

# Chemical and Biological Comparative *in Vitro* Studies of Cinnamon Bark and Lemon Peel Essential Oils

Eman M. Elgendy<sup>1\*</sup>, Hoda S. Ibrahim<sup>2</sup>, Hanaa F. Elmecherry<sup>1</sup>, Amal G. Sedki<sup>1</sup>, Faten U. Mekhemer<sup>3</sup>

<sup>1</sup>Department of Home Economy, Faculty of Specific Education, Mansoura University, Mansoura, Egypt

<sup>2</sup>Department of Nutrition and Food Science, Faculty of Home Economics, Helwan University, Cairo, Egypt

<sup>3</sup>Department of Home Economy, Faculty of Specific Education, Tanta University, Tanta, Egypt

Email: \*eman\_elgendy@hotmail.com

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## Abstract

Cinnamon and lemon are the oldest plants which possess a rising popularity due to their therapeutic potential from centuries. *Cinnamomum zeylanicum* and lemon (*Citrus lemon L.*) have been subjected to extensive research. Their essential oils were extracted by steam distillation from selected plants and their chemical compositions were determined by the GC-MS system. Cinnamon and lemon essential oils were examined for antioxidant activity by ABTS method which showed the ability to inhibit lipid per-oxidation. On the other hand, in antimicrobial investigations, cinnamon and lemon essential oils have inhibitory effect against Gram positive bacteria (*Staphylococcus aureus*) and Gram negative bacteria (*Escherichia coli*) using Muller Hinton agar medium. The essential oils of cinnamon and lemon showed antifungal effects which were tested against (*Candida albicans*). The volatile oil of cinnamon bark has been found to be highly effective against all the tested bacteria and fungi. However, lemon peel essential oil has shown medium inhibition for Gram positive bacteria (*Staphylococcus aureus*). On the other hand, the cytotoxic activities of the essential oils were tested on hepatocellular carcinoma and colorectal carcinoma. Essential oils have shown good activities on the cell lines. Essential oil of cinnamon showed more inhibition rate than essential oil of lemon. This study reported the importance of both cinnamon and lemon volatile oils and recommends that cinnamon and lemon can be used as an active therapy for humans.

## Keywords

Cinnamon, Lemon, Essential Oil, Cinnamaldehyde, Limonene

## 1. Introduction

Many pathways have been done to keep our food healthy. One pathway to inhibit food

spoilage is adding preservatives such as antioxidant, antifungal and antimicrobial substances to food. Artificial preservatives, is one of the oldest techniques which play an important role as antimicrobial, antifungal and antioxidant, but have adverse effects on health [1].

Natural additives are widespread due to health problems caused by different artificial preservatives [2] [3].

Using plants and materials of plant origin such as essential oils, is a suitable way to increase safety, quality and nutritional values for food products [4] [5]. Essential oils obtained from plants are used in many foodstuffs to increase their shelf-life [6]. Essential oils are mixture of volatile compounds with intense scent that are synthesized in several plant organs, including flowers, leaves, fruits, or bark, and stored in epidermis cells [7] [8]. These volatile compounds act as protective substances against microorganisms and herbivores [9]. Essential oils are used as carminative, stomachic, stimulant, aromatic, antiseptic [10] and as a flavoring agent in beverages, foods, cosmetics, and household products [11]. Phenolic compounds and terpenes are the main biologic constituents in the volatile oils [12] [13] [14].

Cinnamon, essential oil is obtained from *Cinnamomum zeylanicum* (Lauraceae), plant. The important constituents of cinnamon essential oil are cinnamaldehyde (60-70%), eugenol (5% - 10%), benzaldehyde, cuminaldehyde [15] [16]. Lemon essential oil (*Citrus lemon* L.) from fruit peel contains limonene, beta pinene and gamma terpinene [11]-[17]. Our study has prompted us to describe the chemical composition of cinnamon and lemon essential oils, to explore and compare their antioxidant, antifungal, antibacterial, antifungal, antioxidant and anticancer activities.

## 2. Materials and Methods

### 2.1. Plant Materials

Cinnamon, *Cinnamomum zeylanicum* Nees (Lauraceae) and Lemon *Citrus limonum* L. were bought from a native market in Egypt. Plant materials consisted of stem bark (cinnamon) and fruit peel (lemon).

