J. Agric. Sci. Mansoura Univ., 33 (12): 8577 - 8590, 2008

IN VITRO ANTIBACTERIAL ACTIVITY OF SOME PLANT ESSENTIAL OILS

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ABSTRACT

In this study we aim to in vitro evaluate the antibacterial activity of 15 plant essential oils against six bacterial species. Antibacterial effects of the selected essential oils were investigated against four gram-negative bacteria (*Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Proteus vulgaris*) and two gram-positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*), using Zone of inhibition (mm) and the MIC of each extract. Zone of inhibition were tested at four different concentrations (1:1, 1:5, 1:10 and 1:20) using disc diffusion method and the MIC of the active essential oils were tested using two fold agar dilution method at concentrations ranging from 0.2 to 15 mg/ml.

Results showed that majority of the oils exhibited wide range of antibacterial activity against the tested strains. Out of 15 essential oils tested, 11 oils were found to be inhibiting one or more strains. Lemon grass, clove, basil and rosemary oils exhibited significant inhibitory effect against both gram-positive and gram-negative bacteria. Lemon grass and clove oils showed promising inhibitory activity even at low concentrations, whereas sage, ginger, spearmint oils were least active against the tested bacteria; but black amomum, eucalyptus, camphor and sweet marjoram oils failed to inhibit any of the tested bacteria. In general, *B. subtilis* was the most susceptible. On the other hand, *K. Pneumoniae* exhibited low degree of sensitivity. Data obtained from MIC confirmed the results obtained from the antimicrobial bioactivity study. Except sage, and ginger oils, the MIC of the other 6 oils were ranged between 0.2-1.5 mg/ml, reached its maximum using rosemary and its minimum using lemon grass oils.

These effects against the tested microorganisms indicated the possible ability of each essential oil as a food preservative. Therefore, it is suggested that further work be performed on food to test the antibacterial properties of these oils.

Keywords: aromatic plants - Microorganisms - Essential oils - Food preservatives.

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INTRODUCTION

For many thousands of years, plant essential oils and extracts have been used in food preservation, pharmaceuticals, alternative medicine and natural therapies *[Jones, Reynolds,1996; Balchin and Deans,1997].* Until recently, essential oils have been studied mostly from the viewpoint of their flavour and fragrance chemistry only for flavouring foods, drinks and other

goods. Actually , however, essential oils and their components are gaining increasing interest because of their relatively safe status, their wide acceptance by consumers, and their exploitation for potential multi-purpose functional use *[Sawamura ,2000 and Ormancey et al., 2001].*

Essential oils (also called volatile oils) are aromatic oily liquids obtained from plant materials (flowers, buds, seeds, leaves, twigs, bark, herbs, wood, fruits and roots). They can be obtained by expression, fermentation or extraction but the method of steam distillation is most commonly used for commercial production *[Van de Braak and Leijten,1999].* Essential oils are complex mixers comprising many single compounds. Chemically they are derived from terpenes and their oxygenated compounds. Each of these constituents contributes to the beneficial or adverse effects. Phenolic components of oils sensitize the phospholipid bilayer of the cell membrane, causing an increase of permeability and leakage of vital intracellular constitutes or impairment of bacterial enzyme systems *[Singh et al., 2002] .*

Many authors, in fact, have reported the antibacterial, antifungal, antiviral insecticidal and antioxidant properties of essential oils *[Burt, 2004 and Kordali et al.,2005.].* Some oils have been used in cancer treatment *[Sylvestre, et al.,2006].* Some other oils have been used in food preservation *[Faid, et al.,1995],* aromatherapy *[Buttner et al.,1996]* and fragrance industries *[Van de Braak and Leijten,1999].* Essential oils such as aniseed, calamus, camphor, citronella, clove, eucalyptus, lavender, lemongrass, mint, nutmeg, rosemary, basil and vetiver have been traditionally used by people for various purposes in different parts of the world . Clove and rosemary oils had shown antibacterial and antifungal activity *[Ouattara et al.,1997]*; Anti-inflammatory activity has been found in basil *[Singh and Majumdar,1999].* Rosemary oils possess antioxidant property *[Calabrese, et al.,1999 and Aruoma, et al.,1996].* Peppermint oil has shown anticancer activity [*Kumar et al.,2004; Arias and Ramon,2005*]. Citronella oil has shown inhibitory effect on biodegrading and storage-contaminating fungi [*De Billerbeck, et al.,2001*]. Lavender oil has shown antibacterial and antifungal activity; it was also found to be effective to treat burns and insect bites [*Cavanagh and Wilkinson, 2002*]. In spite of all the information available on the 16 oils selected for this study, we were not able to find antibacterial activity for all those oils. Given these considerations, this study was undertaken with the intention of finding out the efficacy of these essential oils as antimicrobial agents for commercial purposes.

