Essential Oil Chemical Characterization and Antibacterial Activities of
*Lepidium sativum* Seed

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Abstract

*Lepidium sativum* plants are available abundantly in all part of Ethiopia and traditionally used for the treatment of various ailments. The work done on essential oil chemical characterization and biological activity of this plant are still insufficient reports. Therefore the aim of the present study was to carry out essential oil chemical characterization and antibacterial activities of the seeds extracts and oils of *L. sativum*. The study was conducted by extraction of the seeds with organic solvents n-hexane, dichloromethane, ethyl acetate and methanol. The study performed on extraction of essential oil of the seed through hydrodistillation and investigation of phytochemical constituents of each solvent extract. The n-hexane extract (oil) and the essential oil of the seed extract were analyzed with GC-MS and 11 components were obtained from each types of oil. 7, 10, 13-hexadecatrienoic acid and Indol were the major components of n-hexane extracted and essential oil of the seeds respectively. Moreover both oils were held unsaturated fatty acid, saturated fatty acid and aromatic derivative compounds. The preliminary phytochemical test revealed the presence of alkaloids, flavonoids, glycosides, phenols, saponins, anthraquinnes, and tannins. Antibacterial activities of the essential oil were implemented by disc diffusion method against one Gram positive bacterium *Stphylococcus aureus* and three Gram negative bacteria: *E.coli*, *Proteus mirabilis* and *Klibsiella pneumoniae*. The inhibition zones of the samples were compared with standard drug ceftriazone. The essential oil showed antibacterial activities on all the tested bacteria.

Keywords: *Lepidium sativum*, 7, 10, 13-hexadecatrienoic acid, Indol, phytoconstituents
1. Introduction

*L. sativum* (family *Brassicaceae*) is an annual herb growing up to 80 cm. Seeds is small, oval-shaped about 2 to 3 mm long and 1 to 1.5 mm wide with reddish brown in color. The stem is finely striate, branched and glabrous (hairless, smooth). In some regions, garden cress is known as garden pepper cress, pepper grass or pepper wort. It is a fast growing edible plant botanically related to water cress and mustard and sharing their peppery, tangy flavor and aroma. The main character of *L. sativum* is that it can grow in any type of climate and soil condition with few requirements. Seeds, leaves and roots are economically important, however, the crop is mainly cultivated for seeds. It is an important green vegetable consumed by human beings, most typically as a garnish or as a leafy vegetable [1]. The plant have antiasthmatic, antiscorbutic, aperients, diuretic, stimulant, chemoprotective effect, antidiabetic, antihypertensive, fracture healing properties, hepatoprotective activity, pesticidal activity, antidiarrheal activity [2]. Leaf aqueous extracts of *L. sativum* investigated for anticancer activity on human tongue squamous carcinoma (CAL – 27). The results showed that the plant extract inhibit the growth of CAL-27 cells. The seeds are aperient, diuretic, tonic, demulcent, aphrodisiac, carminative, galactagogue and emmenagogue [3]. Seeds of *L. sativum* are prescribed by many Ayurvedic practitioners in bronchial asthmatic patients [4]. Ethiopian people use different parts of the plants for treatment of various diseases such as skin diseases [5], Malaria [6-8], diarrhoea [7]. Essential oil from seeds of *L. sativum* plant is one of them. The seeds are rich in minerals and vitamins; especially vitamins C, A, B and E [9]. In many parts of Ethiopia a special dish called “Feto Fitfit” is prepared from the seeds [10]. Despite the widespread traditional use of *L. sativum* and the presence of many types and sub-class of chemical compositions in the plant, little work done inside the country. The insecticidal, repellents and antimalarial usage of *L. sativum* in Jabitehnan District, West Gojjam has been reported [11], phytochemical screening and antimicrobial activities of crude extract of *L. sativum* seeds grown in Eastern Ethiopia has also been reported [12], and the Genetic Diversity of *L. sativum* populations from Ethiopia reported [13]. *L. sativum* is a useful medicinal plant. However, its chemical composition has not been carried out extensively in Ethiopia. Therefore, this study is expected to fill the study gap and increase knowledge on the chemical composition of *L. sativum* seed in general.

2. Experimental section

2.1. Plant Material Collection

Seeds of *L. sativum* were collected from Goma woreda, Jimma Zone, South west Ethiopia approximately 410 km from Addis Ababa in November 2016. The plant material was authenticated by a taxonomist Melaku Wendafrash and a voucher specimen (Voucher No: 001) was deposited in the National Herbarium, Department of Biology, Addis Ababa University, Addis Ababa, Ethiopia.