Chemical reagents: All the chemicals and solvents were of pro-analysis purity and were obtained from Sigma (Sigma-Aldrich GmbH, Sternheim, Germany). Gram positive bacteria (*Staphylococcus aureus*), Gram negative bacteria (*Escherichia coli*) and (*Candida albicans*) fungi were obtained from department of pharmacology, Faculty of Pharmacy, Mansoura University. Hepatocellular carcinoma (HePG-2) and colorectal carcinoma (HCT-116) cell lines were obtained from ATCC via Holding company for biological products and vaccines (VACSERA), Cairo, Egypt. The reagents RPMI-1640 medium, MTT, DMSO and 5-fluorouracil (Sigma Co., St. Louis, USA), Fetal Bovine serum (GIBCO, UK).

### 2.2. Separation of Essential Oils

Five hundred grams of dried plant material was subjected to three-hours of steam distillation or steam distillation using a Clevenger-type apparatus. The gained essential oils were dried over anhydrous sodium sulphate and stored in locked and dark bottle at  $-18^{\circ}\text{C}$  till needed .

### 2.3. Gas Chromatography-Mass Spectrometry

Volatile essential oils were analyzed by injecting 1  $\mu$ l sample to GC/MS system "Focus/DSQ II" (Thermo Scientific, USA). Focus Gas chromatograph equipped with non-polar column (5% phenyl polysilphenylene-siloxane): Thermo-TR.5MS with dimensions 30 m length and inner diameter 0.25 mm with film thickness 0.25  $\mu$ m) The column hold at 60°C for 5 min and heated with rate 5°C/min to final temperature 260°C and hold to 5 min. He was used as carrier gas with flow rate 1.2 ml/min, the injection port temperature hold at 250°C. The transfer line and ion source temperatures were 280°C. Ionization of sample components was performed in the EI mode with (70 eV).

### 2.4. Components Identification

The components of essential oil were identified on the basis of comparison of their retention indices and mass spectra with published data [18] [19] and computer matching with WILEY 275 and National Institute of Standards and Technology (NIST 3.0) libraries provided with computer controlling the GC-MS system. The results were also confirmed by the comparison of the compounds elution order with their relative retention indices on non-polar phase reported in the literature [18]. The retention indices were calculated for all volatile constituents using a homologous series of n-alkanes C8 - C16.

### 2.5. Determination of Antioxidant Activity with the 2,2'-Azino-Bis(3-ethyl benzthiazoline-6-sulfonic acid) (ABTS) Assay

ABTS solution (2 mL of 60 mM) was added to each of the two essential oils then MnO<sub>2</sub> solution (25 mg/mL of 3 M) in phosphate buffer (pH 7, 0.1 M). The blend was shaken, centrifuged, filtered. The absorbance (A control) of the resulting green-blue solution (ABTS radical solution) was regulated at ca. 0.5 at 1734 nm. Then, 50 ml of (2 mM) solution of the two oils in spectroscopic grade MeOH/phosphate buffer (1:1) was added. The absorbance (A test) was measured and the lowering in color sharpness was expressed as % inhibition. The % inhibition for each oil is calculated from the following equation [20].

$$\% \text{Inhibition} = (A \text{ control} - A \text{ test}) / A \text{ control} \times 100$$

Ascorbic acid (vitamin C) was applied as standard anti-oxidant (positive control). Blank sample was take place without ABTS and using MeOH/phosphate buffer (1:1) instead of sample. Negative control sample was carried out with MeOH/phosphate buffer (1:1) instead of oil.

### 2.6. In Vitro Anti-Microbial Activity Evaluation

Staphylococcus aureus and Escherichia coli were used to determine the anti-bacterial activity of each essential oil under study using Muller Hinton agar medium. The anti-fungal activity of the studied essential oils was tested against Candida albicans using Sabouraud dextrose agar medium. The anti-bacterial and anti-fungal activities were determined by agar streak dilution method. This method was conducted according to the method of Hawkey and Lewis (1994) [21]. Ciprofloxacin and Fluconazole were used

as standard for anti-bacterial and anti-fungal activities, respectively. The lowest concentration of each essential oil under study, which did not show any growth of tested microorganisms after macroscopic evaluation was determined as the minimal inhibitory concentration (MIC) of this oil.

### 2.7. *In Vitro* Cytotoxicity Activity Evaluation

Hepatocellular carcinoma (HePG-2) and colorectal carcinoma (HCT-116) cell lines were used to determine the inhibitory effect of each essential oil under study on cell growth using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. The MTT method was conducted according to the colorimetric method of Mosmann (1983) [22]. 5-Fluorouracil was used as a standard anticancer drug for comparison. The relative cell viability in percentage was calculated as follows:

$$\begin{aligned} \text{Relative cell viability \%} \\ = (\text{Absorbance of treated cells} / \text{Absorbance of untreated cells}) \times 100 \end{aligned}$$

IC50 value of tested essential oil is the concentration of the tested essential oil that would result in the death of 50% of the cells.

