*Received: 14.09.2025 | Revision: 22.09.2025 | Accepted: 22.09.2025*

*Veterinary Sciences*

doi: 10.31210/spi2025.28.04.19
UDC 616:98:579:547
***ORIGINAL ARTICLE***

**Scientific Progress & Innovations**
https://journals.pdaa.edu.ua/visnyk

28 (4)
2025

# Antibacterial activity of ethanolic clove *Syzygium aromaticum* extract against multidrug-resistant gram-negative bacteria

Amaal M. Khudaier

**Article info**

Correspondence Author
A. M. Khudaier
E-mail:
amaal.m@uokerbala.edu.iq

College of Veterinary Medicine, University of Kerbala,
Karbala, 56001, Iraq

**Citation:** Khudaier, Amaal, M. (2025). Antibacterial activity of ethanolic clove *Syzygium aromaticum* extract against multidrug-resistant gram-negative bacteria. *Scientific Progress & Innovations*, 28(4), 132–135. doi: 10.31210/spi2025.28.04.19

The development of multidrug-resistant (MDR) bacteria is a significant global health concern, making the search for novel antimicrobial drugs crucial. Eugenol, a phenolic compound found in high concentration in *Syzygium aromaticum* (clove), is well known for its broad-spectrum antibacterial properties. The study aimed to evaluate the antibacterial activity of ethanolic clove *Syzygium aromaticum* extract against multidrug-resistant Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*). Bacterial isolates (*E. coli*, *K. pneumoniae*, and *P. aeruginosa*) were obtained from animal with infectious diseases and subsequently confirmed by cultivation on several plates of solidified agar media and incubation at 37 °C for 24 hours. Clove powder was processed into ethanolic extracts using standard maceration and evaporation techniques. Four concentrations of antibacterial activity (50 %, 25 %, 12.5 %, and 6.25 %) were tested using the agar well diffusion method. The results showed a concentration-dependent inhibitory effect, with the highest inhibition zones observed at 50 % concentration: *E. coli* (25 mm), *P. aeruginosa* (24 mm), and *K. pneumoniae* (20 mm). No inhibitory activity was observed at the 6.25 % concentration. Among the three microorganisms studied, the *E. coli* test culture demonstrated the highest sensitivity to ethanolic clove extract at concentrations of 12.5, 25.0, and 50.0 %, with growth inhibition zones of 16.0, 21.0 and 25.0 mm, respectively. In contrast, *K. pneumoniae* demonstrated the smallest inhibition zone (20 mm) at the highest extract concentration (50 %). Furthermore, research indicates that the combined use of clove extract with conventional antibiotics may result in a synergistic antibacterial effect. In conclusion, clove extract shows encouraging antibacterial activity against MDR Gram-negative bacteria and warrants futher research for potential medical application.

**Keywords:** *Syzygium aromaticum*, clove extract, antibacterial activity, multidrug-resistant bacteria, eugenol, *Escherichia coli*, *Pseudomonas aeruginosa, Klebsiella pneumoniae*.

# Антибактеріальна активність екстракту гвоздики *Syzygium aromaticum* відносно грамнегативних мультирезистентних штамів бактерій

Амаал М. Худаєр

Коледж ветеринарної медицини, Університет Кербели,
провінція Кербела, Ірак

Поява мультирезистентних штамів бактерій є глобальною проблемою як гуманної, так і ветеринарної медицини, що робить пошук нових антимікробних препаратів та речовин різного походження надзвичайно важливим. Однією з таких речовин є евгенол. Як відомо, евгенол – це фенольна сполука, що міститься у високій концентрації в *Syzygium aromaticum* (гвоздиці), яка має антибактеріальні властивості широкого спектра дії. Тому, метою дослідження було оцінити антибактеріальну активність етанольного екстракту гвоздики *Syzygium aromaticum* відносно грамнегативних бактерій з множинною лікарською стійкістю (*Escherichia coli*, *Pseudomonas aeruginosa* та *Klebsiella pneumoniae*). Бактеріальні ізоляти для дослідів (*E. coli*, *K. pneumoniae* та *P. aeruginosa*) були отримані від тварин з інфекційними захворюваннями. Екстракт гвоздики отримували з порошку рослини за допомогою стандартних методів мацерації та випаровування. У дослідженнях протестовано чотири концентрації екстракту (50,0 %, 25,0 %, 12,5 % та 6,25 %) за допомогою методу дифузії в агар (метод «колодязів»). Результати показали концентраційно-залежний інгібуючий ефект екстракту отриманого з *Syzygium aromaticum*. Найвищі зони інгібування зафіксовано за використання екстракту в 50,0 % концентрації: *E. coli* (25,0 мм), *P. aeruginosa* (24 мм) та *K. pneumoniae* (20 мм). Серед трьох досліджених мікроорганізмів культура *E. coli* продемонструвала найвищу чутливість до виготовленого екстракту гвоздики за концентрацій 12,5 %, 25,0 % та 50,0 % із зонами інгібування росту 16,0, 21,0 та 25,0 мм відповідно. Водночас, *K. pneumoniae* продемонструвала найменшу зону інгібування (20 мм) при найвищій концентрації екстракту (50,0 %). Таким чином, екстракт гвоздики володіє вираженою антибактеріальною активністю щодо мультирезистентних грамнегативних бактерій, що робить його перспективним у використанні й потребує подальших досліджень.

