755

Egypt. J. Chem. **Vol. 65**, No. 11 pp. 595 - 610 (2022)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Chemical Composition of Essential Oils of *Lavandula angustifolia* and *Lavandula hybrida* Cultivated in Egypt and their Biological Activities

Hanem I. Eldeghedy*[1], Abd El-Nasser G. El-Gendy[1], Amr A. Nassrallah[2], Ahmed M. Aboul-Enein[2] and Elsayed A. Omer[1]

[1] *Medicinal and Aromatic Plants Research Department, National Research Centre, 12622, Dokki, Giza, Egypt*

[2] *Biochemistry Department, Faculty of Agriculture, Cairo University, Giza, 12613, Egypt*

Abstract

This research presents a comparative description of the chemical composition and biological activities of the essential oil (EO) of two species of Lamiacea (Labiateae) that belong to Lavandula genus namely Lavandula angustifolia and Lavandula hybrida. They were cultivated in Egypt for their essential oils. Analyses by GC/MS of the main constituents showed camphor as the main component (28.45%) in L. angustifolia followed by eucalyptol (19.08%) and endo-borneol (17.47%), β-Cymene (7.20%), α-pinene (4.16%) and β-pinene (3.79%). L. hybrida essential oil contained eucalyptol as the major component (51.08%) followed by camphor (24.60%) and α-pinene (5.58%) and β-pinene (4.00%). Both oils showed antioxidant activity with the three used methods. Increasing EOs concentrations increased the antioxidant ability up to 32μg/ml compared to ascorbic acid.

The essential oil of both Lavandula species inhibited all screened bacteria with changeable efficacy except Salmonella enterica which did not show any inhibition zones with L. hybrida EO. The results displayed that the EO of L. angustifolia showed moderate antifungal activities against all the strains. At the same time, the EO of L. hybrida showed moderate activity against only two candida strains.

Both essential oils showed a good anti-inflammatory effect with a promising effect concerning L. angustifolia. L. angustifolia represented the best choice since they have low cytotoxicity on RAW cells (mouse macrophage normal cell line). For the cytotoxicity, the most potent essential oil was L. hybrida, but L. angustifolia did not show any activity. L. hybrida oil was effective against HCT116 (Colon Cancer Cell Line) and PACA2 (Pancreas Cancer Cell Line) then against A431 (Skin Cancer Cell Line) and MCF7 (Breast Cancer Cell Line). The study explored that both oils have low cytotoxicity on normal cell BJ1 (Normal Skin Fibroblast). Regarding the selectivity index, the study concluded that L. hybrida exhibited the most potential effect against A431 cell line and the same pattern was shown in PACA2, MCF7 and HCT116 lines.

Keywords: Lamiacea; essential oil; antioxidant; antimicrobial; anti-inflammatory; cytotoxicity; GC-MS.

Introduction

Natural sources, whether they are plants, marine organisms or micro-organisms, remain an essential source of exploration for natural chemical entities that can be used in drug discovery to treat many health problems. Among the most important current health problems are cancer diseases, inflammation, microbial infections and health problems related to oxidation. From this point of view, many plant species are subjected to re-excavation for new biological activities, studying their chemical components and linking their biological effectiveness to their various constituents.

Genus *Lavandula* belongs to *Lamiaceae* family with about 48 species, and it is mostly distributed from the Atlantic Ocean, through the Mediterranean region to Arabia, North-East Africa and India. It also contains many hybrids, and nearly 400 registered cultivars [1]. *L. angustifolia* (lavender) is one of the best-known and economically valued species and is distributed not only in the Mediterranean region but also in Asia, Middle East and Northern Africa [2]. Lavender EO is commonly employed in perfumery

*Corresponding author e-mail: hanemebrahim400@gmail.com.; (Hanem I. Eldeghedy).

Receive Date: 15 August 2022; Revise Date: 30 August 2022; Accept Date: 31 August 2022.

DOI: 10.21608/ejchem.2022.156395.6769

©2019 National Information and Documentation Center (NIDOC).

and cosmetics, food manufacturing and aromatherapy [3-5].

The major constituents of lavender EO were found to be linalool and linalyl acetate [6-12]. While, Jianu [13] mentioned that the major constituents of lavender essential oil were caryophyllene, beta-phellandrene and eucalyptol, *Lavandula intermedia* and *Lavandula angustifolia* Mill. essential oils are rich in linalool, camphor, and 1,8-cineole [14]. Eldeghedy [15] reported that the major constituents of *L. angustifolia* were tau-cadinol, eucalyptol and 1-borneol. While, Cardia [16] reported that the major constituents of *Lavandula angustifolia* essential oil were 1, 8-cineole (39.83%), borneol (22.63%), and camphor (22.12%).

Dakhlaoui [12] showed that the main components of lavender were Linalool (25.83%), Camphor (17.85%), 1,8-Cineole (16.91%), Endo-borneol (12.79%), Linalyl acetate (7.97%) and Caryophyllene oxide (5.63%). Verma [17] stated that the major constituents of *L. angustifolia* essential oil were linalyl acetate (47.56%), linalool (28.06 %), lavandulyl acetate (4.34 %), α-terpineol (3.75 %), geranyl acetate (1.94%), caryophyllene oxide (1.38%) and 1,8-cineole (1.14 %). While the minor components were β-caryophyllene (0.93%), borneol (0.85 %), epi-α-cadinol (0.70%), nerol (0.59%), terpinen-4-ol (0.56%), β-myrcene (0.55%), limonene (0.55%) and 1-octen-3-ol (0.53%).

L. angustifolia Mill. essential oil had anti-inflammatory activity that includes NO, pro-inflammatory cytokines, and histamine [16]. Lavender essential oil had high antioxidant (IC_{50}DPPH = 48.53 µg/ml and IC_{50}ABTS = 195.84 µg/ml), anticancer (IP=54.07%) and anti-inflammatory (IC_{50} = 64.73 µg/ml) activities [12]. Also, Fathima [18] showed that *L. angustifolia* essential oil had antibacterial activity against *E. coli*, *P. saerogenosa*, *E. faecalis* and *S. aureus* and inhibition zone of these bacteria were 24, 23, 22 and 19mm, respectively.

Lavender essential oil showed antioxidant, anti-inflammatory, analgesic [11], antimicrobial, antifungal and cyto/genotoxic effects [19]. Miastkowska [20] indicated that lavender oil has a strong potential to improve the local, tissue derived pro-inflammatory and pro-regenerative response. *E. coli* was the most sensitive organism among *E. coli, S. aureus, Listeria innocua* to the inhibition effect of lavender oils [8]. Lavender essential oil increased HSP70 expression in LPS-stimulated THP-1 cells, suggesting that the LEO inhibited LPS-induced inflammatory effect might be associated with the expression of HSP70 [21]. Lavender showed good antibacterial activity against *B. subtilis*, *P. fluorescens*, *Xanthomonas campestris*, *Erwinia carotovora* at 300 µg/mL concentration, and *Erwinia amylovora*, *Candida utilis* at 150µg/mL concentration, respectively [22]. Lavender (*L. angustifolia*) essential oil had antimicrobial activity and the highest DPPH radical scavenging activity [23]. Sienkiewicz [7] reported that lavender essential oil had antimicrobial against *Acinetobacter baumannii* and MIC was 10.5-13.0 µL/mL and the IC_{50} of lavender against HMEC-1 and T98G cells being 5.15µl/ml and 2.27µl/ml

Lavandin (*Lavandula hybrida* E.Rev. ex Briq.) is a much larger plant than the *L. angustifolia* and is much appreciated for its EO yield [14]. *L. hybrida* essential oil is readily available in many areas of the world by the fragrance industry and is a common ingredient in soaps, laundry detergents, skin care, perfumes, and cleaning products [24]. *L. hybrida* is used in soaps, perfumes, and washing agents, but is also used as a flavor for food and beverages [4]. GC–MS analysis of the essential oil showed the presence of 26 compounds, of which 89.2% were monoterpenoids and 3.1% were sesquiterpenoids. The most abundant components in the essential oil of *L. hybrida* were linalool (41.6%) and linalyl acetate (23.0%), followed by 1,8-cineole (5.2%) and terpinen-4-ol (4.8%), while lavandulyl acetate (3.2%) and borneol (2.8%) were identified as minor compounds [14]. Bajalan [25] analyzed the chemical composition of *L. hybrida* and the main components were 1,8-cineole (31.64 – 47.94%), borneol (17.11 – 26.14%), and camphor (8.41 – 12.68%). Blažeković [9] mentioned that lavandin essential oil contained linalool (57.1%), linalyl acetate (9.8%) and 1,8-cineole (8.4%). While, Kıvrak [10] found the main compound of *L. hybrida* essential oil was eucalyptol. Eldeghedy [15] reported that the major constituents of *L. hybrida* were eucalyptol and camphor.

