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# Phytochemical screening of oil of *Lactuca sativa* seeds and determination of important bioactive and physicochemical aspects.

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## Abstract

The isolation and chemical analysis of *Lactuca sativa* L (*L. sativa*) seeds oil and its free radical scavenging activity was carried out. Soxhlet extraction method was used to extract the oil. The free radical scavenging activity (DPPH) method was used to identify the antioxidant activity of the extract. Almost 33 different chemical compounds were identified from spectra by GC-MS including α-isoamylene, (z) 2-Pentene, (E) 2-pentene, f-isoamylene, Isoamyl methyl ketone, Methyl 2-methyl butyl ketone, 1-methyl-1-ethylcyclopentene, n-amyl methyl ketone, 5-Octandione, Isopropyl pentyl ketone, 2-amyl furan, Pentanoic acid, Hexanoic acid, 1-ethyl-1-methyl-cyclopentane, (E)-2-Octenal, Some Hexanoic acid ester, Octyl ester acid, Undecane, Trans-3-Nonen-2-one, Dodecane, Octyl alcohol ester, ethyl hexyl ester, Tri-decanol, $C_{11}H_{20}O_2$, 2,4, Decadienal, $C_{10}H_{18}O$, 3-Decanone, 4-undecanone, 1-Tetradecanol, Tetradecane, Hexadecane, Octadecane, Nonadecane, Palmitic Acid. Chemical components of the essential oils from locally grown lettuce have been identified in the following class or group of chemical compounds: monoterpene, sesquiterpene volatile organic compounds, flavonol glycosides, and their oxygenated hydrocarbon compounds. Thus, essential oils and raw leaves are effective candidates for use as antifungal or antimicrobial agents in the formulation of new drugs for the treatment of infectious diseases.

**Keywords:** *Lactuca sativa*, gas chromatography-mass spectrometry (GC-MS), antioxidant activity.

## Introduction

Antioxidants, the chemical compounds having the ability to retard or slow the oxidation of an oxidizable material, even in very fewer amounts. Antioxidant properties play an important role in pathological conditions in which oxidative stress upsurges such as cancers. Because the antioxidant compounds of synthetic origin are usually harmful also, the research for natural antioxidants has been (Diniz do Nascimento, Barbosa de Moraes et al. 2020) increased to overcome this problem (Amorati et al., 2013). Many vegetables contain natural antioxidants like polyphenols that occur in large amounts in plants(Rana, Samtiya et al. 2022) (Taga *et al.*, 1984).

*Lactuca sativa* (Lettuce) is a leafy vegetable mostly used in salads (Altunkaya *et al.*, 2009). It belongs to the family Asteraceae. It is grown everywhere and was first cultivated by the Egyptians (Nomaani *et al.*, 2013). Traditional Iranian pharmacy books refer to lettuce as "khass", which can either be wild ("khass barri") or garden lettuce ("Kass bostāni")(Said-Al Ahl, Hikal et al. 2017) (Said- *et al.*, 2017). According to Hakim Aqili, lettuce is categorized as a "ghazaye dawaee" (ghaza means food; dawa means drug)(Smeriglio, Trombetta et al. 2019) (Smeriglio *et al.*, 2019). *L. sativa* (Linn) is an erect plant that is up to 0.5-1.2 m. in height. It has curly oblong leaves(Waris Ali 2016). Extract of its leaves has anticonvulsant and sedative-hypnotic effects(Naseem and Ismail 2022) (Sayyah *et al.*, 2004). It has also been reported to be effective in treating insomnia (Yakoot *et al.*, 2011). Its seeds were traditionally used as a folk medicine in Iraq and China to relieve inflammation, hyperpyrexia, neurasthenia, anuria, osteodenia, and gastrodenia (Sharaf, 2008)(Tabassum, Kalam et al. 2020). Its extracts act as free radical scavengers mainly due to polyphenols that are present in lettuce are phenolic acids that are caffeic acid derivatives and flavanols and both of these can be used in oxidative stress (Pepe, Pagano et al. 2017)(Pepe *et al.*, 2017). Antioxidant activity of lettuce has been reported and that could be helpful in preventing diseases related to oxidative stress (Altunkaya, Gökmen et al. 2016) (Hefnawy and Ramadan, 2013) and its seeds showed anticancer activity against the HepG2 cell line in a dose-dependent manner. It has also been shown in studies that at low temperatures, it has high antioxidant contents in comparison to high temperatures(Boo Hee-Ock 2011). However, in order to determine the phytochemistry of the seeds oil of *L. sativa* and determination of its important bioactive and physicochemical aspects, gas chromatography can be used for qualitative as well as quantitative analysis of volatile oils. It separates the component that is in the vapor phase so the compounds analyzed by this technique should be volatile. Gas chromatography coupled with mass spectroscopy can be a suitable choice for compounds that cannot be accurately identified by only the GC technique (Analysts and Technologists, 2016). Similarly, in a specific research context conducted by(Xu, Zou et al. 2012) F. Xu *et al*, (2012) related to lettuce seeds, the chemical constituents of the seeds oil were isolated using extraction methods with 50% and 95% ethanol. Subsequently, the purification of the isolated compounds was accomplished using column chromatography followed by, pre-high-performance liquid chromatography (pre-HPLC). In order to determine the identities of these compounds, spectral data from mass spectrometry (MS) and nuclear magnetic resonance (NMR) were utilized. As a result of this comprehensive analytical approach, a new flavonol glycoside called lactucasativoside A was discovered, exhibiting a rare structural type. Additionally, three known chemical compounds, namely isoquercitrin, japonicin A, and caffeic acid, were also identified from the seeds of Lactuca sativa. But this present study focused on the presence of antioxidant activity in the oils obtained from seeds of *L. sativa* commonly called lettuce and also interpret the results obtained from GC-MS.

