

An Exploration of Allium Sativum and Honey As Antimicrobial Agents Against Ten Strains of Fatal Pathogens

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Abstract

In 2019, bacterial infections were estimated to be the second leading cause of death worldwide, responsible for one in eight global deaths (GBD, 2019). More than half of these were caused by bacteria such as *Streptococcus pneumoniae*, *E. coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. Although antibiotics are prescribed for these infections, inadequate access, resistant strains, and differing immune systems render many ineffective. To investigate alternative treatments, a Phase 1 investigation on the antibacterial properties of *Allium sativum* (garlic) was expanded to observe inhibitory properties against ten strains of gram-positive and gram-negative bacteria and garlic's strength when combined with various kinds of honey. Garlic was tested in extract and pure Allicin form and in combination with honey from Iowa, India, and New Zealand.

Results showed that a combination of garlic extract (GE) and Manuka honey (MH) was the most effective against strains of *Pseudomonas*, *E.Coli*, and *Staphylococcus*, with inhibition zones up to 91% as large as those displayed by regular antibiotic treatments for these bacteria. However, this study has limitations, such as the need for clinical trials, incomplete understanding of garlic-honey synergistic mechanisms, uncertainty about pill formulation bioavailability, and testing confined to controlled laboratory conditions. Despite these limitations, the in-vitro results suggest that garlic and honey could serve as alternative treatments to antibiotics for certain bacterial respiratory infections, which would be transformative for individuals who are immuno-compromised, allergic to antibiotics, lack access to them, or cannot afford them. To demonstrate the practicality of this treatment, GE+MH candy and pill formulas were developed, offering a convenient, affordable dual-delivery approach with a smaller risk of resistance development.

Keywords: Garlic, Honey, Microbiology, Drug Resistance, Antibiotic

Introduction

Antibiotic-resistant infections are a rising concern in microbiology, causing 4.95 million of the 13.7 million bacterial infection-related deaths in 2019 (GBD, 2019). The high cost, limited availability, and side effects of current antibiotics often prevent patients from completing their treatment, increasing resistance risk. Furthermore, due to a pressing need for newer, more resilient alternatives, there has been greater interest in natural antimicrobials that could become future antibiotics.

A 2021 study by Bhatwalker found garlic to be a potent antimicrobial that strongly exhibited inhibition against multiple bacterial strains, including drug-resistant ones (Bhatwalker et al., 2021). This is due to the way garlic disrupts bacterial membranes and inhibits essential protein and enzyme production (Bayan, 2014). However, its highly volatile nature makes it difficult to keep stable. In a separate 2021 study, Almasaudi found that honey could be a great stabilizing compound that increases diffusion and enhances the antibacterial properties of most antimicrobials (Almasaudi, 2021). However, very little research has been done to observe the combined effects of garlic and honey.

To fill this research gap, the study focused on an essential research question: Does garlic extract and honey inhibit the growth of different strains of fatal bacterial pathogens with results comparable to standard antibiotic treatments? If so, which combination of garlic and honey is the most effective?

Based on the above background research, it was hypothesized that a combination of garlic extract and Manuka honey would be the most effective out of other combinations in stopping or

slowing the progression of the bacteria due to their combined antibacterial properties.

Methods

Materials

- Garlic essential oil (Gya Labs)
- Allicin (Fisher Scientific)
- Local honey (Bloomington, Iowa)
- Indian honey (Kerala, India)
- Manuka honey (KIVA, New Zealand)
- Penicillin drop disks (Carolina Biological Supply)
- Bacterial strains: *Streptococcus pneumoniae* (NCTC 7465), *Haemophilus influenzae* (8143), *Pseudomonas aeruginosa* (27853, NCTC 10332), *Staphylococcus aureus* (29213, NCTC 8530), *Escherichia coli* (25922, NCTC 9001), *Streptococcus pyogenes*
- Filter paper (Ahlstrom, Size 7.5)
- 10 mL vials with lids
- Glass stir sticks
- Tweezers
- Blood Agar Petri dishes
- Disposable sterile loops
- McFarland Standard saline tubes
- 0.5 ml saline vials
- MicroScan Turbidity meter
- Vortex mixer
- Bacterial incinerator
- Non-CO2 Incubator at 37°C



Fig. 1. Experimental Groups

Procedures

Note: The experiment was conducted in two BSL 2 labs with a biosafety cabinet under the supervision of a lab manager or professor with the proper safety precautions.

