The Effect of Heat Treatment on Chemical Composition and Antioxidant Property of *Lippia citriodora* Essential Oil

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Abstract

Background: In recent years, natural products, especially natural antioxidants, play an important role in various fields. In fact, *Lippia citriodora* is one of the medicinal plants which is cultivated in Iran and it may be has potential antioxidant activity.

Objective: The chemical composition of *Lippia citriodora* essential oil (LCEO) has been determined. The total phenolic content and antioxidative properties of LCEO were determined. Finally, the effect of the thermal treatment (80, 120, 180ºC) for 1, 2 and 3 hours on the antioxidant activity (AOA) and the chemical composition of LCEO were studied.

Methods: The unheated and heated LCEO components were identified by GC/MS. The antioxidant activity (AOA) of LCEO was determined by two complementary test methods, namely DPPH free radical-scavenging and β-carotene bleaching.

Results: LCEO had different chemical components such as R-curcumene (14.1 %), caryophyllene oxide (6.6 %), 6-methyl-5-hepten-2-one (7.1 %), and spathulenol (7.0 %). The IC_{50} of LCEO was compared with that of synthetic antioxidant (BHT). Antioxidant activities of LCEO at different concentrations (0.6- 2.5 mg/ml) were determined by β-carotene bleaching method. The antioxidant activities of LCEO at this range of concentrations were 1.5 - 68.0 %. After heating up to 180ºC, the essential oil showed a significantly higher free radical-scavenger activity and evident changes in its chemical composition.

Conclusion: The study can be further extended to exploit not only the phenolic extracts but also the residual phenolic constituents associated with this herbal medicine as health supplement.

Keywords: Antioxidant activity, *Lippia citriodora*, GC/MS, Chemical composition, Thermal treatment

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Introduction

Oxidation processes are intrinsic in the energy management of all living organisms and are, therefore, kept under strict control by several cellular mechanisms [1]. Factors like stress, aging and pollution cause high level of free radicals in the body which damage DNA and lead to heart-diseases, cancer or stroke. Substances like vitamin E, vitamin C, or ß-carotene act as antioxidant nutrients in the body. Vitamin E and β-carotene protect cell membranes and vitamin C removes free radicals from inside the cell. The interest in antioxidants has been increased because of their high capacity in scavenging free radicals related to various diseases. The oxidation which is mediated by free radical reactions is also responsible for the rancidity of unpreserved food rich in unsaturated fatty acids and natural antioxidants are suggested as a superior alternative for the synthetic ones such as BHA or BHT [2]. These natural antioxidants not only protect food lipids from oxidation, but also may provide health benefits that are associated with preventing damages due to biological degeneration.

The genus *Lippia*, (Verbenaceae), comprises approximately 200 species indigenous to southern and central America and Africa [3]. This herb has wide-spread application in the food, cosmetics and household product industries. The traditional applications of LCEO include the treatment of stomach ailments, coughs, colds and asthma, in addition to its use as a tranquillizer. Recently, the ability of phenolic substances including flavonoids and phenolic acids to act as antioxidants has been extensively investigated [4].

The potential use of LCEO often involves some type of processing, e.g. heating, irradiation, and etc. Processing may cause irreversible modifications to the active substances, affecting their original structure, which may cause important changes in the proposed properties of this essential oil. Oxidation of lipids, which occurs during raw material storage, processing, heat treatment and further storage of final products, is one of the basic processes causing rancidity in food products, leading to their deterioration. Furthermore, products of lipid oxidation may influence other food constituents. Oxidative deterioration can result in alterations of organoleptic characteristics, (e.g., alterations in the taste and aroma of the finished products) making them unacceptable to the consumer. In addition, oxidized lipids may have undesirable effects on the human organism too.

The aims of this work were: (i) determining the chemical composition of LCEO by using GC/MS, (ii) evaluating the antioxidant activities (AOA) of LCEO by using the 2, 2′-diphenyl 1-picrylhydrazyl ($DPPH^{o}$) radical scavenging and β - carotene bleaching (BCB) methods, (iii) and studying the effect of heat treatment (80,120 and 180 ^{o}C) for 1, 2 and 3 hours on AOA (IC_{50}) and the chemical composition of LCEO.

