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Original Article

The chemical composition of compounds in *Lepidium sativum* seeds, sourced from two different origins and extracted using two non-polar solvents, and analyzed using GC/MS

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Abstract

The objective of this research was to explore the compounds present in the n-hexane and petroleum ether extracts of Lepidium sativum seeds obtained from two different sources (Sudan and Saudi Arabia) using Gas Chromatography–Mass Spectroscopy (GC/MS) technique. The qualitative and quantitative analysis of the samples was carried out using Gas Chromatography/Mass Spectrometry (GC/MS). The major constituents identified in the n-hexane extract of the Sudan sample were cis-vaccenic acid (17.03%), 9-octadecenamide, (Z)- (13.03%), gamma-sitosterol (10.14%), beta-tocopherol (7.845%), o-cymene (4.30%), and 9-octadecenoic acid, 1,2,3-propanetriyl ester (4.091%). The major phytochemical constituents identified in the petroleum ether extract of the Sudan sample were gamma-sitosterol (14.09%), beta-tocopherol (12.10%), 9-octadecenoic acid, methyl ester (11.82%), 9,12-octadecadienoic acid (Z,Z), methyl ester (8.40%), and tributyl acetyl citrate (8.26%). The major phytochemical constituents identified in the n-hexane extract of the Saudi sample were

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gamma-sitosterol (13.38%), beta-tocopherol (10.92%), 4-fluorobenzaldehyde (6.26%), octadecanoic acid (6.21%), butylphosonic acid, di(4-octyl) ester (6.17%), and campesterol (6.00%). The major phytochemical constituents identified in the petroleum ether extract of the Saudi sample were gamma-terpinene (26.86%), o-cymene (25.52%), thymol (12.76%), beta-tocopherol (6.88%), beta-pinene (6.01%), and gamma-sitosterol (5.73%).

Keywords: GC/MS analysis, chemical composition, Lepidium sativum, n-hexane and petroleum ether extracts, Sudan, Saudi Arabia.

Introduction

Historically, Lepidium sativum seeds have served various medicinal purposes. They have been utilized as aperients, diuretics, tonics, demulcents, carminatives, galactagogues, and emmenagogues. Moreover, they were prescribed for ailments like throat diseases, uterine tumors, nasal polyps, breast cancer, and to promote lactation in postnatal women. Externally, the seeds were applied as poultices for pain relief, sprains, and treatment of bacterial and fungal infections. Traditional medicinal systems including Unani, Saudi, and Turkish folk medicine have integrated Lepidium sativum for its purported efficacy in treating inflammation, bronchitis, rheumatism, and improving digestion [1].

Lepidium sativum boasts a rich assortment of bioactive compounds, including cardiac glycosides, alkaloids, phenolics, flavonoids, cardiotonic glycosides, coumarins, glucosinolates, carbohydrates, proteins, amino acids, mucilage, resins, saponins, sterols, tannins, volatile oils, triterpenes, sinapic acid, and uric acid. Its extensive pharmacological exploration has unveiled a diverse therapeutic spectrum, encompassing antimicrobial, antidiabetic, antioxidant, anticancer, reproductive, gastrointestinal, respiratory, anti-inflammatory, analgesic, antipyretic, cardiovascular, hypolipidemic, diuretic, central nervous system, and fracture healing properties [1]. GC-MS analysis of Lepidium sativum seed oil from Saudi Arabia reveals numerous components, including β-amyrine, octadecatrienoic acid, octadecenoic acid methyl ester, α-amyrine, eicosenoic acid, and methyl stearate, underscoring its complexity and potential pharmacological significance [2]. Studies from different regions such as Saudi Arabia, India, Egypt, and Iraq consistently highlighted significant phenolic and flavonoid content in various parts of L. sativum, underlining its potential as a source of biologically active compounds. Analysis of L. sativum extracts revealed a diverse range of compounds like glucotropaeolin, sinapine, benzyl isothiocyanate, and

others, with variations observed across geographical regions. Additionally, fatty acid profiling showcased varying percentages of palmitic acid, oleic acid, linoleic acid, and other fatty acids, indicating the nutritional diversity of L. sativum [3]. Isoflavonoids isolated from Lepidium sativum demonstrated hepatoprotective effects against paracetamol-induced toxicity in male rats. Investigations into central nervous system (CNS) activity involved studying the effects of total alkaloids extracted from Lepidium sativum seeds at different doses on various parameters in mice and rats [4].

