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Antimicrobial Activity of *Lavandulla pubescens* Essential Oil From Two Places In Yemen

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ABSTRACT

The present work aims to evaluate the antimicrobial activity of essential oils of two different places of similar *Lavandulla pubescens* (Taiz and Ibb). The antimicrobial properties of essential oils obtained from *L. pubescens* (Taiz) and *L. pubescens* (Ibb) were examined. To evaluate the in vitro antimicrobial activities of these tow aromatic extracts; their in vitro antimicrobial activities were determined by disk diffusion testing. *Bacillus subtilis* (ATCC 6633), *Micrococcus luteus* (ATCC 9341), *Stapylococcus aureus* (ATCC 6538), *Streptococcus pyogens* (ATCC 10541), *Escherichia coli* (ATCC 8739), *Pseudomonas aeruginosa* (ATCC25619), *Salmonella abony* (ATCC 6017), *Bifidobacterium bifidum* (EMCC 1334), and *Lactobacillus acidophilus* (EMCC 1324), were used as standard test bacterial strains, while *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus niger* (ATCC 16404) and *Candida albicans* (ATCC 2019) were used as standard test fungal strains. both *L. pubescens* (Taiz) and *L. pubescens* (Ibb) recorded higher antimicrobial activity against all test microorganisms at their high level (15µl), and were no activity against *P. eruginosa*, with lowest activity against all fungal strain tested at low level(0.6µl). The essential oils considered in this research showed a satisfactory antimicrobial activity. The essential oils could be used for the development of novel systems for food preservation.

KEY WORDS

Antimicrobial activity, Essential oils, Disk diffusion, Plant extracts, Taiz, Ibb.

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1. INTRODUCTION

Biological activity of essential oils depends on their chemical composition determined by genotype and influenced by environmental and agronomic conditions (Edris, 2007; Zouari, 2013). In the last decades, the essential oils and organic extracts of plants have been of great interest as they are the sources of natural products. Essential oils from aromatic and medicinal plants have been known to possess biological activity, notably antibacterial, antifungal and antioxidant activities (Bloligon et al., 2013; Carovic-Stanko et al., 2010). Essential oils are multi component mixtures of organic compounds found naturally in various parts of aromatic plants. They have very complex chemical properties and various efficacy modes. This diversity has given rise to a new branch of phytotherapy called aromatherapy (Delaquis et al., 2002). Essential oils have different biological activities. In the aromatherapy, they are used for their antiseptic properties against infectious diseases of bacterial origin like against the endocanalar bacteria, the vaginal microflora and the fungal microflora (against dermatophytes) (Hamoudi, 2008). However, they also possess cytotoxic properties that close them to disinfectants as antimicrobial agents with large spectrum. Lamiaceae species are considered of high importance because of their use in folk medicine, culinary, cosmetics, flavoring and production of essential oils throughout the world.

To the best of our knowledge, antimicrobial activities of the essential oils from *Lavandula pubescens* from (Taiz and Ibb) with regards to the seasonal and place variation have not yet been reported.

The present work was undertaken with the main objective to investigate the antimicrobial activities of the essential oil isolated from the aerial parts of *L. pubescens* indigenous to Yemen, Taiz and Ibb as affected by different growing seasons along with their antimicrobial activities.

2. MATERIALS AND METHODS

2.1. Plant Material

The aerial parts of *L. pubescens* (Taiz and Ibb) were collected during the period from 2010 to 2012 from Taiz and Ibb. Plant material was identified by Dr. Hassan Ibrahim, Biology Department, Sana,a University, Faculty of Science**.**

2.2. Extraction of Essential Oils

Two hundred gram of plant were subjected to hydrodistillation for approximately three hours using a Clevenger type apparatus (Sharififar et al., 2008). The oil layer was collected. However, in some cases, the distillate aqueous layers were washed with ether to extract any dissolved oils in water. Then, the ether was separated by separatory funnel and evaporated on a water bath at 40°C and the residue essential oil was added to the first collected portion. The essential oils were dried over anhydrous sodium sulfate and the yield was calculated. The yield of oils were determined and divided into aliquots and stored in the fridge at 4^0C (in dark glass container) until being used.