MATERIALS AND METHODS

1. Microorganisms tested

Pure cultures of the bacteria used for the testing purpose were provided from Microbial Dept., National Research Center, Dokki, Giza, Egypt. Four strains of gram-negative bacteria [*Escherichia coli* (ATCC 15753), *Klebsiella pneumoniae* (ATCC 15380), *Pseudomonas aeruginosa* (ATCC 27853), *Proteus vulgaris* (MTCC 1771)] and two strains of gram positive bacteria [*Bacillus subtilis* (MTCC 441) and *Staphylococcus aureus* (ATCC 25923)] were obtained and subcultured in Treptone Soya Agar media (TSA).

The inoculated TSA media were inoculated at 37 ± 1° c for 24 hours, before sensitivity testing and evaluating the efficacy of the studied plants.

2. Plant materials and essential oil extract preparation

Samples of mature fresh rhizomes of ginger (*Zingiber officinale*), fresh whole plant of thyme (*Thymus vulgaris*) and samples of mature fresh green leaves of lemon grass (*Cymbopogon citratus*), basil (*Ocimum basilicum*), rosmary (*Rosmariinus officinalis*), sage (*Salvia officinalis*), clove (*Eugenia aromatica*), peppermint (*Mentha piperita*), sweet marjoram (*Majorana hortensis*), spearmint (*Mentha spicacta*), lavender (*Lavandula angustifolia*); were obtained locally (Medicinal, Aromatic Plant Research Center). On the other hand, powdered commercial samples of camphor (*Cinnamomuum camphora*), nutmeg (*Myristica fragrans e*), eucalyptus (*Eucalyptus globules*), cardamom (*Eletteria cardamomum Maton*) and black amomum (*Granum paradisi*) were obtained from the commercial producers of plant essential oils and aromatic substances (Harraz Company for aromatic substances and fragrance) in Egypt. Freshly-collected samples before use, were identified at the Plant Protection Department, National Research Center,Dokki, Giza, Egypt. The essential oils (EO) tested were extracted by the hydrodistillation method using Clevenger's apparatus [*Bankole,1997*]. The recovered oils were dried over anhydrous sodium sulphate and stored in darkness at 4°C. The filtrate , for each , was used as the test extract. Quality of the oils was ascertained to be more than 98% pure.

3. Antibacterial activity testing

Antibacterial activity of selected essential oils was done by the disk diffusion method, which is normally used as a preliminary check and to select between efficient essential oils. The essential oils were dissolved in 10% aqueous dimethylsulfoxide (DMSO) with Tween 80 (0.5% v/v for easy diffusion) and sterilized by filtration through a 0.45 μm membrane filter. Under aseptic conditions, empty sterilized discs (Whatman no. 1 mm diameter) were impregnated with 50 μL of different concentrations (1:1, 1:5, 1:10, 1:20) of the respective essential oils and placed on the agar surface [NCCLS, 2002]. Paper disc moistened with aqueous DMSO was placed on the seeded petriplate as a vehicle control. A standard disc containing streptomycin (25 μg/ disc) was used as reference control. All petridishes were sealed with sterile laboratory parafilm to avoid eventual evaporation of the test samples. The plates were left for 30 min at room temperature to allow the diffusion of oil, and then they were incubated at 37°C for 18 h. After the incubation period, the zone of inhibition was measured with a calliper and the degree of growth inhibition was evaluated and compared with the growth inhibition results obtained from the control samples Gentamycin sulphate with respect to bacteria. Each assay was performed in triplicates, and mean value was calculated.

4. Statistical analysis

Significant differences between treatments and strains sensitivity and the differences between means of minimum inhibitory concentration (MIC) values,were analysed using the Least Significance Difference (LSD) *[Nissen, 1990].*

5. Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) is defined as the minimum level of essential oil concentration that produces a 90% reduction in the growth (populations) of microbial colonies [*Skandamis et al.,2001*]. Minimum Inhibitory Concentrations (MIC) in the plates containing test oil was judged by comparison with growth in blank control plates. The MICs were determined by the microdilution agar plate method, as the lowest concentration of oil inhibiting visible growth of each organism on the agar plate [*Delaquis et al.,2002*]. Aliquots of 90 ml tempered Trypton Soy Agar (TSA) were agitated vigorously with essential oil to achieve the following final oil concentrations: 3.0, 2.8, 2.6, 2.2, 2.0, 1.5, 1.0, 0.6, 0.5, 0.25, 0.15, 0.10, 0.05 and 0.025 ml/100ml. Approximately 15 ml of each of these mixtures was transferred with 1ml of each inoculum to agar plates. The plates were incubated at 37°C for 24h,and the numbers of colonies were determined. Each assay was performed in duplicates in two separate experimental runs.The effects were compared with that of the standard antibiotic streptomycin at a concentration of 25 *µg/* disc, as a positive reference standard [*Khan and Omotoso, 2003*].

