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Antioxidant, Antibacterial and Cytotoxic effect of *Cymbopogon citratus*, *Mentha longifolia*, and *Artemisia absinthium* essential oils

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Abstract

This study aims to evaluate the benefits of some herbs such as antioxidant, antibacterial, and anti-cancer activities of Lemongrass (*Cymbopogon citratus*), peppermint (*Mentha longifolia*), and wormwood (*Artemisia absinthium*) essential oils (EOs). The chemical composition was identified using the GC-MS technique. The total phenolic content and the antioxidant activities were monitored by radical scavenging assay (DPPH). Furthermore, the antibacterial properties were evaluated. The possible anti-cancer activity was determined *in vitro* against colon (HCT116), breast (MC7) cancer, and normal human lung cell lines. The results showed that the major compounds of lemongrass EO were neral, citral,β-myrcene and camphor, while peppermint were E-Menthone, pulegone, Z-menthone, 1,8 cineole and menthol. Moreover, wormwood EO vital constituents were artemisia ketone, camphor, camphene and α-pinene. Lemongrass and wormwood EOs contain the highest total phenolic content than peppermint. Wormwood EO has the highest antioxidant activity using DPPH (IC_{50}= 0.689%). The inhibitory effect of lemongrass, peppermint, and wormwood EOs was higher against Gram-negative bacteria. Lemongrass and wormwood EOs showed the highest anti-cancer potential against HCT116 (IC_{50}=77.413 and IC_{50}=297.5 µg/ml, respectively). Lemongrass and wormwood EOs effectively inhibited the HCT116 cancer cell line's growth. We recommended using these plants, which act as antioxidants, antibacterial and anti-cancer, in the future.

Keywords: antibacterial activities; anti-cancer activities; antioxidant activities; wormwood; Lemongrass; essential oil; peppermint.

1. Introduction

Herbal medicinal products have been found as herbal supplies such as botanicals, nutraceuticals, and therapeutic products throughout human history in recent years [1]. However, free radical's uncontrolled production contributes to the emergence of many diseases, including cancer, cardiovascular problems, diabetes, and other diseases. The formation of free radicals plays a crucial role in the origin of life and biological evolution. Oxidation is vital for living creatures to produce energy to fuel biological processes [2].

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) in the biologic system, including radical superoxide, hydroxyl, and nitric oxide, can damage DNA, leading to fat-protein oxidation in cells [3]. The human body's antioxidant system can scavenge these radicals to balance oxidation and anti-oxidation [4].

Herbal medicines, including medicinal and aromatic plants, have been found throughout human history as herbal supplements such as plants, nutrients, and drugs [1]. In addition to medicinal and aromatic plants as herbs, their essential oils have also been used in the food and medicinal industries [5].

Cancer is a life-threatening disease that kills 7.6 million people annually, including many types, including breast cancer and colon cancer [6]. Breast cancer has spread in many countries, mainly American and Asian countries; it is the second cause of female deaths [7]. Colon cancer is considered one of the most common types, as eating habits, genetic predisposition, smoking, and other factors are among the causes of cancer [8]. Until now, the standard

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treatment for colon cancer or breast cancer is colectomy or breast resection and chemotherapy, which led to many side effects. Therefore the use of natural products alongside chemotherapy gave promising results [9].

Plant-based natural antioxidants, such as flavonoids, phenolic acids, and tocopherols, have recently gained much attention in preventive and therapeutic medicine. Because of their antioxidant properties, these natural compounds are thought to have anticarcinogenic potential and provide various health benefits [10].

Cymbopogon citratus (Lemongrass) is a tropical plant that originated in Maritime Southeast Asia and has subsequently been introduced to a range of tropical habitats as a member of the Poaceae family. *Cymbopogon citratus* EO possesses various pharmacological activities such as antioxidant, antibacterial, antifungal, anti-yeast, insect repellent activities, cytotoxicity, and anti-cancer properties [11].

Mentha longifolia or peppermint is a member of the Lamiaceae family; it grows in many worldwide temperate regions [12]. The peppermint plant contains a volatile oil that has many medicinal effects. Essential oil of the peppermint plant has many properties, including anti-inflammatory, antioxidant, antimicrobial, fungicide, and others [13].

Artemisia absinthium or wormwood belongs to the family of Asteraceae, known as wormwood, and it is one of the most common family species. Moreover, it contains a high percentage of volatile aromatic oils containing cineole, α-pinene, camphor, camphene, and artemisia ketone [14, 15]. The wormwood EO has antibacterial and antifungal activity [16].

The current study evaluated and compared the potential antioxidant, antibacterial and cytotoxic activity of Lemongrass, peppermint, and wormwood essential oils (EOs). First, the chemical composition of three essential oils was identified using GC-MS instrument. At the same time, the DPPH assay and the total phenolic content test examined the antioxidant power. Then, antibacterial properties were also evaluated against gram-positive and gram-negative bacteria. Finally, different EOs was evaluated against both cancer cell lines (breast cell line (MCF7) and colon cell line (HCT116)) in addition to normal cell line (human lung (wi38)).

