 0.6% respectively).

Table (1): Volatile Oils identified using GLC-techniqe of various extracts [15]

Name %	Distilled Water*	Zamzam water*	L 1**	L 2**	L 3**
a-pinene	17.0	19.8	18.2	15.7	113.2
Linalool	18.6	22.6	20.1	17.1	15.4
Camphor	14.2	17.8	16.4	12.5	10.3
Camphene	2.4	3.9	3.0	2.0	1.4
Limonene	1.7	2.4	2.1	1.1	0.6
Terpinen	11.6	14.8	13.0	10.3	8.3
Myrcene	3.2	4.9	4.0	2.7	1.8

*** Distilled and Zamzam water by Clevenger**

**** (L1, L2, L3) by soxhlet apparatus**

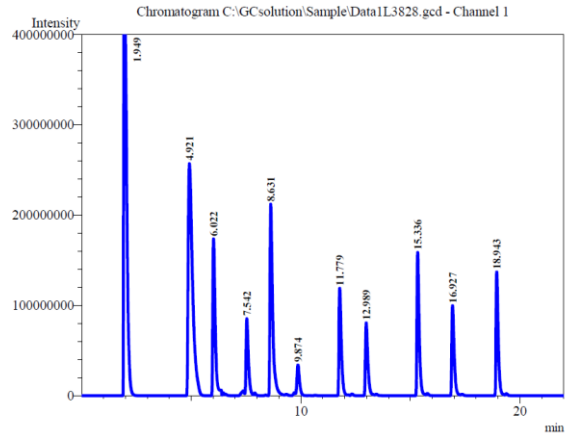

Figure (1): Volatile oils of distilled water using GLC-analysis

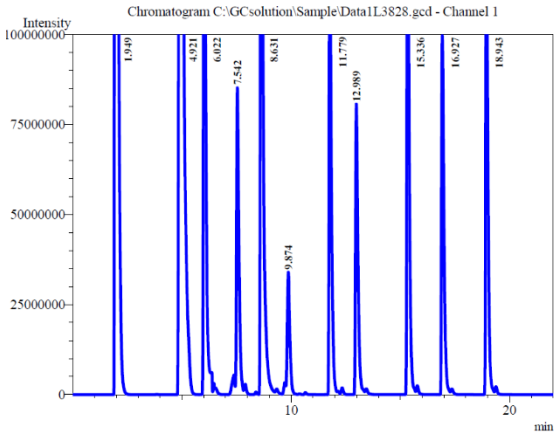

Figure (2): Volatile oils of Zamzum water using GLC-analysis

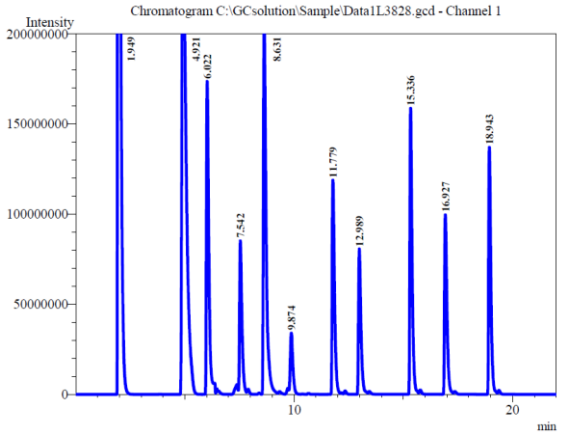

Figure (3): Volatile oils of pet-ether extract of the seeds of *Lepidium stativum* using GLC-analysis

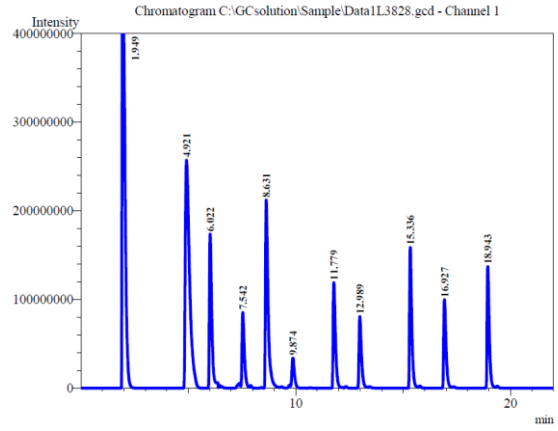

Figure (4): Volatile oils of chloroform extract of the seeds of *Lepidium stativum* using GLC-analysis

