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Free Radical Scavenging Capacity of Extract and Chitosan Nanoparticles loaded with Lepidium Sativum Seeds and Analysis of Bioactive Compounds using HPLC and GC-Mass

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A phytochemical analysis of Lepidium sativum (LS) or garden cress (GC) showed that it included fatty acids, lipids, alkaloids, phenolic compounds, and flavonoids, which are considered biologically active compounds.
Plant samples were taken in Iraq's Hilla City. Using ethanol, the plant extract was extracted from the seeds. Using Folin-Ciocalteu's reagent, the total polyphenol content (TPC) of the extract was identified; using aluminum chloride, the total flavonoid content (TFC) of the extract was found. The in vitro antioxidant for LS extract and nanoparticles (Cs NPs) were determined using DPPH (2,2-diphenyl-1-picrylhydrazyl). HPLC and Gas chromatography and mass spectrometry (GC-MS) analysis were used to detect bioactive compounds.
In LS ethanol extract the highest value was TPC (85.56 mg GAE/100 mg of extract), whereas TFC content was lower (44.58 mg QE/100 g of extract). Chitosan LS nanoparticles (Cs LS NPs) came in second (81.79 ± 6.09%), and LS ethanol extract displayed the greatest percentage of DPPH radical scavenging activity (85.42 5.63%). Compared to ascorbic acid, which has an antioxidant capacity of 122.67 mg/ml, the IC50 values for ethanolic LS extract and Cs LS NPs were substantially higher at 36.27 mg/ml and 52.22 mg/ml, respectively. The findings of the Gas Chromatography-Mass Spectrometry (GC/MS) analysis show various peaks indicating the presence of 31 phytochemical components in seed extract, the peak area of the principal phyto components being 9,2 octadecadienoic acid (30.93%), oleic acid (23.22%), benzenacetonitrile (9.60%), and 1n-Hexadecenoic (9.56%). By using High-Performance Layer Chromatography (HPLC), eugenol from the ethanolic extract of LS seeds was discovered and measured. According to HPLC analysis, the eugenol was

separated on a layer of silica gel and found to have about 362.8 ppm in ethanolic LS extract seeds and 106.0 ppm in Cs LS NPs. Conclusion: These studies will aid in increasing public awareness of LS and offer guidance for future investigations. Additionally, they could help create novel formulations with greater medicinal potential.

Keywords: antioxidant activity; GC-MS analysis; HPLC; Lepidium sativumL; bioactive compounds.

1. Introduction

Lepidium sativum (LS) or garden cress (GC) is considered a nutritional supplement because it contains a variety of micro- and macronutrients, vitamins, and minerals (calcium, iron, and phosphorus (Jagdale et al.,2021). The genus Lepidium, of the Brassicaceae family contains over 3500 species and 370 genera and prefers warm climates, The Mediterranean region is where this family is most found, and it is also found in western and central Asia and parts of North America (Oraby, 2020); (Jonsell et al., 2000). Medicinal plants are boundless reservoirs of molecules possessing diverse biological and pharmacological attributes to treat various illnesses (Chauhan et al., 2012). The LS seeds are rich in important minerals (potassium, phosphorus, calcium, and iron), alkaloids (glucotropaeolin, lepidine, N, N'-dibenzyl urea), fatty acids (oleic and linolenic acids), carotenoids, and phytosterols (sitosterol, campesterol, and avenasterol) (Baregama & Goyal, 2019). ascorbic acid, and tocopherols (Alshammari et al., 2017). riboflavin, glucosinolates (glucotropaeolin and 2-phenyl ethyl glucosinolates) (Jain & Grover, 2018). LS has been demonstrated to have medical benefits, such as improving lactation and boosting libido, and responsiveness [Ranade & Mudgalkar, 2021); (Jabeen et al., 2017). and is found to have antiovulatory (Satyavati, 1984) and antifertility (Falana et al., 2014). After being tested in a variety of solvents, including acetone, glacial acetic acid, petroleum ether, ethanol, chloroform, methanol, benzene, hexane, propanol, and ethyl acetate, the antioxidant activity of LS seed extracts was measured using the reducing power assay. The LS seed ethanol extract was shown to have the highest antioxidant activity (Bansal et al., 2012). According to Kumar et al. (2020), there are significant amounts of flavonoids and phenol compounds in LS seeds. Research utilizing GC/MS for the analysis of medicinal plants has increased significantly. This method has been successfully used to examine volatile essential oils, fatty acids, lipids, and alkaloids, as well as non-polar components (Hussein et al .,2017). Lepidium sativum ethanol extract's bioactive components from the whole plant identified by the GC/MS report revealed the existence of benzyl, oleoyl chloride, cis-9-Hexadecenal, and 3',5'-Dimethoxyacetophenone (7.93%) gamma. -Sitosterol (7.39%) Ethyl (9Z, 12Z): 9,12-Octadecadienoate, n-Hexadecanoic acid (4.19%) gamma. -Tocopherol (4.01%) Benzen,e, (isothiocyanatomethyl)- (3.89%) and ErgostS-5-EN-3-OL, (3.Beta.,24R)- (2.23%), and also the minor compounds were hexadecanoic acid, ethyl ester (1.87%), fumaric acid, 2-dimethylaminoethyl nonyl ester (1.30%), hexadecanoic acid, 2-hydroxyl-1-(hydroxymethyl) ethyl ester (1.14%), and stigmata-5-24 (28)- Dien-3-OL, (3. Beta), respectively (Baregama & Goyal, 2019) ; (Malar et al., 2018).

