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ORIGINAL

Synergistic antibacterial activity of honey–spice combinations against *Staphylococcus aureus* and *Klebsiella pneumoniae*

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The increasing prevalence of antibiotic resistant microorganisms led to renewed interest in exploring natural substances as alternative antimicrobial agents. Honey and commonly available spices have long been used in traditional medicine because of their antimicrobial and antioxidant potential. The present study reveals the medical uses of honey and there combination along with the chosen spices — clove (*Syzygium aromaticum*), black pepper (*Piper nigrum*), turmeric (*Curcuma longa*), and cinnamon (*Cinnamomum* spp.), against two bacterial pathogens, *Staphylococcus aureus* and *Klebsiella pneumoniae*. Aqueous extracts of honey and honey–spice combinations were prepared in a 1:1 ratio and tested at concentrations of 40, 60, 80, and 100 μL by disc diffusion method on Mueller Hinton agar. Standard antibiotics, tetracycline and norfloxacin, served as positive controls. Results indicated concentration dependent bactericidal action for most honey–spice combos. Honey alone demonstrated minimal efficacy against *S. aureus* and no efficacy against *K. pneumoniae*. Among all the spices combination examined, honey–cinnamon showed the significant antibacterial activity against *S. aureus*, compared to the standard antibiotic at higher concentrations. Conversely, honey–clove combination showed stronger inhibitory effects against *K. pneumoniae*, particularly at 100 μL. Statistical analysis was carried (ANOVA) shows that there is a notable difference between diverse treatments ($p < 0.05$). These results highlight that honey-based combinations could serve as efficient antimicrobial agents.

Keywords: honey–spice synergy, antibacterial activity, *Staphylococcus aureus*, *Klebsiella pneumoniae*, clove *(Syzygium aromaticum)*, blackpepper *(Piper nigrum)*, turmeric *(Curcuma longa)*, cinnamon (*Cinnamomum spp.*)

Introduction

Research on plant-derived compounds for medicinal and food-related purposes has been increased in demand for natural alternatives to synthetic antimicrobial agents. The growing occurrence of antibiotic-resistant microorganisms, has drastically decreased the efficiency of several traditional (1). Similarly, the natural substances with anti-bacterial characteristics are being explored as alternative therapeutic agents (2). *Apis mellifera* is a naturally occurring plant that produces honey using floral nectar and plant-derived secretions and has been traditionally employed in wound therapy and infection control. Numerous factors, includes low pH, osmolarity, low water activity and enzymatic hydrogen peroxide generation are linked to its antibacterial activity (3). Collectively, these characteristics contribute to the inhibition of various pathogenic microorganisms and improve wound healing by maintaining a protected and moist environment (4). Spices originate from different parts of plants, including seeds, bark, roots and fruits are widely used for both culinary and medical purposes. In addition to their culinary applications, spices offer substantial antibacterial and antioxidant activity due to the presence of bioactive compounds like phenolics, flavonoids, and essential oils (5). clove, black pepper, Turmeric and cinnamon are most frequently investigated spices for their therapeutic effects. Curcumin, piperine, cinnamaldehyde, and eugenol are major bioactive ingredients responsible for their antimicrobial activity (6, 7).

Staphylococcus aureus and *Klebsiella pneumoniae* are clinically important Gram positive and Gram-negative bacteria, respectively, associated with a wide spectrum of illnesses including cutaneous, respiratory, and urinary tract infections. The rising resistance of these infections to routinely used antibiotics necessitates the identification of new antimicrobial medicines (8). The present research aims to investigate the synergistic antibacterial potential of honey and spices against *S. aureus* and *K. pneumoniae*. The antibacterial activity of honey–spice mixes at different doses was tested using the disc diffusion method and compared with common antibiotics (tetracycline and norfloxacin) as positive controls. The study further tries to determine the most effective honey–spice combination demonstrating improved antibacterial potential.

Methodology

Sample preparation

Honey and spice samples were Prepared as aqueous extracts. Individual spices were finely ground using a sterilized mortar and pestle. All samples were prepared as aqueous extracts under sterile laboratory conditions. Each spice sample was finely powdered using a sterilized mortar and pestle to ensure uniformly. The spice blends were made in a 1:1 (w/w) ratio. Five samples were prepared:

Sample A: Honey,
Sample B: Honey–Turmeric,
Sample C: Honey–Black pepper,
Sample D: Honey–Cinnamon, and
Sample E: Honey–Clove.

Each mixture (1 g) suspended in 1 mL of distilled water and homogenized using a vortex mixer for 2 min.