Material and Methods

Plant material

The plant under investigation (Samples from two sources Omdurman and AL-Madina local markets) were authenticated at the Department of Botany by one author, Prof. Hatil, Hashim ELKamali, Omdurman Islamic University.

Preparation of crude plant extracts

The dried plant material was ground into coarse powder using mortar and pestle. One hundred and fifty grams from the powder were soaked in n-hexane and petroleum ether for three days and then filtered and then weighed and stored.

GC/MS analysis [5]

The qualitative and quantitative analysis of the sample was carried out by using GC/MS technique model (GC/MSQP2010-Ultra) from Japan's Shimadzu Company, with serial number 020525101565SA and capillary column (Rtx-5ms-30mX0.25 mmX0.25um). The sample was injected by using split mode, helium as the carrier gas passed with flow rate 1.61 ml/min, the temperature program was started from 60c with rate 10c/min to 300c as final temperature degree with 5 minutes hold time, the injection port temperature was 300c, the ion source temperature was 200 ℃ and the interface temperature was 250℃. The sample was analyzed by using scan mode in the range of m/z 40-500 charges to ratio. Identification of the sample components was achieved by computer searches in commercial library, the National Institute of Standards and Technology (NIST).

Results and Discussion

GC/MS analysis of *Lepidium sativum* L. (Sudan sample, Hexane extract)

GC/MS chromatogram of n-hexane extract of *L. sativum* seeds (Sudan sample, Hexane extract) (Figure 1 (a)) showed the presence of 32 peaks (compounds) at retension times of 3.436 – 26.751 min. The compounds : cis-vaccenic acid (17.03%), 9-octadecenamide , (Z)- (13.03%) , Gamma-sitosterol (10.14%), Beta-tocopherol (7.845), o-cymene (4.30%) , 9-octadecenoic acid, 1,2,3-propanetriyl ester (4.091%) were found as the major phytochemical constituents.

GC/MS analysis of *Lepidium sativum* L. (Sudan sample, Petroleum ether extract)

GC/MS chromatogram of petroleum ether extract of *L. sativum* seeds (Sudan sample, Petroleum ether extract) (Figure 1 (b)) showed the presence of 30 peaks (compounds) separated at 4.323 – 38.032 min. The compounds : Gamma-sitosterol (14.09%), beta-tocopherol (12.10%), 9-octadecenoic acid, methyl ester (11.82%), 9,12-octadecadienoic acid (Z,Z), methyl ester (8.40%) , tributyl acetyl citrate (8.26%) were found as the major phytochemical constituent.

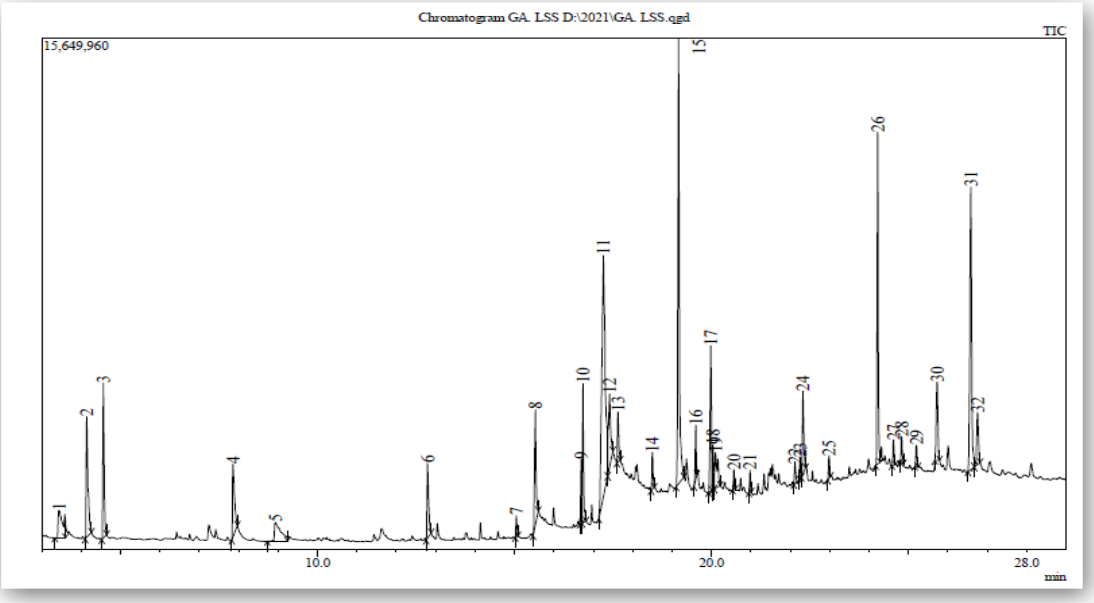

Figure 1 (a): The GC/MS chromatogram of n-hexane extract of *L. sativum* (Sudan

sample, Hexane extract).