2. Experimental

2.1. Chemicals and materials

Fresh Lemongrass, peppermint, and wormwood were bought from medicinal, aromatic, poisonous plants experimental station (farm), Faculty of Pharmacy, Cairo University, Egypt. All chemicals were of analytical grade. DPPH, Folin-ciocalteu reagent, Gallic acid bought from Sigma-Aldrich Chime, Steinheim, Germany. Mueller Hinton broth (Difco) and Nutrient agar were purchased from Sigma (St. Louis, Mo). Pathogenic stock cultures of bacteria, *S.aureus* ATCC 29213, *E. coli* ATCC 25922, and *S.Typhimurium* ATCC 9027 were bought from Microbiological Resources Center (MIRCEN), Faculty of Agriculture., Ain Shams University, Egypt. Three cell lines, normal fibroblasts Homo sapiens, human lung (wi-38) cell line, cancer colon cell line (HCT116), and cancer Breast cell line (MCF7), were obtained from Science Faculty, Al-Azhar University, Egypt.

2.2. Preparation and extraction of essential oils

Fresh plants were washed with distilled water. The essential oils of fresh plants were extracted by distillation of water using a Clevenger Apparatus device for 6 hours [17]. The volatile oil was obtained by passing over anhydrous Na_2SO_4 to strip it of any water, while the oils were kept in sealed glass bottles covered with aluminium foil at 20°C until required.

2.3. Analysis of the essential oil using gas-chromatography-mass spectroscopy (GC-MS)

Agilent Technologies has performed GC–MS analysis of the EOs in the Central Laboratories of the network with gas Chromatography (7890B) and Mass Spectrometer Detectors (5977A), Cairo, Egypt (1987). Hexane-dilute samples (1:19, v/v). The GC has a column HP-5MS (30 mm x 0.25 mm in diameter and 0.25 mm in thickness). Helium was analyzed as a 1,0 ml/min transport gas with a split rate of 1:30, a one µl injection volume and the following temperature program: Helium For one minute, raise 40 °C; raise to 4 °C/min and maintain for six minutes; raise to 210 °C at 4 °C/min and maintain at 1 minute. The injector and detector were maintained at 280°C and 220°C, respectively. Ionization of mass-spectrum (IE) at 70 eV was

achieved with a range of 40-550 m/z and solvent delays of 3 min. identifying the different components was determined compared to the data stored in Wiley and the NIST Mass Spectral Library [18].

2.4. Determination of total phenolic contents (TPC)

TPC was determined by the method described [19] using the Folin-Ciocalteau reagent. Results are shown as milligram gallic acid equivalents per one millilitre of the EO (mg GAE/ml).

2.5. Antioxidant activity

2.5.1. DPPH radical scavenging activity

Different concentrations20, 10, 5, 2.5, 1.25, and 0.625% (v/v) of the plant's EOs were evaluated based on their scavenging activity of the stable free radical (DPPH) [20]. Butylated hydroxytoluene (BHT) was used as a reference standard. The DPPH radical inhibition (percentage) of the samples has been calculated using the following formula:

$$\% \text{ Inhibition} = (Ac\ (0) - AA\ (t)) / Ac\ (0) \times 100$$

Where: Ac (0) is the timing absorption of the control = 0 min.

AA (t) is the antioxidant absorption at a time =30 minutes.

Also, IC_{50} of all EOs samples values were calculated using the formula: y=2.2517x+40.995 for Lemongrass, y=2.1483x+48.525 for wormwood and y=1.4224x+41.009 for peppermint

2.6. Antibacterial activity

2.6.1. Agar Well-diffusion

Antibacterial activities of different EOs were determined using well agar diffusion [21]. Different dilutions were prepared of the essential oils (20, 10, 5, 2.5, 1.25 and 0.625%) and filter sterilized (0.45 µm). About 100 µl of different plant essential oil concentrations were added into the wells, and positive control well containing Gentamicin (10 mcg/disc) as an antibiotic. Plates were kept for 2hr at 4°C to allow antibacterial substance diffusion and incubated at 37°C for 18–24 hrs. The diameter of the inhibition zone was measured (mm). The experiment was repeated triple for each sample, and the average values were recorded.

2.6.2. Minimum inhibitory concentration (MIC)

The antibacterial activity of the plant's EOs was determined by a micro-dilution assay using 96-well plates [22]. Fifty µl of different essential oil dilutions (20, 10, 5, 2.5, 1.25, and 0.625%) and 50µl of bacterial suspension (≅104) were added to all sample mixture. The resulting turbidity was observed after 24 hr. MIC was determined when the growth completely disappears. At least three repetitions were run for each assay.

2.7. Anti-cancer activity

Cytotoxicity of different concentrations of the plant essential oil under test (12.5, 25, 50, 100, 200, 400, 800, and 1000µg/ml) was evaluated via MTT test using normal fibroblasts Homo sapiens, human lung (wi-38) cell line, cancer cell line colon (HCT116) and cancer breast cell line (MCF-7), Al-Azhar University, Egypt [23,24]. The cells were incubated for 24 hours at 37°C in a 5% CO_2 incubator in the presence of various concentrations of the plant essential oil under test (12.5, 25, 50, 100, 200, 400, 800, and 1000µg/ml). Then, the plate was incubated in the presence of 0.5 mg/ml MTT for 4 hours after the media was withdrawn. At 570 nm, absorbance was measured (OD) to determine the number of live cells. The following formulas were used to compute percent cell death:

%Cell Death The effective = [(Control OD – Sample OD)/Control OD] X 100. Concentration to kill 50% of cancer cells (IC_{50}) values was calculated.

