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Research article

Comprehensive phytochemical profiling of Garden Cress (*Lepidium sativum* L.) seeds using UPLC-ESI-MS/MS

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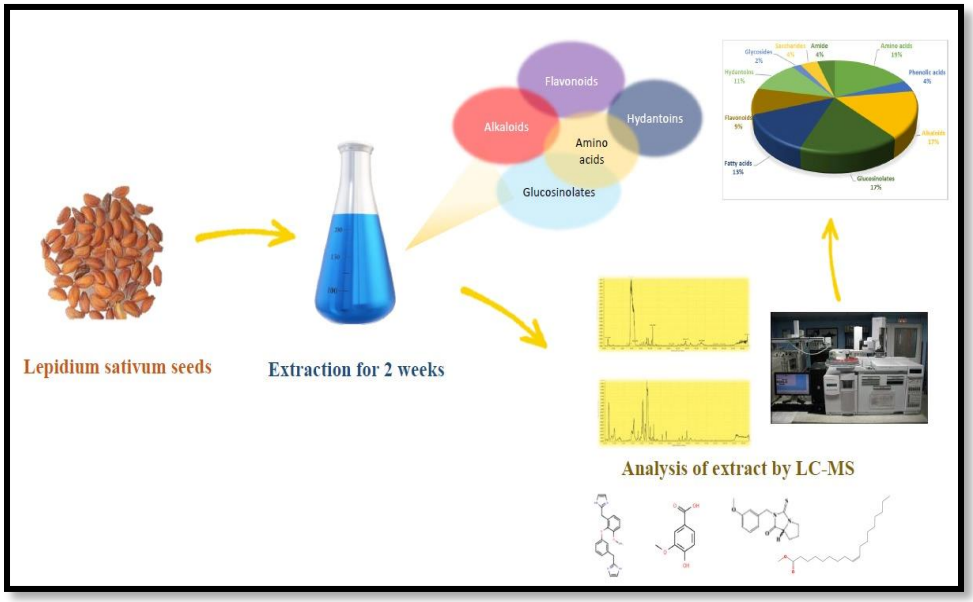
Abstract:



Lepidium sativum or Hab-el Rashad is extensively cultivated as a food supplement to preserve a healthy state of human considering its nutritional and therapeutical properties. Nutritional aspect as it is a valuable source of proteins, carbohydrates, minerals and vitamins. Besides, it has a therapeutic aspect as previous research proved remarkable biological actions including antidiabetic, antimicrobial, antioxidant, anticancer and numerous health-controlling effects. In the current study, *L. sativum* seeds were extracted using 70% ethanol and exposed to ultrahigh-performance liquid chromatography coupled with electrospray ionization tandem mass spectrometry (UPLC-ESI-MS) to probe its phytoconstituents. This analysis resulted in the annotation of 41 metabolites across several chemical categories, including glucosinolates, alkaloids, flavonoids, fatty acids and hydantoin derivatives. Compounds were annotated by comparing their retention times and fragments with those in an in-house collected chemical compounds database, as well as with data from online dictionaries and previous literature. The drive of this study is to extensively profile the active constituents in *L. sativum* seeds extract and demonstrate their mass fragmentation patterns in both positive and negative ionization modes.

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1. Introduction

Hab-el Rashad (*Lepidium sativum*) is an annual, vertical and eatable herbaceous plant [(1)]. It is one of Brassicaceae family members and has been used as a nutritive for a long time. About 175 to 220 worldwide diverse species of *Lepidium* are being grown around the world [(2)]. It is home-grown to southwest Asian ranges and then expanded to other regions such as Western Europe, North America and the Mediterranean [(1)]. Although it is a familiar healthy component that has been added to human diets as sprouts, recent research supported the use of this natural supplement to help alleviating multiple disorders such as hyperglycemia, hypertension [(3)], liver diseases, renal diseases [(4)] and cancer [(5)], as it evokes a cytotoxic effect against hepatocellular carcinoma through different mechanisms as apoptosis [(6)]. *L. sativum* is also believed to be a precious supplier of dietary fibers and additional vital minerals needed for good growing and development [(7)].

Seed extract of *L. sativum* demonstrates an anti-inflammatory, antioxidant [(1)], chemoprotective [(6)], hypolipidemic [(1)], antimicrobial [(8)], antifungal [(9)], hepatoprotective [(10)], antitumor activity and bone fracture healing [(11)]. That is for its fullness of different types of phytochemical classes such as alkaloids, flavonoids, amino acids, terpenoids, phenols [(6)] and glucosinolates [(12)].

