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SHORT COMMUNICATION

Phytochemical characterization and in vitro antioxidant analysis of *Lepidium sativum* seeds

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This research was conducted to examine the phytochemical profile of the ethanolic extract of *Lepidium sativum L*. (garden cress) seeds and to assess the antioxidant activity of the extract with special focus on identification of bioactive compounds that mediate its medicinal effect. Antioxidant activity of Ethanolic seed extract of *Lepidium sativum* was evaluated through the use of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay and the ferric ion (Fe^{3+}) reducing power assay. The identification and characterization of the constituent bioactive in the extract was done by gas chromatography-mass spectrometry (GC-MS) analysis. The seed extract dissolved in ethanol had a significant antioxidant effect, indicated by the ability of the solution to scavenge DPPH radical and the ability to reduce ferric. The GC-MS profiling detected the existence of a few bioactive compounds such as phenolic compounds flavonoids and vitamins which are known to help in antioxidant and anti-inflammatory effects. All of the identified phytochemicals together promote the capacity of *Lepidium sativum* seeds to counter oxidative stress and cellular damages. The results affirm that the *Lepidium sativum* ethanol seed extract is a nutritious source of antioxidant phytochemicals. The traditional medicinal application of garden cress is justified by the observed antioxidant activity and the profile of bioactive compounds that have been identified and that have the potential to make this plant a natural therapeutic measure against oxidative stress-linked diseases.

Keywords: *Lepidium sativum*, garden cress, phytochemical analysis, DPPH, GC-MS, oxidative stress

Introduction

The free radicals are reactive chemical compound with unpaired electrons and as such can easily be involved in redox reactions as oxidants or reductants. Uncontrolled production of free radicals has potential to damage the cellular lipids, proteins, and nucleic acids, which eventually affects the functioning and genetic stability of the cell (1). In response to this, biological systems use antioxidants, which neutralize free radicals by an electron transfer reaction or by transforming them to less reactive and more stable species. Antioxidants can also be indirect in their action, they work by increasing the activity of endogenous antioxidant enzymes and thus help in maintaining cellular redox balance (2).

The disproportion between the production of free radicals and antioxidant defense leads to oxidative stress which is a situation that is strongly related to the occurrence of diseases. Oxidative stress is also caused by environmental and lifestyle factors like air pollution, smoking, alcohol use, hyperglycemia, excessive exercise, excessive polyunsaturated fatty acids, and exposure to radiation (3). Oxidative stress has been a persistent cause of pathogenesis of cardiovascular diseases, chronic obstructive pulmonary disease, chronic kidney disease, neurogenerative disorders,

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FIGURE 1 | Diagnostic plans of significant illnesses related with oxidative stress, such as cardiovascular, neurogenerative, metabolic and cancer diseases.

cognitive decline, visual impairment, and cancer (**Figure 1**). In turn, antioxidant-based interventions can be regarded as the promising strategies in minimizing the hazard of oxidative damage and the risk of the disease (4, 5).

Antioxidants in food, especially those found in plants are a viable and sustainable way of reducing oxidative stress and enhancing health in the long term (6). *Lepidium sativum L.* (garden cress) is an edible herb that belongs to the family of Brassicaceae and is native to Southwest Asia and Egypt. It is treasured as a healthy food and with medicinal value in the region where it is popularly called Halim, Chandrasura, or Holan. The seeds contain significant amounts of proteins, iron, magnesium, calcium, vitamins, and essential fatty acids and can be used in traditional and more modern medical practice (7).

Lepidium sativum is traditionally used to improve lactation, to improve postpartum weakness and to increase overall vitality. Seed preparations, including seed decoctions, and roasted seed preparations in ghee and sugar are usually taken especially by postpartum women, which emphasize their contribution to postnatal recovery and general well-being (8). Ascorbic acid is also a part of the seeds and it helps in strengthening the immune system and defense against the infectious diseases (9). Also, they are rich in vitamin K, which helps keep the bones healthy, forming bone-related proteins like osteocalcin, which might prevent osteoporosis (10).