Materials and Methods

Methods

Gas chromatography

Samples of 0.1 µl were injected for the analysis by capillary gas chromatography. A Hewlett-Packard 5890 gas chromatograph (GC) equipped with a flame ionization detector (FID) and a 30 m × 0.25 mm HP-5 column with 0.25 µm film thicknesses (Hewlett-Packard, CA, USA), was used for this study. The FID and the injector were maintained at 280 and 250**ºC**, respectively. Helium was used as the carrier gas meanwhile

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the flow through the column was 1 ml/min, and the split ratio was set to 5:1. The column was maintained at 60ºC, and then its temperature was increased at a rate of 5ºC /min to 250ºC. For the identification of the compounds, the retention time and retention index were confirmed with commercially available standard compounds by using the external standard method.

Mass spectrometry analysis

Gas chromatography–mass spectrometry (GC/MS) was used for the identification of volatile components in LCEO. A Hewlett-Packard 5890 gas chromatograph (GC), equipped with a 30 m × 0.25 mm HP-5 column with 0.25 µm film thickness, was used. GC/MS analysis was performed on the above-mentioned GC coupled with an Agilent Technologies 5973 Mass system. The other operating conditions were the same conditions which were mentioned above, and mass spectra were taken at 70 eV. Mass range was from 35 – 375 amu, emission current 150 mA. Quantitative data were obtained from the electronic integration of the FID peak areas. The components of the essential oils were identified by comparing their mass spectra and retention indices with those published in the literature and those presented in the MS computer library.

Determination of total phenolic content

Total phenolic content (TPC) of LCEO was determined according to the method described by Makkar et al. [5]. Four hundred µl of sample was taken to the test tube; then, 1.0 ml of Folin–Ciocalteu reagent (diluted 10-fold with distilled water) and 0.8 ml of 7.5 % sodium carbonate were added. After vortexing the reaction mixture, the tube was placed in a dark place for 40 min and the absorption at 765 nm was measured against a blank. The total phenolic content was expressed as mg gallic acid equivalents/g of methanol extract.

DPPH^o test

The stable radical species 2, 2′-diphenyl-1-picrylhydrazyl (DPPH^o), has been widely used for antioxidant capacity screening and estimation due to its clear reaction mechanism, solvent compatibility and the technical simplicity of its assays which requires no special equipment [6]. The purple colored DPPH^o has a strong characteristic absorption at 515 nm and can undergo reactions with hydrogen donating antioxidant compounds to yield the stable yellow DPPH–H molecule easily monitored with UV spectroscopy. The DPPH^o scavenging capacity assay was, therefore, used in this study to evaluate the free radical scavenging capacity of the extract (IC_{50}). The decrease in absorbance at 515 nm was determined continuously at every minute with a Perkin- Elmer UV/Vis model Lambda 2s spectrophotometer until the reaction reached a plateau. The percentage of remained DPPH^o (% DPPH^o-R) was calculated according to the following equation:

$$DPPH^o\text{-}R\ (\%) = [(DPPH^o)_t / (DPPH^o)_{t=0}] \times 100$$

$(DPPH^o)_{t=0}$ and $(DPPH^o)_t$ are concentrations of DPPH^o at t= 0 and t, respectively. The percentage of remaining DPPH^o against the sample/standard ratio was plotted to obtain the amount of antioxidant necessary to reduce the initial concentration of DPPH^o by 50 % (IC_{50}).