2. Material and Methods:

2.1 Plant Material:

The Lepidium sativum seeds were recognized by Dr. Nidaa Abu-Serag Professor of Plant Taxonomy, Biological Department, College of Science, Babylon University. They were bought from a local shop in Hilla City, Iraq.

2.2 Plant Sample Preparation:

By Kamani et al. 2017, a procedure of ethanolic extraction of LS seeds was described. The LS seeds were bought from a shop in Hilla City, Iraq. 500 g of garden cress (GC) seeds were finely ground and completely mixed with 70% ethanol for 24 hours to produce the alcoholic garden cress (GC) seed extract. For 30 to 60 minutes, the covered beaker was continuously stirred. The mixture was filtered, ethanol was added, and it was allowed to rest for a further 12 hours before filtering once more. To dry out the finished product, it was baked at 50 °c. Solutions containing 100, 200, 300, and 400 mg/kg of distilled water were created after the dried extract was weighed.

2.3.Synthesis of chitosan- Cs- plant extract adduct:

according to (Abd El-Ghaffar & Hashem, 2010);(Abd El-Ghaffar& Hashem, 2009), the following procedures were followed to produce the chitosan nanoparticles for the LS seeds.

2.4.Determination of total phenolic compounds:

Total polyphenol content (TPC) was estimated using Folin-Ciocalteu's reagent procedure (Laouini & Ouahrani, 2017). The ethanolic extract's total amount of phenolic components was calculated by measuring the absorbance at 765 nm and using a calibration curve established with gallic acid (Sigma-Aldrich, Germany). Gallic acid equivalent (GAE) is used to express the total amount of phenolic compounds per gram of dry weight.

2.5.Determination of total flavonoid compounds:

The total flavonoid content of crude extract has been determined using the aluminium chloride colourimetric method (Habibatni et al., 2017). A calibration curve was used to determine the total flavonoid content, which later became known as mg rutin equivalent per g of dry weight.

2.6.Biologically Active Compounds assay by HPLC:

A C18-ODS column (250 4.6 mm, 5 m) was used in the high-performance liquid chromatography (HPLC) examination, which was carried out using a SYKAMN HPLC system (Germany). Phenolic chemicals were found using a UV-visible detector at 278 nm Ngamsuk et al., 2019).)

2.7.Determination of DPPH radical scavenging activity:

The procedure outlined by Brand-Williams et al. (1995). was used to test the DPPH radical scavenging activity. Using a UV-VIS spectrophotometer, the colour (from deep violet to bright yellow) changed after 100 minutes of reaction and was measured at 517 nm. As a control, 3.3 mL of ethanol and 0.5 mL of the sample are combined. The control solution was created by combining DPPH radical solution (0.3 mL) with ethanol (3.5 mL). The proportion of scavenging activity (AA%) was calculated according to Mensor et al. (2001).

The DPPH scavenging effect percent was calculated by using the following equation: DPPH scavenging effect (%) or Percent inhibition = A0 - A 1 / A0 × 100. Where A0 was the Absorbance of the control reaction and A1 was the Absorbance in the presence of a test or standard sample.

