Journal of Pharmacy and Pharmacology 8 (2020) 143-150
doi: 10.17265/2328-2150/2020.05.003

Lactuca sativa Stems as the Source of Bioactive Compounds as well as the Leaves

Hanin Nafed Mughrbi and Abdurazag Abdussalam Auzi

Department of Pharmacognosy, Faculty of Pharmacy, University of Tripoli, Tripoli, Libya

Abstract: The stems of *Lactuca sativa* L. are usually not popular as the leaves in preparing the salads and therefore thrown away. The work is intended to compare the phytoconstituents of the leaves and stems extracts. Gas chromatography-mass spectrometry (GC-MS) is applied for the petroleum ether extracts of the leaf and the stem of the selected varieties (*L. sativa* var. *longifolia* L. and *L. sativa* var. *capitata* L.). A total of fifty-eight compounds were identified and quantified from the studied extracts. These compounds have belonged to various categories such as fatty acid, volatile compounds, phytosterols, triterpenes, diterpenes, vitamin E isomers, and others. Triterpenes with phytosterols represented the superior percentage among other categories. Diterpenes were quantified only in the leaves of the studied varieties. The stems exhibited a higher percentage of phytosterols, triterpenes, and volatile compounds rather than the leaves of each variety.

Key words: Fatty acid, GC-MS, leaves, lettuce, phytosterols, stems, triterpenes.

1. Introduction

The prevailing belief about food is that it is just consumed to meet the body requirements and certainly to dumb the sounds of hunger, but nowadays the consumers come to be more educated about the importance of food for health improvement and disease prevention. Lettuce (*Lactuca sativa*) leaves [1] are one of the most consumed vegetables in the leafy group, while the juicy stems are sometimes thrown away. In comparison to the leaves, limited phytochemical researches have been conducted on the stems. Wherefore, this work was intended to analyze and compare the phytoconstituents of petroleum ether extract of both parts (leaf and stem) of two varieties (*L. sativa* var. *longifolia* L. and *L. sativa* var. *capitata* L.).

2. Materials and Methods

2.1 Plant Material Collection and Identification

Two varieties of lettuce were collected in November 2017 from different markets in Tripoli, Libya. The samples have been authenticated by Dr. Mohamed N. Abuhadra, a plant taxonomist at Department of Botany, Faculty of Science, and University of Tripoli. The identified varieties were *Lactuca sativa* var. *longifolia* L. (romaine) and *Lactuca sativa* var. *capitata* L. (iceberg) with voucher numbers (D_1 68107922, D_2 68107921), respectively.

2.2 Preparation of Petroleum Ether Extract

One hundred (100) g of leaves and stems powders of each *L. sativa* variety were macerated in 500 mL of petroleum ether (40-60 °C) for three days at room temperature. After filtration, the filtrates were concentrated by solvent evaporation at room temperature. The yield of extraction was calculated for each extract then the extracts were stored in umber container at 4 °C for further gas chromatography-mass spectrometry (GC-MS) analysis [2].

2.3 GC-MS Analysis of Petroleum Ether Extracts

Each extract was separated by GC-MS-QP2010 Ultra equipment at Putra Malaysia University, Seri Kembangan, Malaysia with experimental conditions as follows: Rxi-5ms fused silica capillary column (30

Corresponding author: Hanin Nafed Mughrbi, MSc, lecturer, research field: pharmacognosy.

m length × 0.25 mm ID × 0.25 μm thickness) was used. The column temperature was initially maintained at 50 °C for 10 min, then gradually raised to 300 °C at the rate 3 °C /min and held for 10 min at 300 °C. Helium was used as a carrier gas with linear velocity 32.4 cm/sec, pressure 37.1 kPa, and flow rate 0.8 mL/min. One (1) μL of the sample was injected in split mode with split ratio 10:1 and the injection temperature was 250 °C. MS system works in EI mode, with ionization potential 70 eV, ionization source temperature 200 °C, interface temperature 250 °C, and mass range 40-700 m/z. Use a quadrupole mass analyzer with scan mode. The composition of *L. sativa* petroleum ether extracts was identified by matching their mass spectra with libraries spectra (WILEY229, NIST11, and FFNSC1.3). The amount of chemical composition is calculated as a percentage of the relative peak area.

3. Results

The percentage of identified categories in petroleum ether extracts of *L. sativa* are presented in Figs. 1-4.