**Ключові слова:** *Syzygium aromaticum*, екстракт гвоздики, антибактеріальна активність, мультирезистентні бактерії, евгенол, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*.

**Бібліографічний опис для цитування:** *Худаєр Амаал М.* Антибактеріальна активність екстракту гвоздики *Syzygium aromaticum* відносно грамнегативних мультирезистентних штамів бактерій. *Scientific Progress & Innovations*. 2025. № 28 (4). С. 132–135.

## Introduction

Multidrug-resistant bacteria are a major cause of mortality associated with infectious diseases, particularly in developing countries [1]. *Syzygium aromaticum* antibacterial, antioxidant, anti-cancer, anti-inflammatory, and anti-diabetic qualities made it significant in medicine [2]. Known more commonly as clove, *Syzygium aromaticum* (family: *Myrtaceae*) is native to the Indonesian islands.

Eugenol is the main component of Clove Essential Oil Composition (CEO), accounting for at least half of the more than 30 compounds that have been identified. Humulene, eugenyl acetate, and caryophyllene make approximately 10 % to 40 % of the residual material. The remaining components are regarded as minor elements because they are present in minor amounts (<10 %) [3]. Due to their antibacterial, antifungal, anti-inflammatory, insecticidal, and antioxidant qualities, as well as their historical use as a flavoring agent and antimicrobial substance in food, the high concentration of eugenol in leaf and bud oil makes it potentially useful in medications [4].

Eugenol, the primary phenolic compound in clove, exhibits multiple antibacterial mechanisms, including disruption of bacterial cell membranes, increased membrane permeability, protein denaturation, and inhibition of biofilm formation [5]. According to reports, they eradicate bacteria without encouraging the growth of resistance [6]. Alternatives to traditional antibiotics must be found immediately since antibiotic-resistant bacteria are becoming more and more common. Potential uses for plant-derived substances (PDSs) include both antibacterial and antibiotic resistance modulation [7]. Clove essential oil and extracts exhibit significant activity against *Pseudomonas aeruginosa*, a multidrug-resistant pathogen [8]. In tropical Asia, clove has traditionally been used to treat various diseases, including cholera, malaria, and tuberculosis. Comparative studies have shown its inhibitory effect on clinical strains of *P. aeruginosa*, as well as its use in the treatment of nausea and vomiting [9].

## The aim of the study

The study aimed to establish the antibacterial activity of ethanolic clove *Syzygium aromaticum* extract against multidrug-resistant Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa, Klebsiella pneumoniae*).

## Materials and methods

*Extraction of Plant Material*

Two hundred fifty milliliters of sterile distilled water were used to dissolve 25 grams of the finely powdered *S. aromaticum* in order to reach a dilution ratio of 1 : 10 (1 g/10 ml). To get the same dilution ratio, another 25 grams of the powder were dissolved in 250 milliliters of 95 % ethanol. The mixtures were shaken intermittently for three days [10]. The solutions were filtered using Whatman No. 1 filter paper. The crude ethanolic extracts of *S. aromaticum* were collected, filtered, evaporated in a water bath, and stored at 4 °C until further analysis [11].

*Collection of test isolates*

The bacterial species used in this study were *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aurogenosa*. The isolates were obtained from animals with infectious diseases and confirmed by culturing on solid agar media followed by incubation at 37 °C for 24 h. A biochemical reaction was then carried out for further identification and confirmation after the colonies had been inspected macroscopically for colonial traits and microscopically using Gram's stain [12].