The ß-carotene bleaching method

A stock solution of *β*-carotene/linoleic acid was prepared as follows: first, 0.5 mg of *β*-carotene was dissolved in 1 ml of chloroform (HPLC grade), then 25 µl of linoleic acid and 200 mg of Tween 40 were added. The chloroform was removed by rotary vacuum evaporator at 45ºC for 4 minutes and 100 ml of

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oxygenated distilled water was added slowly to the semi-solid residue with vigorous agitation, to form an emulsion. Aliquots (3 ml) of the β-carotene/linoleic acid emulsion were mixed with 200 µl of the LCEO and BHT samples dissolved in a solvent, and incubated in a water bath at 50**ºC** for 60 min. Oxidation of the emulsion was monitored spectrophotometrically by measuring its absorbance at 470 nm. Control samples contained 200 µl of solvent instead of LCEO. All determinations were performed in triplicate and results were averaged. The percentage of the inhibition was calculated using the following equation:

$AOA\ (\%) = (A_t - A'_t) / (A'_0 - A'_t) \times 100$

A_t is the absorbance measured in the test sample after incubation for 30 min. A'_0, A'_t are the absorbance of the control measured at t= 0 and 30 minutes, respectively.

Thermal processing of samples

Samples (300 µl) of the essential oil were put in a glass tube with screw cap and incubated for 1, 2 and 3 hours at different temperatures (80, 120 and180**ºC**). At the end of the incubation, the samples were cooled in an ice bath and immediately used to determine their AOA and chemical compositions. All experiments were carried out in triplicate.

Chemicals

Butylated hydroxyl toluene (BHT), 2, 2`-diphenyl-1-picrylhydrazyl ($DPPH^o$), linoleic acid, chloroform, methanol, ethanol and Tween 40 were obtained from Fluka Chemical Co. (Buchs, Switzerland) with highest purity available without further purification. The plant of *L. citriodora* (the voucher specimen is deposited in the herbarium of medicinal plants, Iranian Academic Center for Education, Culture and Research, Karaj, Iran. (Voucher no.11)) was obtained from a research farm of the Institute of Medicinal Plants and Natural Products Research, Golestan, Iran. The essential oil was extracted from the aerial parts and flower heads, respectively, by hydrodistillation method (Clevenger-type apparatus) for 3hours. The oils were dried over anhydrous sodium sulphate and kept at -4 °C until it was used up completely.

Statistical analysis

The data were analyzed statistically, using an analysis of variance (ANOVA) and differences among the means were determined for significance at $P< 0.001$ using the least significance differences (LSD) test (by SAS software). The data are presented as mean ± standard deviation of the three determinations.

Results

Chemical composition and total phenolic content of unheated LCEO

Chemical composition of LCEO at room temperature was determined by GC/MS (Table 1). Forty compounds were identified, representing 94.6 % of the total LCEO. The chemical composition of LCEO has been presented in Table 1 (the identified components present with a concentration >1 % in the analyzed essential oil are listed in Table 1). ar-curcumene (14.1 %) and caryophyllene oxide (6.6 %), isoaromadendrene epoxide (4.0 %), allo-aromadendrene oxide (3.3 %), 1, 8-cineol (4.2 %), E-citral (4.7 %), Z-citral (4.4 %), 6-methyl-5-hepten-2-one (7.1), ß- caryophyllene (3.9 %), limonene (3.8 %), *p*-menth-1-en-8-ol (2.7 %), spathulenol (7.0 %), tau-cadinol (4.4 %), trans-nerolidol (3.1 %) were the main components of LCEO. The total phenolic content of LCEO was 200.0 ± 0.1 mg GAE/g sample.

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Table 1- Chemical composition of LCEO identified by GC/MS[a]

Component	Amount (%)	Kovat`s Index
1-octene-3-ol	1.4	995
6-methyl-5-hepten-2-one	7.1	1004
limonene	3.8	1052
1,8-cineole	4.2	1056
linalool	0.9	1118
trans-carveol	1.6	1206
p-menth-1-en-8-ol	2.7	1217
furfural	1.2	1238
Z-citral	4.4	1265
geraniol	1.7	1274
carvacrol	1.8	1.610
E-citral	4.7	1295
geranyl acetate	1.5	1402
α-copaene	1.3	1413
β -caryophyllene	3.9	1462
ar-curcumene	14.1	1483
α-humulene	1.0	1495
allo-aromadendrene	1.0	1503
trans-nerolidol	3.1	1594
spathulenol	7.0	1627
caryophyllene oxide	6.6	1634
globulol	0.9	1654
tau-cadinol	4.4	1684
isoaromadendrene epoxide	4.0	1704
allo-aromadendrene oxide	3.3	1717