Garzoli [14] showed that *L. hybrida* essential oil had antimicrobial activity against gram-positive (*B. cereus* and *Kocuria marina*) and gram-negative bacteria (*E. coli*, *Acinetobacter bohemicus*, and *P. fluorescens*). Varona [26] mentioned that *L. hybrida* had components with biocide and antiviral properties that could be used as antibiotics. *L. hybrida* essential oil had antimicrobial activity against *E. coli*, *S.*

aureus and *B. cereus*. Bajalan [25] mentioned that *L. hybrida* essential oil illustrated antimicrobial activity against *S. agalactiae*, *S. aureus*, *E. coli*, and *K. pneumoniae*. Blažeković [9] mentioned that *L. hybrida* essential oil had antimicrobial, antifungal and antioxidant activities effective more than *L. angustifolia* and this effect is due to linalool. Kıvrak [10] indicated that the essential oil of *L. hybrida* had the highest value of inhibition in DPPH and ABTS. Furthermore, *Lavandula intermedia* and *L. angustifolia* essential oils, rich in specific constituents such as linalool, camphor, and 1,8-cineole, possessed antibacterial activities against *Listeria monocytogenes*, especially against isolates from a clinical environment [14]. A re-investigation of traditional medicines for the treatment of cancer, inflammation and infectious disease is an attractive vision as the antiseptic qualities of medicinal plants have also been long documented and recorded. Furthermore, there has recently been a revival of concentration in herbal medications due to understanding that there is a lower incidence of adverse reactions to plant preparations compared to synthetic pharmaceuticals. Antimicrobial natural products with high antioxidant contents are particularly attractive as they may treat the symptoms of inflammation as well as block the microbial trigger and thus have pluripotent effects.

The current study was undertaken to explore the main constituents of the essential oil of *L. angustifolia* and *L. hybrida* using GC-MS. In addition, their antioxidant, anti-inflammatory, anticancer and antimicrobial activities were evaluated.

1. Materials and Methods:

1.1. Cultivation and essential oil extraction:

The seeds (to cultivation) of *Lavandula angustifolia* (Lavender), *Lavandula hybrida* (lavandin) were imported from Pharmasaat-Seeds and Plants of Medicinal and Spice Herbs Company and propagated in Elmizan Company. The established seedlings were transplanted in the open field of the Experimental Farm, Sekem Company, El-Adlya Belbeis, EL-Sharkiya Governorate, Egypt (30°35'15.65" N and 31°30'7.20" E) according to Eldeghedy [15] , and herbarium specimens were kept in the NRC Herbarium under the Nos. *L. angustifolia* M138, *L. hybrida* M139. A weight of 10 kg of fresh aerial parts (leaves and branches) from each species was collected in May, 2021. The fresh aerial parts of the plant materials were shade dried which gave 3.2 kg of air-dried materials then cuted into smaller pieces and kept in the dark for extraction of the essential oil. Air dried samples of *Lavandula angustifolia* and *Lavandula hybrida* were separately subjected for hydro-distillation for 3 hours at Clevenger-type apparatus for 3h in order to extract the essential oils according to the Egyptian Pharmacopoeia [27] and Günter [28]. The extracted essential oil of each plant species was separately dehydrated with anhydrous sodium sulphate and kept under conditions of refrigeration for GC-MS and biological activities analyses.

1.2. GC-MS analysis:

In order to determine and identify the main constituents of each essential oil, samples from each oil were subjected for GC-MS analysis using gas chromatography-mass spectrometry instrument of Medicinal and Aromatic Plants Research Department, National Research Centre following the conditions and the specifications of the instrument and used methods mentioned before by Omer [29] and Eldeghedy [15].

1.3. The Biological activities:

1.3.1. Antioxidant capacity determination:

The DPPH free radical scavenging method [30], with modifications as mentioned in Omer [29] was used to assay antioxidant capacity of each essential oil samples.

1.3.1.1. DPPH method:

The DPPH free radical scavenging method [30], with modifications as mentioned in Omer [29] was used to assay antioxidant capacity of each essential oil samples.

1.3.1.2. Ferrous Ion Chelating (FIC) Ability:

The FIC assay was performed using Singh and Rajini's [31] method, with some modifications [31]. 20 times, solutions of 2mM $FeCl_2.4H_2O$ and 5mM ferrozine were diluted. In brief, a solution (1ml) of various antioxidant concentrations (4, 8, 16, and 32mg/ml) was mixed with 1 ml of $FeCl_2.4H_2O$. The reaction was started after 5 minutes of incubation by adding ferrozine (1ml). After vigorous shaking the mixture for 10 minutes, the absorbance of the solution was measured spectrophotometrically at 562 nm. The percentage of inhibition of ferrozine-Fe^{+2}

complex formation was calculated using the following formula:

Chelating effect (percent) = $[(1-A_S)/A_B] \times 100$

Where A_S is the absorbance of the tested sample and A_B is the absorbance of the control sample (which contains $FeCl_2$ and ferrozine)

1.3.1.3. ABTS radical scavenging activity:

The radical scavenging potential of the samples of studied essential oils was determined using a modified method described by Floegel 32. In brief, 700 µl ABTS solution were added to 300µl mixture of different concentrations (4, 8, 16, and 32mg/ml) of each sample. The mixture was then kept to react in the dark at 37°C for 10 minutes. At 734 nm, the absorbance was measured, Each assay was carried out in triplicate and the ABTS radical scavenging percent was calculated using the equation:

% Inhibition = $[(A0 – A1) / A0] \times 100$

(A0 is the $ABTS^{•+}$ absorbance of the control reaction, A1: is the ABTS absorbance of the sample).

1.3.2. Antimicrobial assays of EOs

1.3.2.1. Bacterial and fungal strains:

The reference bacterial strains including *Proteus vulgaris* (ATTC 13315), *Escherichia coli* (ATCC 35218), *Staphylococcus aureus* (ATCC 25923) and *Salmonella enterica* were acquired from the culture collection of the Department of Microbiology and Immunology, National Research Centre, Cairo, Egypt. In addition, a resistant strain of *S. aureus* to most of antibiotics including vancomycin 30µg, oxacillin 5 µg, amoxycillin 10µg, erythromycin 15µg, streptomycin 10µg, cefuroxime sodium 30µg, trimethoprim/ sulphamethozole 25µg, gentamycin 120µg, and rifampicin 5µg were used. Cefoxitin 30µg, cefotaxime 30µg, and colistin sulphate 10µg had a bacteriostatic effect against this strain.

Candida strains (*C. albicans, C. glabrata, C. tropicalis,* and *C. krusei*) were isolated primarily using sabouraud dextrose agar (SDA, Oxoid, UK) and single pure colonies were identified by culturing on rice agar medium with 1% tween 80, germ-tube test for *C. albicans* [33]. Accurate identification and differentiation of *Candida* isolates were achieved with CHROM agar medium, API 20°C Aux and multiplex-PCR [34-36]. All the used fungal strains were isolated according to Khalaf [37] from the culture collection of the Department of Microbiology and Immunology, National Research Centre, Cairo, Egypt.

1.3.2.2. Agar disc diffusion assay:

The extracted EOs was evaluated against bacterial and fungal strains using agar disc diffusion assay [38]. For *S. aureus*, vancomycin (30µg) was used as a positive control, ciprofloxacin 5µg for other bacteria, fluconazole 25µg for *C. albicans*, and DMSO as a negative control. The plates were kept at 37°C for 24 hours (for bacteria) and 28°C for 48-72 hours (for fungi), after which the diameter of the inhibition zone was measured in mm.

1.3.3. Anti-inflammatory activity (nitric oxide assay):

1.3.3.1. Cell line and cell culture:

The American Type Culture Collection was purchased as the source of the murine macrophage cell line (RAW 264.7). USA-sourced cells (ATCC) were grown in DMEM supplemented with 10% FBS, 100U/ml penicillin, 100g/ml streptomycin, and 250g/ml amphotericin B at 37°C in a 5% CO_2 incubator.

1.3.3.2. Anti-inflammatory activity assay:

To study the anti-inflammatory activity of the essential oil of both plant species, the NO generation in LPS-stimulated RAW 264.7 cells was tested.

