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

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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 phytoc onstituents 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 (D1 68107922, D2 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
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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 El 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](image1.png)  
**Fig. 1** The phytoconstituent categories in petroleum ether extracts of L. sativa var. longifolia leaves.

![Fig. 2](image2.png)  
**Fig. 2** The phytoconstituent categories in petroleum ether extracts of L. sativa var. capitata leaves.
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
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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.
### 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 | Leaves % | P. No | R<sub>i</sub> min | Stems % | P. No | R<sub>i</sub> min |
|-----|---------------|--------------|-------------|----------|-------|-----------------|----------|-------|-----------------|
| 1   | Palmitic acid | C<sub>16</sub>H<sub>32</sub>O<sub>2</sub> | 256         | 0.34     | 6     | 51.32           | 1.36     | 6     | 51.1            |
| 2   | Linoleic acid | C<sub>18</sub>H<sub>32</sub>O<sub>2</sub> | 280         | 0.34     | 9     | 57.05           | 6.23     | 8     | 56.6            |
| 3   | Tetramethyleptade can-4-olide | C<sub>23</sub>H<sub>46</sub>O<sub>2</sub> | 324         | 0.27     | 11    | 63.18           | 0.12     | 12    | 63.2            |
| 4   | Methyl docosanoate | C<sub>25</sub>H<sub>50</sub>O<sub>2</sub> | 354         | 0.29     | 15    | 68              | -        | 17    | 68              |
| 5   | Methyl tetracosenoate | C<sub>26</sub>H<sub>52</sub>O<sub>2</sub> | 382         | 0.96     | 20    | 73.16           | -        | 21    | 73.1            |
| 6   | Cerotic acid | C<sub>27</sub>H<sub>54</sub>O<sub>2</sub> | 410         | 1.07     | 24    | 77.97           | 2.02     | 26    | 77.9            |
| 7   | Ethyl linoleate | C<sub>30</sub>H<sub>56</sub>O<sub>2</sub> | 308         | -        | -     | -               | -        | -     | -               |
| 8   | Methyl(Z)-5,11,14,17-tetraicosatetraenoate | C<sub>31</sub>H<sub>56</sub>O<sub>4</sub> | 318         | 0.89     | 22    | 72              | -        | -     | -               |
| 9   | Oleic acid amide | C<sub>18</sub>H<sub>33</sub>N<sub>2</sub>O<sub>2</sub> | 281         | 0.13     | 23    | 74              | -        | -     | -               |
| 10  | α-Glyceryl linoleate | C<sub>22</sub>H<sub>35</sub>O<sub>4</sub> | 354         | 0.76     | 19    | 72.1            | -        | 20    | 72              |

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

| No. | Compound name | Mol. formula | Mol. weight | Leaves % | P. No | R<sub>i</sub> min | Stems % | P. No | R<sub>i</sub> min |
|-----|---------------|--------------|-------------|----------|-------|-----------------|----------|-------|-----------------|
| 1   | 3-Methylbutan | C<sub>6</sub>H<sub>10</sub>O | 86          | 0.05     | -     | -               | -        | -     | -               |
| 2   | 2-Methyl-2-pentanol | C<sub>6</sub>H<sub>10</sub>O | 102         | 0.05     | -     | -               | -        | -     | -               |
| 3   | 2,4-Pentadienal | C<sub>5</sub>H<sub>10</sub>O | 82          | 0.18     | -     | -               | -        | -     | -               |
| 4   | 3,4-Epoxy-2-hexane | C<sub>6</sub>H<sub>10</sub>O | 114         | -        | -     | -               | -        | -     | -               |
| 5   | 2-Pentanol | C<sub>5</sub>H<sub>10</sub>O | 132         | 0.25     | -     | -               | -        | -     | -               |
| 6   | 2,4-Dimethyl-pentane | C<sub>7</sub>H<sub>10</sub>O | 100         | 0.41     | 7     | 14.49           | 0.66     | 3     | 14.92           |
| 7   | Nonanal | C<sub>7</sub>H<sub>14</sub>O | 142         | 0.25     | -     | -               | -        | -     | -               |
| 8   | 2-Pentadecanone | C<sub>15</sub>H<sub>31</sub>O | 268         | 0.58     | -     | -               | -        | -     | -               |
| 9   | Isopropyl 2-methyl butyrate | C<sub>6</sub>H<sub>10</sub>O | 144         | 0.35     | 4     | 7.31            | 0.35     | 1     | 7.29            |
Table 3 to be continued

| No. | Compound name | Mol. formula | Mol. weight | Phytosterols | Leaves | Stems | Leaves | Stems | Leaves | Stems |
|-----|---------------|--------------|-------------|--------------|--------|-------|--------|-------|--------|-------|
| 10  | Furan         | C₈H₁₀O       | 100         | -            | -      | 5     | 7.37   | 0.44  | 2      | 7.35  |
| 11  | 3-Methyl-2-heptanone | C₉H₁₆O   | 128         | -            | -      | 6     | 7.95   | 0.22  | -      | 7.95  |
| 12  | Trans-2,4-Heptadienal | C₇H₁₀O     | 110         | -            | -      | -     | -      | -     | -      | -     |
| 13  | n-Tridecane   | C₁₃H₂₈   | 184         | -            | -      | 9     | 24.80  | 0.13  | -      | -     |
| 14  | 2,4-Decadienal | C₁₈H₃₆O   | 152         | -            | -      | 10    | 25.52  | 0.21  | -      | 25.53 |
| 15  | Eugenol       | C₁₀H₁₄O    | 164         | -            | -      | 11    | 27.43  | 0.27  | -      | 27.43 |