2.8. Statistical analysis

Data are expressed as a mean ± standard error (n = 3). The results were processed by SPSS (ver. 20) as outlined [25], in which a p-value <0.05.

3. Results and discussion

3.1. Chemical composition of Lemongrass, peppermint, and wormwood EOs.

The chemical compounds of EOs were identified using the GC-MS technique. 41 constituents from lemongrass EO were reported, representing 98.21% of the EO, while the remaining unknown portion was 1.79% (Table 1). Moreover, 43 components were detected in peppermint EO, representing 99.55%, besides 0.45% of undetectable percentage (Table 2). On the other hand, it was observed that 57 components were isolated from wormwood EO; these identified compounds represent 97.57% of warm wood EO, while the remaining unknown part was 2.43% (Table 3).

The main components of Lemongrass (Table 1) were neral (19.63%), citral (18.45%), β-Myrcene (7.38%), camphor (6.84%), Endo-Borneol (4.57%) and others, while the minor compounds were D-limonene (1.22%) and 6-Methyl 5-Hepten-z-one (1.1%) and traces of other compounds, so that 41 compounds may be responsible for the EOs bioactivity (antioxidant, antibacterial, or antifungal). The main compounds of lemongrass EO were mixtures of the aldehyde isomers of geranial and neral from the monoterpene citral [26]. The increase in the percentage of these natural antioxidant compounds may be reduced microbial load so that lemongrass oil can be used as a preservative [27].

Table 1.Chemical compounds of lemongrass essential oil identified via GC-MS technique

Chemical compounds	Retention time (min)	Concentration (Area %)
α-Pinene	8.168	3.79
Camphene	8.626	0.8
2,4(10)thujadien	8.826	0.21
2 β-Pinene	9.582	0.29
6Methyl-5Hepten-z-one	10.04	1.1
β-Myrcene	10.228	7.38
3-Carene	10.778	0.34
4-Methylcumene	11.304	0.8
D-Limonene	11.459	1.22
Eucalyptol	11.539	3.87
α-Terpinolene	13.599	0.31
Linalool	14.096	3.3
Chrysanthenone	14.92	0.31
Camphor	15.641	6.84
1,3,4-Trimethyl-3-cyclohexen-1-carboxaldehyde	15.819	0.5
3,7dimethyl 7-octenal	15.973	0.46
2,Norpinanone,3,6,6trimethyl	16.179	0.29
Endo-Borneol	16.408	4.57
E-3-Pinanone	16.683	1.06
Terpinen-4-ol	16.803	0.56
Table 1: continued		
Isogeranial	17.055	2.16
α-Terpineol	17.307	1.12
Myrtenol	17.507	0.4
3-cyclopentene-1-ethanol,2,2,4-trimethyl	17.799	0.57
2-Pinen-4-one	17.982	4.5
Citronellol	18.823	0.94
Citral	19.315	18.45
Geraniol	19.761	2.15
Neral (E-citral)	20.402	19.63
E-verbenol	20.528	0.2
Bornyl acetate	20.665	0.85
Geranyl acetate	23.841	0.74
Methyl eugenol	24.493	0.27
E-Caryophyllene	24.957	1.48
Z- α-Bergamotene	25.46	0.53
α-Humulene	25.998	0.41
Cis-sesquisabinene hydrate	27.293	0.43
Δ-cadinene	28.138	0.23
Caryophyllene oxide	30.021	0.78
Selin-6-en-4 α,ol	31.326	1.16
Diisobutyl phthalate (DIBP)	41.791	3.21

The major compounds of peppermint (Table 2) were trans-menthone (17.1%), pulegone (16.2%), cis-menthone (14.35%), 1, 8-cineole (9.11%), menthol (7.21%) and others .Meanwhile, the minor compounds were L.linalool (0.96%), camphene (0.86%), sabinene (0.87%). Peppermint EO is rich in oxygenated monoterpenes (pulegone, menthone, isopulegole, Isopulegone, and 1, 8-cineole) compounds give it antioxidant properties and antimicrobial properties against many types of bacteria and fungi [28].

Table 2. Chemical compounds of peppermint essential oil identified via GC-MS technique.

Chemical compounds	Retention time (min)	Concentration (Area %)
Santolinetriene	7.379	0.17
α-Pinene	8.168	2.95
Camphene	8.632	0.86
2,4(10)thujadien	8.838	0.09
Sabinene	9.513	0.87
2β-pinene	9.593	2.14
B-Myrcene	10.165	0.98
3-Carene	10.778	0.14
β-Cymen	11.316	0.37
Table 2: continued		
1, 8-cineole	11.567	9.11
Artemisia ketone	12.683	2.42
α-Terpinolene	13.61	0.16
L-Linalool	14.114	0.96
Chrysanthenone	14.932	0.13
Camphor	15.613	4.17
E-Verbenol	15.756	0.53

