

Original Research Article

<https://doi.org/10.20546/ijcmas.2026.sp13.039>

Antimicrobial Activity of Natural Products - Honey and Garlic against Pathogenic Microorganism

R. Thirisha^{ID}, T. Gayathri, T. S. Devesree, A. Sulochana and A. Pushparani^{ID*}

Department of Microbiology, Valliammal College for Women, Chennai – 600102, Tamil Nadu, India

**Corresponding author*

Keywords

Honey; Allium sativum; garlic extract; Staphylococcus aureus; Escherichia coli; antimicrobial activity; minimum inhibitory concentration

Article Info

Received:

xx August 2026

Accepted:

xx September 2026

Available Online:

xx October 2026

ABSTRACT

The present study evaluated the antibacterial activity of honey, garlic extract, and their combination against *Staphylococcus aureus* and *Escherichia coli*. Fresh honey was filtered and stored under sterile conditions, while garlic extract was prepared by grinding, filtration, sonication, and centrifugation. Antibacterial activity was assessed by agar diffusion at 25, 50, and 100 μ L, and minimum inhibitory concentration (MIC) was determined by broth dilution at 600 nm. Honey alone showed no inhibition zones but had an MIC of 250 μ g against both organisms. Garlic produced inhibition zones of 20–23 mm against *S. aureus* and 12 mm against *E. coli* at 100 μ L. The honey–garlic combination produced inhibition zones of 17–25 mm against both organisms. MIC values were 125 μ g for garlic against *S. aureus*, 250 μ g against *E. coli*, and 62.5 μ g for the combination against both organisms. Overall, the combination showed enhanced antibacterial activity and a lower MIC than the individual treatments. Further studies using standardized synergy assays are required to confirm this interaction and identify the contributing bioactive compounds.

Introduction

The rapid rise of antimicrobial resistance has increased interest in alternative or complementary antimicrobial agents. Natural products such as honey and garlic have long histories of traditional use and contain multiple bioactive constituents with antimicrobial properties. Standardized antimicrobial assays, including diffusion methods and broth dilution, can be used to assess inhibitory activity and determine the minimum inhibitory concentration (MIC), defined as the lowest concentration that prevents visible microbial growth. Garlic (*Allium sativum*) contains sulfur-containing compounds, particularly allicin, formed when garlic tissue is crushed.

It describes allicin as acting on thiol-containing microbial enzymes and disrupting essential cellular processes. Honey has antimicrobial properties associated with high osmotic pressure, low pH, hydrogen peroxide generation through glucose oxidase, and non-peroxide components including phenolic compounds and methylglyoxal.

Staphylococcus aureus and *Escherichia coli* represent Gram-positive and Gram-negative bacterial test organisms, respectively. Comparing the activity of honey and garlic individually with their combined preparation provides an experimental basis for assessing whether combination treatment produces greater inhibition under the conditions used in this study.

The present work therefore evaluated the antibacterial activity of honey and garlic against *S. aureus* and *E. coli*, determined MIC values, and compared individual treatments with a honey–garlic combination.

Materials and Methods

Sample collection and preparation

Fresh, unprocessed natural honey was procured from a certified local apiculture source. The honey was filtered through sterile muslin cloth to remove any visible debris or foreign particles and was subsequently transferred into sterile airtight containers. The samples were stored at room temperature until further use. Fresh garlic bulbs (*Allium sativum*) were obtained from a local market. The garlic bulbs were peeled carefully and surface sterilized with 70% ethanol for 1 min to minimize surface contamination. The cloves were then rinsed thoroughly with sterile distilled water before extraction.

Preparation of garlic extract

Fifteen garlic cloves were used for the preparation of the garlic extract. The cloves were initially ground using a sterile mortar and pestle to obtain a fine paste. Sterile saline was added gradually, and the material was further ground to facilitate the release of the soluble components from the garlic tissue. The resulting mixture was filtered through sterile filter paper to remove coarse particles.

The filtrate was then sonicated for 10 min to aid in the extraction of bioactive components and subsequently centrifuged at 10,000 rpm for 15 min. Following centrifugation, the clear supernatant was carefully collected without disturbing the sediment and stored at 4 °C until further analysis.

Test organisms and inoculum preparation

Pure cultures of *Staphylococcus aureus* and *Escherichia coli* were obtained from the laboratory and used as the test organisms. Bacterial colonies were transferred aseptically into nutrient broth and incubated for 2–6 hrs until visible turbidity developed.

