

Antimicrobial Effect of Honey Against Bacteria Isolated from Dental Plaque

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A B S T R A C T

Background: Biofilm formation on the tooth surface is a major health concern that leads to dental plaque and caries formation. Two common bacteria, *Lactobacillus* which is responsible for dental caries and *Pseudomonas aeruginosa* that leads to plaque formation and other periodontal disease, were selected to evaluate antimicrobial activity of honey.

Objective: This study was designed to investigate the antibacterial activity of honey with special emphasis to find the concentration-related effects against *Lactobacillus* and *Pseudomonas aeruginosa* using natural and commercially available honey.

Methodology: Different concentrations of natural and commercial honey (100%, 80%, 60%, 40%, and 20%) were tested to evaluate their antimicrobial activity. Each concentration was tested in triplicate to ensure accuracy and reproducibility of the results. Zones of inhibition (ZOI) were measured in millimeters using a standard ruler, and the average diameter from the three replicates was recorded for each sample. Statistical significance was assessed using t-tests and analysis of variance (ANOVA).

Results: Results indicated that natural honey exhibited stronger antimicrobial activity against *Lactobacillus*, showing a 16 mm zone of inhibition (ZOI), compared to 11 mm for commercial honey. At 100% concentration, the difference in ZOI values between the two honey types was statistically significant ($p < 0.05$). However, at 80%, 60%, and 20% concentrations, the p-values were greater than 0.05, suggesting similar inhibition rates for both honey types. For *Pseudomonas aeruginosa*, natural honey demonstrated a ZOI of 20 mm at 100% concentration and 17 mm at 80%, with p-values less than 0.05 in both cases. This indicates a statistically significant difference in antibacterial activity between natural and commercial honey at these concentrations.

Conclusion: It was concluded that both natural and commercial honey exhibits comparable antibacterial activity against both microbes at certain concentrations. Overall, natural honey demonstrated higher antibacterial potential than artificial honey.

Keywords: Honey, dental plaque, bacterial isolation, *Lactobacillus*, *P. aeruginosa*

Introduction

Dental plaque is a complex oral microbial community composed of biofilm-structured bacterial populations that colonize the mouth and contribute to various health issues.¹ Initially forming as a colorless, sticky film on the teeth, plaque can mineralize over time into tartar (calculus), which appears brown to pale yellow. As a major contributor to oral problems such as cavities and periodontal diseases, dental plaque can lead to tooth decay due to the acidic byproducts of bacterial metabolism.²

The oral microflora comprises diverse bacterial groups, including members of the phylum *Firmicutes*, as well as bacteria that produce lactic acid such as *Streptococcus*, *Lactobacillus*, and *Granulicatella/Abiotrophia* species.³ *Staphylococcus aureus*, *Streptococcus pseudopneumoniae*, *Enterococcus faecalis*, and *Escherichia coli/Shigella flexneri* are the most common bacteria found in dental plaque while *Pseudomonas aeruginosa*, has also been isolated from subgingival plaque, as its presence appears to be limited to subgingival regions and sputum.⁴ *Pseudomonas*, an

opportunistic pathogen, has been broadly studied for its ability to form biofilm in dental caries.⁵

Due to its broad spectrum antimicrobial properties, honey has been used as a remedy in various medical situations.⁶ The chemical composition of honey makes it an inhibitory agent against Gram-positive as well as Gram-negative bacteria. According to recent research, honey has also shown antimicrobial efficacy against bacterial biofilms that are resistant to conventional antibiotics. No documented cases of bacterial resistance to honey has been reported so far.⁷

Various factors like geographical location, climatic conditions and floral source are involved in altering honey antimicrobial efficacy. Honey consists of water and sugars as major components, along with essential minerals, vitamins, and bioactive compounds, such as inhibin, proteins, phenolic antioxidants, and some micronutrients.⁸ Some chemicals, such as flavonoids, carotenoids, phenolic acids, peroxidase, catalase, and ascorbic acid, imparts honey its antioxidant properties.⁹ Multiple mechanisms such as enzymatic activity, water activity osmolarity and low pH are used by honey to produce its antimicrobial effects. Hydrogen peroxide (H₂O₂) is one of major components that possess antimicrobial activity although there are some types of honey referred to as non-peroxide honey (NPA), also indicate significant antimicrobial properties.¹⁰

Many mouth rinses and mouthwashes which help to prevent dental caries and plaque contains Manuka honey due to its antimicrobial potential against oral pathogenic bacteria. Combining different honey varieties can further improve its antimicrobial efficiency. A combination of honey types from *Scaptotrigona bipunctata* (SB) and *Scaptotrigona postica* (SP) has demonstrated ability to reduce the antimicrobial resistance risk of bacteria.¹¹

The broad spectrum antimicrobial properties of honey make its application popular for wound dressing in modern medical practices. Honey effectiveness against some pathogenic and antibiotic resistant bacteria has also been demonstrated by some varieties.¹² Because of antimicrobial potential, honey is gaining recognition as a potential substitute to traditional antibiotics which will help to reduce bacterial resistance.¹⁰

Keeping in mind the antimicrobial properties of honey, this study was conducted to identify the potential of honey as a cost-effective and safer alternative for the treatment of dental caries and plaque, as well as for managing infections due to antibiotic-resistant bacteria.