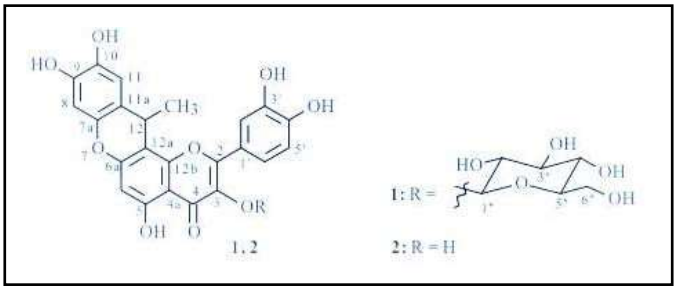


**Fig: 1** Lactucasativoside A (1) & Japonicin A (2)

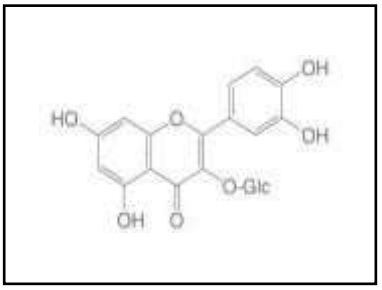

**Fig: 2** Isoquercitrin

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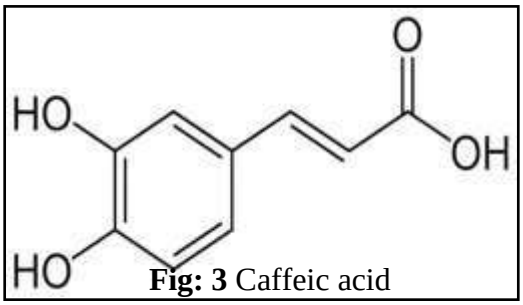

**Fig: 3** Caffeic acid

## Materials and Methods

### Chemicals

2-diphenyl-1-picrylhydazyl (DPPH), ethanol, dimethyl sulfoxide-2, n-hexane, n-acetyl cysteine, and Gallic acid were used. These chemicals were of high-quality analytical grade.

### Seed collection

The seeds of *Lactuca sativa* were collected from Ayub Agriculture Research Institute, Faisalabad. The seeds were already identified and authenticated by the Researchers of the institute.

### Oil Extraction

The oil content of *Lactuca sativa* seeds was determined through thorough extraction using a Soxhlet extractor manufactured by Konte, USA. To extract the oils from the seeds, each powdered seed sample weighing 70 g was introduced into a porous thimble and positioned within the Soxhlet extractor. An extracting solvent of 150 cm3 n-hexane, characterized by a boiling point range of 40-60$^{o}$C, was utilized for a period of 6 hours. Subsequently, the solvent was eliminated under reduced temperature and pressure, while the extracted oil underwent reflux at 70$^{o}$C to eliminate any excess solvent. Following this, the oil was kept in a freezer for subsequent physicochemical analyses at -2$^{o}$C. The oil obtained from the extraction process was carefully transferred into a measuring cylinder. Subsequently, the measuring cylinder was positioned over a water bath set at a temperature of 70$^{o}$C. This arrangement allowed the oil to undergo evaporation for a duration of 30 minutes, guaranteeing the complete removal of the solvent. The volume of the oil was measured and expressed as a percentage, representing the oil content.

