Journal Homepage: - www.journalijar.com
INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)
Article DOI: 10.21474/IJAR01/3676
DOI URL: http://dx.doi.org/10.21474/IJAR01/3676
ISSN NO. 2320-5407

RESEARCH ARTICLE

GC-MS ANALYSIS AND ANTIMICROBIAL ACTIVITY OF FIXED OIL FROM SAUDI *LEPIDIUM SATIVUM* (CRUSIFEREAE) SEEDS.

Abdel Karim M.[1], Sufian A.[2], Kamal M.S.[1] and Inas O.[3].

1. Sudan University of Science and Technology, Faculty of Science, Dept. of Chemistry.
2. Taiba University,Faculty of Science, Dept. of Chemistry.
3. University of Bahri, Faculty of Applied and Industrial Sciences.

Manuscript Info

Manuscript History

Received: 12 January 2017
Final Accepted: 03 February 2017
Published: March 2017

Key words:-
Lipidium sativum, Fixed oil ,GC-MS, Antimicrobial Activity.

Abstract

In this study we planned to identify and quantify the lipid composition of Saudi *Lepidium sativum* seed and to evaluate the potential antimicrobial activity of the extracted fixed oil . GC- MS analysis of the fixed oil revealed the presence of 16 components. Major constituents are :β-amyrin(31.33%) ,9,12,15-octadecatrienoic acid methyl ester(15.97%) ,9-octadecenoic acid methyl ester(11.93%),α-amyrin(9.32%) , 11-eicosenoic acid methyl ester (6.64%), 9,12–octadecadienoic acid (6.03%) , hexadecanoic acid methyl ester(5.24%) . In cup plate agar diffusion bioassay, the oil which was extracted by two different methods (soxhlet and maceration) was evaluated for antimicrobial activity and the effect of .the method of extraction on the the antimicrobial potency was investigated.

Introduction:-

Lepidium sativum (Crusifereae) is an annual plant about 50cm in height. The plant can grow easily tolerating difficult environmental conditions(Sharma and Agrawal ,2011; Wadhwal et.al. ,2012) . It has many branches on the upper parts and white-pinkish flowers(Derek ,1997; Boswell and Sowerby,1863). The plant is genetically related to mustard and watercress and is known in some regions as garden cress, garden pepper cress,pepperwort, pepper grass(Cassidy and Hall ,2002; Staub an Bduchert,2008).

Lepidium sativum L. contains significant amount of iron , calcium , folic acid beside vitamins A and C . It also contains protein(25%) ; leucine (8.21%); glutamic acid(19.3%) and methionin(0.97%) . Seeds mainly contain alkaloids, calcium, iron, carotene, riboflavan, uric acid, phosphorus, thiamine and niacin. Seed oil contains palmitic, linoleic, stearic, behenic, oleic , arachidic and lignceric acids(Hiba and Wasfeih,2014) .

Lepidium sativum is a key species in African system of medicine where it is mainly used against bronchitis. In some Asian communities the plant is used against a wide array of human disorders(Baquar,1989; -Duke et.al,2002).It was reported that garden cress possesses anti-inflammatory, analgesic, anticoagulant(Al-Yahya,1994),diuretic(Patel et.al,2009), antihypertensive (Maghrani et. al.,2005) ,antirheumatic (Ahsan et.al.1989) ,antidiarrheal,antispasmodic, laxative(Rehman et.al.,2011) and hypoglycemic(Patole,1998) properties.

Aqueous extracts of seeds exhibited significant water excretion in spontaneously hypotensive models without any significant change in heart rate.. The petroleum ether extract of seeds showed antimicrobial activity against some

Corresponding Author:- Abdel Karim M.
Address:- Sudan University of Science and Technology, Faculty of Science, Dept. of Chemistry.

standard human pathogens, while oral administration of seeds proved a hypoglycemic effect(Hiba and Wasfeih ,2014).

It was reported that *Lepidium sativum* juice possess chemoprotective effect(Hiba and Wasfeih ,2014).The aqueous methanolic extract of seeds showed important improvement in various parameters of pulmonary function in a clinical experiment(Najeeb-Ur–Rehman et.al.,2012). In another clinical study , the seeds were evaluated for the management of osteoarthritis. Seeds showed considerable relief of joints pain , swelling, stiffness and other symptons associated with osteoarthritis(Nita and pandye,2009).

It seems that *Lepidium sativum* seeds are well tolerated . In clinical experiments of the effect of seed administration on bronchial asthma, none of the test subjects showed the presence of adverse effects or any other problems physically or at hematological profile(www.bioline.org.). Feeding model animals with *Lipidium sativum* seeds (2%w/w) for six weeks was non-toxic , while a dose of 50%(w/w) was lethal(www.worldscientific.com).