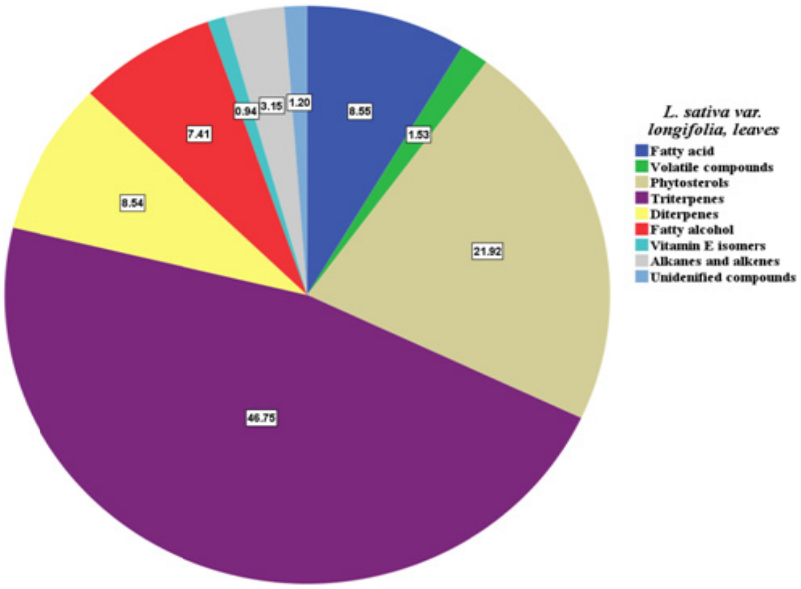

Fig. 1 The phytoconstituent categories in petroleum ether extracts of *L. sativa* var. *longifolia* leaves.

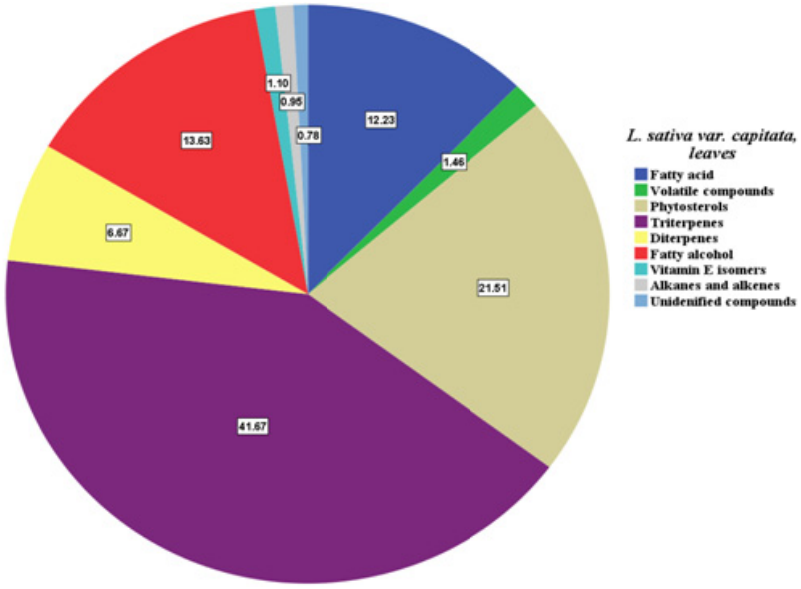

Fig. 2 The phytoconstituent categories in petroleum ether extracts of *L. sativa* var. *capitata* leaves.

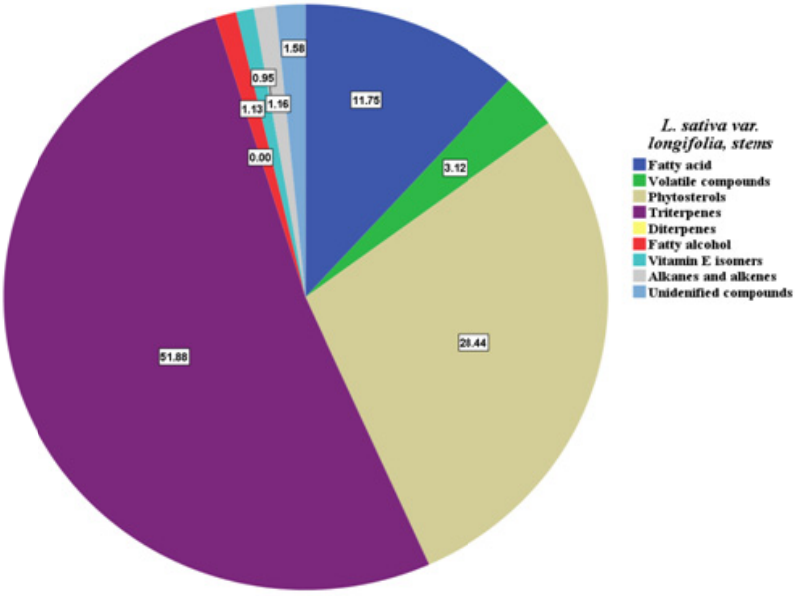

Fig. 3 The phytoconstituent categories in petroleum ether extracts of *L. sativa* var. *longifolia* stems.