Benzaldehyde, 4-fluoro- (3.436 min)[1], o-Cymene (4.138min)[2], gamma.-Terpinene (4.560 min)[3], thymol (7.852 min)[4], Benzene, (isothio cyanatomethyl)- (8.926 min)[5], Butylphosphonic acid, di(4-octyl) ester (12.791 min)[6], Hexadecanoic acid, methyl ester (15.044 min)[7], n-Hexadecanoic acid (15.528 min)[8], 9,12-Octadecadienoic acid (Z,Z)-, methyl ester (16.690 min)[9], 11-Octadecenoic acid, methyl ester (16.735 min)[10], cis-vaccenic acid (17.254 min)[11], Octadecanoic acid (17.412 min)[12], Hexadecanamide (17.622 min)[13], Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanedi1y.ester (18.496 min)[14], 9-Octadecenamide, (Z)- (19.161 min)[15], Oleoyl chloride (19.597 min)[16], 9-Octadecenoic acid, 1,2,3-propanetriyl ester,(E,E, E1)9- (19.978 min)[17], 9,12,15-Octadecatrienoic acid, (Z,Z,Z)- (20.032 min)[18], Decane, 1,9-bis[(trimethylsilyl)oxy]- (20.098 min)[19], Diisooctyl phthalate (20.563 min)[20], Unidentified (20.975 min)[21], 9-Octadecen-1-ol, (Z)- (22.110 min)[22], 18.alpha.-Olean-3.beta.-ol, acetate (22.248 min)[23], Unidentified (22.319 min)[24], Heneicosane (22.982 min)[25], beta-tocopherol (24.214 min)[26], Stigmastan-3,5-diene (24.618 min)[27],17-(1,5-Dimethylhexyl)-10,13-dimethyl-tetrad0ec.6a1hydro-1H cyclopenta[a] phenanthren- (24.822 min)[28], Triacontyl acetate (25.196 min)[29], Campesterol (25.723 min)[30], Gamma-sitosterol (26.578 min)[31], Fucosterol (26.752 min)[32].

