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Evaluation of Bioactive Compounds and Comparison of Antioxidant, Anti-inflammatory and Anti-hemolytic Activities of Essentials Oils from Lemon Peels and Garlic Cloves Extracted by Hydrodistillation

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Abstract

In this study, we extracted essential oils from lemon peel (*Citrus limon L.*) and garlic cloves (*Allium sativum*) by hydrodistillation to investigate and compare their bioactive constituents and biologicals activities. GC-MS analysis shows that main volatile compounds of lemon peel essential oil (LPEO) were terpenes (≈ 29%) such as γ-Terpinene, p-CYMENE, Terpinene 4-acetate, (R)-α-Terpinyl acetate, Sesquiterpene, α-Bergamotene, β-Bisabolene and Limonene-1,2-epoxide. Likewise, the main compounds of garlic clove essential oil (GCEO) were Organosulfur compound (≈ 57%) such as Diallyl Trisulfide, Diallyl disulfide, Diallyl tetrasulfide and Allyl methyl trisulfide. Ferric reducing antioxidant power test is significantly higher in GCEO. However, LPEO is more effective to inhibit DPPH radical, lipids peroxidation and inflammation than GCEO. However, GCEO seems to have a power of reduction of iron and an anti-hemolytic capacity more than LPEO. Terpenes of LPEO may contribute to its antioxidant activities by neutralizing free radicals and have a good anti-inflammatory capacity. Organosulfur compounds of GCEO have a good ability to reduce iron and could explain a good anti-hemolytic activity. This study facilitated the understanding of volatile constituents and antioxidant, anti-inflammatory and anti-hemolytic activities of these essentials oils and contributes in their use in pharmacology, cosmetics and food.

Keywords: *Citrus limon L* essential oil, *Allium sativum* essential oil, GC-MS analyses, Antioxidant activity, Anti-inflammatory activity

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1. Introduction

Lemon and garlic are widely used for their nutritional value and their culinary properties, generally consumed in the form of a fresh or processed product. Their essentials oils contain several bioactive compounds. These essentials oils are important secondary plants metabolites. Secondary metabolites of Citrus essential oils are usually aromatic compounds found in oil glands present in the flowers, leaves, and fruit peels. However, most this essential oil is extracted from fruit peels. This citrus essential oil contains 85–99% volatile and 1–15% non-volatile compounds [1] and their content as well as chemical composition depend on species and extraction methods [2]. Numerous isolated components of some Citrus essential oils (such as D-limonene) have been used individually and have shown various biological activities, especially anti-inflammatory and antioxidant [3]. Likewise, garlic essential oils contain several bioactive compounds that have antimicrobial, antiviral, anti-cancer/anti-proliferative, anti-inflammatory, immune-modulatory, anti-aging, and anti-diabetic activities [4].

Lemon essential oil is extracted from peels, a part of the plant considered as waste, resulting in a waste of resources, is also harmful to the environment, suggesting the importance of its recovery, moreover this oil is rarely used in modern medicine. Similarly, garlic essential oil is studied for these therapeutic virtues in traditional medicine but it is not yet widely used in modern medicine. Both oils have significant therapeutic, nutritional and environmental potential. However, their different composition in bioactive chemical compounds, give them different biological activities. Therefore, the purpose of this study was to determine bioactive chemical compounds of lemon essential oil (*Citrus limon L.*) and garlic essential oils (*Allium sativum*) by GC-MS analyses, and to compare their antioxidant, anti-inflammatory and anti-hemolytic properties.

2. Materiel and Methods

2.1. Plant material preparation

Our study is conducted on the essential oils of two plants, garlic cloves (*Allium Sativum*) and lemon peel (*Citrus Limon*). Garlic and lemon are produced and bought locally. The two plants have been taxonomically identified and authenticated by the Botanical Research Laboratory of our University. After cleaning, garlic cloves and peel lemon are grated. Then the vegetable material of each plant was dried in the shade, at room temperature and kept away from humidity, for 8 days. Isolation of essential oils Samples of 250 g of each material plant were subjected to hydrodistillation at boiling over 3 hours in 2.5 L of distilled water using a Clevenger-type apparatus in accordance with the method recommended by the European Pharmacopoeia Commission [5]. The distillate obtained was extracted with diethyl ether and dried over anhydrous sodium sulphate. The organic layer was then concentrated at 30°C using a rotary evaporator and the resulting essentials oils were stored at -80°C for analysis.

2.2. Volatiles bioactive compounds evaluation

Identification of volatiles components by GC–MS were analyzed and identified using a SHIMADZU (GC MS-TQ8030, Shimadzu Corporation, Kyoto, Japan) chromatograph coupled to mass-spectrometry detector (FTD/BID). The GC-MS system was equipped with a GC, SH-Wax Capillary, 30 m x 0.32 mm x 0.50 µm Column, GC. With the following operating conditions: Column Oven Temp: 50 °C; Injection Temp: 220 °C; Injection Mode: Split; Flow Control Mode: Pressure: 100 kPa; Total Flow: 50 ml/min; Column; Flow: 4. 55 ml/min; Linear Velocity: 77.4 cm/sec; Purge Flow: 3.0 ml/min; Split Ratio: -1.0. Identification of volatile compounds was performed by the device's preconfigured database.