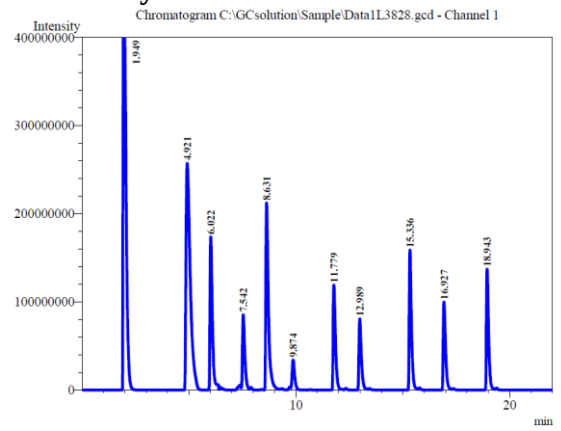

Figure (5): Volatile oils of ethyl acetate extract of the seeds of *Lepidium stativum* using GLC-analysis

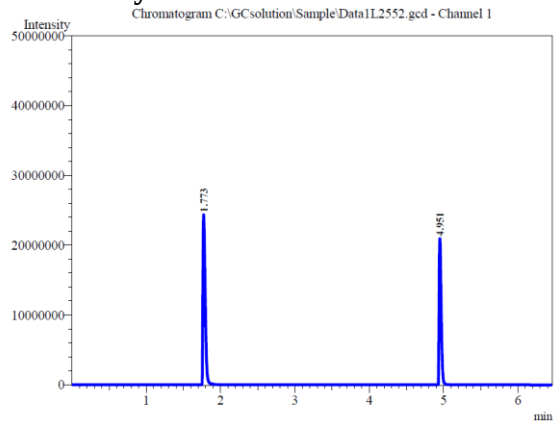

Figure (6): The standard curve for □-pinene essential oil by GLC-analysis

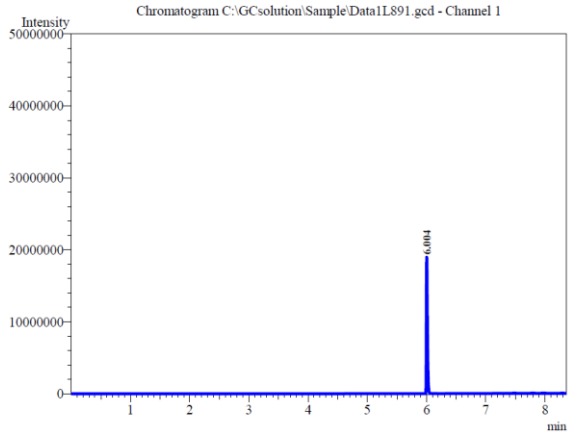

Figure (7): The standard curve for Linalool 0.5% oil by GLC-analysis

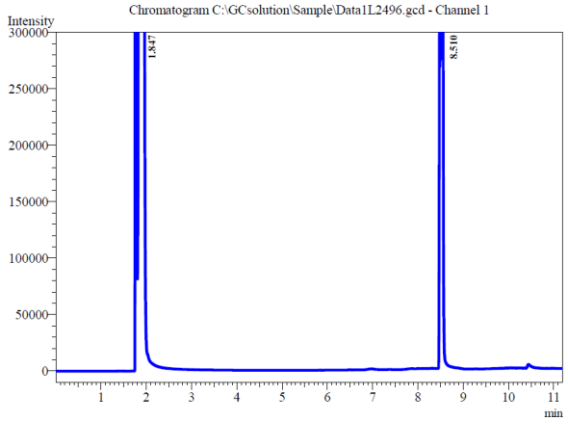

Figure (8): The standard curve for Camphor 0.5% oil by GLC-analysis

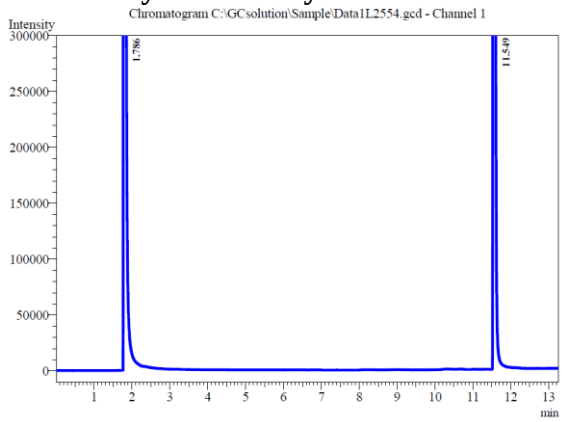

Figure (9): The standard curve for Camphenen 0.5% oil by GLC-analysis

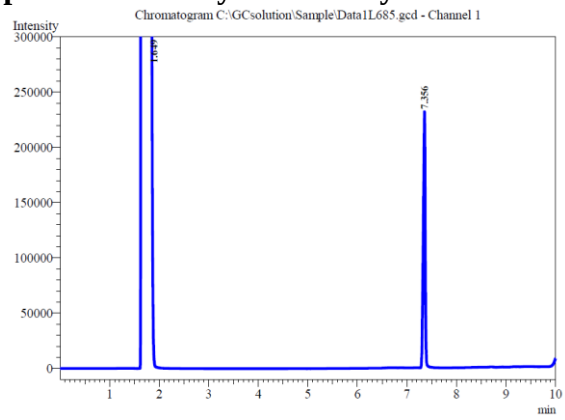