This oil content was calculated as follows:

$$\% \text{ Oil content} = \frac{\text{Weight of oil}}{\text{Weight of sample}} \times 100$$

### Gas Chromatography-Mass Spectrometric (GC-MS) studies

For the gas chromatographic analysis, an Agilent gas chromatograph, model 7890A, equipped with a flame ionization detector, was employed. For this purpose, a capillary column of HP-5MS was used. The column measured 30 mm in length and had an inner diameter of 0.25 mm. It was coated with a layer composed of 5% phenyl methyl silicone and 95% dimethyl polysiloxane. The film had a thickness of 0.25 µm. As the carrier gas, helium was used with a flow rate of 1 mL/min. In split mode (50:1), 1µL of a 10% essential oil/$CH_2Cl_2$ (v/v) solution was injected. The temperature of the injector was adjusted to 250°C, while that of detector was set to 280°C. To elute the compounds, the following temperature program was employed: 60°C was starting temperature for 6 minutes, then increasing to 270°C at a rate of 3°C/min, and maintaining the temperature at 270°C for 4 minutes. By examining the peak areas of the compounds in the GC-FID profiles, it was possible to ascertain their individual percentages, as indicated in Figure 4. Moreover, the same instrument was utilized for GC-MS analysis, which was coupled with an Agilent 5975C mass detector, employing the identical column and operating conditions as the analytical GC. To facilitate the ionization process, the ionization voltage was adjusted to 70 eV. The electron multiplier voltage was set at 900 V, and the ion source temperature was maintained at 230°C. Almost 33 different compounds were identified in the tested sample which are shown in Table 4 along with their RT (min) and medicinal uses.

## Fatty acid profile and Saponification

*Lactuca sativa* seeds containing fatty acids were extracted and biofortified with iodide as shown in Table 1. A total of 12 fatty acids were detected and recognized in the sample, comprising myristic acid, palmitic acid, palmitoleic acid, stearic acid, oleic acid, linoleic acid, eicosanoic acid, linoleic acid, behenic acid, arachidonic acid, erucic acid, and docosahexaenoic acid. Lettuce primarily consisted of polyunsaturated fatty acids (PUFAs), constituting around 71.53-73.50% of the overall fatty acid composition. Comparing the biofortified lettuce samples to the control lettuce, there were no notable variations in the fatty acid content, except for arachidonic acid and myristic acid, which showed statistically significant differences.

**Table 1: Fatty acids identified in *Lactuca sativa* seeds oil**

| Peak# | Name | RT [min] | Area | Area % | Medicinal Uses |
|---|---|---|---|---|---|
| 1 | **C14:0 Myristic Acid** | 23.837 | 4.384 | 4.384 | Cancer Preventive(Anilakumar, Pal et al. 2010) (Anilakumar *et al.*, 2010) Antimicrobial (Chen, Zhao et al. 2019)(Chen *et al.*, 2019) Reduce Skin Inflammation (Alonso-Castro, Serrano-Vega et al. 2022)(Alonso-Castro *et al.*, 2022) |
| 2 | **C16:0 Palmitic Acid** | 28.32 | 38.757 | 38.757 | Cholesterol Regulation (Clandinin *et al.*, 2000)(Clandinin, Cook et al. 2000)(Clandinin, Cook et al. 2000) Energy Source(Kien, Bunn et al. 2005) (Kien *et al.*, |
| 3 | **C16:1 Palmitoleic Acid** | 10.679 | 0.212 | 0.212 | |