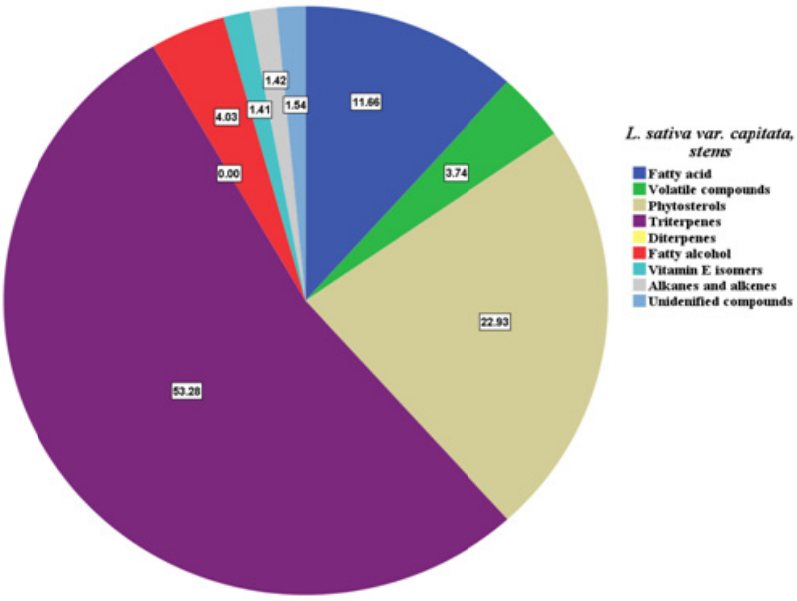

Fig. 4 The phytoconstituent categories in petroleum ether extracts of *L. sativa* var. *capitata* stems.

4. Discussion

This work is considered as the first attempt to recognize the phytochemicals of *L. sativa* stems. The TIC (total ion current) chromatograms as shown in Figs. 5-8 clarified to some extent a similarity in the number, position, and the intensity of the peaks, thus the chromatograms indicated the resemblance in the identified compounds for the leaves and the stems for the studied varieties. A total of fifty-eight compounds were identified and quantified from all the subjected petroleum ether extracts of *L. sativa*. These compounds have belonged to various categories (fatty acids, volatile compounds, phytosterols, triterpenes, diterpenes, fatty alcohol, vitamin E isomers, alkanes, and alkenes).

Table 1 gives the summary of identified fatty acids and their esters including two classifications; saturated and unsaturated fatty acids. Saturated fatty acids such

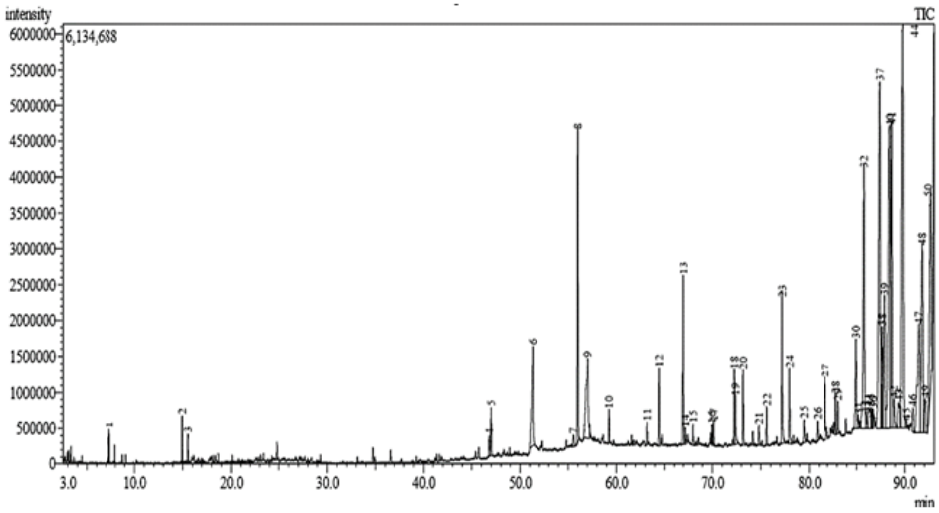

Fig. 5 TIC chromatograms of the identified phytoconstituents in petroleum ether extracts of *L. sativa* var. *longifolia* leaves.