2.3. Essentials oils biological activities evaluation

2.3.1. DPPH scavenging activity

Free radical-scavenging activities of essentials oils were measured using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) as described by Zhao et al., [6]. 0.2 ml of various concentrations of the essential oils in ethanol (ranging 0.05 to 1 mg/ml) was added to 2.3 ml (70 µM ethanol solution of DPPH). After incubation at room temperature, absorbance was read at 517 nm. DPPH radical-scavenging activity was expressed as the inhibition percentage (I%) and calculated using following formula:

I% = ((A blank - A sample)/ (A blank)) x 100, Where:

A blank is the absorbance of the control reaction containing all reagents except the tested compound. A sample is the absorbance of the test compound (essential oils or standard). The IC 50% of the reaction was calculated using a calibration curve representing inhibition percentage against essential oil concentration. Ascorbic Acid were used as reference samples. Test were performed in triplicate. A lower IC50 indicates a higher reducing power.

2.3.2. Thiobarbituric Acid-Reactive Substances (TBARS) assay

The TBARS method was used to assess the ability of essentials oils to inhibit lipid peroxidation. A modified TBARS assay was also used to measure the potential antioxidant capacity of essential oils [7]. Egg yolk homogenate was used as lipid-rich media (10% (w/v) in KCl (1.15%, w/v)). The egg yolk was then homogenized for 30 s, followed by 5 minutes of ultrasonication. 500 µl of homogenate (10% (w/v)) and 100 µl of sample, solubilized in methanol, were added to a test tube and made up to 1 ml with distilled water, followed by addition of 1.5 ml of 20 Acetic acid (pH 3.5) and 1.5 ml of 0. 8% (w/v) 2-thiobarbituric acid (in 1.1% (w/v) sodium dodecyl sulfate). Each essential oil and tested substance was assayed at different concentrations (0.125 to 1 mg/ml). This mixture was vortexed, and heated 1 hour at 95°C for. After cooling, 5 ml butan-1-ol was added to each tube, then the tubes were centrifuged 10 min at 1000 x g. Absorbance of supernatant was read at 532 nm. All of the values were based on the percentage antioxidant index (AI%), whereby the control was completely peroxidized and each oil demonstrated a degree of change; the percentage inhibition was calculated using the formula:

$(1 - T/C) \times 100$, where:

C is the absorbance value of the fully oxidized control and T is the absorbance of the test sample. The results are expressed as IC50 (concentration of essential oil or standard to prevent 50% of lipid oxidation) antioxidant activity as described previously for DPPH. A lower IC50 indicates a higher reducing or inhibiting power.

2.3.3. Essential oil Ferric Reducing Antioxidant Power (FRAP) assay

Reducing powers of each essential oil were assessed according to the method described previously by Alshahrani et al., [8]. Several concentrations of samples (0.1 to 1.5 mg/ml) were mixed with a phosphate buffer and 1% of water solution from potassium ferricyanide. This mixture was maintained at 50°C for 20 minutes, then centrifuged for 10 min at 1000 x g after adding trichloroacetic acid. The supernatant was mixed in the presence of distilled water and $FeCl_3$ solution, then absorbance was measured at 700 nm. Ascorbic acid was used as a standard reference (positive control) with a concentration ranging from 100 to 1500 µg/ml. All tests were carried in triplicates Higher absorbance of the reaction mixture indicated a greater reducing power.

2.3.4. Essential oil Anti-inflammatory activity

Protein denaturation was conducted according Gunathilake et al., [9]. 5 ml of reaction mixture (0.2 ml 1% Bovine serum albumin, 4.78 ml phosphate buffered saline (PBS, pH 6.4), and 0.02 ml of sample (0.125 to 1 mg/ml)) was mixed, incubated 15 minutes at 37 °C in water bath, and then heated 5 minutes at 70 °C. Then turbidity was measured at 660 nm using phosphate buffer solution as a control. The percentage inhibition of the reaction was calculated using the following formula:

Inhibition of denaturation (%) = ((A blank - A sample)/ (A blank)) x 100 Where:

A blank is the absorbance of the control reaction containing all reagents except the tested compound. A sample is the absorbance of the test compound (essential oils or standard). The results are expressed as IC50 anti-denaturation of albumin capacity as described previously for DPPH. Different concentrations of Diclofenac were used as reference samples. All determinations were performed in triplicate. A lower IC50 indicates a higher inhibiting power.

2.3.5. Anti-hemolytic activity

The anti-hemolytic potential of both essential oils was determined by the spectrophotometric procedure as described by Yuan et al., [10] with few modifications. 10 ml of blood from a healthy person voluntary was collected in EDTA vials (10%) and centrifuged for 10 min at 1000 x g. the pellet was washed three times with PBS (0.2 M, pH 7.4) and re-suspending in saline solution (0.9% NaCl). 0.4 ml of each essential oil (0.125 to 1 mg/ml in PBS) were dispensed to 0.4 ml of erythrocytes suspension (4%) and incubated at 37 °C for 5 minutes. Later, 0.2 ml of Hydrogen peroxide (H_2O_2) (0.82 M, PBS) solution was added to the reaction mixture for inducing the membrane lipids peroxidation. The samples were again incubated at a temperature of 37 °C for 4 hours. Then the samples were centrifuged for 10 min at 250 x g and the absorbance of the supernatant was measured at 540 nm.

Relative hemolysis was assessed in comparison with the hemolysis in H_2O_2 treated negative control. In this experiment, quercetin was used as the standard (125-1000 µg/ml) and the blank solution was prepared by adding 0.2 M PBS and 0.82 M H_2O_2. The hemolytic activity was calculated as follows: Anti-hemolytic activity I% = ((Ac-As)/Ac) x100 where Ac is the absorbance of the control (maximum hemolysis); As is the absorbance of samples or standard.