In this study, a very sensitive ultra-performance liquid chromatography (UPLC) is used in analysis of the ethanolic extract of *L. sativum* for its high resolution and increased performance which leads to reduced elution time. In addition, it is connected to electrospray ionization (ESI) mass spectrometry (MS) which has the ability to separate the chemical constituents based on the mass-to-charge ratio [(13)]. So, UPLC-ESI-MS was used to achieve the chemical makeup of *L. sativum* by comparing the fragmentation patterns resulting from the analysis with previous studies.

2. Materials and method

2.1. Preparation of *L. sativum* extract

L. sativum seeds were obtained in November 2022 from well-reputed marketplace in Alexandria, Egypt with a voucher specimen's code of (LS003) deposited in herbarium of the Pharmacognosy Department, Faculty of Pharmacy, Damanhour University and validated for authenticity by Professor Mohamed Abd El-Sattar, Faculty of Agriculture, Alexandria University, Alexandria, Egypt. Initially, the seeds were ground in mixer perfectly and then submerged in ethyl alcohol 70% (International Company for Sup. &Med. Industries, Egypt) at room temperature for couple of weeks. Then, double filtration took place. Finally, a rotary evaporator (Buchi-Rotavapor® R-100) was used to concentrate the filtrate to dry state at 50-55 °C and 100 bar vacuum pressure (vacuum pump v-100) followed by freeze–drying supported with a vacuum device.

2.2. Sample preparation

The dried extract (10 mg) was dissolved in HPLC grade methanol (10 ml), sonicated for sample degassing and filtrated by membrane disc filter (0.2 μm) to prepare (1mg/ml) sample solution.

2.3. UPLC-ESI-MS parameters and conditions

UPLC ACQUITY QSM Waters Corporation, Milford, MA01757 USA system with a quaternary solvent system was used to carry out the analysis with an autosampler and triple quadrupole (QqQ) tandem mass spectrometer (Waters, USA) and linked to electrospray ionization (ESI) source. The chromatographic partition was achieved on a Waters ACQUITY UPLC® BEH C18

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column (1.7 µm particle size) functioning at a temperature of 34 °C and flow rate of 0.2 ml/min. The autosampler injected a volume of (2 µL). The mobile phases as a binary system consisted of ultrapure water +0.1% (v/v) formic acid (Phase A) and acetonitrile + 0.1 % formic acid (phase B). As the sets for the elution gradient were automated as follows: 99% (phase A), 1 %(phase B) at retention time of 0.0–04.0 min, 90% (phase A), 10% (phase B) at 04.0–07.0 min, 80% (phase A), 20% (phase B) at 07.0–09.0 min, 70% (phase A), 30% (phase B) at 09.0–15.0 min, 30% (phase A), 70% (phase B) at 15.0–26.0 min, 100 % (phase B) at 26.0-32.0 min and washing up the step was accomplished at 32.0 min.

Switching to MS parameters and conditions, both ES+ and ES^- ionization modes were utilized, capillary of 3.00 kV, source temperature was 150 °C, desolvation temperature was 400 °C, cone gas flow was 50 L/hr, desolvation gas flow of 600 L/hr and MS mode collision energy was 2.00 kV.

2.4. Annotation of phytoconstituents of *L. sativum*

In-house database of 392 chemical compounds in *Lepidium* species (**Table S1**) was established with the aid of dictionary of natural products (DNP) (https://dnp.chemnetbase.com/chemical/ChemicalSearch.xhtml?dswid=9126) and literature reviews including their chemical nomenclature, chemical formula and molecular weight. In addition, a comparison between our mass fragmentation patterns and molecular ion peaks with standard compounds and preceding studies has been utilized to identify the seeds compounds.

3. Results and discussion

L. sativum seeds are plentiful with several phytochemicals such as alkaloids, glucosinolates, flavonoids, phenols, organic acids and hydantoins [6]. Also, high percentages of amino acids, carbohydrates and minerals are present [8]. For that reason, it can be used for nutritional and therapeutic purposes. Therefore, *L. sativum* has been extensively used as a component added to salads in several traditional cultures. Additionally, the therapeutic properties of *L. Sativum* are attributed to the efficient role of each phytochemical class in maintaining healthy life and helping in the treatment of multiple diseases.

For example, glucosinolates, sulfur and nitrogen-containing compounds are reported to have antagonistic effect in osteoporotic cases through improving bone formation biomarkers and osteocalcin levels [14], antimicrobial effect [15], chemoprotective effect and anti-inflammatory effect [16].

