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RESEARCH ARTICLE

ANTIMICROBIAL ACTIVITY OF ESSENTIAL OIL OBTAINED FROM LEAVES AND ROOTS OF *LAVANDULA PUBESCENS*

Abdullah AA Ali[1*], Hisham MA Alarz[2], Osama AA Amer[2], Bashar HA Masoud[2], Abdulmajeed AY Aljahfli[2], Helmi AA Alselwi[2], Zaid MA Alseraji[3], Abdulnaser SA Aklan[4], Mohammed AN Abbas[4], Hajer AA Qushasha[2]

[1]*Pharmacy Section, Medical Sciences Department, Amran Community College, Republic of Yemen.*
[2]*Pharmacy Department, High Institute of Health Sciences, Sana'a, Republic of Yemen.*
[3]*Pharmacy section, Medical Sciences Department, Sanhan Community College, Republic of Yemen.*
[4]*Pharmacy Department, Al-Hikma University, Republic of Yemen.*

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***Address for Correspondence:**
Dr. Abdullah A. A. Ali, Pharmacy Section, Medical Sciences Department, Amran Community College, Republic of Yemen. Tel-+967-771555216.
E-mail: *abduallah201312@gmail.com*

Abstract

Background: Yemen is home to 23 genera and 23 species of the *Lamiaceae* family. *Lamiaceae* essential oils have shown the strongest antibacterial activity against a variety of pathogens, such as *Candida albicans*, *Staphylococcus aureus*, *Aspergillus fumigatus*, and *Escherichia*. These properties may be attributed to the main constituents of *Lamiaceae* essential oils, such as carvacrol, thymol, p-cymene, 1, 8-cineole, and caryophyllene.
Method: Gram-positive bacteria (*Staphylococcus aureus*, *Streptococcus viridans*.), Gram-negative bacteria (*Klebsiella pneumoniae*, *Escherichia coli*, and *Proteus vulgaris*), and a series of bacteria available in the institute laboratory's stock culture were used for antibiotic sensitivity testing. The dried leaves and roots of the plant were chopped into small pieces, and the essential oil was extracted from each part by hydro distillation for 12 hours using a Clevenger-type all-glass apparatus. The oil was then transferred to a screw-capped glass vial, dried (Na_2SO_4), and kept at 4°C in the dark until analysis.
Results: Higher concentrations of the extract result in stronger inhibition. Gram-positive bacteria were more susceptible to the extract than Gram-negative bacteria, possibly because of differences in their cell wall structure. *Lavandula pubescens* essential oil has strong antimicrobial properties, especially against *Staphylococcus aureus* and *Proteus vulgaris*.
Conclusions: Gram-positive bacteria were more susceptible to the extract than Gram-negative bacteria, possibly because of differences in their cell wall structure. This study supports the potential use of *Lavandula pubescens* as a natural antimicrobial agent, which could be further investigated for pharmaceutical applications. The essential oil of the plant has significant antimicrobial properties, especially against *Staphylococcus aureus* and *Proteus vulgaris*. The antimicrobial effect is concentration dependent, with higher concentrations of the extract leading to stronger inhibition.
Keywords: Antimicrobial activity, essential oils, *Lavandula pubescens*, leaves.

INTRODUCTION

The largest family in the order *Lamiaceae*, with 252 genera and 6700 species, is the mint family of flowering plants, *Lamiaceae* (previously known as *Labiatae*). The *Lamiaceae* family is found almost everywhere, and many of its species are grown for their fragrant foliage and eye-catching blossoms. When it comes to flavor, smell, or therapeutic purposes, the family is very important[1,2]. The *Lamiaceae* family comprises 23 genera and 23 species that are native to Yemen. *Lamiaceae* essential oils have demonstrated the strongest antibacterial activity against a variety of pathogens, such as *Candida albicans*, *Staphylococcus aureus*, *Aspergillus fumigatus*, and *Escherichia coli*[3,4]. These characteristics may be caused by the main constituents of *Lamiaceae* essential oils, including carvacrol, thymol, p-cymene, 1,8-cineole, and caryophyllene[5-7]. Foodborne illnesses are a growing global public health concern due to the rise in microbial resistance to several antibiotics[8]. In developing countries, foodborne microorganisms are the main cause of disease and mortality[9] . The spectrum of foodborne pathogens includes a wide range of

intestinal bacteria, aerobes and anaerobes, viral pathogens, and parasites. *E. coli, Shigella, Salmonella,* and *S. aureus* are regarded as the most prevalent foodborne pathogens[10]. Due to the "back to nature" movement, growing concerns about food safety, and consumer demand for natural products that are good for the environment, naturally occurring antimicrobial compounds are becoming more and more popular. Essential oils have a wide range of antibacterial properties[11], and many plant extracts can be used to preserve food.