In order to determine NO, RAW 246.7 cells were planted in 96-well plates at a density of 10×10^3 cells/well and allowed to develop for 24 hours to measure adherence. The test samples were applied to the cells for one hour, and they were subsequently cultured for 24 hours in new DMEM with 10µg/ml LPS. According to the Griess reaction, the amount of nitrite in the culture media was assessed as a sign of NO production [39]. In a 96-well plate, 100µl of cell culture supernatant was combined with 100µl of Griess reagent for the reaction, and the absorbance at 540nm was recorded using an ELISA reader.

1.3.3.3. Cell culture (seeding and treatment):

The cells of RAW 264.7 macrophage cell line were grown in RPMI1640 media (Roswell Park Memorial Institute) supplemented with 10% heat-inactivated fetal bovine serum and 1% pen/strep. The cells underwent two subcultures in a humidified incubator

with a 5% CO_2 environment at 37°C before the experiment.

1.3.3.4. Procedure:

The following processes were all performed in a biosafety class II level Laminar Flow Cabinet in a sterile environment (Baker, SG403INT, and Sanford, ME, USA). RAW 264.7 cells were suspended in RPMI media and 1×10^5 cells were seeded into each well of 96-well plates and then allowed to grow for 24 hours before being used in studies. The EOs samples were added to the cells at concentrations of 100, 50, 25 and 12.5 g/ml, and they were left to react for an hour. They were then stimulated for an additional 24 hours with 10 g/ml of LPS. The supernatant was smoothly transferred to fresh 96-well plates in order to determine NO.

1.3.3.5. Nitric oxide assay:

By measuring nitrite in the supernatants of cultivated RAW 264.7 cells, nitric oxide production was evaluated. The assay was performed mostly in accordance with the prior description [40]. Using the Griess reagent, the amount of nitrite, a stable metabolite of NO used as an indication of NO generation, in the culture medium was determined after pre-incubating RAW 264.7 cells (1×10^5 cells/ml) with LPS (10µg/ml) for 24 hours (1 percent sulfanilamide and 0.1 percent naphthyl ethylene diamine dihydrochloride in 2.5% phosphoric acid). 50µL of the Griess reagent were combined with 50µl of the cell culture medium. The mixture was then let to sit for 15 min at room temperature, and then the absorbance at 540nm was determined using a microplate reader. In each experiment, the fresh culture medium was used as a blank. The amount of nitrite was calculated from a sodium nitrite standard curve as expressed in the equation:

Nitric Oxide inhibition (%) = (Control −Test) ×100 / Control

1.3.4. Cytotoxic effect on six human cell lines:

The human cell lines that used:

Prostate Cancer (PC3), Pancreatic Cancer(PACA2), Epidermoid Carcinoma (A431), Lung Cancer (A549), Breast Cancer (MCF7), Colon Cancer (HCT116), and Normal Skin Fibroblast (BJ1).

Cell viability was measured as mentioned by to Mosmann [41] by converting yellow MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) to purple formazan in a mitochondrial-dependent reaction.

1.3.4.1. Procedures:

All operations were carried out in a biosafety class II level Laminar Flow Cabinet under a sterile environment (Baker, SG403INT, Sanford, ME, USA). Cells were suspended in RPMI 1640 medium (DMEM for PACA2, A549, PC3, and BJ1), 1% L-glutamine, and 1% antibiotic antimycotic mixture (10,000U/ml Potassium Penicillin, 10,000g Streptomycin Sulfate, and 25µg Amphotericin B). Cells were batch grown for 10 days before being planted at a concentration of $10x10^3$ cells per well in original complete growth media in 96-well microtiter plastic plates for 24 h at 37 ºC under 5% CO_2 using water jacketed carbon dioxide incubator, (Sheldon, TC2323, Cornelius, OR, USA). Cells were incubated either alone (negative control) or with various sample concentrations to give a final concentration of (100-50-25-12.5-6.25-3.125-0.78 and 1.56 µg/ml). Media was aspirated after 48 hours of incubation, 40 µl of MTT salt (2.5 g/ml) was added to each well, and the plate then incubated for a further four hours at 37ºC with 5% CO_2. A volume of 200 µl of 10% sodium dodecyl sulphate (SDS) in deionized water was added to each well and incubated overnight at 37°C to stop the reaction and dissolve the crystals that had formed. A known cytotoxic natural reagent at a concentration of 100 µg/ml was utilized as a positive control since it causes 100% mortality under identical circumstances [42,43]. The absorbance was then measured at 595nm with a reference wavelength of 620nm using a microplate multi-well reader (Bio-Rad Laboratories Inc., model 3350, Hercules, California, USA). The statistical significances were calculated between samples and negative control (cells with the vehicle) using an independent t-test with SPSS 11 program. For dissolution of plant extracts, DMSO was used and its final concentration on the cells was not exceeding than 0.2%. The percentage of change in viability was calculated according to the formula:

% Viability = (Reading of extract / Reading of negative control) -1) x 100

An analysis of probability was carried for IC_{50} determination using SPSS 11 program. The concentration of the samples ranged between (0.78 to 100µg/ml)

2. Results and Discussion:

2.1. The percentage of essential oils :

Essential oil % of *L. angustifolia*in in the airdried herb (leaves and branshes) was 0.6% (v/w), while essential oil % of *L. hybrida* in airdries herb was 2.7% (v/w).

2.2. GC-MS analyses:

The main components of *L. angustifolia* and *L. hybrida* essential oil (EO) analyzed by GC-MS were shown in Table (1). Twenty-nine compounds that represent a total of 99.71% of the compounds annotated have been characterized in the EO of *L. angustifolia*. The ratio of oxygenated compounds is 79.99%, while non-oxygenated compounds represent 19.74% from the identified compounds. In the same time, monoterpene compounds signified 96.97%, while sesquiterpenes corresponded to 2.76% of the identified compounds. The main constituent was reecognised as cmphor that a ratio is up to 28.45% followed by eucalyptol (19.08%), endo-borneol (17.47%), *β*-Cymene (7.20%), *α*-pinene (4.16%) and *β*-pinene (3.79%). These results are in agreement with those of Dakhlaoui [12] and Cardia [16] who found 1,8-Cineole, endo-borneol among the four main compounds in the EO of *L. angustifolia*. Eldeghedy [15] identified the main constituents of *L. angustifolia* EO cultivated in Egypt as tau-cadinol (28.63%), eucalyptol (17.21%) and 1-borneol (12.01%) which agreed with Jianu [13]. In the contrast of these results [5,6,8-12,17], linalool and linalyl acetate were detected as the major constituents of lavender EO.

As shown in Table (1), twenty-nine compounds that represented a total of 99.27% have been identified from essential of *Lavandula hybrida*. The ratio of oxygenated compounds was 85.08%, while non-oxygenated compounds represent 14.26% of the identified compounds. At the same time, monoterpene compounds represented 97.38%, while sesquiterpenes corresponded to 1.96%. The main constituent was eucalyptol that a ratio is up to 51.08% followed by camphor (24.60%), *α*-pinene (5.58%) and *β*-pinene (4.00%).

These results are in accordance with Bajalan [25] who mentioned that 1,8-cineole, borneol, and camphor were the main constituents of *L. hybrida* EO, but Garzoli [14] showed that the major components of *L. hybrida* EO were linalool and linalyl acetate, followed by 1,8-cineole and terpinen-4-ol. These results are close to those of Kıvrak [10] who showed that linalool (28.486%) as the main component followed by eucalyptol (15.650%), *β*-pinene (8.8%), D- germacrene (5.4%), bicyclo-germacrene (2.7%), and (E)-*β*- caryophyllene (2.6%), respectively. Eldeghedy [15] reported eucalyptol and camphor as the major constituents of the EO of *L. hybrida* cultivated in Egypt. Carrasco [44] stated linalool, linalyl acetate, camphor and 1,8- cineol as the predominant metabolites.

2.3. The biological activities

2.3.1. Antioxidant Activity:

The essential oils have a chemical diversity since they are frequently a mixture of chemical classes with varying functional groups, polarity, and chemical actions, may result in disparate results depending on the test used. As a result, a strategy involving multiple assays in the screening effort is ideal [45]. The antioxidant activity can be determined using various methods depending on the sources of free radicals, which work through different mechanisms [46]. It is extremely difficult to evaluate a product's antioxidant activity using a single method. A single method will provide basic information about antioxidant properties, but a combination of methods will describe the antioxidant properties of the investigated natural product in greater detail [47-49]. The antioxidant activities of the essential oils of *L. angustifolia* and *L. hybrida* were determined with three recommended methods; DPPH, FIC and ABTS and the results are shown in Table (3).