Materials and Methods:-

Materials:-

Plant material:-

Lepidium sativum seeds were purchased from the local market-Ryad-Saudi Arabia and authenticated by direct comparison with a herbarium sample.

Instruments:-

A Shimadzo GC-MS-QP2010 Ultra instrument with a RTX-5MS column (30m,length ; 0.25mm diameter ; 0.25 µm, thickness)was used**.**

Methods:-

Extraction of oil:-

Powdered *Lepidium sativum* seeds (400g) were exhaustively extracted with n-hexane (soxhlet).The solvent was removed under reduced pressure and the oil was kept in the fridge at 4^{o}C for further manupulation.Another seed sample (400g) was exhaustively macerated with n-hexane.

Esterification of oil:-

A Methanolic solution of sodium hydroxide was prepared by dissolving (2g) of sodium hydroxide in (100ml) methanol.A stock solution of methanolic sulphuric acid was prepared by mixing (1ml) of concentrated sulphuric acid with(99ml) methanol.

The oil(2ml) was placed in a test tube and (7ml) of alcoholic sodium hydroxide were added followed by(7ml) of alcoholic sulphuric acid. The tube was stoppered and shaked vigorously for five minutes and then left overnight.(2ml) of supersaturated sodium chloride were added,then (2ml) of normal hexane were added and the tube was vigorously shaked for five minutes. The hexane layer was then separated.(5µl) of the hexane extract were mixed with (5ml) diethyl ether . The solution was filtered and the filtrate(1µl) was injected in the GC-MS vial.

GC-MS analysis:-

Lepidium sativum oil was analyzed by gas chromatography – mass spectrometry. A Shimadzo GC-MS-QP2010 Ultra instrument with a RTX-5MS column (30m,length ; 0.25mm diameter ; 0.25 µm, thickness)was used. Helium (purity; 99.99 %) was used as carrier gas. Oven temperature program is given in Table 1, while other chromatographic conditions are depicted in Table 2.

Table 1:- Oven temperature program.

Rate	Temperature (^{o}C)	Hold time (min^{-1})
-	150.0	1.00
4.00	300.0	0.00

Table 2:- Chromatographic conditions.

Column oven temperature	150.0°C
Injection temperature	300.0°C
Injection mode	Split
Flow control mode	Linear velocity
Pressure	139.3KPa
Total flow	50.0ml/ min
Column flow	1.54ml/sec.
Linear velocity	47.2cm/sec.
Purge flow	3.0ml/min.
Spilt ratio	- 1.0

Antimicrobial Assay:-

Preparation of bacterial suspensions:-

One ml aliquots of 24 hours broth culture of the test organisms were aseptically distributed onto nutrient agar slopes and incubated at 37°C for 24 hours.

The bacterial growth was harvested and washed off with sterile normal saline, and finally suspended in 100 ml of normal saline to produce a suspension containing about 108-109colony forming units per ml. The suspension was stored in the refrigerator at 4°C until used. The average number of viable organism per ml of the stock suspension was determined by means of the surface viable counting technique.

Serial dilutions of the stock suspension were made in sterile normal saline in tubes and one drop volumes (0.02 ml) of the appropriate dilutions were transferred by adjustable volume micropipette onto the surface of dry nutrient agar plates. The plates were allowed to stand for two hours at room temperature for the drop to dry, and then incubated at 37°C for 24 hours.

Preparation of fungal suspensions:-

Fungal cultures were maintained on dextrose agar incubated at 25°C for four days. The fungal growth was harvested and washed with sterile normal saline, and the suspension was stored in the refrigerator until used.

Testing for antibacterial activity:-

The cup-plate agar diffusion method was adopted , with some minor modifications, to assess the antibacterial activity. (2ml) of the standardized bacterial stock suspension were mixed with 200 ml of sterile molten nutrient agar which was maintained at 45°C in a water bath. (20 ml) Aliquots of the incubated nutrient agar were distributed into sterile Petri dishes. The agar was left to settle and in each of these plates which were divided into two halves, two cups in each half (10 mm in diameter) were cut using sterile cork borer (No 4), each one of the halves was designed for one of the test solutions. Separate Petri dishes were designed for standard antibacterial chemotherapeutics.