The main goal of this study is to investigate the antibacterial activity of *L. pubescens* leaf oil against both gram positive and gram-negative microorganisms.

MATERIALS AND METHODS

All chemical and solvent was bought from a commercial supplier (Himedia Laboratories, Loba Chemie).

Collection and preparation of sample:

During the rainy season in August 2024, *L. pubescens*, a medicinal *Lamiaceae* species, was gathered from various areas in the Bani Matar District of the Sanaa governorate, Yemen. Using the taxonomic and floristic literature, Dr. Fuad Al-hood of Aden University that was available to specimens identified. Every plant portion that was gathered was cleaned. Additionally, it was allowed to dry fully in a shaded area for two weeks. An electrical mill was then used to grind the dry specimens.

Isolation of the essential oil:

The dried branches and new leaves of the plant were cut into small pieces, and the essential oil was hydro-distilled for 12 hours using a Clevenger-style all-glass apparatus to separate it from each component. Each oil was then transferred to a screw-top glass vial, dried (using Na_2SO_4), and stored at 4°C in the dark until analysis.

Antimicrobial screening:

Antibiotic sensitivity testing was conducted using a number of bacterial strains that were accessible in the institute laboratories' stock culture, including: *S. viridance* and *Staphylococcus aureus* are examples of gram-positive bacteria. Bacteria that are Gram-negative (*K. pneumonia*) *P. vulgaris* and *E. coli*. As a negative control, the previously made essential oil was diluted 1/1, 1/2, and 1/3 v/v in dichloromethane (DCM). Mueller Hinton agar medium injected with the test species' bacterial solution was used to use the agar diffusion method[3]. Discs having a diameter of 5 mm were impregnated with either the control or the oils. The discs were then inserted into the culture medium's surface. Standard antibacterial discs containing gentamycin, and ofloxacin were utilized, respectively. The minimum inhibitory concentrations (MIC) of *L. pubescence* oil against the tested microorganisms were also ascertained by micro dilution method[3], and the diameters of the inhibition zones were measured in millimeters after the plates were incubated at 35–37°C for 24 hours in the case of bacteria.

Statistical analysis

The data were analyzed using SPSS version 21. The results are provided as mean ± SEM. One-way analysis of variance (ANOVA) followed by Turkey's HSD post hoc test was used to compare results across and within the groups. The results were considered significant when $p<0.05$.

RESULTS AND DISCUSSION

The study evaluated the antimicrobial activity of *L. pubescens* essential oil against five bacterial strains using the agar well diffusion method. The results revealed that the essential oil exhibited strong antimicrobial effects, with varying levels of inhibition depending on bacterial species, oil concentration, and bacterial type (Gram-positive vs. Gram-negative). For Gram-positive bacteria, *S. aureus* showed significant sensitivity to the oil extract. At a 1:1 concentration, the oil produced an inhibition zone of 34 mm, out-performing gentamicin (25 mm). As the concentration decreased (1:2 and 1:3), the inhibition zones reduced to 25 mm and 22 mm, respectively, indicating a concentration-dependent effect.

Similarly, *Streptococcus viridance* exhibited inhibition zones of 27 mm (1:1), 22 mm (1:2), and 20 mm (1:3), slightly lower than those observed for *S. aureus* but still demonstrating notable antimicrobial activity. For Gram-negative bacteria, *E. coli* was moderately affected by the oil extract, showing inhibition zones of 22 mm (1:1), 20 mm (1:2), and 19 mm (1:3), slightly lower than the standard antibiotic ofloxacin (25 mm). *K. pneumoniae* showed weaker susceptibility to the oil extract, with inhibition zones of 18 mm (1:1), 15 mm (1:2), and 13 mm (1:3), suggesting a higher resistance level. However, *P. vulgaris* was highly susceptible to the extract, with inhibition zones of 35 mm (1:1), 24 mm (1:2), and 17 mm (1:3), even surpassing ofloxacin (24 mm) at higher concentrations.

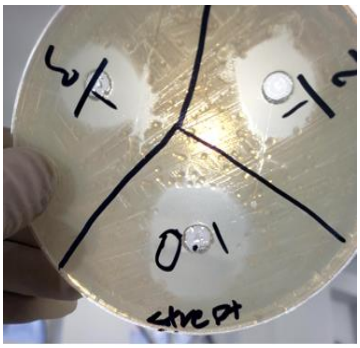
Table1: Antimicrobial activity of the oil of *L. pubescens* as inhibition zone diameter.