Figure (10): The standard curve for Limonin 0.5% oil by GLC-analysis

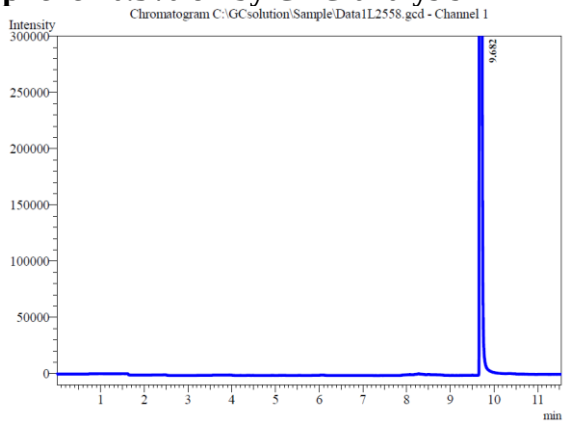

Figure (11): The standard curve for Terpinin 0.5% oil by GLC-analysis

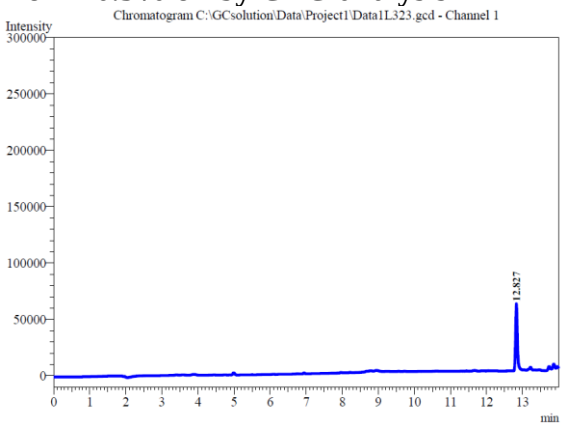

Figure (12): The standard curve for Myarcnin 0.5% oil by GLC-analysis

Table (2): The vatio of fatty acids identified using GLC-technique of various extracts (L1, L2, L3, L4 and L5) [16]

Name %	L 1	L 2	L 3	L 4	L 5
Palmatic	29.2	27.4	22.1	25.0	19.6
Oleic	10.3	8.9	7.0	7.6	6.2
Linoleic	18.4	14.5	10.3	12.0	9.7
Steric	1.7	1.3	0.8	1.1	0.6
Eicosenoic	12.9	10.9	7.6	8.7	7.0
a- Lenolinic	7.6	6.1	4.3	5.7	3.1
butyric	2.5	2.0	1.3	1.6	1.0
undecanoic acid	1.5	1.2	0.8	1.0	0.6