The agar discs were removed, alternate cups were filled with 0.1 ml samples of each test solution using adjustable volume microtiter pipette and allowed to diffuse at room temperature for two hours. The plates were then incubated in the upright position at 37°C for 24 hours. The above procedure was repeated for different concentrations of the test solutions and the standard chemotherapeutics. After incubation, the diameters of the resultant growth inhibition zones were measured in triplicates and averaged.

Results and Discussion:-

GC-MS analysis of *Lepidium sativum* seed oil:-

The GC-MS analysis of *Lepidium sativum* seed oil was conducted and the identification of the constituents was accomplished by comparison with the MS library (NIST) and further confirmed by the observed fragmentation pattern. 80-95% match was observed.

Identification of oil constituents:-

The GC-MS spectrum of the studied oil revealed the presence of 16 components(Table 3).The typical total ion chromatogram(TIC) of the hexane extract is shown in Fig.1.

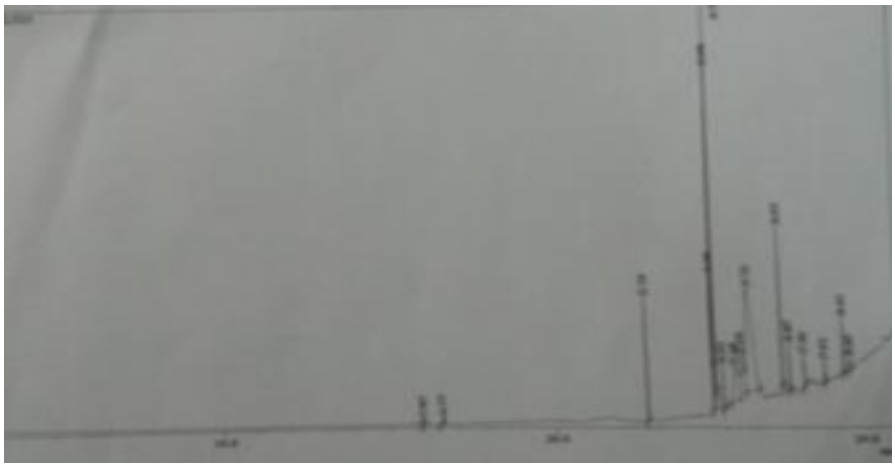

Fig.1:- Total ion chromatograms of hexane extract.

Table 3:- Consituents of *Lepidium sativum* seed oil.

Peak#	R.Time	Area	Area%	Name
1	15.967	103007	0.31	1s,4R,7R,11R-1,3,4,7-Tetramethyltricyclo[
2	16.577	139023	0.42	Butylated Hydroxytoluene
3	22.720	1720641	5.24	Hexadecanoic acid, methyl ester
4	24.640	1978338	6.03	9,12-Octadecadienoic acid (Z,Z)-, methyl e
5	24.696	3914945	11.93	9-Octadecenoic acid (Z)-, methyl ester
6	24.720	5239919	15.97	9,12,15-Octadecatrienoic acid, methyl este
7	24.935	573522	1.75	Methyl stearate
8	25.268	3059739	9.32	.alpha.-Amyrin
9	25.479	826694	2.52	Urs-12-en-24-oic acid, 3-oxo-, methyl ester
10	25.723	10281524	31.33	.beta.-Amyrin
11	26.655	2179660	6.64	11-Eicosenoic acid, methyl ester
12	26.867	633944	1.93	Eicosanoic acid, methyl ester
13	27.282	761783	2.32	9-Octadecenamide, (Z)-
14	27.872	315933	0.96	Phenol, 2,2'-methylenebis[6-(1,1-dimethyl
15	28.423	865133	2.64	13-Docosenoic acid, methyl ester
16	28.605	225495	0.69	Docosanoic acid, methyl ester
		32819300	100.00	

The following major constituents were detected:

β-Amyrine(31.33%):-

The EI mass spectrum of β-amyrine is shown in Fig. 2.The peak at m/z426, which appeared at R.T.25.723 in total ion chromatogram, corresponds $M^+[C_{30}H_{50}O]^+$ .The peak at m/z411 corresponds to loss of a methyl function.

9,12,15-Octadecatrienoic acid (15.97%):-

The EI mass spectrum of 9,12,15-octadecatrienoic acid (as methyl ester)is shown in Fig. 3.The peak at m/z 292, which appeared at R.T.24.720 in total ion chromatogram, corresponds $M^+[C_{19}H_{32}O_2]^+$ .The peak at m/z277 corresponds to loss of a methyl function.