### 2.8. Statistical Analysis

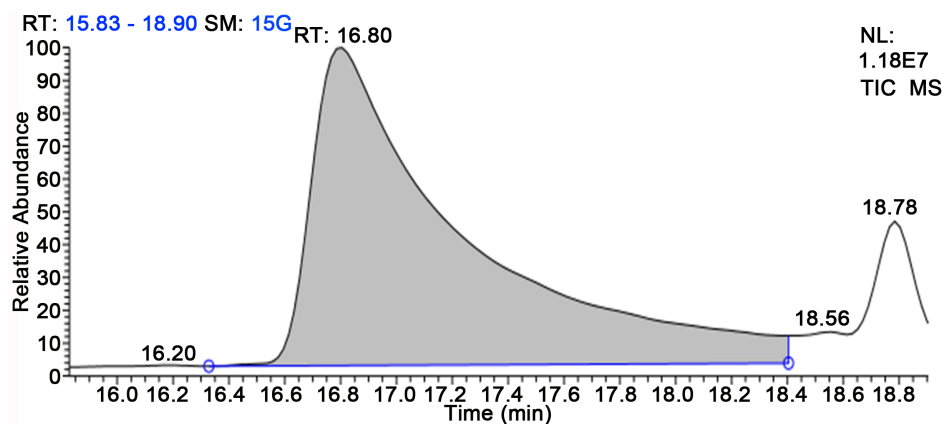
Statistical analysis was done using GraphPad Prism version 3.0 for windows (GraphPad Prism, 1999) [23]. The majority of the data presented in this study reflect the means  $\pm$  deviation (SD). One way analysis of variance (ANOVA) and the Tukey's Multiple Comparison Test were used to determine the differences among the means. The significant difference was set at  $P < 0.05$ .

## 3. Results and Discussion

### 3.1. Phytochemical Analysis

Phytochemical analysis of *Cinnamomum zeylanicum* L. bark essential oil demonstrates the presence with high percent of phenols and terpenoids. Whereas, analysis of *Citrus lemon* L. fruit peel essential oil proved the presence of, terpenoids with high percent [24]. Essential oils are naturally volatile substances, existing in different parts (flowers, leaves, seeds, roots, bark) of all plants with strong aroma. The amount of essential oils in plants different from very small amounts (0.05% - 0.1%) in one while in other up to 20% [25].

Cinnamon bark essential oil obtained by steam distillation. Its yield was found to be 1.5%. The gas chromatography technique of Cinnamon essential oil detected the presence of many substances which could be identified. It has been found main peak at retention time (RT) 16.80 min. with a relative abundance of 64.84% and the molecular formula  $C_9H_8O$  and molecular weight (M.wt = 132.16). The expected substance is trans-cinnamaldehyde (Figure 1). Trans-Cinnamaldehyde [3-phenyl-propenal, II] is the major component of essential oil of cinnamon and its fragments are 132 (M)<sup>+</sup>, 131 (M-1)<sup>+</sup>, 115 (M-1-O)<sup>+</sup>, 103 (M-CHO)<sup>+</sup>, 91 (M-C<sub>2</sub>HO)<sup>+</sup> and 77 (C<sub>6</sub>H<sub>5</sub>)<sup>+</sup>, with base peak 131 m/z. The complete list of the ingredients and their relative abundances are presented in Table 1.