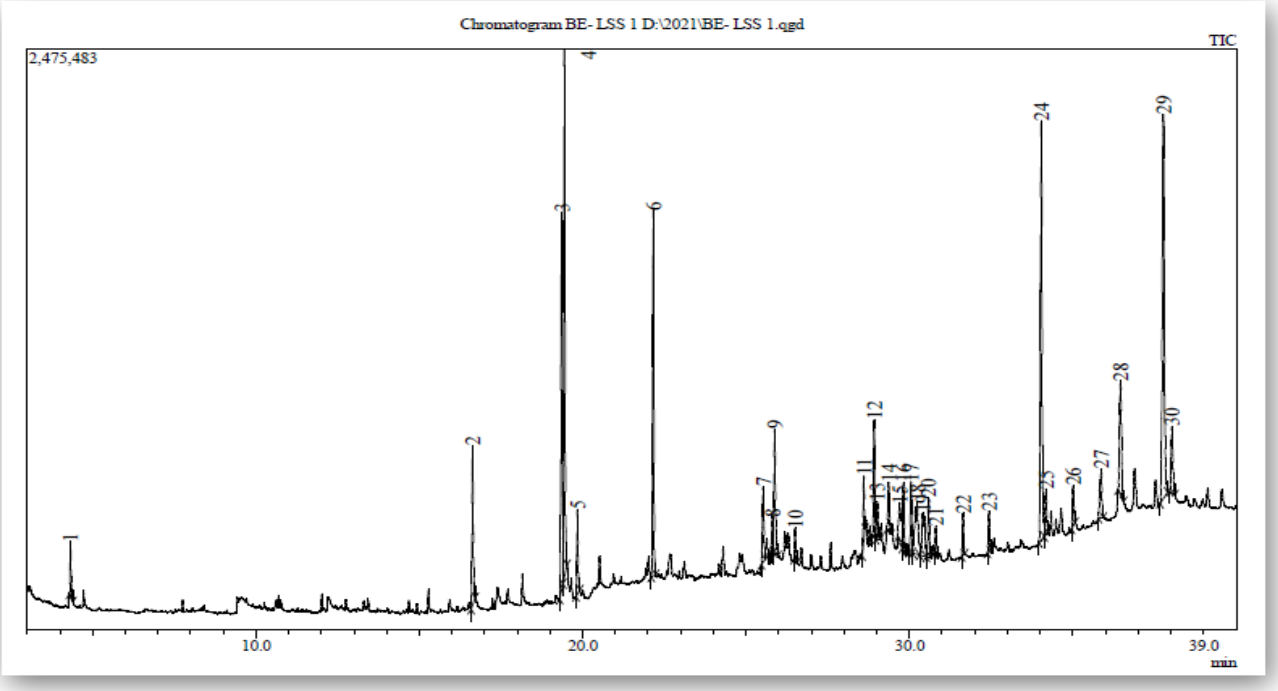

Figure 1 (b): The GC/MS chromatogram of petroleum ether extract of *L. sativum* (Sudan sample, Petroleum ether extract).

D-Limonene (4.323 min)[1], Hexadecanoic acid, methyl ester (16.629 min)[2], 9,12-Octadecadienoic acid (Z,Z)-, methyl ester (19.354 min)[3], 9-Octadecenoic acid, methyl ester, (E)- (19.439 min)[4], Methyl stearate (19.836 min)[5], Tributyl acetylcitrate (22.161 min)[6], 1,3-Dipalmitin trimethylsilyl ether (25.521 min)[7], Decane, 1,9-bis[(trimethylsilyl)oxy]- (25.800 min)[8], Decanoic acid, 2-ethylhexyl ester (25.874 min)[9], Unidentified (26.502 min)[10], Unidentified (28.603 min)[11], Octadecanoic acid, octyl ester (28.918 min)[12], Unidentified (29.013 min)[13], 1,2-Cyclohexanedicarboxylic acid, cyclohexylmeth y2l9 n.3o6n6y l ester (29.366 min)[14], Unidentified (29.690 min)[15], Unidentified (29.815 min)[16], Unidentified (30.047 min)[17], Sulfurous acid, octadecyl 2-propyl ester (30.233 min)[18] , 1,2-Cyclohexanedicarboxylic acid, dinonyl ester (30.404 min)[19], Cyclononasiloxane, octadecamethyl- (30.581 min)[20], Unidentified (30.808 min)[21], Nonacosane (31.639 min)[22],

Unidentified (32.433 min)[23], .beta.-Tocopherol (34.035 min)[24], Tetracosamethyl-cyclododecasiloxane (34.177 min)[25], 17-(1,5-Dimethylhexyl)-10,13-dimethyl-2,3,4,7,8,9 ,3150.,0110,12,13,14,1 554,1563,8127 -tetrad 1ec.0a9h ydro-1H-cyclopenta[a]phenanthren- (35.010 min)[26], Octacosyl acetate (35.866 min)[27], Campesterol (36.460 min)[28], .gamma.-Sitosterol (37.769 min)[29], Fucosterol (38.032 min)[30]

GC/MS analysis of *Lepidium sativum* L. (Saudi sample, Hexane extract)

GC/MS chromatogram of n-hexane extract of *L. sativum* seeds (Saudi sample, Hexane extract) (Figure 2 (a)) showed the presence of 32 peaks (compounds) at retention times of 3.459 – 26.723 min. The compounds : Gamma-sitosterol (13.38%), beta-tocopherol (10.92%), 4-fluro-benzaldehyde (6.26%), octadecanoic acid (6.21%), Butylphosonic acid , di(4-octyl) ester (6.17%) and campesterol (6.00%) were found as the major phytochemical constituents.

GC/MS analysis of *Lepidium sativum* L. (Saudi sample, Petroleum ether extract)

GC/MS chromatogram of petroleum ether extract of *L. sativum* seeds (Saudi sample, Petroleum ether extract) (Figure 2 (b)) showed the presence of 21 peaks (compounds) separated at 3.115 – 38.197 min. The compounds : Gamma-terpinene (26.86%), o-cymene (25.52%) , thymol (12.76%) , beta-tocopherol (6.88%), beta-pinene (6.01%), Gamma-sitosterol (5.73%) were found as the major phytochemical constituents.