9-Octadecenoic acid methyl ester(11.93%):-

The EI mass spectrum of 9-octadecanoic acid methyl ester is shown in Fig. 4.The peak at m/z 296 , which appeared at R.T.24.696 in total ion chromatogram, corresponds M^+ $[C_{19}H_{36}O_2]^+$ .The peak at m/z 264 corresponds to loss of a methoxyl function.

α-Amyrine(9.32%):-

The mass spectrum of α-amyrine is displayed in Fig. 5.The peak at m/z426, which appeared at R.T.25.268 in total ion chromatogram, corresponds $M^+[C_{30}H_{50}O]^+$ .The peak at m/z411 corresponds to loss of a methyl function.

11-Eicosenoic acid(6.64%):-
The mass spectrum of 11-eicosenoic acid is shown in Fig. 6.The peak at m/z324 (R.T.26.655) corresponds $M^+[C_{21}H_{40}O_2]^+$.The signal at m/z292 is due to loss of a methoxyl function.

9,12-Octadecadienoic acid methyl ester(6.03%):-
The EI mass spectrum of 9,12-octadecadienoic acid methyl ester is depicted in Fig. 7. The peak at m/z294 , which appeared at R.T. 24.640 , corresponds $M^+ [C_{19}H_{34}O_2]^+$.The signal at m/z 263 accounts for loss of a methoxyl group.

Hexadecanoic acid(5.24%):-
Mass spectrum of hexadecanoic acid methyl ester is shown in Fig.8.The peak at m/z 270 (R.T. 22.720) corresponds $M^+ [C_{17}H_{34}O_2]^+$.The signal at m/z 239 corresponds to loss of a methoxyl function.

13-Docosenoic acid(2.64%):-
The EI mass spectrum of 13-docosenoic acid is displayed in Fig. 9. The peak at m/z352, which appeared at R.T.28.423 in total ion chromatogram, corresponds $M^+[C_{23}H_{44}O_2]^+$.The signal at m/z320 corresponds to loss of a methoxyl group.

9-Octadecenamide(2.32%):-
The mass spectrum of 9-octadecenamide is shown in Fig.10.The peak at m/z281, which appeared at R.T.27.282 , corresponds $M^+[C_{18}H_{35}NO]^+$.The peak at m/z238 corresponds to loss of acetyl function.

Eicosenoic acid(1.93%):-
The EI mass spectrum of eicosenoic acid is depicted in Fig. 11.The peak at m/z326, which appeared at R.T.26.867 in total ion chromatogram, corresponds $M^+[C_{21}H_{42}O_2]^+$.The peak at m/z295 is due to loss of a methoxyl group.

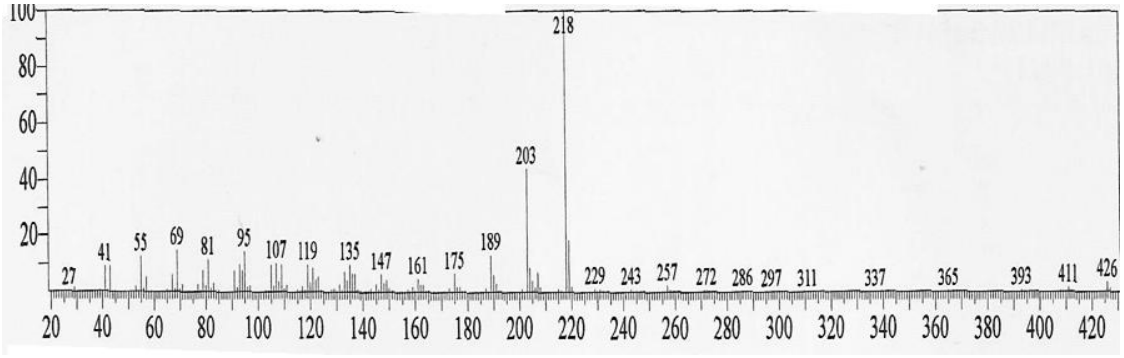

Fig 2:- Mass spectrum of β-amyrine.