**Figure 1.** Chromatogram of the main component (*trans*-cinnamaldehyde) of cinnamon essential oil by GC-MS.

**Table 1.** Chemical composition of cinnamon essential oil.

Retention time (min)	Molecular formula	Compound	Area %
3.78	C <sub>8</sub> H <sub>10</sub>	Ethyl benzene	1.50
16.8	C <sub>9</sub> H <sub>8</sub> O	<i>trans</i> -cinnamaldehyde	64.84
18.56	C <sub>15</sub> H <sub>24</sub>	1,2,4-Metheno-1H-indene, octahydro-1,7 $\alpha$ -dimethyl-5-(1-methylethyl)	1.90
18.78	C <sub>15</sub> H <sub>24</sub>	Copaene	8.48
19.03	C <sub>10</sub> H <sub>12</sub> O <sub>2</sub>	Eugenol	6.72
20.23	C <sub>15</sub> H <sub>24</sub>	Caryophyllene	2.01
21.43	C <sub>15</sub> H <sub>24</sub>	4 $\alpha$ H, 5 $\alpha$ -Eremophila-1(10),11-diene	0.37
21.98	C <sub>15</sub> H <sub>24</sub>	Copaene	1.32
22.75	C <sub>15</sub> H <sub>24</sub>	Cadina-4,9-diene	3.09
23.34	C <sub>15</sub> H <sub>24</sub>	Cadina-3,9-diene	4.46
24.19	C <sub>15</sub> H <sub>22</sub>	Cadala-1(10),3,8-triene	0.44
27.41	C <sub>15</sub> H <sub>24</sub>	$\alpha$ -Guaiene	0.80
28.27	C <sub>15</sub> H <sub>18</sub>	Azulene, 1,4-dimethyl-7-(1-methylethyl)-	0.32
29.41	C <sub>19</sub> H <sub>26</sub> O <sub>6</sub>	Propanoic acid, 2-methyl-, (decahydro-6 $\alpha$ -hydroxy-9 $\alpha$ -methyl-3-methylene-2,9-dioxoazuleno[4,5- $\beta$ ]furan-6-yl)methyl ester,	0.18
29.92	C <sub>26</sub> H <sub>33</sub> NO <sub>2</sub>	Fenretinide	0.40
35.94 - 41.27	C <sub>35</sub> H <sub>70</sub>	17-Pentatriacontene	3.19
		Total identified	100%

Elgendy and Khayyat, 2014 have been proved that *trans* cinnamaldehyde can act as antioxidants. It was trapped the reactive oxygen species (ROS) to give the intermediated epoxides and hydroperoxide derivatives [26].

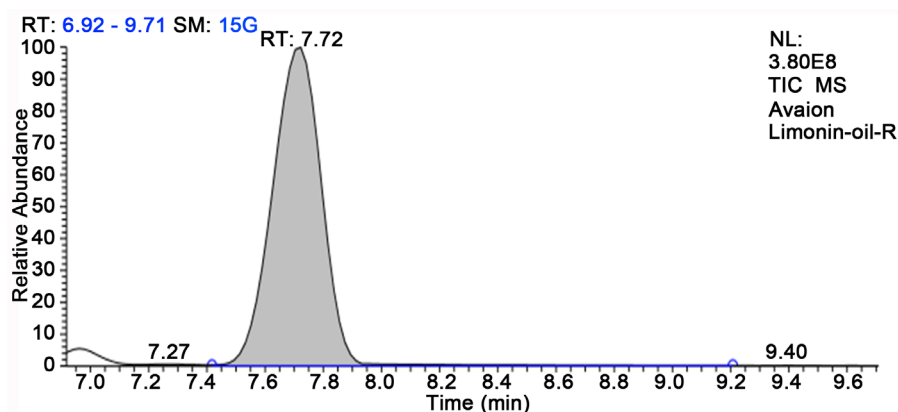
Lemon essential oil is a complex organic compound isolated from citrus fruit peel, through steam distillation process. The yield of its oil was found to be around 1.9%.

The gas chromatography technique of Lemon oil discovered the presence of many substances which could be identified. It has been found main peak at retention time (RT) 7.72 min, with a relative abundance of 86.95% and the molecular formula  $C_{10}H_{16}$  and molecular weight (M.wt = 136.24) (Figure 2). The expected substance is d-Limonene (1-methyl-4-(1-methylethenyl)-cyclohexene IV), is the major component of Lemon essential oil, and its fragments are 136 (M)<sup>+</sup>, 121 (M-CH<sub>3</sub>)<sup>+</sup>, 107 (M-C<sub>2</sub>H<sub>5</sub>)<sup>+</sup>, 93 (M-C<sub>3</sub>H<sub>7</sub>)<sup>+</sup> and 79 (C<sub>6</sub>H<sub>7</sub>)<sup>+</sup>, with base peak 93 m/z. The complete list of the ingredients and their relative abundances are presented in Table 2.

Elgendy, 1998 has been proved that Limonene can act as antioxidants. It was trapped the reactive oxygen species (ROS) to give the intermediated hydroperoxide derivative [27].