2.3.1.1. DPPH Method:

IC_{50} that expressed as the concentration capable of scavenging 50% of the DPPH radical was 19.5 for *L. angustifolia* essential oil and 27.8 for *L. hybrida* essential oil. The radical-scavenging effect of *L. angustifolia* EO (71.16 ± 0.88) at 32 µg/ml and *L. hybrida* EO (56.19 ± 3.00) at 32µg/ml were lower than those of ascorbic acid (89.85 ± 0.30). The values of IC_{50} for DPPH radical scavenging were in the order of: ascorbic acid< *L. angustifolia* < *L. hybrida*.

Table 1. The main identified constituents in the essential oil of *Lavandula angustifolia* and *Lavandula hybrida* as resulted from GC-MS analyses

Compound	RT (min)	Formula	M.W	M/Z	KI_{CAL}	KI_{LIT}	% of *Lavandula angustifolia*	% of *Lavandula hybrida*
Tricyclene	3.71	$C_{10}H_{16}$	136	67, 79, 93, 121	923	926	0.40±0.05	-----
α-pinene	3.91	$C_{10}H_{16}$	136	67, 77, 93, 105, 136	933	939	4.16±0.2	5.58±0.03
Camphene	4.29	$C_{10}H_{16}$	136	67, 79, 93, 107, 121, 136	952	953	2.02±0.19	1.15±0.09
2,4(10)-thujadiene	4.39	$C_{10}H_{14}$	134	65, 91, 105, 103, 119	957	959	0.28±0.07	-----
p-Cymene	4.82	$C_{10}H_{14}$	134	65, 77, 91, 103, 119, 134	976	1020	0.52±0.17	-----
β-Pinene	4.93	$C_{10}H_{16}$	136	53, 69, 93, 107, 121	980	980	3.79±0.20	4.00±0.21
Sabinene	5.05	$C_{10}H_{16}$	136	53, 77, 93, 121, 136	985	976	-----	0.77±0.07
2,3- Dehydro-1,8-cineole	5.29	$C_{10}H_{16}O$	152	67, 79, 94, 109, 124	995	991	0.75±0.09	0.23±0.03
β-Myrcene	5.47	$C_{10}H_{16}$	136	53, 69, 79, 93, 121	1002	991	-----	0.66±0.06
o- Cymene	6.11	$C_{10}H_{14}$	134	51, 77, 91, 119, 134	1026	1026	1.37±0.30	-----
β-Cymene	6.27	$C_{10}H_{14}$	134	77, 91, 103, 119, 134	1032	1020	7.20±0.2	-----
D-Limonene	6.31	$C_{10}H_{16}$	136	53, 68, 79, 93, 107, 136	1034	1024	-----	0.47±0.15
Eucalyptol	6.49	$C_{10}H_{18}O$	154	55, 71, 81, 93, 108, 125, 139	1039	1033	19.08±1.33	51.08±1.17
trans-α-Ocimene	6.95	$C_{10}H_{16}$	136	67, 79, 93, 105, 121	1055	1050	-----	0.51±0.09
ç-Terpinene	7.23	$C_{10}H_{16}$	136	65, 77, 93, 105, 136	1063	1054	0.32±0.06	0.24±0.02
cis-á-Terpineol	7.83	$C_{10}H_{18}O$	154	71, 81, 93, 111, 121	1081	1070	0.44±0.06	0.43±0.02
Fenchone	8.95	$C_{10}H_{16}O$	152	53, 69, 81, 109, 152	1113	1083	-----	0.77±0.07
Linalool	9.72	$C_{10}H_{18}O$	154	55, 71, 80, 93, 121	1135	1098	-----	0.62±0.04
α-Campholenal	9.92	$C_{10}H_{16}O$	152	53, 67, 79, 93, 107	1141	1126	0.54±0.08	0.24±0.08
L-Pinocarveol	10.43	$C_{10}H_{16}O$	152	55, 70, 92, 109, 119	1154	1139	0.72±0.05	-----
(+)-Nopinone	10.57	$C_{9}H_{14}O$	138	55, 67, 83, 95, 109	1158	1140	0.29±0.05	0.15±0.03
Camphor	10.76	$C_{10}H_{16}O$	152	69, 81, 95, 108, 152	1163	1146	28.45±0.97	24.60±0.97
cis-Verbenol	11.67	$C_{10}H_{16}O$	152	6, 81, 91, 109, 119	1185	1147	-----	0.26±0.04
Pinocarvone	11.33	$C_{10}H_{14}O$	150	53, 69, 81, 108, 135	1177	1162	0.27±0.03	0.38±0.04
endo-Borneol	11.73	$C_{10}H_{18}O$	154	59.1, 81.1, 95.1, 110.2, 136.2	1186	1165	17.47±1.13	2.05±0.11
Terpinen-4-ol	11.94	$C_{10}H_{18}O$	154	55.1, 71.0, 93.1, 111.1, 136.1	1191	1177	0.76±0.12	-----
p-Cymen-8-ol	12.29	$C_{10}H_{14}O$	150	65, 77, 91, 115, 135, 150	1199	1183	0.56±0.09	-----
Crypton	12.52	$C_{9}H_{14}O$	138	67, 81, 96, 10, 138	1205	1188	1.29±0.16	-----

Myrtenal	12.70	$C_{10}H_{14}O$	150	59, 79, 91, 121, 135	1210	1193	2.23±0.14	0.65±0.25
Eucarvone	13.42	$C_{10}H_{14}O$	150	65, 79, 91, 107, 135, 150	1229	1201	1.74±0.11	0.28±0.10
Isobornyl formate	13.76	$C_{12}H_{20}O_2$	182	67, 95, 109, 136, 152	1238	1285	0.40±0.0.05	0.14±0.02
α-Terpineol	13.89	$C_{10}H_{18}O$	154	59, 79, 93, 121, 136	1241	1207	-----	0.62±0.05
Verbenone	14.01	$C_{10}H_{14}O$	150	79, 91, 107, 135, 150	1244	1204	-----	0.41±0.03
p-Cumic aldehyde	14.78	$C_{10}H_{12}O$	148	82, 91, 105, 119, 133, 148	1263	1239	1.08±0.09	-----
p-Menth-1-en-3-one	15.19	$C_{10}H_{18}O$	152	67, 82, 95, 110, 137, 152	1272		0.30±0.03	----
carvone	15.72	$C_{10}H_{14}O$	150	54, 67, 82, 93, 108	1284	1242	----	0.28±0.10
Caryophyllene	22.15	$C_{15}H_{24}$	204	69, 79, 91, 105, 120, 133	1442	1454	----	0.30±0.03
ç-Cadinene	25.01	$C_{15}H_{24}$	204	79, 91, 105, 119, 133, 161	1512	1513	0.40±0.03	-----
δ-EIemene	26.00	$C_{15}H_{24}$	204	67, 93, 105, 121, 136	1539	1535	-----	0.88±0.05
Caryophyllene oxide	27.82	$C_{15}H_{24}O$	220	69, 79, 91, 105, 121, 135	1585	1581	1.04±0.09	0.87±0.06
tau-Cadinol	30.24	$C_{15}H_{26}O$	222	81, 105, 119, 161, 204	1651	1640	1.32±0.09	0.57±0.10
tau-Muurolol	32.48	$C_{15}H_{26}O$	222	55, 79, 95, 105, 121	1611	1642	-----	0.13±0.03
6-epi-shyobunol	33.86	$C_{15}H_{26}O$	222	55, 67, 84, 93, 121	1751	1689	-----	0.32±0.06
Oxygenated compounds	-----	-----	-----	-----	-----	-----	79.99	85.08
Non-Oxygenated compounds	-----	-----	-----	-----	-----	-----	19.74	14.26
Monoterpenes	-----	-----	-----	-----	-----	-----	96.97	97.38
sesquiterpenes	-----	-----	-----	-----	-----	-----	2.76	1.96
Total of identified compounds	-----	-----	-----	-----	-----	-----	99.71	99.34

2.3.1.2. FIC Method:

Metal chelation may provide significant anti-oxidative benefits by delaying metal-catalyzed oxidation. Analysis of metal ion-chelating properties using the FIC method revealed that both of the EOs were capable of chelating iron (II) and did so in a concentration-dependent manner (Table 2). The EOs of *L. angustifolia* and *L. hybrida* were quite similar to ascorbic acid in chelating of iron (II). The highest concentration of *L. angustifolia* EO showed the highest iron-chelating ability (53.90% at 32 μg/ml). This activity was followed by the *L. hybrida* (53.27 at 32μg/ml). The values of IC_{50} of FIC radical were 14.3 for *L. angustifolia* oil and 16.0 for *L. hybrida* oil, while was 8.04 for ascorbic acid. In other words, the values of IC_{50} were in the order of: ascorbic acid< *L. angustifolia*< *L. hybrida*.