a Other minor components not shown here

The antioxidant activity (AOA) of LCEO determined by ß-carotene bleaching method

The rate of β-carotene bleaching can be slowed down in the presence of antioxidants [7]. So, the antioxidant activity of the LCEO in comparison with that of a synthetic antioxidant, namely BHT was evaluated. Fig. 1 shows that the AOA of LCEO over the range 0.6- 2.5 mg/ml and BHT (0.1 and 0.2 mg/ml).

The antioxidant activity of heated and unheated LCEO using DPPH^o method

In this study, antioxidant activity of LCEO was measured by the model of scavenging the stable DPPH^o radical which is a widely used method to evaluate antioxidant activity in a relatively short time compared to the other methods should be pointed out [8]. The antioxidant activity of LCEO tested by the DPPH^o model system and the antioxidant activity of LCEO was IC_{50} = 3.20 ± 0.15 µg/ml. BHT showed a higher antioxidant activity (IC_{50} = 0.04 ± 0.01 µg/ml) than LCEO.

Discussion

The chemical composition of *Lippia chevalieri* grown in France [9]; *Lippia origanoides* H.B.K [10]; *Lippia citriodora* H.B.K [11] have been previously reported by others. The essential oil of *Lippia chevalieri* (grown in France) contains ß-caryophyllene (27 %), elemol (22 %) and caryophyllene oxide (9%) as major components. In the present study, the relative amount of ß-caryophyllene was found to be (3.9 %), and caryophyllene oxide (6.6 %), whereas elemol

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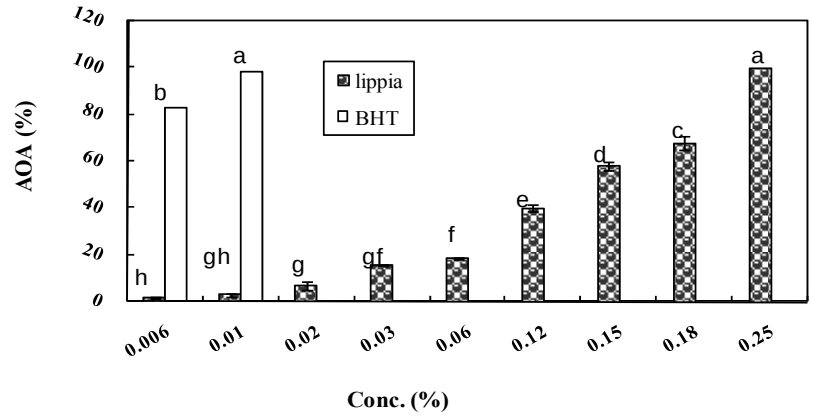


Fig 1- The antioxidant activity of LCEO as measured by ß-carotene bleaching method

was not detected. The essential oil of *Lippia origanoides* H.B.K composed *p*-cymene (10.3 %), γ-terpinene (4.1 %), linalool (2.5 %), thymyl methyl ether (2.2 %), thymol (18.5 %) and carvacrol (38.6 %). In the present study, the relative amount of *p*-cymene was found to be (0.5 %), γ-terpinene (0.3 %), linalool (0.9 %), carvacrol (1.8 %), whereas thymol methyl ether and thymol were not detected. The essential oil of LCEO H.B.K. (*Verbenaceae*) contained limonene (5.8 %), neral (24.5 %), geranial (38.7 %) and ß-caryophyllene (1.8 %) as major components. In the present study, the relative amount of limonene was found to be (3.8 %), neral (0.4 %), geranial (0.7 %) and ß-caryophyllene (3.9 %). The difference among chemical compositions of the essential oils widely depends on production conditions, such as climate, soil, harvest date, storage time, variety and cultivar factors. Generally, LCEO is characterized by the presence of aliphatics, terpenoids and aromatics compounds. Among them, the aromatics and terpenoids are the main portions of the investigated essential oil (such as curcumene (14.1 %)). Oxygenated monoterpenes and sesquiterpenes are shown to be the main group of terpenoids among the chemical composition of the LCEO. The percentage of the oxygenated terpenes such as α-terpinene, ß-terpinene was lower than that of others.