Calculation of IC50 concentration The concentration of extract corresponding to 50 percent inhibition (IC50) was calculated with the help of the curve of DPPH percentage against the concentration of extract. In triplications of concentrations, each sample was ascorbic acid was taken as standard.

2.8.Identification of the compounds in the extracts by GC-MS:

The bioactive compounds in the ethanol extract made from LS seeds were discovered using gas chromatography, and they were identified using mass spectrometry. The mass spectrum was interpreted using the "National Institute of Standards and Technology" (NIST, USA) database. In the database, the composition of the more than 62,000 known compound patterns was given as a percentage based on peak concentration. Bioactive compounds were identified based on GC retention times and by matching the spectra with standard values using computer software (Martins et al., 2001).

3. Results:

3.1. plant extracts have amounts of total polyphenol and flavonoids:

The equivalent of gallic acid on the dry extract weight was used to calculate the overall concentration of phenolic compounds. The result was 85.56 mg GAE/100 gm Table (1). Total flavonoid amounts of the extract were expressed as mg/100gm quercetin equivalent. The result was 44.58 mg quercetin/100gm. The ethanol extract of LS had higher phenolic contents than the flavonoid contents.

Table 1: Total phenolic compounds and total flavonoid constituents of ethanolic LS extract

No	Name	Con (mg / 100 gm)
1	Total phenolic content	85.56
2	Total flavonoid content	44.58

3.2. Plant extracts showed significant antioxidant activity by reducing DPPH radical :

The antioxidant activity of the extract was assessed using the DPPH free-radical scavenging experiment. (Table 2) displays the mean percentage of DPPH free-radical scavenging activity at various extract concentrations. The highest level of DPPH free-radical scavenging activity was found in the LS (85.42%) and Cs LS NPs (81.79%). IC50 value, or the quantity needed to scavenge 50% of DPPH free radicals antioxidant capacities of LS extract and Cs LS NPs 36.27 mg/ml, and 52.22 mg/ml respectively. The IC50 values of extracts were statistically different from the ascorbic acid (AA), whose IC50 value was 122.67 mg/ml (Fig. 1). The ethanolic LS extract showed the lowest IC50 value, which indicated its strongest antiradical activity.

