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Research Article

ANTIBACTERIAL EFFECTS OF *CITRUS LIMON* AND *LAVANDULA ANGUSTIFOLIA* ESSENTIAL OILS

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ABSTRACT. Essential oils have been largely used for their antibacterial, antifungal and insecticidal properties. Thus this study evaluated the *in vitro* antibacterial activity of essential oils from lemon and lavender on 30 bacterial strains of *Escherichia coli* and *Staphylococcus aureus* either isolated from animals (cows and sheep) or obtained from reference strains provided by the Biotechnology and Animal Reproduction from the Laboratory of the Institute of Veterinary Sciences from Blida. The sensitivity of the strains was tested *in vitro* by aromatogram, using the two essential oils separately as well as their associations. Our results showed that lemon essential oil had a bacteriostatic effect on the tested strains of *E. coli*, while a high percentage of them was extremely sensitive to lavender oil, and more than half ones were extremely sensitive to the oil blend at a concentration of 100µl/disc. As for *S. aureus*, all the tested strains were extremely sensitive to the two essential oils as well as to their association. Lavender and/or lemon essential oils proved effective against strains of *E. coli* and *S. aureus*.

Keywords: *Lemon, Lavender, Essential oils, Aromatogram, Escherichia coli, Staphylococcus aureus*

INTRODUCTION

The spread of drug-resistant microbial pathogens is one of the most serious threats to successful treatment of infectious diseases. Overuse of antibiotics is the most important factor contributing to emergence of resistant microbes. Therefore, there is an urgent need to develop new alternative compounds to decrease the problem of microbial resistance[1]. Essential oils (EOs), also called volatile oils, are concentrated natural extracts derived from plants, which have been used as alternative medicines since the late twelfth century, and became more widespread in the second half of the sixteenth century. Recent studies have investigated the use of EOs as therapeutics for infectious diseases. EOs are volatile and complex natural products derived from the secondary metabolism of plants and can be found in the different plant parts. A lot of EOs are known to exert antimicrobial activity, but the mechanism of action of which is often not entirely understood. EOs exhibit antimicrobial potential against a large number of Gram-negative and Gram-positive bacteria and as far as we know, they destroy bacteria without acquisition of resistance [2].¶

Among the EOs, lemon oil has significant medicinal uses. Aside food uses, lemon is cultivated mainly for its alkaloids, which exert anti-cancer activities and has

antibacterial potential. The flavonoids from citrus fruits cuts have broad biological activity, including antibacterial, antifungal, antidiabetic, antiviral ones [3].
Essential oil of lavender is known as one of the most popular EOs that can be extracted from several lavender plant species, highly cultivated worldwide. The zones of lavender culture are Europe, Middle East, Asia and North Africa [4]. Lavender oil, traditionally used as an antiseptic agent, is now predominantly used as a relaxant, carminative, and sedative in aromatherapy [5].
Thus this study aimed to evaluate in vitro the antibacterial activity of essential oils from lemon and lavender on bacterial strains of *Escherichia coli* and *Staphylococcus aureus*.

MATERIAL AND METHODS

Material

Two EOs were tested for their antibacterial properties, i.e., lemon (*Citrus limon*), and lavender (*Lavandula angustifolia*). They were obtained by distillation.
Gram-positive (*S. aureus)* and gram-negative (*E. coli*) bacteria species were tested for their sensitivity to the EOs. Fifteen strains of each were acquired from the Animal Reproduction Biotechnology Laboratory from Veterinary Institute of Blida University as well as reference strains (*S. aureus*: ATCC 25923 and E. coli: ATCC 25922). They were maintained in Tryptic Soy Broth (TSB) medium with glycerin at -70 ° C.

Methods

Antibacterial activity was assessed according to the aromatogram method. The oils were added at a volume of 50 or100 μL of pure EOs (lavender or lemon oil) (when tested individually) and of their combination 50: 50 w/w.