*Standardization of Inoculum*

Colonies from overnight cultures of the test organisms were transferred using a sterile inoculating loop into tubes containing approximately 2.0 mL of sterile normal saline (0.9%). The turbidity was adjusted to match the 0.5 McFarland standard [13].

*Extract Working Concentrations Preparation*

The greatest stock concentration, 100 mg/mL, was obtained by dissolving one gram of the crude extract in ten milliliters of 2 % Dimethyl Sulfoxide (DMSO) in a test tube. Serial dilutions were prepared using distilled water to obtain concentrations of 100, 50, 25, 12.5, and 6.25 mg/mL.

*Determination of Antibacterial Activity*

The antibacterial effectiveness of the *S. aromaticum* extracts was assessed using the agar well diffusion method, which was initially demonstrated by [13]. A sterile swab was used to inoculate the Mueller-Hinton agar with the modified bacterial suspensions after it had been prepared and solidified. After that, the agar was allowed to stand for 15 minutes. Then, four 4 mm wells were drilled using a sterile cork tool. The ethanol extract was added to 0.2 milliliters at varying doses (50, 25, 12, and 6.25 milligrams per milliliter). Plates were incubated at 37 °C for 24 h. Antibacterial activity was evaluated by measuring the diameter of inhibition zones (mm).

## Results and discussion

The study's findings demonstrated that the ethanolic extract of cloves (*Syzygium aromaticum)* exhibited concentration-dependent antibacterial activity. The inhibition zones were highest at 50%, followed by 25 % and 12.5 %, while no inhibition was observed at 6.25 % (***Table 1*** and ***Fig. 1***).

This aligns with the findings of [14], who reported a similar trend where higher concentrations of clove extract produced significantly larger inhibition zones against *E. coli*, *P. aeruginosa*, and *Salmonella* species. Their study showed inhibition zones up to 27 mm using ethanolic extract concentrations ranging from 100 to 500 mg/mL.

**Table 1**
Diameter of inhibition zones produced by ethanolic clove extract against test isolates

| Test isolates | Concentration ethanolic extract of cloves, % | | | | Mean±SD |
|---|---|---|---|---|---|
| | 50.0 | 25.0 | 12.5 | 6.25 | |
| *Escherichia coli* | 25 mm | 21 mm | 16 mm | 0 | 15.50 ± 10.37 |
| *Klebsiella pneumoniae* | 20 mm | 18 mm | 15 mm | 0 | 13.25 ± 8.38 |
| *Pseudomonas aeruginosa* | 24 mm | 17 mm | 15 mm | 0 | 14,00 ± 9.71 |

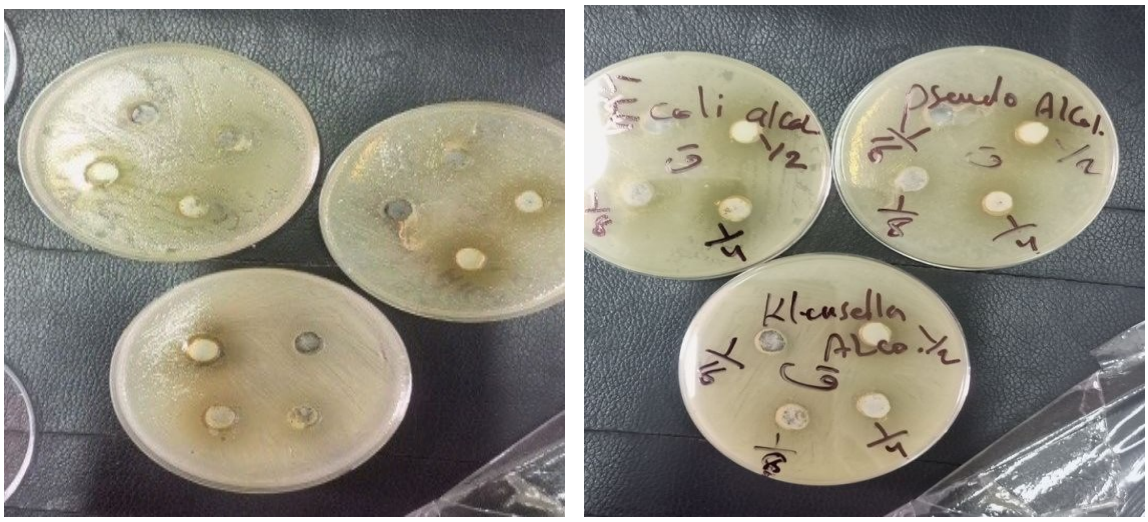


**Fig. 1.** showed the Clove ethanol extract's inhibitory zone in relation to the test isolates

Among the tested bacteria, *E. coli* showed the highest sensitivity (25 mm at 50 %), followed by *P. aeruginosa* (24 mm) and *K. pneumoniae* (20 mm). These results align with previous studies [15], which found that *E. coli* and *P. aeruginosa* were significantly inhibited by clove extract with inhibition zones between 18–26 mm at higher concentrations.

