fortune Journals

Original Research Article

ARCHIVES OF MICROBIOLOGY & IMMUNOLOGY

ISSN: 2572-9365

Antibacterial Activity and Antibiofilm of Essential Oils of Clove and Thyme Against Clinical Isolates of Multidrug-Resistant *Acinetobacter Baumannii* Associated with Nosocomial Infections

Nihal EZZARIGA[*, 1], Oumaima ZOUHARI[2], Amal RHARS[1], Asmaa WAKRIM[3], Mohamed AGHROUCH[4], Zohra LEMKHENTE[1]

Abstract

In recent years, *Acinetobacter baumannii* infections have drawn significant medical interest. This opportunistic pathogen often causes nosocomial infections. The antibiotic resistance in *A. baumannii* biofilms complicates treatments, necessitating new control strategies. This study evaluated the antibacterial and antibiofilm activities of clove and thyme essential oils. Seven multidrug-resistant *A. baumannii* strains, including six clinical isolates and one reference strain (ATCC 19606), were tested. Essential oils demonstrated strong antibacterial effects, with inhibitory zone diameters (IZDs) of 14-31 mm for *Syzygium aromaticum* and 17-39 mm for *Thymus vulgaris*. MIC values ranged from 0.78 to 12.5µl/ml for *S. aromaticum* and 0.19 to 12.5µl/ml for *T. vulgaris*. All strains formed biofilms, and the oils effectively inhibited biofilm formation. These results suggest that clove and thyme oils could offer new strategies for managing nosocomial infections.

Keywords: Acinetobacter baumannii, Nosocomial infections, Clove (*Syzygium aromaticum*), Thyme (*Thymus vulgaris*), Antibacterial activity, Antibiofilm activity

Introduction

Acinetobacter baumannii is a Gram-negative opportunistic bacterium with a remarkable ability to colonize humans. It is now a major nosocomial pathogen due to its resistance to conventional antibiotic treatments and its persistence in hospital environments [1]. Classified as a priority pathogen by the World Health Organization (WHO) since 2017, *A. baumannii* represents a global health threat, particularly due to its resistance to carbapenems, which are often considered the last therapeutic option against multidrug-resistant infections [2]. Infections caused by multidrug-resistant *A. baumannii* strains are particularly difficult to treat and are associated with higher mortality rates than those caused by other resistant pathogens [3]. Its ability to form biofilms on hospital surfaces and persist in healthcare settings promotes its spread, especially in intensive care units [4]. These biofilms, composed of complex microbial structures surrounded by an extracellular matrix rich in polysaccharides, provide *A. baumannii* with increased protection against antimicrobial agents, making the treatment of biofilm-associated infections particularly challenging [5,6]. Thus, the persistence of this bacterium and its ability to cause severe nosocomial infections refractory to treatment make it a major public health concern.

Affiliation:

[1]Faculty of Medicine and Pharmacy of Agadir, University Ibn Zohr, Agadir 80060, Morocco.

[2]Municipal Office of Cleaning of Agadir, Morocco.

[3]Private University of Health and Sciences of Agadir, Morocco.

[4]Hassan II Regional Hospital Center of Agadir, Morocco.

***Corresponding author:**
Nihal EZZARIGA, Faculty of Medicine and Pharmacy of Agadir, University Ibn Zohr, Agadir 80060, Morocco.

Citation: Nihal EZZARIGA, Oumaima ZOUHARI, Amal RHARS, Asmaa WAKRIM, Mohamed AGHROUCH, Zohra LEMKHENTE. Antibacterial Activity and Antibiofilm of Essential Oils of Clove and Thyme Against Clinical Isolates of Multidrug-Resistant *Acinetobacter Baumannii* Associated with Nosocomial Infections. Archives of Microbiology and Immunology. 9 (2025): 90-97.

Received: February 28, 2025
Accepted: March 03, 2025
Published: March 10, 2025

Nihal EZZARIGA, et al., Arch Microbiol Immunology 2025
DOI:10.26502/ami.936500213

In response to this issue, essential oils (EOs), such as those of *Syzygium aromaticum* and *Thymus vulgaris*, are gaining increasing interest due to their antimicrobial properties. They contain bioactive compounds, such as eugenol and thymol, capable of disrupting bacterial cell membranes, altering enzymatic processes, and inducing cell lysis. Moreover, these compounds can penetrate the protective barrier of biofilms, disperse them, and directly target bacterial cells. Thanks to these properties, EOs are increasingly studied as potential alternatives to conventional antibiotics, with a reduced risk of resistance development. They are particularly used for wound disinfection, treatment of skin and respiratory infections, and the management of nosocomial infections caused by multidrug-resistant strains such as *A. baumannii* [7,8]. Thus, the present study aims to evaluate the antibacterial and antibiofilm activity of *Syzygium aromaticum* and *Thymus vulgaris* essential oils against clinical isolates of multidrug-resistant *A. baumannii*, collected from the hospital environment of the intensive care units of the Regional Hospital Center of Agadir, Morocco.

Materials and Methods

Essential oils

The essential oils of *Syzygium aromaticum* (clove) and *Thymus vulgaris* (thyme) were evaluated for their antibacterial and antibiofilm activities. These oils were extracted through hydrodistillation and were subsequently purchased from a commercial supplier. The oils were solubilized in a 10% dimethyl sulfoxide (DMSO) solution to prepare working stock solutions.

