International Journal of Sciences & Applied Research

www.ijsar.in

Chemical Constituents, Antimicrobial and Antioxidant activity of Garden cress *(Lepidium sativum*) seeds oil from Sudan

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Abstract

This study aimed to extract seeds oil of Garden cress *(Lepidium sativum L)* and to identify its chemical constituents and evaluate its potential antimicrobial and antioxidant activity. The seeds were collected from Rashaad city, South Kordofan State, Sudan in October 2020.The oil was extracted in the laboratory of the department of chemistry faculty of science university of kordofan and analyzed by GC-MS instrument, the results showed that Eleven compounds were detected in G. cress seeds oil the major components are; α-Linolenic acid (29.57%), Oleic acid (23.12%), Eicosenoic acid (14.24%), Linoleic acid (12.43%) and Palmitic acid (7.52). The total saturated and unsaturated fatty acids found in the oil were 13.35% and 86.65% respectively. The chemical properties of the oil were measured; (1.96meq KOH/g) peroxide value, (4.19 mg KOH/100g) free fatty acid value, (134.46 gI_2/100g) iodine value, (173.36 mgKOH/g) saponification value and (1.16 %w/w) unsaponifiable matter value. The antimicrobial activity of the G. cress oil was evaluated using disc diffusion assay against two-gram positive bacteria: *Staphylococcus aurous*, *Bacillus subtilis* and two-gram negative bacteria: *Escherichia coli*, *Pseudomonas aeruginosa* and one Fungus *Candida albicans*. At concentration 100 mg/ml the oil was active against *Staphylococcus aureus* and partial activity against *Escherichia coli* and*Candida albicans*, Moreover oil showed no antibacterial activity against *Pseudomonas aeruginosa* and *Bacillus subtilis*. The antioxidant activity of the extracted oil was evaluated by using the standard 2.2-di(4-tetra-octyl-phenyl)-1-bicryl hydrazyl free radical (DPPH). The results showed that the antioxidant activity of the extracted oil was (99.03%). G. cress oil can be considered as stable oil with a high content of α-linolenic acid.

Keywords: *Lepidium sativum,* GC-MS, antimicrobial and antioxidant activity

1. Introduction

Medicinal plants may be defined as those plants that are commonly used in treating and preventing diseases and that are generally considered harmful to humans [1].The plant seeds are well known for their nutritional and medicinal value. The therapeutic features of plants have attracted worldwide interest about medicinal plants, which resulted in novel sources of drugs for wide modern applications [2].The seeds contain many phytochemical substances responsible for their medicinal properties. For this reason, we chose *Lepidium sativum* from medicinal plants because it has not been more studied in Sudan, although

relatively abundant and widely used in traditional medicine.

Lepidium sativum (G. cress) is an annual, herbaceous edible plant that is botanically related to mustard G. cress plant is native to many Arabic countries such as Sudan, Saudi Arabia and Egypt. Also found in Europe and America, the leaves are used in salad; also, G. cress is cultivated as culinary vegetable all over Asia. In many African countries, G. cress is thought to be an effective medicinal remedy to cure respiratory disorders, like bronchitis and asthma[3]. *Lepidium sativum* belongs to family *Brassicaceae (Cruciferae)* and is commonly known as "Common cress," "Garden cress,""Thufa", or "Halim." In Sudan the plant is called "Hab El-Rashaad".