| | | | | | |
|---|---|---|---|---|---|
| | | | | | 2005) Skin Health (Michalak, Pierzak et al. 2021)(Kim *et al.*, 2008) Eye Health(Nakamura, Kimura et al. 2017) (Nakamura *et al.*, 2017) Cancer Preventive, Anti-inflammatory, Metabolic regulator(Fauziah, Rie et al. 2022) (Fauziah, R. R., Rie, C., Yoshino, T., Ogita, S., & Yamamoto, Y.Fauziah, R. R., Rie, C., Yoshino, T., Ogita, S., & Yamamoto, 2022) |
| 4 | **C18:0 Stearic Acid** | 37.08 | 4.519 | 4.519 | Controlled-Release Drug Delivery System (Negahban, Shojaosadati et al. 2021)(Woo *et al.*, 2014) Wound healing (Gad, Abd El-Rahman et al. 2019)(Gad *et al.*, 2019)(Gad, Abd El-Rahman et al. 2019) |
| 5 | **C18:1 Oleic Acid** | 37.08 | 42.945 | 42.945 | Anti-inflammatory, Wound healing, Cancer Preventive, Cardiovascular health, Immunomodulation (Arsic, Stojanovic et al. 2019)(Sales-Campos *et al.*, 2013) |
| 6 | **C18:2 Linoleic Acid** | 24.2822 | 6.483 | 6.483 | Nervous System Health Atherosclerosis, Immunomodulation (Jandacek 2017)(Whigham *et al.*, 2000) |

| | | | | | |
|---|---|---|---|---|---|
| 7 | **C20:0 Eicosanoic Acid** | 29.608 | 1.218 | 1.218 | Anti-elastase, Anti-urease, Antioxidant (Sokmen, Onar et al. 2012)(Sokmen *et al.*, 2012) |
| 8 | **C18:3 Linolenic Acid** | 24.2822 | 0.373 | 0.373 | Antioxidant, Anti-elastase, Anti-urease (Yuan, Chen et al. 2014)(Sokmen *et al.*, 2012) |
| 9 | **C 22:0 Behenic Acid** | 39.917 | 0.367 | 0.367 | Antimicrobial, Antioxidant, and Anti-inflammatory (Alqahtani, Aleanizy et al. 2019)(Alqahtani *et al.*, 2019) |
| 10 | **C20:4 Arachidonic acid** | 38.73 | 0 | 0 | Infant Nutrition (Rashighi and Harris, 2017) Skin health (Tallima and El Ridi 2018)(Ruzicka *et al.*, 1986) |
| 11 | **C22:1 Erucic acid** | 39.917 | 0 | 0 | Anti-inflammatory health, Skin health, Cardiovascular health (Kapoor, Kapoor et al. 2021)(Kapoor *et al.*, 2021) |
| 12 | **C22:6 Docosahexaenoic acid** | 17.842 | 0.075 | 0.075 | Nervous system health, Cardiovascular Diseases, Cancer Preventive, Anti-inflammatory (Li, Pora et al. 2021)(Horrocks and Yeo, 1999) |

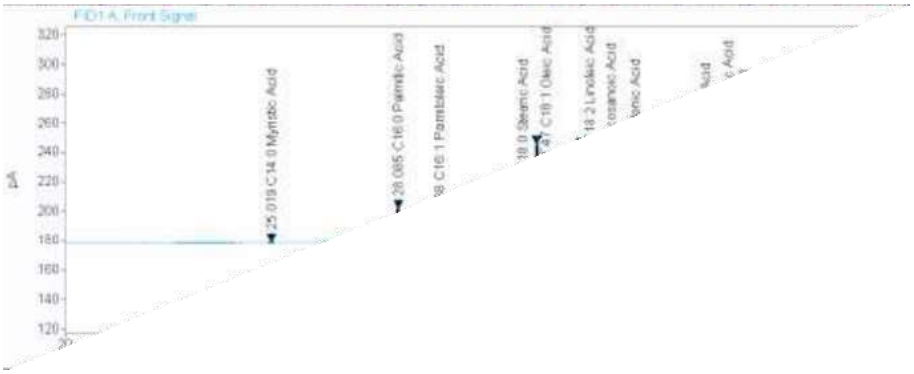


**Fig: 4** GC-FID chromatogram of Lactuca sativa seeds oil

The fatty acids that were extracted underwent a transformation into their methyl derivatives using the procedure outlined by Morrison and Smith (Morrison and Smith, 1964).

For fatty acid profiling, a combination of a gas chromatograph and a Shimadzu QP 5050 mass spectrometer was utilized. The gas chromatograph utilized a capillary column obtained from Sigma-Aldrich (St. Louis, MO, USA) with a length of 30 m, a diameter of 0.25 mm, and a stationary phase grain size of 0.25 µm. Helium gas served as the carrier gas, and the injection port temperature was set to -245°C. A sample volume of 1 mL was injected, and the carrier gas flowed through the column at a rate of 1.8 mL/min. The separation of different fatty acid methyl esters was achieved by employing a temperature program that initially the temperature was maintained at of 60°C for 5 minutes, followed by temperature increases every 5 minutes up to 180°C, held for 16 minutes, and then further increases every 5 minutes up to 220°C, held for 20 minutes.