The detoxification and protective properties of *Lepidium sativum* seeds have been demonstrated in the context of experimental studies, both the restoration of liver and kidney functioning in the models of exposure to aluminum as well as the sheltering of the hepatic cells against the effects of oxidative stress through the use of hydrogen peroxide (11, 12). The seeds have an excellent omega-3 to omega-6 ratio of fatty acids, which is beneficial to the cardiovascular system and to cholesterol levels (13). There are also emerging signs of possible effects on glycemic control, lipid metabolism, and anticancer activity as well as an increase in antioxidant status and iron levels (14–16).

Due to the negative outcomes that are linked to the long consumption of synthetic drugs, plant-based treatments are becoming more noticeable. Against this backdrop, the current research paper intends to assess the phytochemical makeup and antioxidant capacity of *Lepidium sativum* seeds whereby, gas chromatography-mass spectrometry (GC-MS) analysis will be used to determine the important bioactive compounds that contributed to the antioxidant activity.

Materials and methods

Preparation and collection of plant material

The seeds of *Lepidium sativum L.* (garden cress) were organically produced and were purchased in one of the local markets of Chennai, Tamil Nadu, India (**Figure 2**). The seeds were rinsed with a running water in order to get off any remaining dust and any other extraneous material, then left to dry in the air and afterwards crushed into a nice powder-like substance using a mechanical grinder (**Figure 3**). The fine material was placed in non-porous bottles and taken through additional extraction processes.

Chemicals and reagents

Analytical grade chemicals and reagents were used in the study. Ethanol and other reagents were bought at Sisco Research Laboratories (SRL), India. 2,2-diphenyl-1-picrylhydrazyl (DPPH) was purchased from HiMedia Laboratories, India.

Preparation of seed extracts

Ethanolic extract

The Soxhlet method of extraction was carried out using ethanol as the solvent with the powdered *Lepidium sativum* seeds. The involved amount of seed powder (approximately 20 g) was weighed properly and dissolved in a 250 mL of 99.9% ethanol at a constant temperature (40–50 °C). This extraction was repeated until enough extract was acquired. A rotary evaporator with 40 °C heating temperature was used



FIGURE 2 | *Lepidium sativum* seeds to be extracted.



FIGURE 3 | *Lepidium sativum* powdered seed sample.

to concentrate the resulting filtrate under low pressure. The extract was dried in 4 °C and then was stored to be subjected to subsequent analysis.

Aqueous extract

50 gm of grinded seed residue was resolved in 50 mL of distilled water and left to stay overnight. Whatman No. 1 filter paper was used to filter the mixture and a clear filtrate was obtained that was stored at 4 °C till use.

Percentage yield of extracts

After extraction, solvents were evaporated and dried extracts put in a desiccator. The level of extracts obtained was evaluated by the following formula:

$$\text{Extract yield (\%)} = \frac{W_2(\text{Weight of the extract obtained in grams})}{W_1(\text{Weight of the plant material in grams})} \times 100$$

W2 is the weight of the dried extract (g) and W1 is that of the plant material (g).

Qualitative screening

Phytochemical quantitative analysis

The aqueous and ethanolic seed extracts of *Lepidium sativum* were qualitatively phytochemically screened by performing routine qualitative assays to identify whether they contained major bioactive constituents. The ferric chloride test was used to determine the presence of phenolic compounds, whereas the alkaline reagent test and the lead acetate test were used to determine the presence of flavonoids (17). The tests conducted by Mayer and Wagner screened alkaloids (18). The Salkowski test was applied to determine the presence of terpenoids, whereas the froth test was conducted to determine the presence of saponins (19). The Liebermann Buchhard reaction was used to identify steroids (20, 21), and the ferric chloride test was the method that detected tannins (18). The DNPH test was used to identify vitamin C, the chloroformammonium test was used to identify glycosids, and the Molisch test was used to identify carbohydrates, the Biuret test was used to identify proteins, and the lead acetate test was used to identify the free amino acids.