Bacterial strains

The antibacterial and antibiofilm activities of the essential oils were tested against *Acinetobacter baumannii* strains, including one reference strain (ATCC 19606) and six clinical isolates associated with nosocomial infections. The clinical isolates were obtained from the following sources: one strain from protected distal sampling (PDS), three strains from cytobacteriological examinations of urine (CBU), and two strains from catheters. All isolates were sourced from patients in intensive care units and were confirmed to be *A. baumannii* through standard microbiological identification techniques.

Antibacterial activity

Antibacterial activity was assessed using the disk diffusion method [9]. Sterile filter paper discs (6 mm diameter) were impregnated with 10 µL of each essential oil and placed on Mueller-Hinton agar plates inoculated with bacterial suspensions standardized to 10^6 CFU/mL. The plates were incubated at 37°C for 24 hours, and the inhibition zone diameters (IZDs) were measured in millimeters. Bacterial sensitivity was classified as follows [10]:

Non-sensitive: IZD ≤ 8 mm Sensitive: 9 mm ≤ IZD ≤ 14 mm

Very sensitive: 15 mm ≤ IZD ≤ 19 mm Extremely sensitive: IZD ≥ 20 mm

Minimum Inhibitory Concentrations (MIC) and Minimum Bactericidal Concentrations (MBC)

Minimum Inhibitory Concentrations (MIC, µL/mL) and Minimum Bactericidal Concentrations (MBC) were determined using the broth microdilution method in 96-well microplates [11]. Serial dilutions of the essential oils were prepared in nutrient broth, and bacterial suspensions standardized to 10^6 CFU/mL were added. After 24 hours of incubation at 37°C, the MIC was recorded as the lowest concentration that inhibited visible bacterial growth. The MBC was identified as the lowest concentration at which no bacterial growth occurred upon subculturing onto agar plates. The MBC/MIC ratio was calculated to evaluate the bactericidal effect of HEs if MBC/MIC = 1-2 or its bacteriostatic effect if MBC/MIC = 4-16 [12]. All experiments were performed in triplicate, and results were expressed as mean values.

Antibiofilm activity

The biofilm formation was evaluated by the TCP (Tissue Culture Plate) method. The bacterial suspensions were inoculated into 96-well microplates, and after incubation for 24 hours at 37°C, the planktonic phase was gently removed, the wells were washed and dried, and then stained with purple crystal. The optical density of the biofilms formed was measured at 550 nm. Biofilm production was classified as negative, weak, moderate and strong based on the optical density threshold value, calculated according to the following formula [13].

Threshold value DO = DO of the negative control (3 × standard deviation of the DOs of the negative control) The criteria used were: DOm ≤ Threshold value DO Non-biofilm-former

Threshold value DOm ≤ 2 × Threshold value DO Low biofilm

2 × Threshold value DOm ≤ 4 × Threshold value DO Moderate biofilm-former

DOm 4 × Threshold value DO High biofilm formation

With: DOm: the average of the optical density of the three wells.

Finally, the inhibition of the initial attachment of bacterial cells was studied using a method adapted from Bazargani and Rohloff [14]. Semi-logarithmic dilutions of the essential oils corresponding to the MIC, MIC/2 and MIC/4 values were applied in 96-well microplates, followed by the addition of bacterial suspensions. After incubation at 37°C for 8 hours,

Nihal EZZARIGA, et al., Arch Microbiol Immunology 2025
DOI:10.26502/ami.936500213

the wells were rinsed, dried and then stained with purple crystal. Absorbance was measured at 590 nm to calculate the percentage inhibition of bacterial attachment compared to positive controls.

%Inhibition = (Mean OD control - Mean OD sample) ×100 / The Mean OD control

Statistical analysis

The values were obtained as mean ± standard deviation (SD) from three repetitions. Optical density values obtained for the different bacterial strains were organized in an Excel table, and the statistical analysis was carried out using an analysis of variance. The results were analyzed using Excel, where a p-value of less than 0.05 was considered significant.

Results

Antibacterial activity of essential oils

Diameter of inhibition zones

The essential oils of *Syzygium aromaticum* and *Thymus vulgaris* showed significant antibacterial activity against *A.baumannii* strains, measured by the diameters of the inhibition zones (± SD) (Table 1). *Thymus vulgaris* exhibits varying antibacterial properties against different strains of *Acinetobacter baumannii*. *Syzygium aromaticum* shows inhibition zones (IZD) ranging from 14 ± 1.2 mm for strain N6 (sensitive) to 31 ± 1.5 mm for strain N2 (extremely sensitive), with notable effectiveness against strains ATCC 19606, N2, and N4. *Thymus vulgaris* demonstrates more pronounced activity, with IZDs ranging from 16 ± 1.5 mm to 39 ± 2.1 mm, and "extremely sensitive" effectiveness against strains ATCC 19606, N1, N2, N3, and N4. In comparison, *T. vulgaris* appears more effective, but S. aromaticum still performs well, particularly against strains ATCC 19606, N2, and N4. These results suggest that both essential oils have significant antibacterial potential against *A. baumannii*, although their mechanisms of action or intrinsic effectiveness may differ.

