Original Article

# Antimicrobial Activity of Clove Oil Extract against *A. baumanii* and its Impact on Gene Expression

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## Abstract

**Background:** *Acinetobacter baumannii*, a pathogen with clinical significance, can cause a range of well-known nosocomial infections, the most important examples of which are septicemia, pneumonia, and dermatitis. This bacterium is known for its multi-drug resistance, posing a significant health concern. The bacteria were isolated and identified from clinical samples obtained from patients who presented to a burns hospital in Baghdad, Iraq. **Objectives:** The study aimed to isolate and characterize *A. baumannii* from clinical samples, evaluate the antimicrobial activity of clove oil extract against these isolates, and examine gene expression patterns using real-time polymerase chain reaction (PCR). **Materials and Methods:** A total of 100 clinical isolates of *A. baumannii* were identified using morphological, microscopic, and biochemical methods, along with the Vitek II system. On growth media like MacConkey agar, the isolates displayed classic phenotypic features of *A. baumannii*. **Results:** Biochemical tests confirmed catalase production, while oxidase, methyl red, Voges–Proskauer, urease, gelatinase, and indole tests stained negative. Vitek II provided additional species-level confirmation for the isolation of *A. baumannii*. Clove oil extract showed antimicrobial activity, with minimal inhibitory concentration values ranging from 10 to 0.01 mg/mL. Real-time PCR revealed diverse gene expression patterns, highlighting the genetic responses of *A. baumannii* to environmental stressors. The study confirms the phenotypic and genetic characteristics of *A. baumannii* from clinical samples and demonstrates the potential of clove oil as an antibacterial agent. These findings enhance our understanding of the bacterial responses to environmental stress and could inform new therapeutic strategies. **Conclusion:** This study provides valuable insights into the antibacterial properties of clove oil against *A. baumannii* and advances our knowledge of the genetic and phenotypic features of this pathogen, potentially aiding in the development of new treatment approaches.

**Keywords:** *Acinetobacter baumannii*, clove oil, MIC, real-time PCR, Vitek II

## Introduction

One of the major and well-known causes of infections in hospitals and globally are those diagnosed infections that can be caused by multidrug-resistant (MDR) Gram-negative bacteria. In order to protect and prevent against such diseases, it is very necessary to monitor such cases of resistance to pathogenic antimicrobials. Gram-negative aerobic cocci *Acinetobacter baumannii* can cause a range of well-known nosocomial infections, the most important examples of which are septicemia, pneumonia, and dermatitis. It can be seen continuously and repeatedly in hospitals,[1] especially in the well-known intensive care units (ICUs). Bacteria, including those resistant to antibiotics (e.g., beta-lactams and quinolones), are already resistant, due in part to processes such as efflux pumping, enzyme inactivation[2,3] and biofilm formation system; virulent

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DOI: 10.4103/MJBL.MJBL_666_24

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**Submission:** 16-Jun-2024 **Accepted:** 17-Feb-2025 **Published:** 30-Sep-2025



**How to cite this article:** Al-Dulaimi TH, Abdul-Husin IF, Al-Musawi AMS, Al-Jayid RD, Kamil SS, Kataa BR, *et al*. Antimicrobial activity of clove oil extract against *A. baumanii* and its impact on gene expression. Med J Babylon 2025;22:904-8.

factors such as colicin V synthetase, aerobactin siderophores, and cytotoxic necrosis factor actually serve to augment their pathogenicity and efficacy. Resistance to *A. baumannii* results mainly from the production of extended-spectrum beta-lactamases, and these are already coded for by genes such as blaTEM, blaSHV, and e blaCTX. One of the most critical reasons that drastically increase resistance to beta-lactam bacteria is the much lower binding affinity between penicillin receptors and beta-lactams.[2]

Detection of virulence and antibiotic resistance genes in clinical isolates aids in tracking the transmission of *A. baumannii* infections. Natural phytocompounds with enzyme-mimicking properties are essential in combating MDR Gram-negative bacteria and their biofilms. Herbal medicines offer potential sources for discovering new treatments for severe diseases.[4,5]

The study aimed to detect the potential of clove oil as an antibacterial agent against *A. baumannii.*

