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# Comparative Evaluation of Purity and Antioxidant Effects of Commercial and Laboratory Essential Oils of *Cinnamomum zeylancium*

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### Authors' contributions

This work was carried out in collaboration between all authors. Author AJJ designed the study and wrote the protocol. Authors MA and BS performed the statistical analysis, wrote the first draft of the manuscript and managed the analyses of the study. Author SM managed the literature searches. All authors read and approved the final manuscript.

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## ABSTRACT

**Background:** According to the proof of antioxidant and antibacterial effects of cinnamon essential oil, nowadays several commercial companies around the world have extracted and distributed cinnamon essential oil with one hundred percent purity.

**Objectives:** This study has been designed to evaluate chemical composition and antioxidant activity of commercial cinnamon essential oil compared with pure essential oil obtained in laboratory.

**Materials and Methods:** Laboratory cinnamon essential oil was extracted by hydro distillation

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method and the commercial essential oils were purchased from two different companies. GC/MS analyses were done to find out the chemical compositions and finally the antioxidant activity of essential oils was determined using three different methods including Fe II chelating test, Reducing power and Antiradical effect (DPPH).

**Results:** The main components identified in laboratory cinnamon essential oil were Cinnamaldehyde (77%), Cinnamaldehyde Dimethyl Acetate (6.6%), Alpha- copaene (6%) and Delta-cadinene (3%). The result of GC/MS showed that the components of the laboratory essential oil were different from those of commercial ones, so that in the two commercial essential oils Cinnamaldehyde, trans caryophyllene, linalool and eugenol were the main components. Regarding the antioxidant activity of metal chelating test, the laboratory essential oil with the inhibitory effect of 17.1% was stronger than two commercial essential oils with the inhibitory effect of 9% and 6.2% for highest concentration ( $P<0.05$ ). But laboratory essential oil was statistically weaker than commercial essential oils with the  $IC_{50}$  of 22449  $\mu\text{g/ml}$  compared with 5708  $\mu\text{g/ml}$  and 4230  $\mu\text{g/ml}$  in DPPH assay, as well as absorption values of 0.15 compared with 0.2 and 0.22 for highest concentration in reducing power assay, respectively ( $P<0.05$ ).

**Conclusion:** Generally, the analysis of the chemical composition of essential oils proved that laboratory essential oil and commercial essential oils were extracted from different parts of plant and commercial essential oils showed stronger antioxidant activities than laboratory essential oil. This evidence could be due to low levels of phenolic and monoterpene components in laboratory cinnamon essential oil.

*Keywords: Cinnamomum zeylancium essential oil; laboratory; commercial; purity; antioxidant activity.*

## 1. INTRODUCTION

Food oxidation, in particular oils, reduces nutritional value, chemical and organoleptic quality of food and causes cancer, diabetes and cardiovascular disease in consumers [1]. Therefore, the problem of oxidative instability and its prevention through the addition of antioxidants is important for the sustainability of food and health [2,3]. Research studies conducted on food industry in the world shows that the major volume of the researches in recent years has focused on the issue of health foods and healthy compounds, as well as natural preservatives, which reflects the global desire for and acceptance of these compounds [4-8]. The use of synthetic antioxidants is controversial, despite the fact that they are effective during thermal processes and under storage conditions, because of the potential for toxicity from the food safety point of view. So that the strongest synthetic antioxidants such as TBHQ in Japan, Canada and Europe are not allowed to be used, and Butylated Hydroxy Anisole (BHA) has been also excluded from the list of compounds that are generally recognized as safe (GRAS) [9]. The Medicine and Therapy effects of cinnamon have been proven in the past, and people have long been using this plant as a food flavor. Considering the proof of anti-oxidant and anti-bacterial effects of cinnamon essential oil in several studies carried out, the essential oil can be used as a food flavor and preservative [10].

Accordingly, today, various commercial companies have been extracting and distributing cinnamon essential oil [11-13]. These essential oils are sold in glass containers and as 100% pure, but due to their volume and price, there has been doubt about the purity and effectiveness of these essential oils compared to the extracted essential oil in laboratory. Therefore, the purpose of this research is to evaluate the essential oil of cinnamon in terms of the chemical compositions and antioxidant activity compared to pure essential oil prepared in the laboratory.