2.3.1.3. ABTS Method:

L. angustifolia EO showed the highest antioxidant inhibition (90.67% at 32 μg) compared to EO of *L. hybrida* and ascorbic acid (Table 2), with no statistically significant differences with positive control (ascorbic acid), followed by *L. hybrida* EO (86.00 % at 32μg/ml). The IC_{50} values ranged from 0.01 for *L. angustifolia* to 1.78μg for *L. hybrida* and were in the order of: *L. angustifolia*< ascorbic acid < *L. hybrida.*

The antioxidant activity of these essential oils may be attributed to the high contents of oxygenated compounds mainly Eucalyptol, camphor and endo-Borneol. In the same manner, Carrasco [44] reported that *L. hybrida* essential oil had antioxidant activity due to linalool and linalyl acetate while anti-inflammatory activity was due to linalool and camphor. The antioxidant capacity of essential oils is a biological property of great concern since they may be used to preserve foods from the hazard effects of oxidants [50].

Table 2. Antioxidant activities of *Lavandula angustifolia* and *Lavandula hybrida* essential oils measured by DPPH, Metal-chelating (Ferrozine) and ABTS assay

Essential oil	DPPH % inhibition				IC50[a]
	4µg/ml	8µg/ml	16µg/ml	32µg/ml	
L. angustifolia	22.91± 0.84	31.08 ± 1.09	44.26± 3.50	71.16± 0.88	19.50
L. hybrida	9.79±0.59	18.36±1.00	32.30± 3.80	56.19± 3.00	27.80
Ascorbic acid	8.562±2.090	25.372±0.847	58.105±1.794	89.846±0.296	16.6
FIC % inhibition					
L. angustifolia	44.93±1.06	46.80±1.42	51.64±0.19	53.90±1.01	14.30
L. hybrida	43.05±0.90	47.72±0.38	51.64±0.85	53.27±0.62	16.00
Ascorbic acid	44.86±3.17	50.50±1.12	52.01±0.75	54.00±0.28	8.04
ABTS % inhibition					
L. angustifolia	81.90 ±1.21	86.48± 0.54	87.71± 0.38	90.67± 0.79	0.01
L. hybrida	60.29 ± 1.29	63.24± 1.39	77.90± 3	86.00 ± 1.57	1.78
Ascorbic acid	85.95±1.94	94.86±0.15	95.00±0.25	95.38±0.08	0.1653

[a] Concentration (µg/ml) for 50% inhibition for DPPH, (µg/ml) for a 50% chelating effect for FIC and (µg/ml) for 50% inhibition for ABTS

The essential oils being also able of scavenging free radicals and play a significant role in some diseases prevention such as brain dysfunction, cancer, heart diseases and immune system decline. Increasing evidences has projected that these diseases may result from cellular damage caused by free radicals [51,52].

These results are in good agreement with the antioxidant capacity of essential oils reported by Saleh [53]. 17 species belonging to the *Lamiaceae* family possessed effective antioxidant activity. The active compounds detected by DPPH/TLC method included phenols, non-phenols and oxygenated or non-oxygenated compounds. In terms of free radical-scavenging activity, *L. angustifolia* essential oil had the highest activity; however, it did not have the highest ABTS-scavenging effect. Although linalool and linalyl acetate were the major components of this essential oil, limonene had similar activity to *L. angustifolia* in the DPPH method. The predominant limonene in *Citrus x limon* (L.) oil did not correspond to the best activity [54].

2.3.2. Antimicrobial Activity:

The antibacterial potentialities of the EOs of *L. angustifolia* and *L. hybrida* were assayed using agar disc diffusion protocol and the results are summarized in Table (3) including inhibition zones (IZ), and minimum inhibition concentration (MIC). The antibacterial properties are the resultant of the major and minor components in the essential oil [13]. The data showed that EOs inhibited all screened bacteria with changeable efficacy except *Salmonella enterica* that did not show any inhibition zones with *L. hybrida* EO. *Lavandula hybrida* displayed similar results with a moderate activity against *E. coli* (ATCC 35218) with IZ: 20mm, and *S. aureus* (ATCC 25923) with IZ: 13 mm and a low antibacterial effect on *P. vulgaris* (ATCC 13315) with IZ: 8mm and *S. aureus* (resis.) with IZ: 7 mm and did not show any effect on *S. enterica*. Otherwise, *L. angustifolia* EO showed a strong antibacterial activity against *P. vulgaris* (ATCC 13315) (IZ: 13mm), *E. coli* (ATCC 35218) (IZ:

12mm), *S. aureus* (ATCC 25923) (IZ: 13 mm), *S. aureus* (resis.) (IZ: 12 mm) and *S. enterica* (IZ: 8mm).

Table 3. Inhibition zone (IZ mm) and MIC µg/ml of *Lavandula angustifolia* and *Lavandula hybrida* essential oils against different strains of bacteria

Essential oils / strains	*L. angustifolia*		*L. hybrida*		VA 30 µg (IZ mm)	CIP 5 µg (IZ mm)	Am 10 µg (IZ mm)
	IZ mm	MIC µg/ml	IZ mm	MIC µg/ml			
***P. vulgaris* (ATCC 13315) -**	13±0.25	10	8±0.15	10	NA	30	13
***E. coli* (ATCC 35218) +**	12±0.15	5	20±0.15	5	NA	30	NA
***S. aureus* (ATCC 25923) -**	13±0.15	10	13±0.20	10	15	NA	NA
***S. aureus* (resis.) -**	12±0.22	10	7±0.20	10	Resis. *	NA	NA
***S. enterica* +**	8±0.10	30	NA	NA	NA	NA	10

*Resis.: resistant, ** Values are the average (n = 3) of the inhibition zone diameter (mm) ± standard deviation, b minimum inhibitory concentrations, MIC: maximum inhibition (no growth at all), VA: Vancomycin, CIP: Ciprofloxacin, AM: amoxycillin, NA: no activity.

The selected antibiotics showed a varied activity against the bacterial strains. Vancomycin with 30µg showed an activity against *S. aureus* (ATCC 25923) (ZI: 15mm), while it was inactive against *S. aureus* (resis.). The ciprofloxacin 5µg exhibited the maximum inhibition on *S. entrica*, but did not show any activity against *P. vulgaris* (ATCC 13315) and *E. coli* (ATCC 35218) (IZ 30 mm). On the other hand, amoxycillin at 10µg was active against *S. aureus*, *P. vulgaris* (ATCC 13315) (IZ: 13mm) and *S. entrica* (IZ: 10 mm).

The antifungal activities of *L. angustifolia and L. hybrida* were examined and the results as inhibition zone (IZ mm) and their MIC (µg/ml) are listed in Table (4). The results displayed that *L. angustifolia* EO had a moderate antifungal activity against all the strains with IZs ranging from 12-16mm compared to fluconazole 25 µg. The *L. angustifolia* EO exhibited antifungal activity against the five *Candida* strains as *C. krusei* and *C. tropicalis* (IZ: 16mm for both), *C. albicans* ((IZ: 15 mm), *C. glabrata* and *C. albicans* (ATCC 10231) (IZ: 12mm for both). *C. albicans* and *C. glabrata* were the most sensitive strains for *L. angustifolia* EO at MIC of 2.5µg/ml followed by *C. albicans, C. krusei* and *C. tropicalis* (MIC: 5µg/ml).

Lavandula hybrida EO didn't show any effect against *C. glabrata, C. albicans* and *C. tropicalis*, but inhibited the growth of *C. krusei* and *C. albicans* (ATCC 10231) with IZ of 12 and 9 mm, respectively. All these data were evaluated with the respecting of the reference drug (fluconazole 25µg) with inhibition zones ranging from 18 to 26µg/ml.

Generally, the results indicated that the bioactivity properties of the essential oils are related to the synergistic effects of its diverse major and minor components as mentioned by You [55]. The mechanism of anti-*candida* action of the essential oil could possibly be due to an increase in yeast membrane permeability and disrupting the normal membrane transport by affecting membrane ATPase [56].