2.4. Statistical analyses

All data are presented as means ± SD of 3 tests per sample. Statistical analysis was carried out by STATISTICA (version 4.1; Statsoft, Tulska, Okla). The significance of differences was analyzed by one-way analysis of variance followed by Tukey honestly significant difference test. Statistical significance was set at $p < 0.05$: (*) vs Lemon peel essential oil, (#) vs Garlic clove essential oil; at $p < 0.01$: (**) vs Lemon essential oil, (##) vs Garlic essential oil. Essential oils biologicals activities are assessed by one-way analysis of variance followed by Tukey honestly significant difference test, and followed by Principal components analysis (PCA) carried by XLSTAT software.

3. Results and Discussion

3.1. Extraction yields of essential oils

The essential oils extraction yields were 0.516% for lemon peel and 0.46% for garlic cloves. Our results are different in comparison with other studies, for example, the study by Herrera-Calderon et al., [11] revealed a value of 0.78% (v/dry weight) with garlic cloves (*Alium sativum*). Similarly, study by Himed et al., [12]; revealed an extraction yields of 1.24% with lemon peel essential oil (Citrus limon). These differences in yields obtained can be explained by the influence of several factors, such as the origin of the plant, the season, environmental factors and the stage of maturity (immature, semi-mature or mature), or the extraction method [13].

Main volatile compounds present in the essential oils. In the GS-MS analysis results, we presented the main compounds which have peaks with area and height > 0.15 %. The representative GC-MS profile of the lemon essential oil is shown in Table 1 and Figure 1a. Major constituents in lemon essential oil representing an Area of 78.67% and a Height of 81.28%. These are, Monoterpenes (≈ 38% of the components) such as, γ-Terpinene, p-Cymene, Terpinene 4-acetate, (R)-α-Terpinyl acetate, p-Menth-8-ene-1,2-diol (is a Limonene-1,2-diol). Sesquiterpene (6. 52%) such as α-Bergamotene and Caryophyllene oxide. And other compounds like Dehydrogeraniol (1.47%), β-Bisabolene (7.75%), 3,7-Dimethylocta-2,6-dienyl acetate (6.57 %), Neryl acetate (3.03%) and Limonene-1,2-epoxide (1.27%). However, main compounds in garlic essential oil representing an area of 57.03% and a height of 50.27%. These are, Organosulfur compounds (≈57%) such as Diallyl disulfide (10.73), Allyl methyl trisulfide, s-Trithiane (0.94%), Diallyl Trisulfide (31.16%), L-5-Propylthiomethylhydantoin (0.64%), 5-Methyl-1,2,3,4-tetrathiane (4.46%), 3,4-Dihydro-2H-thiopyran (2.8%), Diallyl tetrasulfide (3.30%), Cyclopentene, 3-methyl-1-(trimethylsilyloxy) (1.97%) and other compounds such as Monoterpene, p-Cymene (3.42%), cis-Carvyl acetate (0.82%); 2-Methoxy-3-allylphenol (0.73%) and 1,3-Bis(trimethylsilyloxy) propane (≈ 7.8%). And, 1-Methyl-1,4-cyclohexadiene (1.72 %) (Table 2 and Figure. 1b).

GS-MS analysis shows the existence of three chemical components in common in the two essential oils, p-Cymene and Dehydrogeraniol (majority in lemon essential oil), and Diallyl trisulfide (majority in Garlic essential oil) (Table 1 and 2). Our results agree with some studies with minor differences. For example, Ganiyu et al., [14] study identified in lemon peel essential oil (Citrus limon) the presence of sabinene (4.18) %, limonene (53.08%), α-pinene (3.82%), β-pinene (9.53%), myrcene (3.33%), neral (4.70%), geranial (3.34%), 1,8-cineole (3.38%), linalool (3.70%), borneol (5.58%), α-terpineol (0.25%), terpinen-4-ol (0.23%), linalyl acetate (1.48%) and β-caryophyllene (1.49%). El Aboubi et al., [15] study carried out on the Citrus limon peel essential oil from three Moroccan regions, reveal that monoterpene compounds constituted the main important fraction (monoterpene hydrocarbons, oxygenated monoterpenes). Moreover, D-limonene was found to be the main monoterpene constituent in all essential oils in the following concentrations: 53.44% (essential oil -1), 49.37% (essential oil -3) and 48.56% (essential oil -2), followed by β-pinene 18.29% (essential oil -3), 17.78% (essential oil -2) and 17.37 (essential oil -1), and γ-terpinene 12.84% (essential oil -3), 12.81% (essential oil -1) and 12.33% (essential oil -2).

Other monoterpenes with a percent less than 17% were found in all essentials oils such as α-pinene, sabinene, β-myrcene, fenchol alcohol, and geranial. Aguilar-Hernández et al., [16] identified in lemon peel essential oil (Citrus limon) monoterpenes (20 compounds such as α-Thujene, α-Pinene, Sabinene, α-Terpinene); sesquiterpenes (3 compounds: trans-Caryophyllene, trans-α-Bergamotene and β-Bisabolene); aldehydes (2 compounds Octanal and Nonanal) and esters (Neryl

acetate). Herrera-Calderon et al., [11] study identified in garlic oil (*Allium sativum*) the diallyl trisulfide as the major component (44.21%) of the volatile chemicals, followed by diallyl disulfide (22.08%), methyl allyl trisulfide (9. 72%) and 2-vinyl-4H-1, 3-dithiine (4.78%). Moreover, Mnayer et al., [17] identified in garlic oil (*Allium sativum*) as major components, diallyl disulfide (37.90%), diallyl trisulfide (28.06%), allyl methyl trisulfide (7.26%), diallyl sulfide (6.59%), diallyl tetrasulfide (4.14%) and allyl methyl disulfide (3.69%). The difference in volatile compounds with the other studies can be explained by the different extraction methods used to obtain the essential oil, such as conventional or non-conventional techniques [18] or might be related with external factors such as regions [15], temperature, soil composition, climate conditions, environmental stress, ecosystem, and altitude [19, 11].