**Table 2.** Chemical composition of lemon oil.

Retention time (min)	Molecular formula	Compound	Area %
3.65	$C_{16}H_{34}O$	2-Hexadecanol	0.08
5.02	$C_{10}H_{16}$	3-Carene	0.45
6.45	$C_{10}H_{16}$	$\alpha$ -Pinene	0.45
6.96	$C_{10}H_{16}$	$\alpha$ -Phellandrene	4.59
7.72	$C_{10}H_{16}$	D-Limonene	86.95
9.40	$C_{10}H_{16}$	$\rho$ -Mentha-1,4(8)-diene	0.19
10.14	$C_{10}H_{18}O$	<i>cis</i> -Geraniol	0.33
10.83	$C_{10}H_{16}$	Allo-Ocimene	1.28
11.74	$C_{10}H_{18}O$	$\alpha$ -Citronellal	1.16
12.84	$C_{10}H_{18}O$	Isoborneol	0.35
13.45	$C_{10}H_{18}O$	$\rho$ -Menth-1-en-8-ol	3.10
14.94	$C_{13}H_{22}O_2$	$\alpha$ -Terpinyl propionate	0.08
16.10	$C_{10}H_{16}O$	$\alpha$ -Citral	0.13
18.78	$C_{15}H_{24}$	Copaene	0.05
20.23	$C_{15}H_{24}$	Caryophyllene	0.05
21.45	$C_{28}H_{48}O$	Cholestan-3-ol, 2-methylene-,	0.05
22.23	$C_{15}H_{26}O$	Longiborneol	0.16
22.55	$C_{15}H_{24}$	4 $\alpha$ H,5 $\alpha$ -Eremophila-1(10),11-diene	0.21
23.33	$C_{15}H_{24}$	$\alpha$ -Guaiene	0.09
31.18	$C_{10}H_{16}$	Allo-Ocimene	0.03
34.85	$C_{30}H_{50}O_2$	Betulin	0.04
35.73	$C_{30}H_{50}O$	Lupeol	0.04
36.13	$C_{20}H_{28}O_6$	1H-2,8 $\alpha$ -Methanocyclopenta[a]cyclopropa[e]cyclo-decen-11-one	0.06
36.76	$C_{20}H_{28}O_3$	6 $\alpha$ -Hydroxymethandienone	0.06
		Total identified	100%



**Figure 2.** Chromatogram of the main component (Limonene) of lemon essential oil by GC-MS.

### 3.2. Antioxidant Activity of Essential Oils

It is recognized that free radicals are implicated in the lipid per-oxidation reactions and represent a major role in many chronic diseases such as heart and cancer diseases [28]. Therefore, the capability to scavenge free radicals is an important antioxidant feature to decrease cell damage. In many studies *in vitro* the essential oils exhibited noticeable antioxidant activity [29] [30].

#### Choice of the Method for Determination of Antioxidant Activities

Estimation of antioxidant activity needs use of different methods [31] [32]. ABTS, RANCIMAT and TBARS can be quoted as comparatively simple methods that can be used to gauge the antioxidant activity of essential oils [33]-[39].

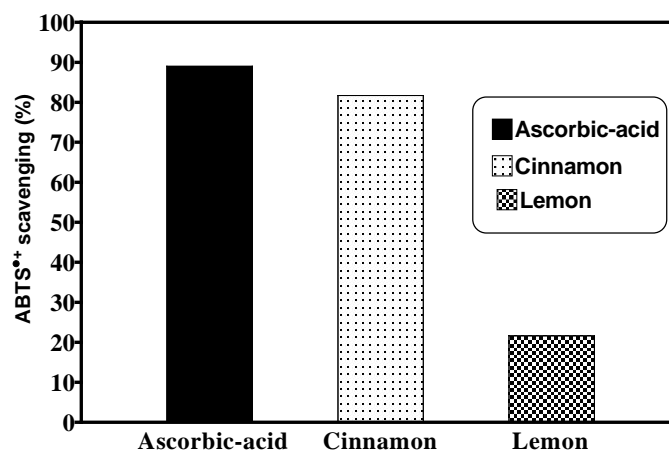
TBARS assay cannot evaluate the antioxidant activity of cinnamon essential oil, because *trans*-cinnamaldehyde is the main component of oil, which strongly interacted with the thiobarbituric acid used in the assay, developing a yellow color [40].

On the other hand, although the RANCIMAT test is commonly used in the food industry and governmental analytical laboratories [41] [42], RANCIMAT test is not convenient for such measurement, because air inserted inside hot systems (oil) during measurement evaporates essential oils samples formerly and retards measurements.

### 3.3. Anti-Oxidant Activity Screening Assay ABTS

Antioxidant activities of essential oils from redolent plants are due to the active or major constituents, in them. This may be also, due to the minor constituents among them [43]. In this study, the antioxidant activities are determined. It has been suggested that the two essential oils, *i.e.*, cinnamon and lemon, could be used as a prospective origin of natural antioxidants with wide applications in food products. The antioxidant activity of cinnamon and lemon essential oils are mainly due to the high content of cinnamaldehyde and limonene respectively. The Stronger activity is indicated by a higher antioxidant index determined by ABTS method. Cinnamon and lemon essential oils were examined for antioxidant activity which showed the ability to inhibit lipid per-oxidation. They proved powerful anti-oxidative activity in the lipid per-oxidation test. The results are summarized in **Table 3**.