## Materials and Methods

A total of 100 clinical isolates of *A. baumannii* were collected from infected patients who constantly receive drug treatment in the burn department of the Medical City, Baghdad, Iraq. At the same time, the Vitek II analysis system was used in addition to the most important morphological, chemical, and microscopic tests in order to characterize and complete the examination process of these isolates with great accuracy. Microscopic laboratory analyses, including the process of Gram staining to evaluate the shape and structure of the studied bacterial cells and their organization with the type of response studied and the observation of colony morphology on blood agar, were among the most important bacteriological procedures already used for identifying *A. baumannii*.[6,7] Here, the inability of these *A. baumannii* colonies to ferment lactose has already been demonstrated by their unique and distinct properties on MacConkey agar, a well-known selective medium that allows the development of Gram-negative bacteria. Additional biochemical testing using the API 20E diagnostic kit and the VITEK2 Compact System was conducted to confirm the results of microscopic inspection and culture diagnosis.

**Solutions and culture medium** (both conventional and differential): The solutions were produced in accordance with the instructions provided by the manufacturers.

### Minimal inhibitory concentration (MIC) assay:

In accordance with the Clinical and Laboratory Standards Institute standards, the MIC assay was performed in a 96-well polystyrene plate using the broth microdilution method. The MIC value was determined by recording the lowest concentration of each fraction that did not show any observable growth. The activity was confirmed by determining the MIC values at least twice. To determine the cell viability, using blue dye, and to test cell health, we employed a resazurin solution.

### Extraction of clove oil extract:

Oil extract from cloves (*Syzygium aromaticum*) was prepared using the Clevenger method. Ground cloves (10 g) were mixed with 100 mL of ethanol and distilled. The distillate was evaporated to obtain eugenol as a pale yellow oil.

### Preparation of antimicrobial agents:

To prepare 1.5 mL microcentrifuge tubes with a final concentration of 10 mg/mL of oil extract, the tubes were filled with distilled water and mixed to a stock solution. Concentrations ranging from 10 to 0.01 mg/mL were achieved by preparing two-fold serial dilutions of the stock solution in a 96-well plate using Muller–Hinton broth.

### Inoculum preparation:

Inoculum preparation involved taking bacteria or other microorganisms from an overnight agar culture and placing them in tubes with Muller–Hinton broth. The concentration of the broth culture was decreased to around $10^8$ CFU/mL by adjusting its turbidity to a 0.5 McFarland Standard and then diluting it to 1:100. These methods were performed to conduct tests and identify bacterial isolates, determine the antimicrobial activity, and assess the efficacy of clove oil extract against bacterial growth.

### Molecular Assay:

Quantitative reverse transcription polymerase chain reaction (qPCR): To extract total ribonucleic acid (RNA) from a bacterial culture, cells were pelleted and lysed using lysozyme. The lysate was then treated with FARB buffer, followed by centrifugation and ethanol precipitation. RNA was purified using a FARB Mini Column, washed, and eluted with RNase-free water. The eluted RNA was converted to complementary deoxyribonucleic acid (cDNA) using random primers and reverse transcriptase. After the end of the known incubation period, cDNA was already amplified by qPCR using forward and reverse primers specific for the applicable target gene, as well as a reference gene for normalization. An initial activation, denaturation, and annealing phase was included of the qPCR programmed. The ΔΔCT method was used to compute the fold changes by comparing the target gene's cycle threshold (CT) to the reference gene's CT. Gene expression investigations can benefit from this simplified protocol's RNA extraction, cDNA synthesis, and qPCR analysis capabilities.

## Ethical approval

The ethical committee of the Department of Parenting, College of Women's Sciences at Babylon University, authorized the study. Patients were informed about their participation in the study and received this information orally. According to the document, the local ethics committee reviewed and approved the study protocol, study data, and consent. According to document number 28, a local ethics commission reviewed and gave its approval to the study protocol, subject information, and permission on July 11, 2024.