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

| No. | Compound name | Mol. formula | Mol. weight | Phytosterols | Leaves | Stems | Leaves | Stems | Leaves | Stems |
|-----|---------------|--------------|-------------|--------------|--------|-------|--------|-------|--------|-------|
| 1   | Campesterol   | C₂₅H₄₈O     | 400         | 30           | 84.96  | 85.04 | 84.93  | 85.01 | 2.93   |       |
| 2   | β-Stegmasterol| C₂₉H₄₈O     | 412         | 32           | 85.8   | 85.96 | 85.79  | 85.88 | 6.59   |       |
| 3   | Ergo-22-en-3-ol| C₂₅H₄₈O   | 400         | 33           | 86.03  | 86.1  | 86.1   | 86.06 | 0.39   |       |
| 4   | β-Sitosterol  | C₂₉H₅₀O     | 414         | 37           | 87.43  | 87.78 | 87.4   | 87.56 | 9.42   |       |
| 5   | Stigmastanol  | C₂₉H₅₀O     | 416         | 38           | 87.65  | 87.65 | 87.65  | 87.56 | 9.42   |       |
| 6   | Ergostanol    | C₂₈H₄₈O     | 402         | -            | -      | 85.23 | 85.23  | -     | -      | -     |
| 7   | Chondrillast-7-enol | C₂₉H₅₀O   | 414         | 42           | 88.89  | 88.89 | 88.89  | 88.89 | 10.54  |       |

Triterpenes

| No. | Compound name | Mol. formula | Mol. weight | Triterpenes | Leaves | Stems | Leaves | Stems | Leaves | Stems |
|-----|---------------|--------------|-------------|-------------|--------|-------|--------|-------|--------|-------|
| 1   | 12-Oleanen-3-y acetate, (3. alpha.)- | C₃₀H₅₀O     | 468         | 40           | 88.42  | 88.42 | 88.42  | 88.52 | 10.54  |       |
| 2   | Germanicol    | C₃₀H₅₀O     | 426         | 41           | 88.66  | 88.97 | 88.66  | 88.8  | 7.53   |       |
| 3   | Germanicene   | C₃₀H₅₀O     | 410         | 48           | 91.78  | 92.09 | 91.78  | 91.93 | 9.27   |       |
| 4   | α-Amyrin      | C₃₀H₅₀O     | 426         | 44           | 89.78  | 90.07 | 89.78  | 89.9  | 10.28  |       |
| 5   | β-Amyrin      | C₃₀H₅₀O     | 426         | 47           | 91.43  | 91.53 | 91.43  | 91.51 | 7.34   |       |
| 6   | Mortenol      | C₃₀H₅₀O     | 426         | 50           | 92.65  | 92.65 | 92.65  | 92.76 | 4.48   |       |
| 7   | Betulin       | C₃₀H₅₀O₂    | 442         | -            | -      | 81.88 | 81.88  | 81.91 | 0.65   |       |
| 8   | Squalene      | C₃₀H₅₀O     | 410         | 22           | 75.6   | 75.6  | 75.6   | 75.6  | 1.82   |       |

Diterpenes

| No. | Compound name | Mol. formula | Mol. weight | Diterpenes | Leaves | Stems | Leaves | Stems | Leaves | Stems |
|-----|---------------|--------------|-------------|------------|--------|-------|--------|-------|--------|-------|
| 1   | Phytol        | C₂₀H₄₀O     | 296         | 8          | 56     | 3.97  | 56     | 3.97  | -      | -     |
| 2   | Phytol acetate| C₂₁H₄₂O     | 338         | 39          | 87.9   | 3.09  | 87.9   | 3.09  | -      | -     |

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 | Fatty alcohol | Leaves | Stems | Leaves | Stems | Leaves | Stems |
|-----|---------------|--------------|-------------|---------------|--------|-------|--------|-------|--------|-------|
| 1   | 1-Eicosanol   | C₂₀H₄₀O     | 298         | 13           | 66.97  | 2.29  | 66.97  | 2.29  | 14     | 66.96 |
| 2   | 1-Octadecanol | C₁₈H₃₈O     | 270         | 18           | 72.26  | 1.03  | 72.26  | 1.03  | -      | -     |
| 3   | Lignocerol   | C₂₄H₄₈O     | 354         | 17           | 70.11  | 0.29  | 70.11  | 0.29  | -      | -     |
| 4   | 1-Heptacosanol-heptafluoro butyrate | C₁₇H₃₅F₁₀O₂ | 592         | 23           | 77.19  | 2.07  | 77.19  | 2.07  | 26     | 77.18 |
| 5   | 1-Docosanol   | C₂₄H₄₈O₂    | 368         | -            | -      | -     | -      | -     | 18     | 70.08 |

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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-decadional, 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, geranicol, geranicene, 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.

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