The antibacterial activity of clove is mainly attributed to its bioactive constituents, particularly eugenol, which disrupts bacterial membranes, inhibits enzyme activity, and impairs biofilm formation. Eugenol has been shown to inhibit fimbrial gene expression and curli formation in *E. coli*, significantly reducing bacterial adhesion [16]. In *P. aeruginosa*, clove extract interferes with quorum-sensing pathways essential for virulence and biofilm production [17].

In addition, recent studies also indicate synergistic effects when clove extract is combined with conventional antibiotics. The combination of clove extract with imipenem or amoxicillin–clavulanic acid significantly reduced the minimum inhibitory concentration (MIC) against multidrug-resistant *P. aeruginosa*, suggesting its potential role as an adjuvant therapy [18–20]. Overall, the data suggest that clove extract holds promising antibacterial potential, particularly against Gram-negative pathogens. The findings encourage more research into its mechanisms of action and potential therapeutic uses, as well as supporting earlier investigations.

## Conclusions

Studies have shown that clove extract exhibits pronounced antibacterial activity against multidrug-resistant Gram-negative bacteria. It was found that the expression of antimicrobial properties depends on the concentration of the ethanolic extract of cloves. The largest inhibition zones were observed at 50 % concentration: *E. coli* (25 mm), *P. aeruginosa* (24 mm), and *K. pneumoniae* (20 mm). At 6.25 %, no inhibition was observed. Among the three microorganisms studied, the *E. coli* test culture demonstrated the highest sensitivity to ethanolic clove extract at concentrations of 12.5, 25.0, and 50.0 %, with growth inhibition zones of 16.0, 21.0 and 25.0 mm, respectively.

## Conflict of interest

The author state that there is no conflict of interest.

## References

1. Taati Moghadam, M., Khoshbayan, A., Chegini, Z., Farahani, I., & Shariati, A. (2020). Bacteriophages, a new therapeutic solution for inhibiting multidrug-resistant bacteria causing wound infection: lesson from animal models and clinical trials. *Drug Design, Development and Therapy*, 14, 1867–1883. https://doi.org/10.2147/dddt.s251171
2. Lau, K. Y., & Rukayadi, Y. (2015). Screening of tropical medicinal plants for sporicidal activity. *International Food Research Journal*, 22 (1), 421–425.
3. Haro-González, J. N., Castillo-Herrera, G. A., Martínez-Velázquez, M., & Espinosa-Andrews, H. (2021). Clove essential oil (*Syzygium aromaticum* L. Myrtaceae): Extraction, chemical composition, food applications, and essential bioactivity for human health. *Molecules*, 26 (21), 6387. https://doi.org/10.3390/molecules26216387
4. Velluti, A., Sanchis, V., Ramos, A. J., Egido, J., & Marín, S. (2003). Inhibitory effect of cinnamon, clove, lemongrass, oregano and palmarose essential oils on growth and fumonisin B1 production by Fusarium proliferatum in maize grain. *International Journal of Food Microbiology*, 89 (2–3), 145–154. https://doi.org/10.1016/s0168-1605(03)00116-8