3.2. Biological activities of essentials oils

3.2.1. DPPH, TBARS assay and Ferric reducing antioxidant power (FRAP)

The antioxidant activity of the examined essential oils was estimated by the DPPH and TBARS methods which are presented in Table 3, columns 1 and 2. For better comparison of the antioxidant properties of each essential oil, the results are expressed as IC50. The DPPH test shows the following IC50, 0.14±0.02 mg/ml for lemon essential oil, 0.50±0.13 mg/ml for garlic essential Oil and 0.31±0.01mg/ml for ascorbic acid. Similarly, the ability to inhibit lipid peroxidation (TBARS) reveals the following IC50, 0.134±0.039 for lemon essential Oil, 0.169±0.015mg/ml for garlic essential Oil and 0.129±0.007mg/ml for ascorbic acid. It means that, DPPH radical scavenging capacity is significantly increased in lemon essential oil compared to garlic essential Oil by +72% ($p<0.01$). Likewise, TBARS reduction capacity is significantly increased in lemon essential oil compared to garlic essential Oil by +21% ($p<0.05$). However, lemon essential oil has similar antioxidant capacity as compared to ascorbic acid. According to the Figure 2 and at a concentration of 500µg/ml, the Ferric reducing antioxidant power (FRAP) test reveals that ascorbic acid represents the highest absorbance value of 2.601, followed by garlic essential oil (1.981) and lemon essential oil (1.801). A higher absorbance indicates a greater reducing power, ascorbic acid has the highest reducing power followed by that of the garlic essential oil and finally lemon essential oil has the least significant reducing power.

3.2.2. Anti-inflammatory and anti-hemolytic activities

Anti-inflammatory activity is significantly increased in lemon essential oil (IC50: 0.27±0.01mg/ml) compared to garlic essential oil (IC50: 0.42±0.01 mg/ml) by +36% ($p<0.01$). However, Diclofenac (IC50: 0.03±0.01 mg/ml) represents the most important anti-inflammatory activity compared to lemon oil and garlic essential oils by +89 and +93%, respectively ($p<0.01$) (Table 3). However, anti-hemolytic activity is significantly increased in garlic essential Oils (IC50: 0.29±0,01mg/ml) compared to those of lemon peel (IC50: 0.21±0.01 mg/ml) by +27% ($p<0.05$). And a significant increase with Quercetin (IC50: 0.03±0.01mg/ml) compared to lemon and garlic essential oils by + 89 and + 85%, respectively ($p<0.01$) (Table 3).

To compare and understand the correlation between the biological activities of the two oils, a PCA analysis was carried out. Biological activities PCA of the two essential oils (figure 3) revealed, along the PC1 axis, which represents 80.42% of the observations, formation of two clusters. On the left, a cluster represented by lemon essential oil and on the right a cluster represented by garlic essential oil. For the quantitative variables, which are the biological activities of the oils, there is formation of a plot represented by DPPH, TBARS and anti-inflammatory activities, which correlate negatively with the lemon essential oil, showing that lemon essential oil inhibit these parameters. On the other hand, garlic essential oil negatively correlated with hemolysis and FRAP, which forms the second plot, indicating a strong anti-hemolytic potential and iron reduction of garlic essential oil. Briefly, PCA analysis shows that lemon essential oil inhibits DPPH radical, lipid peroxidation (TBRAS) and inflammation better than garlic essential oil. On the other hand, garlic essential oil inhibits hemolysis and high reducing capacity to reduce iron better than lemon essential oil (figure 3).

Our results agree with some studies, for example the study by Herrera-Calderon et al., [11] shows that garlic essential oil has a DPPH radical scavenging capacity expressed in IC50 at 0.124 ± 0.0023 mg/ml, reflects a lower capacity than lemon essential oil. Ganiyu et al., [14] study show that lemon peels essential oil inhibits induced lipid peroxidation (TBARS) in rat's brain homogenates showing good antioxidant capacity. The antioxidant (DPPH scavenging capacity) and the protective effect against lipid peroxidation observed by lemon essential oil may be due to the nature of the bioactive compounds it contains, as we found with CG-MS analysis, which indicated a strong presence of monoterpenes, γ-terpinene and terpinene. Indeed, Lu et al., [20] study found that two lemon monoterpenes, γ-terpinene and terpinolene, displayed strong DPPH scavenging abilities and exhibited 80% inhibition of lipid peroxidation (TBARS), indicating good potency against oxidation and lipid peroxidation. Likewise, the chemical structure of bioactive compounds of lemon essential oil (for example flavonoids) indicates that they act as free radical scavengers, oxygen scavenging agents and antioxidant hydrogen donors, thus preventing the formation of oxygen free radicals and protecting against cellular damage [21]. Likewise, the presence of monoterpenes in lemon essential oil may contribute to its antioxidant activity by neutralizing free radicals [22]. In the FRAP assay, the presence of the electron donor in the sample would result in the reduction of Fe^{3+} to Fe^{2+}. The formation of reduced ions increases the absorbance of the sample, which indicates the reductive ability of the sample [23]. Garlic essential oil (Allium sativum) has significant iron reducing power, this antioxidant activity could be attributed to the organosulfur compounds of this essential oil such as diallyl polysulphides) [24].