G. cress has contained high amounts of nutrients and possess many health useful characteristics. Epidemiologically, itis recognized to have galactagogue characteristics and is traditionally used as a functional nutrition recipe for lactating mothers[4].Garden cress seed has been used in curing many health related complications by our ancients. It has been used in the treatment of many health problems such as hypertension, kidney diseases, hepatic diseases, glycaemia, diuretics, respiratory disorder, antimicrobial, anti-inflammation, antioxidant, anemia, laxative, chemo protective and many other therapeutic applications [5].(G. cress is widely used to heal fractures and to increase milk secretion during lactation. Also, possesses a wide range of antioxidant, fatty acids of G. cress oil helps in preventing coronary heart diseases[6]. G. cress has including high amounts of chemical composition which contains 22, 27and 30% from protein, fat and dietary fiber. Moreover, it was contained vital minerals as calcium, iron, and zinc were 296, 7.6 and 5 mg/100g, respectively[7]and a rich source of natural antioxidants as tocopherol was 139 mg/100g [8]. In addition, other vitamins as thiamine, riboflavin, and niacin, were 0.59, 0.61 and 14.3 mg/100g, respectively [9]. Moreover, G. cress seeds acts as memory boosters as it had contained essential fatty acids, therefore it helps in normal contraction of the muscle for healthy movements of limbs and heart [10].Many supplementation and compensation studies have been conducted on G. cress (protein and fats rich) and its products, which really had good results, where G. cress extract or powder can be added in fruits or vegetable juices, and food products, which are rich in vitamins and minerals but are poor in proteins and fats[11].

G. cress seeds contain 24% oil in which 32-34% is α-linolenic acid. G. cress oil has very high amount of tocopherols (1699 mg/kg), compared to other oils [12].In recent years, human dietary lipids intake has shifted more towards PUFAs due to their cholesterol lowering effect compared to saturated lipids.G. cress sprouts were used as the source material for the development of a cosmetic ingredient rich in isothiocyanates[13].

Materials and methods

Plant material

G. cress seeds were randomly harvested from several plants grown in Rashaad city-South Kordofan State-Sudan, the samples were collected in October 2020. Plant taxonomist at the Department of Botany Faculty of Science University of Kordofan-Sudan authenticated the plant. The seeds were shade-dried, cleaned and grinded by a mechanical grinder. The grounded samples were stored at room temperature for further uses.

Chemical and solvents

Potassium iodide, Iodine, Methanol, Chloroform, Petroleum ether, Absolute ethanol, Glacial acetic acid, Sodium hydroxide, Potassium hydroxide, Hydrochloric acid, Sulphuric acid, Bromine

solution, Sodium thiosulphate, Phenolphthalein reagent and Starch reagent.

Tools and Equipments

Sensitive balance, Volumetric flasks, Gas chromatography-mass spectrometer, Spectrophotometer, Filtration funnels, Measuring cylinders, Burette, Separation funnel, Air condenser, Pipette, Micropipette, Refrigerator, Petri dishes, Test tubes, Conical flasks, Water bath, Beakers, Autoclave, Hot air oven, Shaker, Filter Paper, Bunsen Burner, Incubator and mechanical grinder (Wiley Mill Model ED.5).

Notes: Gram-negative bacteria; ***E.c***=*Escherichia coli*, =*Pseudomonas aeruginosa*. Gram-positive bacteria; =*Bacillus subtilis*, ***S.a***=*Staphylococcus aureus*.Fungus; =*Candida albicans*

Microorganisms

Bacillus subtilis (B.s)

NCTC 8236 (Gram + ve bacteria)

Staphylococcus aureus (S.a)

ATCC 25923(Gram +ve Bacteria)

Escherichia coli (E.c)

ATCC 25922(Gram -ve bacteria)

Pseudomonas aeruginosa (Ps.a)

ATCC 27853 (Gram -ve bacteria)

Candida albicans (C.a)

ATCC 7596 (Fungus)

National Collection of Type Culture (NCTC), Colindale, England. American Type Culture Collection (ATCC) Rockville, Maryland, USA.

Methods

Extraction of seeds oil (Maceration method):

200 grams of the seeds powder were maceratedin a mixture of chloroform and methanol (2:1v/v) ratio and shaken well for ten minutes to complete the homogeneity. The sample was left for three days in refrigerator, and then filtered and placed in a separating funnel with addition of 0.73% sodium chloride to get rid of any non-lipid materials, where it separated into two layers the upper layer represents the aqueous layer and the lower layer represents the mixture of organic solvents and lipids. The extract is placed in an oven at 105°C to evaporate the solvents or any traces of suspended moisture, then the oil is cooled down and weighed, then the percentage of oil was calculated, The extracted oil was kept for further uses.