6. Identification of oil components

The most potent oils, (lemon grass, clove, basil and rosemary oils) were analyzed using GC/MS. GC-MS analysis of the volatile oil was performed on a Varian gas chromatograph interfaced to a Finnigan SSQ 7000 mass selective detector (MSD) with ICIS V2.0 data system for MS identification of the GC components. The column used was a DB-5 (J & W Scientific, Folsom, CA) cross-linked fused silica capillary column (30 m, 0.25 mm i.d.) coated with polydimethylsiloxane (0.5 µm film thickness). The oven temperature was programmed from 40°C for 3min, isothermal, then heating by 5°C/min to 250°C, and held for 2 min at 250°C. Injector temperature was 220°C and the volume injected was 0.5µl. Transition-line and ion source temperatures were 250° and 150°C, respectively. The mass spectrometer had a delay of 3 min to avoid the solvent peak and then scanned form m/z 40 to m /z 400. Ionization energy was set at 70 eV. Identifications were based on comparison with the MS computer library (NIST-Software package, Finnigan), and on the respective retention indices. The separated components were identified by matching them with the National Institute of standards and Technology (NIST) mass spectral library data, comparison of the Kovat's index with those of authentic components and with published data [*Adams, 1995*]. The quantitative determination was carried out by peak area integration.

RESULTS AND DISCUSSION

1- Zone of inhibition of the tested essential oils against tested microorganisms.

The antimicrobial activity of many essential oils has been previously reviewed and classified as strong, medium or weak [*Zaika, 1988*]. In this study, the most of selected essential oils under investigation showed considerable anti-bacterial activity (specially, at the concentration 1:1), with

varying magnitudes, against six bacterial species (Tables 1and 2). In our study, out of 15 essential oils tested, 12 showed antibacterial activity against one or more bacteria. The most potent oils against all the bacterial species tested were, lemongrass oil, clove oil, basil oil and rosemary oil. The zone of inhibition above 7 mm in diameter was taken as positive result. Even though earlier studies have reported better antimicrobial activity for eucalyptus oil [*Cimanga, et al.,2002 and Takarada et al., 2002*], our study showed no inhibitory activity of eucalyptus in addition to black amomum and camphor oils. Both gram-positive and gram-negative bacteria were sensitive to the potent essential oils. *P. Aeruginosa* were inhibited by 10 oils, followed by *S. aureus* (8 oils) , *P. vulgaris* and *E. coli* (7 oils), *K. pneumoniae* (6 oils) *and B. subtilis* (4 oils). In general lemongrass oil showed significant inhibitory effect against *P. aeruginosa* (33.3 mm), *B. subtilis* (29.9 mm), *P. vulgaris* (29.4 mm), *K. Pneumoniae* (27.5 mm) and *S. aureus* (20.8 mm). Moderate effects were seen in clove oil, basil oil and rosemary oil. There was no inhibition of growth with black amomum oil, eucalyptus oil and camphor oil or the vehicle control (10% DMSO).

Table 1: Antibacterial activity of 15 essential oils and Streptomycin against tested microorganisms, using disc diffusion method (at concentration, 1:1).