## 2. MATERIALS AND METHODS

### 2.1 Reactants

Ferrous chloride ( $\text{FeCl}_2 \cdot \text{H}_2\text{O}$ ), Ferric chloride ( $\text{FeCl}_3 \cdot \text{H}_2\text{O}$ ), Trichloroacetic acid ( $\text{CCl}_3 \cdot \text{COOH}$ ) and Potassium ferrocyanide ( $\text{K}_4\text{Fe}(\text{CN})_6$ ) were purchased from Merck Co. (3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine-4,4-di-sulfonic acid sodium salt) and DPPH (1 and 1-diphenyl-2-picryl-hydrazyl) and methanol were purchased from Sigma Co.

### 2.2 Preparation of Essential Oils

**Laboratory essential oil:** Cinnamon Essential Oil was prepared by Hydro Distillation method and Clevenger apparatus method. For this purpose, 100 g of powdered cinnamon bark was put in a distillation flask with distilled water, then

the distillation flask was linked to Clevenger apparatus device; the distillation continued until nothing was added to the volume of essential oil. Finally 1 ml of essential oil (1% yield) was obtained. Subsequently, the extracted essential oil was dehydrated with sodium sulfate and stored after being transferred to the container in the refrigerator until it was used [14].

**Commercial cinnamon essential oil:** Commercial essential oils of cinnamon were purchased with two different brand from the local market in Tehran, Iran.

### **2.3 Analyze the Chemical Compounds of the Essential Oils**

The chemical compounds of essential oils were identified by GC/MS gas chromatography (GC/MS) in Medicinal Herb Medicine Laboratory, University of Tehran. GC/MS device had a 30-meter-long capillary column, internal diameter of 250 micrometers and internal layer thickness 0.25 micrometers with a temperature of 50 to 265 degrees Celsius and a gradual increase of 2.5 degrees per minute and column maintenance at 265 degrees Celsius for 30 minutes. The injection room temperature was 250°C and the helium carrier gas was 1.5 milliliters per minute. The EI detector had ionization energy of 70 electron volts and the temperature of the ionization source was 250°C. The spectra was identified using their Retention Index (RI). The essential oil compounds were identified using inhibitory indicators and studying the mass spectra of compounds by comparing their standard mass spectra available in computer libraries and authoritative references [15].

### **2.4 Assess Antioxidant Activity of the Essential Oils**

The antioxidant activity of each of the essential oils was assessed using three lab methods: metallic chelating, reducing power, and anti-radical effect test (DPPH).

#### **2.4.1 Metal chelating capacity test**

Intermediary elements with double capacity play an important role as a catalyst in oxidative processes and lead to the formation of hydroxyl radicals and destructive reactions of hydro peroxide [16]. These stages can be delayed or deactivated by chelating iron ion. This test was performed using Ding et al. [17] method. To perform this experiment, 400 µl of different

concentrations of essential oils (300 to 5000 µg / ml) was prepared, then 50 µl of a solution of 2 mM iron II and 200 µl of solution of 5 mM ferrozine were added to each of the concentrations. At this stage, the solutions became red, which involved the formation of ferrozine-iron complex and, after 10 minutes incubating at room temperature, optical absorption of the samples was read in a spectrophotometer at a wavelength of 562 nm against methanol and finally the percentage of inhibition of the red ferrozine-iron complex formation by essential oils was calculated using the following formula, which I (%) is the percentage of inhibition, AC is the absorption amount of the control solution and AS is the sample absorption rate [17].

$$I(\%) = (Ac-As/Ac) \times 100$$

#### **2.4.2 The reducing power test**

Iron reduction (III) is often used as a criterion for electron donation ability. The measurement of the reducing power was done according to the method of Oyaizu et al. [18]. To perform this test, different concentrations of essential oils (2 to 10 mg / ml) were prepared first, and then 1000 µl of phosphate buffer solution (pH = 7) and 1000 µl of 1% Furosianide solution were added to each concentration. The complex was incubated for 20 minutes at 50°C. Then, 1000 µl of 10% trichloroacetic acid solution was added and centrifuged for about 10 minutes with 2800 rounds. At the next stage, the upper phase was mixed with 1000 µl of distilled water and 1000 µl of iron chloride III 0.1% and then the absorption was read at a wavelength of 700 nm against the standard solution (control) [18].