The disk diffusion method was used in the present study. An overnight culture of each microbial strain was adjusted to 0.5 McFarland standards (10^8 CFU/ml). 500 µL of the suspension were spread over agar plates containing Mueller Hinton Agar (MHA).
Under aseptic conditions, empty sterilized discs (Whatman 6 mm in diameter) were impregnated with pure essential oil and placed on the agar surface. The inoculated plates were incubated at 37°C for 24 hours. Antimicrobial activity was evaluated by measuring the diameter of inhibition (mm) against the test organisms. The experiment was repeated in duplicate for all of the test strains.
The sensitivity to the oils was classified by the diameter of the inhibition zone as per the procedure of Mutai et al [6]:
No inhibition: inhibition diameter is less than 10 mm
Slight inhibition : total diameter between 10 and 16 mm
Moderate inhibition : total diameter between 17 – 20 mm
Strong inhibition : total diameter between 21 – 29 mm
Very strong inhibition : total diameter larger than 30 mm

Table 1: *Antibacterial effects (inhibition diameter) of lemon and/or lavender essential oils against Escherichia coli strains*

Stocks	**Lemon D (mm)**		**Lavender D (mm)**		**Mixture (lemon-lavender)**	**D (mm)**
	50µl	**100 µl**	**50µl**	**100 µl**	**50µl**	**100 µl**
S1	17.5	22.5	22	30	14	17.5
S2	15	21.5	40	27.5	15	22.5
S3	16	18	17.5	23	21	22.5
S4	15	20	13.5	21.5	16	21
S5	21	18.5	16	23.5	135	16.5
S6	21	18	20	20	20	22.5
S7	21	18.5	23	29	21	25.5
S8	12.5	22	19	25	115	20
S9	13.5	20	19	25.5	16	23.5
S10	16	18	32.5	30	15.5	18.5
S11	0	12.5	15	21.5	11.5	15.5
S12	0	12	14	16	12.5	16.5
S13	0	11.5	15.5	16.5	14	17
S14	0	13	15	20	15	20
S15	6	15	13	19	12	15
ATCC 25922	25	36	19	35	20	39

S: strain; ATCC 25922: reference strain

Table 2. *Antibacterial effects (diameter of inhibition) of lemon and lavender essential oils against Staphylococcus aureus strains*

Stocks	**Lemon D (mm)**		**Lavender D (mm)**		**Mixture (Lemon-lavender) D (mm)**	
	50µl	**100 µl**	**50µl**	**100 µl**	**50µl**	**100 µl**
S1	30	40	30	40	25	30
S2	25	28	32	42	30	40
S3	30	40	40	50	35	45
S4	31	37	40	50	30	42
S5	29	22	39	40	32	40
S6	30	40	33	45	32	42
S7	40	45	45	50	29	38
S8	35,5	48.5	31	47	27	39
S9	30	42	40	50	38	40
S10	29	40	42	50	40	48
S11	33.5	39	41	49	30	40
S12	30	34	47	46	32	42
S13	35	40	40	50	39	44
S14	42.5	48	44	47	39	45
S15	40	44	42	47	41	47
ATCC 25923	30	41	39	46	24	31

