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Evaluation of the antibacterial potency of *Thymus algeriensis* essential oil against nosocomial bacterial strains

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Abstract

Introduction. The emergence of multi-drug resistant bacteria, and the toll healthcare-associated infections have been causing globally with the development of human therapy increased the overall interest in alternative natural prevention and treatment strategies. Aromatic extracts generally and essential oils specifically were proposed as potential prevention, or complementary therapy against bacterial infections, especially the nosocomial ones.

Objectives. The *Thymus* genus, with its various species, was subject to investigation worldwide, and this study explored the in-vitro antibacterial activity of the formerly chemically identified Algerian northern central *Thymus algeriensis*.

Method. Three bacterial strains were employed (i.e., *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli*) based on their prevalence in Algerian health institutions.

Results. The analysis revealed the sensitivity of Escherichia coli and Staphylococcus aureus to the essential oil activity, while Pseudomonas aeruginosa was resistant. The results comparison to local and regional research groups' outputs suggests that Linalool, a major component of the present study essential oil, is a main vector of the antibacterial activity, and its quantitative variation results in drastic activity shifts.

Conclusions. This work highlighted the selective sensitivity of *Escherichia coli* and *Staphylococcus aureus* to *Thymus algeriensis* essential oil and insist on the chemical identification of local aromatic species before aromatherapy implementation.

Keywords: Essential oils; Antibacterial activity; *Thymus algeriensis*; Nosocomial infections; Aromatogram.

1. Introduction

The use of essential oils for therapeutic purposes has generated increasing interest as potential complementary therapy against bacterial infections, particularly nosocomial, or healthcare-associated infections (HCAIs)(1); These arose as global major health concerns causing the death of 1 out of each 17 patients acquiring HCAIs among the estimated 1.7 million cases(2). In light of the emergence of multi-drug resistant (MDR) bacteria, the economic and clinical stress they generate on health systems(3), and the continuous efforts seeking novel prevention/treatment strategies(4,5), the use of essential oils bears the premise to offer a cost-effective, wide scope primary prevention strategy(1,6,7).

In this context, the *Thymus* genus with its various species has attracted significant attention due to its antibacterial properties reported by various authors scattered through spread locations. (8–11)

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Based on the hypothetical antibacterial activity of *Thymus Sp* extracts (12), the present study object to evaluate the in-vitro activity of the Algeria northern central *Thymus algeriensis* essential oil qualitatively and quantitatively on three bacterial strains selected based on their pathogenicity, availability, and role in nosocomial infections in Algeria(13) and globally(14) (i.e., *Staphylococcus aureus, Pseudomonas aeruginosa,* and *Escherichia coli*).

2. Materials and Methods

2.1. Sample collection and preparation

The plant material was gathered in November, specifically during the dry season. Healthy wild specimens were selected, and the upper stem portion was cut. These specimens were found to be growing at an altitude of 150 meters in the Bougara region of Blida, Algeria (latitude: 36.4718377, longitude: 3.1077277).

To prepare the material for extraction, it was first spread out and allowed to dry naturally in a well-ventilated chamber with amber lighting. This drying process lasted 3 months. Importantly, the integrity of the stems was carefully maintained throughout the entire drying period(15).

2.2. Species Identification

The sample was identified through a comprehensive analysis involving macroscopic and microscopic examination, as well as gas chromatography coupled with mass spectrometry chemotype determination(15).

2.3. Antibacterial activity assessment

The following experimentations were conducted at the microbiology laboratory of Hussein Dey University Hospital Center – Algiers – according to the Clinical and Laboratory Standards Institute (CLSI) guidelines on antimicrobial susceptibility testing.

2.4. Test strains isolation

American Type Culture Collection (ATCC) strains of *Staphylococcus aureus* (ATCC 25923), *Pseudomonas aeruginosa* (ATCC 27853), and *Escherichia coli* (ATCC25922) were invested as test samples for the antibacterial activity evaluation.

Apparent colonies of three strains were sampled from the ATCC petri dishes via a sterile Pasteur pipette and seeded promptly into nutrient agar. The four (4) quarters dilution method was used. The freshly seeded petri dishes were incubated at 37°C for 24 hours for further experimentations.

2.5. Aromatograms

From the reisolated strains, using a Pasteur pipette sterilized with the blue flame of the Bunsen burner, the most individualized colonies were sampled and diluted into physiological water. The obtained solution's turbidity was adjusted to 0.5 McFarland. A sterile swab was soaked with the bacterial suspension then spin against the wall of the tube to remove any excess before inoculation into a Muller Hinton agar. The tight streaks method was repeated three times with successive 60 degrees (°) rotations of the petri dish. Last, to ensure a homogeneous distribution of the bacterial suspension, the swab was used to seed the petri dish walls. The seeded dishes were set to rest near the Bunsen burner during the preparation of the antibiogram discs.

Six (6) millimetres (mm) diameter cellulose discs cut on Whatman No. 1 paper were used to prepare the aromatogram discs; These were impregnated with *Thymus algeriensis* essential oil extracted and conserved as priorly described (15) then placed on the freshly seeded Petri dishes and incubated 24 hours at 37 °C. The dilution ratios and evaluation techniques were adapted according to the below-described methodologies (3).