Table 1: Main volatile compounds of Lemon peel essential oil by GC-MS analyses

	Name	Formula	R.Time	Area (%)	Height (%)
1	γ-Terpinene	$C_{10}H_{16}$	3.721	13.65	19.39
2	p-Cymene	$C_{10}H_{14}$	4.125	14.70	20.14
3	1-p-Menthene	$C_{10}H_{18}$	4.301	0.71	0.90
4	Methy lheptenone	$C_8H_{14}O$	5.396	0.24	0.28
5	4-Thujanol	$C_{10}H_{18}O$	8.012	0.29	0.26
6	Diallyl disulfide	$C_6H_{10}S_2$	8.265	0.65	0.60
7	trans-Sabinene hydrate	$C_{10}H_{18}O$	9.841	0.36	0.24
8	d-linalool	$C_{10}H_{18}O$	10.031	0.71	0.67
9	trans-α-Bergamotene	$C_{15}H_{24}$	10.134	0.49	0.34
10	α-Bergamotene	$C_{15}H_{24}$	10.514	4.92	3.66
11	Terpinene 4-acetate	$C_{32}H_{46}O_2$	11.003	1.58	1.36
12	trans-p-Mentha-2,8-dienol	$C_{10}H_{16}O$	11.679	0.36	0.28
13	β-Santalene	$C_{15}H_{24}$	11.903	0.24	0.17
14	(E)-β-farnesene	$C_{15}H_{24}$	12.663	0.65	0.39
15	Dehydrogeraniol	$C_{10}H_{16}O$	12.777	1.47	1.31
16	1,8-Menthadien-4-ol	$C_{10}H_{16}O$	12.987	0.51	0.30
17	(R)-α-Terpinyl acetate	$C_{12}H_{20}O_2$	13.212	1.40	1.27
18	β-Bisabolene	$C_{15}H_{24}$	13.723	7.75	6.00
19	3,7-Dimethylocta-2,6-dienyl acetate	$C_{12}H_{20}O_2$	13.930	6.57	5.34
20	cis-Carvyl acetate	$C_{12}H_{18}O_2$	14.274	0.22	0.17
21	1,3-Methano-5bH-cyclobuta[cd]pentalen-5b-ol, octahydro	$C_{10}H_{14}O$	14.381	0.38	0.30
22	Neryl acetate	$C_{12}H_{20}O_2$	14.607	3.03	2.73
23	β-Elemene	$C_{15}H_{24}$	14.795	0.45	0.28
24	Diallyl trisulfide	$C_6H_{10}S_3$	14.982	1.05	0.84
25	cis-Carvyl acetate	$C_{12}H_{18}O_2$	16.272	0.62	0.58
26	p-Cymen-8-ol	$C_{10}H_{14}O$	16.614	0.53	0,28
27	cis-Carvyl acetate	$C_{12}H_{18}O_2$	16.911	0.47	0.39
28	Caryophyllene oxide	$C_{15}H_{24}O$	18.787	1.60	1.23
29	1,1,7-Trimethyl-4-methylenedecahydro-1H-cyclopropa[e]azulen-7-ol-, (1aR-(1a.alpha.,4a.alpha.,7.beta.,7a.beta.,7b.alpha.))	$C_{15}H_{24}O$	21.893	0.98	0.80
30	Methyl 9,10-dihydroxyoctadecanoate	$C_{19}H_{38}O_4$	22.455	0.47	0.37
31	Eugenol	$C_{10}H_{12}O_2$	22.908	0.80	0.62
32	1,3-Dioxolane, 2,2-dimethyl-4,5-di-1-propenyl	$C_{11}H_{18}O_2$	23.067	0.56	0.30
33	α-Bisabolol	$C_{15}H_{26}O$	23.834	0.47	0.41
34	Limonene-1,2-epoxide	$C_{10}H_{16}O$	24.216	1.27	1.12
35	p-Menth-8-ene-1,2-diol	$C_{10}H_{18}O_2$	24.491	0.29	0.24
36	Citronellic acid	$C_{10}H_{18}O_2$	24.640	0.33	0.22
37	p-Menth-8-ene-1,2-diol	$C_{10}H_{18}O_2$	25.001	6.35	6.24
38	Tridecyl 1-adamantanecarboxylate	$C_{24}H_{42}O_2$	28.198	0.91	0.75
39	5-Hydroxymethyl-1,1,4a-trimethyl-6-methylenedecahydronaphthalen-2-ol	$C_{15}H_{26}O_2$	28.283	0.65	0.52
	% of total identified volatile compounds			**78,67%**	**81,28%**

Main compounds which peaks with area and height > 0.15% are presented. R. Time: retention time

Table 2: Main volatile compounds of garlic essential oil by GC-MS analyses

	Name	Formula	R.Time	Area (%)	Height (%)
1	1-Methyl-1,4-cyclohexadiene	C_7H_{10}	3.720	2.22	4.07
2	p-Cymene	$C_{10}H_{14}$	4.126	3.42	5.97
3	Diallyl disulfide	$C_6H_{10}S_2$	8.294	10.73	13.08
4	Allyl methyl trisulfide	$C_4H_8S_3$	10.574	2.13	2.25
5	s-Trithiane	$C_3H_6S_3$	13.627	0.94	0.60
6	Cis-Carvyl acetate	$C_{12}H_{18}O_2$	13.743	1.06	0.75
7	Dimethyl tetrasulfide	$C_2H_6S_4$	13,817	0.57	0.46
8	Dehydrogeraniol	$C_{10}H_{16}O$	13,935	0.68	0.49
9	Diallyl Trisulfide	$C_6H_{10}S_3$	15.024	27.04	24.49
10	Diallyl Trisulfide	$C_6H_{10}S_3$	15.197	4.12	1.67
11	L-5-Propylthiomethylhydantoin	$C_7H_{12}N_2O_2S$	19.206	0.64	0.46
12	5-Methyl-1,2,3,4-tetrathiane	$C_3H_6S_4$	19.596	4.46	2.12
13	3,4-Dihydro-2H-thiopyran	C_5H_8S	21.703	1.16	0.60
14	Diallyl tetrasulfide	$C_6H_{10}S_4$	22.005	3.30	2.11
15	Cyclopentene, 3-methyl-1-(trimethylsilyloxy)	$C_9H_{18}OSi$	22.611	1.97	1.91
16	α-Bromonaphthalene	$C_{10}H_7Br$	22.745	0.27	0.13
17	2-Methoxy-3-allylphenol	$C_{10}H_{12}O_2$	22.911	0.94	0.87
18	Silane, [[5,5-dimethyl-4-methylene-2-(trimethylsilyl)-1-cyclopenten-1-yl]methoxy]trimethyl	$C_{15}H_{30}OSi_2$	25.079	0.36	0.38
19	Propane, 1,1'-thiobis[3-(methylthio)	$C_8H_{18}S_3$	25.222	0.23	0.20
20	3,4-Dihydro-2H-thiopyran	C_5H_8S	28.112	1.64	1.69
21	1,3-Bis(trimethylsilyloxy) propane	$C_9H_{24}O_2Si_2$	28.360	7.79	8.06
	% of total identified volatile compounds			75,68%	72,38%