Sample Preparation

1. The filter paper was hole-punched into 6 mm disks and sterilized by heating on high in a standard 2.45 GHz microwave for 3 minutes.
2. Vials were labeled with abbreviations (Garlic Extract = GE, Allicin = A, Local Honey = LH, Indian Honey = IH, Manuka Honey = MH, Saline Control = SC, Penicillin = P).
3. Added 0.5 grams of test substances or 0.25 grams of each component for combinations into labeled vials. (eg. 0.5g GE for pure garlic extract and 0.25g GE + 0.25g MH for a 1:1 ratio for the combination of garlic extract and manuka honey)
4. Mixed with a glass stir stick.
5. 10-15 disks were placed in each vial with tweezers
6. Stored in a cool, dark place for 18-24 hours (Figure 3).



Fig. 2. Sterilizing Vials



Fig. 3. The prepared vials

Bacteria Plating

1. Parent bacterial strains were obtained from the labs
2. New blood agar Petri dishes were labeled.
3. Under the biosafety hood, disposable inoculation loops were used to subculture bacteria onto agar plates using the streak plate method (Sanders, 2012) (Figure 4).
4. Plates were incubated at 37°C for 24 hours.



Fig. 4. Researcher streaking plates

Experiment set-up and sample plating (after 24 hours)

1. New blood agar plates were labeled into four quadrants (Figure 5).
2. Bacterial dilutions were made by mixing bacteria from subcultured plates into a 0.5 ml saline solution using a vortex mixer (Figure 6).
3. The dilution was adjusted to a concentration of 0.11 NTU (Nephelometric Turbidity Units) using a turbidity meter referencing a 0.5 McFarland standard (Figure 7).
4. Agar plates were swabbed using the Kirby-Bauer method.
5. Pre-soaked disks were placed on corresponding quadrants with metal-tipped tweezers.
6. Steps 2-8 were repeated for each bacterial strain.
7. Plates were incubated at 37°C for 24 hours.



Fig. 5. Pattern for petri dishes divided into four equal quadrants



Fig. 6. Vortex Machine



Fig. 7. Turbidity meter

Data collection (24 hours later)

1. The petri dishes were transferred to the biosafety hood.
2. The diameter of the inhibition zone around each drop disk was measured in millimeters and recorded in a spreadsheet along with additional observations.

Over the span of the study, nine trials were conducted at both sites, testing various bacteria, methods, and materials to strengthen the study, enhance results, and reduce variables.

Garlic-Honey Drop and Capsule procedure

After promising results, garlic extract and Manuka honey drops and capsules were made to demonstrate the research's practicality and effectiveness using the same 1:1 ratio used in this study.



Fig. 8. GE+MH drops



Fig. 9. GE+MH capsules

Results

By comparing the average inhibition zone for each sample across the strains, the study found that garlic extract, Manuka honey, and a combination of both displayed significant average inhibition in *Pseudomonas aeruginosa* (Figure 10), *E. coli* (Figure 11), and *Staphylococcus aureus* (Figure 12).

Allicin showed intermediate inhibition but was less effective than garlic extract. As expected, the saline control had no inhibition, while penicillin showed some inhibition for its typical bacteria but was less effective than anticipated.

To fully understand the effectiveness of the experimental groups, each experimental group's inhibition zones were compared with susceptibility parameters found for common antibiotics (NIH, 2021) in tables in Figures 13 and 14.

The one-way ANOVA results showed that combining garlic extract and Manuka honey significantly inhibited bacterial growth across several strains, including *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *E. coli* (all $p < 0.001$). The F-statistics were notably high (e.g., $F = 19.08$ for *P. aeruginosa* and $F = 178.67$ for *Staphylococcus aureus*), indicating substantial differences between the treatment groups. Additionally, the large effect sizes (e.g., $\eta^2 = 0.98$ for *Staphylococcus aureus*) suggest a strong impact of the treatment on bacterial inhibition.