#### **2.4.3 The antiradical effect test DPPH**

The DPPH radical scavenging activity of essential oils was measured as described earlier by Chang et al. (2001). Free radicals' inhibition is one of the most recognized mechanisms by which antioxidant compounds can inhibit lipid oxidation. DPPH by taking the antioxidant compounds' electron changed the color from yellow to purple that it depends entirely on the concentration of essential oils. In this method, different concentrations of essential oil (100 to 6000 micrograms per ml) were prepared first, then to each of the concentrations 5 ml of DPPH % 0/004 solution was added and, after 30 minutes incubating at room temperature, the optical absorption of the samples was read in a

spectrophotometer at 517 nm against methanol. And finally, the radical capacity reduction was calculated using the following formula which (I) % is the reduction percentage in radical capacity, AC is the amount of absorption of the control solution, and AS is the amount of absorption of the sample [19].

$$I (\%) = (Ac-As/Ac) \times 100$$

In this test, the results were reported as IC<sub>50</sub> (a concentration of essential oils that could reduce the free radical of DPPH by 50% µg / ml) and compared with each other.

## 2.5 Statistical Design and Data Analysis

All tests were performed with three replications and the results were reported as mean ± SD. To compare the different groups, ANOVA and Tukey tests were used with software Sigma Stat and a significant level ( $P < 0.05$ ) was defined

## 3. RESULTS AND DISCUSSION

### 3.1 Analyze the Chemical Compounds of Cinnamon Essential Oil

By examining the results of the GC/MS analysis of the essential oil prepared in the laboratory, it was determined that 17 different components of the essential oil were identified, the highest percentage was respectively related to the compounds of 77% Cinnamaldehyde, 6.63% Cinnamaldehyde Dimethyl Acetate, 6% Alpha-copaene and 3% Delta-cadinene. The results of the GC/MS analysis of commercial essential oils showed that 15 chemical compounds were identified from each of the essential oils, with the highest percentage of compounds in commercial essential oil (1) respectively as 76% Cinnamaldehyde, 3.68% Trans caryophyllene, 2.37% Linalool and 2.3% Eugenol and in commercial essential oil (2) respectively as 72% Cinnamaldehyde, 3.45% Trans-caryophyllene, 2.23% Linalool and 1.98% eugenol.

By comparing the results of cinnamon essential oils' analysis in this study with the study results of Parganagam et al. (2001) it was seen that compounds such as 1-8-cineole, 3-caron, palmitic acid and caryophyllene oxide extracted from commercial essential oils were respectively special for the roots, leaves, blooms and leaves of cinnamon, and these compounds are not present in the lab essential oil extracted from cinnamon bark [20]. This suggests that the origin of commercial essential oils is not just the cinnamon bark and other parts of the plant such as leaves, roots and flowers have been also used in the preparation of essential oils.

According to the results of the GC/MS analysis of the essential oils, also a comparison was made from the viewpoints of various chemical groups (monoterpene, sesquiterpene, monoterpenol, aldehyde and phenol) for the essential oils that the result of this comparison is given in Table 1.

### 3.2 The Chelating Power Test

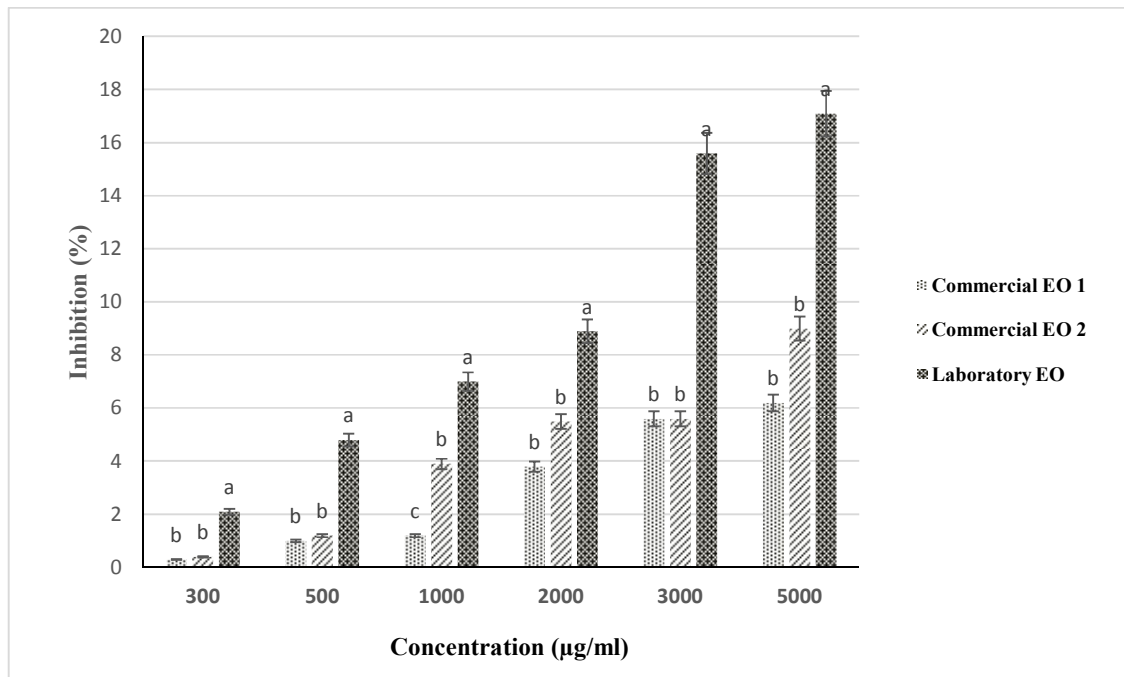
Intermediate elements such as iron have the capability to form free radicals from the peroxides based on Fenton's reactions.