## Results

The result of the present study showed the colonies of *A. baumannii* (small, circular, regular pale colonies, and non-fermented lactose sugar grown on MacConkey agar at 37°C for 24 h). In the current investigation, a range of biochemical tests were conducted to characterize *A. baumannii* isolates. These tests revealed positive results for the catalase test, indicating the presence of the catalase enzyme, as evidenced by the release of oxygen bubbles when the hydrogen peroxide reagent was added. This morphology is in line with earlier descriptions of *A. baumannii*. However, the isolates tested negative for the oxidase, methyl red, Voges–Proskauer, urease, and gelatinase tests, as well as for indole production. The absence of a yellow ring after the addition of Kovacs reagent to the peptone water medium signified the bacteria's inability to produce the tryptophanase enzyme, necessary for indole formation.

MICs were determined to assess the antimicrobial activity of MIC E against the tested *A. baumannii* bacteria. MICs of the extract ranged from 10 to 0.01 mg/mL. At a concentration of 0.31 mg/mL, growth inhibition of *A. baumannii* isolates was observed in 37.5% (3/8) of cases. Similarly, concentrations of 0.15 mg/mL inhibited growth in 37.5% (3/8) of isolates, while concentrations of 0.075 mg/mL inhibited growth in 25% (2/8) of isolates, as shown in Figure 1.

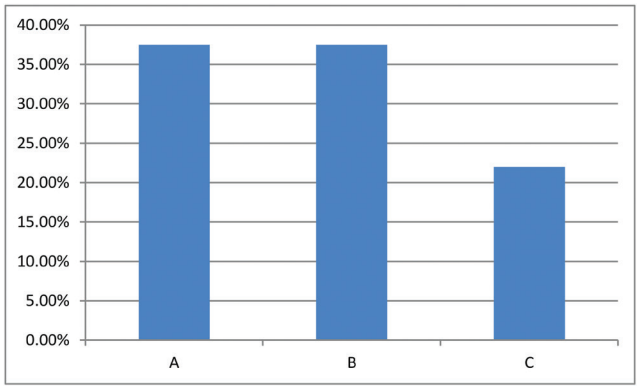


**Figure 1:** Inhibition percentage of clove oil against *Acinetobacter baumannii*. (a: 0.31, b: 0.15, c: 0.075)

## Discussion

*A. baumannii* isolates are widely distributed in various environments and resistant to antibiotics, which is considered a public health concern. Hospitals are considered a major reservoir for bacterial infections. Bacteria were detected in many hospital-acquired infections such as ventilator-associated pneumonia, endocarditis, meningitis, urinary tract infections, bloodstream infections, pneumonia, wounds, and soft tissue infections, especially in the ICU and burn unit.[8] Also, several studies have found that this bacterium causes diseases and has been isolated from the respiratory system, burns, wounds, and urine in hospitals in Babylon Governorate.[9,10]

**Table 1: Qualitative study by real-time polymerase chain reaction**

| Result | Cp. Hex | Cp. Fam | ID of the tube | Number of the well |
|---|---|---|---|---|
| + | | 34.2 | Sample 1 (qpcr) | A1 |
| + | | 33.7 | Sample 2 (qpcr) | A2 |
| + | | 33.4 | Sample 3 (qpcr) | A3 |
| + | | 33.8 | Sample 4 (qpcr) | A4 |
| + | | 34.1 | Sample 5 (qpcr) | A5 |
| + | | 34.1 | Sample 6 (qpcr) | A6 |
| + | | 32.5 | Sample 7 (qpcr) | A7 |
| + | | 30.7 | Sample 8 (qpcr) | A8 |
| + | | 28.2 | Sample 9 (qpcr) | A9 |
| + | | 28.6 | Sample 10 (qpcr) | A10 |
| + | | 27.2 | Sample 11 (qpcr) | A11 |
| + | | 25.6 | Sample 12 (qpcr) | A12 |
| + | | 24.5 | Sample 13 (qpcr) | B1 |
| + | | 24.5 | Sample 14 (qpcr) | B2 |
| + | | 24.4 | Sample 15 (qpcr) | B3 |
| + | | 24.4 | Sample 16 (qpcr) | B4 |