Examples of observed inhibition zones across different bacteria in different trials:

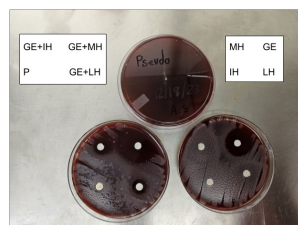


Fig. 10. Trial 1 results on *Pseudomonas*

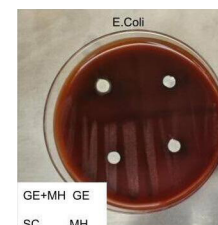


Fig. 11. Trial 3 results on *E. coli*

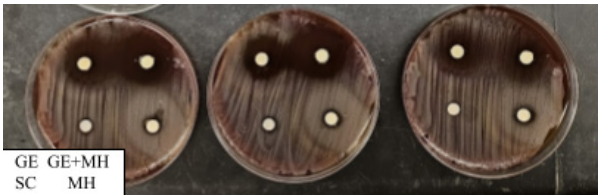


Fig. 12. Trial 7 results on *Staphylococcus aureus*



Fig. 13. Results from Trial 2 on *Haemophilus*

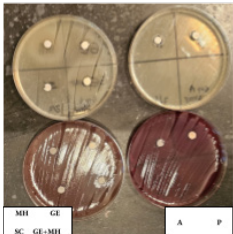


Fig. 14. Trial 9 results on Group A Strep

Trial Number	Picture of prepared Petri Dishes	Notes
Trial 1		Pilot testing on <i>Haemophilus</i> <i>Pseudomonas</i> <i>Strep pneumo</i>
Trial 2		Testing on <i>Haemophilus</i> <i>Pseudomonas</i> <i>Strep pneumo</i> with a turbidity of 0.06 NTU, half of before. Just the Manuka Honey was tested due to a lack of results from local or Indian honey.
Trial 3		Testing on <i>Staphylococcus</i> <i>E.Coli</i>
Trial 4		Testing on <i>Staphylococcus</i> <i>Pseudomonas</i> <i>Strep pneumo</i>
Trial 5		A purity test of the four bacteria being tested at this lab was conducted to look for any secondary contamination from the bacteria themselves.

Trial Number	Picture of prepared Petri Dishes	Notes
Trial 6		Testing on <i>Staphylococcus</i> <i>Pseudomonas</i> <i>Strep pneumo</i> <i>E.Coli</i> At both 0.06 and 0.11 NTU.
Trial 7		Testing on <i>Staphylococcus</i> <i>Pseudomonas</i> <i>Strep pneumo</i> <i>E.Coli</i> Garlic Extract, Manuka Honey, Garlic Extract + Manuka Honey, and the saline control were tested on the four bacteria with three replications of each plate. This was done at the second lab, and a throat culture was done from the researcher's throat to see how GE+MH reacts with regular microbiota.
Trial 8		Testing on <i>Staphylococcus</i> <i>Pseudomonas</i> <i>Strep pneumo</i> <i>E.Coli</i> Continued testing at the second lab explored the different effects of nutrient agar and blood agar on bacteria growth and test subject diffusibility.
Trial 9		Testing on <i>Staphylococcus</i> <i>Pseudomonas</i> <i>Strep pyogenes</i> <i>E.Coli</i> - Group A Strep Final replicability testing was done at lab one to confirm the results seen in all previous trials.

Fig. 15. Table summarizing the nine trials.

	<i>P. aeruginosa</i>	<i>H. influenzae</i>	<i>S. pneumoniae</i>	<i>Staph. Aureus</i>	<i>E.coli</i>	Group A <i>Strep</i>
Susceptibility Parameter	IZ > 14	IZ > 12	IZ > 14	IZ > 14	IZ > 14	IZ > 15
Garlic extract	I	R	R	S	R	I
Allicin	R	R	R	R	R	R
Manuka Honey	R	R	R	R	R	R
Local Honey	R	R	R	R	R	R
Indian Honey	R	R	R	R	R	R
Garlic Extract + Manuka Honey	S	I	S	S	S	I
Garlic Extract + Local Honey	R	R	R	R	R	R
Garlic Extract + Indian Honey	I	R	R	R	R	R
Penicillin	R	R	S	S	R	R

S = Susceptible
I = Intermediate
R = Resistant
IZ = Inhibition Zone (mm)

Fig. 16. Analysis of average inhibition zones of sample groups across the first six trials in comparison to international standards on the bacteria strains tested at lab site 1.

	<i>P. aeruginosa</i>	<i>S. pneumoniae</i>	<i>Staph. aureus</i>	<i>E.coli</i>	Throat Culture
Susceptibility Parameter	IZ > 14	IZ > 14	IZ > 14	IZ > 14	Visual Analysis
Garlic extract	I	I	I	R	R
Manuka Honey	R	R	R	R	S
Garlic Extract + Manuka Honey	S	S	S	S	S
Saline Control	R	R	R	R	R

Fig. 17. Analysis of average inhibition zones of sample groups from trials 7-9 in comparison to international standards on the bacteria strains tested at lab site 2.