Essential oils	*S. aureus*	*S. viridiance*	*E. coli*	*K. pneumoniae*	*P. vulgaris*
Disc diffusion assay					
1:1	34±0.37	35±0.12	22±0.58	18±0.63	27± 0.25
1:2	25±0.44	24±0.14	20±0.12	15±0.37	22±0.37
1:3	22±0.25	17±0.40	19±1.2	13±0.37	20±0.58
Positive control					
Gentamycin	25±0.44				25±0.44
Ofloxacin		24± 0.34	25± 0.14	24±0.34	

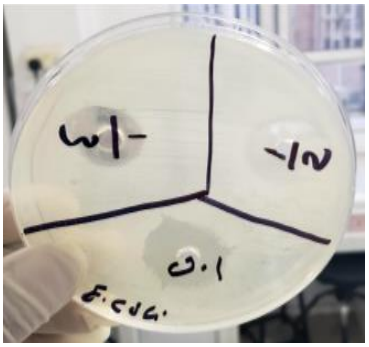
Inhibition zone diameter (mm)**;** Results are Mean±SD of triplicate values.

6-10 mm: no activity; 12-15 mm: low activity; 16-19 mm: good activity; above 19 mm: significant activity.

S. aureus

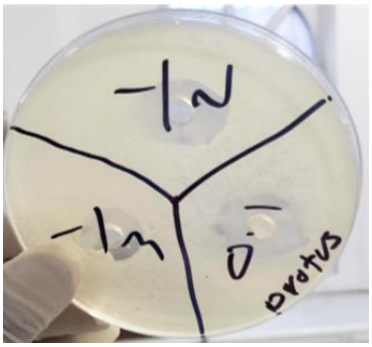


S. viridance



E. coli

K. pneumonia



P.vulgaris

Figure 1: Antimicrobial activity of essential oil against different bacteria.

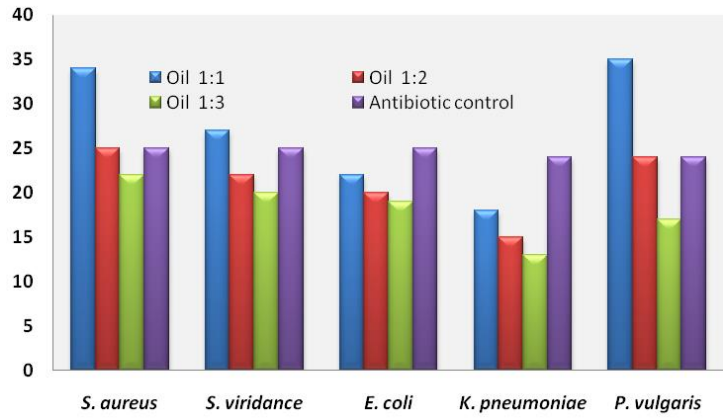


Figure 2: Antimicrobial activity of *L. pubescens* oil against antibiotics.

Overall, the results indicate that *L. pubescens* essential oil has strong antimicrobial potential particularly against Gram-positive bacteria. Its effectiveness appears to be concentration-dependent, with higher concentrations yielding stronger inhibition. While Gram-negative bacteria showed some resistance, *P. vulgaris* was highly susceptible to the oil. These findings suggest that *L. pubescens* essential oil could be a potential natural antimicrobial agent, warranting further studies to explore its full pharmaceutical applications.

Limitation of study:

The effect of the oil on human cells was not checked Future studies should conduct cytotoxicity assays to determine safe concentrations. The study assumes the antimicrobial activity is due to carvacrol, thymol, p-cymene, and 1,8-cineole ,but the exact composition was not confirmed using GC-MS analysis. The study only tested a few bacterial strains. Future research should evaluate its effects on fungi (e.g., *C. albicans* and viruse). Essential oils degrade over time. Stability tests should be conducted to determine the shelf life of the extract under different conditions (light, temperature, humidity).

CONCLUSIONS

Significant antibacterial activity is exhibited by the essential oil of *L. pubescens*, especially against *Proteus* sp. and *S. aureus.* The antimicrobial impact is concentration dependent, with stronger inhibition occurring at higher extract concentrations. Perhaps as a result of variations in the structure of their cell walls, Gram-positive bacteria were more vulnerable to the extract than Gram-negative bacteria. The possible utility of *L. pubescens* as a natural antibacterial agent is supported by this study and may be explored further for use in pharmaceutical applications.

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AUTHOR'S CONTRIBUTION

Ali AAA: writing original draft. **Alarz HMA:** writing methodology. **Abbas MAN**: investigation. **Alseraji ZMA:** formal analysis. **Aklan ASA:** data curation. **Alselwi HAA:** lab prepration. **Amer OAA:** conceptualization. **Masoud BHA:** writing, review and editing. **Qushasha HAA:** writing. **Aljahfli AAY:** review and editing. Final manuscript was checked and approved by all authors.

DATA AVAILABILITY

Data will be `made available on request.

CONFLICT OF INTEREST

None to declare.

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