The total phenolic content of some selected culinary herbs and spices were determined in Finland [12]. These data were showed that the total phenolic content of LCEO is more than that of culinary herbs and spices. The AOA of the LCEO at the concentration of 2.5 mg/ml was similar to that of BHT at the concentration of 0.2 mg/ml ($P < 0.001$). The antioxidant activity of LCEO was related to that of phenolic and terpenic components such as spathulenol, 6-methyl-5-hepten-2-one and 1, 8-cineole. In addition, LCEO is a good antioxidant. It can be attributed to OH (such as spathulenol, 6-methyl-5-hepten-2-one and 1, 8-cineol) fraction in the oil. In the literature, there are many reports indicating the antioxidant potential of the spathulenol and carvacrol [13]. So the key role of phenolic compounds and terpenoids as scavengers of free radicals has been emphasized in several reports.

The inhibitory effect of this essential oil on lipid peroxidation was determined by the β-carotene/linoleic acid bleaching test and loss of the yellow color of β-carotene due to its reaction with radicals which are formed by linoleic acid oxidation in an emulsion. This

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test simulates the oxidation of the membrane lipid components in the presence of antioxidants inside the cells. β-carotene/linoleic acid test measures the capacity to inhibit the formation of conjugated diene hydroperoxide arising from linoleic acid oxidation.

Antioxidant activities of *P. endlicherianum*, *H. cappadocicum* and *V. wiedemannianum* were determined by ß-carotene bleaching method. In the ß-carotene/linoleic acid system (2 mg/ml of ethanolic extract) oxidation of linoleic acid was effectively inhibited by *P. endlicherianum* (72.6 ± 3.0 %), followed by *H. cappadocicum* (55.1 ± 2.33 %) and *V. wiedemannianum* (52.5 ± 3.1 %) extracts [13]. In comparison, the antioxidant activity of LCEO (1.8 mg/ml of EO in methanol, 68.0 ± 0.6 %) was more than *H. cappadocicum*, *V. wiedemannianum* (at 2 mg/ml of extracts). Compounds containing hydrogen atoms in the allylic and/or benzylic positions may show a better activity in this test because of a relatively easy abstraction of hydrogen atom from these functional groups by peroxy radicals formed in the test circumstances. Occurrence of the compounds with allylic and/or benzylic hydrogen's such as terpenoids and steroids were also reported in the stachys genus plants [14].

The addition of the essential oil to the DPPH^{o} solution caused a rapid decrease in the absorption at 517 nm. The degrees of discoloration indicate the radical scavenging capacity of the essential oil. The effect of the antioxidant on DPPH^{o} radical scavenging is thought to be due to their hydrogen donating ability [15]. The radical scavenging effect of the studied essential oil (by DPPH^{o} method) increased with an increase in its concentration. The antioxidant activities of LCEO were examined using DPPH^{o} free radical-scavenging method after heating the essential oil at different temperatures (80, 120 and 180 ^{o}C) for 1, 2 and 3 hours. As shown in Table 2, by increasing the heating time (from 1 to 3 hours), the AOA of LCEO has been increased significantly at three tested temperatures ($p < 0.001$). Also, by increasing the temperature, the AOA of LCEO has been increased but, as seen, the AOA of the EO was higher than 180 ^{o}C at 120 ^{o}C. This behavior can be due to a decrease in the amounts of 6-methyl 5-hepten-2-one and AR-curcumene and an increase of ß- caryophyllene (Table 3). To studying this behavior, the chemical compositions of heat treated LCEO have been determined by GC/MS. To simplify the experiment, we have chosen seven major components, namely 6-methyl-5-hepten-2-one, 1, 8-cineol, *Z*- and *E*-citral, ß- caryophyllene, AR-curcumene and spathulenol (Table 3).