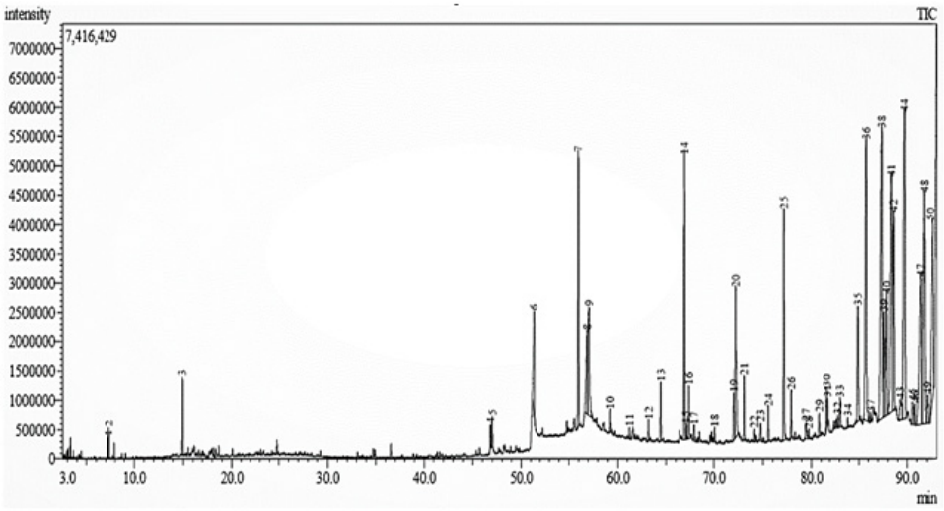

Fig. 6 TIC chromatograms of the identified phytoconstituents in petroleum ether extracts of *L. sativa* var. *capitata* leaves.

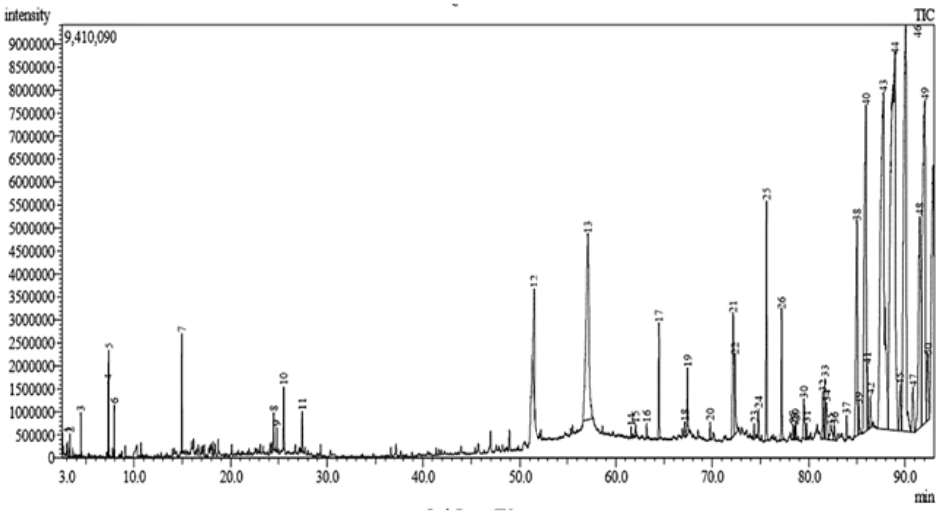

Fig. 7 TIC chromatograms of the identified phytoconstituents in petroleum ether extracts of *L. sativa* var. *longifolia* stems.

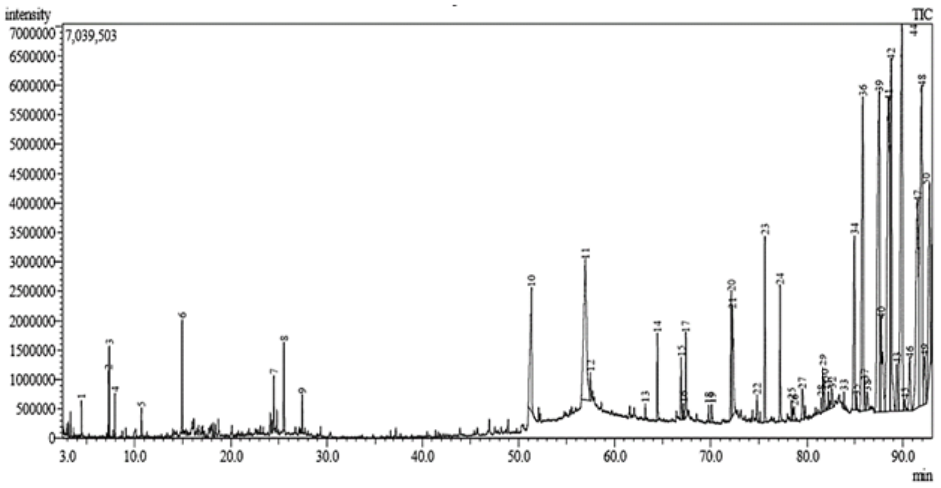

Fig. 8 TIC chromatograms of the identified phytoconstituents in petroleum ether extracts of *L. sativa* var. *capitata* stems.