Antibacterial assay

Mueller-Hinton agar plates were used to measure antibacterial activity by using the disc diffusion method (9). Sterile filter paper discs (6 mm diameter) were soaked with the sample volumes of 40, 60, 80, and 100 µL. Agar plates were uniformly swabbed with bacterial cultures, and the soaked discs were placed at equidistant positions. Tetracycline and norfloxacin discs served positive controls for two pathogens, respectively.

Preparation of bacterial cultures

Pure cultures of *S. aureus* and *K. pneumoniae* were sub-cultured in nutrient broth and then incubated at 37 °C for 24 h to yield actively developing bacterial suspensions. All media and glassware were sterilized before to use.

FIGURE 1 | Zone of inhibition in *Staphylococcus aureus* (*S. aureus*) (Sample - A).

Incubation and analysis

At 37 °C all plates were incubated in an inverted position for 24 h. Anti-bacterial potential was determined by measuring the zone of inhibition (mm) around each disc.

Results

Staphylococcus aureus

The antibacterial activity of honey alone and honey–spice combinations against *S. aureus* showed concentration-dependent zone of inhibition. **Sample A** (**Figure 1**) Very minimal antibacterial activity was observed. The zone of inhibition gradually increased from 0.2 mm at 40 µL to 1 mm (100 µL), **Sample B** (**Figure 2**) showed moderate activity at lower concentration with 1 mm zone of inhibition at (40 µL) and 3 mm at (60 µL). No zone of inhibition was noted at 80 µL, while 2 mm zone of inhibition was observed at 100 µL. **Sample C** (**Figure 3**) showed no zone of inhibition at 40, 60, and 80 µL. Detectable antibacterial activity was observed only at the highest concentration tested at 100 µL (5 mm). **Sample D** (**Figure 4**) revealed low but consistent zone of inhibition increasing from 0.2 mm (40 µL) to 1 mm (100 µL). As expected, the standard antibiotic (Positive control) showed the largest zone of inhibition (11 mm), **Sample E** (**Figure 5**) showed no zone inhibition at 40 and 60 µL and moderate activity was noted at 80 and 100 µL (1 mm each) (**Table 1**).

Klebsiella pneumoniae

The antibacterial activity against *K. pneumoniae* varied among the honey-spice samples. **Sample A** (**Figure 6**) and

FIGURE 2 | Zone of inhibition in *S. aureus* (Sample - B).

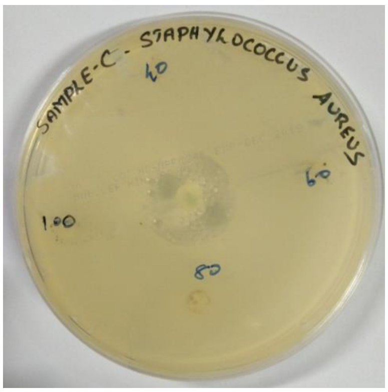

FIGURE 3 | Zone of inhibition in *S. aureus* (Sample - C).

FIGURE 4 | Zone of inhibition in *S. aureus* (Sample - D).

Sample B (**Figure 7**) showed no zone of inhibition at any tested concentration (40–100 μL), while the positive control produced a 9 mm zone. **Sample C** (**Figure 8**) exhibited zone of inhibition at 60 and 100 μL (2 mm each). **Sample D** (**Figure 9**) showed zones of inhibition of 1, 5 and 2 mm at 60, 80 and 100 μL, respectively, with a 12 mm positive control. **Sample E** (**Figure 10**) exhibited zones of inhibition of 5, 2 and 4 mm at 40, 60 and 80 μL (**Table 2**).

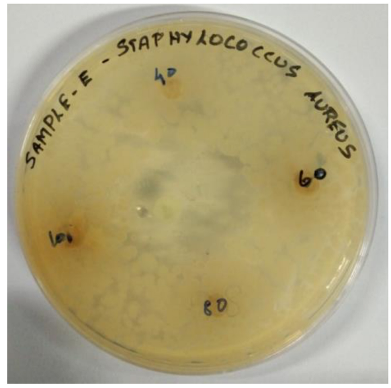

FIGURE 5 | Zone of inhibition in *S. aureus* (Sample - E).

TABLE 1 | Zone of inhibition (*S. aureus*).