Table 2- The changes of AOA (%) of heated LCEO determined by DPPH^{o} method[a]

Temperature / Time (h)	80 ^{o}C	120 ^{o}C	180 ^{o}C
1	6.4 ± 0.2 ^{i}	52.2 ± 0.3 ^{d}	36.9 ± 0.1 ^{g}
2	33.8 ± 0.5 ^{h}	59.4 ± 0.6 ^{c}	50.2 ± 0.4 ^{e}
3	43.9 ± 0.7 ^{f}	65.0 ± 0.1 ^{a}	62.1 ± 0.2 ^{b}

a Data are expressed as means ± s.d. and different letters in the table show significant difference between them at $p < 0.001$

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Table 3- Effect of heat treatment on chemical composition of LCEO (main components (%))[a]

Heating Temperature	Heating time								
	1h			2h			3h		
Component	80 °C	120 °C	180 °C	80 °C	120 °C	180 °C	80 °C	120 °C	180 °C
6-methyl-5-hepten-2-one	10.8±0.2^{ab}	10.6±0.3^{bc}	6.1±0.2^{e}	11.6±0.3^{a}	9.8±0.8^{c}	5.9±0.2^{e}	10.7±0.3^{ab}	10.7±0.5^{ab}	7.1±0.3^{d}
1,8-cineole	6.1±0.3^{a}	5.0 ±0.1^{bcd}	4.0±0.1^{de}	3.7±0.2^{e}	5.5±0.4^{abc}	4.6±0.5^{cde}	5.9±0.7^{ab}	4.2±0.5^{de}	4.4±0.6^{de}
Z-citral	6.2±0.2^{bc}	5.5±0.3^{d}	6.3±0.2^{b}	7.7±0.3^{a}	5.5±0.1^{cd}	6.4±0.4^{b}	5.4±0.4^{cd}	5.6±0.3^{cd}	4.8±0.2^{e}
E-citral	9.7±0.4^{abc}	8.6±0.4^{c}	10.8±0.7^{a}	10.1±0.5^{a}	8.7±0.3^{c}	10.1±0.5^{a}	9.9±0.8^{ab}	9.0±0.4^{bc}	9.1±0.1^{d}
β-caryophyllene	2.8±0.1^{dc}	2.1±0.8^{d}	3.6±0.2^{ab}	3.6±0.2^{ab}	3.1±0.2^{bc}	3.8±0.2^{ab}	3.8±0.4^{ab}	2.0±0.1^{d}	3.9±0.1^{a}
AR-curcumene	15.3±0.6^{a}	15.2±0.5^{a}	9.8±0.5^{bc}	10.8±0.5^{b}	15.2±0.3^{a}	10.3±0.2^{bc}	9.2±0.8^{c}	15.1±0.3^{a}	14.6±0.5^{a}
spathulenol	11.3±0.3^{a}	10.6±0.3^{a}	11.0±0.7^{a}	11.5±0.4^{a}	10.8±0.7^{a}	10.6±0.4^{a}	11.2±0.2^{a}	10.9±0.7^{a}	6.9±0.3^{b}

a Data are expressed as means ± s. d. of three experiments and were analyzed by the Student's t-test for paired data ($p< 0.001$). Data with different letters in the same row are significantly different at $p< 0.001$