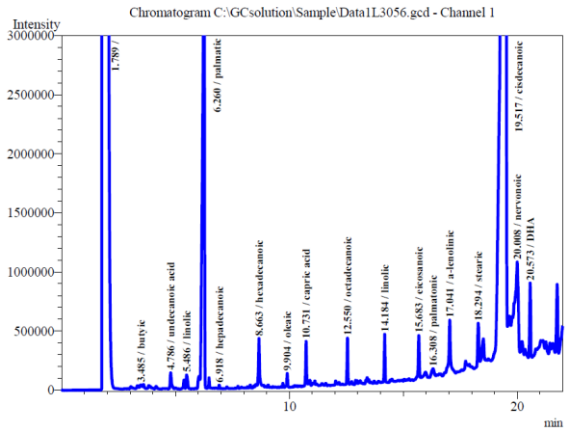

Figure (13): The standard curve of fatty acids compounds

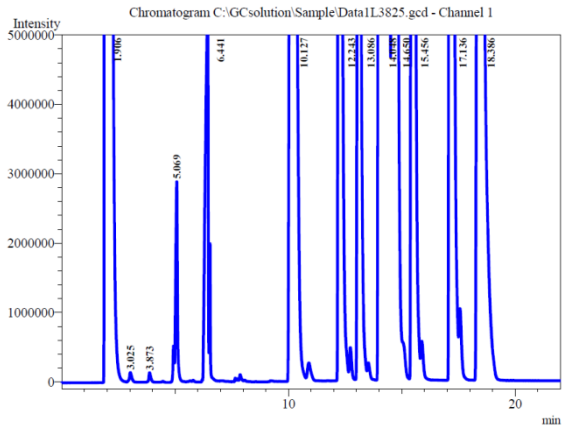

Figure (14): The fatty acid compounds from the saporified pet.ether extract of *Lepidium sativum* seeds

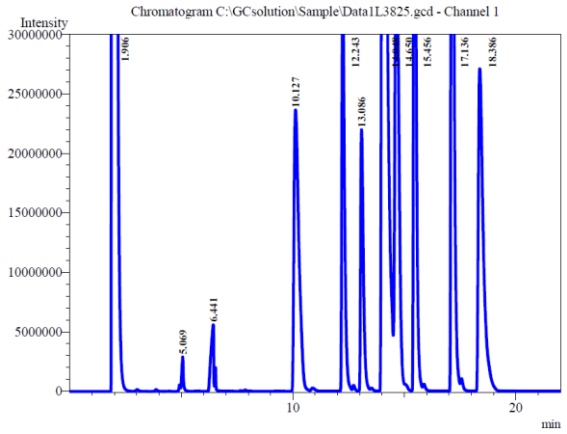

Figure (15): The fatty acid compounds from the saporified chloroform extract of *Lepidium sativum* seeds

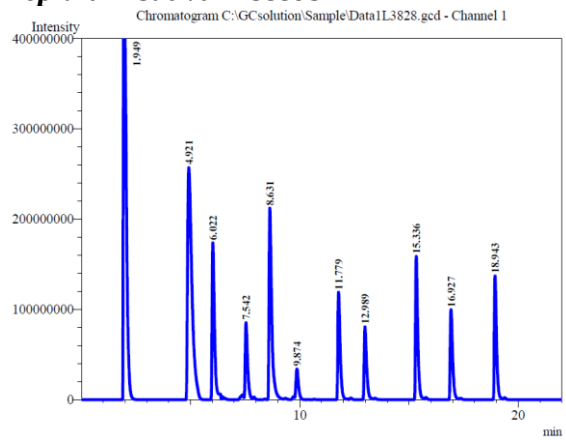

Figure (16): The fatty acid compounds from the saporified ethyl acetate extract of *Lepidium sativum* seeds

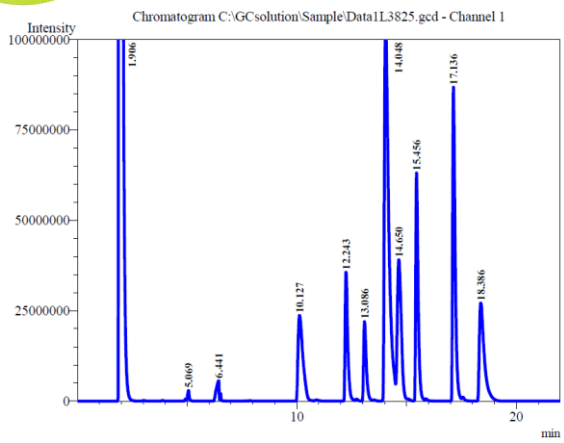

Figure (17): The fatty acid compounds from the saporified Ims extract of *Lepidium sativum* seeds

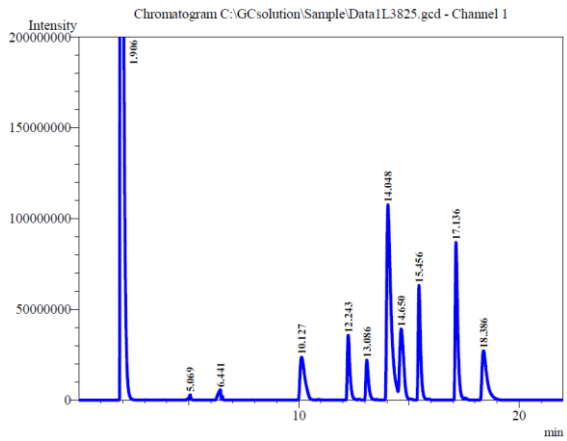

Figure (18): The fatty acid compounds from the saporified hot equeous extract of *Lepidium sativum* seeds

Conclusion

From the results (Tables nd Figureures) is confirmed that *Lepidium sativum* seeds are among the plants rich in volatile oil compounds and fatty acids because the seeds are as factory region belong to the secondary metabolisim compounds, including volatile oils and fatty acid compounds.