Name of oil	Zone of inhibition (mm) a, b, c, d, e					
	S. aureus	*B. subtilis*	*K. pneumoniae*	*P. vulgaris,*	*P. aeruginosa*	*E. coli*
Basil	$21^{Aa} \pm 0.5$	$-^{Bb}$	$10.9^{Cc} \pm 0.5$	$8.9^{Cc} \pm 0.7$	$25^{Ca} \pm 0.3$	$15^{Ca} \pm 0.5$
Black amomum	$-^{Ba}$	$-^{Ba}$	$-^{Ba}$	$-^{Ba}$	$-^{Ba}$	$-^{Ba}$
Camphor	$-^{Ba}$	$-^{Ba}$	$-^{Ba}$	$-^{Ba}$	$-^{Ba}$	$-^{Ba}$
Cardamom	$30^{Ca} \pm 0.3$	$-^{Bb}$	$14^{Cc} \pm 0.2$	$-^{Bb}$	$-^{Bb}$	$13^{Cc} \pm 0.2$
Spearmint	$-^{Ba}$	$-^{Ba}$	$-^{Ba}$	$-^{Ba}$	$8.2^{Db} \pm 0.1$	$-^{Ba}$
Clove	$30^{Ca} \pm 0.5$	$14.3^{Cb} \pm 0.4$	$12^{Cb} \pm 0.3$	$16.5^{Dc} \pm 5$	$27^{Ca} \pm 0.9$	$22^{Ad} \pm 0.6$
Eucalyptus	$-^{Ba}$	$-^{Ba}$	$-^{Ba}$	$-^{Ba}$	$-^{Ba}$	$-^{Ba}$
Ginger	$-^{Ba}$	$-^{Ba}$	$-^{Ba}$	$8.5^{Cb} \pm 0.17$	$10^{Db} \pm 0.5$	$-^{Ba}$
Lavender	$-^{Ba}$	$12.5^{Cb} \pm 0.45$	$-^{Ba}$	$11.2^{Cb} \pm 0.5$	$12.1^{Db} \pm 0.9$	$-^{Ba}$
Lemon grass	$33^{Ca} \pm 0.7$	$18.5^{Db} \pm 0.7$	$10.3^{Cc} \pm 0.6$	$16.6^{Db} \pm 0.3$	$35^{Ea} \pm 0.4$	$27^{Dd} \pm 0.8$
Peppermint	$8.1^{Da} \pm 0.5$	$10.6^{Ca} \pm 0.5$	$-^{Bb}$	$8.5^{Ca} \pm 0.2$	$12.1^{Da} \pm 0.5$	$-^{Bb}$
Rosemary	$28^{Ea} \pm 0.3$	$-^{Bb}$	$10.4^{Cc} \pm 0.13$	$14.5^{Dc} \pm 0.8$	$30^{Fa} \pm 0.4$	$21^{Ad} \pm 0.5$
Sage	$12^{Da} \pm 0.5$	$-^{Bb}$	$-^{Bb}$	$-^{Bb}$	$8.5^{Da} \pm 0.1$	$10^{Ca} \pm 0.5$
Sweet marjoram	$-^{Ba}$	$-^{Ba}$	$-^{Ba}$	$-^{Ba}$	$-^{Ba}$	$-^{Ba}$
Thyme	$12.6^{Da} \pm 0.4$	$-^{B}$	$10.1^{Ca} \pm 0.8$	$-^{Bb}$	$20.1^{Ac} \pm 0.3$	$9.4^{Ca} \pm 0.2$
Streptomycin**	$20.9^{Aa} \pm 0.5$	$26.9^{Ab} \pm 0.5$	$20.9^{Aa} \pm 0.9$	$18.4^{Aa} \pm 0.7$	$16.9^{Aa} \pm 0.2$	$20.2^{Aa} \pm 0.1$
Vehicle control	$-^{Ba}$	$-^{Ba}$	$-^{Ba}$	$-^{Ba}$	$-^{Ba}$	$-^{Ba}$
LSD = 4.02						

(a) EC, *Escherichi coli*; PM, *Proteus mirabilis;* KP, *Klebsiella pneumoniae;* ENC, *Enterobacter cloacae;* ST, *Salmonella typhi;* PA, *Pseudomonas aeruginosa;* AH,*Acinetobacter haemolyticus;* SA, *Staphylococcus aureus;* ES, *Enterococcus* sp. and CA, *Candida albicans.* Values = mean of 3 readings. All values expressed as mean + SE

(b) Including diameter of the filter paper disc. (c) Means value of three independent experiments.

(d) Values are mean inhibition zone(mm) ± S.D of three replicates.

(e) Different capital litters in columns between means denote significant difference between treatment in the same treatment at (p>0.05) and vise versa. Denote. But the different small litters in raws between means denote significant change between strains in the same teatment and vise versa; - no activity.

**** Streptomycin (25 μg/ disc) as a positive reference standard**

The antibacterial activity of the essential oils could be due to the presence of some active constituents in the oils like, phenolic, alcoholic and aldehydic contents which were reported as antimicrobial substances *[Sacchetti et al.,2005].*

Findings of our GC-MS study revealed geranial and neral to be the major constituent of lemon grass oil (Table 3), the antibacterial activity of lemon grass oil was probably due to their major component, geranial and neral and their properties could be multiple. These findings with lemon grass are similar to those reported by earlier studies [*Handique and Singh1990; Adegoke and Odesola,1996 and Shaaban,2005*]. On the other hand, several studies have shown that clove and rosemary oils had strong and consistent inhibitory effects against various pathogens[*Aureli et al.,1992 and Matan et al.,2006*].