Determination of minimum inhibitory and bactericidal concentrations

Minimum inhibitory (MIC) and bactericidal (BMC) concentrations of *Syzygium aromaticum* (Table 2) and *Thymus vulgaris* (Table 3) essential oils were determined to evaluate their efficacy against *A.baumannii* strains. The determination of the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of *Syzygium aromaticum* (Table 2) and *Thymus vulgaris* (Table 3) essential oils reveals their efficacy against *Acinetobacter baumannii*. For *Syzygium aromaticum*, MIC values range from 0.78 µL/mL (N5, N6) to 12.5 µL/mL (N3, N4), and MBC values range from 0.78 µL/mL to 25 µL/mL. Strains N5 and N6 exhibit a bactericidal effect (MIC = MBC = 0.78 µL/mL), whereas others, such as ATCC 19606 and N3, show bacteriostatic activity (high MBC/MIC ratio). For *Thymus vulgaris*, MIC values range from 0.19 µL/mL (N1) to 12.5 µL/mL (N4, N6), and MBC values range from 0.39 µL/mL to 50 µL/mL. Strains N1, N2, and N5 demonstrate a bactericidal effect, while N3, N4, and N6 show bacteriostatic effects. In conclusion, both essential oils exhibit significant antibacterial activity against *A. baumannii*. *Syzygium aromaticum* is particularly effective against N5 and N6, while *Thymus vulgaris* acts more strongly on N1 and N2. The MBC/MIC ratios highlight differences in their mechanisms of action.

Table 1: Antibacterial activity of EHs Against seven strains of *A.baumannii* ± SD

Bacterial strains	*Syzygium aromaticum*		*Thymus vulgaris*	
	DIZ (mm)	Germ sensitivity	DIZ (mm)	Germ sensitivity
ATCC 19606	29 ± 0.7	extremely sensitive	32 ± 1.2	extremely sensitive
N1	17 ± 0.9	Very sensitive	36 ± 2.6	extremely sensitive
N2	31 ± 1.5	extremely sensitive	37 ± 1.2	extremely sensitive
N3	15 ± 0.3	Very sensitive	29 ± 2.3	extremely sensitive
N4	19 ± 0.6	Very sensitive	39 ± 2.1	extremely sensitive
N5	19 ± 0.7	Very sensitive	17 ± 2.3	Very sensitive
N6	14 ± 1.2	sensitive	16 ± 1.5	Very sensitive

Abbreviations: diameter of the inhibition zone (DIZ) ± SD and n=3.

Table 2: Minimum inhibitory and bactericidal concentrations of *Syzygium aromaticum* essential oil against *A.baumannii* strains.

Bacterial strains	*Syzygium aromaticum*			
	MIC (ul/ml)	MBC (ul/ml)	MBC/MIC	Effects
ATCC 19606	3,12	25	8	Bacteriostatic
N1	6,25	12,5	2	Bactericide
N2	1,56	12,5	8	Bacteriostatic
N3	12,5	25	4	Bacteriostatic
N4	6,25	25	4	Bacteriostatic
N5	0,78	0,78	1	Bactericide
N6	1,56	1,56	1	Bactericide

Abbreviations: MIC: minimum inhibitory concentration; MBC: minimum bactericidal concentration

Citation: Nihal EZZARIGA, Oumaima ZOUHARI, Amal RHARS, Asmaa WAKRIM, Mohamed AGHROUCH, Zohra LEMKHENTE. Antibacterial Activity and Antibiofilm of Essential Oils of Clove and Thyme Against Clinical Isolates of Multidrug-Resistant *Acinetobacter Baumannii* Associated with Nosocomial Infections. Archives of Microbiology and Immunology. 9 (2025): 90-97.

Nihal EZZARIGA, et al., Arch Microbiol Immunology 2025
DOI:10.26502/ami.936500213

Table 3: Minimum inhibitory and bactericidal concentrations of *Thymus vulgaris* essential oil against *A.baumannii* strains.

Bacterial strains	*Thymus vulgaris*			
	MIC (ul/ml)	MBC (ul/ml)	MBC/MIC	Effects
ATCC 19606	3.12	12.5	4	Bacteriostatic
N1	0.19	0.39	2	Bactericide
N2	3.12	6.25	2	Bactericide
N3	6.25	25	4	Bacteriostatic
N4	12.5	50	4	Bacteriostatic
N5	0.39	1.56	4	Bacteriostatic
N6	12.5	50	4	Bacteriostatic

Abbreviations: MIC: minimum inhibitory concentration; MBC: minimum bactericidal concentration

Antibiofilm activity of essential oils

Biofilm formation

The potential for biofilm formation and antibiotic resistance of six strains of *A. baumannii* isolated from different clinical sites were evaluated (Table 4) to better understand their resistance and behaviour in the presence of the essential oils studied. The table highlights the biofilm-forming potential and antibiotic resistance profiles of six *A. baumannii* strains from different clinical sites, revealing a strong correlation between biofilm formation and antimicrobial resistance. Five strains (N°2 to N°6) exhibit strong biofilm-forming ability and multidrug resistance, while the weak biofilm-forming strain (N°1) shows comparatively lower resistance, particularly to imipenem, norfloxacin, and gentamicin. Resistance to carbapenems (ertapenem and meropenem) is universal, with imipenem effective only against N°1. Fluoroquinolones (ciprofloxacin and levofloxacin) exhibit widespread ineffectiveness, with norfloxacin effective against strains N°1 and N°5. Among aminoglycosides, amikacin and gentamicin show limited efficacy, and tobramycin resistance is universal. The strong biofilm-forming capacity of most strains underscores their role in enhancing resistance, complicating treatment options and emphasizing the need for tailored strategies, including the exploration of antibiofilm agents and combination therapies, to combat these resilient strains.