In this test, the antioxidant activity of the essential oils is evaluated based on their chelating properties. The chelating properties of the essential oils are such that the antioxidant compounds contained in the essential oils with the iron ion form a complex and prevent producing a red ferrozine-iron complex. The results of the chelating power test are shown in Fig. 1.

As it is observed, by increasing the concentration of the essential oils, the chelating properties are also increased, and the lab essential oil of cinnamon significantly ( $P < 0.05$ ) in all concentrations has a greater chelating power than commercial essential oils, and no significant difference is observed between the two commercial essential oils in this regard ( $P > 0.05$ ).

**Table 1. Comparison of different chemical compounds of cinnamon lab essential oil and two commercial essential oils**

Chemical compound	Lab essential oil	Commercial essential oil 1	Commercial essential oil 2
Monoterpene	<0.1	1.9	2.11
Sesquiterpene	9.43	3.68	3.45
Monoterpenol	<0.1	3.57	3.16
Aldehyde	77.11	76	72
Phenol	<0.1	2.3	2.68



**Fig. 1. Comparison of the chelating power of commercial and laboratory essential oils of cinnamon**

Ding et al. [17] have pointed to the good chelating effects of sesquiterpene compounds. Given that the percentage of sesquiterpene compounds in the lab essential oils is higher than the two commercial essential oils (Table 2), this could be due to the stronger lab essential oil in terms of the chelating properties.

### 3.3 The Reducing Power

In this method, the reducing power of the essential oil is measured by the reduction of iron III to iron II with the ability to donate electrons. Iron III reduction is often used as an indicator of electron donation activity, which forms an important mechanism for antioxidant action. Increasing the optical absorption in the mixture caused by the intensity of the green color produced by the production of iron II (ferruginum) will mean increasing the reducing (antioxidant) power. The results of the reducing power test are shown in Fig. 2.

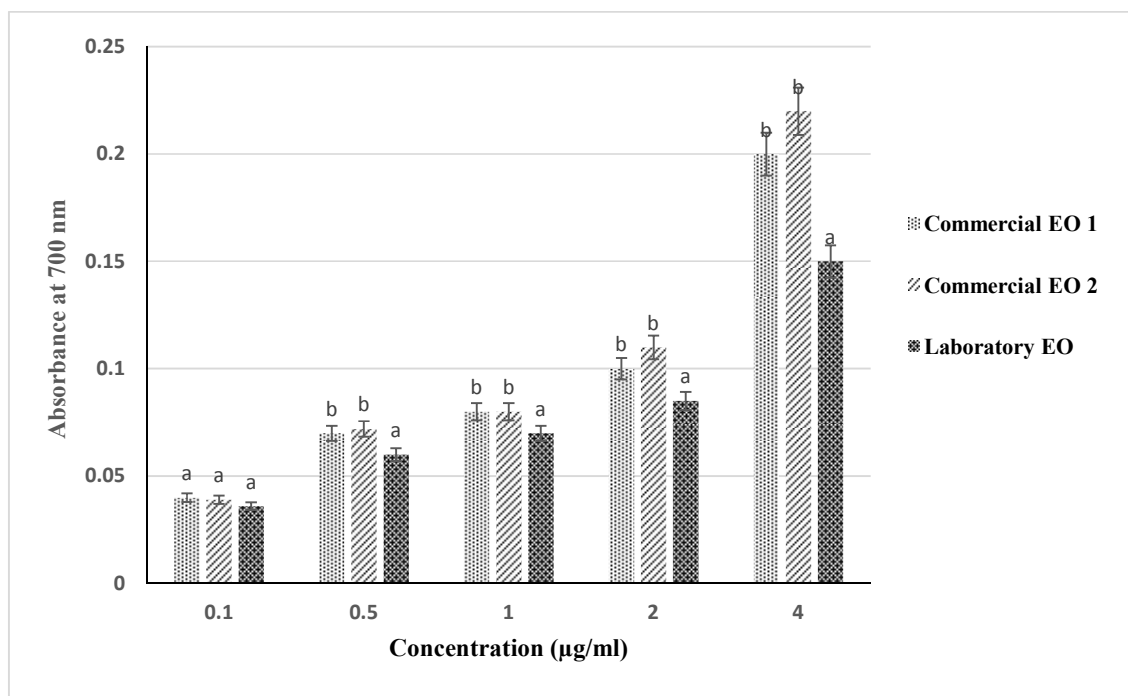
As shown in Fig. 2, according to the amount of reported absorption if the concentration of essential oils is increased, the reducing power is increased; as well as the reported absorption values indicate that the reducing activity of the