Main compounds which peaks with area and height > 0.15% are presented. R. Time: retention time

Table 3: DPPH free radical scavenging, TBARS reductive, anti-inflammatory and anti-hemolytic activities of lemon and garlic essentials oils and their standards

IC50 (mg/ml)	DPPH	TBARS	Anti-Inflammatory activity	Anti Hemolytic activity
Lemon essential oil	0.14 ± 0.02	0.134±0.039	0.27±0.01	0.29±0,01
Garlic essential oil	0.50 ±0.13**	0.169±0.015*	0.42±0.01**	0.21±0,01*
Ascorbic Acid	0.31±0.01$^{\#}$	0.129±0.007$^{\#}$		
Diclofenac			0.03±0.01$^{**,\#\#}$	
Quercetin				0.03±0,01$^{**,\#\#}$

IC50: median inhibition concentration. NB: A lower IC50 indicates a higher reducing or inhibiting power. Data are presented as means ± SD, n=3 and analyzed by two-way ANOVA followed by Tukey post-hoc test. $^{*}P< 0.05$ vs Lemon essential oil, $^{\#}P< 0.05$ vs Garlic essential oil, $^{**}P< 0.01$ vs Lemon essential oil, $^{\#\#}P< 0.01$ vs Garlic essential oil

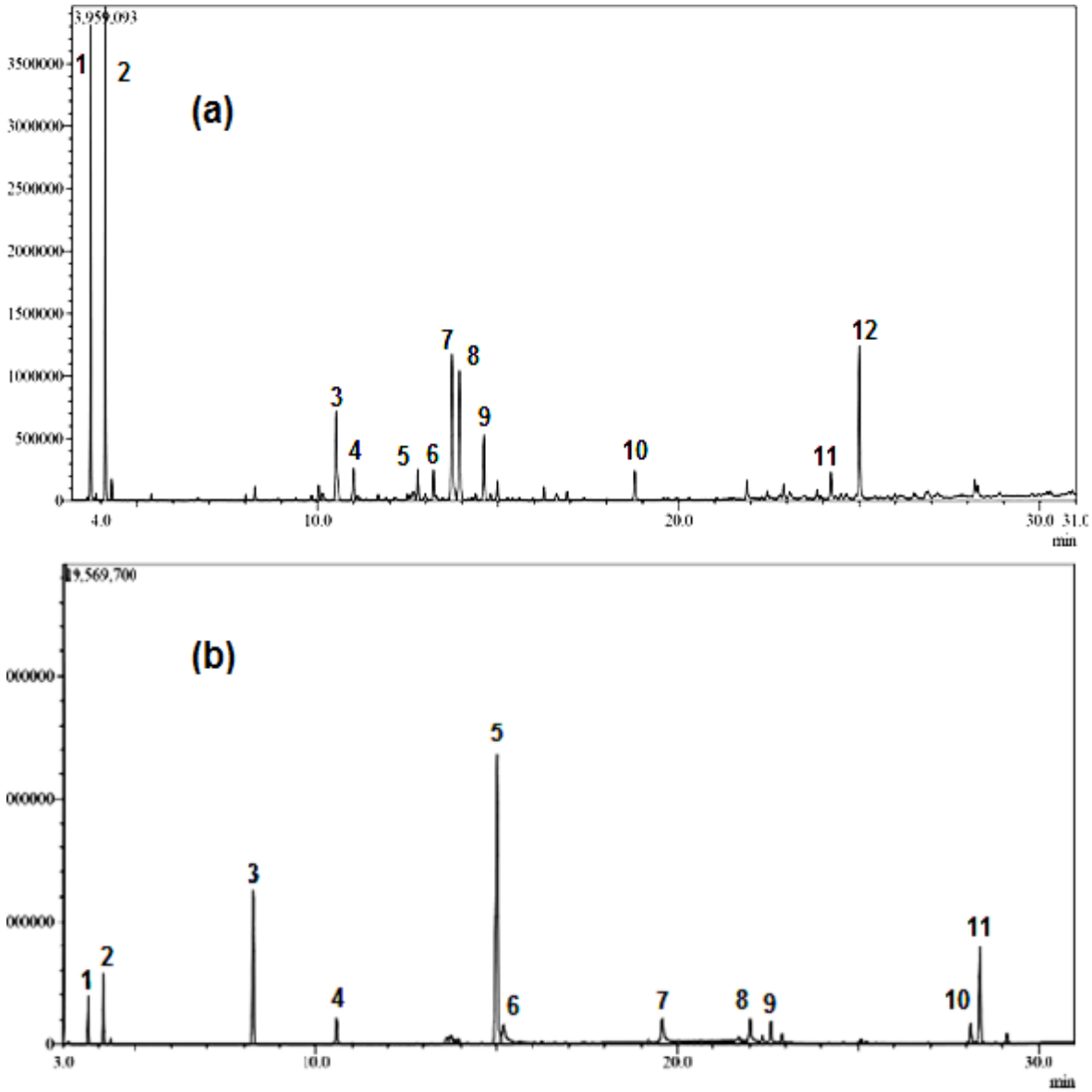

Figure 1: GC-MS chromatogram of main volatile compounds of essentials oils with labeling of the most intensive peaks