2.3. Determination of Antimicrobial Activities

2.3.1. Microorganisms

The tested bacteria were obtained from Yemeni Pharmacovigilance Center (*Bacillus subtilis* (ATCC 6633), *Micrococcus luteus* (ATCC 9341), *Stapylococcus aureus* (ATCC 6538), *Streptococcus pyogens* (ATCC 10541), *Escherichia coli* (ATCC 8739), *Pseudomonas aeruginosa* (ATCC25619), *Salmonella abony* (ATCC 6017), *Bifidobacterium bifidum* (EMCC 1334), and *Lactobacillus acidophilus* (EMCC 1324), Four fungal species were tested in this work, namely: *Aspergillus flavus* and *Aspergillus fumigatus* were obtained from the Modern National Laboratory Yemen while *Aspergillus niger* (ATCC 16404) and *Candida albicans* (ATCC 2019) were obtained from Yemeni Pharmacovigilance Center.

2.3.2. Antimicrobial Assay

Screening of essential oils for antibacterial and antifungal activity were done by the disk diffusion method (Prabuseenivasan et al., 2006; Bouddine et al., 2012). Essential oils were done with different levels at 15µl, 10µl, 5µl, 2.5µl, 1.25µl and 0.6µl.

10µg/ml of gentamicin and clotrimazole were used as positive control (El-Malti et al., 2007) for bacteria and fungi strains, respectively.

2.4. Statistical Analysis

Results were expressed as mean ± standard error of the mean (SEM) was calculated according to (Snedecore and Cochran, 1967). If the different between the arithmetical means of data is less than L.S.D.0.05, the result is insignificant. If the different is more than L.S.D.0.05 the results is significant. The data were statistically analyzed using SAS 9 Second Edition (for Windows) programmer (SAS 2002).

3. RESALTS AND DISCUSSION

The yield and physical properties of essential oils obtained from tow places Taiz and Ibb are shown in table (1). Data in table (1) show that the highest yield of essential oils was obtained from *L. pubescens* (Taiz) (1ml (1.03g) / 100g),while *L. pubescens* was (Ibb) (1ml (0.97g) / 100g). Refractor index for essential oils was *L. pubescens* (Taiz) (1.4936), *L. pubescens* (Ibb) (1.4944), while density of volatile oils were *L. pubescens* (Taiz) (1.03g\cm^3), *L. pubescens* (Ibb)

(0.97g\cm^3). pH of essential oils of *L. pubescens* (Taiz) was (5.4), while *L. pubescens* (Ibb) was (4.1). Different several workers showed different yields of oils (0.1 to 0.25%) (Pirbalouti and Mohammed, 2013) , and (0.13 to 1.02%) (Derwich et al., 2011).

3.1. Antimicrobial Activity of Essential Oils

Many microorganisms, which cause damage to human health, exhibit drug resistance due to inadequate use of antibiotics. Thus, there is a need for the discovery of new substances from natural sources, including plants. The in vitro antimicrobial activity by the agar disc diffusion method of the essential oils resulted in a range of growth inhibition pattern against pathogenic microorganisms.

3.2. Antibacterial Activity

The antibacterial of *Lavandulla pubescens* oils (Taiz and Ibb) results represent in (Table 2).

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Table 1. The yield and Physical properties of essential oils from *L. pubescens* (Taiz and Ibb) grown in Yemen

Plant (Essential oil)	Reign	Refractor index	Density g\cm^3	pH	Yield of ml	%	Yield of g
Lavandulla pubescens	Taiz	1.4936	1.03	5.4	1.0	1%	1.03
Lavandulla pubescens	Ibb	1.4944	0.97	4.1	1.0	1%	0.97

Table 2. The antibacterial of *Lavandulla pubescens* oils (Taiz and Ibb)