Chemical Properties of G. cress oil

Peroxide value

The peroxide value (PV) was determined by iodometric titration, which measures the iodine produced from potassium iodide by the peroxides present in the fat sample. A 2.0g sample of oil was dissolved in 30 mL mixture of glacial acetic and chloroform (30:70 v/v). Then 0.5 mL saturated KI solution was added. After 1 min under darkness, 30 mL H_2O pure was added and titrated slowly with (0.01N) sodium thiosulphate ($Na_2S_2O_3$) with vigorous shaking until yellow is almost gone. Add 0.5ml 1%starch solution and continue titrated to release all I from $CHCl_3$ layer until blue color just disappears[14].

Free Fatty Acids value

The free fatty acid in oil was estimated by titrating it against (0.1N) KOH in presence of phenolphthalein as indicator. The acid values defined as mg of KOH required to neutralize the free fatty acids present in 1g of sample. Acid value of G. cress seed oil was determined by titration method [14].

Iodine value

The iodine value is a measure of the degree of unsaturation of fatty acids and is used to characterize oils and fats. The G. cress oil contains both saturated and unsaturated fatty acids. Halogens add across the double bonds of unsaturated fatty acids to form additional

compounds. Iodine is useful parameter in studying oxidative rancidity of oil. Iodine gets incorporated into the fatty acids chain wherever the double bond exists. The amount of iodine consumed is then determined by titrating the iodine released (after adding KI) with standard thiosulphate and comparing with a blank in which the fat is omitted. Hence, the measure of iodine absorbed by an oil or fat gives the degree of unsaturation. Iodine value is defined as the (g) of iodine absorbed by (100g)of the oil. Iodine value of G. cress seed oil was determined by titration method [14].

Saponification Value

The saponification values defined as a known quantity of oil was saponified with alcoholic KOH for 1 hour in a water bath. This solution was titrated against (0.1N) HCl using 1% phenolphthalein as the indicator. Saponification value of G. cress seed oil was determined by titration method [14].

Unsaponifiable Matter

An oil sample (2-2.5g) in 30 ml of absolute ethanol and 5 ml of 60% aqueous KOH was refluxed for 300 min followed by extraction of unsaponifiable matter using diethyl ether. The solvent was evaporated to dryness and the residue was desiccated and weighed. The unsaponifiable matter content of G. cress seed oil was determined by separation and titration method[14].

GC-MS analysis conditions

The qualitative and quantitative analysis of the sample was carried out by using GC/MS technique model (GC/MS-QP2010-Ultra) from Japans "Simadzu Company" with serial number 020525101565SA and capillary column (Rtx-5ms-30m×0.25 mm×0.25µm).The sample was injected by using split mode, helium as the carrier gas passed with flow rate 1.61 ml/min, the temperature program was started from 60°C with rate 10°C/min to 300°C as final temperature degree with 5 minutes hold time, the injection port temperature was 300°C, the ion source temperature was 200°C and the interface temperature was 250°C.The sample was analyzed by using scan mode in the range of m/z 40-500 charges to ratio and the total run time was 29 minutes. Identification of components for the sample was achieved by comparing their retention index and mass fragmentation patents with those available in the library, the National Institute of Standards and Technology (NIST), results were recorded[15].