Figure-1a. *Lemon essential oil**:1** γ-Terpinene (**RT**=3.721, Area%: 13.65%). **2:** p-Cymene (**RT**=4.125, Area%: 14.70%). **3:** α-Bergamotene (**RT**=10.514, Area%: 4.92%). **4:** Terpinene 4-acetate (**RT**= 11.003, Area%: 1.58%). **5:** Dehydrogeraniol (**RT**= 12.777, Area%: 1.47%).**6:** (R)-α-Terpinyl acetate (**RT**=13.212, Area: 1.40%:). **7:** β-Bisabolene (**RT**= 13.723, Area%: 7.75%). **8:** 3,7-Dimethylocta-2,6-dienyl acetate (**RT**=13.930, Area%:6.57%). **9:** Neryl acetate (**RT**= 14.607, Area%: 3.03%). **10:** Caryophyllene oxide (**RT**= 18.787, Area%: 1.60%). **11:** Limonene-1,2-epoxide (**RT**= 24.216, Area%: 1.27%). **12:** p-Menth-8-ene-1,2-diol (**RT**=25.001, Area%: 6.35%).*

Figure-1b. *Garlic essential. **1:** 1-Methyl-1,4-cyclohexadiene (**RT**= 3.720, Area%: 2.22%**). 2:** p-Cymene monoterpene (**RT**= 4.126, Area%: 3.42%). **3:** Diallyl disulfide (**RT**=8.294, Area%: 10.73%). **4:** Allyl methyl trisulfide (**RT**=10.574, Area%: 2.13%); **5:** Diallyl Trisulfide (**RT**=15.024, Area%: 27.04); **6:** Allitridin (Diallyl Trisulfide) (**RT**=15.197, Area%: 4.12%); **7:** 5-Methyl-1,2,3,4-tetrathiane (**RT**=19.596, Area%: 4.46%); **8:** Diallyl tetrasulfide (**RT**=22.005, Area%: 3.30%); **9:** Cyclopentene, 3-methyl-1-(trimethylsilyloxy) (**RT**= 22.611, Area%: 1.97%); **10:** 3,4-Dihydro-2H-thiopyran (**RT**=28.112, Area%: 1.64%); **11:** 1,3-Bis(trimethylsilyloxy) propane (**RT**= 28.360, Area%: 7.79%).*

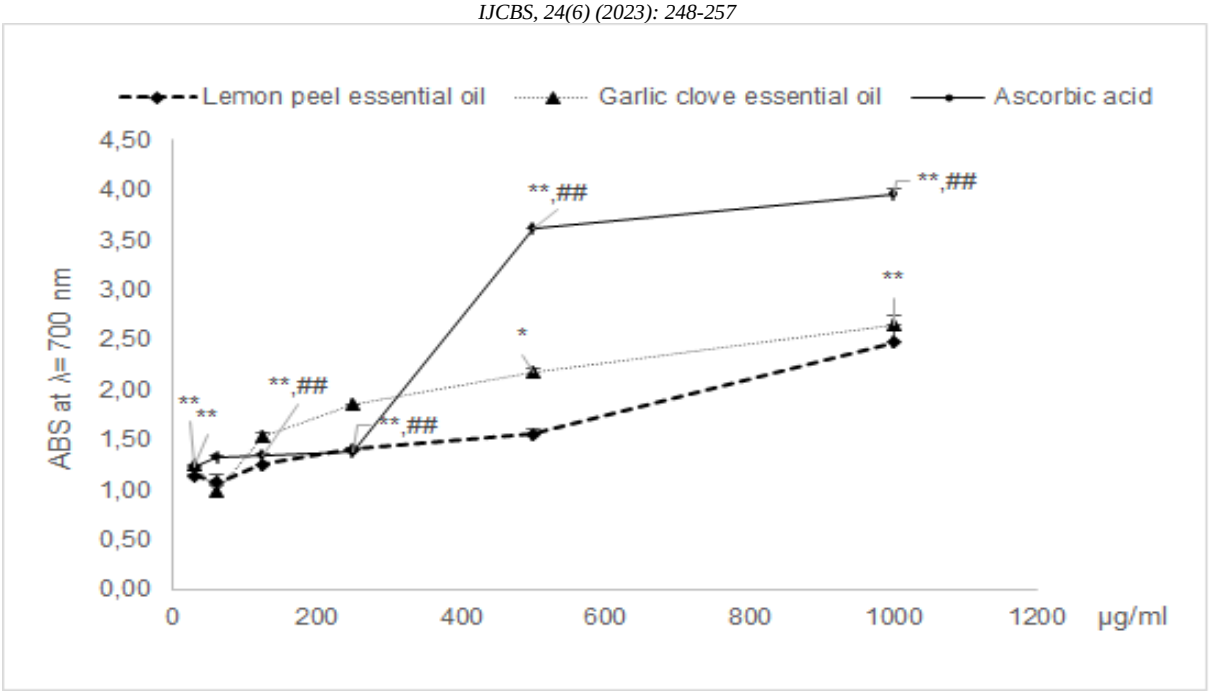

Figure 2: Ferric ion reducing antioxidant power (FRAP) of essentials oils of lemon and garlic
Higher absorbance of the reaction mixture indicated greater reducing power.
Data are presented as means±SD and analyzed by two-way ANOVA followed by Tukey post-hoc test.
**P< 0.05 vs Lemon essential oil, #P< 0.05 vs Garlic essential oil*
***P< 0.01 vs Lemon essential oil, ##P< 0.01 vs Garlic essential oil*