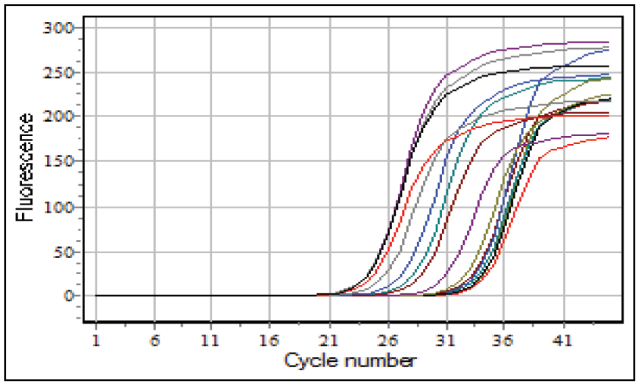


**Figure 2:** Dependence of FAM channel fluorescence on cycle threshold

**Table 2: Qualitative analysis by real-time polymerase chain reaction (for the reference gene)**

| Result | Cp. Hex | Cp. Fam | ID of the tube | Number of the well |
|---|---|---|---|---|
| + | | 23.4 | Sample 1 (qpcr) | A1 |
| + | | 23.8 | Sample 2 (qpcr) | A2 |
| + | | 22.1 | Sample 3 (qpcr) | A3 |
| + | | 21.5 | Sample 4 (qpcr) | A4 |
| + | | 21.0 | Sample 5 (qpcr) | A5 |
| + | | 23.5 | Sample 6 (qpcr) | A6 |
| + | | 21.5 | Sample 7 (qpcr) | A7 |
| + | | 23.2 | Sample 8 (qpcr) | A8 |
| + | | 21.8 | Sample 9 (qpcr) | A9 |
| + | | 22.6 | Sample 10 (qpcr) | A10 |
| + | | 21.9 | Sample 11 (qpcr) | A11 |
| + | | 22.4 | Sample 12 (qpcr) | A12 |
| + | | 22.4 | Sample 13 (qpcr) | B1 |
| + | | 23.3 | Sample 14 (qpcr) | B2 |
| + | | 22.9 | Sample 15 (qpcr) | B3 |
| + | | 22.9 | Sample 16 (qpcr) | B4 |

In the present investigation, a range of biochemical tests were conducted to characterize *A. baumannii* isolates and determine the antimicrobial activity of MIC E against the tested bacteria. All studied samples tested positive in the qPCR, with this "+" sign placed in the "Result" column. The Cp values, ranging from 25.6 to 34.2, indicate the actual point at which the target sequence was detected, while at the same time, lower values found indicate a higher abundance of the studied sequence. These results indicate that the studied DNA, which is at most a specific part or gene from *A. baumannii*, was detected in all analyzed studied samples. The combination of differences in Cp values may be due to actual differences in the actual initial concentration of the target sequence between these samples, as shown in Table 1 and Figure 2.

In the Table 2 and Figure 3, all the studied samples actually tested positive in the qPCR test, indicating the presence of the *A. baumannii* target sequence. Meanwhile, Cp values, ranging from 21.0 to 23.8, indicate varying levels of target sequence abundance across the studied