5. Elbestawy, M. K. M., El-Sherbiny, G. M., & Moghannem, S. A. (2023). Antibacterial, antibiofilm and anti-inflammatory activities of eugenol clove essential oil against resistant *Helicobacter pylori*. *Molecules*, 28 (6), 2448. https://doi.org/10.3390/molecules28062448
6. Kavanaugh, N. L., & Ribbeck, K. (2012). Selected Antimicrobial essential oils eradicate *Pseudomonas* spp. and *Staphylococcus aureus* biofilms. *Applied and Environmental Microbiology*, 78 (11), 4057–4061. https://doi.org/10.1128/aem.07499-11
7. Al-Tawalbeh, D. M., Alawneh, J. M., Momani, W., & Mayyas, A. (2025). Comparative antibacterial activity of clove extract against Pseudomonas aeruginosa. *BMC Complementary Medicine and Therapies*, 25 (1), 7. https://doi.org/10.1186/s12906-024-04740-7
8. Garba, L., Lawan, H. S., Puma, H. U., Abdullahi, M. M., Yusuf, I., & Mukhtar, M. D. (2019). Phytochemical screening and *in vitro* bacteriostatic effects of *Syzigium aromaticum* (clove) extracts on clinical bacterial isolates. *Journal of Biochemistry, Microbiology and Biotechnology*, 7 (1), 5–9. https://doi.org/10.54987/jobimb.v7i1.445
9. Oteng Mintah, S., Asafo-Agyei, T., Archer, M.-A., Atta-Adjei Junior, P., Boamah, D., Kumadoh, D., Appiah, A., Ocloo, A., Duah Boakye, Y., & Agyare, C. (2019). Medicinal plants for treatment of prevalent diseases. *Pharmacognosy - Medicinal Plants*, 17, 1–9. https://doi.org/10.5772/intechopen.82049
10. Kubmarawa, D., Khan, M. E., & Shuaibu, A. (2012). Comparative phytochemical screening and biological evaluation of n-hexane and water extracts of Acacia tortilis. *Research in Pharmaceutical Biotechnology*, 4 (2), 18–23. https://doi.org/10.5897/rpb11.029
11. Sanusi, S. B., Audu, Y., Hamza, I., Usman, A., & Makama, P. (2019). Phytochemical analysis and antibacterial activities of ginger (*Zingiber officinale*) collected from different parts of Kaduna state against selected bacteria isolated from wound. *Science World Journal*, 14 (4), 62–65.
12. Chesebrough, M. (2005). *Medical laboratory manual for tropical countries. Part 2. Second Edition*. New York: Cambridge University Press
13. Sanusi, S. B., Lawal, S. M., Usman, A., Musa, F. M., & Ardo, B. (2022). Phytochemical analysis and antibacterial activity of stem bark extracts of *Detarium microcarpum* against bacteria causing gastrointestinal tract infections in humans. *Dutse Journal of Pure and Applied Sciences*, 8 (1b), 82–89. https://doi.org/10.4314/dujopas.v8i1b.10
14. Shehu, I., Sanusi, S. B., & Saka, H. K. (2023). Study on antibacterial activity of clove (*Syzygium aromaticum*) crude extract against *Staphylococcus aureus*, *Escherichia coli*, *Salmonella* sp. and *Pseudomonas* sp. *Science World Journal*, 18 (1), 97–100.
15. Bisht, D., Faujdar, S., & Sharma, A. (2020). 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 (1), 180–186. https://doi.org/10.4103/jfmpc.jfmpc_908_19
16. Devi, K. P., Nisha, S. A., Sakthivel, R., & Pandian, S. K. (2010). Eugenol (an essential oil of clove) acts as an antibacterial agent against Salmonella typhi by disrupting the cellular membrane. *Journal of Ethnopharmacology*, 130 (1), 107–115. https://doi.org/10.1016/j.jep.2010.04.025
17. Musthafa, K. S., Balamurugan, K., Pandian, S. K., & Ravi, A. V. (2012). 2,5-Piperazinedione inhibits quorum sensing-dependent factor production in *Pseudomonas aeruginosa* PAO1. *Journal of Basic Microbiology*, 52 (6), 679–686. https://doi.org/10.1002/jobm.201100292
18. Abbas, M., Gururani, M. A., Ali, A., Bajwa, S., Hassan, R., Batool, S. W., Imam, M., & Wei, D. (2024). Antimicrobial properties and therapeutic potential of bioactive compounds in Nigella sativa: A review. *Molecules*, 29 (20), 4914. https://doi.org/10.3390/molecules29204914
19. Hemaiswarya, S., & Doble, M. (2009). Synergistic interaction of eugenol with antibiotics against Gram negative bacteria. *Phytomedicine*, 16 (11), 997–1005. https://doi.org/10.1016/j.phymed.2009.04.006
20. Mahboub, R., & Memmou, F. (2016). Antimicrobial properties of 6-bromoeugenol and eugenol. *International Letters of Natural Sciences*, 53, 57–64. https://doi.org/10.56431/p-4u2geq

**ORCID**

Amaal M. Khudaier https://orcid.org/0009-0009-2207-4266