2- Minimum Inhibitory Concentration (MIC) of selected essential oils against tested microorganisms.

The MIC values for essential oils were presented in Table (2). Minimum inhibitory concentration (MIC) for selected five oils ranged from 0.2 to 2.0 ml/100 ml (Table 2). This study revealed that lemongrass oil showed maximum activity with MIC values ranging from 0.2-0.35 ml/100 ml followed by clove oil with MIC values ranging from 0.3-0.55 ml/100 ml and rosemary oil with MIC values ranging from 0.3-0.6 ml/100 ml against all the tested strains, where as remaining oils showed moderate MIC values. The standard streptomycin had MIC values varying between 0.025 – 0.50 ml/100 ml. Data exhibited that standard antibiotic Streptomycin had stronger bioactive effect on the tested bacteria compared with all the selected plant extracts, at least 3 to 4 folds more than sage exctract (Table 2).

Table 2: : Minimum Inhibitory Concentration (MIC) of selected essential oils (mg/100 ml) and Streptomycin (25 µg/ disc) against tested microorganisms, using disc diffusion method.

Name of oil	MIC ml/100 ml),a,b					
	S. aureus	*B. subtilis*	*K. pneumoniae*	*P. vulgaris,*	*P. aeruginosa*	*E. coli*
Basil	0.75^{Ba}±0.05	-^{Bb}	-^{Bb}	-^{Bb}	0.60^{Bc}±0.04	0.50^{Bc}±0.01
Clove	0.30^{Ca}±0.03	-^{Bb}	-^{Bb}	-^{Bb}	0.50^{Bc}±0.02	0.55^{Bc}±0.03
Lemon grass	0.25^{Ca}±0.07	-^{Bb}	-^{Bb}	-^{Bb}	0.20^{Ca}±0.01	0.35^{Ca}±0.04
Rosemary	0.35^{Ca}±0.07	-^{Bb}	-^{Bb}	-^{Bb}	0.30^{Ca}±0.05	0.60^{Bc}±0.04
Sage	1.80^{Da}±011	-^{Bb}	-^{Bb}	-^{Bb}	1.50^{Dc}±0.07	2.00^{Dd}±0.08
Streptomycin	0.50^{Aa}±0.05	0.025^{Ab}±0.08	0.30^{Ab}±0.03	0.30^{Ab}±0.05	0.30^{Ab}±0.03	0.30^{Ab}±0.03
LSD = 0. 11						

Each assay was performed by duplication

(a) EC, *Escherichi coli*; PM, *Proteus mirabilis;* KP, *Klebsiella pneumoniae;* ENC, *Enterobacter cloacae;* ST, *Salmonella typhi;* PA, *Pseudomonas aeruginosa;* AH,*Acinetobacter haemolyticus;* SA, *Staphylococcus aureus;* ES, *Enterococcus* sp. and CA, Candida albicans. Values = mean of 3 readings. All values expressed as mean + SE

(b) Different capital litters in columns between means denote significant difference between treatment in the same treatment at (p>0.05) and vise versa. But the different small litters in raws between means denote significant change between strains in the same teatment and vise versa; - no activity.

**** Streptomycin (25 µg/ disc) as a positive reference standard**

Similar MIC values (0.25 ml/100ml) for lemon grass against *S. aureus*, *Pseudomonas aeruginosa*, *E. Coli*, were reported [*Adegoke and Odesola,1996*]. On the other hand, [*Sacchetti et al.,2005*] studied MIC values for lemon grass and found that concentration between 0.2 and 0.3 ml/100 ml were microbiocidal, and this is in agreement with our results.

Results obtained by [*Elgayyar et al.,2001*] may support our results with basil oil. They found that coriander and basil were highly inhibitory to *E. coli* O157:H7 (MIC approximately 0.25–0.50 ml/100 ml).

Data obtained from disc diffusion method and MIC indicate that lemon grass and clove oils inhibited all microorganisms tested. Four bacteria appeared to be more sensitive to these oils. Gram positive bacteria were found to be more susceptible than Gram negative bacteria. This could be due to the fact that the cell wall of Gram positive bacteria is less complex and lack the natural sieve effect against large molecules due to the small pores in their cell envelope [*Elgayyar et al.,2001* and *Moreira et al., 2005*]. The observed resistance of the tested organisms could be due to cell membrane permeability or due to other genetic factors.