2.3.3. Anti-inflammatory Activity:

If essential oils can scavenge some free radicals, they can also act as an anti-inflammatory agents, because the oxidative burst that occurs in various cells (monocytes, neutronphils, eosinophils, and macrophages) is one of the inflammatory responses, as mentioned by Huang [46]. The effects of EOs on NO production in RAW 264.7 cells were measured to evaluate EOs anti-inflammatory activity. As well known, NO is a small molecule that play a part in signaling involved in a wide range of pathophysiological processes, particularly a series of processes related to inflammation [57]. When an inflammatory stimulus starts, the production of NO elevated, that intervene the pro-inflammatory effect. However, increase in NO in the cells can be injurious

and can lead to several inflammatory diseases [58]. Therefore, the study of the effect of EOs on NO production is being used as a research method to confirm its ability to regulate inflammation. Nitric oxide is a commonly parameter used as a marker for macrophages that are activated by inducing lipopolysaccharide. Application of the different essential oils at the rate of 100, 50µg/ml after the application of LPS resulted in inhibition percentage but these concentrations were toxic. Therefore, we treated the RAW264.7 macrophage cells with essential oils of *L. angustifolia* and *L. hybrida* at concentration 25µg/ml, to avoid cytotoxicity of EO, for 24 h prior.

Table 4. Inhibition zone (IZ mm) and MIC µg/ml of the EOs of *Lavandula angustifolia* and *Lavandula hybrida* essential oils against some *Candida* strains

Essential oils / strains	*L. angustifolia*		*L. hybrida*		fluconazole 25 µg (IZ mm)
	IZ (mm)	MIC µg/ml	IZ (mm)	MIC µg/ml	
C. glabrata	12	2.5	NA	NA	21
C. albicans	15	5	NA	NA	20
C. tropicalis	16	5	NA	NA	18
C. krusei	16	5	12	2.5	26
C. albicans (ATCC 10231)	12	2.5	9	2.5	23

Furthermore, 0.02% ethanol and 100µM of dexamethasone each with 10µg/ml LPS was respectively used as the negative and positive controls. Treatment of cells with LPS (10µg/ml) for 20 hours increased the production of nitric oxide. However, treatment of LPS-induced cells with nontoxic concentrations of EO were reduced. The NO inhibition percentage was performed with 25µg/ml (non-toxic concentration) of *L. angustifolia* and *L. hybrida* essential oils to result in 24.5 and 43%, respectively (Table 5). The cell viability of RAW cells as affected by *L. angustifolia* and *L. hybrida* essential oils at 25 µg/ml were 97.3 and 87.4, respectively (Table 5). In the same pattern, LPS and dexamethasone gave 97.3 and 79.3% cell viability, respectively. The results displayed in Table (5) explore that *L. hybrida* oils showed the promising anti-inflammatory. In this concern, Silva [11] reported the anti-inflammatory property of *L. angustifolia* essential oil and this essential oil increased HSP70 expression in LPS-stimulated THP-1 cells, implying that the LEO-induced inflammatory effect may be linked to HSP70 expression. Horváth [59] stated that *L. angustifolia* EO was more effective than eucalyptus EO and concluded that it may be suitable for use as an adjunct to intra-vesical therapy. After the preparation of the appropriate pharmaceutical formulation from the oil, their anti-inflammatory effect could well complement glycosaminoglycan-regenerative therapy in the urinary bladder.

Table 5. Anti-inflammatory, Nitric oxide inhibition and Cell viability of *Lavandula angustifolia* and *lavandula hybrida* essential oil at 25µg/ml

Essential Oil	% Nitric oxide inhibition at 25µg/ml	% Cell viability of RAW cell line at 25µg/ml
***L. angustifolia* (Lavender)**	24.5±0.49	97.3±0.89
***L. hybrid* (Lavandin)**	43±0.78	87.4±1.2
LPS	------	97.3±0.78
Dexamethasone (+ve)	94.1±0.78	79.3±0.51

2.3.4. Cytotoxicity effect:

A) Primary screening:

The essential oils have been evaluated regarding their potentiality against several human cancer cell lines at 100µg/ml. In this study, DMSO was used as control while, doxorubicin was used as the positive control. The results of the applied essential oils are shown in Table (6).One hundred ppm (100µg/ml) of all studied essential oils were tested for the ability to inhibit the growth of the used 6 cancer cell lines (PC3, PACA2, A431, A549, MCF7, and HCT116) as well as on normal skin fibroblast cell line (BJ1). The essential oil of *L. hybrida* was more potent than *L. angustifolia*. *L. hybrida* oil was effective against HCT116, MCF7, and PACA2 (100% inhibition), and A431 (81.7%), while *L. angustifolia* essential oil was slightly cytotoxic and ineffective for the studied cell lines. The value of IC_{50} of *L. hybrida* essential oil and doxorubicin (+ve control) against different cell lines was calculated and shown in Fig (1).

B) Secondary screening:

The most promising essential oils were subjected to secondary screening to calculate their IC_{50} and selectivity index. The results displayed in Table (7) indicated that *L. hybrida* was the promised essential oil compared to *L. angustifolia* oil with IC_{50} 57.3, 74.4, 57.6 and 30.5 for PACA2, A431, MCF7 and HCT116, respectively. So, *L. hybrida* represented the most promising oil on PACA2, MCF7 and HCT116 cell lines. Thus, the promising essential oils were screened for their safety on the normal cell line BJ1 and their selectivity index were calculated and illustrated in Table (8). The results indicated that the two essential oils have low cytotoxicity on normal cell BJ1. In the field of selectivity index that displayed in Table (10), we found that *L. hybrida* is promising in A431 cell line and the same pattern was showed in PACA2, MCF7 and HCT116.

Table 6. Cytotoxicity percentage of *L. angustifolia* and *L. hybrida* essential oils at 100 ppm

Cell line / EOs	PC3 Prostate Cancer	PACA2 Pancreatic Cancer	A431 Epidermoid Carcinoma	A549 Lung Cancer	MCF7 Breast Cancer	HCT116 Colon Cancer
L. angustifolia	6.2±0.06	12.5±0.3	2.4±0.01	9.3±0.7	2.5±0.01	24.3±1.2
L. hybrida	24.3±2.5	100±zero	81.7±2.8	23.4±1.3	100±zero	100±zero
DMSO Control (0.5%)	1	1	1	5	3	1
Negative control	0	0	0	0	0	0
Positive Control	100	100	100	100	100	100

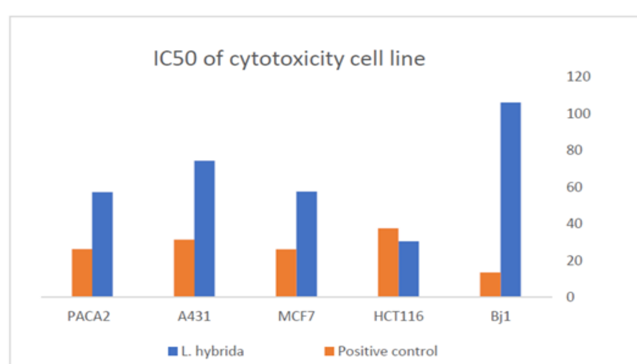

Fig 1. IC_{50} of cytotoxicity cancer cell and normal cell line BJ1 (µg/ml)

3. Conclusion

From the results that were presented and discussed, and from the aforementioned summary, it is possible to consider the essential oil *L. hybrida* as encouraging natural entity to be used in the exploration of natural medicines. It is important to subject it for further biological studies on experimental animals before using them to reach new effective drug entities, especially as anti-inflammatory and anti-tumor agents.

*Corresponding author e-mail: ewiesfawzy@yahoo.com.; (ggggggggggggggggggg).
EJCHEM use only: Received date here; revised date here; accepted date here
DOI: 10.21608/EJCHEM.2019.6778.1566
©2019 National Information and Documentation Center (NIDOC)

Table 7. IC_{50} of cytotoxicity cancer cell lines (µg/ml)

Essential oils	IC_{50} of cytotoxicity cancer cell lines (µg/ml)				
	PACA2	**A431**	**MCF7**	**HCT116**	**Bj1**
L. hybrida	57.3±2.7	74.4±3.2	57.6±2.4	30.5±1.2	106.2±4.2
Positive control	26.2±1.2	31.5±2.2	26.1±1.3	37.6±2.4	13.5±0.6

Table 8. Selectivity index of *L. angustifolia* and *L. hybrida* essential oils on normal cell (BJ1) and IC_{50} of (Bj1)

Essential oils	*L. hybrida*
IC_{50} (µg/ml)	106.2
Selectivity index for PACA2	1.853
Selectivity index for A431	1.427
Selectivity index for HCT116	3.48
Selectivity index for MCF7	1.84

4. Contributions:

Conceptualization, Elsayed A. Omer and Ahmed M. Aboul-Einen.; Methodology, Hanem I. Eldeghedy, Elsayed A. Omer, AbdelNassaer G. El-Gendy, Ahmed M. Aboul- Enein and Amr A. Nassrallah.; Software, Hanem I Eldeghedy, Elsayed A. Omer, AbdelNassaer G. El-Gendy.; Validation, Elsayed A. Omer and Ahmed M. Aboul-Einen and Amr A. Nasrallah.; Writingoriginal draft preparation, Elsayed A. Omer, Abdel Nassaer G. El-Gendy; Writing-review and editing, Hanem I. Eldeghedy, Visualization, Ahmed M. Aboul-Enein and Elsayed A. Omer All authors have read and agreed to the published version of the manuscript.