Bacteria	P-Value	F Statistic	Effect Size (Eta ²)
<i>P. aeruginosa</i>	P < 0.001	19.08	0.81
<i>S. pneumoniae</i>	P < 0.001	23.58	0.91
<i>H. influenzae</i>	P < 0.001	46.57	0.96
<i>Staph. aureus</i>	P < 0.001	178.67	0.98
<i>E. Coli</i>	P < 0.001	17.19	0.89
Group A <i>Strep</i>	P < 0.05	16.48	0.92

Fig. 18. One-Way Anova Analysis for Inhibition of experimental groups across tested bacteria

Discussion

The results of this study supported the original hypothesis and were consistent with previous research done by Bhatwalker and Almasaudi. Garlic's effectiveness increased by 63% when combined with Manuka honey (Figure 18), and the combination achieved inhibition zones of up to 91.5% of the size of those produced by standard antibiotics (Figure 19). This effective solution is also globally affordable for all ages and body types, with a low material cost of \$0.05 per drop and \$0.18 per capsule.

Average Inhibition Zones for Garlic, Manuka Honey and Garlic and Manuka Honey

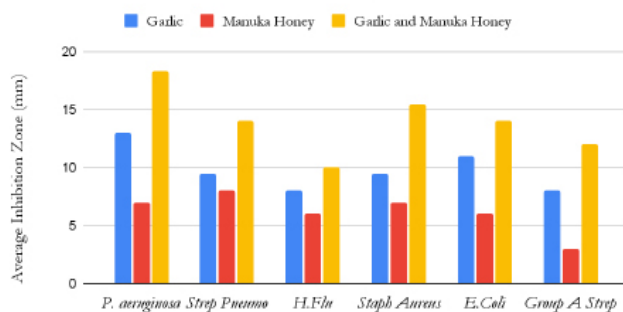


Fig. 19. Comparison of GE, MH, and GE+MH using average inhibition zones

Garlic and Manuka Honey Inhibition Zones in Comparison to Antibiotics

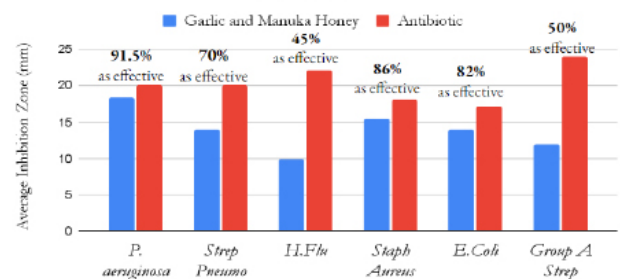


Fig. 20. Comparison of GE+MH and common antibiotics

Conclusion

This study offers a promising exploration of the synergistic antimicrobial properties of garlic and honey, an area yet to be widely examined in the literature.

Garlic (*Allium sativum*) and Manuka honey (*Leptospermum scoparium*) were the most effective in inhibiting bacterial growth, particularly against strains of *E. coli*, *Pseudomonas*, and *Staphylococcus*. These results are exciting because they are effective against notoriously resistant strains of bacteria and because research suggests these bacteria may not mutate against different types of honey and garlic due to their composition and variance (Almasaudi, 2021).

For immunocompromised patients who frequently face recurrent infections and diminishing antibiotic options, a natural, affordable

alternative with virtually no side effects or allergies could be transformative. This research highlights the potential of garlic and honey as a versatile, cost-effective remedy that can be consumed as a food supplement or medication without any drawbacks.

This study demonstrates promising laboratory results; however, several important limitations should be acknowledged. The primary limitation is that the experiments were conducted only in laboratory conditions (in vitro), and human trials (in vivo studies) are necessary to confirm whether these findings translate to actual therapeutic benefits in people. Another significant area for improvement is understanding how garlic and honey interact to produce their combined effects. While positive results were observed, the exact mechanisms behind their interaction require further investigation. Additionally, it has yet to be determined how effectively the human body can absorb and use these compounds in pill form. The rate at which these pills dissolve and release their active ingredients could significantly affect how well the treatment works. To address these limitations, future studies will examine the biological mechanisms behind garlic and honey's interaction, test how well the body absorbs the pill formulation, and conduct carefully designed clinical trials to verify our laboratory findings in human subjects.

By advancing this research, garlic, and honey have the potential to emerge as transformative, natural alternatives in the fight against

antibiotic resistance, offering an affordable and accessible solution to a growing global health challenge.

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