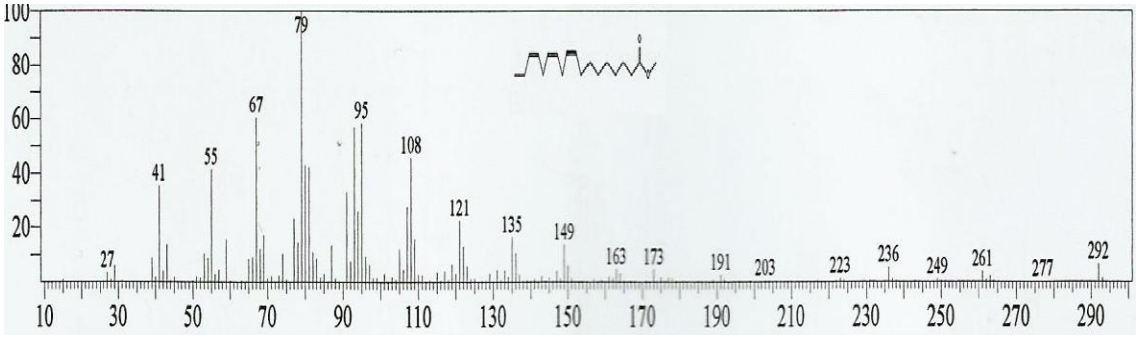

Fig 3:- Mass spectrum of 9,12,15-octadecatrienoic acid methyl ester.

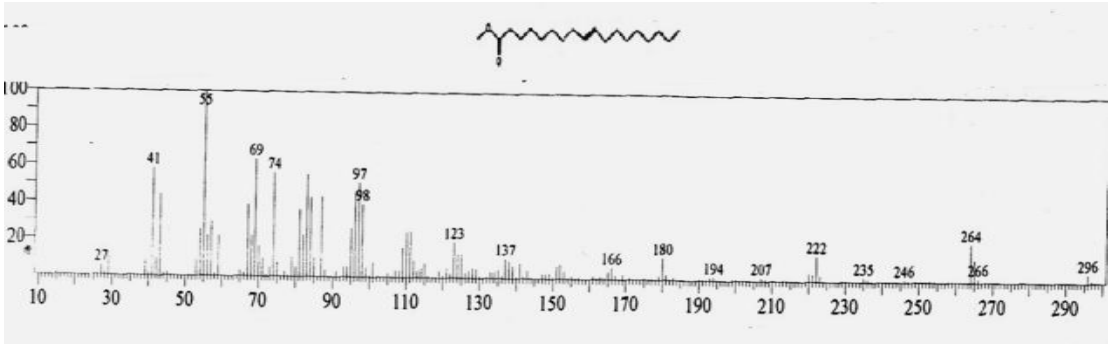

Fig 4:- Mass spectrum of 9-octadecanoic acid methyl ester.

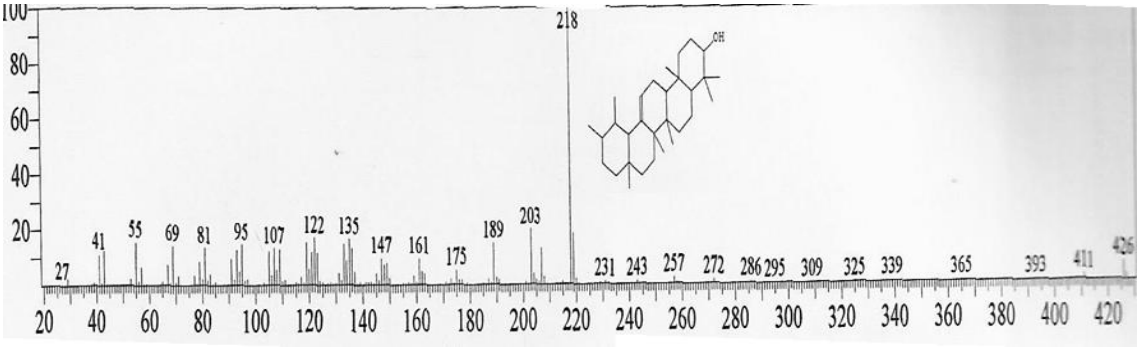

Fig 5:- Mass spectrum of α-amyrine.

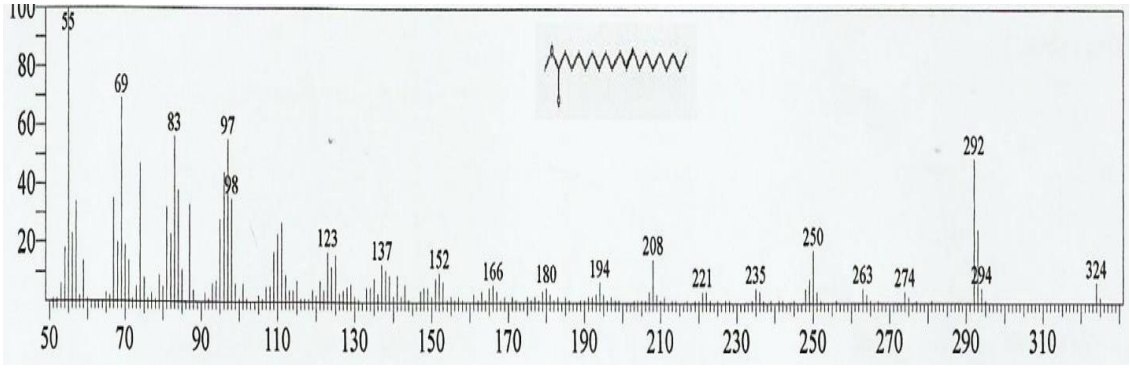

Fig 6:- Mass spectrum of 11-eicosenoic acid.