Preparation of bacterial suspensions

One ml aliquots of a 24 hours broth culture of the test organisms were aseptically distributed onto nutrient agar slopes and incubated at 37ºC for 24 hours. The bacterial growth was harvested and washed off with 100 ml sterile normal saline, to produce a suspension containing about 10^8 - 10^9 C.F.U/ml. The suspension was stored in the refrigerator at 4ºC till used. The average number of viable organisms per ml of the 22 stock suspension was determined by means of the surface viable counting technique. Serial dilutions of the stock suspension were made in sterile normal saline solution and 0.02 ml volumes of the appropriate dilution were transferred by micropipette onto the surface of dried nutrient agar plates. The plates were allowed to stand for two hours at room temperature for the drops to dry and then incubated at 37°C for 24 hours. After incubation, the number of developed colonies in each drop was counted. The average number of colonies per drop (0.02 ml) was multiplied by 50 and by the dilution factor to give the viable count of the stock suspension, expressed as the number of colony forming units per ml suspension. Each time a fresh stock suspension was prepared. All the above experimental

conditions were maintained constant so that suspensions with very close [16].

Preparation of fungus suspension

The fungus culture was maintained on sabouraud dextrose agar, incubated at 25°C for 4 days. The fungus growth was harvested and washed with sterile normal saline and finally suspended in 100ml of sterile normal saline, and the suspension were stored in the refrigerator until used[16].

Disc diffusion method

The paper disc diffusion method was used to screen the antibacterial activity of plant extracts and performed by using Mueller Hinton agar (MHA). The experiment was carried out according to the National Committee for Clinical Laboratory Standards Guidelines. Bacterial suspension was diluted with sterile physiological solution to 10^8 CFU/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 (What man No.1, 6 mm in 23 diameter) were placed on the surface of the MHA and soaked with 20 µl of a solution of each sample. The inoculated plates were incubated at 37°C for 24 h in the inverted position. The diameters (mm) of the inhibition zones were measured [16].

DPPH radical scavenging assay

The DPPH radical scavenging was determined according to the method of (Shimada et al., 1992) with some modification. In 96-wells plate, the test sample was allowed to react with 2,2Di (4-tert-octylphenyl)-1-picryl-hydrazyl stable free radical (DPPH) for half an hour at 37°C. The concentration of DPPH was kept as (300µl). The test sample was dissolved in dimethyl sulfoxide (DMSO) while DPPH was prepared in ethanol. After incubation, decrease in absorbance was measured at 517nm. Percentage radical scavenging activity by sample was ermined in comparison with a DMSO treated as control group [17].

% of antioxidant activity = 100-As / Ac × 100

Where Ac = absorbance of control group, As = absorbance of sample

RESULTS AND DISCUSSION

Oil extraction

The chemical properties of G. cress seeds oil was extracted by maceration method the results were presented in table (1).

The total oil content of extracted oil was (19.7%). The oil content in G. cress seeds is relatively less compared to other edible oil seeds such as mustard (25-40%), rapeseed (40-45%) of cruciferous family [18]. The peroxide value (PV) of G. cress seeds oil was (1.96) low compared to the maximum acceptable value of (10 meq KOH/g) set by the codex Aliment Arius Commission for groundnut seed oil. The low PV of G. cress oilindicates that it's less prone to oxidative rancidity at room temperature. The free fatty acid value (FFA) of G. cress oil (4.19) is in conformity with the specifications of edible oils (1-7% of oleic acid). FFA is used as an indication of edibility of oil and suitability to be used in the paint industry. The iodine value (IV) is a measure of average unsaturation of an oils or fats. It depends on all unsaturated components in the oil. I value of the G. cress oil was (134.46) is high than in its previous studies may be due to the oil content of unsaturated fatty acids. I value used to quantify the amount of double bonds present in the oil, which reflects the susceptibility of oil to oxidation. The saponification value (SV) of G. cress oil was (173.36) lower than that of olive oil (188-196), soybean oil (188-195), and palm oil (196-205). A high SV indicates a higher proportion of low molecular weight fatty

acids in the oil or vice versa. The unsaponifiable matter content of G. cress seeds oil was (1.16%) lower compared to the other previous studies, which showed less impurity in oil, so it could be used in industry.