Quantitative analysis

Estimation of total phenolic content

The Folin-Ciocalteu technique was used to estimate the total phenolic content though some slight modifications were made (22). In a single drop, a 100 µL ethanol (1 mg/mL) was added to 900 µL extract (1 mg/mL) and 1 mL Folin-Ciocalteu reagent (1:10 dilution). The mixture was incubated in dark for 5 min after which, 1 mL of 20% Na_2CO_3 was added. The absorbance at 765 nm was recorded. The outcome was presented in form of mg gallic acid equivalents per gram of dry sample (mg GAE/g).

Estimation of total flavonoid content

The total flavonoid content was estimated with the help of a bioassay based on the color change of a solution into a pink-brown color and the duration required to change the solution to a pink-brown color.

The total flavonoid content was calculated following the aluminum chloride colorimetric method (23). Methanol, sodium nitrite, aluminum chloride and sodium hydroxide were added together to the extract (500 µL, 1 mg/mL). The absorbance was measured at 510 nm, and the findings were reported as mg quercetin equivalent to every gram of dry sample (mg QE/g).

Estimation of ascorbic acid

The titrimetric procedure was used to estimate the ascorbic acid quantity using 2, 6-dichlorophenol indophenol dye (24, 25). The endpoint was realized when a pale pink color appeared. The standard formula was used to determine the results and they were quantified in mg/100 g of sample.

In vitro antioxidant assays

DPPH radical scavenging assay

The ethanolic seed extract was tested using the DPPH free radical scavenging assay by following the method proposed by Burits and Bucar (26). The extracts with concentration of 10–60 μg /mL were incubated with DPPH solution in the dark for 30 min. The absorbance at 517 nm was measured using ascorbic acid as a standard. The percentage of inhibition was thus calculated.

Ferric reducing power assay

The reduction power was calculated with the technique identified by Yildirim et al. (27). Phosphate buffer and potassium ferricyanide were then incubated with extracts, which were then followed by trichloroacetic acid, and ferric chloride. The absorbance was recorded at 700 nm with the ascorbic acid as the reference.

GC-MS analysis

A GC-7890/MS-5975C system (Agilent Technologies, USA) was used to perform a GC-MS analysis of the ethanolic seed extract. The column was separated on an Elite-1 fused silica capillary column (30 m × 0.25 mm ID × 1 μm film thickness). The carrier gas, which was set at 1.51 mL/min, was helium. The energy of ionization was 70 eV, and scan range was 40–1000 m/z. The retention time and mass spectral comparison of the compounds were used to identify them (28).

Statistical analysis

All the experiments were done in triplicates. Findings are given in the form of mean ± standard deviation (SD).

Results and discussion

Percentage yield of seed extracts

The extraction yield by two different solvents differed (**Table 1**; **Figure 4**). The percentage yield of extract by ethanol (6.33) was more than the extract by aqueous solution (4.30), which means that the extractive power of the ethanol was more effective than water in extracting soluble components of the *Lepidium sativum* seed powder. This is in line with the solvent polarity effect on extraction efficiency with ethanol having the ability to dissolve a wider variety of phytochemicals in comparison to water.

TABLE 1 | Percentage yield of ethanolic and aqueous seed extracts of *Lepidium sativum*.

S. no	Contents	Percentage yield
1.	Ethanol extract	6.33%
2.	Aqueous extract	4.30%

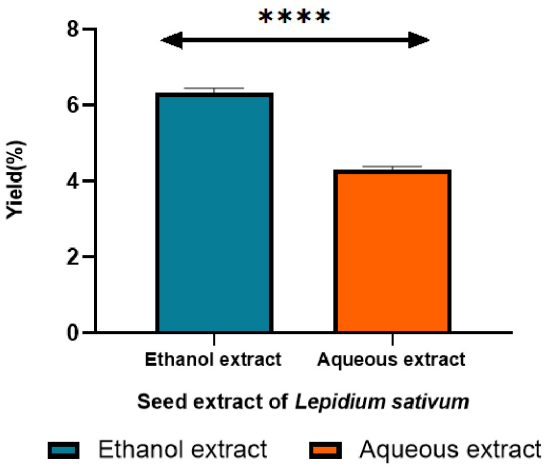


FIGURE 4 | Comparative extraction yield of ethanolic and aqueous seed extracts of *Lepidium sativum*. **** represents $p < 0.0001$, indicating a statistically significant difference in yield between ethanol and aqueous extracts.