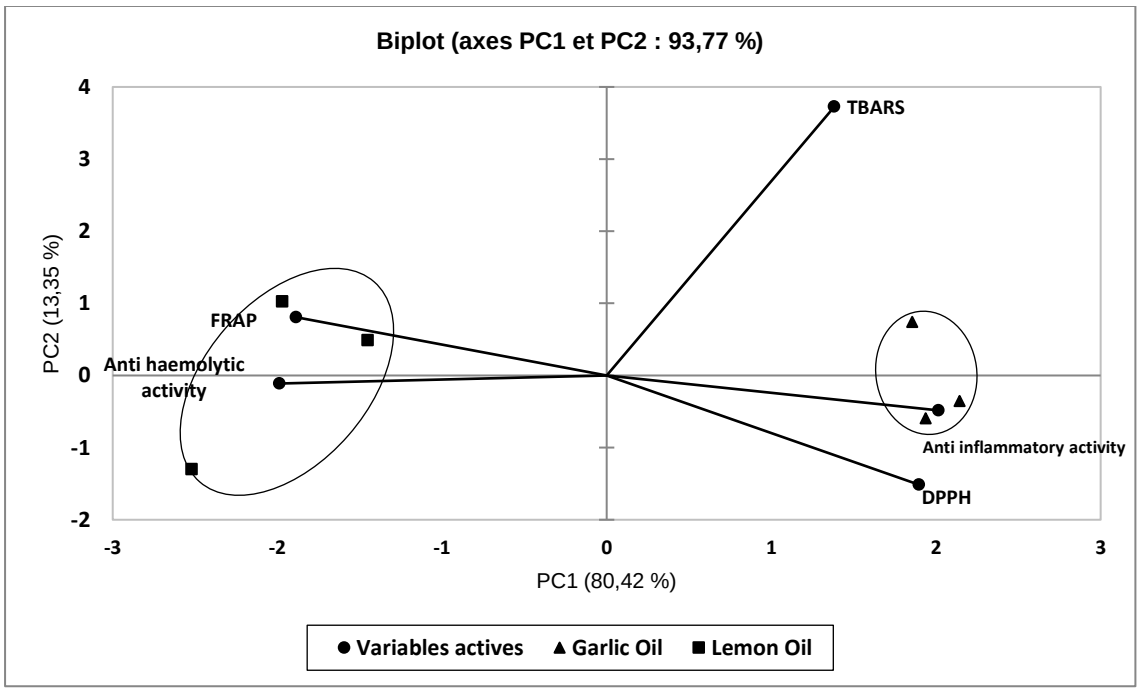

Figure 3: Principal components analysis (PCA) plot of biochemical parameters measured in the essentials oils of Lemon and Garlic

The strong anti-inflammatory activity observed with lemon essential oil may be due the bioactive compounds such as γ-terpinene and linalool [25]. Protein denaturation is well studied in the literature, and it is due to an inflammation

mechanism [26]. Molecules that are able to inhibit proteinase and protein denaturation can be considered anti-inflammatory compounds. The bioactive compounds of lemon essential oil may contribute to their anti-inflammatory properties. A previous study demonstrated that peel lemon essential oil possessed a significant anti-inflammatory capacity [26], this activity is mostly due to their bioactive compounds, such as monoterpenes, D-limonene. Other bioactive compounds, such as α-pinene and α-terpinene are also responsible for this anti-inflammatory capacity, probably acting by synergism with D-limonene [15]. Our results agree with Azantsa et al., [27] study, which found that Allium sativum extract (97.87%, $p < 0.05$) has greater anti-hemolytic activity compared to Citrus sinensis extract (87.7%).

Erythrocytes are vulnerable to oxidative stress due to their high content of polyunsaturated lipids and transition metals (particularly iron) that act as a catalyst of free radicals' generation via the Fenton reaction. Iron is associated with the oxidative degradation of membrane lipids and in the subsequent hemoglobin [28]. The anti-hemolytic activity test may be then taken as a tool to validate the interaction between the constituent's essentials oils and the biological entities at cellular level. The major constituents of the garlic essential Oil are organosulfur compounds such as Diallyl disulfide, Diallyl Trisulfide (10.73%), Allyl methyl trisulfide (2.13%) and s-Trithiane (0.94%). This organosulfur compounds, which revealed a good reducing power of iron with, FRAP test, could explain this good anti-hemolytic activity observed. Indeed, organosulfur compounds seem to reduce the iron present in hemoglobin by transferring an electron to it.

4. Conclusions

Lemon and garlic essentials oils contain different bioactive compounds. Lemon essential oil is rich in Terpenes, while garlic essential Oil is rich in Organosulfur compound. Lemon essential oil has significant anti-inflammatory activity, but garlic essential Oil has significant anti-hemolytic activity. However, the two essential oils have different antioxidant capacities. Lemon essential oil seems to be better to reduce DPPH free radical and the lipid peroxidation and garlic essential Oil has a high reducing capacity to reduce iron (FRAP). Both essential oils seem interesting for medicinal or cosmetic application.

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