E-Menthone	16.116	17.1
Z-Menthone	16.482	14.35
Menthol	16.78	7.21
Isopulegone	16.832	0.7
d-isomenthol	17.055	0.5
α-Terpineol	17.346	1.26
Myrtenol	17.547	0.1
Levoverbenone	17.959	1.92
β-citronellol	18.748	0.09
Pulegone	19.132	16.2
Piperitone	19.527	1.83
E-Citral	20.104	1.42
Bornyl acetate	20.597	0.33
Piperitenone	22.405	0.83
E-Caryophyllene	24.951	1. 33
Humulene	26.004	0.3
D-Germacrene	26.856	1.29
γ- Muurolene	27.858	0.42
Z-Calamenene	28.121	0.16
Nerolidol	29.363	0.11
Caryophyllene oxide	30.015	0.64
Epicubenol	31.182	0.21
tau.-Cadinol	32.235	2.05
tau.-Muurolol	32.825	0.14
α-Bisabolol	34.238	1.43
Diisobutyl phthalate	41.774	1.42
5-(7a-Isopropenyl-4,5-dimethyl-octahydroinden-4-yl)-3-methyl-pent-2-en-1-ol	48.463	0.16

On the other side, the major component of wormwood (Table 3) were Artemisia ketone (12.4%) ,camphor (11.49%), D-germacrene (6.5%), camphene (4.28%), α-pinene (3.13%), and 1,8-cineole (2.42%).Minor components were β-Myrcene (2.28%), linalool (1.38%), sabinene (1.38%), cis-chrysanthemol (0.73%), caryophyllene oxide (0.71%), α-terpineol (0.7%), pulegone (0.3%) and other traces components. Wormwood EO contains many vital activities, including remarkable antioxidant activity and cytotoxicity against cancer cells because it contains artemisinin compounds [29].

Table 3. Chemical compounds of wormwood essential oil identified via GC-MS technique.

Chemical compounds	Retention time (min)	Concentration (Area %)
Santolinatriene	7.384	2.55
Tricyclene	7.762	0.26
α-Pinene	8.174	3.13
Camphene	8.655	4.28
Sabinene	9.519	1.38
2 β- Pinene	9.604	2.19
β-Myrcene	10.177	2.28
Yamogi alcohol	10.503	0.87
3-Carene	10.777	0.22
2-Carene	11.018	0.13
β-Cymene	11.31	0.73
Mentha-1,7(8)-diene	11.464	3.01
1, 8-cineole	11.538	2.42
γ- Trepinene	12.551	0.19
Artemisia ketone	12.849	12.4
Hotrienol	13.032	0.27
Artemisia alcohol	13.49	0.59
α-Terpinolene	13.627	0.47
L-linalool	14.119	1.38
Chrysanthenone	14.937	0.26
Camphor	15.71	11.49
z-Chrysanthemol	16.276	0.73
endo-Borneol	16.414	2.89
3-pinanone	16.688	0.68
Terpinen-4-ol	16.808	0.45
3,6-Octadienal, 3,7-dimethyl	17.037	0.16
3,9-Epoxy-p-mentha-1,8(10)-diene	17.117	0.48
α-Terpineol	17.301	0.7
Myrtenol	17.501	0.56
3-Cyclopentene-1-ethanol, 2,2,4-trimethyl	17.77	0.38
Levoverbenone	17.97	2.87
Cyclohexane, 1,1,4,4-tetramethyl-2,5-dimethylene	18.863	0.34
Pulegone	18.983	0.3
Citral	19.086	2.24
Geraniol	19.544	0.25
E-Citral	20.127	2.35
Bornyl acetate	20.596	0.55
α.-Copaene	23.555	0.18
β-Elemene	24.093	0.93
Methyleugenol	24.499	0.18
Isolongifolol	24.59	0.21
E-Caryophyllene	24.951	0.96
Table3: continued		
α –Humulene	26.002	0.32
α-cedrene	26.851	2.62
Germacrene-D	26.908	6.5

Tau-Cadinol acetate	28.144	0.31
E-α-Bisabolene	28.722	0.74
E- Nerolidol	29.386	1.38
7-Oxabicyclo[4.1.0]heptane, 2,2,6-trimethyl-1-(3-methyl-1,3-butadienyl)-5-methylene	29.574	0.39
Caryophyllene oxide	30.026	0.71
Junenol	31.302	0.35
Isospathulenol	31.743	0.52
Bisabolol oxide B	32.842	0.8
α.-Bisabolol	34.358	0.63
α.-Bisabolol	34.438	6.17
1,6-Dioxaspiro[4.4]nona-2,8-diene, 7-(2,4-hexadiynylidene)	39.571	0.26
Diisobutyl phthalate	41.785	1.98

3.2. Total phenolic content of Lemongrass, peppermint and wormwood Eos

The data recorded in (Table 4) showed that the highest TPC content was recorded (156.29 and 156.23 mg GAE/ml) for Lemongrass and wormwood leaves EO, followed by peppermint 127.22 mgGAE/ml, respectively. Different plants in our study contain a high phenolic compound, which acts as a potent antioxidant activity. The Lemongrass, peppermint and wormwood EOs have high TPC.

Table 4. Total phenolic content (TPC) of Lemongrass, peppermint, and wormwood essential oils.