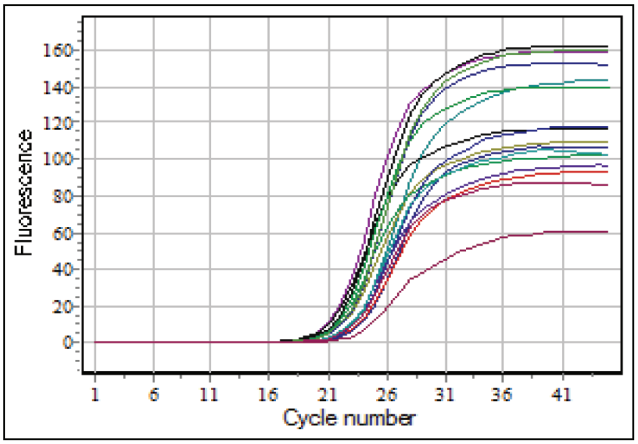


**Figure 3:** Dependence of FAM channel fluorescence on the cycle threshold (for reference gene)

samples. When starting to discuss a reference gene for qPCR analysis, it is certainly important to select an already stable, constitutively expressed gene that directly serves as an internal control for normalization. Here, commonly used reference genes include GAPDH.[11-13] Even if the reference gene is not mentioned in the data obtained, it is very necessary to incorporate one into qPCR research to take into account these differences in sample handling, RNA integrity, and known amplification efficacy. At the same time, this largely ensures that the quantification of gene expression is accurate and reliable. Both *A. baumannii* and *Pseudomonas* have known reference genes, as confirmed by Zhang *et al*.[11] Clove oil contains numerous active compounds that are linked to its antibacterial properties. The main ones include eugenol (48%–89%), beta-caryophyllene (5%–22%), and eugenyl acetate (0.4%–22%). Alpha-humulene is also present in trace amounts. The quantity of these components varies depending on a number of factors, including the kind of plant, the portion used, genetics, agricultural climate, soil type, and techniques for effective extraction of oils.[14] The action of eugenol increases the transport of potassium ions and ATP from the cells, and its hydroxyl group binds to proteins and inhibits the activity of enzymes.[15]

Additionally, numerous investigations have evaluated the antibacterial activity of plant essential oils against *A. baumannii* as in[16-18]. The essential oils of *O. decumbens, E. caryophyllata,* and *T. copticum* oils possess the highest antibacterial activity against clinical isolates of *A. baumannii* in the study by Mahboubi *et al*.[19] Many studies have been conducted using chamomile extract as an antibacterial agent against these antibiotic-resistant bacteria, and they have confirmed the effectiveness of these extracts against them using various extraction methods.[8,20-22]

Burn wound infections are among the most serious issues that may arise after a burn injury;[23] most bacterial burn infections are due to bacterial resistance to antibiotics,

which has become a major and serious challenge in hospitals. This requires the conduction of many new and ongoing studies to identify potential updates in the patterns of bacteria causing burn infections.[24] So, essential oils and plant extract can be used as an alternative treatment of *A. baumannii*.

## Conclusion

As we know, middle-income countries were critical in evaluating the antimicrobial effectiveness of MIC E against *A. baumannii*. Through this study, MIC values obtained in our study vary from 10 to 0.01 mg/mL, which indicates different efficacy of the extract against tested bacteria growing. In particular, 37.5% of the isolates acted to reduce growth at a concentration above those tested with (0.31 mg/mL and 0.15 mg/mL), while another one decreased growth even at low concentrations; an IC30 or as little inhibitory on acyl-CoA desaturase activity only reduced cell survival by about ~25%. Increased research into the efficacy of antimicrobial drugs against *A. baumannii* is warranted, and our study adds to the existing body of information in this area.

### Financial support and sponsorship

Nil.

### Conflicts of interest

There are no conflicts of interest.