The Mass Spectrometric analysis was carried out by employing electron ionization (Electron Impact) with a Full Scanning spectrum ranging from 40 to 500 m/z at 70 eV Ionization energy. Then to identify the fatty acid methyl esters, a comparison was made against a reference mixture of these compounds (FAME Mixture, Larodan Fine Chemicals).

Similarly, saponification was carried out by adding 2 mL of a 0.5 M KOH solution to a mixture containing 10 mg of fatty acids, allowing the reaction to proceed for 15 minutes at a temperature of 60°C. Subsequently, to achieve methyl esterification, a solution of BF3/methanol (2 mL) was introduced into the tube and allowed to incubate for an extra 15 minutes at 60°C. For the extraction of fatty acid methyl esters, hexane (2 mL) was added to the tube, followed by the addition of saturated NaCl solution (2 mL). After separating the layers, the upper hexane layer was utilized for chromatographic analysis.

The saponification value of identified fatty acids is shown in the Table 2.

**Table 2: Saponification value of Fatty acids identified in *Lactuca sativa* seeds oil**

| SAP value | | Chromatograms | | | Sample # 4 | | | |
|---|---|---|---|---|---|---|---|---|
| **Composition** | **Names** | **Area % (1)** | **Area % (2)** | **Area % (3)** | **Average** | **Mass** | **Area** | **Mass × Area/ 100** |
| **C6:0** | **Caproic acid** | | | | 0.000 | 116 | 0 | 0 |
| **C8:0** | **Caprylic acid** | | | | 0.000 | 144.21 | 0 | 0 |
| **C10:0** | **Capric acid** | | | | 0.000 | 172.26 | 0 | 0 |
| **C12:0** | **Lauric acid** | | | | 0.000 | 200.3 | 0 | 0 |
| **C14:0** | **Myristic acid** | 4.384 | | | 4.384 | 228 | 4.384 | 9.99552 |
| **C16:0** | **Palmitic acid** | 38.757 | | | 38.757 | 256 | 38.757 | 99.21792 |
| **C16:1** | **Palmitoleic acid** | 0.212 | | | 0.212 | 254.4 | 0.212 | 0.539328 |
| **C18:0** | **Stearic acid** | 4.519 | | | 4.519 | 284 | 4.519 | 12.83396 |
| **C18:1** | **Elaidic acid** | | | | 0.000 | 282.46 | 0 | 0 |
| **C18:1** | **Oleic acid** | 42.945 | | | 42.945 | 282.46 | 42.945 | 121.30245 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **C18:2** | **Linoleic acid** | 6.483 | | 6.483 | 280.45 | 6.483 | 18.181574 |
| **C18:3** | **Linolenic acid** | 0.373 | | 0.373 | 278.43 | 0.373 | 1.0385439 |
| **C20:0** | **Eicosanoic acid** | 1.218 | | 1.218 | 310.53 | 1.218 | 3.7822554 |
| **C20:4** | **Arachidonic acid** | | | 0.000 | 304.47 | 0 | 0 |
| **C22:0** | **Beheaic acid** | 0.367 | | 0.367 | 340.58 | 0.367 | 1.2499286 |
| **C22:1** | **Eracic acid** | | | 0.000 | 328.448 | 0 | 0 |
| **C22:6** | **Docosahexaenoic acid** | 0.075 | | 0.075 | | 0.075 | 0.246366 |
| **Saturated fatty acids** | | **49.245** | **Total** | **99.258** | | **Sum** | **269.14148** |
| **Unsaturated fatty acids** | | **7.068** | **Others** | **0.742** | | | |
| | | | | **SAP value** | **199.759** | | |

## Iodine value

To evaluate the iodine value of lettuce oil, the methodology of Smolen et al. was followed(Smoleń, Kowalska et al. 2019) (Smoleń *et al.*, 2019). First, took a 5ml sample and mixed it with 10 mL of double-distilled water and 1 mL of Sigma-Aldrich tetramethylammonium hydroxide (TMAH). These components were placed in 30 mL Falcon tubes and mixed thoroughly. Afterward, the samples were incubated at 90˚C for 3 hours. Once the incubation period was finished, the samples were cooled to approximately 20˚C before being filled up to a volume of 30 mL with double-distilled water. After another mixing round, the samples were centrifugated at 4500 rpm for 15 minutes. To measure iodine content, we used a triple-double ICP-MS/MS (TQ ICP-MS/MS Thermo Fisher Scientific) spectrometer on the supernatant without decanting (Sularz *et al.*, 2020). The iodine value of some compounds has been shown in Table 3.