Plant essential oils	Total phenolic content (mg GAE)
Lemongrass	156.29^{a}±4.94
Peppermint	127.22^{b}±0.54
Wormwood	156.23^{a} ±0.89
*LSD	10.09

Results expressed as mg Gallic acid equivalent per 1mL of essential oil. All values represented as mean ± SD (n=3); *LSD: least significant difference. Different superscripts in the same column mean significant difference (P<0.05).

3.3. Antioxidant properties

3.3.1. DPPH radical scavenging

The EO of wormwood recorded higher scavenging activity than the lemongrass and peppermint, giving IC_{50} of 0.689%, 4%, and 6.329% in order, as shown in (Table.5). The radical DPPH is a stable free radical at room temperature, with an alcoholic dark purple colour. The interaction between this radical and antioxidant results in a reduction in its absorbent intensity, which is the basis of antioxidant action measurement [30]. The free radical scavenging behaviour of the samples examined is suggested by the decolouration and the three volatile oils obtained have potent activity in removing free radicals. Also, the Lemongrass, peppermint and wormwood essential oils contain a high percentage of antioxidant activities [27]. It was thought that the antioxidant activity is due to several phytochemical compounds, which were studied on the effects of antioxidants using the peroxidation assay. It was found that α – pinene and camphor have the highest antioxidant activity [31].

3.4. Antibacterial activities

3.4.1. Agar well diffusion

Inhibition zones of Lemongrass, peppermint, and wormwood essential oils against *E.coli*, *S. Typhimurium*, and *S.aureus* recorded (35mm, 24mm & 30.6mm), (33.5mm, 17.66 mm & 26.5mm), and (25mm, 16.3mm & 24mm), respectively (Fig.1). Lemongrass had the highest microbial activity, followed by wormwood then peppermint. The components of volatile oils differ according to the surrounding environment and the different region

Table 5. DPPH radical scavenging activity (%) of lemongrass, peppermint and wormwood essential oils.

Plants essential oils	% inhibition DPPH						
	Different concentrations (%)						
	0.625	1.25	2.5	5	10	20	IC_{50} (%)
Lemongrass	37.99^{b}±0.77	41.88^{b}±0.18	48.24^{b}±0.99	53.54^{b}±0.43	70.92^{a}±1.15	82.06^{b}±0.25	4
Peppermint	35.52^{c}±0.39	41.35^{b}±0.51	46.29^{b}±0.21	52.73^{b}±0.14	60.35^{b}±0.18	65.82^{c}±0.25	6.329
wormwood	46.78^{a}±0.07	49.05^{a}±0.71	55.08^{a}±0.29	64.32^{a}±0.59	70.39^{a}±0.41	90.12^{a}±0.18	0.689
*LSD	1.73	1.77	2.10	1.48	2.47	0.78	$^{\#}$0.21

All values represented as mean ± SD (n=3);* LSD: least significant difference. Different superscripts in the same column mean significant difference (P<0.05). $^{\#}$ IC_{50} of butylated hydroxytoluene (BHT) as a reference standard.

The essential oils of the plants have a content of an antimicrobial activity. Phenolic compounds have proved to be a significant contributor to the preventive effects of diseases and treatment with aromatic EO to increase food and food product nutritional quality and provide preservatives against foodborne pathogens. Clear areas of inhibition were observed on the high concentration of EO plates [32].

3.4.2.Minimum inhibitory concentration (MIC)

MICs of Lemongrass against *S.aureus*, *E.coli*, and *S. Typhimurium* were 5, 0.625, and 0.625%, respectively (Table 6). Meanwhile, wormwood EO against the same bacteria was the same (5%). Peppermint showed 10, 5 & 5%, respectively. The terpene EO components, including α-terpineol, linalool, eucalyptol, and α-pinene, are effective in biological activities, including antimicrobial and antioxidant activities, which can be used as a useful reference for the food industry [33].

Phenolic compounds can inhibit and remove free radicals and can inhibit the growth of bacteria. These activities have been associated with the phenolic content, as there is a relationship between the total phenolic content and the measured activities. We can say that there is a correlation between antibacterial activity and antioxidant activity [34, 35].

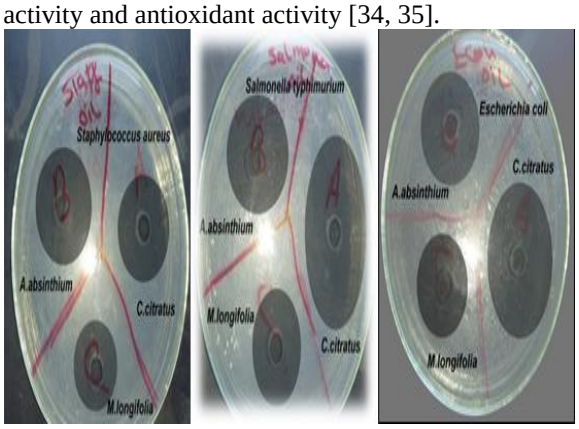

Figure 1. Inhibition zone (mm) of lemongrass, wormwood, and peppermint essential oils indicating the inhibition of the growth of the three microorganisms ;(a) *Staphylococcus aureus*, (b) *Salmonella Typhimirium*, (c) *Escherichia coli.*

Table 6. The minimum inhibitory concentration of different essential oils (%) against indicator strains.