2.6. Qualitative analysis

During this first screening, the aromatograms were conducted by applying discs impregnated with the undiluted essential oil on the three (3) selected strains, then the inhibition diameter (ID) measurements in millimetres (mm) were interpreted according to Menna and Sethi (1994)(16) scaling system, described as follows:

- Strong growth inhibition (+++): ID > 28 mm, corresponds to an extremely sensitive strain.
- Moderate growth inhibition (++): ID = 16-28 mm, corresponds to a very sensitive strain.
- Light growth inhibition (+): ID = 10-16 mm, corresponds to a sensitive strain.

- Absence of inhibition (-): ID < 10 mm, corresponds to a resistant strain.

All the diameters were measured from the exposure discs centre to the periphery of the inhibition zone.

2.7. Quantitative analysis

The sensitive, very sensitive, and extremely sensitive strains were selected and subjected to quantitative analysis to determine the dose-inhibition response.

The pure essential oil was sequentially diluted four (4) times observing a 1:1 ratio of active material and dilution solution for each point. The newly prepared discs were applied to freshly seeded Muller Hinton agar, divided into four (4) frames corresponding to the four (4) dilutions in order to compare the inhibitory effects while reducing the seeding variability effects on the test. The readings were performed after 24 hours of incubation at 37 °C. Menna and Sethi's (1994)(16) scaling was used for the interpretation.

3. Results and Discussion

3.1. Aromatograms and qualitative analysis

The results of the isolated strains and aromatograms are displayed in **Figure 1**.

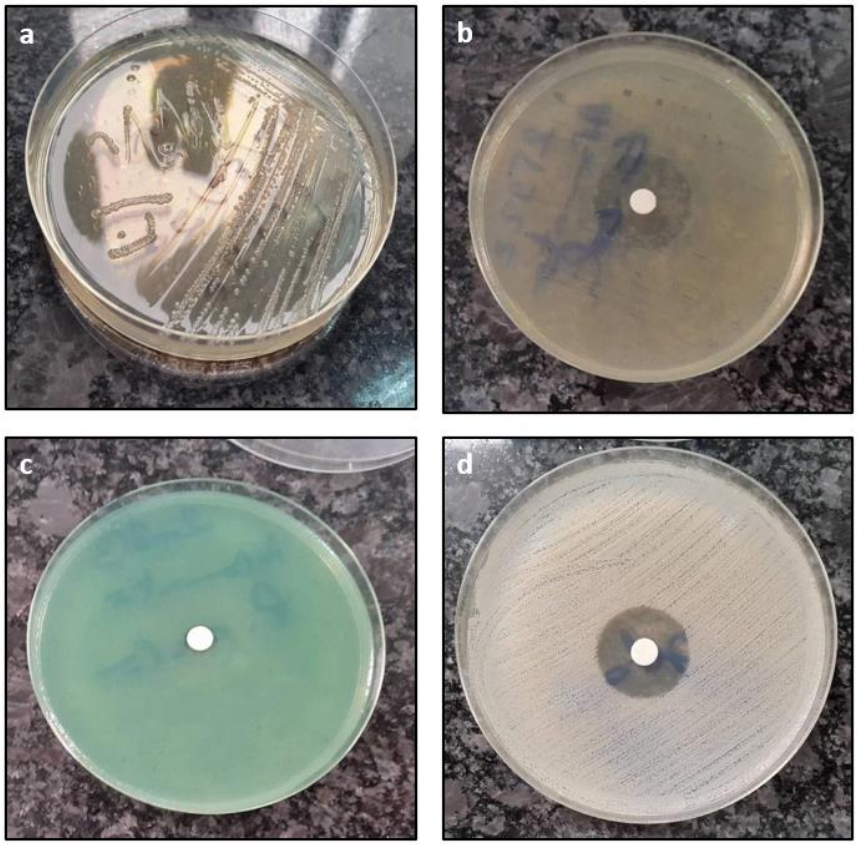

Figure 1 Bacterial strains isolation and aromatograms results. (a) ATCC 25922 *Escherichia coli* isolated strain. (b) ATCC 25922 *Escherichia coli* aromatogram. (c) ATCC 27853 *Pseudomonas aeruginosa* aromatogram. (d) ATCC 25923 *Staphylococcus aureus* aromatogram.

The results of the qualitative analysis after metrics measurement are reported in **Table 1**:

Table 1 Qualitative antibacterial activity of the essential oil of *Thymus algeriensis.* [a] - the diameter of the growth inhibition zone in millimetres includes the disc diameter (6mm).