Table 1 The identified and quantified fatty acids and their esters in petroleum ether extracts of *L. sativa.*

No.	Compound name	Mol. formula	Mol. weight	*L. sativa* var. *longifolia*						*L. sativa* var. *capitata*					
				Leaves			Stems			Leaves			Stems		
				P. No.	R_t min	%	P. No.	R_t min	%	P. No.	R_t min	%	P. No.	R_t min	%
1	Palmitic acid	$C_{16}H_{32}O_2$	256	6	51.32	2.54	12	51	1.36	6	51.1	2.13	10	51	2.73
2	Linoleic acid	$C_{18}H_{32}O_2$	280	9	57.05	3.24	13	57	6.23	8	56.6	2.42	11	57	5.48
3	Tetramethylheptadecan-4-olide	$C_{21}H_{40}O_2$	324	11	63.18	0.27	16	63	0.12	12	63.2	0.28	13	63	0.15
4	Methyl docosanoate	$C_{23}H_{46}O_2$	354	15	68	0.29	-	-	-	17	68	0.24	-	-	-
5	Methyl tetracosanoate	$C_{25}H_{50}O_2$	382	20	73.16	0.96	-	-	-	21	73.1	0.8	-	-	-
6	Cerotic acid	$C_{27}H_{55}O_2$	410	24	77.97	1.07	21	72	2.02	26	77.9	0.6	-	-	-
7	Ethyl linoleate	$C_{20}H_{36}O_2$	308	-	-	-	-	-	-	9	57	3.31	12	58	0/31
8	Methyl(Z)-5,11,14,17-eicosantetraenoate	$C_{21}H_{34}O_2$	318	-	-	-	22	72	0.89	-	-	-	-	-	-
9	Oleic acid amide	$C_{18}H_{35}O$	281	-	-	-	23	74	0.13	-	-	-	-	-	-
10	α-Glyceryl linoleate	$C_{21}H_{38}O_4$	354	-	-	-	-	-	-	19	72.1	0.76	20	72	1.65

Table 2 The identified and quantified volatile compounds in petroleum ether extracts of *L. sativa.*

No.	Compound name	Mol. formula	Mol. weight	*L. sativa* var. *longifolia*						*L. sativa* var. *capitata*					
				Leaves			Stems			Leaves			Stems		
				P. No.	R_t min	%	P. No.	R_t min	%	P. No.	R_t min	%	P. No.	R_t min	%
1	3-Methylbutanal	$C_5H_{10}O$	86	-	-	-	1	2.58	0.05	-	-	-	-	-	-
2	2-Methyl-2-pentanol	$C_6H_{14}O$	102	-	-	-	2	3.33	0.05	-	-	-	-	-	-
3	2,4-Pentadienal	C_5H_6O	82	-	-	-	3	4.47	0.18	-	-	-	1	4.46	0.16
4	3,4-Epoxy-2-hexanone	$C_6H_{10}O_2$	114	-	-	-	-	-	-	-	-	-	3	7.37	0.46
5	2-Pentanol	$C_7H_{16}O_2$	132	1	7.35	0.25	-	-	-	-	-	-	-	-	-
6	2,4-Dimethyl-pentane	C_7H_{16}	100	2	14.94	0.41	7	14.95	0.66	3	14.92	0.72	6	14.93	0.74
7	Nonanal	$C_9H_{26}O$	142	3	15.53	0.25	-	-	-	-	-	-	-	-	-
8	2-Pentadecanone	$C_{18}H_{36}O$	268	5	47.00	0.58	-	-	-	5	46.98	0.35	-	-	-
9	Isopropyl 2-methyl butyrate	$C_8H_{16}O_2$	144	-	-	-	4	7.31	0.35	1	7.29	0.16	2	7.30	0.34

Table 3 to be continued

10	Furan	$C_6H_{12}O$	100	-	-	-	5	7.37	0.44	2	7.35	0.21	-	-	-
11	3-Methyl-2-heptanone	$C_8H_{16}O$	128	-	-	-	6	7.95	0.22	-	-	-	4	7.95	0.23
12	Trans-2,4-Heptadienal	$C_7H_{10}O$	110	-	-	-	-	-	-	-	-	-	5	10.70	0.20
13	n-Tridecane	$C_{13}H_{28}$	184	-	-	-	9	24.80	0.13	-	-	-	-	-	-
14	2,4-Decadienal	$C_{10}H_{20}O$	152	-	-	-	10	25.52	0.41	-	-	-	8	25.53	0.72
15	Eugenol	$C_{10}H_{12}O$	164	-	-	-	11	27.43	0.27	-	-	-	9	27.43	0.32