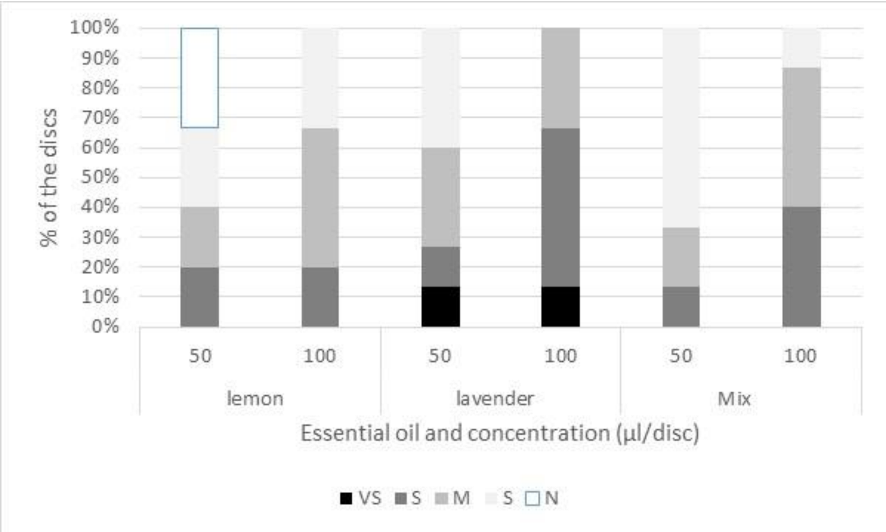
S: strain; ATCC 25923: reference strain

Statistical Analysis

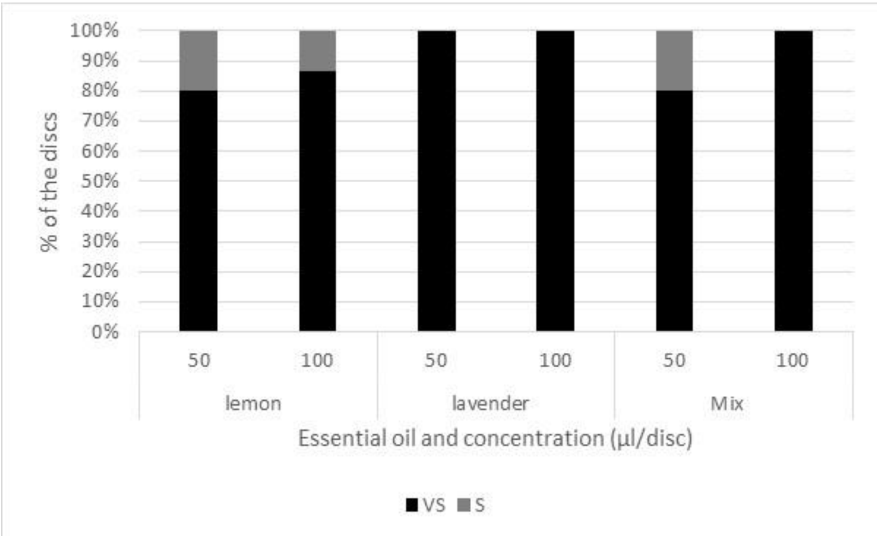
The statistical study aimed to explore the effect of the two essential oils and their mixtures on the diameter of inhibition, depending on the bacteria species.
The model included the factors: bacteria (*E. coli* or *S. aureus*), EO concentration (50µl or 100µl), and EO combination (lavender or lemon or both).
The inhibition diameters were not distributed normally, which made unfeasible the application of parametric tests such as ANOVA. For this purpose, a nonparametric analysis of variance was carried out according to Kuskall Wallis test.
The significance level was fixed at 5%.

RESULTS AND DISCUSSION

The effects of the EO concentrations and their combination on inhibition diameter classes of *E. coli* and *S. aureus* strains are illustrated in Fig. 1 and Fig. 2. Highly significant differences were observed between the oils concentrations ($p < 0.005$). In addition, the effects of oil type and/or associations) were very highly significant ($p < 0.001$). The inhibition diameter differed very highly significantly according to the bacterial species ($p < 0.001$).



Levels of inibition: VS-very strong; S-strong; M-moderate; S-slight; N-no
Fig. 1. *Distribution of Escherichia coli inhibitory effects (aromatogram method) of essential oils according to their concentrations and combinations*



Levels of inhibition: VS-very strong; S-strong
Fig. 2. *Distribution of Staphylococcus aureus inhibitory effects (aromatogram method) of essential oils according to their concentrations and combinations*