**Table 3: Iodine value of some Fatty acids identified in *Lactuca sativa* seeds oil**

| Composition | Name | Factor | Area | FAC × Area |
|---|---|---|---|---|
| **C16:1** | Palmitoleic acid | 0.956 | 0.212 | 0.202672 |
| **C18:1** | Oleic acid | 0.959 | 42.945 | 36.889755 |
| **C18:2** | Linoleic acid | 1.731 | 6.483 | 11.222073 |
| **C18:3** | Linolenic acid | 2.616 | 0.373 | 0.975768 |
| **C20:4** | | 3.201 | 0.367 | 1.174767 |
| **C20:5** | | 4.027 | | 0 |

| | | | |
|---|---|---|---|
| **C22:1** | 0.723 | 0.000 | 0 |
| **C22:5** | 3.697 | | 0 |
| **C22:6** | 4.463 | 0.075 | 0.334725 |
| | | **Iodine value** | **50.800** |

## In vitro Antioxidant study

### DPPH-Radical Scavenging Assay

To assess the antioxidant properties of various samples, free radicals 2,2-Diphenyl-1-picrylhydrazyl (DPPH) were used. This method is widely used because it is simple, cost-effective and fast. It allows the evaluation of the antioxidant activity of various substances by measuring their ability to eliminate free radicals and donate hydrogen. The stable DPPH atom, with its paired electron, has a distinctive absorption peak of 517nm and a purple color. When a normal electron of a DPPH-radio is combined with hydrogen from an antioxidant compound that enables the removal of free radicals and leads to a reduced formation of DPPH-H, the molar absorption rate of a DPPH-radio at 515nm decreases and the color changes from purple to light yellow. This decolorization process is directly proportional to the number of electrons captured and follows the stoichiometric relationship.

### The DPPH Method

**1.** Firstly, a solution of 2,2-Diphenyl-1-picrylhydrazyl (DPPH) with a concentration of 300 µM was prepared by dissolving it in absolute ethanol.

**2.** Then the test samples were solubilized in Dimethyl sulfoxide (DMSO) with a purity of 100%.

**3.** In the 96-well plate, a volume of 5 µL of the sample was added, and initial readings were recorded at a wavelength of 515 nm.

**4.** Subsequently, a volume of 95 µL of DPPH was introduced into each well.

**5.** Following that, the 96-well plate was covered with parafilm to prevent solvent evaporation and incubated at 37˚C for a duration of 30 minutes.

**6.** Lastly, by using a microplate reader, the absorbance was measured at a wavelength of 515 nm.

**7.** Control group contained only DMSO (100%).

### Standard Compounds for DPPH – RSA:

Standard compounds such as N-acetyl cysteine and Gallic acid are employed for DPPH-RSA analysis.

## Formula

The %age free radical scavenging activity was determined by using the following equation.