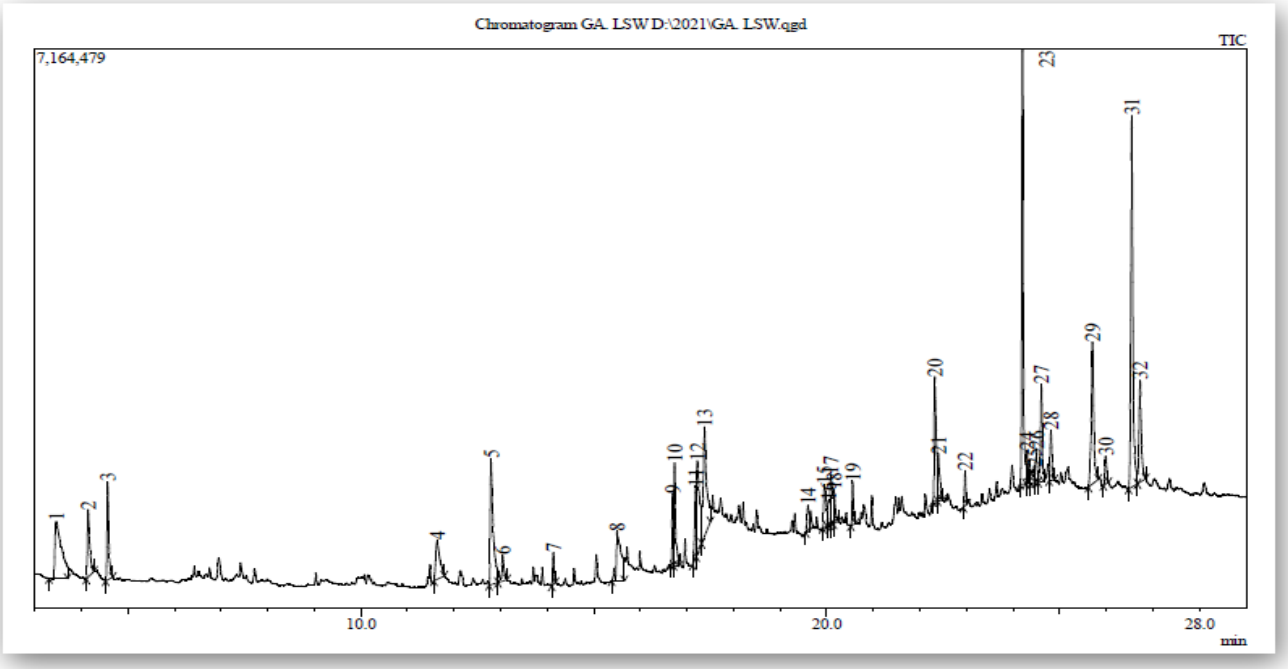

Figure 2 (a): The GC/MS chromatogram of n-hexane extract of *L. sativum* (Saudi sample, Hexane extract)

Benzaldehyde, 4-fluoro- (3.459 min)[1], o-cymene (4.143 min)[2], Gamma-Terpinene (4.562 min)[3], Diethylphthalate (11.632 min)[4], Butylphosphonic acid, di(4-octyl) ester (12.790 min)[5], Cyclohexanone, 5-butyl-3,3,5-trimethyl- (13.041 min)[6], Phytol, acetate (14.179 min)[7], n-Hexadecanoic acid (15.498 min)[8], Methyl 10-trans,12-cis-octadecadienoate (16.687 min)[9], 11-Octadecenoic acid, methyl ester, (Z)- (16.731 min)[10], cis-9-Hexadecenoic acid (17.180 min)[11], 3-Tetradecyn-1-ol (17.221 min)[12], Octadecanoic acid (17.374 min)[13], Oleoyl chloride (19.595 min)[14], 1,3-Dipalmitin trimethylsilyl ether (19.948 min)[15], 9,12,15-Octadecatrien-1-ol, (Z,Z,Z)- (20.035 min)[16], Decane, 1,9-bis[(trimethylsilyl)oxy]- (20.087 min)[17], Octadecanoic acid, 2-hydroxy-1,3-propanediyl ester (20.153 min)[18], Diisooctyl phthalate (20.554 min)[19], 9-Octadecenamide, (Z)- (22.317 min)[20], Unidentified (22.395 min)[21], Heneicosane (22.968 min)[22] , .beta.-

Tocopherol (24.198 min)[23], Tetracontane, (24.298 min)[24] Obscurinervan-21-one, 6,7-didehydro-16-methoxy- 2224-.m44e5th yl-, (22.a 8lp5h2a8.0)- (24.445 min)[25], Lup-20(29)-en-3-ol, acetate, (3.beta.)- (24.495 min)[26], Stigmastan-3,5-diene (24.604 min)[27], 17-(1,5-Dimethylhexyl)-10,13-dimethyl-hydro-1H-cyclopenta[a]phenanthren- (24.810 min)[28], Campesterol (25.702 min)[29], Stigmasterol (25.984 min)[30], Gamma-sitosterol (26.545 min)[31], Fucosterol (26.723 min)[32].