Determination of biofilm inhibition

Inhibition of biofilm formation, was determined to evaluate the efficacy of *Syzygium aromaticum* (Figure 1) and *Thymus vulgaris* (Figure 2) essential oils against *A.baumannii* strains. The percentages of inhibition obtained illustrate the effectiveness of these oils in preventing bacterial fixation. Both figures demonstrate the antibiofilm effects of *Syzygium aromaticum* and *Thymus vulgaris* essential oils against *Acinetobacter baumannii*, showing a concentration-dependent inhibition (MIC, MIC/2, MIC/4). In both cases, the highest inhibition occurs at MIC, with the ATCC reference strain exhibiting the strongest response, followed by clinical isolates No. 1 and No. 4, which also show significant inhibition. Other isolates (No. 2, No. 3, No. 5, and No. 6) display moderate to low sensitivity, with reduced inhibition at lower concentrations. The variations in response suggest strain-specific differences in biofilm formation capabilities. These findings highlight the potential of both essential oils as promising antibiofilm agents, though their effectiveness varies among bacterial isolates.

Discussion

The risk of antibiotic-resistant hospital-acquired infections is particularly severe in intensive care units and other hospital settings, posing a significant threat to patient health [15]. Multidrug-resistant bacteria such as *Acinetobacter baumannii* and *Pseudomonas aeruginosa* are major contributors to these infections [16]. All the isolates analyzed exhibited resistance to one, two, or all three classes of antibiotics (carbapenems, fluoroquinolones, and aminoglycosides), classifying them as multidrug-resistant and associated with nosocomial infections. These findings align with previously reported

Table 4: Biofilm formation in *A. baumannii* strains and their antibiotic resistance profiles

Site of isolation	N°	Biofilm formation	Resistance profiles								
			Carbapenem			Fluoroquinolones			Aminoglycosides		
			ETP	MEM	IPM	NOR	CIP	LEV	AK	TOB	GEN
CBU	N°1	**WEAK**	R	R	S	S	R	R	R	R	R
Catheter	N°2	**STRONG**	R	S	R	R	R	R	R	R	R
PDS	N°3	**STRONG**	R	R	R	S	S	R	R	R	R
CBU	N°4	**STRONG**	R	R	R	S	S	R	R	R	R
Catheter	N°5	**STRONG**	R	S	R	S	R	S	R	R	R
CBU	N°6	**STRONG**	R	R	R	R	R	R	R	R	R

Abbreviations: CBU: cytobacteriological examination of urine. PDS: Protected distal sampling. ETP: ertapenem. MEM: meropenem. IMP: imipenem. Nor: norfloxacin. CIP: ciprofloxacin. LEV: levofloxacin. AK: amikacin. TOB: tobramycin. GEN: gentamicin. R: Resistant. S: Sensitive.

Citation: Nihal EZZARIGA, Oumaima ZOUHARI, Amal RHARS, Asmaa WAKRIM, Mohamed AGHROUCH, Zohra LEMKHENTE. Antibacterial Activity and Antibiofilm of Essential Oils of Clove and Thyme Against Clinical Isolates of Multidrug-Resistant *Acinetobacter Baumannii* Associated with Nosocomial Infections. Archives of Microbiology and Immunology. 9 (2025): 90-97.

Nihal EZZARIGA, et al., Arch Microbiol Immunology 2025
DOI:10.26502/ami.936500213

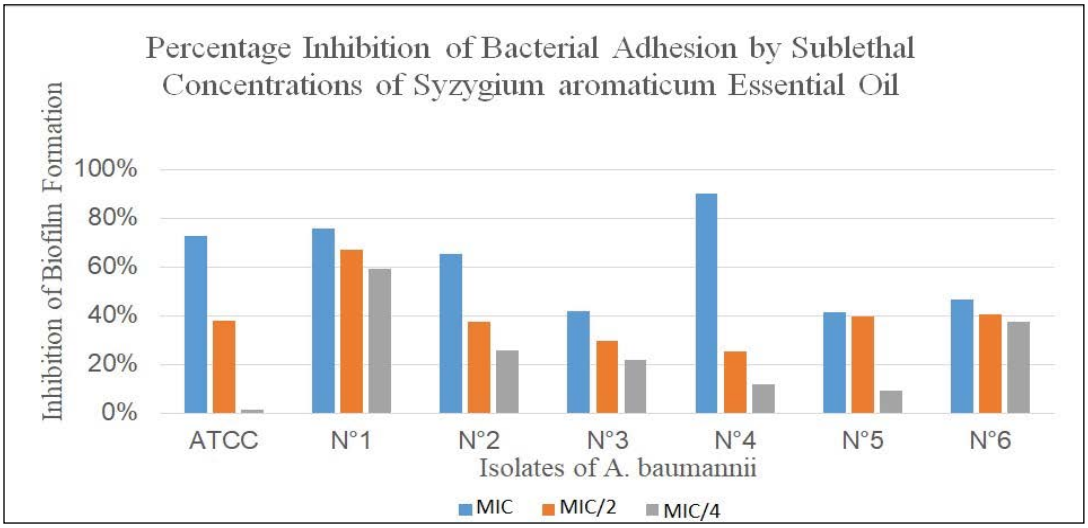

Figure 1: Percentage inhibition of *A. baumannii* fixation by sublethal concentrations of *Syzygium aromaticum* essential oil.