Table 3 The identified and quantified phytosterols, triterpenes, and diterpenes in petroleum ether extracts of *L. sativa.*

No.	Compound name	Mol. formula	Mol. weight	*L. sativa* var. *longifolia*						*L. sativa* var. *capitata*					
				Leaves			Stems			Leaves			Stems		
				P. No.	R_t min	%	P. No.	R_t min	%	P. No.	R_t min	%	P. No.	R_t min	%
Phytosterols															
1	Campesterol	$C_{28}H_{48}O$	400	30	84.96	1.84	38	85.04	3.63	35	84.93	2.44	34	85.01	2.93
2	*β*-Stegmasterol	$C_{29}H_{48}O$	412	32	85.8	5.94	40	85.96	7.33	36	85.79	7.27	36	85.88	6.59
3	Ergo-22-en-3-ol	$C_{28}H_{48}O$	400	33	86.03	0.32	41	86.1	0.61	-	-	-	37	86.06	0.39
4	*γ*-Sitosterol	$C_{29}H_{50}O$	414	37	87.43	10.18	43	87.78	14.85	38	87.4	9.33	39	87.56	9.42
5	Stigmastanol	$C_{29}H_{52}O$	416	38	87.65	2.31	-	-	-	39	87.61	2.17	40	87.77	2.54
6	Ergostanol	$C_{28}H_{50}O$	402	-	-	-	39	85.23	0.37	-	-	-	35	85.23	0.21
7	Chondrillast-7-enol	$C_{29}H_{50}O$	414	42	88.89	0.76	-	-	-	-	-	-	-	-	-
Triterpenes															
1	12-Oleanen-3-yl acetate, (3. alpha.)-	$C_{30}H_{50}O$	468	40	88.42	9.49	-	-	-	41	88.36	7.5	41	88.52	10.54
2	Germanicol	$C_{30}H_{50}O$	426	41	88.66	7.25	44	88.97	20.34	42	88.6	4.55	42	88.8	7.53
3	Gemanicene	$C_{30}H_{50}$	410	48	91.78	5.71	49	92.09	9.8	48	91.75	7.29	48	91.93	9.27
4	*α*-Amyrin	$C_{30}H_{50}O$	426	44	89.78	11.84	46	90.07	12.08	44	89.71	9.33	44	89.9	10.28
5	*β*-Amyrin	$C_{30}H_{50}O$	426	47	91.43	4.7	48	91.53	5.49	47	91.39	6.41	47	91.51	7.34
6	Mortenol	$C_{30}H_{50}O$	426	50	92.65	6.05	-	-	-	50	92.6	5.53	50	92.76	4.48
7	Betulin	$C_{30}H_{50}O_2$	442	-	-	-	34	81.88	0.62	-	-	-	30	81.91	0.65
8	Squalene	$C_{30}H_{50}$	410	22	75.6	0.49	25	75.62	1.98	24	75.55	0.45	23	75.61	1.82
Diterpenes															
1	Phytol	$C_{20}H_{40}O$	296	8	56	3.97	-	-	-	7	55.84	3.7	-	-	-
2	Phytol acetate	$C_{22}H_{42}O$	338	39	87.9	3.09	-	-	-	40	87.86	2.88	-	-	-

Table 4 The identified and quantified fatty alcohol, vitamin E isomers, alkanes, alkenes, and others in petroleum ether extracts of *L. sativa* quantified phytosterols, triterpenes, and diterpenes in petroleum ether extracts of *L. sativa*.

No.	Compound name	Mol. formula	Mol. weight	*L. sativa* var. *longifolia*						*L. sativa* var. *capitata*					
				Leaves			Stems			Leaves			Stems		
				P. No.	R_t min	%	P. No.	R_t min	%	P. No.	R_t min	%	P. No.	R_t min	%
Fatty alcohol															
1	1-Eicosanol	$C_{20}H_{42}O$	298	13	66.97	2.29	-	-	-	14	66.96	4.17	15	66.96	0.61
2	1-Octadecanol	$C_{18}H_{38}O$	270	18	72.26	1.03	-	-	-	-	-	-	-	-	-
3	Lignocerol	$C_{24}H_{50}O$	354	17	70.11	0.29	-	-	-	20	72.23	3.38	21	72.26	1.9
4	1-Heptacosanol-heptafluoro butyrate	$C_{31}H_{55}F_7O_2$	592	23	77.19	2.07	26	77.18	1.09	25	77.16	3.39	24	77.19	1.37
5	1-Docosanol	$C_{24}H_{48}O_2$	368	-	-	-	-	-	-	18	70.08	0.23	-	-	-