Furthermore, phenolic compounds have many actions as they possess antioxidant activity [17], antibacterial, anticancer [18] and osteoprotective effect [14]. In addition to these classes, flavonoids are a large group of compounds that are widely present in *Lepidium* species and they have cytotoxic effect, antioxidant effect and bactericidal effect [6].

Besides, imidazole alkaloids and N-hydroxypyridine derivatives are nitrogenous compounds that are found as a characteristic phytochemical class in *L. sativum* extract. They have many pharmacological actions as antioxidant [6], anticancer [19] and hormonal activity [20].

Worth noting that hydantoins and their sulphur-analogues thiohydantoins are chemical components that are very characteristic of *Lepidium* species that possess remarkable activity against multiple disorders as they act as anticarcinogenic [21], hypolipidemic and antimicrobial agent [22].

The base peak (BP) chromatograms of UPLC-ESI-MS analysis of ethanolic extract of *L. Sativum* seeds in both positive and negative mode of ionization are represented in **Fig. 1** which demonstrated the richness of the extract with phytoconstituents of various polarities.

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Additionally, **Table 1** represents the components annotated from *L. sativum* extract arranged according to their elution order and accompanied by their molecular formula, chemical class and fragmentation pattern.

The pie chart **(Fig. 2)** represents the relative distribution of phytochemical classes of the identified compounds in ethanolic extract of *L. sativum* seeds, where amino acids class was the most enriched one (19%), followed by glucosinolates (17%), alkaloids (17%), fatty acids (13%), hydantoins (11%) and flavonoids (9%).