The performed extraction yield of essential oil by hydrodistillation from 100 g of dried seed powder of *L. sativum* was also 2.0 g

2.2. Chemicals and Reagents

The study was conducted using the following chemicals: n-hexane (Ranchem industry, India) dichloromethane (Carlo Erba reagents, France), ethyl acetate (Atico, India), methanol (Carlo Erba reagents, France), chloroform (Blulux Laboratories Pvt. Ltd., India), distilled water, hydrochloric acid, ammonia, ferric chloride, sulfuric acid, Wagner’s reagent, silica gel particle size (200-400 mesh).
2.3. Apparatus

The list of apparatus that were used in this research: mortar, pestle, rotary evaporator (model R1001-VNAC 220,50HZ, China), round bottom flasks, measuring cylinders, drying oven, filter paper (Whatmann No. 1), separatory funnels, Water bath, Clevenger type apparatus, beakers, spatula, conical flasks, vials, refrigerator and tong. GC-MS which is Agilent technologies 7820A GC system with Agilent technologies 5977E MSD, USA. A highly polar capillary column (30 m × 0.25 mm× 0.25 μm film thickness) of HP-88 coated with poly(dimethylsiloxane) was used to separate the FAMEs. Analytical conditions were; injector and detector temperature: 250 and 260 °C respectively. The oven temperature was programmed from 60 °C to 325/350 °C at rate of 6 °C/min. Helium gas was employed as the carrier gas at 1 mL/min flow rate; source 70 eV. Injected volume in the GC-MS was 1μL.

2.4. Extraction of seed powder of *L. sativum*

The dry plant seed was powdered to suitable size by using mortar and pestle to make it ready for extraction. First 500 g of powdered *L. sativum* seed was soaked in n-hexane (2 L) for 72 hrs and shaken occasionally, then, filtered with Whatman No. 1 filter paper and concentrated using rotary evaporator at 40 °C to furnish 84.9 g of pale-yellow oil and it was kept in refrigerator at 4 °C until analysis. The solvent free marc was then soaked in 2 L of dichloromethane for 72 hrs, filtered, concentrated and 31.2 g of grey yellow oil was obtained. The marc after dichloromethane extract was soaked again in 2 L of ethyl acetate for 72 hrs, filtered and concentrated following the same ways as the previous two extractions and 15.8 g of yellow jelly substance was obtained. Finally, the last marc was soaked for 72 hrs in methanol (2 L), filtered and concentrated using rotary evaporator at 40 °C, and afforded 10.2 g of dark reddish jelly crude.

2.5. Extraction of essential oil

Dried and pulverized seeds of *L. sativum* (100 g) was suspended in distilled water (350 mL) and placed in a round bottom flask in Clevenger type apparatus and hydrodistilled for 4 hrs. The procedure was repeated four times with new sample to increase the yield and 400 mL hydrodistilled product were collected. Then, the collected amount was divided into three separatory funnels and mixed with chloroform (equal volume), shaken well and stayed for 24 hrs to clearly separate the organic and water layer. The organic layer, contained the essential oil separated from water was denser than the water layer and taken position at the bottom of the funnel. Finally, the organic layer was collected in flask. The
2.6. Preparation of fatty acid methyl ester (FAME)

The popular and rapid method for preparation of FAME from oils is base catalyzed trans-methylation by using methanol [14]. This converts the non-volatile fatty acids in the oil into volatile methyl esters that can be easily analyzed by gas chromatography. The trans-esterification was done as described below. In to a 50 mL bottom flask, one gram of essential oil was weighted and dissolved in 10 ml of methanolic sodium hydroxide solution (prepared by mixing NaOH in methanol) and added a boiling aid. The condenser was fitted to the mixture containing round bottom flask. The content was refluxed at 60 °C for 5 min. The mixture was placed in a water bath at 50 °C for 1hr reflux, then stopped boiling and removed the condenser, then cooled it to room temperature. The reaction was stopped by adding small portion of saturated NaCl solution and the solution was swirled gently several times. 20 mL of n-hexane was added to the solution, and then the mixture was transferred to the separatory funnel and shaken for a few minutes, then allowed the mixture to settle for some time till it formed layers. The distinct upper layer of methyl ester in n-hexane was separated carefully in a volumetric flask and anhydrous Na2SO4 was added to remove any trace of water of the mixture. Then the mixture was filtered with standard Grade 4 qualitative filter paper. Finally, the solvent (n-hexane) was removed from the FAMEs sample using rotary evaporator. The esterified sample was prepared 10ppm in HP capped vial by serial dilution method from 200 ppm stock solution for GC-MS analysis or injection and the components of the oil were identified on the basis of the comparison of retention time and m/z of the compounds with the same compounds found in the NIST 14.L Library.