Table 4 to be continued

Vitamin E isomers															
1	α-Tocopherol	$C_{29}H_{50}O_2$	430	-	-	-	36	82.69	0.17	-	-	-	32	82.7	0.36
2	β-Tocopherol	$C_{29}H_{50}O$	430	-	-	-	47	90.6	0.75	45	90.54	0.55	46	90.72	1
3	γ-Tocopherol	$C_{28}H_{48}O_2$	416	26	80.85	0.33	-	-	-	29	80.82	0.37	-	-	-
4	α-Tocopherol-quinone	$C_{29}H_{50}O_3$	446	28	82.85	0.59	-	-	-	32	82.8	0.16	-	-	-
Alkanes and alkenes															
1	Neophytadiene	$C_{20}H_{38}$	278	4	46.8	0.28	-	-	-	4	46.79	0.3	-	-	-
2	Triacontane	$C_{30}H_{62}$	422	25	79.47	0.38	30	79.46	0.59	27	79.43	0.33	27	79.48	0.53
3	Tetracontane	$C_{40}H_{82}$	562	34	86.29	0.74				34	83.86	0.13	-	-	-
4	Nonacosane	$C_{29}H_{60}$	408	27	81.71	1.02	33	81.69	0.53	-	-	-	-	-	-
5	*n*-Eicosane	$C_{20}H_{42}$	282	7	55.54	0.23	-	-	-	-	-	-	-	-	-
6	1-Eicosene	$C_{20}H_{40}$	280	36	86.74	0.35	-	-	-	-	-	-	-	-	-
7	Tetratriacontane	$C_{34}H_{70}$	478	-	-	-	-	-	-	-	-	-	29	81.7	0.48

Mol: molecular, (-): not detected, P. No.: peak number, R_t: retention time, %: percentage of compound.

as palmitic acid, oleic acid, methyl tetracosanoate, methyl docosanoate, 4,8,12,16-tetramethylheptadecan-4-olide, and cerotic acid while the linoleic acid is classified as an unsaturated fatty acid [3]. In this study, most of the identified fatty acids were saturated fatty acids. Palmitic and linoleic acids represent the superior content relative to other fatty acids in all the studied extracts. The results of this work agree with that reported by Kim et al. [4], who have analyzed the fatty acids content of *L. sativa* var. *longifolia* and *L. sativa* var. *capitata* leaves using GC with fatty acids standards. On the other side, the seeds of *L. sativa* exhibited a higher percentage of different types of unsaturated fatty acids in comparison to the leaves [3].

The identified volatile compounds from the *L. sativa* extracts are listed in Table 2. Generally, the percentage of the volatile compounds in all studied extracts was less than other detected categories while the higher percentage of volatile compounds has been quantified in the stems of both varieties rather than the leaves. In the present study, some of the volatile compounds such as 3-methylbutanal, 2,4-pentadienal, 2-pentanol, nonanal, 2-pentadecanone, furan, 2,4-decadienal, and eugenol were randomly detected in either the leaves or the stems of both varieties, and they have been detected by Deza-Durand and Petersen [5] in the leaves of *L. sativa* var. *capitata*. The content and the type of volatile compounds are influenced by many internal and external factors. Internal factors in the plant can be genetics, plant part, and physiological and biochemical pathway; whereas, the external factors include cultivation and environmental conditions [6].

The identified phytosterols, triterpenes, and the diterpenes are listed in Table 3. Triterpenes followed by phytosterols displayed the greatest percentage together (about 75%) among other identified categories of all the studied extracts; however, the higher percentage has been observed in the stems of both varieties. The detected phytosterols in all studied extracts were campesterol, β-stigmasterol, and γ-sitosterol. γ-Sitosterol represents the highest content while the main identified triterpenes, such as α-amyrin, β-amyrin, germanicol, germanicene, and betulin. The triterpenes and phytosterols' findings of this study confirmed the findings of Elsharkawy and Alshathly [7] that have been conducted on the hexane extract of *Lactuca steriolla* leaves. Triterpenes and phytosterols are one of the promising bioactive secondary metabolites that have been reported to possess several pharmacological activities. Specifically, triterpenes have been reported as anticancer, antiulcer, and anti-inflammatory [8]. Phytosterols are considered as a

cholesterol-lowering agent by its interference with cholesterol absorption in small intestines [9]. Hence, *L. sativa* leaves and stems can be suggested for patients suffering from hypercholesterolemia. Phytol is the only detected diterpene in a moderate percentage in the leaf's extracts. Many studies have tested the activity of phytol against free radicals, inflammation, anxiety, and depression, in addition to its applications in the cosmetics field [10, 11].