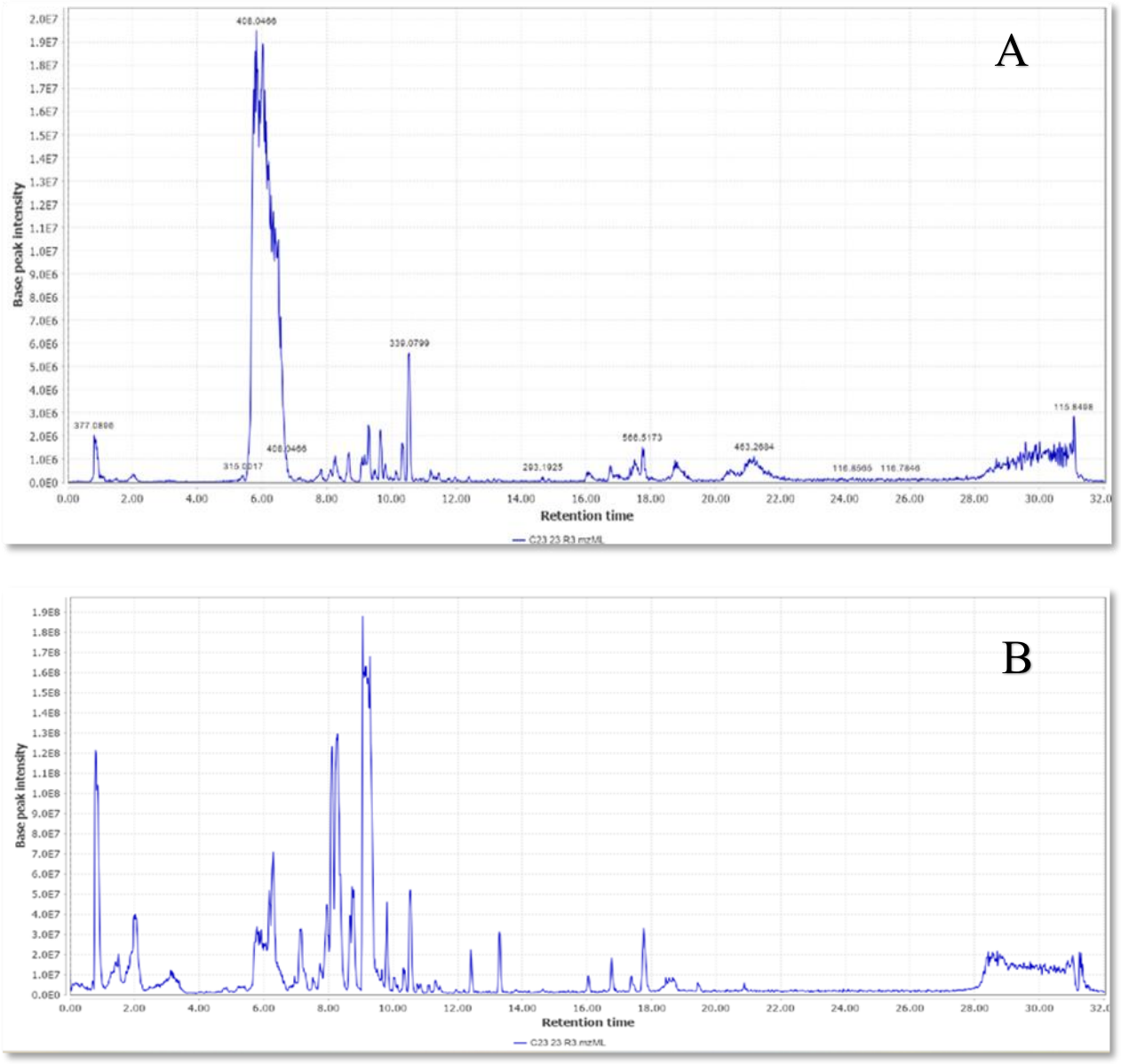

Fig. 1: UPLC-ESI-MS base peak chromatograms of *L. sativum* seeds ethanolic extract .in negative (A) and positive (B) ionization mode.

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Table 1: Identified phytoconstituents in *L. sativum* seed extract by UPLC-ESI-MS in both negative and positive ionization modes.

No.	Rt (min.)	Identified compounds	Molecular ion peak	Molecular Formula	MS/MS Product Ions	Chemical class	Reference
1	0.09	Valine	$[M+H]^+$ 118.15	$C_5H_{11}NO_2$	74	Amino acid	(23)
2	0.75	Cysteine	$[M+H]^+$ 122.16	$C_3H_7NO_2S$	78	Amino acid	(23)
3	0.78	Hexose	$[M+H]^+$ 181.16	$C_6H_{12}O_6$	131, 120, 103	Monosaccharide	(24)
4	0.80	Glycine	$[M+H]^+$ 76.07	$C_2H_5NO_2$	32	Amino acid	(23)
5	0.90	Aspartic acid	$[M-H]^-$ 132.09	$C_4H_7NO_4$	88	Amino acid	(23)
6	0.92	Niacin	$[M+H]^+$ 124.11	$C_6H_5O_2N$	80	Amino acid	(25)
7	1.23	Serine	$[M+H]^+$ 106.09	$C_3H_7NO_3$	62	Amino acid	(23)
8	1.49	Proline	$[M+H]^+$ 116.13	$C_5H_9NO_2$	72	Amino acid	(23)
9	2.02	Semilepidinoside A	$[M+H]^+$ 337.35	$C_{16}H_{20}N_2O_6$	175	Glycoalkaloid	(14)
10	2.02	Rosmarinic acid	$[M-H]^-$ 359.29	$C_{18}H_{16}O_8$	197, 179	Phenolic acid	(26)
11	5.40	Glucotropeolin	$[M+H]^+$ 410.40	$C_{14}H_{19}NO_9S_2$	327, 309, 224, 165	Glucosinolate	(27)
12	5.82	Benzyl glucosinolate	$[M-H]^-$ 408.42	$C_{14}H_{18}NO_9S_2$	276, 242	Glucosinolate	(28)
13	6.50	Apetalumoside A	$[M-H]^-$ 801.67	$C_{34}H_{42}O_{22}$	639, 477, 315	Flavonoid glycoside	(29)
14	6.94	Macaridine	$[M+H]^+$ 216.25	$C_{13}H_{13}NO_2$	198, 169, 152	Alkaloid	(24)
15	7.18	Lepidine B/E/F	$[M+H]^+$ 347.39	$C_{20}H_{18}N_4O_2$	174	Alkaloid	(14)
16	7.24	Benzyl isothiocyanate	$[M+H]^+$ 150.22	C_8H_7NS	92	Isothiocyanate	(30)
17	7.29	Acetylbenzylamide	$[M+H]^+$ 164.17	$C_9H_9NO_2$	105, 77	Amide	(31)
18	7.75	Dithiohexoside	$[M-H]^-$ 389.42	$C_{12}H_{22}O_{10}S_2$	227, 195, 91	Thioglycoside	(32)
19	8.24	Lepidine AK	$[M+H]^+$ 361.42	$C_{21}H_{20}N_4O_2$	188	Alkaloid	(14)
20	8.25	Lepidimoic acid	$[M-H]^-$ 321.26	$C_{12}H_{18}O_{10}$	276	Disaccharide	(32)
21	9.27	Macathioamide A	$[M-H]^-$ 283.37	$C_{16}H_{16}N_2OS$	240, 212, 168	Thioamide	(33)
22	9.30	Kaempferol-7-hexoside	$[M+H]^+$ 433.38	$C_{21}H_{20}O_{10}$	271, 151, 133	Flavonoid	(26)
23	9.56	Meyeniin C	$[M+H]^+$ 309.42	$C_{14}H_{16}N_2O_2S_2$	265, 235, 185	Thiohydantoin	(24)
24	9.66	Apigenin-7-hexoside	$[M+H]^+$ 433.40	$C_{21}H_{20}O_{10}$	271, 227	Flavonoid glycoside	(26)