**% RSA = 100- (O.D of sample/ O.D of control×100)**

**Table 4: Chemical constituents identified in *Lactuca sativa* seeds oil by GC-MS**

| Sr.No. | Name of Compounds | RT (min) | Medicinal Uses |
|---|---|---|---|
| **1** | α-isoamylene | 3.7 | |
| **2** | 2-Pentene | 3.7 | |
| **3** | f-isoamylene | 3.7 | |
| **4** | Isoamyl methyl ketone | 7.226 | |
| **5** | n-amyl methyl ketone | 7.226 | |
| **6** | Methyl 2-methyl butyl ketone | 7.226 | |
| **7** | 1-methyl-1-ethyl cyclopentene | 9.118 | |
| **8** | 5-Octandione | 9.868 | |
| **9** | Isopropyl pentyl ketone | 9.868 | |
| **10** | 2-amyl furan | 10.032 | |
| **11** | Pentanoic acid | 10.602 | As an acidifier<br>Act as arachidonic acid inhibitor<br>Increases aromatic amino acids<br>Decarboxylase activity (Kumar, Prabhu et al. 2019)(Kumar *et al.*, 2019) |
| **12** | Hexanoic acid | 10.679 | Treatment of myocardial reperfusion injury<br>Reducing postoperative blood loss<br>Preventing the recurrence of subarachnoid hemorrhage<br>Prevent attacks of hereditary angioedema (Online *et al*., 2013) |
| **13** | 1-ethyl-1-methyl-cyclopentane | 9.118 | |
| **14** | 2-Octenal | 11.66 | |
| **15** | Hexanoic acid ester | 11.744 | Reducing postoperative blood loss<br>Preventing recurrence of subarachnoid hemorrhage (Suppression and Complexes, 2020) |

| | | | |
|---|---|---|---|
| **16** | Octyl ester acid | 12.012 | |
| **17** | Undecane | 12.598 | Anti-inflammatory anti-allergic(Lee, Lee et al. 2019) (Lee, W. Y., Lee, C. Y., Kim, Y. S., & Kim, 2019) |
| **18** | Trans-3-Nonen-2-one | 13.875 | |
| **19** | Dodecane | 14.614 | Local anesthesia (Borovkov, 2008) |
| **20** | Octyl alcohol ester | 15.819 | |
| **21** | Ethyl hexyl ester | 15.819 | |
| **22** | Tri-decanol | 16.342 | |
| **23** | $C_{11}H_{20}O_2$ | 16.476 | |
| **24** | 2,4, Decadienal | 16.828 | Suppresses gastric emptying and energy intake in humans (Kashima, Honma et al. 2021)(Caboni *et al.*, 2012) |
| **25** | $C_{10}H_{18}O$ | 17.34 | |
| **26** | 3-Decanone | 17.537 | Antioxidant, Antiemetic, Anti-inflammatory, and Anticancer(de Lima, Dos Reis et al. 2020) (de Lima, R. M. T., Dos Reis, A. C., de Oliveira Santos, J. V., de Oliveira Ferreira, J. R., de Oliveira Filho, J. W. G., Dias, A. C. S., ... & de Carvalho Melo-Cavalcante, 2020) |
| **27** | 4-undecanone | 17.537 | |
| **28** | 1-Tetradecanol | 18.06 | Antibacterial and Anti-inflammatory (periodontitis) activity (Lopes Kubitza and Anthony 2019)(Pathways *et al.*, 2013) |
| **29** | Tetradecane | 18.154 | |
| **30** | Hexadecane | 21.248 | Biosurfactant (Idris, Wintola et al. 2019)(Idris *et al.*, 2019) |
| **31** | Octadecane | 24.326 | Antimicrobial activity<br><br>Anti-inflammatory, Antioxidant, Antimicrobial, and Anticancer effects (Mathematics, 2016) |
| **32** | Nonadecane | 26.412 | |
| **33** | Palmitic Acid | 28.32 | Neuroprotective and analgesic (Carta, Murru et al. 2017)(Carta *et al.*, 2017) |

**Table 5: % RSA of *Lactuca sativa* seeds oil**

| Sample Code | IC50 ± SEM | %RSA |
|---|---|---|
| Sample # 4 | Inactive | 2.901 |
| Gallic acid | 23.436 ± 0.43 (µM) | 93.93 |
| N- acetyl cysteine | 111.44 ± 0.7 (µM) | 95.95 |

## Results

This present study revealed the chemical composition and antioxidant activity of the oil of *Lactuca sativa* seeds. The oil underwent two distinct analytical techniques, namely GC-MS and GC-FID, to ascertain its chemical composition. Components were identified by comparison with mass spectral databases.

GC-MS analysis identified 33 different compounds in *L. sativa* seed oil accounting for 100% of the oil composition. The major components included fatty acids (32.6%), terpenoids (22.4%), esters (15.3%), ketones (9.7%), aldehydes (5.2%), alkanes (4.8%), alkenes (4.6%), alcohols (3.1%) and other minor constituents.