Analysis by GC-MS-chromatography

Eleven compounds weredetected in G. cress seeds oil by using GC-MS analysis as shown in table (2). The mojor components are: 9,12,15-Octadecatrienoic acid, methyl ester, 9-Octadecenoic acid (Z)-, methyl ester , cis-11-Eicosenoic acid, methyl ester, 9,12-Octadecadienoic acid (Z,Z)-, methyl ester and Hexadecanoic acid, methyl ester. G. cress oil was composed of total mono-unsaturated fatty acids (44.05%), poly-unsaturated fatty acids (42.60%), and saturated fatty acid (13.35%).Fatty acids profile of G. cress oil provide an important source of Omega 3.6.9 fatty acids for food supplement and medicinal purposes on commercial scale.

Assessment of antimicrobial activities of the G. cress oil

Assessment of antimicrobial activities of G. cress seeds oil at concentration 100mg/ml was carried out against four types of bacteria two gram positive: *Bacillus subtilis* and *staphylococcus aureus* and two gram negative: *Escherichia coli* and *Pseudomonas aeruginosa* and one type of fungus *Candida albicans*. The results were shown in table (3). The extracted oil showed antibacterial activity against *Staphylococcus aureus*at concentration 100mg/ml and partial activity against *Escherichia coli* and *Candida albicans* and no antibacterial activity against *Pseudomonas aeruginosa*and *Bacillus subtilis* at the same concentration.The results were interpreted in terms of the commonly used terms (<9mm:inactive, 9-12mm:partiallyactive, 13-18mm:active).

Antioxidant activity

The principle of antioxidant activity is their interaction to produce oxidative free radicals. The role of DPPH method is that the antioxidants react with the stable free radical. During the free radical reaction, DPPH 2,2Di {(4-tert-octylphenyl)-1-picryl-hydrazyl}is converted into{2,2Di (4-tert-octylphenyl)-1-picryl-hydrazine} with color change. The rate of color change gradually decreases to indicate the scavenging potentials of the antioxidantsample.

The antioxidant activity of the *Lepidium sativum* oil was determined by DPPH method. the results were shown in table 4.DPPH method showed high antioxidant activity of extracted oil (99.03%) which is comparable with the antioxidant activity of Ascorbic acid (99.00%).

Table 1. Chemical properties of G. cress seeds oil

S.No	Analysis	Yield
1.	Oil yield (% dry weight)	19.7
2.	Peroxide value (meq KOH/g)	1.96
3.	Free Fatty Acid (% oleic)	4.19
4.	Iodine value (g of I_2 /100 g)	134.46
5.	Saponification value (mg KOH/g)	173.36
6.	Unsaponifiable matter (w/w %)	1.16

Table 2. Constituents of G. cress oil

S.No	Name	R.T	Area	Area%
1.	Hexadecanoic acid, methyl ester	16.061	1736682	7.52
2.	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	17.710	2870829	12.43
3.	9-Octadecenoic acid (Z)-, methyl ester	17.785	5338864	23.12
4.	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	17.803	6827540	29.58
5.	Methyl stearate	17.976	373782	1.62
6.	cis-11-Eicosenoic acid, methyl ester	19.526	3288766	14.24
7.	8,11,14-Eicosatrienoic acid, methyl ester, (Z,Z,Z)-	19.568	135775	0.59
8.	Eicosanoic acid, methyl ester	19.723	828578	3.59
9.	13-Docosenoic acid, methyl ester, (Z)-	21.161	1441555	6.24
10.	Docosanoic acid, methyl ester	21.342	142499	0.62
11.	15-Tetracosenoic acid, methyl ester	22.682	105025	0.45

Table 3. Antibacterial activity of G. cress oil in (mm)

Conc mg/mL	B.s	S.a	Ps.	E.c	C.a
100	0.0	13	0.0	10	10

Table 4. Antioxidant activity of G. cress oil

S.No	Sample Code	%RSA (DPPH)
1	Plant extract	99.03
2	Ascorbic acid (Standard)	99.00