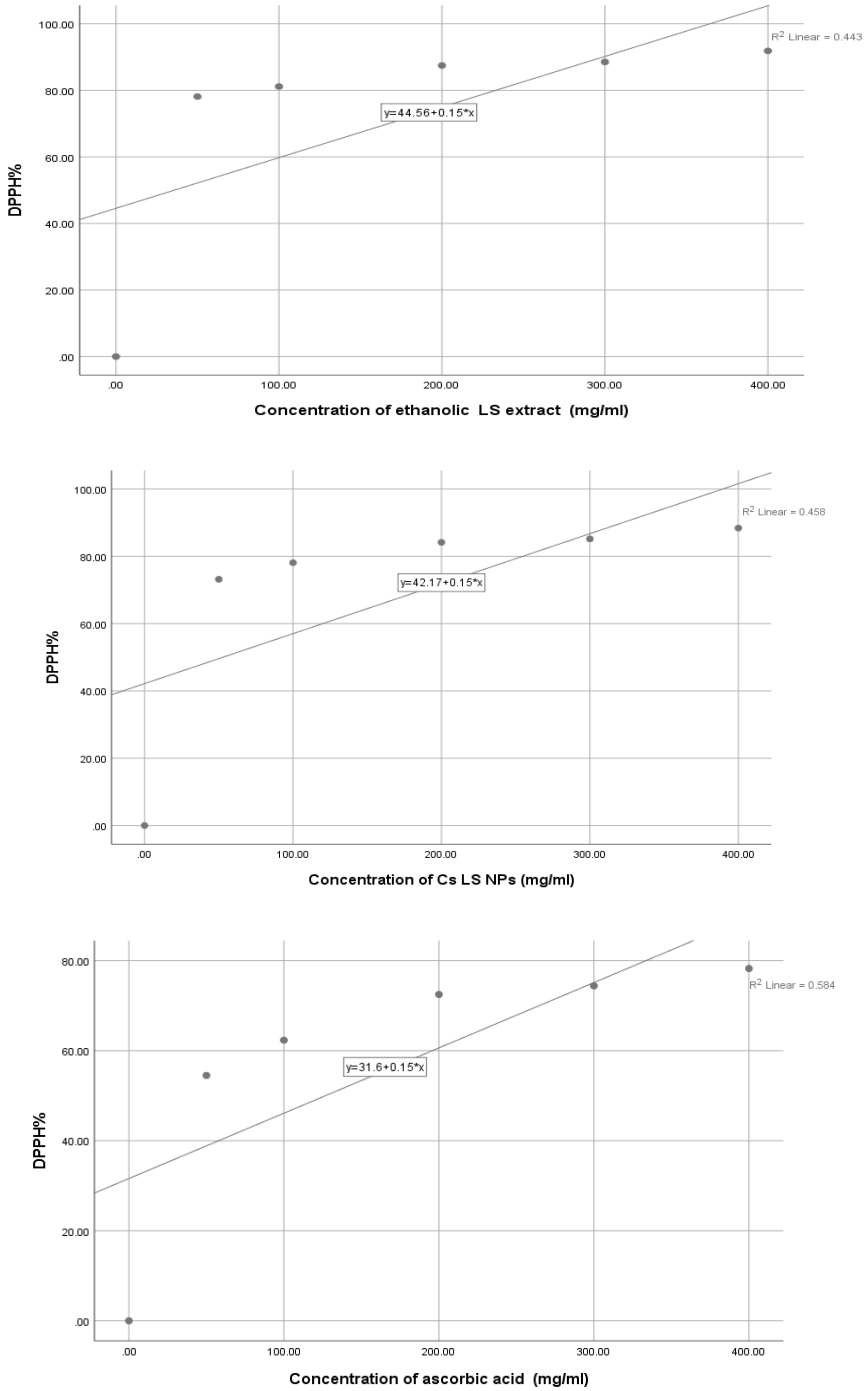

Figure 1: Free radical scavenging activity of different concentrations of ethanolic LS extract, Cs LS NPs, and ascorbic acid against DPPH

Table 2: DPPH%, IC50 of the ethanolic LS extract, Cs LS NPs, and standard ascorbic acid

Sample	DPPH%	IC50 mg\ml	R^2
Ethanolic *LS* extract	85.42±5.63 [a]	36.27	0.443
Cs *LS NPs*	81.79±6.09 [a]	52.22	0.458
Ascorbic acid	68.39±9.74 [b]	122.67	0.584

Abbreviation :DPPH,2,2-Diphenyl-1-picrylhydrazyl,IC50 concentration(mg/ml)/inhibition, R^2 coefficient of determination.

3.3 Estimation of eugenol content by HPLC:

The accurate HPLC analysis was employed as an appropriate technique for the identification and quantification of phenolic compounds in ethanolic LS extract and Cs LS NPs (Figure 2). Retention periods and UV spectra measured analytes. From the analysis of phenolic compounds, eugenol ethanolic LS extract (362.8 ppm) and Cs LS NPs (106.0 ppm) were the most abundant molecules in the ethanolic LS extract Table (3).