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Bacteria strains	Essential oils (µl)												Gentamicin
	Lavandulla pubescens (Taiz)						*Lavandulla pubescens* (Ibb)						
	15	10	5	2.5	1.2	0.6	15	10	5	2.5	1.2	0.6	
	Inhibition zone in mm												10µg/mg
***Bacillus subtilis* (ATCC 6633)**	22.0±0.0	17.0±0.0	17.0±0.0	17.0±0.0	16.0±0.0	0.0±0.0	18.0±0.0	17.0±0.0	17.0±0.0	8.0±0.0	5.0±0.0	0.0±0.0	17.0±0.0
***Micrococcus luteus* (ATCC 9341)**	17.0±0.0	16.3±0.5	13.0±0.0	13.0±0.0	6.0±0.0	1.0±0.0	22.0±0.0	18.0±0.0	14.0±0.0	8.6±1.5	7.0±0.5	1.0±0.0	14.0±0.0
***Stapylococcus aureus* (ATCC 6538)**	15.3±0.5	13.6±0.5	13.0±0.0	8.0±20	5.3±0.5	5.0±1.0	18.0±0.0	14.3±0.0	14.0±0.0	13.6±0.5	13.3±0.5	3.0±0.0	25.0±0.0
***Streptococcus pyogens* (ATCC 10541)**	12.0±0.0	8.3±0.0	8.0±0.0	7.0±0.0	6.0±0.0	0.0±0.0	13.6±0.5	8.0±0.0	8.0±0.0	7.0±0.0	0.0±0.0	0.0±0.0	6.0±0.0
***Escherichia coli* (ATCC 8739)**	12.3±1.1	8.0±0.0	4.6±0.5	3.6±0.5	3.0±0.0	1.0±0.5	8.0±0.0	7.3±0.7	3.3±0.5	4.0±0.0	0.0±0.0	0.0±0.0	17.0±0.0
***Pseudomonas aeruginosa* (ATCC 25619)**	4.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	2.6±0.5	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	10.0±0.0
***Salmonella abony* (ATCC 6017)**	21.0±0.0	17.3±1.1	13.0±0.0	8.0±0.0	7.6±1.1	4.0±1.7	21.0±0.0	18.0±0.0	15.0±0.0	4.0±0.0	2.6±0.5	0.0±0.0	11.0±0.0
***Bifidobacterium bifidum* (EMCC 1334)**	19.0±0.0	13.0±0.0	12.0±0.0	10.0±0.0	6.0±0.0	1.3±0.0	16.0±0.0	13.0±0.0	12.0±0.0	6.0±0.0	4.0±0.0	4.3±0.5	8.0±0.0
***Lactobacillus acidophilus* (EMCC 1324)**	25.0±0.0	15.0±0.0	12.0±0.0	10.0±0.0	7.0±0.5	2.6±0.5	16.0±0.0	13.0±0.0	12.0±0.0	5.0±0.0	4.0±0.0	0.0±0.0	7.0±0.0

Lavandulla pubescens oils (Taiz and Ibb) at its highest level have shown high antibacterial against *Bacillus subtilis*, but it were significantly higher than the positive control (gentamycin), the high activity of these oils could be attributed to the high content of carvacrol was 20.6 and 70.0% (Al.maqtari et al., 2014).These results agreement with (Rodrigues et al., 2013). Regarding the *L. pubescens* oils (Taiz and Ibb) obtained from leaves, it has shown similar high antibacterial activities against *S. aureus* at their high concentration. (Yalcin,2010; Benbelaid et al., 2012) who found that lavender oil has high antibacterial activity against the growth of *S. aureus*. Different results reported by (Serban et al., 2011) who found that lavender oils has moderately activities against *S. aureus*. Taiz and Ibb oils show antibacterial activity against *Salmonella* (Pasoua et al., 2005), and exhibited high activity against *Lactobacillus acidophilus*. These finding are differ to (Pasoua et al., 2005).