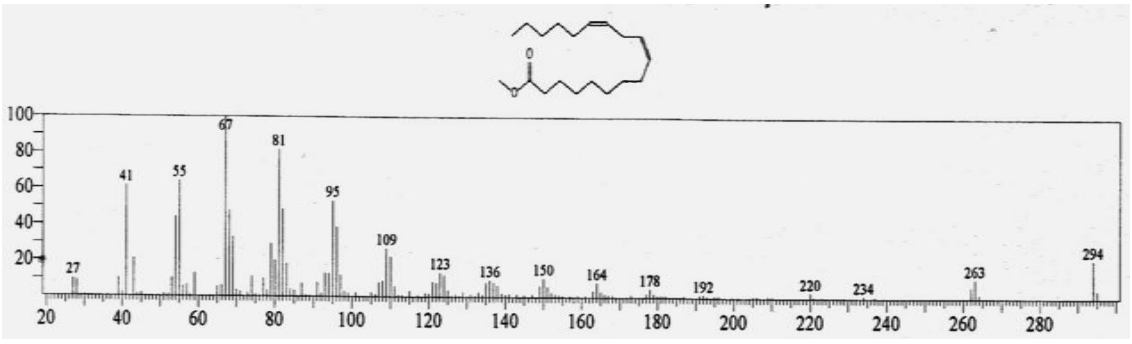

Fig 7:- Mass spectrum of 9,12-octadecadienoic acid methyl ester.

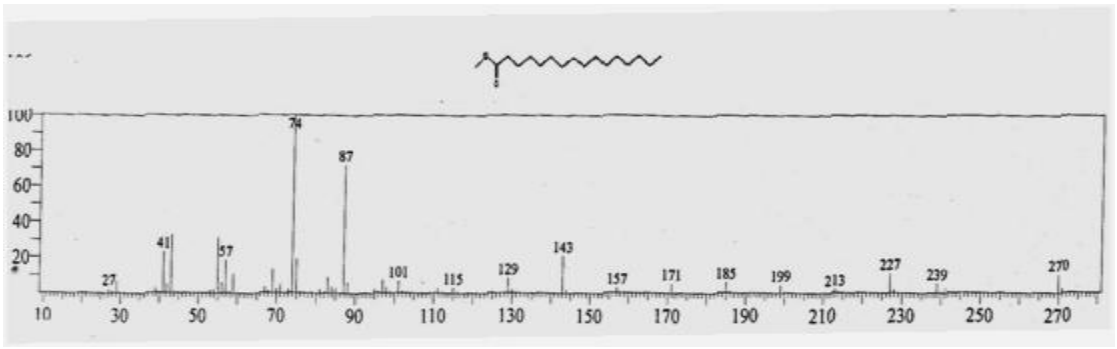

Fig 8:- Mass spectrum of hexadecanoic acid methyl ester.

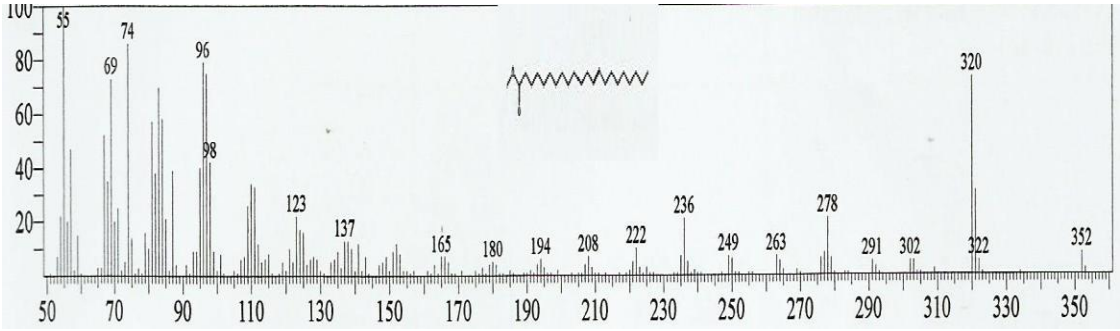

Fig 9:- Mass spectrum of 13-docosenoic acid.