Table 3: Biologically active compounds of ethanolic LS extract and Cs LS NPs

No	Name	Con of eugenol (ppm)
1	Ethanolic *LS* extract	362.8
2	Cs *LS* NPs	106.0

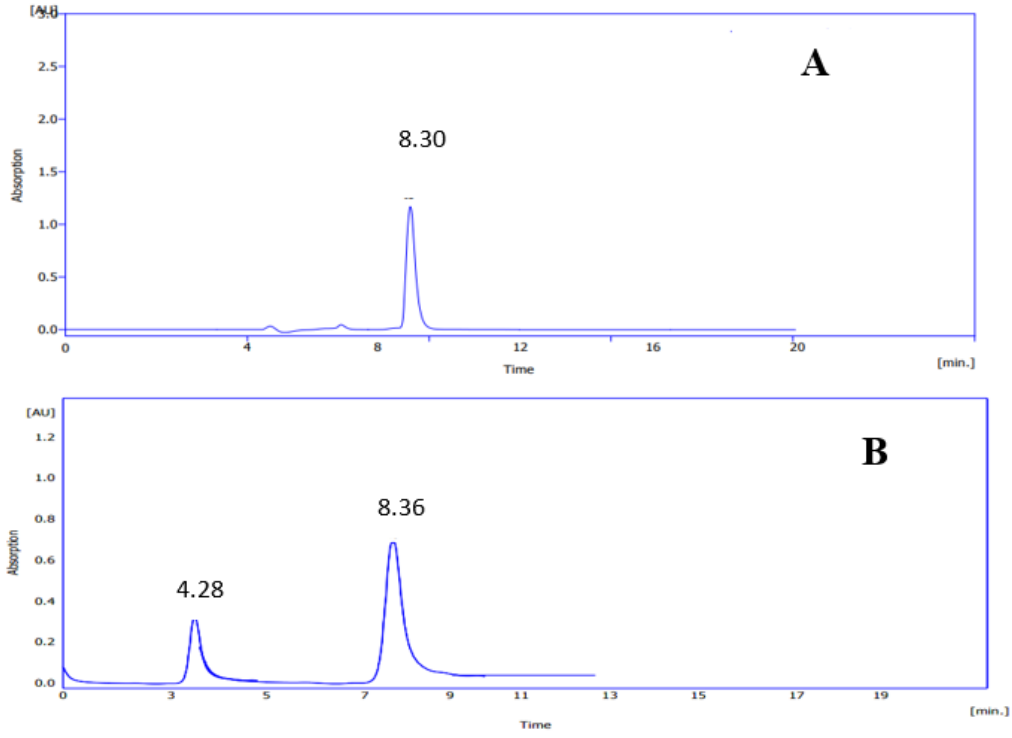

Figure 2: HPLC chromatograms of ethanolic LS extract (A) and Cs LS NPs (B) measured at 278 nm wavelengths

GC/MS analyses LS ethanolic extracts:

In the GC-MS profile, around 31 peaks were seen in LS ethanolic extract, but 3.4. GC/MS analyses LS ethanolic extracts:

In the GC-MS profile, around 31 peaks were seen in LS ethanolic extract, but only 11 compounds exhibited a high percentage of peak (Table 4). Based on abundance, two major compounds identified were 9,12 octadecadienoic acid (30.93 %) at the retention time (Rt 62.026 min) and oleic acid (23.22%, Rt 62.22 min), Peak number 1 was identified as benzenacetonitrile (9.60%, Rt 23.188 min), hexadecenoic (9.56, Rt 55.725), 2-Cyclohexen-1-one, 2-methyl-5 (7.32%, Rt 28.229 min), Octadecanoic acid (4.14%, Rt 62.709 min), Octadecenoic acid, methyl ester (2.17 %, Rt 60.429 min), Benzyl isothiocyanate (1.59 %, Rt 30.93 min), 2,5-Cyclohexadiene-1,4-dione, 2,3,5-trimethyl-(1.85 %, Rt 41.871 min), Cyclopropaneoctanal, 2-octyl-(1. 20 %, Rt 57.045min) and 2-Furancarboxaldehyde (1.01 %, Rt 27.571 min).

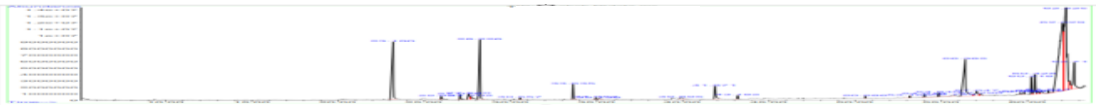

Figure 3: GC-MS chromatogram of ethanolic extract of Lepidium sativum

Table 4: Detail of compounds identified by GC-MS analysis of ethanolic extract of Lepidium sativum seeds

