Phytochemistry, Antioxidant, and Lipid Peroxidation Inhibition of the Essential Oils of *Lavandula Officinalis* L. in Iran

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**ABSTRACT**

The aim of present study was to evaluation the antioxidant potential of *Lavandula officinalis* on the basis of the chemical compositions of oils obtained by hydrodistillation. In the case of *L. officinalis*, 13 compounds were identified representing the 96.53% of the total oil. The major constituents of the oil were described as α-pinene (20.14%), camphor (14.36%), menthol (32.51%), and 1,8-cineole (20.14%). The oils were also subjected to screening for their possible antioxidant activity by using 2,2-diphenyl-1-picrylhydrazyl assays. 1,8-Cineole and menthol showed appreciable antioxidant activity in 2,2-diphenyl-1-picrylhydrazyl test. Antioxidant activity guided fractionation of the oil was carried out by The TLC-bioautography screening and fractionation resulted in the separation of the main antioxidant compound which were identified as 1,8-cineole (48%) and menthol (39%).

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**Introduction**

The antioxidant activity (AA) of fruits is noteworthy, several associations have been made between fruit and vegetable intake and a reduced risk of cancer and incidences of chronic and degenerative diseases.\[1\] Oxidation reactions and the decomposition of oxidation products are major causes of deterioration of various food products. To prevent these processes, antioxidants are widely used as additives in some foods. Currently, scientific research reveals that the antioxidant property of the plant extracts gives beneficial effect to human health.\[2,3\] Essential oils have therapeutic uses in human medicine due to its anticancer, antinociceptive, antiphlogistic, antiviral, antibacterial, and antioxidant properties.\[4,5\] Aromatic and medicinal plants are known to produce certain bioactive molecules which react with other organisms in the environment, inhibiting bacterial or fungal growth.\[6–8\] *Lavandula officinalis* L. is indigenous to Southern Europe and is sometimes found growing wild in the Mediterranean area between the coast and the lower mountain slopes. Leaves and flower of *L. officinalis* have the highest amount of essential oils.\[9,10\] Iran is one of the richest countries of the world in terms of having a substantial number of different medicinal plants species grown in various ecological conditions. The present study was performed to investigate components of essential oils *L. officinalis* and to evaluate the efficacy of chemically characterized *L. officinalis* essential oil as antioxidant agent.

**Materials and Methods**

**Plant Materials**

The plant materials (fresh) were collected from Ilam, Iran in 2013-2014 (March–April). Voucher specimens were identified by Mr. Esmaeili and deposited, under the number 48, in the private
herbarium of Dr F. Esmaeili. The *L. officinalis* aerial parts were ground and the resulting powder was subjected to hydrodistillation for 3 h in an all glass Clevenger-type apparatus according to the method recommended by the European Pharmacopoeia.\(^{(11)}\) The obtained essential oils were dried over anhydrous sodium sulphate and after filtration, stored at +4°C until tested and analyzed.

**Oil Isolation and Identification of the Oil Components**

Compositions of the essential oils of the aerial parts of *L. officinalis* were determined by gas chromatography (GC) analysis, and gas chromatography–mass spectrometry (GC–MS; replicated three times). GC analysis was done on a an Agilent Technologies 7890 GC equipped with FID and a HP-5MS 5% capillary column (30.00 m × 0.25 mm, 0.25 µm film thicknesses). Oven temperature was kept at 60°C for 4 min initially, and then raised at the rate of 4°C/min to 260°C. Injector and detector temperatures were set at 290 and 300°C, respectively. Helium with purity 99.999% was used as carrier gas at a flow rate of 2 mL/min, and 0.1 µL samples were injected manually in the split mode. Peaks area percents were used for obtaining quantitative data. The EI-MS operating parameters were as follows: ionization voltage, 70 eV; ion source temperature, 200°C. Retention indices were calculated for all components using a homologous series of \(n\)-alkanes (C\(_5\)–C\(_{24}\)) injected in conditions equal to samples ones. Identification of oil components was accomplished based on comparison of their retention times with those of authentic standards and by comparison of their mass spectral fragmentation patterns (WILLEY/ChemStation data system).\(^{(12)}\)

**Total Phenolic Determination**

Total phenolic contents in aerial parts *L. officinalis* were determined by the Folin–Ciocalteu method.\(^{(13)}\) The total phenolic content was expressed as gallic acid equivalents (GAE; mg per g oil).