Qualitative phytochemical bioimpedance

Phytochemicals are secondary metabolites that cause various biological effects such as antioxidant, antimicrobial, antifungal, and anticancer effects and could help with the protection of chronic diseases and environmental toxins (29). Qualitative screening of *Lepidium sativum* seed extracts in the current study demonstrated that there were significant differences between the ethanolic and aqueous fractions (**Table 2**). The phenols, alkaloids, flavonoid, tannin, terpenoid, saponins, glycosides, and ascorbic acid were detectable in the ethanolic extract with a relatively stronger degree of observed phenol, with some of the constituents being observed in the aqueous extract with lower strength. On the whole, the findings indicate that ethanol was more effective in the elution of bioactive compounds than water. The availability of phenols and flavonoids is especially the case since these groups of molecules are commonly linked to antioxidant activity. The same has been observed before (30). Secondly, it has been reported that secondary metabolites like flavonoids are associated with several pharmacological activities including antioxidant, anti-inflammatory, anti-viral, cytotoxic and anti-cancer (31). The phytochemical profile presented in this case is consistent with previous results, which indicate phenols, alkaloids, flavonoids, tannins, terpenoids, and saponins in *Lepidium sativum* seed extracts (32).

TABLE 2 | Qualitative phytochemical profile of ethanolic and aqueous seed extracts of *Lepidium sativum*.

S. no	Bioactive compounds	Ethanol extract	Aqueous extract
1.	Phenols	++	+
2.	Flavonoids	++	+
3.	Quinones	-	-
4.	Tannins	++	+
5.	Terpenoids	+	-
6.	Steroids	-	-
7.	Saponins	+	-
8.	Glycosides	+	-
9.	Ascorbic acid	++	+
10.	Alkaloids	++	+
11.	Carbohydrates	-	-
12.	Proteins	-	-
13.	Amino acids	-	-

TABLE 3 | Total phenolic content, flavonoid content, and ascorbic acid levels in ethanolic and aqueous seed extracts of *Lepidium sativum*.

S. no	Contents	Ethanol extract (mg/g)	Aqueous extract (mg/g)
1.	Phenols	26.9 ± 0.23	13.5 ± 0.05
2.	Flavonoids	22.3 ± 0.03	10.0 ± 0.02
3.	Ascorbic acid	10.5 ± 0.72	9.5 ± 0.02

Determination of total phenolics, flavonoid and ascorbic acid

Ethanol and aqueous seed extracts were quantitatively assayed as regards the total phenolic content, total flavonoid content and ascorbic acid (**Table 3**; **Figure 5**). Ethanolic extract had better amounts of total phenolics (26.9 ± 0.23 mg GAE/g), flavonoids (22.3 ± 0.03 mg QE/g), and ascorbic acid (10.5 ± 0.72 mg/100 mL) than the aqueous extract (13.5 ± 0.05 mg GAE/g; 10.0 ± 0.02 mg QE/g; and 9.5 ± 0 It is well known that phenolic compounds and flavonoids have a high free-radical scavenging capacity (33), and their increased presence in the ethanolic fraction correlates with a more potent antioxidant.

The change in solubility and extraction efficiency of phytochemicals especially phenolics and flavonoids can be explained by differences between ethanol and aqueous extracts. The solvent effect that has been observed justifies the need to use the right extraction solvents to recover the maximum of antioxidant constituents. Similar effects of solvents on recovery of phytochemicals have been observed on other plant systems (34). Similar tendencies in the contents of the phenolics and flavonoids were also found in a related study on *Lepidium sativum* seeds (35), which suggests the reproducibility of the findings.