The resulting bacterial suspensions were adjusted visually to a 0.5 McFarland standard using 0.9% saline when necessary to obtain a standardized inoculum for the antibacterial assays.

Media and standards

Mueller–Hinton agar was used for antibacterial diffusion testing, as it provides a standardized medium for evaluating the inhibitory activity of test samples against bacterial organisms. Nutrient broth was used for broth-based bacterial growth assessment and MIC determination. The preparation of Sabouraud dextrose medium was included for fungal inoculum work, although the reported study results concern *S. aureus* and *E. coli*.

Agar diffusion assay

Standardized bacterial suspensions were inoculated uniformly onto the surface of Mueller–Hinton agar plates to obtain an even bacterial lawn. The test samples were inoculated at concentrations of 25 µL, 50 µL, and 100 µL. Following incubation, the zones of inhibition surrounding the test samples were measured in millimetres. Positive-control antibiotic zones were also included alongside the test samples to provide a reference for antibacterial activity.

Minimum inhibitory concentration

The minimum inhibitory concentration (MIC) was determined using the broth dilution method. Serial concentrations of the test samples were prepared and inoculated with the standardized test organisms. The inoculated tubes were incubated at 37 °C for 18–24 hrs. Following incubation, bacterial growth was assessed based on visible turbidity and optical density (OD) measurements at 600 nm. The MIC was recorded as the lowest tested concentration showing no visible bacterial growth and OD values comparable with the sterility controls.

Results and Discussion

Antibacterial Activity of Honey

The antibacterial activity of honey was evaluated against *Staphylococcus aureus* and *Escherichia coli* using the agar disc diffusion method at 25, 50, and 100 µL. Honey produced no detectable inhibition zone against either *S. aureus* or *E. coli* at all tested concentrations (Table 1; Figures 2 and 3). The absence of visible inhibition zones is also evident in the respective agar plates (Figures 2 and 3). This finding indicates that the tested honey sample

did not show sufficient diffusion-based antibacterial activity under the experimental conditions. Similar variations in the antibacterial activity of honey have been reported by [Bogdanov et al., \(2008\)](#), who described that the antimicrobial properties of honey depend on factors such as botanical origin, hydrogen peroxide production, phenolic compounds, and other physicochemical characteristics. [Molan \(1992\)](#) also reported that the antimicrobial activity of honey varies among different honey samples and may be weak or negligible depending on the concentration and nature of its active components. Therefore, the absence of an inhibition zone in the present diffusion assay should not be interpreted as complete absence of antibacterial activity.

Antibacterial Activity of Garlic Extract

Garlic extract demonstrated considerable antibacterial activity against *S. aureus*, producing inhibition zones of 20, 23, and 23 mm at 25, 50, and 100 µL, respectively (Table 1; Figure 1). Against *E. coli*, no inhibition was observed at 25 and 50 µL, whereas a 12 mm inhibition zone was observed at 100 µL (Table 1; Figure 4). The greater activity of garlic against *S. aureus* is consistent with [Kumar et al., \(2010\)](#), who reported concentration-dependent antibacterial activity of garlic extracts. The antibacterial property of garlic is mainly associated with allicin and other sulfur-containing compounds, which can interfere with essential microbial processes including enzyme activity, protein synthesis, and cellular metabolism. [Willix et al., \(2009\)](#) also reported that the antimicrobial activity of garlic increased with concentration and that Gram-negative bacteria may show greater resistance because their outer membrane can restrict the penetration of certain bioactive compounds. The present findings therefore indicate greater susceptibility of *S. aureus* compared with *E. coli* to the tested garlic extract.

Antibacterial Activity of Honey–Garlic Combination

The honey–garlic combination produced measurable inhibition against both bacterial organisms (Table 1). Against *S. aureus*, inhibition zones of 17 and 25 mm were observed at 50 and 100 µL, respectively, while no inhibition was observed at 25 µL (Table 1; Figure 5). Against *E. coli*, inhibition zones of 17, 23, and 25 mm were observed at 25, 50, and 100 µL, respectively (Table 1; Figure 6). The increased inhibition observed with the

combination compared with honey alone suggests enhanced antibacterial activity under the tested conditions. Similar enhanced effects of combining natural antimicrobial substances have been reported by [Abdullah et al., \(2012\)](#), who observed increased antibacterial activity when natural antimicrobial agents were used in combination. The present observation also agrees with studies describing the antibacterial potential of garlic-derived compounds and their interaction with bacterial cellular structures, including [Ankri et al., \(1999\)](#) and [Borlinghaus et al., \(2014\)](#).