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25	10.12	Glucobrassicanapin	[M-H]$^-$ 385.39	$C_{12}H_{20}NO_9S_2$-	97	Glucosinolate	(34)
26	10.34	Gallocatechin	[M+H]$^+$ 307.27	$C_{15}H_{14}O_7$	179, 125	Flavonoid	(35)
27	10.36	Cyclomethyltryptophan	[M+H]$^+$ 217.24	$C_{12}H_{12}N_2O_2$	144, 113	Amino acid	(24)
28	10.51	Sinapoyl malate	[M-H]$^-$ 339.27	$C_{15}H_{16}O_9$	223	Cinnamic acid derivative	(36)
29	10.52	Glucobrassicin	[M-H]$^-$447.46	$C_{16}H_{20}N_2O_9S_2$	367, 206	Glucosinolate	(37)
30	10.84	Macahydantoin A	[M+H]$^+$ 261.35	$C_{14}H_{16}N_2OS$	184, 105	Thiohydantoin	(24)
31	11.31	Macahydantoin C	[M+H]$^+$ 247.26	$C_{13}H_{14}N_2O_3$	170, 107	Hydantoin derivatives	(24)
32	13.29	Macahydantoin D	[M+H]$^+$ 231.26	$C_{13}H_{14}N_2O_2$	154, 91	Hydantoin derivatives	(24)
33	16.78	Meyeniihydantoin A	[M+H]$^+$ 261.29	$C_{14}H_{16}N_2O_3$	230, 184, 121	Hydantoin	(24)
34	17.37	4-Methoxyglucobrassicin	[M+H]$^+$ 479.50	$C_{17}H_{22}N_2O_{10}S_2$	399, 237	Glucosinolate	(37)
35	17.43	3,4,5-Trimethoxybenzyl glucosinolate	[M+H]$^+$ 500.52	$C_{17}H_{25}NO_{12}S_2$	482, 420, 258	Glucosinolate	(37)
36	21.16	Myristoleic acid	[M-H]$^-$ 225.34	$C_{14}H_{26}O_2$	181	Fatty acid	(38)
37	22.87	Alpha-linolenic acid	[M-H]$^-$ 277.40	$C_{18}H_{30}O_2$	233	Fatty acid	(38)
38	31.28	9,11,14-Eicosatrienoic acid	[M-H]$^-$ 301.25	$C_{20}H_{34}O_2$	257	Fatty acid	(38)
39	31.45	Stearic acid	[M-H]- 283.50	$C_{18}H_{36}O_2$	239	Fatty acid	(38)
40	31.45	Methyl isoheptadecanoate	[M+H]$^+$ 285.50	$C_{18}H_{36}O_2$	254, 252, 74	Fatty acid ester	(32)
41	31.66	Nervonic acid	[M-H]$^-$ 365.60	$C_{24}H_{46}O_2$	321	Fatty acid	(38)