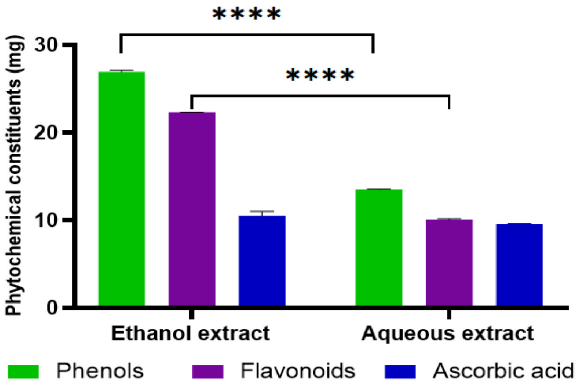


FIGURE 5 | Total phenolic and flavonoid contents of ethanolic and aqueous seed extracts of *Lepidium sativum*. **** represents $p < 0.0001$, indicating a statistically significant difference between ethanol and aqueous extracts for phenols, flavonoids, and ascorbic acid.

TABLE 4 | In vitro antioxidant activity of ethanolic seed extract of *Lepidium sativum* determined by 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging and ferric reducing power assays.

S. no	Concentration (µg/ml)	DPPH (% of inhibition)	FRAP (% of reduction)
		Lepidium sativum seed extract	
1.	20	9.04 ± 1.31	13.55 ± 0.28
2.	40	31.07 ± 1.45	15.70 ± 0.12
3.	60	48.86 ± 1.86	31.93 ± 0.09
4.	80	62.59 ± 0.91	34.55 ± 0.04
5.	100	64.86 ± 0.55	40.11 ± 0.03
6.	120	80.84 ± 1.22	49.82 ± 0.03

In vitro antioxidant activity

A radical scavenging assay was performed using DPPH Radicals according to the guidelines of a previous study by Wan et al. (36). The DPPH assay determined the reactive oxygen species (ROS) scavenging activity of the ethanolic *Lepidium sativum* seed extract. The extract showed an increment in DPPH radical scavenging activity with concentrations and the highest scavenging activity of 80.84 ± 1.22% being at 120 µg/mL (**Table 4**; **Figure 6**). The ion chromatography (IC) of the result was 61.4 µg/mL and compared to the standard. This observed activity is due to the capability of the extract to diminish the stable DPPH radical to a non-radical form, thereby implying the presence of significant antioxidant activity. The same case has been reported in a number of medicinal plants using DPPH-based antioxidant activity (37, 38).

Ferric (Fe^{3+}) reducing power assay

Reducing power was identified against the capability of the extract in reducing Fe^{3+} to Fe^{2+} and then the development of the ferro-ferric complex. The concentration-dependent