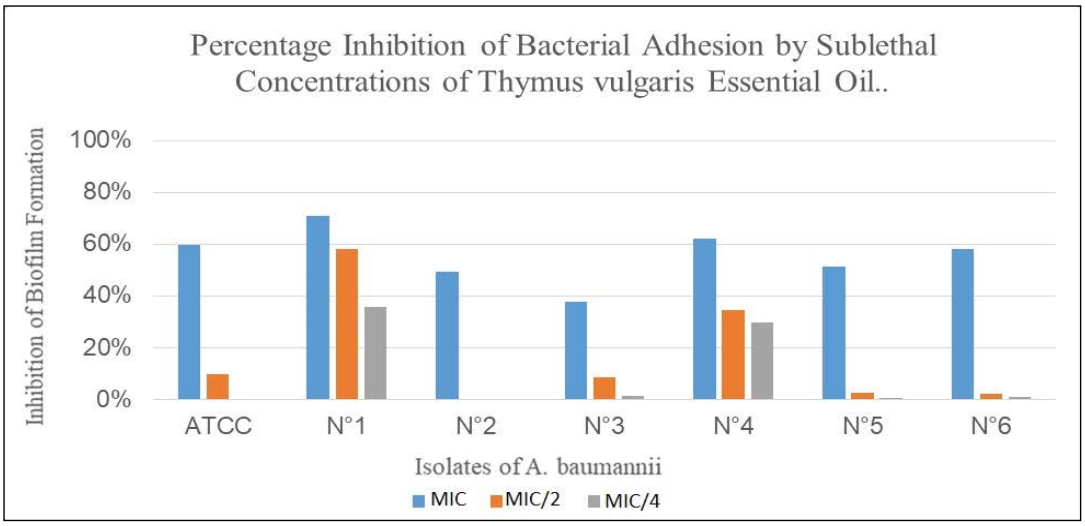

Figure 2: Percentage inhibition of *A.baumannii* fixation by sublethal concentrations of *Thymus vulgaris* essential oil.

studies. Plants have long been a valuable source of bioactive compounds, offering promising alternatives for biomedical applications. Essential oils contain highly concentrated bioactive molecules capable of exerting antimicrobial effects at low concentrations against multidrug-resistant bacteria [15]. Our results demonstrated that both essential oils exhibited strong activity against all *A. baumannii* strains, with inhibition zone diameters (IZD) ranging from 14 to 31 mm for *Syzygium aromaticum* (Myrtaceae) and from 17 to 39 mm for *Thymus vulgaris* (Lamiaceae), classifying them as highly to extremely sensitive. These findings are consistent with previous research, such as the study by Al Janabi and Asaad [17], which reported a 30 mm IZD for *S. aromaticum* essential oil against *Acinetobacter* strains. Similarly, Zeshan [18] observed an IZD of 15.4 mm, while Haddouchi and Benmansour [19] reported an IZD of 34 mm for *T. vulgaris* essential oil against *A. baumannii*. Additionally, Boukhatem et al. [20] recorded a 16 mm IZD against *A. baumannii* strains.

These findings corroborate those of Nzeako et al. [21], who highlighted the antimicrobial properties of clove and thyme essential oils, attributing their activity to the presence of bioactive compounds such as thymol, eugenol, flavones, phenolic monoterpenoid glycosides, and aliphatic alcohols. Determination of minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) confirmed the potent antibacterial activity of *S. aromaticum* and *T. vulgaris* essential oils against clinical multidrug-resistant *A. baumannii* strains. The MIC and MBC values exhibited variability, demonstrating both bacteriostatic and bactericidal effects depending on the strain and the essential oil used. For

Nihal EZZARIGA, et al., Arch Microbiol Immunology 2025
DOI:10.26502/ami.936500213

S. aromaticum, MIC values ranged from 0.78 to 12.5 µl/ml, while MBC values varied between 0.78 and 25 µl/ml. The oil exhibited bacteriostatic activity against *A. baumannii* ATCC 19606, N2, N3, and N4 strains, whereas it showed bactericidal activity against strains N1, N5, and N6. These results align with the findings of Zeshan [18], who reported MIC values between 2 and 6.25 µl/ml for *S. aromaticum* essential oil against *A. baumannii*. Similarly, Faujdar et al. [22] reported a MIC of 0.78 mg/ml and an MBC of 0.39 mg/ml for all *A. baumannii* isolates. For *T. vulgaris*, MIC values ranged from 0.19 to 12.5 µl/ml, while MBC values varied between 0.39 and 50 µl/ml. The oil exhibited bacteriostatic activity against *A. baumannii* ATCC 19606, N3, N4, N5, and N6 strains, while it showed bactericidal activity against strains N1 and N2. These results are consistent with previous research by Saghi et al. [23], which demonstrated a MIC of 0.44 µg/ml for *Thymus syriacus* against *A. baumannii*, as well as the findings of Safi et al. [24], who observed a MIC of 0.04 µl/ml for *T. vulgaris* against Gram-negative bacteria including *A. baumannii*.