According to the scale of Mutai et al. [6], the main dose-effect of lemon EO on *E. coli* was a moderate inhibition on 20% of the strains with 50 µl/disc and on 47% of the strains with 100 µl/disc. This indicates that bacteria were middly sensitive to the EO and mainly due to increasing dose. Man et al. [7] reported that micellar lemon oil showed good minimum inhibitory concentration on all bacteria. Few studies on lemon oil were published, compared with other extracts. Though, it was shown that lemon oil exerts antibacterial and antioxidant properties, present cytoprotective activity and is parasitic repellant [8,9]. Our results also confirm that lemon essential oil had activities on all bacteria, possibly due to limonene and pinenes [10]. Ekawati and Darmaono [11] reported that at concentrations of 80 to 500 mg/ml, lemon EO inhibited the growth of sensitive bacteria over diameters of 10.5 to 14.5 mm. At doses of 900 to 1000 mg/ml, the diameters reached 16 to 18.7 mm

The highest concentration of lavender EO was necessary to induce a strong inhibitory activity on *E. coli*. The EO was strongly inhibiting on 13% of the strains alone, with 50 µl/disc and the effect increased to reach activity on 53% of the strains tested with 100 µl/disc. These results are in agreement with that observed by Umezu et al. [12]. They showed that EO of lavender had a stronger activity on *E. coli* compared to that of lemon. Little information on antimicrobial effects of lavender EO has been published. Its main constituents are linalool and linalyl acetate, which account for more than 50%, followed by smaller amounts of sesquiterpenes and terpenes [12]. Lavender has a strong antiseptic effect against antibiotic resistant strains of *S. aureus* [13, 14]. Lavender is also active against strains of *E. Coli* [15].

The EO mixture used in this experiment showed dose-effects similar to that observed with the EO taken separately, i.e., increases in moderate, strong or very strong effect with higher dose. In addition the combination of the two EOs was very strongly inhibitory on 100% of *S. aureus* strains at 100 µl Therefore, the obtained results show that both essential oils could be needed.

El Jabri et Hossain [16] reported the chemical composition of theTurkish and Indian lemon fruits essential oils and their antimicrobial activity against four pathogenic bacterial strains such as *S. aureus* or *E. coli*. Trepenoids and their oxygenated derivatives are the main components of essential oil. These compounds have a strong inhibitory potential on pathogenic bacterial strains. Current results suggest that certain forms of colloidal or micellar suspension of oils may aid in antimicrobial control, particularly those that contain high amounts of terpenes and terpenoids (such as oregano, thyme, or lemon oil) that are active against many bacterial species, including highly resistant ones [11, 12]. An important characteristic of essential oils and their components is their hydrophobicity, which enable them to partition lipids of bacterial cell membrane and mitochondria, disturbing cell structures and rendering them more permeable [17].

The mode of action of essential oils depends primarily on the type and characteristics of the active components, in particular their hydrophobic property, which allows them to penetrate into the phospholipid double layer of bacterial cell membrane. This can induce a change in the conformation of the plasma membrane accompanying loss of chemiosmotic control [18] [19]. This is the most likely origin of the lethal action of the EOs. However, EOs can also inhibit the synthesis of DNA, RNA, proteins and polysaccharides [20]. Noteworthy, some EOs flavor also have be reported to present bacteria killing potency [21].

Essential oils may become reliable alternatives in the fight against excessive or inadequate uses of antimicrobials and against multidrug-resistant strains. Further studies are needed in order to identify the appropriate way to deliver active antimicrobial compounds. Thus, based on of the most recent literature, new therapeutic protocols against resistant infectious diseases, based on essential oils, are expected [19, 20].

CONCLUSION

Essential oils of lavender or lemon showed varying degrees of antibacterial activity against Gram-positive (*E. coli*) and Gram-negative bacteria (*S. aureus*). The efficacy of essential oils of lavender or lemon against these microorganisms may provide a scientific basis for prevention and treatment of bacterial infections caused by pathogenic bacteria which have developed resistance to antibiotics. The results of this study present these oils as candidates to explore new alternative antibacterial agents to combat pathogenic microorganisms.

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