2.7. Preliminary phytochemical screening test on the crude extracts of the seed of L. sativum

The preliminary phytochemical screening test for each of the crude extracts (n-hexane, dichloromethane, ethyl acetate and methanol) of the seeds of L. sativum were done according to the method [15, 16] to analyze the presence of phytochemical constituents namely: tannins, saponins, alkaloids, flavonoids, glycosides, anthraquinones, and phenols.

2.7.1. Test for alkaloids (Wagner's test)

Each of the extracts (2 mL) was treated with Wagner's reagents (Iodine and potassium iodide in 100 mL water) and formation of reddish brown precipitate was observed.

2.7.2. Test for flavonoids (Sulfuric acid test)

Each of the extracts (2 mL) was treated with concentrated H2SO4 and the formation of orange color was observed.

2.7.3. Test for tannins (Ferric chloride test)

Each of the extracts (2 mL) was added with alcohol and treated with neutral ferric chloride solution and greenish color was observed.

2.7.4. Test for saponins (Foam test)

A small amount of extract was shaken vigorously with water and persistent foam was observed.

2.7.5. Test for anthraquinones (Borntrager's test)

To 0.5 g of crude extract, 5 mL of chloroform was added and shaken and then filtered. 0.5 mL of ammonia solution was added to the filtrate and the mixture was shaken well and the violet color was observed.

2.7.6. Test for phenols (Ferric chloride test)

Each of the crude extracts (0.5 g) were put in test tube and treated with few drops of FeCl3 and black color was observed.
2.7.7. **Test for glycosides (Borntrager's test)**

In the first test tube, 1 mL of H$_2$SO$_4$ was added to 1 mL of the extract solution. The mixture was boiled in a water bath and then filtered to the second test tube. Filtrate was then shaken with equal volume of chloroform and kept to stand for 5 min. Then lower layer of chloroform was shaken with half of its volume with dilute ammonia and the formation of rose pink to red color was observed.

2.8. **Antibacterial activity tests**

The essential oil of *L. sativum* seed were evaluated *in vitro* for antibacterial activity tests by using the disc diffusion method [17]. The antibacterial activities of all samples were tested against four test microorganisms such as one Gram-positive test bacterium *Staphylococcus aureus* and three Gram-negative bacteria: *Escherichia coli*, *Proteus mirabilis* and *Klebsiella pneumoniae*. The essential oil was used at a concentration of 1 mg/mL. Discs (6 mm diameter) were prepared using Whatman No. 1 filter paper and sterilized by autoclaving at 121 °C. The blank sterile discs were placed on the inoculated Mueller Hinton Agar (MHA) surface and impregnated with 20 μL of stock solution. The plates were incubated at 37 °C for 24 hrs. The activities of the samples against all bacteria were recorded by measuring the inhibition zone and compared the results with positive control drug for the experiment, ceftriaxone. Zone of inhibition for the standard antibiotic (ceftriaxone) at 20 μL was 23 mm.

3. **Results and Discussion**

3.1. **Yields of Extract**

The solvents that were used during the extraction, the mass of the extracts and percent yield of each of the crude extracts were tabulated in Table 1.

| Solvent          | Mass of extract obtained in (g) | Percentage yield |
|------------------|--------------------------------|------------------|
| n-hexane         | 84.9 pale-yellow oil            | 16.98            |
| Dichloromethane  | 31.2 grey oil                   | 6.24             |
| Ethyl acetate    | 15.8 yellow jelly               | 3.16             |
| Methanol         | 10.2 dark reddish jelly         | 2.04             |

The performed extraction yield of essential oil by hydrodistillation from 100 g of dried seed powder of *L. sativum* was also 2.0 g