5- Ethical Committee Approval:

The approval from Ethical Committee of The Medical Research in the National Research Centre regarding this research was taken under the **No. of (1342032022) for 2022.**

6. Funding:

This research was financially supported by the National Research Centre through project **No. 2/6/1**.

7. Conflicts of interest:

The authors declare that no conflict of interest.

8. References:

1. Upson, T. and Andrews, S. (2004). The Genus *Lavandula*. A Botanical Magazine Monograph Royal Botanic Gardens, Kew, 442.
2. Bailey, L.H. and Bailey, E.Z. (1976). A Concise Dictionary of Plants Cultivated in the U.S. and Canada. Hortus Third “Revised by Staff of the L. H. Bailey Hortium”. The Macmillan Publishing Company. New York.
3. Cavanagh, H.M.A. and Wilkinson, J.M. (2002). Biological activities of lavender essential oil. Phyther. Res., 16, 301-308.
4. Goncalves, S. and Romano, A. (2013). In vitro culture of lavenders (*Lavandula* spp.) and the production of secondary metabolites. Biotechnol. Adv., 31, 166-174.
5. Prusinowska, R. and Śmigielski, K.B. (2014). Composition, biological properties and therapeutic effects of lavender (*Lavandula angustifolia* L). A review. Herba Polonica, 60, 2, 56– 66.
6. Zheljazkov, V.D., Astatkie, A. and Hristov, A.N. (2012). Lavender and hyssop productivity, oil content, and bioactivity as a function of harvest time and drying. Industrial Crops and Products, 36, 1, 222-228.
7. Sienkiewicz, M., Głowacka, A., Kowalczyk, E., Wiktorowska-Owczarek, A., Jóźwiak- Bębenista, M. and Łysakowska, M. (2014). The Biological Activities of Cinnamon, Geranium and Lavender Essential Oils. Molecules, 19, 12, 20929-20940.

8. Romeo, F.V., Luca, S.D., Piscopo, A. and Poiana, M. (2008). Antimicrobial Effect of Some Essential Oils. Essential Oil Research, 20:4, 373-379.

9. Blažeković, B., Yang, W., Wang, Y., Li, C., Kindl, M., Pepeljnjak, S. and Vladimir-Knežević, S. (2018). Chemical composition, antimicrobial and antioxidant activities of essential oils of *Lavandula* × *intermedia* 'Budrovka' and *L. angustifolia* cultivated in Croatia. Industrial Crops and Products, 123, 173-182.

10. Kıvrak, Ş. (2018). Essential oil composition and antioxidant activities of eight cultivars of Lavender and Lavandin from western Anatolia. Industrial Crops & Products, 117, 88–96.

11. Silva, G.L.D., Luft, C., Lunardelli, A., Amaral, R.H., Melo, D.A.D.S., Donadio, M.V.F., Nunes, F.B., Azambuja, M.S.D., Santana, J.C., J.C., Moraes, J.C., Mello, R.O., Cassel, E., Pereira, M.A.D.A. and Oliveira, J.R.D. (2015). Antioxidant, analgesic and anti-inflammatory effects of lavender essential oil. Anais da Academia Brasileira de Ciências, 87, 2 Suppl., 1397-1408.

12. Dakhlaoui, S., Wannes, W.A., Sari, H., Hmida, M.B., Frouja, O., Limam, H., Tammar, S., Bachkouel, S., Jemaa, M.B., Jallouli, S., Hessini, K. and Msaada, K. (2022). Combined Effect of Essential Oils from Lavender (*Lavandula officinalis* L.) Aerial Parts and Coriander (*Coriandrum sativum* L.) Seeds on Antioxidant, Anti-diabetic, Anti-cancer and Anti inflammatory Activities. J. Essent. Oil-Bear. Plants 25, 1, 188 –199.

13. Jianu, C., Pop, G., Gruia, A.T. and Horhat, F.G. (2013). Chemical composition and antimicrobial activity of essential oils of lavender (*Lavandula angustifolia*) and lavandin (*Lavandula x intermedia*) grown in Western Romania. Int. J. Agric. Biol., 15, 772-776.

14. Garzoli, S., Turchetti, G., Giacomello, P., Tiezzi, A., Masci, V.L. and Ovidi, E. (2019). Liquid and Vapour Phase of Lavandin (*Lavandula X intermedia*) Essential Oil: Chemical Composition and Antimicrobial Activity. Molecules, 24, 15, 2701.

15. Eldeghedy, H.I., El-Gendy, A.G., Nassrallah, A.A., Aboul-Enein, A.M. and Omer, E.A. (2022). Comparative Chemical Profiles of *Lavandula* Species Essential Oils Grown in Egypt and others from France and Australia: Evidence from Chemometric Analysis. J. Essential Oil-Bearing Plants, 25, 52-63.

16. Cardia, G.F.E., Silva-Filho, S.E., Silva, E.L., Uchida, N.S., Cavalcante, H.A.O., Cassarotti, L.L., Salvadego, V.E.C., Spironello, R.A., Bersani-Amado, C.A. and Cuman, R.K.N. (2018). Effect of Lavender (Lavandula angustifolia) Essential Oil on Acute Inflammatory Response. Evidence-Based Complementary and Alternative Medicine, 10.

17. Verma, R.S., Rahman, L.U., Chanotiya, C.S., Verma, R.K., Chauhan, A., Yadav, A., Singh, A. and Yadav, A.K. (2010). Essential oil composition of Lavandula angustifolia Mill. cultivated in the mid hills of Uttarakhand, India. J. Serb. Chem. Soc., 75, 3, 343–348.

18. Fathima, F., Vishnu Priya, V., and Geetha, R. V. (2015). Evaluation of antimicrobial activity of lavender oil against selected bacterial pathogens: An in vitro study. Journal of Chemical and Pharmaceutical Research, 7, 12, 624-626.

19. Mesic, A., Mahmutović-Dizdarević, I., Tahirović, E., Durmišević, I., Eminovic, I., Jerković-Mujkić, A. and Bešta-Gajević, R. (2019). Evaluation of toxicological and antimicrobial activity of lavender and immortelle essential oils. Drug and Chemical Toxicology, 8, 190-197.

20. Miastkowska, M., Kantyka, T., Bielecka, E., Kałucka, U., Kami´nska, M., Kucharska, M., Kilanowicz, A., Cudzik, D. and Cudzik, K. (2021). Enhanced Biological Activity of a Novel Preparation of Lavandula angustifolia Essential Oil. Molecules, 26, 2458.

21. Huang, M.Y., Liao, M.H., Wang, Y.K., Huang, Y.S. and Wen, H.C. (2012). Effect of Lavender Essential Oil on LPS-Stimulated Inflammation. The American Journal of Chinese Medicine, 04, 845-859.

22. Ciocarlan, A., Lupascu, L., Aricu, A., Dragalin, I., Popescu, V., Geana, E.I., Ionete, R.E., Vornicu, N., Duliu, O.G., Hristozova, G. and Zinicovscaia, I. (2021). Chemical Composition and Assessment of Antimicrobial Activity of Lavender Essential Oil and Some By-Products. Plants, 10, 1829.

23. Valková, V., Dˇúranová, H., Galoviˇcová, L., Vukovic, N.L., Vukic. M. and Kaˇcániová, M. (2021). In Vitro Antimicrobial Activity of Lavender, Mint, and Rosemary Essential Oils and the effect of their Vapours on Growth of Penicillium spp... Molecules, 26, 3859.

24. Varona, S., Martín Á., Cocero, M.J. (2009). Formulation of a natural biocide based on lavandin essential oil by emulsification using modified starches. Chemical Engineering and Processing, 48, 1121-1128.