3- GC-MS analysis of some essential oils

***3-a:* Major chemical compounds of Lemon Grass oil (GC-MS analysis)**

The constituents percentage of the essential oil is shown in Table (3). Eleven compounds were identified in the oil, accounting for about 95% of the total oil. The major content identified (GC-MS) compounds were Geranial (37.71%), Neral (33.17%), Myrcene (12.87%), Linalool (3.59%), Carveol (2.34%) and Limonene (1.22%). These data are in agreement with those obtained by [*Sacchetti et al.,2005*].The antimicrobial activity of the oil could be due to Geranial and Citral isomers. Also, terpene alcohols such as linalool were reported as antimicrobial substances [*Pattnaik et al.,1997 And Osawa et al.,1999*]. Linalool has been demonstrated to have a strong inhibitory effect against 17 bacteria and 10 fungi [*Pattnaik et al.,1997*].

Table (3): Chemical constitution of the essential oil of Lemon Grass (*Cymbopogon citratus*) using gas chromatography-mass spectrometry (GC-MS).

No.	Rt[a] (min)	KI[b]	Conc.	Compound
1	5.85	985	1.15	Methyl-5-hepten-2-2one
2	8.64	991	12.87	Myrcene
3	9.45	1031	1.22	Limonene
4	11.92	1098	3.59	Linalool
5	13.11	1100	1.16	Octadienal 2,2-dimethyl-3,4-
6	13.46	1114	0.39	Thujone
7	14.25	1217	2.34	Carveol
8	16.22	1240	33.17	Neral
9	17.55	1270	37.71	Geranial
10	19.33	1383	1.20	Geranyl acetate
11	20.34	1409	0.39	Cedrene

(a) **Retention time (min) Conc.%: The percent of concentrations based on peak area integration.**

(b) **Confirmed by comparison with Kovat's index on DB5 column (Adams, 1995).**

In addition, citral showed appreciable antimicrobial activity against Gram-positive, Gram –negative bacteria and some fungi *[Onawunmi,1989]*. The essential oil may also be rich in terpenes such as myrcene, limonene and Citral and antimicrobial activities have been found with some terpenes of plant origin [*Kubo et al.,1993*].

3-b: Major chemical compounds of Clove oil (GC-MS analysis)

In comparison with the herbs under investigation the dry clove buds oil showed very low number of separated volatile constituents which are reported along with their relative concentrations in Table 4.

Table 4: Chemical composition of Clove *(Syzygium aromaticum L.)* essential oil.

Peak No.	Components	KI*	Concentration (relative area%)	Method of identification**
1	**Eugenol**	1356	92.01***	MS, St, KI
2	**Methyl eugenol**	1401	0.21	MS, St
3	***β*-Caryophelene**	1414	0.34	MS, St
4	***α*-Farnesene**	1508	0.09	MS, St
5	**Eugenyl acetate**	1530	7.53	St,KI

* Compounds listed according to their retention time on DB5 column Kovats index.
** Compounds identified by GC-MS (MS) and/or by Kovats index on DB5 (KI) and/or by comparison of MS and KI of standard compounds (St) run under similar GC- MS conditions
*** Values are average of two experiments.

3-c: Major chemical compounds of rosemary oil (GC-MS analysis)

Identification of the volatile components of the hydrodistilled essential oil of rosemary was carried out by comparison of their mass spectra and retention times with those of reference standards (Aldrich Co.,) and published data. The results are presented in Table (5). Thirty five components were identified including monoterpene hydrocarbons (9), oxygenated monoterpene (17) and sesquterpenes (9).

3-d: Major chemical compounds of basil oil (GC-MS analysis)

The hydrodistilled oil of basil dry leaves was subjected to GC and GC-MS analysis. Identification of the volatile components of the essential oil was carried out by comparison of their mass spectra and retention times with those of reference standards (Aldrich-Co.,) and published data [*Adams, 1995*]. Table (6) shows the percentage composition of the identified volatile components along with the identification methods. Thirty two components were positively identified in basil essential oil including monoterpene hydrocarbons (5), oxygenated monoterpene hydrocarbons (16), sesquiterpene hydrocarbons (9) and oxygenated sesquiterpene hydrocarbons (2).