	Compound Name	RT(min)	Chemical Formula	Molecular Weight	Area (%)	Base (m|Z)
1	Benzeneacetonitrile	23.188	C_8H_5NO	131.1314	9.60	90.10
2	Cyclohexanone, 2-methyl-5-(1-met..	25.983	$C_{10}H_{16}O$	152.2334	0.19	95.10
3	2-Cyclohexen-1-ol, 2-methyl-5-(1..	27.068	$C_{10}H_{16}O$	152.2334	0.48	84.10
4	2-Furancarboxaldehyde	27.571	C5H4O2	96.09	1.01	126.10
5	5-Hydroxymethylfurfural	27.703	C_6H_6O	126.1100	0.34	126.10
6	2-Cyclohexen-1-one, 2-methyl-5	28.229	$C_{10}H_{16}O$	152.2334	7.32	108.20
7	Phenol, 5-methyl-2-(1-methylethyl)-	30.555	$C_{12}H_{16}O_2$	192.2542	0.22	150.05
8	Benzyl isothiocyanate	33.635	C_8H_5NOS	163.196	1.59	149.05
9	Tetradecane	34.950	$C_{14}H_{30}$	198.3880	0.20	71.20
10	Benzeneacetamide	35.138	C_8H_9NO	135.1632	0.30	92.10
11	3,4-Altrosan	39.894	$C_6H_{10}O_5$	162.14 g/mol	0.45	60.10
12	2,5-Cyclohexadiene-1,4-dione, 2,3,5-trimethyl-	41.871	C9H10O2	150.17 g/mol	1.85	165.10
13	Hexadecane	43.180 min A	$C_{16}H_{34}$	226.4412	0.30	71.20
14	Octadecane	50.587	$C_{18}H_{38}$	254.4943	0.17	71.10
15	Z-5-Nonadecene	53.165	$C_{19}H_{38}$	266.5050	0.20	83.10
16	Docosane	54.016	$C_{22}H_{46}$	310.6006	0.30	71.10
17	Hexadecanoic acid, methyl ester	54.839	$C_{17}H_{34}O_2$	270.4507	0.42	87.10
18	-8-Azabicyclo[3.2.1]octan-3-ol, 6-methoxy-8-methyl	55.725	$C_8H_{15}NO$	141.2108	0.25	57.10
19	n-Hexadecanoic acid	56.382	$C_{16}H_{32}O_2$	256.4241	9.56	60.10

20	**Cyclopropaneoctanal, 2-octyl-**	**57.045**	**$C_{26}H_{50}O_2$**	**394.6740**	**1.20**	**67.10**
21	**Eicosane**	**57.291**	**$C_{20}H_{42}$**	**282.5475**	**0.35**	**55.10**
22	**9,12-Octadecadienoic acid (Z,Z)-**	**59.583**	**$C_{18}H_{32}O_2$**	**280.4455**	**0.19**	**59.583**
23	**9,12-Octadecadienol**	**59.800**	**$C_{18}H_{34}O$**	**266.4620**	**0.21**	**81.10**
24	**9,12-Octadecadienoic acid (Z,Z)-, methyl ester**	**60.217**	**$C_{19}H_{34}O_2$**	**294.4721**	**1.56**	**81.10**
25	**9-Octadecenoic acid, methyl ester**	**60.429**	**$C_{19}H_{36}O_2$**	**296.4879**	**2.17**	**69.10**
26	**9,17-Octadecadienal**	**60.617**	**$C_{18}H_{32}O$**	**264.4461**	**0.43**	**67.10**
27	**14-methyl-(Z)-8-hexadecen-1-ol**	**60.817**	**$C_{17}H_{34}O$**	**254.4513**	**0.58**	**81.10**
28	**Octadecanoic acid, methyl ester**	**61.212**	**$C_{19}H_{34}O_2$**	**294.4721**	**0.27**	**87.10**
29	**9,12-Octadecadienoic acid (Z,Z)-**	**62.029**	**$C_{18}H_{32}O_2$**	**280.4455**	**30.93**	**81.10**
30	**Oleic Acid**	**62.229**	**$C_{18}H_{34}O_2$**	**282.4614**	**23.22**	**69.20**
31	**Octadecanoic acid**	**62.709**	**$C_{18}H_{36}O_2$**	**284.4772**	**4.14**	**55.10**

4. Discussion

Finding trustworthy, natural antioxidant sources is crucial. In particular, phenolic chemicals, flavonoids, tannins, and anthocyanins in plants are natural antioxidants that are both safe and active in biology. As a result, the number of studies on the possible antioxidant effects of plant extracts or isolated compounds obtained from In this study, we delve into the analysis of ethanolic LS extract and Cs LS NPs using High-performance liquid chromatography (HPLC) (Hwang et al., 2013). cation of specific polyphenols present in these extracts, shedding light on their potential health benefits and therapeutic applications. Garden cress seed holds a wealth of biologically active compounds that can have a profound impact on our health. In particular, eugenol was the most abundant. A similar proportion of biologically active compounds was confirmed by a previous study healthy fatty acids and natural antioxidants like vitamins A, E, and eugenol are abundant in garden cress seed, which helps defend cells from damage from free radicals (Singh et al., 2015). Antioxidant properties of eugenol and isoeugenol, one of its isomers, have been studied. Eugenol's antioxidant activity can form complexes with reduced metals, according to the findings of the analysis of the two substances' biological mechanisms. Isoeugenol inhibits the synthesis of the iron-oxygen chelate complex, the main contributor to lipid peroxidation (Ito et al, 2005). Ogata et al.(2005) discovered that the suppression of lipid peroxidation at the level of initiation is the mechanism by which eugenol exerts its antioxidant activity. As a consequence of many metabolic processes, active substances, also known as secondary metabolites, are created in plants. they are known for their biological or medicinal properties and are crucial in many defence mechanisms (Mazumder et al., 2016).