Cinnamon and lemon oils have antioxidant activities. Cinnamon oil showed higher inhibitory anti-oxidant activity than lemon oil (**Figure 3**).



**Figure 3.** Anti-oxidant activity screening of the essential oils under study by ABTS method.

**Table 3.** Anti-oxidant activity screening of the essential oils under study by ABTS method.

Sample	2,2'-azino-bis(3-ethyl benzthiazoline-6-sulfonic acid) (ABTS)	
	Absorbance	*ABTS <sup>+</sup> scavenging (%)
Control of ABTS	0.508	-
**Ascorbic-acid	0.056	89.0%
Cinnamon oil	0.093	81.7%
Lemon oil	0.398	21.6%

\* ABTS<sup>+</sup> scavenging (%) =  $(1 - \text{absorbance of sample at } 734 \text{ nm} / \text{absorbance of control at } 734 \text{ nm}) \times 100\%$ ;

\*\*Ascorbic acid (vitamin C) was used as standard anti-oxidant (positive control).

### 3.4. Antimicrobial and Antimycotic Activities in Terms of MIC ( $\mu\text{g/mL}$ )

#### Anti-Microbial Activity Evaluation *in Vitro*

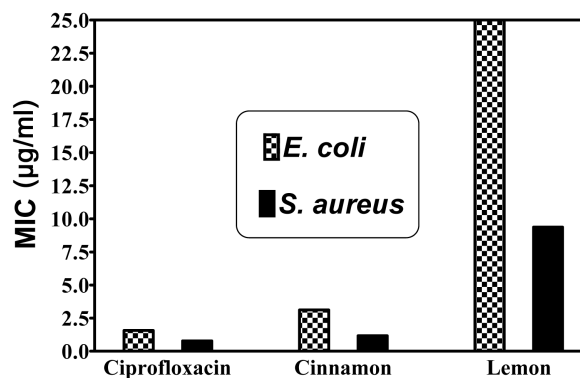
Essential oils of cinnamon and lemon submit the potential not only to resist infection, but also to prevent the outgrowth of microbe.

Cinnamon bark essential oil has cinnamaldehyde as a major ingredient was chosen for this study due to its various chemical compositions. Lemon citrus peel essential oil has limonene as a major ingredient was chosen for comparative study between them. According to Inouye et al., essential oil of cinnamon bark has antibacterial effects on major respiratory pathogens such as *Streptococcus pneumoniae* and *Streptococcus pyogenes* [44] [45].

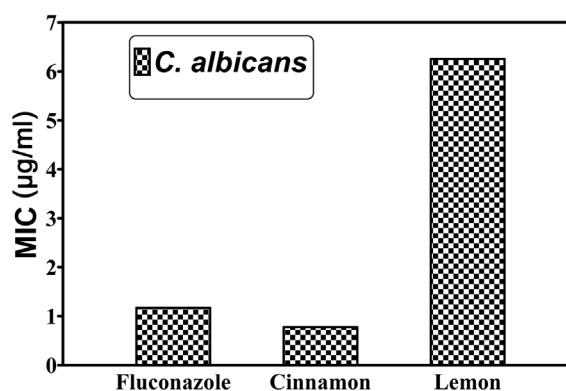
Cinnamon and lemon essential oils have strong inhibitory effect against Gram positive bacteria (*Staphylococcus aureus*) and Gram negative bacteria (*Escherichia coli*) using Muller Hinton agar medium (Oxoid) [46]. On the other hand, The essential oils of cinnamon, and lemon showed antifungal effects which were tested against (*Candida albicans*). The lowest concentration of each essential oil under study, which did not show any growth of tested microorganisms after macroscopic evaluation, was determined as the minimal inhibitory concentration (MIC) of this oil. Comparative study has been done of the antimicrobial activities of cinnamon and lemon essential oils. The results were obtained in **Figure 4** and **Figure 5**.

Cinnamon essential oil demonstrated larger effect than lemon. Lemon peel essential





**Figure 4.** Effect of the essential oils under study on *Escherichia coli* and *Staphylococcus aureus*.



**Figure 5.** Effect of the essential oils under study on *Candida albicans*.

oil has shown good inhibition only for Gram positive bacteria (*Staphylococcus aureus*). Ciprofloxacin and Fluconazole were used as standard for anti-bacterial and anti-fungal activities, respectively (Table 4).