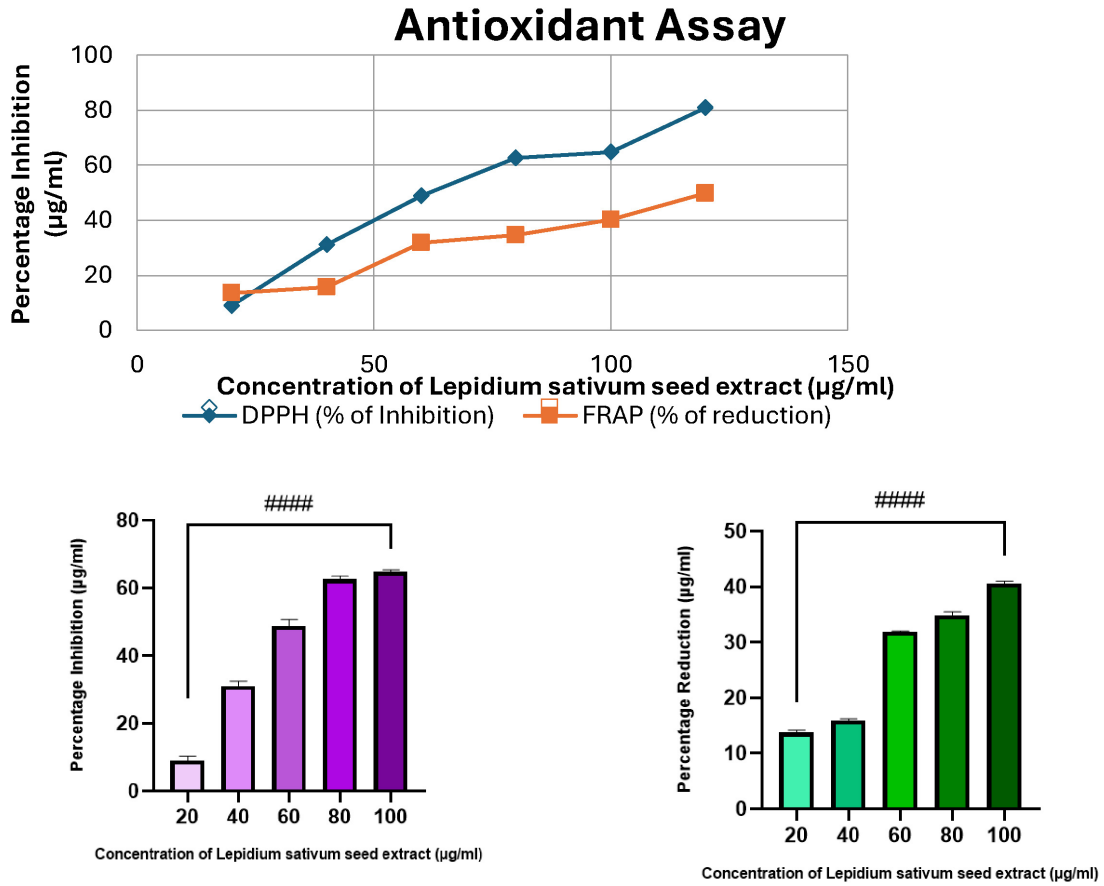


FIGURE 6 | Antioxidant activity of ethanolic seed extract of *Lepidium sativum* assessed by DPPH radical scavenging and ferric reducing power assays. #### $p < 0.0001$, indicating a statistically significant increase in inhibition activity and reduction activity with increasing concentration of *Lepidium sativum* seed extract.

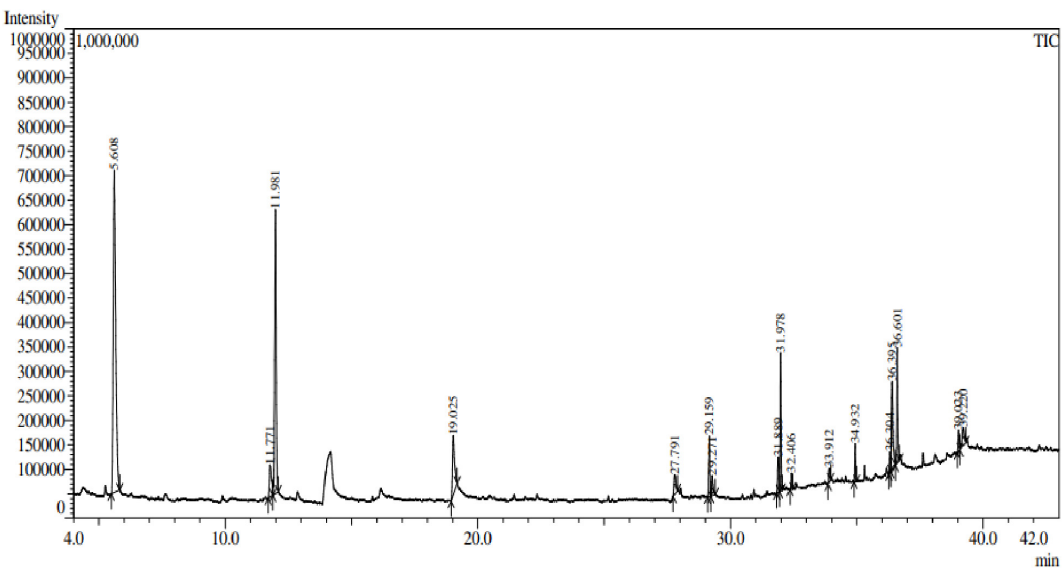


FIGURE 7 | Gas chromatography-mass spectrometry (GC-MS) chromatogram of ethanolic seed extract of *Lepidium sativum*.