S. no.	Conc.	***S. aureus*** **zone of inhibition (mm)**				
		Sample				
		A	**B**	**C**	**D**	**E**
1.	40 μL	0.2	1	0	2	0
2.	60 μL	0.5	3	0	3.5	0
3.	80 μL	0.7	0	0	7	1
4.	100 μL	1	2	5	15	1
5.	Positive control	9	9	9	11	10

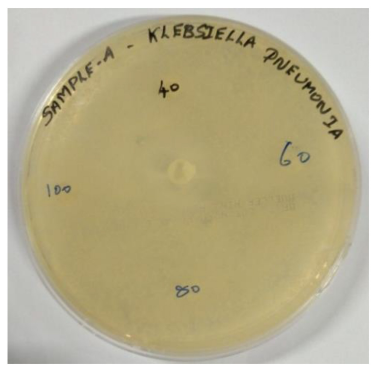

FIGURE 6 | Zone of inhibition in *K. pneumonia* (Sample - A).

Statistical analysis

One-way ANOVA was employed to assess the variation within and between the groups. A statistically significant F value was observed ($p < 0.05$) (**Table 3**). Accordingly, the null hypothesis was rejected, demonstrating that the different spices significantly affected the antibacterial activity against *S. aureus*.

The differences among and within the experimental groups was calculated using a one-way ANOVA. The analysis showed a statistical significant F value ($p < 0.05$) (**Table 4**). Therefore, the tested spices exert a significant influence on antibacterial activity against *K. pneumoniae*.

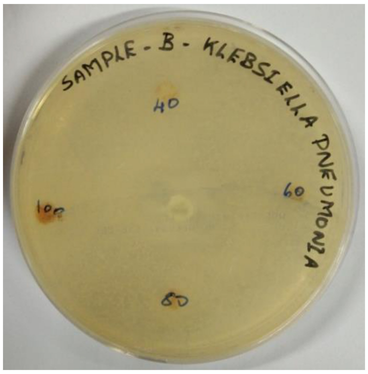

FIGURE 7 | Zone of inhibition in *K. pneumonia* (Sample - B).

FIGURE 8 | Zone of inhibition in *K. pneumonia* (Sample - C).

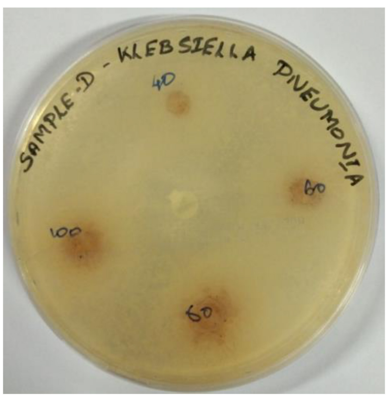

FIGURE 9 | Zone of inhibition in *K. pneumonia* (Sample - D).

FIGURE 10 | Zone of inhibition in *K. pneumonia* (Sample - E).

TABLE 2 | Zone of inhibition (*Klebsiella pneumoniae*).

S. no	Conc.	*K. pneumoniae* zone of inhibition (mm)				
		Samples				
		A	B	C	D	E
1.	40 µL	0	0	0	0	5
2.	60 µL	0	0	2	1	2
3.	80 µL	0	0	0	5	4
4.	100 µL	0	0	2	2	9
5.	Positive control	9	9	9	12	11

TABLE 3 | Mean Zone of inhibition (mm) ± standard deviation (*S. aureus*).

SAMPLES	MEAN ± S.D
SAMPLE - A	0.6 ± 0.33
SAMPLE - B	1.25 ± 1.32
SAMPLE - C	1.25 ± 2.49
SAMPLE - D	6.8 ± 5.8
SAMPLE - E	0.5 ± 0.57

Discussion

Natural plant-based products have historically played a significant role and their antimicrobial potential continues to attract scientific interest. In this work, the anti-bacterial activity of honey and honey–spice combinations against *S. aureus* and *K. pneumoniae* was assessed using the disc diffusion method. Honey alone demonstrated inhibitory efficacy against *S. aureus* at higher concentrations but was ineffective against *K. pneumoniae*, a tendency often reported in Gram-negative bacteria due to their complex outer membrane (10). Among the spice combinations, honey–cinnamon demonstrated the strongest antibacterial activity against *S. aureus*, while honey–clove was most effective against *K. pneumoniae*. The observed effects can likely be explained by the combined or synergistic interactions of honey's physicochemical qualities and bioactive phytochemicals present in spices, such as cinnamaldehyde and eugenol, which affect bacterial cell wall integrity (11). The stronger activity shown by standard antibiotics validates the experimental design used in this study. Similar findings about the increase of honey's efficacy with botanical additives have been described in recent literature (12). Variations in antibacterial activity may be attributable to changes in microbial susceptibility and the precise concentration of active ingredients in the tested spices.

TABLE 4 | Mean zone of inhibition (mm) ± standard deviation (*K. pneumoniae*).