3.2. **Phytochemical Screening**

The result revealed that alkaloids and saponins were found in all solvent extracts and flavonoids did not show positive test in n-hexane, dichloromethane and ethyl acetate extracts. More phytochemical constituents were obtained in the methanol crude extract when compared with other crude extracts and this implemented that, the seeds of *L. sativum* held polar compounds. Results that were obtained from the phytochemical screening tests of n-hexane, dichloromethane, ethyl acetate and methanol crude extracts of *L. sativum* seeds are given in Table 2.

| No | Constituents   | n-hexane | dichloromethane | ethyl acetate | methanol |
|----|----------------|----------|-----------------|--------------|----------|
| 1  | alkaloids      | +        | +               | +            | +        |
| 2  | flavonoids     | -        | -               | -            | +        |
| 3  | tannins        | +        | -               | -            | +        |
| 4  | saponins       | +        | +               | +            | +        |
| 5  | anthraquinones | -        | -               | +            | +        |
| 6  | phenols        | -        | +               | +            | +        |
| 7  | glycosides     | -        | -               | +            | +        |

Key: + = present and - = absent
3.3. Result of GC-MS Analyses of n-hexane Extracted Seed Oil of L. sativum

The components of the oil were identified using GC-MS analysis after converted in to FAME. The GC-MS spectrum of the seed oil components were given as in Table 3.

The result obtained from the GC-MS analysis indicated that, the n-hexane extracted seed oil of L. sativum contained 11 different types of components. The major identified components in these oils were 7,10,13-hexadecatrienoic acid unsaturated fatty acid in agreement with other observations [18]. A recent study demonstrated that the main constituents of L. sativum oil are β-amyrin, 9,12,15-octadecatrienoic acid methyl ester, 9-octadecenoic acid methyl ester, α-amyrin, 11-eicosenoic acid methyl ester, 9,12-octadecadienoic acid, and hexadecanoic acid methyl ester [19]. These qualitative and quantitative differences in the chemical composition of essential oils could be attributed to several factors such as geographical location [20], climatic effects of the plants, harvest season, nature of the soil, age of the plant parts, the part of the plant used [21], and time of collection [22] with consequent influence on biological activities [23]. It is well known that L. sativum oil is an abundant source of omega-3 which makes it suitable for use as a food supplement and for medicinal purposes [24]. The seeds oil was also containing saturated fatty acid (hexadecanoic acid), nitrogen containing aromatic derivative compounds and nitrogen containing hydrocarbons.

3.4. Result of the GC-MS Analysis of the Essential Oil of L. sativum

The components of the essential oil were identified using GC-MS analysis. The GC-MS chromatogram of the seed oil components were given as in Table 4.

Table 3: GC-MS analysis of n-hexane extracted seed oil of L. sativum.

| Retention time (min) | Compound name | Molecular formula | Molecular weight (g/mol) | Area |
|----------------------|---------------|-------------------|--------------------------|------|
| 6.313                | Acetamide,2-fluoro- | C₇H₆FNO          | 77                       | 0.67 |
| 12.351               | 1-methylcyclohexylamine | C₉H₁₅N          | 199                      | 0.56 |
| 13.632               | Methylpent-4-enylamine | C₉H₁₄N          | 99                       | 1.01 |
| 15.934               | Benzenehexanamine,4-fluoro- | C₈H₁₄NO₂        | 185                      | 1.33 |
| 16.291               | 1-propanamine,N,2-dimethyl- | C₃H₇N          | 87                       | 1.06 |
| 18.900               | Hexadecanoic acid, methyl ester | C₁₆H₃₂O₂   | 270                      | 15.14|
| 21.679               | Acetamide,N-aminocarbonyl)-2-chloro- | C₈H₁₃NO₂ | 136                     | 4.9  |
| 21.796               | 7,10,13-Hexadecatrienoic acid, methyl ester | C₁₆H₃₂O₂ | 264                      | 64.42|
| 22.230               | Benzenehexanamine,2-fluoro-beta,5-dihydroxy-n-methyl- | C₁₆H₃₂NO₂ | 185                      | 3.74 |
| 25.695               | Amphetamine | C₁₆H₂₃N          | 135                      | 5.79 |
| 26.288               | 1-Butanamine, N-methyl | C₈H₁₈N          | 87                       | 1.38 |