ATCC Strain	***Escherichia coli***	***Staphylococcus aureus***	***Pseudomonas aeruginosa***
Inhibition Diameter (mm)[a]	24	21	<6
Sensitivity Grade (Meena and Seth 1994)	Very Sensitive (++)	Very Sensitive (++)	Resistant (-)

Compared with the study of Dob et al., (2006)(17) evaluating the antibacterial activity of the Algerian northeastern *Thymus algeriensis,* surprising differences were noted. While we have found the investigated essential oil to be active on *Escherichia coli* and *Staphylococcus aureus,* and not on *Pseudomonas aeruginosa,* Dob et al., (2006) found no activity on the three species. Although the major component of the two species' essential oil was revealed to be Linalool, its relative abundance was superior in the central *Thymus algeriensis* (i.e., 69.2% compared to 43.3% in Dob et al., (2006) study), suggesting that Linalool is a main vector of the species antibacterial activity on the tested strains.

It is noteworthy that the Moroccan variant of *Thymus algeriensis* characterised by a dominant camphor and α-pinene phenotype (27.7%, and 20.5% respectively) were found to be active on *Escherichia coli* and *Staphylococcus aureus* by Amarti et al., (2010)(18). This observation, combined with Dob et al., (2006) results might indicate that *Thymus algeriensis* essential oil, through its variable components, can convey an antibacterial activity through differential, and potential synergic mechanisms.

Linalool has been reported bacterial membrane disruptor through oxidative stress(19) while Thymol was suggested to disturb the bacterial cellular integrity through permeabilising the membrane(20).

3.2. Quantitative analysis

Table 2 Quantitative antibacterial activity of the essential oil of *Thymus algeriensis.* [a] - the diameter of the growth inhibition zone in millimetres includes the disc diameter (6mm).

Essential oil concentration dilution ratio	**ATCC strain**		**Interpretation**	
	Escherichia coli	*Staphylococcus aureus*	*Escherichia coli*	*Staphylococcus aureus*
	Inhibition Diameter (mm) [a]			
1:2	24	27	Very sensitive	Very sensitive
1:4	34	24	Extremely sensitive	Very sensitive
1:8	30	14	Extremely sensitive	Sensitive
1:16	11	8	Sensitive	Resistant

Figure 2 and **Table 2** display the result of the quantitative analysis of the tested essential oil by the dilution and sic diffusion method.

A selective activity on *Escherichia coli* can be noted as the strain remained sensitive to the lowest dilution ratio, while a resistance of *Staphylococcus aureus* was noted at the lowest concentration.

These results emphasize the critical role the essential oil chemotype has on the species activity as the Algerian northeastern *Thymus algeriensis* showed no activity against neither *Escherichia coli* nor *Staphylococcus aureus*, while the Moroccan species did(17,18).

Although non-chemically determined, Messaoudi et al., (2019)(11) Investigated the effect of methanolic and ethanolic extract of the Algerian southwestern *Thymus algeriensis* and concluded on a stable inhibitory action towards *Escherichia coli* and *Staphylococcus aureus.*

Interestingly, the present results highlight the shift analytical determination of the chemical print of aromatic species had on prior reports on the general inactivity of essential oils on Gram-negative and positive bacteria(21, 22).

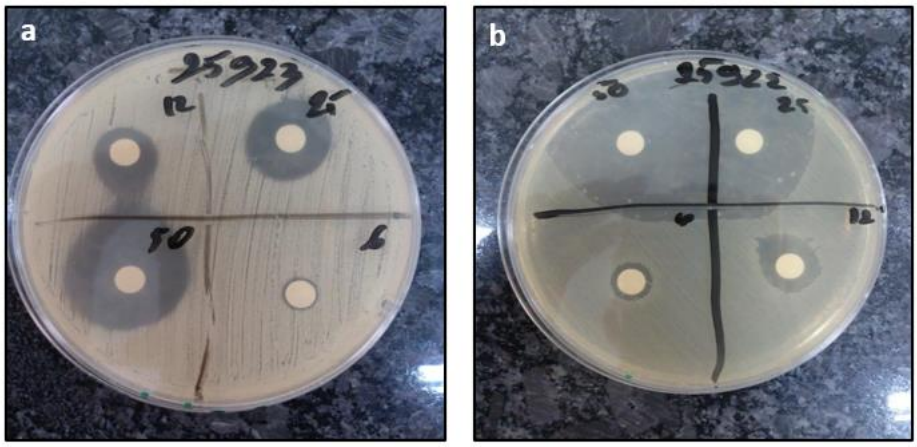

Figure 2 Results of the quantitative analysis of the antibacterial activity of *Thymus algeriensis* on (a) ATCC 25923 *Staphylococcus aureus*, and (b) ATCC 25922 *Escherichia coli*.

4. Conclusion

The current study, aside from revealing the promising potential of *Thymus algeriensis* essential oil as a prevention strategy against common HCAIs responsible bacteria in Algeria, highlights the importance of chemical identification of local aromatic species prior to therapeutic investment as compositional difference may induce dramatic activity, or efficiency discrepancies. Similarly, the standardisation of chemotype determination and activity testing among laboratories appears to grow as a fundamental necessity. Finally, although the promising nature of aromatherapy against MDR bacterial infection, further inherent toxicity evaluations are needed to ensure its proper implementation.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors and all co-authors declare that they have no conflicts of interest in connection with this document.

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