Prolonged use of medical devices significantly increases the risk of infectious complications, leading to extended hospital stays and higher healthcare costs. Consequently, most patients requiring medical devices experience negative outcomes due to treatment failures [13]. *A. baumannii* accounts for approximately 20% of intensive care unit infections [25]. Our study confirms that all tested *A. baumannii* strains demonstrated biofilm-forming capacity. Notably, isolates from catheters in intensive care units exhibited strong biofilm formation, whereas strain N1, isolated from a different biological sample (CBU), formed a weak biofilm. Our findings reveal that all studied *A. baumannii* isolates exhibited resistance to one, two, or all three antibiotic classes (carbapenems, fluoroquinolones, and aminoglycosides) and formed biofilms, with five strains (83.3%) demonstrating strong biofilm formation, while only one strain (N1) displayed weak biofilm formation (16.7%). A study in India also established a correlation between antibiotic resistance and biofilm formation, showing that multidrug-resistant *A. baumannii* strains formed more biofilm than antibiotic-sensitive strains [26]. Carbapenems, a subclass of beta-lactam antibiotics, have been considered an effective treatment option, particularly imipenem. However, increasing resistance to imipenem and ciprofloxacin has been widely reported [27]. Interestingly, strain N1, which exhibited weak biofilm formation, was more susceptible to imipenem, suggesting a potential inverse relationship between biofilm formation and antibiotic susceptibility. This observation aligns with findings by Gedefie et al. [28], who noted a statistically significant correlation between *A. baumannii* biofilm formation and imipenem resistance.

Krystova and colleagues [34] studied the effect of *Thymus vulgaris* essential oil on various typical bacterial strains. The antibiofilm activity test showed that the bacterial biofilm was reduced by 53% after exposure to the lowest concentration of thyme EO, and use at higher concentrations resulted in a reduction in structure by 76%. There was a case where the anti-adhesion activity did not depend on the concentration used, as for the Ab6 strain where MIC/2 had the same percentage inhibition to eliminate the adherent cells as the MIC itself. This finding suggests that the antibacterial effect is not solely responsible for the inhibition of adhesion. These results are consistent with those found by Kerekes et al. [35], when biofilms were significantly inhibited by EOs at MIC/2 concentration. The antibiofilm activity of essential oils of the *Myrtaceae* family against multidrug-resistant *A. baumannii* has been reported for several species. For example, *Cinnamomum zeylanicum* essential oil (cinnamon) showed significant inhibition of *A. baumannii* biofilms (80%) [37]. Similarly, essential oils of *Pimenta dioica* and *Pimenta racemosa* demonstrated notable antibiofilm activity against strains of *A. baumannii*, with inhibition rates of 85% [38].

The *Lamiaceae* family has also demonstrated antibiofilm activity against antibiotic-resistant *A. baumannii* in several species. *Mentha pulegium L.* [8], *Ziziphora tenuior L.* [7], and *Salvia glutinosa L.* [39] have all shown promising results. *M. pulegium* was observed to damage biofilms formed by *A. baumannii* strains, with inhibition rates ranging from 26% to 91% [8]. Similarly, the minimum biofilm inhibitory concentration of *Z. tenuior* essential oil affected *A. baumannii* biofilms at rates between 51% and 84% [7]. Additionally, *S. glutinosa* essential oil exhibited antibacterial efficacy with MICs between 1.25 and 2.5 µl/ml and MBCs between 5 and 10 µl/ml, along with notable antibiofilm activity against *A. baumannii*, with minimum biofilm inhibitory concentrations ranging from 0.3 to 2.5 µl/ml [39]. The antibacterial therapeutic potential of thyme and clove is based on their major active compounds, thymol and eugenol [21,32,34]. In summary, future studies should focus on deepening the understanding of the major active components of thyme and clove essential oils, their specific mechanisms of action against biofilms through molecular biology approaches, and the optimization of their clinical application to effectively combat nosocomial infections caused by *A.baumannii.*

Conclusion

In conclusion, this study highlights the importance of antibiotic resistance and biofilm formation in *Acinetobacter baumannii*, as well as their role in the persistence of nosocomial infections. It also emphasizes the therapeutic potential of clove and thyme essential oils, which exhibit antimicrobial and antibiofilm properties. These findings pave the way for new therapeutic approaches aimed at complementing conventional antibiotic treatments, particularly in the face of the growing threat of multidrug-resistant bacteria.

Citation: Nihal EZZARIGA, Oumaima ZOUHARI, Amal RHARS, Asmaa WAKRIM, Mohamed AGHROUCH, Zohra LEMKHENTE. Antibacterial Activity and Antibiofilm of Essential Oils of Clove and Thyme Against Clinical Isolates of Multidrug-Resistant *Acinetobacter Baumannii* Associated with Nosocomial Infections. Archives of Microbiology and Immunology. 9 (2025): 90-97.