SAMPLES	MEAN ± S. D
SAMPLE - A	0
SAMPLE - B	0
SAMPLE - C	1 ± 1.15
SAMPLE - D	2 ± 2.16
SAMPLE - E	5 ± 2.94

Conclusion

Based on the findings, honey and their spices like turmeric, black pepper, cinnamon and clove possess both bacteriostatic and bactericidal action against the investigated pathogens. Honey, when coupled with different spices, may be considered as a potential antibacterial against both Gram positive and Gram-negative bacteria (12). The antibacterial potential of honey with cinnamon, found to be more effective than other samples against the bacteria *S. aureus*. The honey with clove combination was found to be more effective than other samples against *K. pneumoniae*.

The bioactive compounds present in spices contribute to their ability to inhibit bacterial growth due to the numerous bioactive components found in both the honey and the spices (13).

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References

1. Murray CJ, Ikuta KS, Sharara F, Swetschinski L, Aguilar GR, Gray A, et al. Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. *Lancet.* (2022) 399(10325):629–55. doi: 10.1016/S0140-6736(21)02724-0
2. Ferrer-Luque CM, Solana C, Aguado B, Ruiz-Linares M. Antimicrobial activity and cytotoxicity of nonsteroidal anti-inflammatory drugs against endodontic biofilms. *Antibiotics.* (2023) 12(3):450. doi: 10.3390/antibiotics12030450
3. Almasaudi S. The antibacterial activities of honey. *Saudi J Biol Sci.* (2021) 28(4):2188–96. doi: 10.1016/j.sjbs.2020.10.017
4. Combarros-Fuertes P, Fresno JM, Estevinho MM, Sousa-Pimenta M, Tornadijo ME, Estevinho LM. Honey: another alternative in the fight against antibiotic resistance bacteria? *Antibiotics.* (2020) 9(11):774. doi: 10.3390/antibiotics9110774
5. Sulieman AME, Abdallah EM, Alanazi NA, -Dra A, Jamal A, Idriss H, et al. editors. Spices as sustainable food preservatives: a comprehensive review of their antimicrobial potential. *Pharmaceuticals.* (2023) 16(10):1451. doi: 10.3390/ph16101451
6. Raduly FM, Raditoiu V, Raditoiu A, Purcar V. Curcumin: modern applications for a versatile additive. *Coatings.* (2021) 11(5):519. doi: 10.3390/coatings11050519
7. Sani AA, Pereira AFM, Furlanetto A, Sousa DSM, Zapata TB, Rall VLM, et al. Inhibitory activities of propolis, nisin, melittin and essential oil compounds on *Paenibacillus alvei* and *Bacillus subtilis*. *J Venom Anim Toxins Incl Trop Dis.* (2022) 28:e20220025. doi: 10.1590/1678-9199-jvatitd-2022-0025
8. Braun HG, Perera SR, Tremblay YD, Thomassin JL. Antimicrobial resistance in *Klebsiella pneumoniae*: an overview of common mechanisms and a current Canadian perspective. *Can J Microbiol.* (2024) 70(12):507–28. doi: 10.1139/cjm-2024-0032
9. Balouiri M, Sadiki M, Ibnsouda SK. Methods for in vitro evaluating antimicrobial activity: a review. *J Pharm Anal.* (2016) 6(2):71–9. doi: 10.1016/j.jpha.2015.11.005
10. Cianciosi D, Forbes-Hernández TY, Afrin S, Gasparrini M, Mantane P, Danila MW, et al. Phenolic compounds in honey and their associated health benefits: a review. *Molecules.* (2018) 23(9):2322. doi: 10.3390/molecules23092322
11. Jaktaji RP, Ghalamfarsa F. Antibacterial activity of honeys and potential synergism of honeys with antibiotics and alkaloid extract of *Sophora alopecuroides* plant against antibiotic-resistant *Escherichia coli* mutant. *Iran J Basic Med Sci.* (2021) 24(5):623. doi: 10.22038/ijbms.2021.54224.12179
12. Kieliszek M, Piwowarek K, Kot AM, Wojtczuk M, Roszko M, Bryła M, et al. Recent advances and opportunities related to the use of bee products in food processing. *Food Sci Nutr.* (2023) 11(8):4372–97. doi: 10.1002/fsn3.3411
13. Yadav P, Lalwani S, Chanchal DK, Mittal N, Chaudhary JS. Exploring the synergistic antimicrobial potential of garlic and honey: a comprehensive review. *Curr Drug Ther.* (2025) 20(5):689–98. doi: 10.2174/0115748855287440240529080448