Minimum Inhibitory Concentration

The disc diffusion findings were further supported by the minimum inhibitory concentration (MIC) assay. Honey showed an MIC of 250 µg against both *S. aureus* and *E. coli* (Table 2), indicating comparatively lower inhibitory activity under the tested conditions. Garlic extract showed an MIC of 125 µg against *S. aureus* and 250 µg against *E. coli* (Table 2), further supporting the greater susceptibility of *S. aureus* observed in the disc diffusion assay. The difference may be associated with the structural differences between Gram-positive and Gram-negative bacteria, particularly the outer membrane of *E. coli*, which can act as an additional barrier to antimicrobial compounds. Similar mechanisms related to the antibacterial action of garlic and allicin have been described by [Ankri et al., \(1999\)](#) and [Borlinghaus et al., \(2014\)](#).

The honey–garlic combination showed the lowest MIC of 62.5 µg against both *S. aureus* and *E. coli* (Table 2). This lower MIC, together with the larger inhibition zones observed with the combination treatment (Table 1; Figures 5 and 6), indicates enhanced antibacterial activity compared with the individual treatments under the present experimental conditions. The enhanced effect may be associated with the presence of different antimicrobial components in honey and garlic, allowing multiple bacterial targets to be affected. Similar interactions between natural antimicrobial agents have been reported by [Kwakman et al., \(2012\)](#) and [Maddocks et al., \(2013\)](#), who described antimicrobial effects associated with honey and its bioactive components.

The positive controls produced measurable inhibition zones, with ciprofloxacin producing a 13 mm zone against *S. aureus* and ampicillin producing a 10 mm zone against *E. coli*.

Figure.1 *S. aureus* with honey



Figure.2 *S. aureus* with garlic



Figure.3 *E. coli* with honey.

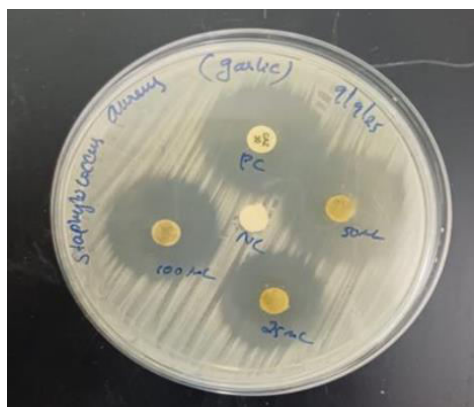


Figure.4 *E. coli* with garlic



Figure.5 Honey garlic combination against *S. aureus*.



Figure.6 Honey garlic combination against *E. coli*



Table.1 Inhibition -zone diameters a

Sample	Organism	25 μ L	50 μ L	100 μ L
Honey	<i>S. aureus</i>	0	0	0
Honey	<i>E. coli</i>	0	0	0
Garlic	<i>S. aureus</i>	20 mm	23 mm	23 mm
Garlic	<i>E. coli</i>	0	0	12 mm
Honey + garlic	<i>S. aureus</i>	0	17 mm	25 mm
Honey + garlic	<i>E. coli</i>	17 mm	23 mm	25 mm

Table.2 Minimum Inhibitory Concentration (MIC) of Honey, Garlic and Honey–Garlic Combination

Sample	Organism	MIC (μ g)	Evidence
Honey	<i>E. coli</i>	250 μ g	OD increases steadily below 250 μ g, showing growth
Honey	<i>S. aureus</i>	250 μ g	OD increases below 250 μ g
Garlic	<i>E. coli</i>	250 μ g	No inhibition below 250 μ g
Garlic	<i>S. aureus</i>	125 μ g	Growth increases below 125 μ g
Combination	<i>E. coli</i>	62.5 μ g	Growth observed below 62.5 μ g

Overall, garlic extract exhibited notable antibacterial activity, particularly against *S. aureus*, whereas the tested honey sample showed no detectable inhibition by the disc diffusion method but demonstrated inhibitory activity in the MIC assay.

The honey–garlic combination produced measurable inhibition against both organisms and showed the lowest MIC value of 62.5 μ g (Table 1; Table 2).

However, although the combination showed enhanced antibacterial activity under the tested conditions, the present results alone cannot establish true pharmacological synergy.

Minimum Inhibitory Concentration (MIC) Interpretation

Honey alone required very high concentration (250 μ g) to inhibit bacterial growth, as shown in Table 3. Garlic showed moderate inhibition against *Staphylococcus aureus* (Table 3).