Acknowledgments

First of all, All praises be to Almighty God. Then I owe deep and sincere gratitude to my kind supervisor Prof. Dr. Ayad C. Khorsheed I appreciate his constant encouragement from the selection of the topic of the thesis till the final touches. His non-stop invaluable advice, and brilliant supervision help me to finish my work within time. Many thanks go Dr. Nassir A. Daod Also, gratitude is extended to the Department of Biology, College of Education for Girls, University of Mosul for the logistic help it has provided.

References

1) AL-Rawi, A. and chakravarty H.L. (1964). Medicinal Plants of Iraq, Bagdad, Ira

2) Anone (1981). An integrateal system of classification of flowering plants. Columbia University Press, New York, USA.

3) Arthur, I. V. (1972). Practical organic chemistry including qualitative organic analysis 3rd

4) Chatoui, K. A.; Talbaoui, M.; Aneb, Y.; Bakri, H. H. and Tabyaoui, M. (2016). Phytochemical screening antioxidant, and antibacterial activity of *lepidium sativum* seeds from morocco. J. mater Environ. Sci. 7(8), 2938- 2946.

5) Doke, S. and Guha, M. (2014). Garden cress (*Lepidium sativum* L.) seed- An important medicinal source: A review. J. Nat. Prod. Plant. Resour. 4.69- 80.

6) Fulwah, Y.A.; Aleanizy, F. S.; Mahmoud, A. Z.; Farshori, N. N.; Alfaraj, R.;

7) Al-Sheddi, E. S. and Alsarra, I. A. (2019). Chemical composition and antimicrobial, antioxidant, and anti-inflammatory activities of Lepidium sativum seed oil. Saudi J. Biol. Sci. 26(5), 1089-1092.

8) Gokavi, S. S.; Malleshi, N. G. and Guo, M. (2004). Chemical composition of graden cress (*Lepidium sativum*) seeds and its fractions and use of bran as a functional ingredient plant. Food. Hum. Nutr. 59. 105- 111.

9) Harborne, J. B. (1973). "Phytochemical Methods. 2nd ed., chapman Hall, Atlanta, USA.

10) Hcinik, S. Y.; Jor D. and Bov, Z. (2013). Chemical composition of the essential oil of rosemary (*Rosmarinus officinalis* L.) of Tunisian origin. Chem. Asian J. 25(5):2601-2603.

11) Honggen, Z.; Zhenyu, W. and Oscar, L. (2015). Development and validation of a GC-F10 method for quantitative analysis of lleic acid and related fatty acids. J. Pharm. Anal. 5(4):223-230.

12) Husseni, J.; Imad, H.; Hameed, H. and Hadi, M. Y. (2017). IOP Conf. Ser.: Mater. Sci. Eng.

13) Jan, S. A.; Shinwari, Z. K.; Rabbani, M. A.; Niaz I. A. and Shah, S. H. (2017). Assessment of quantitative agro-morphological variations among Brassica rapa diverse populations. Pak. J. Bot. 49 (2): 561- 567.

14) Kumar, V. and Yadav, H. K. (2019). Assessment of genetic diversity in *lepidium sativum* L. using inter simple sequence repeat (ISSR) marker. Physio mole. Bio. Plant, 25, 399- 406.

15) Parsa, A. (1959). Medicinal plants and drugs of plant origin in Iran. Quallitas plantar materiae vegetabilles 5: 375- 394.

16) Raval, N. (2016). A comprehensive review of *lepidium sativum* linn, atraditional medicinal plant, world. J. pharm. Sci. 5: 1593- 1660.

17) Wadhwa, S.; Panwar, M. S.; Agrawal, M.; Saini, N. and Patidar, L. N. (2012). A review on pharmcognostical study of *lepidum sativum*. Adv. Res. Pharmaceuticals Bio. 2: 316- 323.

18) Warwick, S. I.; Fracis A. and Al- Shehbaz, I. A. (2006). Brassicaceae, species checklist and database on CD- Rom. Plant syst. Evol., 259: 249- 258.