Bacteria strains	Lemongrass	Wormwood	Peppermint
E. coli	0.625	5	5
S.Typhimurium	0.625	5	5
S. aureus	5	5	10

3.5. Anti-cancer activities

In vitro cytotoxic activity of different EOs types against two cancer cell lines (MCF-7 and HCT116) beside one normal (Wi38) cell line was used assessed by MTT. Different concentrations were used (12.5, 25, 50, 100, 200, 400, 800, 1000 µg/ml) at incubation period of 24h.

Results showed a dose-dependent decrease in cancer cell line survival for the three EOs over the test concentration range. Lemongrass EO possess the highest cytotoxic activity 94.23% at 1000 µg/ml (IC_{50}=77.413 µg/ml) against HCT116 cell line, while the lowest cytotoxic activity was against MCF-7 (IC_{50}= 317.40 µg/ml), whereas wi38 normal cell line has the moderate effect between the two-cancer cell line (IC_{50}= 176.3 µg/ml) as shown in Fig. 2

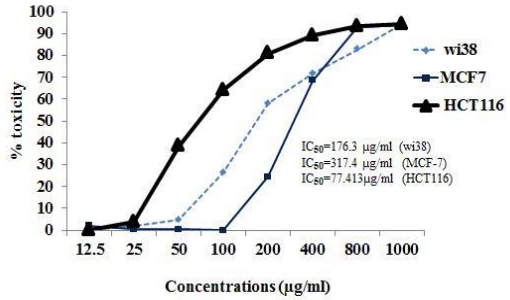

Figure 2. Cytotoxic activity (%) of lemongrass essential oil against wi38, MCF-7, and HCT116 cell lines.

Moreover, Fig.3 showed that wormwood essential oil against HCT116 possesses the highest cytotoxic activity IC_{50}=297.5 µg/ml, while its cytotoxicity effect was equal against MCF-7 and wi38 with IC_{50} of =506.18 and 506.11 µg/ml, respectively.

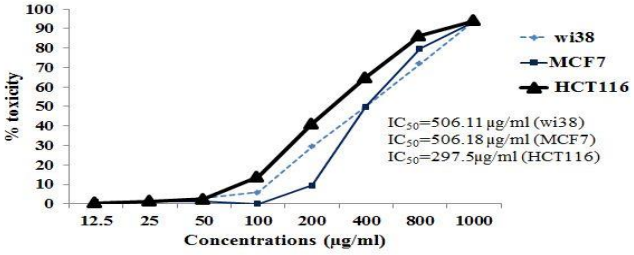

Figure 3. Cytotoxic activity (%) of wormwood essential oil against wi38, MCF-7, and HCT116 cell lines.

On the other hand, peppermint showed a very weak effect against HCT116, MCF-7, and wi38 cell lines, as shown in Fig.4. The wormwood, peppermint, and lemongrass EO have a cytotoxic effect on various cancer cell lines [36, 37]. Moreover, essential oils rich in α-pinene and β-myrcene also presented anti-cancer properties along with their antimicrobial and antioxidant properties, indicating their auxiliary therapeutic role in treating cancer. Until now, few studies have reported on the effectiveness of essential oils and their chemical components as a natural resource used for treatment [38].

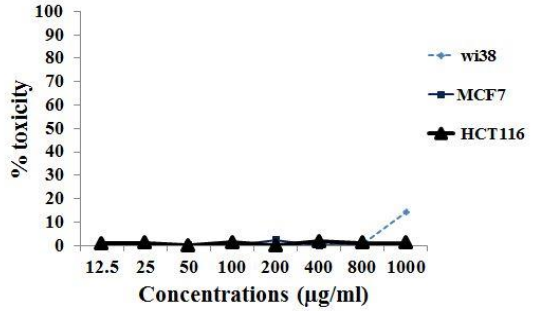

Figure 4. Cytotoxic activity (%) of peppermint essential oil against wi38, MCF-7 and HCT116 cell lines.

4. Conclusions

The current study showed that plant's volatile oils have multiple active properties, including antioxidant, antibacterial and anti-cancer activity. Lemongrass and wormwood EOs, with high phenolic content, effectively reduced the *in vitro* free radical activity, suggesting a novel natural antioxidant role originating from these medicinal plants. Moreover, the results also showed that lemongrass EO has the highest antibacterial capacity, allowing its EOs as a preservation agent. Furthermore, the lemongrass and wormwood EOs had the highest selectivity and toxicity in targeting cancer cell line (HCT116) than the normal cell line (wi38). Thus, these plants had registered antioxidant, antibacterial, and anticarcinogenic potential for the future development of potent antioxidants and anticarcinogenic treatments.

5. Conflicts of interest

No potential conflict of interest was reported by the authors.