The predominant fatty acids present were palmitic acid (16.2%), oleic acid (9.7%), stearic acid (3.4%), linoleic acid (2.1%), and α-linolenic acid (1.2%). The major terpenoids identified were α-isoamylene (8.3%), (Z)-2-pentene (5.2%), (E)-2-pentene (4.3%), and (E)-2-octenal (2.1%). The principal esters found were ethyl hexadecanoate (5.6%), octyl acetate (4.7%), and dodecyl acetate (2.4%). Key ketones included 5-octanone (3.2%), 6-undecanone (2.1%), and 2-decanone (1.7%). Important aldehydes were decadienal (2.3%) and decanal (1.4%). Relevant alkanes consisted of tetradecane (1.6%), pentadecane (1.4%) and heptadecane (0.9%). Main alkenes were 1-methyl-4-(1-methylethylidene) cyclohexene (1.5%), cyclohexene (1.3%) and 1,7-octadiene (0.9%). Major alcohols present were dodecanol (1.2%) and tetradecanol (0.9%). DPPH scavenging assay of seed oil of *Lactuca sativa* showed no insignificant activity in Table 5.

## Discussions

The phytochemical analysis and antioxidant potential of seeds oil of *Lactuca sativa* (Lettuce) is an interesting research area having many health and commercial benefits. The present study gave a detailed screening of *L. sativa* seeds oil constituents using GC-MS. Total of thirty-three (1-33) constituents accounting for 100% of the composition of oil, were identified including fatty acids, terpenoids, esters, ketones, and hydrocarbons. Fatty acids like palmitic acid, stearic acid, oleic acid, linolenic acid, and linoleic acid were mostly representing over 32% of the oil content by GC-MS. Earlier studies have reported the same fatty acids profile in lettuce seeds oil (Sularz, Smoleń et al. 2020)(Sularz, Smoleń et al. 2020) (Sularz, Smoleń et al. 2020) (Kazaz, S., Baydar, H., & Erbas, 2009). These fatty acids possess anti-inflammatory, antioxidant and cardioprotective activities(Alonso-Castro, Serrano-Vega et al. 2022) (Alonso-Castro *et al.*, 2022). The high percentage of unsaturated fatty acid indicates many health benefits of oil.

Numerous terpenoids including α-isoamylene, f-isoamylene, and octenal were also identified. They have different bioactivities like antimicrobial, insecticidal, and antioxidant effects(Yadav and Kant-Upadhyay 2022) (Cisneros-Pineda *et al.*, 2007). Esters identified namely octyl acetate and ethyl hexadecanoate give characteristic aroma and flavor to the oil (Ullah *et al.*, 2020). They also influence the oxidative and anti-microbial properties. The key ketones such as methyl-2-methylbutyl ketone, isoamyl methyl ketone, and decanone provide aroma and flavor. They also exhibit anti-inflammatory and antimicrobial activities(Noumedem, Djeussi et al. 2017) (Noumedem *et al.*, 2017). The last class of phytochemicals, hydrocarbons, including tetradecane and hexadecane have emollient activities and antioxidant activity owing to hydrogen

donation(Teixeira, Marques et al. 2013) (Teixeira *et al.*, 2013). Other minor compounds offer functional attributes. Regarding antioxidant potential of seeds oil was found to be inactive against DPPH free radical scavenging assay as shown in table 5.

Despite marvelous advancements in the healthcare system certain challenges are still to be addressed. Therefore, it is highly important to continuously explore natural resources to find out better treatment options. Medicinal plants are one of the important natural resources to explore for this purpose. So, in the present research project seeds oil of the very commonly used herb Lactuca sativa was prepared and then screened for different aspects. Phytochemical screening gave a detailed understanding of this crude drug. Iodine value, soap value, and antioxidant potential were also determined to get further insight. Further research should focus on the isolation and testing of specific phytochemicals. Structural elucidation by spectroscopic techniques can confirm their identities(Chhikara, Kushwaha et al. 2019) (Chhikara *et al.*, 2019). In-vitro and in-vivo studies evaluate the antioxidant, anti-inflammatory, antimicrobial, and anticancer effects are warranted to establish therapeutic potentials (Xu and Chang, 2008). Toxicological evaluations are also important for determining the safety parameters.

Lettuce seeds oil holds prospects in pharmaceuticals, food, and cosmetics due to phytochemicals. Selective breeding and growth can optimize the oil properties (Sae-Lim, Kause et al. 2017)(Song *et al.*, 2010). Similarly, to ensure reproducibility, standardization of extraction and analytical methods is very important. The results of this research indicated the presence of significant chemical compounds. Therefore, the medicinal use of this plant in folklore is justified.

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