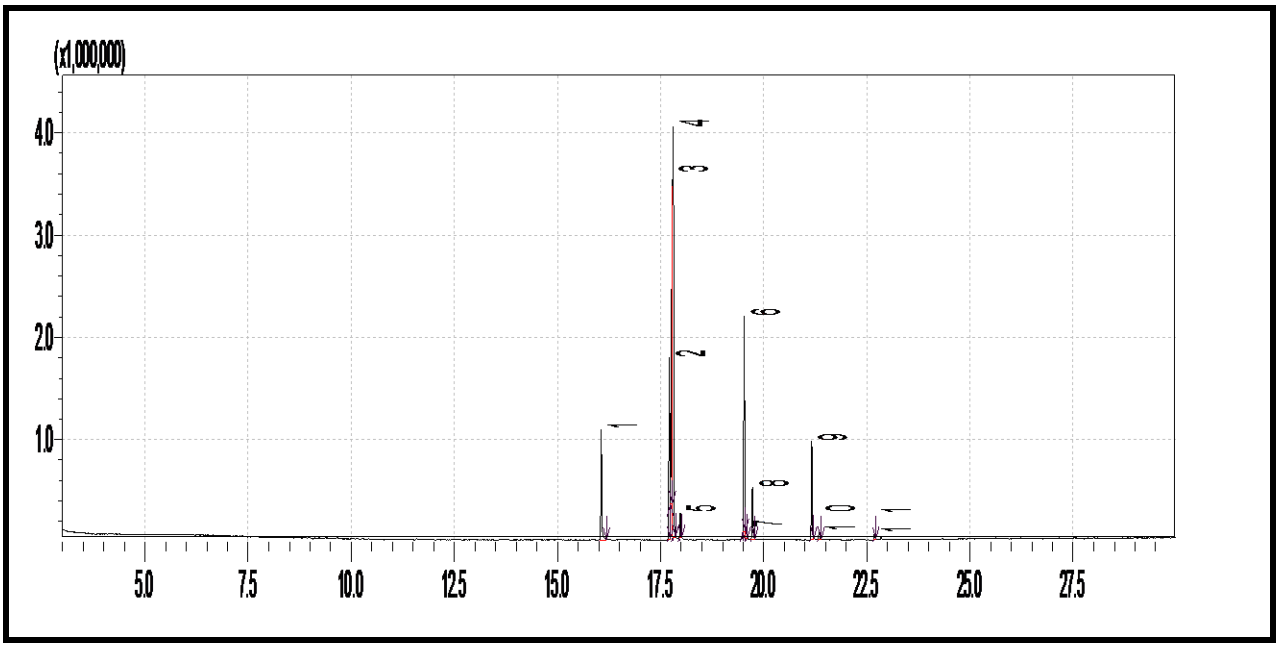

Figure 1. Gas chromatogram of G. cress seeds oil.