The amount of *E*- and *Z*-citral in heated LCEO at 80 °C (*E*-citral (9.7 %) and *Z*-citral (6.2 %)) is more than those of LCEO at room temperature (*E*-citral (4.7 %), *Z*-citral (4.4 %)). Citral consists of two geometrical isomers, neral and geranial, and is highly susceptible to acid-promoted and oxidative degradation. Consequently, the AOA of heated LCEO was decreased. On the other hand, the amounts of spathulenol (7.0 %), AR-curcumene (14.1 %), 1, 8-cineol (4.2 %) and 6-methyl 5-hepten 2-one (7.1 %) at room temperature were less than spathulenol (11.3 %), AR-curcumene (15.3 %), 1, 8-cineol (6.1 %) and 6-methyl 5-hepten 2-one (10.8 %) at 80**°C**. Therefore, terpenoids compounds in heated LCEO have been increased in comparison to the unheated LCEO. As observed in Tables 2 and 3, LCEO showed thermal stability and high free radical-scavenging activity. Moreover, heat treatment had no considerable effect on it. Heating LCEO up to 120 °C has not changed the amount of 6-methyl 5-hepten 2-one, β-caryophyllene and 1, 8-cineol components significantly. Conversely, when essential oil has been heated at 120**°C**, LCEO showed a significantly higher free radical-scavenging activity which may be due to a marked loss of the *E*-citral content and an evident increase in the AR-curcumene content. IC_{50} value of the LCEO has been compared with other essential oils and BHT in Table 4. As shown in Table 4, radical scavenging power of LCEO is higher than that of activities reported in other papers [13, 16, 17]. Oxidation of lipids, which occurs during raw material storage, heating process and further storage of final products, is one of the basic processes causing rancidity in food products, leading to their deterioration. Oxidative deterioration can result in alterations of organoleptic characteristics, e.g., taste and aroma, in the finished products, making them unacceptable to the consumer. In addition, oxidized lipids may have undesirable effects on the human organism. Thus, due to health protection and economic reasons, many investigations have been undertaken with the aim to enhance the stability of lipids and lipid-containing products. The application of antioxidants is one of the technically simplest ways of reducing fat oxidation.

Tomaino et al., (2005) studied the effect of thermal processing on some spice essential oils at 80, 100, 120, 180**°C** [18]. Heating basil,

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Table 4- Comparison of DPPH radical scavenging capacity (IC_{50} value) of LCEO with some natural and synthetic antioxidants

Sample	IC_{50}	Reference
LCEO	3.20 ± 0.15 µg mL^{-1}	This work
Petroselinum crispum	80.21 ± 3.41 (mg mL^{-1})	[16]
Cyclotrichium origanifolium.	17100 ± 0.5 (µg mL^{-1})	[17]
Thymus sipyleus subsp. *sipyleus* var. *sipyleus*	2670 ± 0.5 (µg mL^{-1})	[7]
BHT	0.04 ± 0.01 (µg mL^{-1})	This work

cinnamon, clove, oregano and thyme oils (up to 180 ^{o}C) did not influence either their antioxidant activities by the DPPH^{o} method or their chemical composition. Conversely, when heated at 180 ^{o}C, nutmeg oil showed a significantly higher free radical-scavenging activity, together with a marked loss of α-pinene, ß-pinene and sabinene, and an evident increase in safrole and myristicin contents. Thus, one could consider that the observed higher free radical-scavenging capacity of the nutmeg oil might be related to a heating-induced increase in the content of these two components [18]. However, due to their complex composition, the correlation between antioxidant activity and the components present in the oil is difficult to establish. Ketones, aldehydes and alcohols are active but with differing specificity and levels of activity, which is related to the present functional group but it is also associated with hydrogen-bonding parameters in all cases [19]. High AOA of LCEO may relate to much stability of spathulenol and AR- curcumene while heat treatment was done on LCEO. Jovanovic et al., (1999) indicated that hydrogen abstraction from the methylene CH_2 group is responsible for the remarkable antioxidant activity of curcumin [20]. The phenolic OH is the most preferable group for the proton loss from the one-electron oxidized species. The stability of the resultant phenoxyl radicals, therefore, imparts greater ability for curcumin to scavenge the oxidizing free radicals. This finally resulted in a much greater ability to inhibit free radical-induced lipid peroxidation and the antioxidant activity. The resonance-stabilized radicals can undergo loss of the second hydrogen from the second phenolic OH group, producing a diradical. This diradical may be converted into stable products [21].

Conclusion

The study concludes that LCEO poses antioxidant property. Unlike synthetic antioxidants, LCEO is safe and can be added to the foodstuffs without side effects (the addition of synthetic antioxidants is limited under food laws and regulations). However, further investigation such as other antioxidant testing methods, toxicological test and so on as well as the antioxidant activity mechanism is warranted. These studies can be useful as a starting point for further application of LCEO in food preparations.

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