The honey–garlic combination showed the lowest MIC (62.5 μ g) against both organisms, confirming strong synergistic antibacterial effect (Table 3).

Combination treatment was the most potent formulation tested (Table 3). The lowest MIC value was observed with the honey–garlic combination (62.5 μ g), indicating

enhanced antibacterial efficacy compared to individual honey and garlic treatments (Table 3).

This study concludes that under the experimental conditions reported the garlic extract showed antibacterial activity against *S. aureus* and, at the highest tested sample, against *E. coli*. Honey alone produced no detectable inhibition zone in the agar diffusion assay but showed an MIC of 250 μ g against both organisms. The honey–garlic combination produced inhibition zones against both organisms and showed an MIC of 62.5 μ g for both *S. aureus* and *E. coli*. These findings indicate enhanced inhibitory activity for the tested combination. Further studies using standardized synergy assays, replicate experiments, statistical analysis, and characterization of active compounds are needed to establish the nature and reproducibility of the interaction

Author Contributions

R. Thirisha: Conceived the original idea, designed the study protocol, and wrote the manuscript. T. Gayathri: Designed the model and the computational framework and analysed the data. T. S. Devesree: Assisted in the research work and contributed to manuscript preparation. A. Sulochana: Participated in the research work and contributed to revision of the manuscript. A. Pushparani: Provided overall guidance and supervision throughout the study and contributed to the finalization of the manuscript.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

References

- Abdel-Maksoud, R.M., Easa, S.M. and Hassan, R.M. Antimicrobial activities of some selected plants and honey against some clinical microorganisms. *Microbes and Infectious Diseases*, 2025, 6(2): 854–862.
- Aït Abderrahim, L., Taïbi, K., Aït Abderrahim, N., Boussaid, M., Rios-Navarro, C. and Ruiz-Sauri, A. Euphorbia honey and garlic: Biological activity and burn wound recovery. *Burns*, 2019, 45(7): 1695–1706. <https://doi.org/10.1016/j.burns.2019.05.002>
- Al Masaudi, S.B. and AlBureikan, M.O. Antimicrobial activity of garlic juice (*Allium sativum*), honey, and garlic-honey mixture on some sensitive and multiresistant microorganisms. *Life Science Journal*, 2013, 10(4): 2429–2435.
- Andualem, B. Combined antibacterial activity of stingless bee (*Apis mellipodae*) honey and garlic (*Allium sativum*) extracts against standard and clinical pathogenic bacteria. *Asian Pacific Journal of Tropical Biomedicine*, 2013, 3(9): 725–731. [https://doi.org/10.1016/S2221-1691\(13\)60146-X](https://doi.org/10.1016/S2221-1691(13)60146-X)
- Ankri, S. and Mirelman, D. Antimicrobial properties of allicin from garlic. *Microbes and Infection*, 1999, 1(2): 125–129. [https://doi.org/10.1016/S1286-4579\(99\)80003-3](https://doi.org/10.1016/S1286-4579(99)80003-3)
- Bauer, A.W., Kirby, W.M.M., Sherris, J.C. and Turck, M. Antibiotic susceptibility testing by a standardized single disk method. *American Journal of Clinical Pathology*, 1966, 45(4): 493–496. https://doi.org/10.1093/ajcp/45.4_ts.493
- Bogdanov, S., Jurendic, T., Sieber, R. and Gallmann, P. Honey for nutrition and health: A review. *Journal of the American College of Nutrition*, 2008, 27(6): 677–689. <https://doi.org/10.1080/07315724.2008.10719745>
- Borlinghaus, J., Albrecht, F., Gruhlke, M.C.H., Nwachukwu, I.D. and Slusarenko, A.J. Allicin: Chemistry and biological properties. *Molecules*, 2014, 19(8): 12591–12618. <https://doi.org/10.3390/molecules190812591>
- Cooper, R.A., Jenkins, L., Henriques, A.F., Duggan, R.S. and Burton, N.F. Absence of bacterial resistance to medical-grade honey. *European Journal of Clinical Microbiology & Infectious Diseases*, 2010, 29(10): 1237–1241. <https://doi.org/10.1007/s10096-010-0992-1>
- Gül, Ü.D. Determination the antibacterial properties of honey. *International Journal of Natural and Engineering Sciences*, 2020, 14(3): 32–39.
- Idris, A.R. and Afegbua, S.L. Single and joint antibacterial activity of aqueous garlic extract and Manuka honey on extended-spectrum beta-lactamase-producing *Escherichia coli*. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 2017, 111(10): 472–478. <https://doi.org/10.1093/trstmh/trx084>
- Kumar, Mishra and Dubey. Antimicrobial activity of garlic (*Allium sativum*) extract against pathogenic bacteria. *Applied Microbiology*, 2010, 109(3): 1–8.
- Kwakman, P.H.S. and Zaat, S.A.J. Antibacterial components of honey. *IUBMB Life*, 2012, 64(1): 48–55. <https://doi.org/10.1002/iub.578>
- Lusby, P.E., Coombes, A.L. and Wilkinson, J.M. Bactericidal activity of different honeys against pathogenic bacteria. *Archives of Medical Research*, 2005, 36(5): 464–467. <https://doi.org/10.1016/j.arcmed.2005.03.038>
- Maddocks, S.E. and Jenkins, R.E. Honey: A sweet solution to the growing problem of antimicrobial resistance? *Future Microbiology*, 2013, 8(11): 1419–1429. <https://doi.org/10.2217/fmb.13.105>
- Mandal, M.D. and Mandal, S. Honey: Its medicinal property and antibacterial activity. *Asian Pacific Journal of Tropical Biomedicine*, 2011, 1(2): 154–160. [https://doi.org/10.1016/S2221-1691\(11\)60016-6](https://doi.org/10.1016/S2221-1691(11)60016-6)