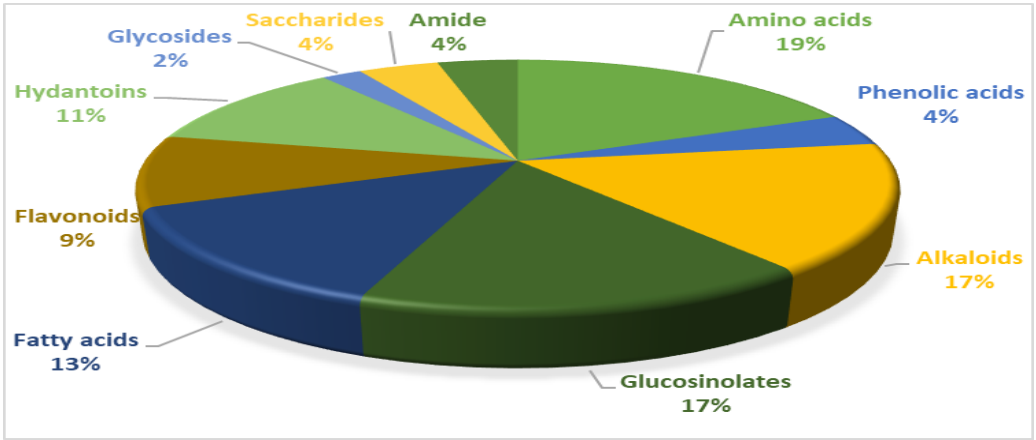

Fig. 2. A pie chart demonstrating the distribution of different phytochemical classes in ethanolic extract of *L. sativum.*

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3.1. Characterization of amino acids

Amino acids constitute a vast classification of compounds as well they manage the chemical composition of *Lepidium* extract by a percentage reaching 39% signified as essential and non-essential amino acids [39]. So *L. sativum* complete seeds are highly sponsored by researchers for the achievement of wellness owing to their high nutritional value [1].

Through our present analysis, we could identify 8 amino acids. Among them, compounds **(1, 4, 5, 6, 7 and 8)** were positively identified as valine, glycine, aspartic acid, niacin, serine and proline, respectively by inferencing to literature data with a $[M+H]^+$ at m/z 118.15, 76.07, 124.11, 106.09 and 116.13 for valine, glycine, niacin, serine and proline, respectively and clear fragment peaks at m/z 74, 32, 80, 62 and 72, respectively indicating that they all share the loss of 44 Da that symbol to CO_2. For compound **(5)**, serine showed a $[M-H]^-$ peak at m/z 132.09 and a fragment at m/z 88 showing the loss of CO_2 corresponding to 44 Da [23].

3.2. Characterization of fatty acids

Fatty acids represent a broad class of compounds and are symbolized in saturated and unsaturated forms. It is demonstrated that these components employ an important action in controlling different inflammatory diseases [40].

In our analysis, we could identify five fatty acids and one fatty acid ester in *L. sativum* extract. One of them, compound **(36)** is confirmed to be myristoleic acid, by referring to literature data with a molecular ion peak of $[M-H]^-$ at m/z 225.34 and a fragment at m/z 181 explained by loss of CO_2 [38]. In addition, compounds **(37, 38, 39 and 41)** were proved to be alpha-linolenic acid, 9,11,14-eicosatrienoic acid, stearic acid and nervonic acid, respectively. They have been undertaken in the same manner of fragmentation pattern with a molecular ion peak of $[M-H]^-$ at m/z 277.40, 301.25, 283.50 and 365.60, respectively. They gave intense fragment peaks at m/z 233, 257, 239 and 321, respectively with the loss of 44 Da represented in CO_2 [38].

Compound **(40)** is believed to be methyl isoheptadecanoate, as it evoked a molecular ion peak of $[M+H]^+$ at m/z 285.50 and a fragment ion peak at m/z 254 due to the loss of (OCH_3) moiety. Besides, it undergoes McLafferty rearrangement to give its recognizable base peak at m/z 74 as a presence of $[C_2H_5COOH]^+$ and another one at m/z 252 as a presence of $[C_{16}H_{32}CO]^+$ moiety [32].

3.3. Characterization of glucosinolates

Glucosinolates represent a major group of characteristic compounds in *L. sativum* extract, including glucotropeolin, benzyl glucosinolate, glucobrassicanapin, glucobrassicin, 4-methoxyglucobrassicin and 3,4,5-trimethoxybenzyl glucosinolate. According to literature, glucosinolates have a high antioxidant action through chasing free radicals [28]. New studies discovered that they are responsible for cancer cells suicidal action [41].

In current study, compounds **(29)** and **(34)** with a $[M-H]^-$ at m/z 447.46 and $[M+H]^+$ at m/z 479.50 were evidently characterized as glucobrassicin and 4-methoxyglucobrassicin, respectively by comparing with literature data [37]. Their spectra provided typical fragment ion peaks at m/z 367 and 399, respectively due to early loss of SO_3 (80 Da) and additional fragment ion peak at m/z 206 and 237, respectively owing to loss of (162 Da) as aglycone part. In addition, compound **(12)** with $[M-H]^-$ at m/z 408.42 and fragmentation ion peaks at m/z 276 and 242 indicating ($C_6H_{12}O_8S_2$) and ($C_6H_{10}O_8S$) moieties, respectively [37].