Table 5 : Chemical composition of Rosemary (*Rosmarinus officinalis L.*) essential oil

Peak No.	Components	KI*	Concentration (relative area%)	Method of identification**
1	Tricyclene	925	0.21***	MS, KI
2	ά-Thujene	931	0.16	MS,KI
3	α-Pinene	939	7.21	MS, St, KI
4	Camphene	950	6.01	MS, ST
5	β-Pinene	980	4.07	MS, St
6	Myrcene	991	0.19	MS, St, KI
7	1,8-Cineole	1033	13.46	MS, St, KI
8	α-Terpinene	1060	0.28	MS,KI
9	*cis*-Sabinene hydrate	1070	0.20	MS, St
10	Terpinolene	1088	1.11	MS, St, KI
11	p-cymene	1089	10.33	MS,KI
12	linalool	1098	3.37	MS, St, KI
13	α-Thujone	1105	0.8	MS,KI
14	Camphore	1134	11.03	MS, St
15	*trans*-Verbenol	1145	1.17	MS,KI
16	Borneol	1160	1.47	MS, St, KI
17	4-Terpineol	1176	1.83	MS, St
18	α-Terpineol	1189	6.73	MS, St
19	Borneol acetate	1285	3.39	MS, St
20	Iso borneol acetate	1287	0.37	MS, KI
21	Thymole	1292	5.63	MS,KI
22	Carvacrol	1299	1.73	MS,KI
23	*cis*-Pinocarvyl acetate	1309	0.38	MS,KI
24	α-Terpinyl acetate	1351	0.44	MS
25	Eugenol	1356	5.74	MS, St, KI
26	Methyl eugenol	1401	6.33	MS, St
27	β-Caryophelene	1414	6.33	MS, St
28	α-Humulene	1454	0.28	MS
29	γ-Muurolene	1477	0.23	MS
30	Germacrene D	1480	0.69	MS, St
31	α-Furnesene	1508	0.77	MS, St
32	*trans*-Calamene	1530	0.55	MS, St
33	Spathulenol	1575	0.50	MS,KI
34	Caryophelene oxide	1577	2.01	MS,KI
35	Carotol	1595	1.38	MS

Compounds listed according to their retention time on DB5 column.

* Kovats Index.

** Compounds identified by GC-MS (MS) and/or by Kovats index on DB5 (KI) and/or by comparison of MS and KI of standard compounds (St) run under similar GC-MS conditions

*** Values are average of two experiments.

Table 6 : Chemical composition of Basil *(Ocimum basilicum)* essential oil.

Peak No.	Components	KI*	Concentration*** (relative area %)	Method of identification**
1	α-Pinene	939	0.40	MS, St, KI
2	Comphene	953	0.038	MS, St
3	Sabinene	970	0.56	MS, St
4	β-Pinene	980	0.21	MS, St
5	Myrcene	991	0.43	MS, St, KI
6	1,8-Cineole	1033	8.7	MS, St, KI
7	α-Terpinolene	1088	3.0	MS, St, KI
8	Linalool	1098	19.48	MS, St, KI
9	Fenchol	1115	0.18	MS, St
10	Camphor	1143	0.24	MS, St
11	Borneol	1163	0.23	MS, St, KI
12	4- Terpineol	1176	0.15	MS, St
13	α-Terpineol	1189	0.27	MS, St
14	Methyl chavical	1198	25.00	MS, St, KI
15	Fenchyl acetate	1223	2.08	MS, St
16	Linalool acetate	1250	0.86	MS, St
17	Bornyl acetate	1280	0.72	MS, St
18	δ- Elemene	1339	0.18	MS
19	Eugenol	1355	3.09	MS, St, KI
20	*cis*- Jasmone	1388	2.99	MS, St
21	Methyl eugenol	1401	9.54	MS, St
22	β- Caryophelene	1405	3.89	MS, St
23	α-*trans*-bergamotene	1435	1.77	MS
24	α-Guaiene	1441	0.43	MS
25	α- Humulene	1455	1.71	MS
26	γ- Muurolene	1477	1.93	MS
27	Germacrene D	1488	4.1	MS, St
28	α- Farnesene	1510	0.69	MS, St
29	γ- Cadinene	1520	0.27	MS, St
30	Calamene	1530	1.48	MS, St
31	Germacerene B	1556	1.62	MS
32	γ-Cadinol	1600	3.6	MS

Compounds listed according to their retention time on DB5 column

* Kovats index

** Compounds identified by GC-MS (MS) and/or by Kovats index on DB5 (KI) and/or by comparison of MS and KI of standard compounds (St) run under similar GC-MS conditions.

*** Values are average of two experiments.

Conclusion

From this study it can be concluded that many essential oils possess antibacterial activity. Essential oils of lemongrass, clove and rosemary oils were found to be inhibiting both gram-positive and gram-negative bacteria. Lemongrass, clove, rosemary and basil oils have the most potential bactericidal properties. They can be a good source of antibacterial agents and can also be further exploited as an alternative food preservatives. We believe that the present investigation together with previous studies provide support to the antibacterial properties of lemongrass, clove, rosemary and basil oils.