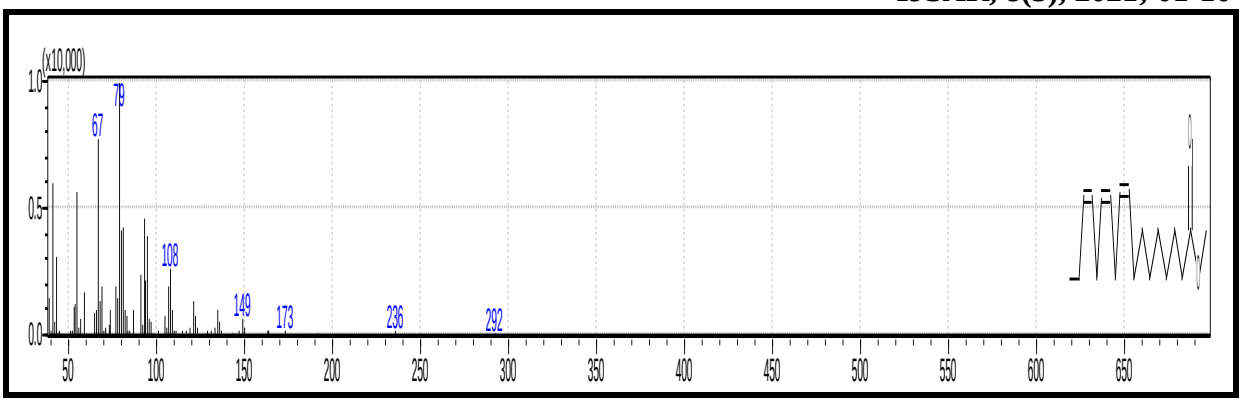

Figure 2. Mass spectrum of 9,12,15-Octadecatrienoic acid, methyl ester,(Z,Z,Z)-

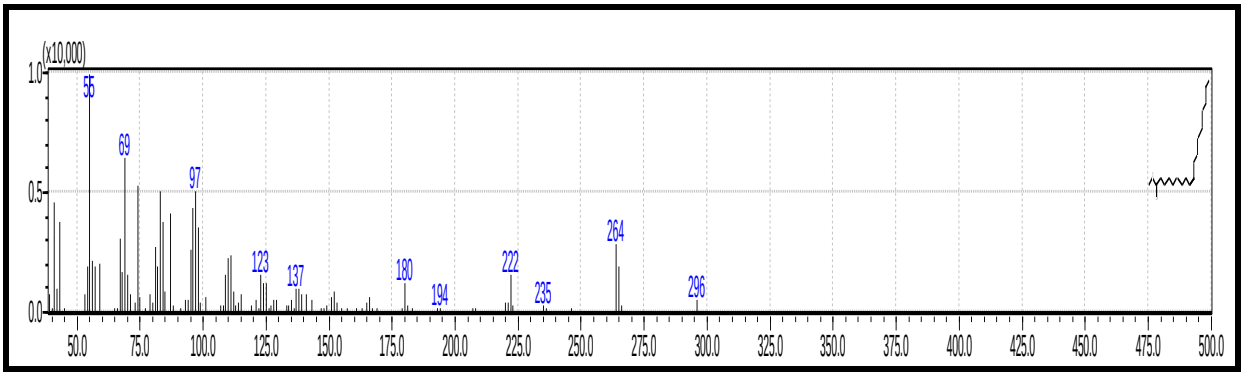

Figure 3. Mass spectrum of 9-Octadecenoic acid (Z)-, methyl ester

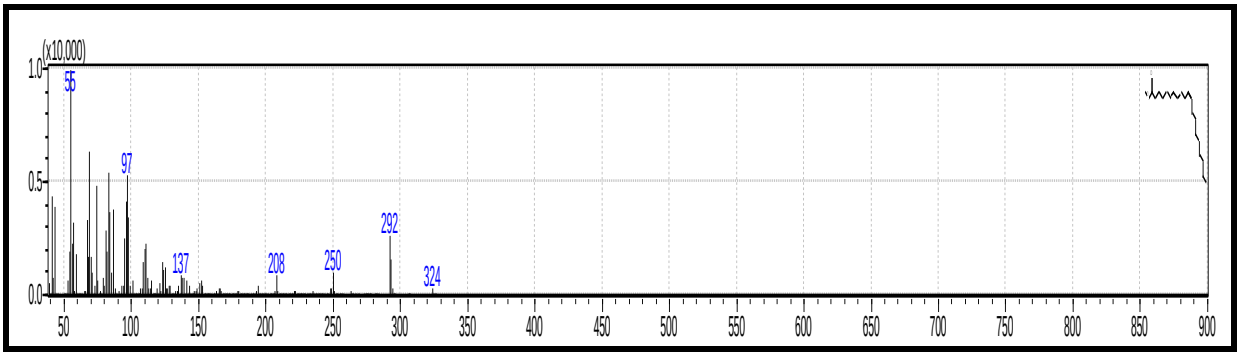

Figure 4. Mass spectrum of cis-11-Eicosenoic acid, methyl ester

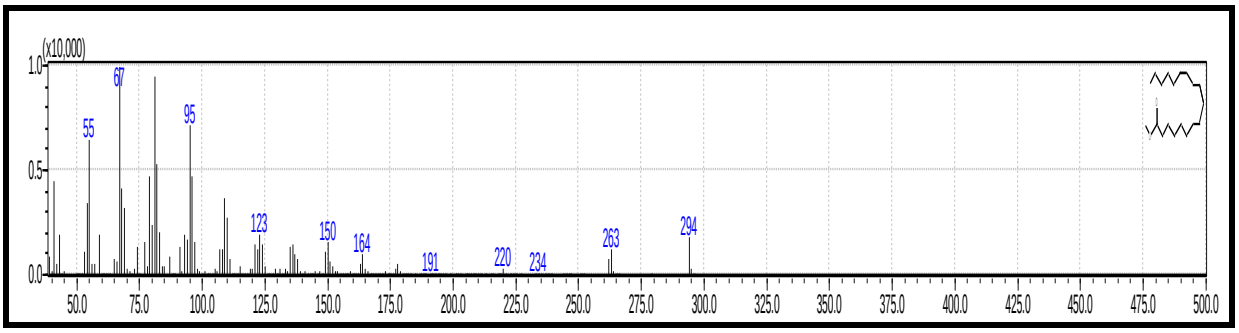

Figure 5. Mass spectrum of 9,12-Octadecadienoic acid (Z,Z)-, methyl ester

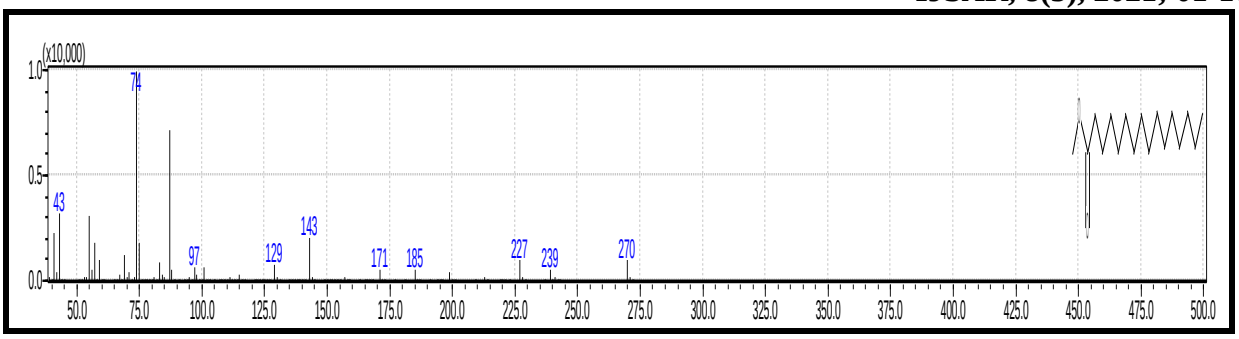

Figure 6. Mass spectrum of Hexadecanoic acid, methyl ester

Conclusion

In the present investigation, the overall results from the GC-MS analysis of *Lepidium sativum* seeds oil showed 11 components the major components are: α-Linolenic acid, Oleic acid, Eicosenoic acid, Linoleic acid and Palmitic acid. In addition, the oil has potential antimicrobial and antioxidant activity.

Acknowledgment:

Authors gratefully acknowledge the members of the University of Medical Sciences and Technology laboratories, Medicinal and Aromatic Plants Research Institute, National Center for Research, Khartoum, Sudan for their valuable help. Our thanks extended to the Deanship of Post Graduate Studies and Scientific Research, University of Kordofan.

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