6. References

1. Ekor, M.Th. growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. *Frontiers in pharmacology*, 4, p.177 (2014).
2. Guenane, H., Mechraoui, O., Bakchiche, B., Djedid, M., Gherib, A. and Benalia, M., Antibacterial, antioxidant activities and mineral content from the algerian medicinal plants. *Scientific Study & Research. Chemistry & Chemical Engineering, Biotechnology, Food Industry*, 21(2), pp.175-194 (2020).
3. Li, S., Tan, H.Y., Wang, N., Zhang, Z.J., Lao, L., Wong, C.W. and Feng, Y., The role of oxidative stress and antioxidants in liver diseases. *International journal of molecular sciences*, 16(11), pp.26087-26124 (2015).
4. Wang, F., Li, Y., Zhang, Y.J., Zhou, Y., Li, S. and Li, H.B.,. Natural products for the prevention and treatment of hangover and alcohol use disorder. *Molecule*s, 21(1), p.64. (2016)
5. Edris, A.E., Pharmaceutical and therapeutic potentials of essential oils and their individual volatile constituents: a review. Phytotherapy Research: An *International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives*, 21(4), pp.308-323.(2007)
6. Roy, A., Ahuja, S. and Bharadvaja, N.,. A review on medicinal plants against cancer. *Journal of plant sciences and Agriculture Research*, 2, p.1008 (2017).
7. Jemal, A., Siegel, R., Ward, E., Hao, Y., Xu, J., Murray, T., &Thun, M. J., Cancer statistics, 2008. CA: *a Cancer Journal for Clinicians*, 58, 71–96 (2008).
8. Siegel, R., DeSantis, C. and Jemal, A., Colorectal cancer statistics, 2014. CA: *a cancer journal for clinicians*, 64(2), pp.104-117 (2014).
9. Jose, J., & Rao, P. G. M., Pattern of adverse drugreactions notified by spontaneous reporting in an Indiantertiary care teaching hospital. *Pharmacological Research*, 54, 226–233(2006).
10. Eloff, J. N., It is possible to use herbarium specimens to screen for antibac-terial components in some plants. *Journal of Ethnopharmacology*, 67(3), 355-360(1999).
11. Almeida, K.B., Ramos, A.S., Nunes, J.B., Silva, B.O., Ferraz, E.R., Fernandes, A.S., Felzenszwalb, I., Amaral, A.C.F., Roullin,

V.G. and Falcão, D.Q., PLGA nanoparticles optimized by Box-Behnken for efficient encapsulation of therapeutic *Cymbopogon citratus* essential oil. Colloids and Surfaces B: *Biointerfaces*, 181, pp.935-942 (2019).

12. Hussain, A.I., Anwar, F., Nigam, P.S., Ashraf, M. and Gilani, A.H.,. Seasonal variation in content, chemical composition and antimicrobial and cytotoxic activities of essential oils from four *Mentha* species. *Journal of the Science of Food and Agriculture*, 90(11), pp.1827-1836. (2010).
13. Mkaddem, M., Bouajila, J., Ennajar, M., Lebrihi, A., Mathieu, F. and Romdhane, M., Chemical composition and antimicrobial and antioxidant activities of *Mentha* (*longifolia* L. and *viridis*) essential oils. *Journal of food science*, 74(7), pp.M358-M363 (2009).
14. Radulović, N.S., Randjelović, P.J., Stojanović, N.M., Blagojević, P.D., Stojanović-Radić, Z.Z., Ilić, I.R. and Djordjević, V.B.,. Toxic essential oils. Part II: Chemical, toxicological, pharmacological and microbiological profiles of *Artemisia annua* L. volatiles. *Food and Chemical Toxicology*, 58, pp.37-49 (2013)
15. Pino-Otín, M.R., Val, J., Ballestero, D., Navarro, E., Sánchez, E. and Mainar, A.M., Impact of *Artemisia absinthium* hydrolate extracts with nematicidal activity on non-target soil organisms of different trophic levels. *Ecotoxicology and environmental safety*, 180, pp.565-574 (2019).
16. Ćavar, S., Maksimović, M., Vidic, D. and Parić, A., 2012. Chemical composition and antioxidant and antimicrobial activity of essential oil of *Artemisia annua* L. from Bosnia. *Industrial Crops and Products*, 37(1), pp.479-485 (2012).
17. Giray, E.S., Kırıcı, S., Kaya, D.A., Türk, M., Sönmez, Ö. and Inan, M.,. Comparing the effect of sub-critical water extraction with conventional extraction methods on the chemical composition of Lavandula stoechas. *Talanta*, 74(4), pp.930-935 (2008).
18. Sandra, P. and Bicchi, C.,. Capillary Gas Chromatography in essential oil anylysis. (1987).
19. Spanos, G.A. and Wrolstad, R.E.,. Influence of processing and storage on the phenolic composition of Thompson seedless grape juice. *Journal of agricultural and food chemistry*, 38(7), pp.1565-1571 (1990).
20. Brand-Williams, W., Cuvelier, M.E. and Berset, C.,. Use of a free radical method to evaluate antioxidant activity. *LWT-Food science and Technology*, 28(1), pp.25-30 (1995).
21. Olurinola, P.F., 1996. A laboratory manual of pharmaceutical microbiology. *Idu, Abuja, Nigeria*, 69 pp.1-05. (1996).
22. Clinical and Laboratory Standards Institute (CLSI), Document ILA24-A: Fluorescence Calibration and Quantitative Measurement of Fluorescence Intensity; Approved Guideline. *Wayne, Pennsylvania*, pp.19087-1898(2003).
23. Alley, M.C., Scudiero, D.A., Monks, A., Hursey, M.L., Czerwinski, M.J., Fine, D.L., Abbott, B.J., Mayo, J.G., Shoemaker, R.H. and Boyd, M.R.,. Feasibility of drug screening with panels of human tumor cell lines using a microculture tetrazolium assay. *Cancer research*, 48(3), pp.589-601 (1988)
24. Van de Loosdrecht, A.A., Beelen, R.H.J., Ossenkoppele, G., Broekhoven, M.G. and Langenhuijsen, M.M.A.C.,. A tetrazolium-based colorimetric MTT assay to quantitate human monocyte mediated cytotoxicity against leukemic cells from cell lines and patients with acute myeloid leukemia. *Journal of immunological methods*, 174(1-2), pp.311-320. (1994).
25. Steel, R.G. and Torrie, J.H.,. Principles and procedures of statistics: a biometrical approach. McGraw-Hill (1986).
26. Gonzales, A.P.P.F., Yoshioka, E.T.O., Mathews, P.D., Mertins, O., Chaves, F.C.M., Videira, M.N. and Tavares-Dias, M.,. Anthelminthic efficacy of *Cymbopogon citratus* essential oil (Poaceae) against monogenean parasites of Colossoma macropomum (Serrasalmidae), and blood andhistopathologicaleffects. Aquaculture, 52 8, p.735500 (2020).
27. Hartatie, E.S., Prihartini, I., Widodo, W. and Wahyudi, A., May. Bioactive Compounds of Lemongrass (*Cymbopogon citratus*) essential oil from different parts of the plant and distillation methods as natural antioxidant in broiler meat. *In IOP Conference Series: Materials Science and Engineering*, 532(1), 012018 (2019).
28. Ertaş, A., Gören, A.C., Haşimi, N., Tolan, V. and Kolak, U., 2015. Evaluation of Antioxidant, Cholinesterase Inhibitory and Antimicrobial Properties of *Mentha longifolia* subsp. noeana and Its Secondary Metabolites. *Records of Natural Products*, 9(1), pp.105-115 (2015).
29. Nigam, M., Atanassova, M., Mishra, A.P., Pezzani, R., Devkota, H.P., Plygun, S., Salehi, B., Setzer, W.N. and Sharifi-Rad, J.,