two commercial essential oils of cinnamon was significantly higher than the lab essential oil ( $P < 0.05$ ).

In a study by Chua et al. [21] and Abdul Wahhab et al. [22], it has been proven that antioxidant power is directly related to the percentage of phenolic and monoterpenic compounds in the essential oils. In this regard, Wang et al. [23] also pointed to the high reducing power of phenolic and monoterpenic compounds of the essential oils. It seems, the presence of higher percentage of phenolic and monoterpenic compounds in the commercial essential oils compared to the lab essential oils (Table 2) has caused the potential for greater reducing power of these essential oils.

### 3.4 The Antiradical Effect Test DPPH

In this test, DPPH free radicals react with antioxidant compounds and are inhibited [1,19]. The reduction of DPPH molecules is directly related to the number of hydroxyl groups. Hydroxyl groups by denoting H into free DPPH radicals converts them from purple diphenylpicrylhydrazine to yellow diphenylhydrazine [24], and finally the results are expressed as the percentages of a reduction in



**Fig. 2. Comparison of antioxidant properties between the two commercial and pure cinnamom essential oils based on the reducing power**

the adsorption of DPPH solutions in the presence of essential oils in relation to DPPH-essential oil free solution (control solution) and as  $IC_{50}$  (a concentration of essential oils which can reduce DPPH free radical by 50%  $\mu\text{g} / \text{ml}$ ). The results of this test are shown in Table 2.

**Table 2. The inhibitory concentration of 50% ( $IC_{50}$ ) of commercial and laboratory essential oils of cinnamom in DPPH test (Mean  $\pm$  SD)**

Sample	$IC_{50}$ ( $\mu\text{g}/\text{ml}$ )
Lab cinnamom essential oil	22449 $\pm$ 256
Commercial cinnamom essential oil 1	5708 $\pm$ 42
Commercial cinnamom essential oil 2	4230 $\pm$ 31

According to the Table 2, the lab essential oil with  $IC_{50}$ = 22449  $\pm$  256 showed a weaker anti-radical activity than commercial essential oils 1 and 2 with  $IC_{50}$ = 425708  $\pm$  42 and  $IC_{50}$ = 4230  $\pm$  31, respectively.

In numerous studies conducted by Roberto and Barata [25], Hector et al. [26], Kamkar et al. [27] and Lane [28], it has been proven that phenolic and monoterpenic compounds have anti-radical activity and finally high anti-oxidant properties.

Saleh et al. [29] also from their studies concluded that monoterphenol compounds and monoterpenic hydrocarbons identified from essential oils, like phenolic compounds, played an important role in the antiradical effect of essential oils, and a linear relationship is found between them. In this regard, Hang et al. [23] and Wang [30] in their studies on cinnamom bark have mentioned high antiradical effects of phenolic compounds extracted from cinnamom essential oils. According to Table 1, the higher anti-radical effect of commercially available cinnamom essential oils in DPPH test can be attributed to higher phenolic and monoterpenic compounds of these essential oils.

#### 4. CONCLUSION

In the production of commercial cinnamom essential oils, no counterfeit has been made and no specific compounds have been added to it, but for the essential oil extraction all parts of cinnamom plant have been used, which not only did not reduce the antioxidant properties but also increased it. It can be the economic justification of manufacturers by this method. Also, according to the results of the tests carried out in this study, it has been proved that only one method cannot be used to evaluate the antioxidant property, but

we should use two or more methods simultaneously.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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