Compound **(25)** with a $[M-H]^-$ at m/z 385.39 and a fragment ion peak at m/z 97, matches the HO_4S^{-1} moiety, a characteristic ion fragment which reflects the common

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fragmentation manner of glucosinolates. This pattern gives an indication to glucobrassicanapin [34]. Related to compound **(35)**, the spectrum shows $[M+H]^+$ at m/z500.52 and product ions at m/z 482 due to a neutral loss of H_2O by 18 Da, at m/z 420 corresponding to the loss of SO_3 by 80 Da and at m/z 258 related to the subsequent loss of glucose moiety. So this compound could be recognized as 3,4,5 trimethoxybenzylglucosinolate [37].

3.4. Characterization of alkaloids

Alkaloids are among the most important phytochemicals that have diverse therapeutic actions. *L. sativum* extract is distinguished by having a large set of alkaloids as lepidines [1] which are responsible for anti-inflammatory effect of *L. sativum* extract [42].

The LC-MS analysis of *L. sativum* extract revealed the following alkaloids: macaridine, lepidine B/ E/ F and lepidine AK, as well as the glycoalkaloid semilepidinoside A. The chromatograms showed compound **(14)** with a molecular ion $[M + H]^+$ at m/z 216.25 and fragment ion peaks at m/z 198 and m/z 169 as a result of the loss of an H_2O molecule then HCO group, correspondingly. The extra neutral loss of the NH_3 group from the later ion (m/z 169) produced the fragment at m/z 152. Moreover, the dissection of C_6H_5, $C_9H_6NO_2$, C_7H_7, and $C_7H_7NO_2$ groups from the initial ion was noticed [24] which proves the compound is macaridine. The identified alkaloids included a glycoalkaloid compound **(9)** which exerts a molecular ion peak of $[M + H]^+$ at m/z 337 and showed further predominant fragment ion peak at m/z 175 by loss of 162 Da which is indicative of hexosyl moiety and confirming its elucidation as semilepidinoside A [14]. Furthermore, a molecular ion peak of $[M + H]^+$ at m/z 347 and a fragment ion peak at m/z 174, proof of loss of 173 Da which is indicative of ($C_{10}H_9N_2O$) moiety and compound **(15)** is claimed to be lepidine B/ E / F [14]. Besides, a molecular ion peak of $[M + H]^+$ at m/z 361.41 and a fragmentation ion peak at m/z 188 that indicate the loss of 173 Da representing the elution of ($C_{10}H_9N_2O$) group and compound **(19)** is likely to be lepidine AK [14].

3.5. Characterization of hydantoins derivatives

Three hydantoin derivatives were detected in our analysis as compound **(31),** macahydantoin C, exhibits a molecular ion peak in the positive ion mode as $[M + H]^+$ at 247.26. Spectral data showed fragments at m/z 170 and 107, proofing loss of C_6H_5 and $C_6H_8N_2O_2$ groups, respectively [24]. In addition, compound **(32)** is recognized as macahydantoin D with a molecular ion peak of $[M+H]^+$ at m/z 231.26 and a predominant fragment ion peaks at 154 and 91, indicating loss of $C_6H_6N_2O_3$ and $C_6H_8N_2O_2$ groups, respectively [24]. Compound **(33)**, meyeniihydantoin A, appeared in the positive ion mode of $[M + H]^+$ at m/z 261.29. The chromatogram showed fragments at m/z 230, 184 and 121, indicating loss of OCH_3, C_6H_5 and $C_6H_8N_2O_2$ groups, respectively [24]. Compound **(30)** is known to be macahydantoin A, a thiohydantoin derivative, that has been detected in positive ionization mode with a $[M + H]^+$ at m/z 261.35. In the MS/MS analysis of macahydantoin A, the characteristic fragment ions at m/z 184 and 105 are due to the neutral loss of C_6H_5 (77 Da) and $C_6H_8N_2OS$ (156 Da) [24]. Compound **(23)** is recognized to be meyeniin C, a thiohydantoin derivative, that has been detected in positive ionization mode as $[M + H]^+$ at 309.42. The MS/MS analysis showed the fragment ions at m/z 265, 235 and 185 were produced as neutral loss of the following groups, C=S, C_3H_6S and C_5H_2NOS, respectively [24].