25. Bajalan, I., Rouzbahani, R., Pirbalouti, A.G. and Maggi, F. (2017). Chemical Composition and Antibacterial Activity of Iranian *Lavandula hybrida*. Chem. Biodivers,14, 7, 9-22.
26. Varona, S., Rojo, R.S.R., Martín, A., Cocero, M.J., Serra, A.T., Crespo, T. and Duarte, C.M.M. (2012). Antimicrobial activity of lavandin essential oil formulations against three pathogenic food-borne bacteria. Industrial Crops and Products, 24, 243-250.
27. Egyptian pharmacopoeia (1984). Cairo General Organization for Government Printing Offices , 3rd ed., 867p, Cairo, Egypt.
28. Güenther, E. (1995). The Essential Oils: V. Nostrand Ed., New York, 236p.
29. Omer, E., Elshamy, A., El Gendy, A., Cai, X., Sirdaarta, J., White, A. and Cock, I.E. (2016). Cakile maritima Scop. extracts inhibit the growth of some bacterial triggers of autoimmune diseases: GC-MS analysis of an inhibitory extract. Pharmacognosy Journal, 8, 4.
30. Brand-Williams, W., Cuvelier, M.E., Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. LWT - Food Science and Technology, 28, 1, 25-30.
31. Singh, N. and Rajini, P.S. (2004). Free radical scavenging activity of an aqueous extract of potato peel. Food Chem., 85, 611–616.
32. Floegel, A., Kim, D.O., Chung, S.J., Koo, S.I. and Chun, O.K. (2011). Comparison of ABTS/DPPH assays to measure antioxidant capacity in popular antioxidant-rich US foods. Food Composition and Analysis, 24, 7, 1043-1048.
33. Marinho, S.A., Teixeira, A.B., Santos, O.S., Cazanova, R.F., Ferreira, C.A.S., Cherubini, K. and Oliveira, S.D.D., (2010). Identification of *Candida* spp. by phenotypic tests and PCR. Brazilian Journal of Microbiology 41: 286-294.
34. Gündeş, S.G., Gulenc, S. and Bingol, R. (2001). Comparative performance of fungichrom I, candifast and API 20C aux systems in the identification of clinically significant yeasts. Journal of Medical Microbiology 50: 1105-1110.
35. Hermansyah, H., Adhiyanti, N., Julinar, Rahadiyanto, K.Y. and Susilawati (2017). Identification of *Candida* species by assimilation and multiplex-PCR methods. Journal of Chemical Technology and Metallurgy, 52, 6, 1070-1078.
36. Ozcan, K., Ilkit, M., Ates, A., Turac-Bicer, A. and Demirhindi, H. (2010). Performance of Chromogenic *Candida* agar and CHROM agar *Candida* in recovery and presumptive identification of monofungal and polyfungal vaginal isolates. Medical Mycology, 48, 29-34.
37. Khalaf, D.D., Soliman, M.M.H. and Mansour, A.S. (2021). Conventional and molecular identification of mycotic mastitis caused by *Candida* in farm animals. International Journal of Veterinary Science, 10, 1, 64-68.
38. Lorian, V. (2005). Antibiotics in Laboratory Medicine; Lippincott Williams & Wilkins. Baltimore, PA, USA.
39. Wu, S.J., Liu, P.L., Ng, L.T. (2008). Tocotrienol-rich fraction of palm oil exhibits anti-inflammatory property by suppressing the expression of inflammatory mediators in humanmonocytic cells. Mol Nutr Food Res., 52, 8, 921–929.
40. Yoon, W.J., Kim, S.S., Oh, T.H., Lee, NH.and Hyun, C.G. (2009). Abies koreana essential oil inhibits drug-resistant skin pathogen growth and LPS-induced inflammatory effects of murine macrophage. Lipids, 44, 471 – 476.
41. Mosmann, T. (1983). Rapid colorimetric assays for cellular growth and survival: Application to proliferation and cytotoxicity assays. J. Immunol Methods, 65, 55-63.
42. Thabrew, M.I., Hughes, R.D. and Mcfarlane, I.G. (1997). Screening of hepatoprotective plant components using a HepG2 cell cytotoxicity assay. J. Pharm Pharmacol, 49, 11, 1132-1135.
43. El-Menshawi, B.S., Fayad, W., Mahmoud, K., El-Hallouty, S.M., El-Manawaty, M., Olofsson, M.H. and Linder, S. (2010). Screening of natural products for therapeutic activity against solid tumors. Indian Journal of Experimental Biology, 48, 258-264.
44. Carrasco, A., Martinez-Gutierrez, R., Tomas, V. and Tudela, J. (2016). Lavandin (*Lavandula* × *intermedia* Emeric ex Loiseleur) essential oil from Spain: determination of aromatic profile by gas chromatography–mass spectrometry, antioxidant and lipoxygenase inhibitory bioactivities. Natural Product Research, 30, 10, 1123–1130.
45. Sacchetti, G., Maietti, S., Muzzoli, M., Scaglianti, M., Manfredini, S., Radice, M. and Bruni, R. (2005). Comparative evaluation of 11 essential oils of different origin as functional antioxidants, antiradicals and antimicrobials in foods. Food Chem., 91, 621– 632.
46. Huang, D.J., Ou, B.X., Prior, R.L. (2005). The chemistry behind antioxidant capacity assays. J.

Agric. Food Chem., 53, 6, 1841−1856.

47. Cız, M., Cızova´, H., Denev, P., Kratchanova, M., Slavov, A., Lojek, A. (2010). Different methods for control and comparison of the antioxidant properties of vegetables. Food Control, 21, 4, 518–523.

48. Viuda-martos, M., El Gendy, A.G.S., Sendra, E., Fernandez-lopez, J., Abd el razik, K. A., Omer, E.A. and Perez-alvarez, J.A. (2010). Chemical Composition and Antioxidant and Anti-Listeria Activities of Essential Oils Obtained from Some Egyptian Plants. J. Agric. Food Chem., 58, 9063-9070

49. Perez-Jimenez, J., Arranz, S., Tabernero, M., Dıaz-Rubio, M. E., Serrano, J., Gon˜i, I. and Saura-Calixto, F. (2008). Updated methodology to determine antioxidant capacity in plant foods, oils and beverages: Extraction, measurement and expression of results. Food Res. Int. 41, 274–285.

50. Maestri, D.M., Nepote, V., Lamarque, A.L., Zygadlo, J.A. (2006). Natural products as antioxidants, Imperato, F., editor. Phytochemistry: Advances in Research. Research Signopost, Kerala, India,105–135.

51. Aruoma, O. I., (1998). Free radicals, oxidative stress and antioxidants in human health and disease. J. Am. Oil Chem. Soc., 75, 199–212.

52. Kamatou, G.P.P. and Viljoen A.M. (2010). A review of the application and pharmacological properties of α-bisabolol and α-bisabolol-rich oils. J. Am. Oil Chem. Soc., 87, 1–7.

53. Saleh, M.A., Clark, S., Woodard, B., Deolu-Sobogun, S. A., (2010). Antioxidant and free radical scavenging activities of essential oils. Ethn. Dis., 20, 78–82.

54. Yang S.A., Jeon S.K., Lee E.J., Shim C.H. and Lee I.S. (2010). Comparative study of the chemical composition and antioxidant activity of six essential oils and their components. Natural Product Research, 24, 2, 140–151.

55. You, C. X., Wang, Y., Zhang, W. J., Yang, K., Wu, Y., Geng, Z. F., Chen, H.P., Jiang, H.Y., Du, S.S., Deng, Z.W. and Liu, Z. L. (2014). Chemical constituents and biological activities of the Purple *Perilla* essential oil against *Lasioderma serricorne*. Industrial Crops and Products, 61, 331-337.

56. Hristova, Y., Wanner, J., Jirovetz, L., Stappen, I., Iliev, I., and Gochev, V. (2015). Chemical composition and antifungal activity of essential oil of *Hyssopus officinalis* L. from Bulgaria against clinical isolates of *Candida* species. Biotechnology & Biotechnological Equipment, 29, 3, 592-601.

57. Sharma, J. N., A. Al-Omran and S. S. Parvathy, (2008). Role of nitric oxide in inflammatory diseases. Inflammopharmacology, 15, 252–259.

58. Horváth, A., Pandur, E., Sipos, K., Micalizzi, G., Mondello, L., Böszörményi, A., Birinyi, P. and Horváth, G. (2022). Anti-inflammatory effects of lavender and eucalyptus essential oils on the *in-vitro* cell culture model of bladder pain syndrome using T24 cells. BMC Complementary Medicine and Therapies, 22, 119.