#### Cytotoxicity Assay

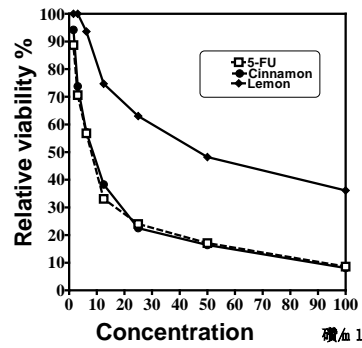
##### Hepatoprotective Activity Evaluation *in Vitro*:

##### Cell Cultures and Anti-Proliferative Activity Assay

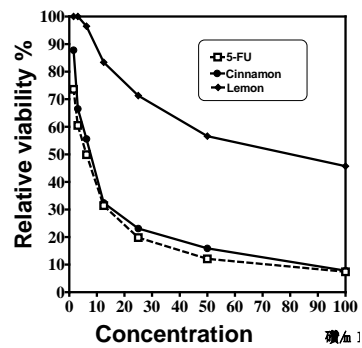
A great feature of essential oils if they used on a long-term, is not associated with genotoxic risk. Some of them have antimutagenic activity that could well be connected to an anticarcinogenic activity [47].

Hepatocellular carcinoma (HePG-2) and colorectal carcinoma (HCT-116) are perennial cell line used in scientific study. They are the most popular applied human cell lines [48] [49]. The cytotoxic activities of the essential oils were tested on Hepatocellular carcinoma and colorectal carcinoma. Essential oil submitted good activities on the cell lines. Essential oil of cinnamon showed more inhibition rate than essential oil of lemon (Tables 5-7 and Figures 6-9).

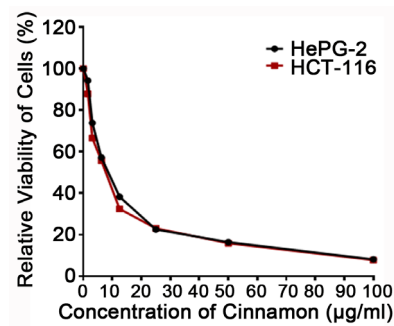
The IC<sub>50</sub> values against HePG2 indicated that the cytotoxicity of each essential oil decreased when their concentration increase. IC<sub>50</sub> value of cinnamon essential oil was very strong whereas, IC<sub>50</sub> value of lemon essential oil was moderate. On the other hand, The IC<sub>50</sub> values against HCT-116 indicated that it was very strong in case of cinnamon essential oil, whereas, in case of lemon essential oil, it was weak (Table 8 and Figure 10).



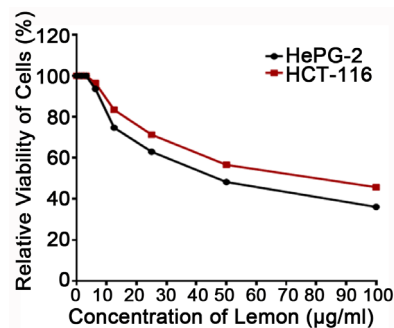
**Figure 6.** Effect of the essential oils under study on relative viability % of hepatocellular carcinoma cell line (HePG-2).



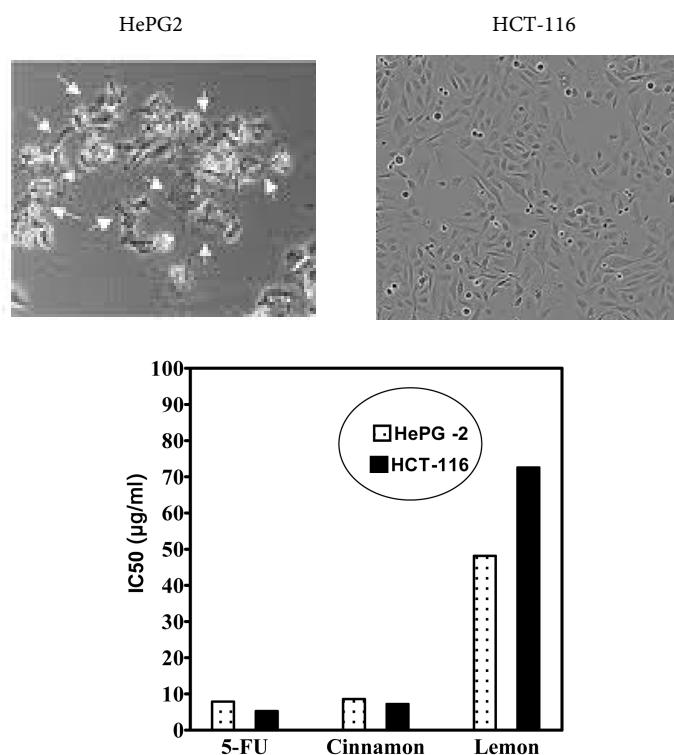
**Figure 7.** Effect of the essential oils under study on relative viability % of colorectal carcinoma cell line (HCT-116).