Nihal EZZARIGA, et al., Arch Microbiol Immunology 2025
DOI:10.26502/ami.936500213

References

1. Perez F, Hujer AM, Hujer KM, et al. Global Challenge of Multidrug-Resistant Acinetobacter baumannii. *Antimicrobial Agents and Chemotherapy* 51 (2007).
2. Tacconelli E, Carrara E, Savoldi A, et al. Discovery, Research, and Development of New Antibiotics: The WHO Priority List of Antibiotic-Resistant Bacteria and Tuberculosis. *The Lancet Infectious Diseases* 18 (2018): 318-327.
3. Babiker A, Clarke LG, Saul M, et al. Changing Epidemiology and Decreased Mortality Associated with Carbapenem-resistant Gram-negative Bacteria 2000–2017. *Clinical Infectious Diseases* 73 (2021): 4521-4530.
4. Baranzelli A, Wallyn F, et Nseir S. Infections bronchopulmonaires à *Stenotrophomonas maltophilia* et à *Acinetobacter baumannii*. *Revue de Pneumologie Clinique* 69 (2013): 250-59.
5. Jain R, et Danziger LH. Multidrug-Resistant *Acinetobacter* Infections: An Emerging Challenge to Clinicians. *Annals of Pharmacotherapy* 38 (2004): 1449-1459.
6. Singh S, Singh SK, Chowdhury I, et al. Understanding the Mechanism of Bacterial Biofilms Resistance to Antimicrobial Agents. *The Open Microbiology Journal* 11 (2017): 53-62.
7. Çelik C, Tutar U, Karaman İ, et al. Evaluation of the Antibiofilm and Antimicrobial Properties of *Ziziphora Tenuior L.* Essential Oil Against Multidrug-Resistant *Acinetobacter Baumannii*. *International journal of pharmacology* 12 (2016): 28-35.
8. Tutar U, Çelik C, Karaman İ, et al. Anti-Biofilm and Antimicrobial Activity of *Mentha Pulegium L* Essential Oil against Multidrug-Resistant *Acinetobacter Baumannii*. *Tropical Journal of Pharmaceutical Research* 15 (2016): 1039-1046.
9. Shunying Z, Yang Y, Huaidong Y, et al. Chemical composition and antimicrobial activity of the essential oils of *Chrysanthemum indicum*. *Journal of Ethnopharmacology* 96 (2005): 151-58.
10. Ponce AG, Fritz R, del Valle C, et al. Antimicrobial activity of essential oils on the native microflora of organic Swiss chard ». *LWT - Food Science and Technology* 36 (2003): 679-684.
11. Achmit M, Aoussar N, Mellouki F, et al. Activité antibactérienne et inhibitrice du biofilm in vitro de l'huile essentielle de sciure de bois de *Tetraclinis articulata* (vahl) contre les isolats cliniques de *Staphylococcus aureus* associés au cathéter. *Biotechnology* 3 (2021): 1-5.
12. Ploy MC, Poyart C, Cattoir V, et al. *Bactériologie médicale : Techniques usuelles*. Elsevier Health Sciences (2016).
13. Folliero V, Franci G, Dell'Annunziata F, et al. Evaluation of Antibiotic Resistance and Biofilm Production among Clinical Strain Isolated from Medical Devices. *International Journal of Microbiology* (2021): 9033278.
14. Bazargani MM, et Rohloff J. Activité antibiofilm des huiles essentielles et extraits de plantes contre les biofilms de *Staphylococcus aureus* et *Escherichia coli*. *Food Control* 61 (2016): 156-164.
15. Exner M, Bhattacharya S, Christiansen B, et al. Antibiotic resistance: What is so special about multidrug-resistant Gram-negative bacteria? *GMS Hygiene and Infection Control* 12 (2017): 05.
16. Motbainor H, Bereded F, et Mulu W. Multi-Drug Resistance of Blood Stream, Urinary Tract and Surgical Site Nosocomial Infections of *Acinetobacter Baumannii* and *Pseudomonas Aeruginosa* among Patients Hospitalized at Felegehiwot Referral Hospital, Northwest Ethiopia: A Cross-Sectional Study. *BMC Infectious Diseases* 20 (2020): 92.
17. Al-Janabi M, et Asaad JI. Antibacterial Activity of Cloves Seeds (*Syzygium Aromaticum*) And Cinnamon Bark Essential Oils. *NVEO - natural volatiles & essential oils journal* 8 (2021): 131-41.
18. Zeshan MQ, ashraf M, Omer MO, et al. Antimicrobial Activity of Essential Oils of *Curcuma Longa* and *Syzygium Aromaticum* against Multiple Drug-Resistant Pathogenic Bacteria. *Tropical Biomedicine* 40 (2023): 174-182.
19. Haddouchi F, et Benmansour A. Huiles essentielles, obtentions, utilisations et activités biologiques. Application à deux plantes aromatiques. *Les technologies de laboratoire* 3 (2008).
20. Boukhatem MN, Ferhat MN, Kameli A, et al. Valorisation de l'essence aromatique du Thym (*Thymus vulgaris L.*) en aromathérapie anti-infectieuse. *International Journal of Innovation and Applied Studies* 8 (2014): 1418-1431.
21. Nzeako BC, Al-Kharousi ZS, Al-Mahrooqui Z. Antimicrobial activities of clove and thyme extracts. *Sultan Qaboos Univ Med J* 6 (2006): 33-39.
22. Faujdar SS, Bisht D, et Sharma A. Antibacterial activity of *Syzygium aromaticum* (clove) against uropathogens producing ESBL, MBL, and AmpC beta-lactamase: Are we close to getting a new antibacterial agent? *Journal of Family Medicine and Primary Care* 9 (2020): 180-186.
23. Saghi H, Bahador A, Dastjerdi F, et al. Hassan Asadolahi, Mehdi Neyshaboori, Esmaeili Mohammad, Maryam