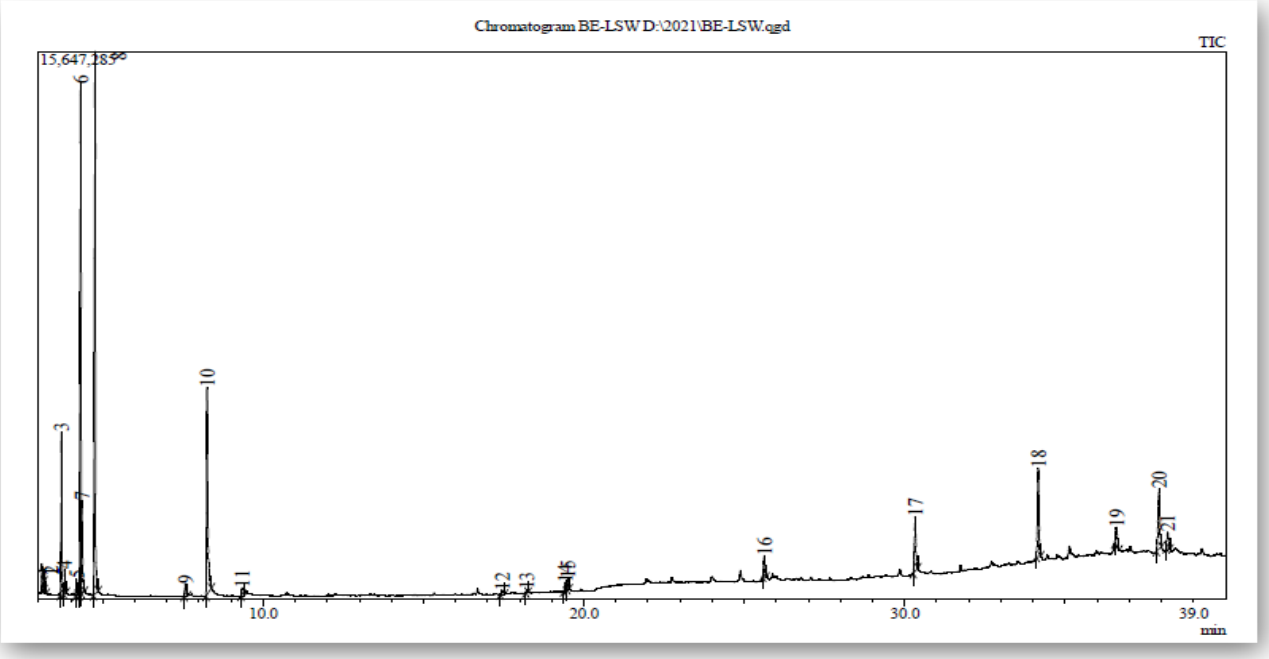

Figure 2 (b): The GC/MS chromatogram of petroleum ether extract of *L. sativum* (Saudi sample, Petroleum ether extract)

Bicyclo [3.1.0]hex-2-ene, 2-methyl-5-(1-methylethyl) – (3.115 min)[1], .alpha.-Pinene (3.200 min)[2], .beta.-Pinene (3.710 min)[3], .beta.-Myrcene (3.813 min)[4], (+)-4-Carene (4.185 min)[5], o-Cymene (4.313 min)[6], D-Limonene (4.350 min)[7], .gamma.-Terpinene (4.763 min)[8], Thymoquinone (7.573 min)[9], Thymol (8.252 min)[10], Benzene, (isothio- cyanatomethyl)- (9.351 min)[11], Dibutyl phthalate (17.446 min)[12], Octadecanal (18.218 min)[13], 9,12-

Octadecadienoic acid (Z,Z)-, methyl ester (19.418 min)[14] , 9-Octadecenoic acid, methyl ester, (E)-, (19.501 min)[15] 9-Octadecenoic acid, 1,2,3-propanetriyl ester, (E,E, E2)5- (25.618 min)[16], 13-Docosenamide, (Z)-, (30.324 min)[17] .beta.-Tocopherol (34.164 min)[18], Campesterol (36.598 min)[19], .gamma.-Sitosterol (37.929 min)[20], Fucosterol (38.197 min)[21]..