**Figure 8.** Effect concentration of cinnamon essential oil on relative viability % of hepatocellular carcinoma cell line (HePG-2) and colorectal carcinoma cell line (HCT-116).



**Figure 9.** Effect concentration of lemon essential oil on relative viability % of Hepatocellular carcinoma cell line (HePG-2) and colorectal carcinoma cell line (HCT-116).



**Figure 10.** Cytotoxic activity of the essential oils under study against colorectal carcinoma (HCT-116) and hepatocellular carcinoma (HePG-2) cell lines. IC<sub>50</sub> (µg/ml): 1 - 10 (very strong), 11 - 20 (strong), 21 - 50 (moderate), 51 - 100 (weak).

**Table 4.** Antimicrobial activity of the tested essential oils.

Sample	<i>Minimum inhibitory concentration</i> MIC (µg/ml)		
	<i>E. coli</i>	<i>S. aureus</i>	<i>C. albicans</i>
Cinnamon oil	3.12	1.17	0.78
Lemon oil	25.0	9.37	6.25
Ciprofloxacin	1.56	0.78	...
Fluconazole	...	...	1.17

**Table 5.** Relative viability % of hepatocellular carcinoma cell line (HePG-2) and colorectal carcinoma cell line (HCT-116) after treating by the tested essential oils.

Concentration µg/ml	5-FU		Cinnamon oil		Lemon oil	
	HePG-2	HCT-116	HePG-2	HCT-116	HePG-2	HCT-116
100	8.6	7.4	8.1	7.8	36.1	45.7
50	17.1	12.1	16.4	15.9	48.2	56.6
25	24.0	19.8	22.5	23.1	63.0	71.3
12.5	33.1	31.4	38.3	32.4	74.7	83.4
6.25	56.8	49.9	57.2	55.6	93.6	96.5
3.125	70.6	60.5	73.8	66.5	100	100
1.56	88.7	73.6	94.2	87.8	100	100

5-FU: 5-Fluorouracil was used as a standard anticancer drug for comparison.

**Table 6.** Relative viability % of hepatocellular carcinoma cell line (HePG-2) after treating by the tested essential oils.

Concentration $\mu\text{g/ml}$	5-FU	Cinnamon essential oil	Lemon essential oil
100	8.6	8.1	36.1
50	17.1	16.4	48.2
25	24.0	22.5	63.0
12.5	33.1	38.3	74.7
6.25	56.8	57.2	93.6
3.125	70.6	73.8	100
1.56	88.7	94.2	100

5-FU: 5-Fluorouracil was used as a standard anticancer drug for comparison.

**Table 7.** Relative viability % of colorectal carcinoma cell line (HCT-116) after treating by the tested essential oils.

Concentration $\mu\text{g/ml}$	5-FU	Cinnamon essential oil	Lemon essential oil
<b>100</b>	7.4	7.8	45.7
<b>50</b>	12.1	15.9	56.6
<b>25</b>	19.8	23.1	71.3
<b>12.5</b>	31.4	32.4	83.4
<b>6.25</b>	49.9	55.6	96.5
<b>3.125</b>	60.5	66.5	100
<b>1.56</b>	73.6	87.8	100

5-FU: 5-Fluorouracil was used as a standard anticancer drug for comparison.

**Table 8.** Cytotoxic activity of essential oils against human tumor cells.

Compounds	<i>In vitro</i> cytotoxicity IC50 ( $\mu\text{g/ml}$ )•	
	HePG2	HCT-116
5-FU	7.9 $\pm$ 0.28	5.3 $\pm$ 0.31
Cinnamon	8.6 $\pm$ 0.49	7.2 $\pm$ 0.72
Lemon	48.2 $\pm$ 3.10	72.6 $\pm$ 4.16

•IC50 ( $\mu\text{g/ml}$ ): 1 - 10 (very strong). 11 - 20 (strong). 21 - 50 (moderate). 51 - 100 (weak) and above 100 (non-cytotoxic); •5-FU = 5-fluorouracil.

#### 4. Conclusion

Since medicinal plants are important for pharmacological studies and medication progress, cinnamon and lemon essential oils are found very safe in cytotoxicity *in vitro*. Our study proved that antioxidant activity of essential oils was related to their chemical composition. The results obtained from this study showed that cinnamon and lemon essential oils can be considered good sources of natural antioxidants and can be used as natural food additives. This may be referred either to high proportion of the major ingredients or to cooperation between different oil constituents, so could be incorporated in the diet.

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