2019. Bioactive compounds and health benefits of Artemisia species. *Natural Product Communications*, 14(7), p.1934578-19850354 (2019).

30. Bouaziz, A., Abdalla, S., Baghiani, A. and Charef, N., Phytochemical analysis, hypotensive effect and antioxidant properties of Myrtus communis L. growing in Algeria. *Asian Pacific Journal of Tropical Biomedicine*, 5(1), pp.19-28 (2015).
31. Wang, C.Y., Chen, Y.W. and Hou, C.Y., Antioxidant and antibacterial activity of seven predominant terpenoids. *International Journal of food properties*, 22(1), pp.230-238 (2019).
32. Singh, P., Kumar, R., Prakash, O., Kumar, M., Pant, A.K., Isidorov, V.A. and Szczepaniak, L., Reinvestigation of chemical composition, pharmacological, antibacterial and fungicidal activity of essential oil from *Mentha longifolia* (L.) Huds. *Research Journal of Phytochemistry*, 11, pp.129-141(2017).
33. Zengin, H. and Baysal, A.H., 2014. Antibacterial and antioxidant activity of essential oil terpenes against pathogenic and spoilage-forming bacteria and cell structure-activity relationships evaluated by SEM microscopy. *Molecules*, 19(11), pp.17773-17798(2014).
34. Farag, R.S., Daw, Z.Y., Hewedi, F.M. and El-Baroty, G.S.A., Antimicrobial activity of some Egyptian spice essential oils. Journal of food protection, 52(9), pp.665-667 *(1989).*
35. Bahri-Sahloul, R., Fredj, R.B., Boughalleb, N., Shriaa, J., Saguem, S., Hilbert, J.L., Trotin, F., Ammar, S., Bouzid, S. and Harzallah-Skhiri, F.,. Phenolic Composition and Antioxidant and Antimicrobial Activities of Extracts Obtained from Crataegus azarolus L. var. aronia (Willd.) Batt. Ovaries Calli. *Journal of Botany*. (2014).
36. Fitsiou, E. and Pappa, A., Anti-cancer activity of essential oils and other extracts from aromatic plants grown in Greece. *Antioxidants*, 8(8), p.290 (2019).
37. Trang, D.T., Hoang, T.K.V., Nguyen, T.T.M., Van Cuong, P., Dang, N.H., Dang, H.D., Nguyen Quang, T. and Dat, N.T., 2020. Essential oils of Lemongrass (*Cymbopogon citratus Stapf*) induces apoptosis and cell cycle arrest in a549 lung cancer cells. *BioMed Research International*, ID 5924856, 8 pages (2020).
38. El-Abid, H., Amaral, C., Cunha, S.C., Augusto, T.V., Fernandes, J.O., Correia-da-Silva, G., Teixeira, N. and Moumni, M., Chemical composition and anti-cancer properties of *Juniperus oxycedrus* L. essential oils on estrogen receptor-positive breast cancer cells. *Journal of Functional Foods*, 59, pp.261-271 (2019).