Nihal EZZARIGA, et al., Arch Microbiol Immunology 2025
DOI:10.26502/ami.936500213

Meskini, et Davoud Esmaeili. Antibacterial Effects of Herbal Compounds against *Acinetobacter baumannii* Isolated from Hospital of Tehran, Iran. *Global Journal of Infectious Diseases and Clinical Research* 1 (2015): 018-020.

24. Safi M, Al-Hallab L, Al-Abras R, et al. Efficacy of Some Antibiotics and Essential Oils Against *Acinetobacter Baumannii*: An *in Vitro* Study. *Avicenna Journal of Clinical Microbiology and Infection* 7 (2020): 1-7.

25. Garnacho-Montero J, et Timsit JF. Managing *Acinetobacter baumannii* infections. *Current Opinion in Infectious Diseases* 32 (2019): 69-76.

26. Muzafar A, Qayoom S, Pai V, et al. Biofilm Formation and Multidrug Resistance in Nosocomial Isolates of *Acinetobacter*. *Indian Journal of Microbiology Research* 5 (2018): 425-429.

27. Avila-Novoa MG, Solís-Velázquez OA, Rangel-López DE, rt al. Biofilm Formation and Detection of Fluoroquinolone- and Carbapenem-Resistant Genes in Multidrug-Resistant *Acinetobacter Baumannii. Canadian Journal of Infectious Diseases and Medical Microbiology* (2019): 3454907.

28. Gedefie A, Demsis W, Ashagrie M, et al. *Acinetobacter baumannii* Biofilm Formation and Its Role in Disease Pathogenesis: A Review. *Infection and Drug Resistance* 14 (2021): 3711-3719.

29. Di Domenico EG, Farulla I, Prignano G, et al. Biofilm Is a Major Virulence Determinant in Bacterial Colonization of Chronic Skin Ulcers Independently from the Multidrug Resistant Phenotype. *International Journal of Molecular Sciences* 18 (2017): 1077.

30. Mohamed EA, Raafat MM, Mohamed RS, et al. *Acinetobacter baumannii* biofilm and its potential therapeutic targets. *Future Journal of Pharmaceutical Sciences* 9 (2023): 82.

31. Kowalczyk A, Przychodna M, Sopata S, et al. Thymol and Thyme Essential Oil—New Insights into Selected Therapeutic Applications. *Molecules* 25 (2020): 4125.

32. Alibi S, Ben Selma W, Ramos-Vivas J, et al. Anti-oxidant, antibacterial, anti-biofilm, and anti-quorum sensing activities of four essential oils against multidrug-resistant bacterial clinical isolates. *Current Research in Translational Medicine* 68 (2020): 59-66.

33. Kavanaugh NL, et Ribbeck K. Selected Antimicrobial Essential Oils Eradicate *Pseudomonas spp.* and *Staphylococcus aureus* Biofilms. *Applied and Environmental Microbiology* 78 (2012): 4057-4061.

34. Kryvtsova MV, Salamon I, Koscova J, et al. Antimicrobial, antibiofilm and biochemichal properties of *thymus vulgaris* essential oil against clinical isolates of opportunistic infections. *Biosystems Diversity* 27 (2019): 270-275.

35. Kerekes EB, Vidács A, Takó M, et al. Anti-Biofilm Effect of Selected Essential Oils and Main Components on Mono- and Polymicrobic Bacterial Cultures. *Microorganisms* 7 (2019): 345.

36. Karameşe M, et Özgür D. The antibacterial and antifungal activities of commonly used herbal oils. *Journal of Experimental and Clinical Medicine* 37 (2020): 47-51.

37. Ganić T, Vuletić S, Nikolić B, et al. Cinnamon Essential Oil and Its Emulsion as Efficient Antibiofilm Agents to Combat *Acinetobacter Baumannii*. *Frontiers in Microbiology* 13 (2022).

38. Ismail MM, Samir R, Saber FR, et al. Pimenta Oil as a Potential Treatment for *Acinetobacter Baumannii* Wound Infection: In Vitro and In Vivo Bioassays in Relation to Its Chemical Composition. *Antibiotics* 9 (2020): 679.

39. Tutar U. Study of the effect of essential oil of *Salvia glutinosa L.* on microbial biofilm formation by clinical isolates of *Acinetobacter baumannii*. *AIP Conference Proceedings* 1726 (2016).