Gamma-terpinene (p-Mentha-1,4-diene; Crithmen), it shows as strong antioxidant activity. Is widely used in the pharmaceutical, cosmetics, and perfume industry. It possesses anti-inflammatory activity [6]. O-cymene ((2-isopropyltoluene; o-cymol), it uses as a solvent, metal polish additive, and intermediate for resin manufacturing. It used for metal degreasing [7].

Thymol is used as an antioxidant, anti-inflammatory, analgesic, antispasmodic, antibacterial, antifungal, antiseptic, antitumor [8].

Inhibitory effect of beta-pinene on the growth of potential infectious endocarditis causing Gram-positive bacteria [9].

Octadecanoic acid (Stearic acid) is used in oral pharmaceutical formulations, it is mainly used in oral formulations as a tablet lubricant although it may also be used as a binder [10]. It used in the production of detergents, soaps and cosmetics such as shampoos and shaving cream products. Antidabetic and antioxidant agent [11].

Cis-vaccenic acid is a bacterial biomarker – on the origin of cis-vaccenic acid photodegradation products in the marine environment [12].

9-octadecenamide, (Z)- is used as adhesive and sealant chemical lubricants and lubricant assistive processing aids, food packing, fuels and related products, plastic and rubber products and construction materials [13]. 9-octadecenoic acid, 1,2,3-propanetriyl ester is used in cosmetics, as emollient, skin conditioning and viscosity controlling [14].

Table 1 summarizes the major chemical compounds in two different sources (Sudan sample and Saudi sample) extracted by n-hexane and petroleum ether solvents.

Table 1. Major chemical compounds in the *Lepidium sativum* obtained from two different sources extracted by two non-polar solvents

Major compound	**n-Hexane (%)**		**Petroleum ether (%)**	
	Sudan sample	**Saudi Sample**	**Sudan Sample**	**Saudi Sample**
Cis-vaccenic acid	17.03	-	-	-
9-octadecenamide,(Z)-	13.03	4.37	-	-
Gamma-sitosterol	10.14	13.38	14.09	5.73
Beta-tocopherol	7.85	10.92	12.10	6.88
o-cymene	4.30	2.56	-	25.52
9-octadecenoic acid, 1,2,3-propanetriyl ester (E,E,E1)-	4.09	-	-	-
4-fluorobenzaldehyde	-	6.26	-	-
Octadecanoic acid (Stearic acid)	3.36	6.21	1.89	-
Butylphosonic acid, di (4- octyl) ester	2.39	6.17	-	-
campesterol	-	6.00	3.99	1.65
n-hexadecanoic acid (Palimitic acid)	3.66	4.32	-	-
3-tetradecyn-1-ol	-	4.83	-	-
stigmastan-3,5-diene	0.60	2.82	-	-
Fucosterol	1.92	4.11	2.18	1.36
Gamma-terpinene	3.91	-	-	26.86
Thymol	3.06	-	-	12.76
Beta-pinene	-	-	-	6.01
D-limonene	-	-	0.97	4.03
13-docosenamide (Z)-	-	-	-	4.03
9-octadecenoic acid,	-	-	11.82	-

methyl ester (E) (Elaidic acid)				
9,12-octadecadienoic acid (Z,Z), methyl ester (Linoleic acid, methyl ester)	--	-	8.40	-
Tributyl acetyl citrate	-	-	8.26	-
Hexadecanoic acid, methyl ester (Palimatic acid, methyl ester	-	-	3.57	-
9,12,15-Octadecatrienic acid (Z,Z,Z)- Linolenic acid	1.27	0.93	-	-

Major chemical classes in the *Lepidium sativum* obtained from two different sources extracted by two non-polar solvents (Table 2):

(a) n-hexane extract, Sudan sample:

Ten fatty acids compounds were identified in the extract (36.08%), the result revealed that cis-vaccenic acid (17.03%) was found as a major component followed by 9-octadecenoic acid, 1,2,3-propanetriyl ester (E,E,E) (4.09%) and palmitic acid (n-hexadecanoic acid) (3.66%).

Four phytosterol compounds were identified in the extract (15.47%), the result revealed that Gamm-sitosterol (10.14%) was found as a major compound followed by campesterol (2.81%).