ferric reduction of the ethanolic extract has a maximum of 49.82 ± 0.03% at 120 µg/mL (**Table 4**; **Figure 6**). The RC50 was 120.43 µg/mL. In this test, the greater the absorbance, the greater the reducing power which is one indicator of antioxidant potential commonly used. The diminutive mechanisms of antioxidants have been widely credited to the capacity of antioxidant molecules to donate electrons, which is in line with

TABLE 5 | GC-MS-identified compounds in the ethanolic seed extract of *Lepidium sativum* with retention time and area percentage.

S. no	RT	Area (%)	Name of the compound	Molecular weight	Molecular formula
1.	5.60	38.53	Benzyl nitrile	117	C_8H_7N
2.	11.77	3.35	Sulfurous acid, cyclohexylmethyl hexadecyl ester	402	$C_{23}H_{46}O_3S$
3.	11.98	19.98	Benzene, (isothiocyanato 4-methyl)	149	C_8H_7NS
4.	19.02	5.99	3',5'-dimethoxyacetophenone	180	$C_{10}H_{12}O_3$
5.	27.79	1.58	Benzyl 2-cynoacetamide	174	$C_{10}H_{10}N_2O$
6.	29.15	3.13	Hexadecanoic acid, ethyl ester	284	$C_{18}H_{36}O_2$
7.	29.27	1.34	8-amino-4-[3-hexoxy]-6-methoxy-3-methyl quinoline	288	$C_{17}H_{24}N_2O_2$
8.	31.88	2.25	n-propyl 9,12-octadecadienoate	322	$C_{21}H_{38}O_2$
9.	31.97	6.04	Dichloroacetic acid, tridec-2-ynyl ester	307	$C_{15}H_{24}Cl_2O_2$
10.	32.40	0.63	Octadecanoic acid, ethyl ester	312	$C_{20}H_{40}O_2$
11.	33.91	0.63	Hexadecanoic acid, 1-(hydroxymethyl) 1,2-ethanediyl ester	568	$C_{35}H_{68}O_5$
12.	34.93	1.54	Ethyl oleate	310	$C_{20}H_{38}O_2$
13.	36.30	0.94	9,12-octadecadienoyl chloride	298	$C_{18}H_{31}ClO$
14.	36.39	6.15	9,12,15-octadecatrienoic acid, 2,3-dihydroxypropyl ester (Z,Z,Z)	352	$C_{21}H_{36}O_4$
15.	36.60	5.14	O-ethyl S-2-dimethylaminoethyl ethylphosphonotiolate	225	$C_8H_{20}NO_2PS$
16.	39.02	1.02	9-octadecenoic acid, 1,2,3-propanetriyl ester, (E,E,E)	884	$C_{57}H_{104}O_6$
17.	39.22	1.76	Nonanoic acid, 9-(3-hexenylidenecyclo propylidene), 2 hydroxyl 1(hydroxy methyl 1) ethyl ester (Z,Z,Z)	352	$C_{21}H_{36}O_4$

TABLE 6 | Reported biological activities of selected compounds identified in the ethanolic seed extract of *Lepidium sativum*.