Acknowledgements

We thank the Department of Microbiology, National Research Center, Dokki, Giza, Egypt, for providing the bacterial cultures. We are thankful to Mr. Mohamed Bedear for helping in achieving this work .

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النشاط المعاكس لبعض الزيوت النباتية العطرية ضد بعض السلالات البكتيرية معملياً

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استهدفت هذه الدراسة تقييم نشاط خمسة عشر زيتا عطريا نباتيا ضد ستة أنواع من البكتيريا ، منها أربعة انواع سالبة لجرام هى : ايشرشيا كولاي ، كليبسيللا بينيومونيا، سيدوموناس ايرجينوزا و بروتس فولجارس ، واثنان من البكتيريا الموجبة لجرام هى: باسيللس سبتلس، ستافلوكوكس ايريس. وجميع السلالات المختبرة سواء الموجبة أو السالبة معرفه بمقدرتها علي إنتاج سموم ميكروبية وبأنها كائنات غير مرغوبة فى الأغذية.

والنباتات موضوع الدراسة تم جمعها من الأسواق المحلية واستخلص محتواها الزيتي وقد تم استخدام هذه المستخلصات. بصورتها الخام للتعرف علي تأثيرهاالتثبيطي على كل السلالات البكتيرية المختبرة عن طريق: قياس مساحة التثبيط ، كذلك تم دراسة "أقل تركيز تثبيطى MIC" للمستخلصات الزيتية الخمسة عشر علي السلالات البكتيرية علي السواء.

ودلت النتائج علي تفاوت درجات النشاط التثبيطى للمستخلصات المستخدمه حيث لوحظ أن إحدى عشر زيتا من بين الخمسة عشر زيتا ، كان لها تأثير تثبيطى على واحدة أو أكثر من السلالات البكتيرية المختبرة، وكان أعلاها نشاطا مثبطا هى زيوت حشيشة الليمون والقرنفل والريحان وحصالبان ، وهو ما انعكس علي مساحات التثبيط للنموات البكتيرية علي السواء .وقد أوضحت النتائج أيضا أن زيوت حشيشة الليمون والقرنفل كان لها تأثير واضح على جميع السلالات البكتيرية المختبرة، حتى مع التركيزات المنخفضة المستخدمة منها، وفي المقابل لوحظ أن لمستخلصات المريميه والزنجبيل والنعناع التي أظهرت نشاطا محدودا للغاية علي جميع السلالات بصفه عامه. أما مستخلصات تين الفيل، eucalyptus ، والكافور والبردقوش، فلم يكن لها أى تأثير يذكر على أى من السلالات البكتيرية المختبرة.

وبالنسبة للنتائج المتحصل عليها من أقل نشاط تثبيطى MIC، فقد لوحظ أنه فيما عدا مستخلصات المريميه والزنجبيل فأن الفعل التثبيطى لوحظ عند تركيزات تتراوح بين ٠,٢ - ١,٥ ملليجرام لكل ملليلتر من مستنبتات الأختبار، كان حدها الأدنى عند المعاملة بمستخلص حصالبان وحدها الأعلى عند المعاملة بحشيشة الليمون.

وهذه التأثيرات ضد السلالات المختبرة تؤكد إمكانية إستخدام هذه الزيوت كمواد حافظة ،ولكن هذا يتطلب مزيدا من البحث بالتجربة على بعض الأغذية.

These effects against the tested microorganisms indicated the possible ability of each essential oil as a food preservative. Therefore, it is suggested that further work be performed on food to test the antibacterial properties of these oils.

التأثير المثبط لمستخلصات

أظهرت النتائج أن التأثير المثبط للمستخلصات المائية لهذه التوابل على كلا من النمو الفطرى و كذا انتاج التوكسين - كان أضعف كثيرا بالمقارنة بمثيله فى حالة الاستخلاص بمخلوط الماء والميثانول . كما أوضحت النتائج أن أعلى معدل تثبيط للنمو الفطرى وانتاج التوكسين كان عند اضافة أى من المستخلصات تحت الاختبار - وذلك نسب ٥% . كما لوحظ أيضا أن فطر الاسبرجيليس أو كريشيس كان الاكثر حساسية للتأثيرالمثبط بواسطة المستخلصات تحت الاختبار

ولذلك فانه من الضرورى أن يتم اختبار تأثير هذه النباتات على الاغذية لتعظيم الاستفادة من هذه النتائج.

ويستخلص من هذه الدراسة هذه الدراسة ان مستخلصات نبات اززز يمكن استخدامها كاضافات غذائية لمنتجات الالبان لما لها من فوائد غذائية وصحية ومنع تكوين النموات المنتجة للسموم.