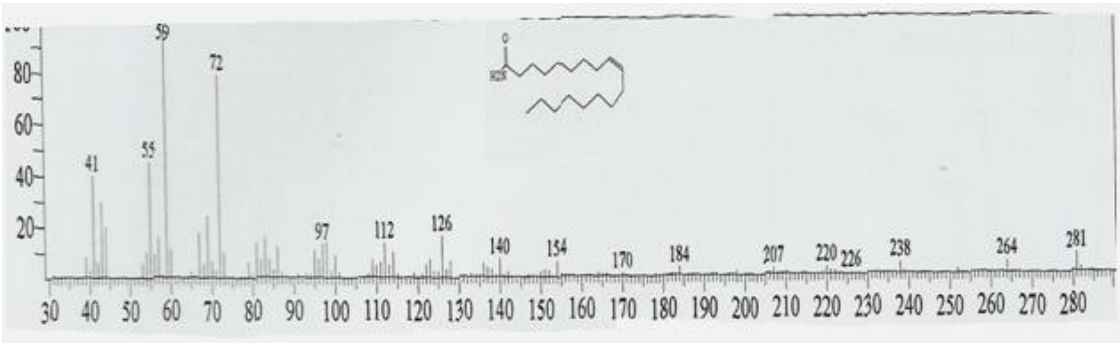

Fig 10:- Mass spectrum of 9-octadecenamide.

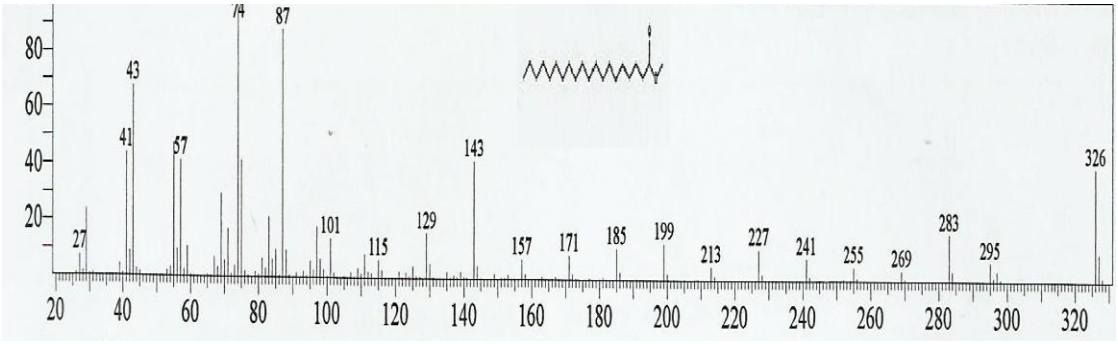

Fig 11:- Mass spectrum of eicosenoic acid methyl ester.

Antimicrobial assay:-

Lepidium sativum seed oil was extracted by two different methods (A; soxhlet and B ; maceration) to evaluate the effect of heating(soxhlet) on the antimicrobial potency of the extracted oil. Table (4)shows that the oil extracted by maceration is partially active against *Escherichia coli* at concentrations of 10 and 50 µg/ml , while the soxhlet

sample was devoid of activity at these concentrations. At 50 µg/ml both samples were partially active against *Klebsiella pneumonia.* Furthermore,the soxhlet sample exhibited partial activity against *Bacillus subtilis* at a concentrations of 10 and 50µg/ml while the macerated sample was inactive at these concentrations .However, both samples were inactive against *Staphylococcus aureus* at test concentrations. It seems that the potency of such extracts depends largely on the type of test organism and the concentration of the sample. Similar trends were observed for the minimum inhibition concentration(Table 5).

Table 4:- Minimum Inhibition Zone (mm)

		A			B			Amp	Kan	Strip		Nys
Gram	Strain	5	10	50	5	10	50	5	10	10	10	10
-ve	Escherichia coli	-	-	-	-	10±0.4	12±0.3	10±0.4	15±0.4	19±0.3	25±0.4	0
-ve	*Klebsiella pneumoniae*	-	9±0.4	10±0.4	7±0.0	8±0.3	10±0.4	9±0.5	10±0.4	19±0.0	26±0.4	0
-ve	Acinetobacter baumannii	-	10±0.5	11±0.4	8±0.0	10±0.4	10±0.7	-	12±0.3	19±0.3	12±0.3	0
-ve	Pseudomonas aureginosa	-	-	-	-	7±0.4	8±0.4	-	12±0.3	14±0.3	20±0.5	0
+ve	*Staphylococcus aureus*	-	-	-	-	-	-	-	15±0.5	17±0.5	15±0.5	0
+ve	Bacillus subtilis	-	11±0.4	11±0.3	-	-	-	9±0.0	13±0.4	19±0.4	30±0.4	0
C	Candida albicans	7±0.0	7±0.4	7±0.5	7±0.4	9±0.3	11±0.6	8±0.4	N	16±0.5	N	12±0.5
F	Aspergillus flavus	7±0.0	9±0.4	11±0.4	-	8±0.0	8±0.3	-	N	15±0.5	N	15±0.5