GC-MS Analysis of ethanolic extract of Lipidium sativum: Total 31 peaks were formed in the GC-MS spectrum of ethanolic extract of LS shown in Fig. 2. In the spectral matching of ethanolic LS extract spectra with NIST database to identify constituent compounds, The list is given in Table 2 with their retention time, molecular formula, molecular weight, and the elution time of compounds with their base mass-to-charge ratio (m/z). The major components identified at various RTs were as 9,12-Octadecadienoic acid (Z, Z)- 5-(RT 62.029; area 30.93%); Oleic Acid, (RT 62.229; peak area 23.22 %); Benzeneacetonitrile, (RT 23.188; peak area 9.60%); n-Hexadecanoic acid (56.382; peak area 9.56 %); 2-Cyclohexen-1-one, 2-methyl-5 (RT 28.229 1; peak area7.32%) 7.32%), Octadecanoic acid (RT 62.709; peak area 4.14%). Moreover, Octadecenoic acid, methyl ester (RT 60.429; peak area 2.17%); Benzyl

isothiocyanate (RT 33.635; peak area 1.59%);2-Furancarboxaldehyde (RT 27.571; peak area 1.01%) in this study, have been identified volatile organic compounds such as benzeneacetonitrile, benzyl isothiocyanate, and benzene acetamide are products of degradation of glucosinolates, which are composed of sulfate and nitrogen molecules, These compounds have nutritional importance and have a therapeutic role as anti-inflammatory, antioxidant and anti-cancer (Painuli et al., 2022) . El-Gendy,2021 identified the 10 major compounds including benzyl nitrile, 2,3,4-trimethoxycinna-mic acid, 5-hydroxy-methyl furfural, and furfural .In general, GC functions as a type of medicine food to benefit from many health advantages and to fend off a wider range of conditions. Since they are the primary inducers of carcinogen-detoxifying enzymes, isothiocyanates are the most significant biochemical agents in terms of their impact on human health. One of the strongest isothiocyanates is benzyl isothiocyanate (BITC), which is abundant in garden cress (Williams et al.,2009). Based on quantitative research, it can be determined that the ethanolic extract seeds have a significant content of volatile organic chemicals. These chemicals' concentrations are influenced by extraction methods, solvents, and raw materials reported by Nortjie et al. (2022).

Our study revealed The garden cress seed has a high quantity of linolenic acid (30.93%) and oleic acid (23.22%), as well as higher amounts of saturated and unsaturated fatty acids. It is also particularly high in omega-3 fatty acids, which are good for your health. The seeds of L. sativum are rich in (Z, Z)-9,12-octadecanoic acid (stearic acid) and (9,12,15-octadecadienoic acid (oleic acid), both of which have been shown to have antioxidant properties

(Tian et al ., 2018). The evaluation of fatty acids was done for three L. sativum seed oil extracts produced by the cold press extraction method, the Soxhlet extraction method, and the supercritical carbon dioxide extraction method.

According to the investigation's findings, linoleic acid was the fatty acid with the highest concentration. Oleic acid had the lowest concentration, at 2.8%, in all seed oil extracts (measured at 34–35% (Diwakar et al., 2010). The main fatty acid in L. sativum seeds is -linolenic acid (ALA), although there are also varying amounts of oleic, palmitic, stearic, arachidic, linoleic, and lignoceric acids, as well as behenic and -sitosterol (Chatoui et al., 2020). GC-MS investigations have been used more frequently recently for the examination of medicinal plants since they have shown to be a good technique for the analysis of non-polar components and volatile essential oils, fatty acids, lipids, and alkaloids (Sharifi-Rad et al., 2021).

5. Conclusion :

In this study, the antioxidant potential and content of physiologically active chemicals in L. sativum were examined. Our research shows that plant seeds are a reliable source of eugenol, fatty acids, and amino acids, and have the capacity to function in vitro as antioxidants. Their high concentration of phenolic chemicals may be the cause of this.

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facilities.

Conflict of interest:

The authors declare that there is no conflict of interest.

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