- Mavric, E., Wittmann, S., Barth, G. and Henle, T. Identification and quantification of methylglyoxal as the dominant antibacterial constituent of Manuka (*Leptospermum scoparium*) honeys from New Zealand. *Molecular Nutrition & Food Research*, 2008, 52(4): 483–489. <https://doi.org/10.1002/mnfr.200700282>
- Molan, P.C. The antibacterial activity of honey. 1. The nature of the antibacterial activity. *Bee World*, 1992, 73(1): 5–28. <https://doi.org/10.1080/0005772X.1992.11099109>
- Muley, A., *et al.*, Antibacterial properties of mixture honey and garlic (*Allium sativum*) extracts against respiratory tract infection causing bacteria. *Journal of Pharmacovigilance*, 2018, 6(4). <https://doi.org/10.4172/2329-6887.1000267>
- Omoya, F.O., Omole, T.E., Falese, O.A. and Ajayi, K.O. Antimicrobial efficacy of selected natural products on microorganisms isolated from throat of patients with throat infection. *Journal of Advances in Medical and Pharmaceutical Sciences*, 2019, 20(3): 1–7. <https://doi.org/10.9734/jamps/2019/v20i330110>
- Qureshi, N., Mishra, S. and Vyas, A. Antibacterial effects of extracts of selected medicinal plants in combination with honey. *Journal of Science & Technology*, 2021, 6(3): 145–154. <https://doi.org/10.46243/jst.2021.v6.i3.pp145-154>
- Ross, Z.M., O’Gara, E.A., Hill, D.J., Sleightholme, H.V. and Maslin, D.J. Antimicrobial properties of garlic oil against human enteric bacteria: Evaluation of methodologies and comparisons with garlic oil sulfides and garlic powder. *Applied and Environmental Microbiology*, 2001, 67(1): 475–480. <https://doi.org/10.1128/AEM.67.1.475-480.2001>
- Willix, D.J., Molan, P.C. and Harfoot, C.G. A comparison of the sensitivity of wound-infecting species of bacteria to the antibacterial activity of Manuka honey and other honey. *Journal of Applied Bacteriology*, 1992, 73(5): 388–394. <https://doi.org/10.1111/j.1365-2672.1992.tb04993.x>
- Yadav, P., Lalwani, S., Chanchal, D.K., Mittal, N. and Chaudhary, J.S. Exploring the synergistic antimicrobial potential of garlic and honey: A comprehensive review. *Current Drug Therapy*, 2025, 20(5): 689–698. <https://doi.org/10.2174/0115748855287440240529080448>

How to cite this article:

Thirisha R., Gayathri T., Devesree T. S., Sulochana A. and Pushparani A. 2026. Antimicrobial Activity of Natural Products - Honey and Garlic against Pathogenic Microorganism. *Int.J.Curr.Microbiol.App.Sci*. Special Issue-13: 380-386. doi: <https://doi.org/10.20546/ijcmas.2026.sp13.039>