**Total Flavonoid Determination**

Total flavonoid contents in aerial parts *L. officinalis* were measured as described previously.\(^{(14)}\) The total flavonoid content was calculated as rutin equivalents (mg per g oil).

**AA**

The efficacy of the essential oils to scavenge 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals was evaluated using a spectrophotometry method.\(^{(15,16)}\) Briefly, a 50 µL volume of various dilutions of each sample was mixed with 5 mL of 0.004% methanol solutions of DPPH followed by 30 min incubation at ambient temperature. Thereafter, absorbance values of the sample were recorded against control at 517 nm. The inhibition percentages were measured using Eq. (1). The antioxidants activity of the test samples in concentration providing 50% inhibition, were considered as IC\(_{50}\) (µg/mL).

\[
\text{Inhibition percent} = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \times 100 \quad (1)
\]

Butylhydroxyanisole (BHA) and ascorbic acid were used as positive controls. All experiments were repeated three times and the average results and standard deviations were calculated.

**Rapid Screening for Antioxidants**

For screening of antioxidant compounds in aerial parts of *L. officinalis* essential oil, the TLC-bioautography method was carried out.\(^{(17,18)}\) The diluted oil (1:20 in methanol) was spotted on silica gel sheets (silica gel 60 F254 TLC plates) and developed in \(n\)-hexane-ethyl acetate (9:1). Plates
were sprayed with the methanolic solution of DPPH (0.2%). The active constituents were detected as yellow spots on a violet background. Only zones where their color turned from violet to yellow within the first 30 min (after spraying) were taken as positive results.

**Activity Guided Fractionation of the Essential Oil for Antioxidants**

For the isolation and identification of the active compounds in the essential oil, TLC was performed using the conditions previously described. The regions showing DPPH scavenging activity were scrapped off then, they were eluted with chloroform. All resulting constituents were analyzed by GC–MS and also tested for their antioxidant activities.

**β-Carotene-Linoleic Acid Model System (β-CLAMS)**

The β-CLAMS method by the peroxides generated during the oxidation of linoleic acid at elevated temperature. The AA of the extracts was evaluated in term of β-carotene blanching using the following formula: AA (%) = [(A0 – A1)/A0] × 100. where A0 is the absorbance of the control at 0 min, and A1 is the absorbance of the sample at 120 min. The results are expressed as IC50 values (µg/mL). All samples were prepared and analyzed in triplicate.

**Reducing Power and Lipid Peroxidation Inhibition**

The ability of the extracts to reduce Fe3+ was assayed by the method of Oyaizu. One milliliter of aerial parts of *L. officinalis* essential oil and new component were mixed with 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of 1% K3Fe(CN)6. After incubation at 50°C for 25 min, 2.5 mL of 10% trichloroacetic acid was added and the mixture was centrifuged at 650 g for 10 min. Finally, 2.5 mL of the upper layer was mixed with 2.5 mL of distilled water and 0.5 mL of 0.1% aqueous Fe Cl₃. The absorbance was measured at 700 nm. The mean of absorbance values were plotted against concentration and a linear regression analysis was carried out. Increase absorbance of the reaction mixture indicated increased reducing power. EC50 value (µg/mL) is the effective concentration at which the absorbance was 0.5 for reducing power. Ascorbic acid was used as positive control. Lipid peroxidation inhibition was determined by Shirwaikar et al. Ascorbic acid and trolox was used for comparison.

**Statistical Analysis**

The results are presented as mean ± SD and statistically analyzed by one-way analysis of variance (ANOVA) followed by Duncan’s test.