3.3. Antifungal Activity

The antifungal activity of *Lavandulla pubescens* oils (Taiz and Ibb) results represent in (Table 3 **)**

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Table 3. The antifungal activity of *Lavandulla pubescens* oils (Taiz and Ibb)

Fungal strains	Essential oils (µl)												Clotrim-azole
	Lavandulla pubescens (Taiz)						*Lavandulla pubescens* (Ibb)						
	15	10	5	2.5	1.2	0.6	15	10	5	2.5	1.2	0.6	10µg/mg
	Inhibition zone in mm												
Aspergillus flavus	39.0±0.0	25.0±0.0	23.0±0.0	4.0±0.0	2.0±0.0	0.0±0.0	39.0±00	24.0±0.0	20.0±0.0	4.0±0.0	2.0±0.0	0.0±0.0	7.0±0.0
Aspergillus umigatus	39.0±0.0	30.0±0.0	19.0±0.0	18.0±0.0	17.0±0.0	2.0±0.0	31.0±0.0	23.0±0.0	18.0±0.0	13.0±0.0	13.0±0.0	1.5±0.0	3.0±0.0
Aspergillus niger **(ATCC 16404)**	29.0±0.0	4.3±0.0	3.0±0.0	1.0±0.0	7.0±0.0	0.0±0.0	36.0±0.0	8.0±0.0	5.0±0.0	3.0±0.0	2.0±0.0	0.0±0.0	2.0±0.0
Candida albicans **(ATCC 2019)**	30.0±0.0	30.3±0.0	25.0±0.0	20.0±0.0	7.0±0.0	2.0±0.0	39.0±0.0	25.0±0.0	20.0±0.0	6.0±0.0	5.3±0.5	1.3±0.5	4.0±0.0

The data indicated that volatile oils of *L. pubescens* (Taiz), and *L. pubescens* (Ibb) showed high antifungal activities against the growth of *A. niger*. The present work revealed that, *L. pubescens* (Taiz), and (Ibb) have strong antifungal effects and even more effective than the positive control. In Romania (Serban et al., 2011) observed that *Lavandula hybrida* oil showed high antifungal activities against *C. albicans* by well agar diffusion. The high antibacterial activities of *L. pubescens* (Taiz) and *L. pubescens* (Ibb) volatile oil could be attributed to the high contents of carvacrol was 20.6% and 70% respectively.

The effectiveness of carvacrol as a natural antimicrobial is well established; however, the mechanism of action is less understood and is believed to be associated with damage to the cell membrane. The phenolic component of carvacrol has prompted research focused on its effect on structural and functional damage to cellular membranes (Sivropoulou et al., 1996; Ultee et al., 2000).

Ultee et al., (2002); Rattanachaikunsopon and Phumkhachorn (2009) showed that carvacrol destabilizes the cytoplasmic membrane and in addition, acts as a proton exchanger, thereby reducing the pH gradient across the cytoplasmic membrane. The resulting collapse of the proton motive force and depletion of the ATP pool eventually lead to cell death of bacterial cell. Chemical control of fungal pathogens remains as one of the main measures of reducing the incidence of post harvest diseases in various fruits and vegetables. However, due to the development of new strains of pathogens, many of these synthetic chemicals are gradually becoming ineffective (Tagne and Nguefack, 2000). In addition, due to their possible carcinogenicity, teratogenicity, high and acute toxicity, long degradation periods, environmental pollution and side effects on human beings the use of synthetic chemicals for controlling post harvest deterioration of food commodities is restricted (Lingk, 1991). The increasing recognition and importance of fungal infections and the difficulties encountered in their treatment have stimulated the search for alternatives for synthetic chemical fungicides. Spoilage and poisoning of foods by fungi is a major problem, especially in developing countries. *Aspergillus* is the most Important fungi causing spoilage of foodstuffs in Africa (Nickelsen and Jakobsen, 1997). Current study supports the traditional use of aromatic plants as antimicrobial agents.

4. CONCLUSION

These results confirm the use of this plant by the ancient people as a medicinal plant with antiseptic effects since it has an interesting effect on a variety of microbial species such as *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Canidida albicans* . These strains are the species involved in nosocomial infections as well as *Listeria monocytogenes* and *Bacillus cereus* implicated in food poisoning, which explains the use of this plant in traditional medicine.

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