Other categories are presented in Table 4 including fatty alcohol, vitamin E isomers, alkanes, and alkenes. A higher fatty alcohol quantity has been notified in the leaves of both varieties. Fatty alcohol is assumed as the main ingredient in the moisturizing creams, especially for the dry skin [12]. A minor percentage of vitamin E isomer (α, β, and γ) has been detected in all the studied extracts. *L. sativa* var. *longifolia* leaves showed the greatest alkanes and alkenes content in comparison to other extracts.

5. Conclusions

The leaves and the stems of *L. sativa* are a valuable source of diverse phytochemicals, which have belonged to several categories. The stems of each variety implied a higher percentage of phytosterols, triterpenes, and volatile compounds rather than the leaves. Phytol and its salt were detected only in the leaves. Consequently, it is advisable to consume the leaves as well as the stems of *L. sativa* and never discard the stems.

Acknowledgement

Special thanks go to Putra Malaysia University for their assistance and cooperation especially, Prof. Khozirah Shaari.

References

[1] Alavi, S. A. 1983. *Flora of Libya: Asteraceae*. Athawrah Alarabiya Publisher.

[2] Chung, M., Nagella, P., Ahn, Y. S., Kim, S. J., and Ahmad, A. 2011. "Composition of the Essential Oil and Petroleum Ether Extract of *Lycium chinense* Miller Fruits and Antioxidant Activity of Its Several Extracts."*J Med Plant Res* 5 (25): 5973-81. doi: 10.5897/JMPR11.285.

[3] Afsharypuor, S., Ranjbar, M., Mazaheri, M., Shakibaei, F., and Aslani, A. 2018. "Analysis of Fatty Acid Composition of Crude Seed Oil of *Lactuca sativa* L. by GC-MS and GC Methods." T*rends Pharm Sci* 4 (2): 95-8. doi: 10.1111/tips.v4i2.177.

[4] Kim, M. J., Moon, Y., Kopsell, D. A., Park, S., Tou, J. C., and Waterland, N. L. 2016. "Nutritional Value of Crisphead 'Iceberg' and Romaine Lettuces (*Lactuca sativa* L.)." *J Agric Sci* 8: 1-10. doi:10.5539/jas.v8n11p1.

[5] Deza-Durand, K. M., and Petersen, M. A. 2011. "The Effect of Cutting Direction on Aroma Compound and Respiration Rate of Fresh-Cut Iceberg Lettuce (*Lactuca sativa* L.)." P*ostharvest Bio Technol* 61 (1): 83-90. doi.org/10.1016/j.postharvbio.2011.02.011.

[6] Moghaddam, M., and Mehdizadeh, L. 2017. "Chemistry of Essential Oils and Factors Influencing Their Constituents." In *Soft Chem Food Fermentation*, 379-419. doi.org/10.1016/b978-0-12-811412-4.00013-8.

[7] Elsharkawy, E., and Alshathly, M. 2013. "Anticancer Activity of *Lactuca steriolla* growing under Dry Desert Condition of Northern Region in Saudi Arabia." *Nat Sci Res* 3: 5-18.

[8] Dzubak, P., Hajduch, M., Vydra, D., Hustova, A., Kvasnica, M., Biedermann, D., Markova, L., Urbanc, M., and Sarek, J. 2006. "Pharmacological Activities of Natural Triterpenoids and Their Therapeutic Implications." *Nat Product Rep* 23: 394-411. doi.org/10.1002/chin.200637244.

[9] Segura, R., Javierr, C., Lizarraga, A., and Ros, E. 2006. "Other Relevant Components of Nuts: Phytosterols, Folate and Minerals." *Br J Nutr* 96 (2): 36-44. doi.org/10.1017/bjn20061862.

[10] De Santos, C. C. M. P., Salvadori, M. S., Mota, V. G., Costa, L. M., De Almeida, A. C. C., De Oliveira, G. A. L., Costa, J. P., De Sousa, D. P., De Freitas, R. M., and De Almeida, R. N. 2013. "Antinociceptive and Antioxidant Activities of Phytol *In-Vivo* and *In-Vitro* Models." *Neuroscience J*, 1-9. doi.org/10.1155/2013/949452.

[11] Costa, J. P., Da Silva Oliveira, J., Rezende, L. M., and Freitas, R. 2015. "Phytol a Natural Diterpenoid with Pharmacological Applications on Central Nervous System: A Review." *Biotechnol*. 8 (3): 194-205. doi.org/10.2174/1872208308031506051627 45.

[12] Kraft, J. N., and Lynde, C. W. 2005. "Moisturizers: What They Are and a Practical Approach to Product Selection." *Skin Ther Lett.* 10 (5): 1-12.