## References

1. Ahmed SM, Yaseen KH, Mahmood MM. Molecular evaluation of *Acinetobacter baumannii* in a sample of Iraqi patients: Clinical and environmental study. HIV Nurs 2022;22:121-6.
2. Cayô R, Rodríguez MC, Espinal P, Fernández-Cuenca F, Ocampo-Sosa AA, Pascual A, *et al*. Analysis of genes encoding penicillin-binding proteins in clinical isolates of *Acinetobacter baumannii*. Antimicrob Agents Chemother 2011;55:5907-13.
3. Radhi SH, Al-Charrakh AH. Occurrence of MBLs and carbapenemases among MDR and XDR *Acinetobacter baumannii* isolated from hospitals in Iraq. Indian J Public Health Res Dev 2019;10:668-74.
4. Hemraj V, Diksha S, Avneet G. A review on commonly used biochemical test for bacteria. Innovare J Life Sci 2013;1:1-7.
5. Mahmoud A, Afifi MM, El Shenawy F, Salem W, Elesawy BH. Syzygium aromaticum extracts as a potential antibacterial inhibitors against clinical isolates of *Acinetobacter baumannii*: An in-silico-supported in-vitro study. Antibiotics (Basel, Switzerland) 2021;10:1062.
6. Mahon CR, Lehman DC, Manuselis G. Textbook of Diagnostic Microbiology-e-Book. Elsevier Health Sciences; 2018.
7. Rakovitsky N, Lurie-Weinberger MN, Hameir A, Wulffhart L, Keren Paz A, Schwartz D, *et al*. Phenotypic and genomic characterization of nine string-positive carbapenem-resistant *Acinetobacter baumannii* isolates from Israel. Microbiol Spectrum 2023;11:e0300222.
8. Al-ammery RKA, Chabuck ZAG. Naturally derived efflux pump inhibitor among tetracycline-resistant *Acinetobacter baumannii* isolates. Med J Babylon 2024;21:57-64.
9. Alzaidi JR. Prevalence of OXA genes responsible for carbapenem-resistance among *Acinetobacter baumannii* isolated from clinical samples in Iraq. Med J Babylon 2023;20:632-7.
10. Kareem IN, Al-Hasnawy HH, Ali JA. Role of some proteins in resistance of clinical *Acinetobacter baumannii* isolates to imipenem. Int J Health Sci 2022;6:15118-27.
11. Zhang J, Liu J, Wang Y, Yu D, Sui Q, Wang R, *et al*. Profiles and drivers of antibiotic resistance genes distribution in one-stage and two-stage sludge anaerobic digestion based on microwave-H2O2 pretreatment. Bioresour Technol 2017;241:573-81.
12. Shan W, Kan J, Cai X, Yin M. Insights into mucoid Acinetobacter baumannii: A review of microbiological characteristics, virulence, and pathogenic mechanisms in a threatening nosocomial pathogen. Microbiol Res 2022;261:127057.
13. Oliveira TA, Santiago MB, Santos VH, Silva EO, Martins CH, Crotti AE. Antibacterial activity of essential oils against oral pathogens. Chem Biodivers 2022;19:e202200097.
14. Khalil AA, Rahman U, Khan MR, Sahar A, Mehmood T, Khan M. Essential oil eugenol: Sources, extraction techniques and nutraceutical perspectives. RSC Adv 2017;7:32669-81.
15. Gill AO, Holley RA. Mechanisms of bactericidal action of cinnamaldehyde against Listeria monocytogenes and of eugenol against *L. monocytogenes* and *Lactobacillus sakei*. Appl Environ Microbiol 2004;70:5750-5.
16. Łysakowska M, Denys A, Sienkiewicz M. The activity of thyme essential oil against Acinetobacter spp. Open Life Sci 2011;6:405-13.
17. Duarte A, Ferreira S, Silva F, Domingues FC. Synergistic activity of coriander oil and conventional antibiotics against *Acinetobacter baumannii*. Phytomedicine 2012;19:236-8.
18. Jazani NH, Zartoshti M, Babazadeh H, Ali-Daiee N. Antibacterial effects of *Artemisia dracunculus* essential oil on multi-drug resistant isolates of *Acinetobacter baumannii*. J Bacteriol 2011;1:31-6.
19. Mahboubi M, Kazempour N, Taghizadeh M. The antibacterial activity of some essential oils against clinical isolates of *Acinetobacter baumannii*. Songklanakarin J Sci Technol 2014;36:513-9.
20. Ali E, Ibrahim K, Ismail M. Activity of Flower and Callus Extracts of *Matricaria chamomilla* L. in the Treatment of Experimentally Induced Skin Infections in Mice; 2009.
21. Ismail I, Ibrahim K, Lateef N. Effect of flower extracts of *Matricaria chamomilla* L. on some bacteria causing eye infections. Diyala J Pure Sci 2011;7:96-107.
22. Jafarzadeh M, Moghaddam M, Bakhshi D. Antimicrobial activity of three plant species against multi-drug resistant *E. coli* causing urinary tract infection. J Herb Med 2020;22:100352.
23. Alwan ZHO, Lilo RA, Al-Taee ZM, Mohsen LY, Al-Alaq FT. Detection of exopolysaccharides (algD, pelF, and pslD) genes in burn wound Pseudomonas aeruginosa isolates. Med J Babylon 2023;20:595-9.
24. Kadhim TA, Al Sa'ady AT, Khayoon HA, Hameed DM, Hussain SS, Kareem AS, *et al*. The bacterial contamination in the burn unit of Al-Hussain Teaching Hospital at Al-Samawa City, Iraq. Med J Babylon 2023;20:357-61.