**Results and Discussion**

**Chemical Composition of *L. Officinalis* EO**

The chemical compositions of *L. officinalis* essential oil are shown in Table 1. Thirteen compounds representing 96.56% of *L. officinalis* essential oil were identified. The organic compounds detected in the aerial parts oils, were linalool oxide (0.34%), α-pinene (20.14%), α-terpinene (0.87%), borneol (3.45%), camphor (14.36%), menthol (32.51%), eucarvone (0.45%), terpinolene (0.71%), b-caryophyllene (0.14%), viridiflorol (0.7%), 1,8-cineole (20.114%), β-pinene (1.36%), and linalool (1.36%). The chemical compositions revealed that this leaves had compositions relatively similar to those of other *L. officinalis* essential oils analyzed by Rostami et al. *L. officinalis* oils and extraction contain more than 100 compounds, with the two major constituents being linalool and linalylacetate. The different qualitative and quantitative chemical compositions of these EOs with respect to previous
investigations could be related first and foremost to the different environmental conditions, genetics (degree of hybridization), geographical origin, and harvest period.

**Extraction Yield, Total Phenolic Contents, and Total Flavonoid Contents**

As shown in Table 2, the extraction yield of *L. officinalis* was 121.74 ± 07 mg per g oil. The total phenolic and total flavonoid contents were 164.75 ± 01 and 191.27 ± 02 mg per g oil. These results showed that the total phenolic and total flavonoid contents have an obvious variation in various concentrations.

**AA**

The DPPH is a stable free radical, which has been widely accepted as a tool for estimating the free radical scavenging activities of antioxidants. The results presented in Table 3 revealed that *L. officinalis* EO and its main constituents exhibited a remarkable activity. In particular, 1,8-cineole exhibited clearly a higher activity (11.04 ± 0.31 µg/mL) followed by menthol (11.54 ± 0.04 µg/mL; Table 3), while the activities of other terpenoid was weak (α-pinene and Camphor). The positive controls BHT and ascorbic acid exhibited IC\(_{50}\) values equal to 12.00 ± 0.01 µg/mL and 12.07 ± 0.08 µg/mL, respectively. Table 3 depicts the inhibition of β-carotene bleaching by the *L. officinalis* EO. The IC\(_{50}\) value was 11.84 ± 0.11 µg/mL. The reducing power of *L. officinalis* EO, expressed as CE\(_{50}\), was clearly more significant than that of the positive control BHA and AA. As the EO presented a significant AA in the assays and bioautography test, it was subjected to the TLC for isolation of the active compounds. Components identified and their AA relative percentages have been shown in Table 4. According to these results, there is a relationship between total phenolic contents and AA.

**Table 1.** Chemical compositions of *Lavandula officinalis*.

| Components       | %  |
|------------------|----|
| 1. Linalool oxide| 0.34 |
| 2. α-Pinene      | 20.14 |
| 3. α-Terpinene   | 0.87 |
| 4. Borneol       | 3.45 |
| 5. Camphor       | 14.36 |
| 6. Menthol       | 32.51 |
| 7. Eucarvone     | 0.45 |
| 8. Terpinolene   | 0.71 |
| 9. β-Caryophyllene| 0.14 |
| 10. Viridiflorol | 0.7 |
| 11. 1,8-Cineole  | 20.14 |
| 12. β-Pinen      | 1.36 |
| 13. Linalool     | 1.36 |
| Total            | 96.53 |

RI: Retention indices relative to C\(_4\)–C\(_{25}\) n-alkanes on the HP-5 column.

**Table 2.** Extraction yields, total phenolic contents, and total flavonoid contents of *L. officinalis* extracts.

| Extract     | Extraction yield\(^a\) | Total phenolic\(^b\) | Total flavonoid\(^c\) |
|-------------|-------------------------|----------------------|-----------------------|
| *L. officinalis* | 121.7 ± 07               | 164.75 ± 01          | 191.27 ± 02           |

\(^a\)Expressed as mg of extract per gram dry material;
\(^b\)Expressed as mg of gallic acid per gram dry extract;
\(^c\)Expressed as mg of rutin per gram dry extract (water).
According to the obtained results, *L. officinalis* EO and its main component significantly inhibited the formation of TBARS in brain homogenates in a concentration dependent manner (Table 5). The suppressive power on the lipid peroxidation of 1,8-cineole and menthol were found to be the most potent (91.01 ± 0.04 µg/mL and 89.21 ± 0.09 µg/mL). Ascorbic acid and trolox showed significant suppressive power on lipid peroxidation in mice brain homogenate with IC$_{50}$ value of 84.14 ± 0.06 and 85.21 ± 0.02 µg/mL (Table 5). Phenolics are important components of the human diet due to their potential AA, their capacity to diminish oxidative stress induced tissue damage resulted from chronic diseases and their potentially important properties such as anticancer activities.[24–26]