Name of the compound	Biological activity
9-octadecenoic acid, 1,2,3-propanetriyl ester, (E,E,E)	Anti-spasmodic and immune modulators (46)
9,12,15-octadecatrienoic acid, 2,3-dihydroxypropyl ester (Z,Z,Z)	Anti-inflammatory and CNS depressant activity (47)
Hexadecanoic acid, 1-(hydroxymethyl) 1,2-ethanediyl ester [2-hydroxy-1-[(palmitoyloxy)methyl] ethyl palmitate]	Antibacterial, antifungal, and nematicidal activities (48)
Nonanoic acid, 9-(3-hexenylidenecyclopropylidene), 2 hydroxyl 1(hydroxy methyl 1) ethyl ester (Z,Z,Z)	Antioxidant, antimicrobial, antiproliferative activity (49)
3',5'-dimethoxyacetophenone	Antioxidant and cytotoxic activities (50)
Ethyl oleate	Antibacterial activity, antioxidants, anti-diabetics, anti-inflammatory (51)

the described description of changing color with the increasing antioxidants concentration (39). The overall findings of the DPPH and reducing power effect are in favor of antioxidant activity of the ethanolic seed extract, which may be linked to its fortified phenolic and flavonoid constituents.

GC-MS profiling of ethanolic seed extract

Analysis of the ethanol *Lepidium sativum* seed extract by GC-MS identified 17 compounds (**Table 5**; **Figure 7**). The compounds were identified by comparing retention times and mass spectra of the compounds against the entries in the National Institute of Standards and Technology (NIST) library. Compounds that were detected in large amounts were benzyl nitrile (RT 5.60; 38.53% area) and benzene (isothiocyanato 4-methyl) (RT 11.98; 19.98% area) among other compounds found in minor amounts. **Table 5** indicates the retention time, area percentage, molecular weight, and molecular formula of all the compounds.The bio-relevance of chosen compounds is summarized in **Table 6**. Benzene (isothiocyanato 4-methyl), benzyl isothiocyanate (BITC) is a substance that has been tested to have anticancer potential,

including as a chemo - preventive agent against pancreatic cancer (40). Mechanically, isothiocyanates, including BITC, were proposed to carry out anticancer activity by inducing p21/WAF1 (41). Moreover, 3, 5-dimethoxyacetophenone has been stated as a natural occurring ketone which has antioxidant effects (42). Antioxidant, antiviral, and antibacterial effects have been linked to the estrogens of octadecanoic acid (43). The derivative of a saturated fatty acid is hexadecanoic acid (ethyl ester) that is said to have antibacterial, anticancer, and antioxidant actions (44). More so, 9-(3-hexenylidenecycloprolylidene)-2-hydroxy-1-(hydroxymethyl) ethyl ester of nonanoic acid (Z, Z, Z) has been found to be antioxidant, cytotoxic, anticancer, and antiproliferative (45). The GC-MS profile in combination with each other contributes to the presence of numerous bioactive components that could be involved in detected antioxidant activity of the ethanol seed extract.

Conclusion

The results of the current paper prove that *Lepidium sativum* seed extracts are characterized by a wide variety of bioactive phytochemicals, which can be associated with antioxidant activity and free radical-scavenging capacity. Ethanol was the best solvent compared to water in extracting these bioactive constituents, thus showing higher yields and antioxidant ability. GC-MS analysis revealed that few compounds with antioxidant properties were present in the ethanolic seed extract thus justifying the medical interest of *Lepidium sativum.*

The identified phytochemicals, especially the phenolic compounds, flavonoids, and analogs have a possible role to play in the observed antioxidant activities and has clear ability to prevent oxidative stress-related health conditions. These findings reveal the potential use of *Lepidium sativum* seeds as a natural source of antioxidants that may be used in developing nutraceutical and pharmaceutical products. Additional research are however, essential to understand the actual mechanisms behind the action of the identified bioactive compounds. Moreover, their efficacy and safety cannot be validated in vivo and clinical assessments are required before they can be recommended to apply them therapeutically and clinically.

Data availability statement

Data included in this article are referenced in the article.

Author contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Ramya Raveendran, Fahima Tasneem and Asmita Amirthakumar. The first draft of the manuscript was written by Ramya Raveendran and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Conflict of interest

The authors declare that they have no conflict of interest.

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