-ve: gram negative, +ve: gram positive, C: colony forming, F: filamentous, -: no activity.

Table 5:- Minimum inhibition concentration(µg/ml).

		A			B			Amp.	Kan.	Strip.	Nys.
Gram	Strain	100	250	500	100	250	500	10	10	10	10
-ve	Escherichia coli	R	R	R	R	R	I	S	S	S	N
-ve	Klebsiella pneumoniae	R	R	R	R	R	R	S	S	S	N
-ve	Acinetobacter baumannii	R	R	R	R	R	I	S	S	S	N
-ve	Pseudomonas aureginosa	R	R	R	R	R	R	S	S	S	N
+ve	Staphylococcus aureus	R	R	R	R	R	R	S	S	S	N
+ve	Bacillus subtilis	R	R	I	R	R	R	S	S	S	N
C	Candida albicans	R	R	R	R	R	I	N	S	N	S
F	Aspergillus flavus	R	R	I	R	R	R	N	S	N	S

-ve: gram negative, +ve: gram positive, C: colony forming, F: filamentous,

References:-

1. **Sharma**,S. and Agrawal,A.(2011),*Indian Journal of Natural Products and Resources*,292.
2. **Wadhwal**,S.,Panwarl,M.S.,Agrawal,A., Sain,N., Patidar,L.N.(2012) , *ARPB* ,2(IV).
3. **Derek**,B., " Vegetables of Canada " , NRC Research Press , Canada(1997).
4. **Boswell**,J.T. and Sowerby,J.(1863), " English Botany" , Published by Robert Hardwicke, P215.
5. **Cassidy**,F.G. and Hall,J.H.(2002) " Dictionary of Amerrican Regional English " , Harvard University Press, P97.
6. **Staub**,J.E., Buchert,E.(2008) " Exceptional Herbs for Your Garden " , Published by Gibbs Smith, UK.
7. **Hiba**,F. and Wasfeih,N.,A.(2014) , Review Article –Lepidium sativum – See at: https://www.researchgate.net/publications/262914046.
8. **Baquar**,S.R. "Medicinal and Poisonous Plants of Pakistan" , Printas Karachi, Pakistan, (1989).
9. **Duke**,J.A., M. J. Bogenschutz-Godwin,M.J., J. Ducelliar,J and P. A. K. Duke,P.A.K, " Handbook of Medicinal Herbs", CRC Press, Boca Raton, USA(2002).
10. **Al-Yahya**,M.A., Mossa,J.S.,Ageel,A.M., and Rafatullah,S.(1994) *Phytomedicine*,**1**,155.
11. **Patel**,U. Kulkarni,M., Undale,V. and Bhosale,A.(2009), *Tropical Journal of Pharmaceutical Research*, **8**(3), 215.
12. **Maghran**i,M., Zeggwagh,N.A., Michel,J.B. and Eddouks, M. ,(2005) ,. *Journal of Ethnopharmacology***,100**(1-2), 193.
13. **Ahsan**,S.K., M. Tariq, M. , Ageel,M., Al-Yahya,A. and A. H. Shah,A.H.(1989), *International Journal of Crude Drug Research*, **27**(4) 235.
14. **Rehman**,N.,Mehmood,M.H.,Alkharfy,K.M. and Gilani,A.H.(2011), *Journal of Ethnopharmacology*, **134**, 878 .
15. **Patole**,A.P.(1998) , *Journal of Medicinal and Aromatic Plant Sciences*, **20**, 1005.
16. **Najeeb**-Ur–Rehman,Arif-Ullah K.,Khalid,M. and Anwarul-Hassan,G." Evidence-Based Complementary and Alternative Medicine",Vol.2012(2012).
17. **Nita,**D., pandye,T.N.(2009), *AYA*,**30**(2),15.
18. http://www.bioline.org.br/pdt?pt06009.
19. http://www.worldscientific.com/doi/abs/10.1142/s0192415x99000458.