### Conclusions

During recent years, the plant has received an increased attention due to its remarkable AA in the food industry. Our results show that *L. officinalis* oil and one of its main compounds, 1,8-cineole, may be a source of antioxidant drugs for the food, cosmetic, and pharmaceutical industries.

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**Table 3.** Antioxidant activity of EO extract from *L. officinalis*: DPPH free radical scavenging activity (expressed as IC$_{50}$ values: µg/mL), and β-carotene bleaching test. Reducing power was expressed as EC$_{50}$ values (µg/mL). Butylhydroxyanisole and ascorbic acid were used as positive controls.

| Tested compounds | IC$_{50}$ (µg/mL) |
|------------------|-------------------|
| *L. officinalis* EO | 12.07 ± 0.31 µg/mL |
| α-Pinene | 14.07 ± 0.11 µg/mL |
| Camphor | 12.18 ± 0.65 µg/mL |
| Menthol | 11.54 ± 0.04 µg/mL |
| 1,8-Cineole | 11.04 ± 0.31 µg/mL |
| *L. officinalis* EO (β-Carotenes IC$_{50}$ µg/mL) | 12.84 ± 0.11 µg/mL |
| *L. officinalis* EO (Reducing power EC$_{50}$ µg/mL) | 11.69 ± 0.04 µg/mL |
| BHA (IC$_{50}$ µg/mL) | 12.00 ± 0.01 µg/mL |
| AA (IC$_{50}$ µg/mL) | 12.07 ± 0.08 µg/mL |

Values are mean ± S.D. of three replications; *IC$_{50}$ values have been presented with their respective 95% confidence limits.

**Table 4.** Components identified and their antioxidant activity relative percentages.

| Compounds | % |
|-----------|---|
| α-Pinene | 3 |
| Camphor | 5 |
| Menthol | 39 |
| 1,8-Cineole | 48 |

Values are given as mean ± SD (n = 3); Means in each column followed by different letters are significantly different (p < 0.05).

**Table 5.** Lipid peroxidation inhibition of EO extract from *L. officinalis* and its main samples (expressed as IC$_{50}$ values: µg/mL). Trolox and ascorbic acid were used as positive controls.

| Tested compounds | IC$_{50}$ (µg/mL) |
|------------------|-------------------|
| *L. officinalis* EO | 82.21 ± 0.35 µg/mL |
| α-Pinene | 61.21 ± 0.03 µg/mL |
| Camphor | 75.21 ± 0.07 µg/mL |
| Menthol | 89.21 ± 0.09 µg/mL |
| 1,8-Cineole | 91.01 ± 0.04 µg/mL |
| Trolox | 85.21 ± 0.02 µg/mL |
| AA | 84.14 ± 0.06 µg/mL |

Experiments were carried out in triplicate and the results are expressed as mean ± SD.

**Lipid Peroxidation Inhibition**

According to the obtained results, *L. officinalis* EO and its main component significantly inhibited the formation of TBARS in brain homogenates in a concentration dependent manner (Table 5). The suppressive power on the lipid peroxidation of 1,8-cineole and menthol were found to be the most potent (91.01 ± 0.04 µg/mL and 89.21 ± 0.09 µg/mL). Ascorbic acid and trolox showed significant suppressive power on lipid peroxidation in mice brain homogenate with IC$_{50}$ value of 84.14 ± 0.06 and 85.21 ± 0.02 µg/mL (Table 5). Phenolics are important components of the human diet due to their potential AA, their capacity to diminish oxidative stress induced tissue damage resulted from chronic diseases and their potentially important properties such as anticancer activities.[24–26]
conclusion, the use of naturally occurring agents in this popular traditional plant in preserving and flavoring of various food products is recommended.

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