Four ester compounds were identified in this extract (3.76%), the result revealed that butyl phosphonic acid, di (4-octyl) ester (2.39%) was found as a major component followed by triacontyl acetate (0.55%). Whereas three terpenes compounds were identified in extract (11.27%), the result revealed that o-cymene (4.30%) was found as a major component followed by Gamma-terpinene (3.91%) and thymol (3.06%).

(b) Petroleum ether extract , Sudan sample:

Eight alkane compounds were identified in petroleum ether extract, Sudan sample (7.52%), the result revealed that cyclononasiloxane, octadecamethyl (1.58%) was formed as major component followed by unidentified compound C18H54O9Si9 (1.45%).

Six fatty acids compounds were identified in this extract (31.63%), elaidic acid, methyl ester (9-octadecenoic acid, methyl ester, E (11.82%) was found as major component followed by linoleic acid, methyl ester (8.40%).

Seven esters compounds were identified in this extract (11.94%), sulfurous acid, octadecyl 2-propyl ester (3.17%) was found as a major component followed by 1,2- cyclohexane dicarboxylic acid, dinonyl ester (2.35%). Whereas three phytosterols compounds were identified (20.26%), Gamma-sitosterol (14.09%) was formed as a major component followed by campesterol (3.99%).

(c) n-hexane extract, Saudi sample:

Eight fatty acids compounds were identified in this extract (19.6%), the result revealed that stearic acid (6.21%) was found as a major component followed by palmitic acid (4.32%).

Five phytosterol compounds were identified in this extract (27.21%), the result revealed that Gamma-sitosterol (13.38%) was found as a major component followed by campesterol (6.00%) and fucosterol (4.11%).

Five ester compounds were identified (12.95%), the result revealed that butyl phosphonic acid, di (4-octyl) ester (6.17%) was found as a major component followed by diethyl phthalate (2.66%).

Four terpenes compounds were identified in this extract (7.05%), the result revealed that Gamma-terpinene (2.91%) was found as a major component followed by o-cymene (2.56%).

(d) Petroleum ether extract, Saudi sample:

Eight terpenes compounds were identified in the petroleum ether extract, Saudi sample (77.01%), the result revealed that Gamma-terpinene (26.86%) was found as a major

component followed by o-cymene (25.52%) and thymol (12.7%).

Three fatty acids compounds were identified in this extract (2.54%), the result revealed that 9-octadecenoic acid 1,2,3-propanetriyl ester (E,E,E 2) (1.43%) was formed as major component. Whereas three phytosterols compounds were identified in this extract (8.74%), Gamma-sitosterol (5.73%) was found as a major component.

Table 2. Major chemical classes in the *Lepidium sativum* obtained from two different sources extracted by two non-polar solvents

Chemical Class	**n-Hexane (%)**		**Petroleum ether (%)**	
	Sudan sample	**Saudi Sample**	**Sudan Sample**	**Saudi Sample**
Terpenes	3(11.27)	4(7.05)	1(0.97)	8(77.01)
Fatty acids	10(36.08)	8(19.6)	6(31.63)	3(2.54)
Alkenes	-	-	-	1(1.58)
Phytosterols	4(15.47)	5(27.21)	3(20.26)	3(8.74)
Alcohols	2(0.83)	1(4.83)	-	-
phenols	-	-	-	1(0.4)
Ketones	-	2(1.97)	-	1(1.01)
Aldehydes	1(2.74)	1(6.26)	-	1(0.15)
Amides	3(16.47)	1(4.37)	-	4(.03)
Vitamin E	1(7.84)	1(10.92)	1(12.10)	1(6.88)
Alkanes	2(2.43)	3(3.32)	8(7.52)	-
Esters	4(3.76)	5(12.95)	6(11.94)	-
Ethers	-	-	1(2.12)	-
Carboxylic acids	-	-	1(8.26)	-

Conclusion

The analysis of n-hexane and petroleum ether extracts of Lepidium sativum seeds

obtained from two different sources (Sudan and Saudi Arabia) in this study is an attempt to gain a better understanding of the primary and secondary metabolites profile, which would be beneficial in evaluating the therapeutic potential of this important, valuable medicinal and nutritive plant, and to develop phytochemical standards for quality control purposes. The present study revealed that the terpenes, fatty acids, and phytosterol derivatives compounds in the studied plant may be explored for manufacturing industrial products.

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