3.6. Characterization of flavonoids

Compound **(22),** kaempferol-7-hexoside, produced a molecular ion peak of $[M+H]^+$ at m/z 433.38 with a fragment ion peak at m/z 271 which indicated the loss of sugar part

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($C_6H_{10}O_5$). Further fragmentation to kaempferol part gives two peaks at 151 and 133 as a result of Retro Diels-Alder reaction (RDA) [43], [26].

Compound **(24),** apigenin-7-hexoside, showed a molecular ion peak of $[M+H]^+$ at m/z 433.40 and produced a fragmentation ion peak at m/z 271 as it represents the loss of sugar part ($C_6H_{10}O_5$) by 162 Da. Another fragment ion peak appeared at m/z 227 due to the loss of CO_2 by 44 Da [26].

Compound **(26),** gallocatechin, exhibited a molecular ion peak in the positive mode of ionization of $[M+H]^+$ at m/z 307.27 and a base peak at m/z 125 as a proof of pyrogallol moiety ($C_6H_5O_3$) presence and another fragment ion peak at m/z 179 as a result of ($C_9H_7O_4$) moiety [35].

Compound **(13)** is identified as apetalumoside A, evoked a molecular ion peak of $[M-H]^-$ at m/z 801.67 and a fragmentation ion peak at m/z 639 as a result of glucose moiety loss. Another two fragment peaks at m/z 477 and 315 due to (RDA) reaction [29].

3.7. Characterization of miscellaneous compounds

Compound **(10)**, rosmarinic acid, with a $[M-H]^-$ at m/z 359.29 fragment ion peaks at m/z 197 and 179 of the two main components of rosmarinic acid: 2-OH derivative of hydro caffeic acid and caffeic acid, respectively [26].

In the analysis, compound **(16)** produced a molecular ion peak of $[M+H]^+$ at m/z 150.22 and a predominant fragment ion at m/z 92 as a result of the loss of SCN group. According to the literature, the compound is benzyl isothiocyanate [30].

Compound **(17),** acetylbenzylamide, produced a molecular ion peak in positive ionization mode of $[M+H]^+$ at m/z 164.17 and it lost NH_2 group, resulting in stable benzoyl cation ($C_6H_5CO^+$) with a fragment ion peak at m/z 105 which then went through further fragmentation to the phenyl moiety ($C_6H_5^+$) with fragment ion peak at m/z 77 [31].

Compound **(28),** sinapoyl malate, showed a molecular ion peak of $[M - H]^-$ at m/z 339.07 and the fragment mass at m/z 223 indicating the presence of sinapic acid with a 116 Da loss as characteristic malic acid [36].

Compound **(20)** is identified as lepidimoic acid, as it has produced a molecular ion peak of $[M-H]^-$ at m/z 321.26 and a fragmentation ion peak at m/z 276 as a result of 45 Da loss, indicating the departure of (COOH) group [32].

Compound **(21)** is recognized as macathioamide A, as it produced a molecular ion peak of $[M-H]^-$at m/z 283.37 and a fragment ion peak appeared at m/z 240 due to the loss of (CONH) group, further fragmentation occurred and a peak appeared at m/z 212 as a result of C=O loss by 28 Da, followed by successive loss of C=S moiety by 44 Da and the peak appeared at m/z 168 [33].

4. Conclusion

With the aid of UPLC-ESI-MS, we could identify 41 different phytoconstituents in *L. sativum* seeds extract diverting between fatty acids, amino acids, alkaloids, glucosinolates and flavonoids and could identify their mass fragmentation patterns in both positive and negative modes. It is worth mentioning that the current study is the first one to confirm the presence of hydantoins and thiohydantoins in *L. sativum* species. This extensive chemical profiling gives the basis for upcoming research to experiment with the action of *L. sativum* seed extract on other health-controlling effects on both in-vivo and in-vitro trials and to provide more undoubted verification for its effectiveness.

Authors' contributions

Sarah A. Elsayed: experimental, original draft writing and editing.

Eman Shawky: visualization, editing, reviewing and approving the final manuscript.

El Moataz Bellah El Naggar: editing and reviewing and approving the final manuscript.

Reham S. Ibrahim: visualization, editing, reviewing and approving the final manuscript.

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Data availability
All analyzed data in this study are available upon e-mailing the authors.

Ethical approval and consent to participate
Not applicable

Highlights

- *Lepidium sativum* is a perfect candidate to treat multiple diseases.
- UPLC-ESI-MS technique was used to separate and identify phytochemicals in the extract.
- Hydantoins such as macahydantoin A, C, and D are characteristic compounds of *Lepidium*.
- Five chemical classes were detected as amino acids, glucosinolates, and fatty acids.

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