Table 4: The GC-MS analysis of the essential oil of L. sativum

| Retention time (min) | Compound name | Molecular formula | Molecular weight (g/mol) | Area |
|----------------------|---------------|-------------------|--------------------------|------|
| 8.702                | Indole | C₅H₅N          | 117                      | 63.78|
| 11.756               | Thiocyanic acid, phenylmethyl ester | C₅H₅NS         | 149                      | 5.23 |
| 12.052               | Benzene, (isothiocyanatooethyl)- | C₇H₇N          | 149                      | 6.95 |
| 12.352               | Tetradecane | C₇H₁₅NO         | 198                      | 0.49 |
| 13.631               | pentadecane | C₉H₁₄N          | 212                      | 0.68 |
| 15.931               | 2H-Azezip-2-one,hexahydro-1-methyl- | C₁₀H₁₈NO       | 127                      | 0.17 |
| 16.292               | Methyl tetradecanoate | C₁₀H₂₂O₂        | 242                      | 1.07 |
| 18.898               | Hexadecanoic acid, methyl ester | C₁₆H₃₂O₂       | 270                      | 7.36 |
| 21.682               | 1-Methyl-2-phenoxymethylamine | C₁₄H₂₂NO      | 151                      | 1.42 |
| 21.782               | 9-Octadecanoic acid (Z)-, methyl ester | C₁₆H₃₂O₂      | 296                      | 10.67|
| 22.232               | Methyl stearate | C₁₆H₃₂O₂       | 298                      | 2.18 |

Table 5: Result of inhibition zone of tested samples against bacteria

| No | Sample | Concentration (mg/mL) | Staphylococcus aureus (mm) | Escherichia coli (mm) | Proteus mirabilis (mm) | Kibsiella pneumoniae (mm) |
|----|--------|-----------------------|--------------------------|----------------------|-----------------------|--------------------------|
| 1  | Essential oil | 1                     | 60                       | 45                   | 40                    | 18                       |
| 2  | Ceftriaxone | 0.03                  | 23                       | 23                   | 23                    | 23                       |
The result revealed, the plant seed contained 11 different types of components. Indol was a major component of the essential oil of *L. sativum* seed. Indole derivatives constitute an important class of therapeutic agents in medicinal chemistry including antihypertensive, antiproliferative, antiviral, antitumor, analgesic, anti-inflammatory, antimicrobial, antifungal activities, etc. [25]. Therefore essential oil of *L. sativum* seed has diverse pharmacological activities. Moreover, the analysis result showed, the seeds oil was also contained saturated and unsaturated fatty acids, nitrogen and sulphur containing aromatic derivative compounds, saturated hydrocarbons, nitrogen and oxygen containing aromatic derivative compound and nitrogen containing ketone.

### 3.5. Result of Antibacterial activity tests

The result revealed essential oil of *L. sativum* showed great antibacterial activities against gram positive bacterium; *Staphylocous aureus* and in all tested gram negative bacteria recording higher inhibition zone. 0.03 mg/mL concentration of ceftriaxone, standard medicine (positive control) was used and its inhibition zone 23 mm in all of the tested strains. 20μL of each of the tested sample was taken and added to the 6mm whole made on the disc using a micropipette. A previous study conducted by [26] showed that petroleum ether, aqueous, and methanolic extracts of *L. sativum* seed obtained from Sudan exhibit antimicrobial activity against six opportunistic microrganisms: *S. aureus*, *E. coli*, *K. pneumoniae*, *Proteus vulgaris*, *P. aeruginosa*. Recently, another study conducted in Ethiopian the crude extract from *L. sativum* seeds exhibited antimicrobial properties against tested bacteria (*E. coli*, *S. typhi*, *B. subtilis*, and *S. aureus*) [12]. Therefore our studies resemble with other studies.

### 4. Conclusion

GC-MS analysis of the hydrodistilled seed oil and the n-hexane extract oil of *L. sativum* seed showed the presence of 11 components in each type of oil. 7,10,13-hexadecatrienoic acid, and Indole was identified as the major compounds in LSH and LSE respectively. In addition, the result of preliminary phytochemical screening test indicated more phytoconstituent present in the methanol extract than other solvent extracts (n-hexane, dichloromethane, ethyl acetate). Antibacterial activities were performed using disc diffusion method and the result showed that, LSO were active against tested bacteria, suggesting its broad-spectrum antimicrobial activity.

### Abbreviation

- **LSH**- Lepidum Sativum n-hexane Extract
- **LSE**- Lepidum Sativum Essential oil
- **LSO**- Lepidum Sativum oil
- **FAME**- Fatty Acid Methyl Ester

### Conflict of Interest

The authors declare there is no conflict of interest.

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