Materials and Methods

This study was conducted at Department of Zoology, University of Lahore, Sargodha, Pakistan. The natural honey used in this study was collected from Mianwali local beekeepers while commercial honey was obtained from local market of Sargodha, Pakistan. Both samples were stored at room temperature. Prior to experimentation, serial dilutions were prepared using distilled water to achieve final concentrations of 20%, 40%, 60%, 80%, and 100%.

Dental plaque samples were collected from 10 individuals using sterile cotton swabs. The swabs were gently rubbed on the molars and premolars to collect plaque and were immediately sealed and refrigerated at 4 °C until further processing.

Under aseptic conditions, the plaque samples were inoculated onto selective media: MRS agar was used for the isolation of *Lactobacillus* spp. and *Pseudomonas* agar was used for the isolation of *Pseudomonas aeruginosa*. The plates for *Lactobacillus* were incubated at 37 °C for 24 hours while *Pseudomonas* agar at 42 °C for 24 hours.

The antibacterial effect of both honey types at all five concentrations was evaluated using the agar well diffusion assay. The organisms (*Lactobacillus* and *Pseudomonas aeruginosa*) were uniformly spread on L-agar plates. Wells were carefully bored into the agar surface, 100 µL of honey sample was added to individual wells. The inoculated plates were incubated for 24 hours at 37 °C. Following incubation, the inhibition zones around each well were measured in millimeters reflecting antibacterial efficacy.¹³

Each assay was conducted five times for both bacterial strains and for each honey type. Unpaired t-tests were used to evaluate the antibacterial activities between natural and commercial honey. One-way ANOVA was done to compare the effectiveness of different honey concentrations. All results were presented as mean ± standard deviation (SD), with the level of significance defined at $p < 0.05$.

Results

The inoculation of plaque samples onto MRS agar plates resulted in the selective growth of *Lactobacillus* species, as this medium supports the growth of *Lactobacillus* exclusively. The colonies appeared as clear to whitish cream-colored formations. Similarly, plaque samples cultured on *Pseudomonas* agar formed light yellowish colonies. Both *Lactobacillus* and *P. aeruginosa* growth was suppressed by the application of both natural and commercially available honey. The results demonstrated that natural honey exhibits superior antibacterial activity compared to commercially available honey. At all five

tested concentrations, the two honey types showed significantly different levels of antibacterial efficacy, with natural honey consistently demonstrating greater inhibitory effects.

The mean zone of inhibition (ZOI) at 100% concentration produced by natural honey against *Pseudomonas aeruginosa* was 20 mm, while for *Lactobacillus* it was 16 mm. A p-value of less than 0.05 indicated a statistically significant difference in the antibacterial activity between natural honey and commercial honey. At 80% concentration, natural honey remained effective against both bacteria, producing a mean ZOI of 17 mm for *P. aeruginosa* and 13 mm for *Lactobacillus*. At 60% concentration, the mean ZOI for both bacterial species was 12 mm. At 40% concentration, natural honey exhibited a mean ZOI of 9.4 mm against *Lactobacillus* and 9 mm against *P. aeruginosa*. Even at the lowest tested concentration of 20%, measurable antibacterial activity was observed, with a mean ZOI of 7 mm for *P. aeruginosa* and 9 mm for *Lactobacillus* (Table I).

Organism	Honey Concentration	Zone of Inhibition in mm (Mean±S.D)	p-value
<i>Lactobacillus</i>	100%	16±2	0.001
	80%	13±1.58	0.03
	60%	12±1.41	0.04
	40%	9±1.41	0.09
	20%	9.4±1.51	0.004
<i>P.aeruginosa</i>	100%	20±2.54	<0.05
	80%	17.25±1.87	<0.05
	60%	12±1.58	0.001
	40%	9±1.41	1
	20%	7±0.70	0.17

At a concentration of 100%, commercial honey exhibited a larger zone of inhibition (ZOI) against *Lactobacillus* compared to *Pseudomonas aeruginosa*. At 60% concentration, the mean ZOI was 10 mm for *Lactobacillus* and 8 mm for *P. aeruginosa*. When tested at 40% concentration, commercial honey produced a mean ZOI of 9 mm against *Lactobacillus* and 8 mm against *P. aeruginosa*. At the lowest tested concentration of 20%, the mean ZOI was 7 mm for both bacterial strains. These findings are summarized in Table II.

The antibacterial activity of natural honey was found to be significantly greater than that of the commercial honey. In the case of *Lactobacillus*, statistically significant differences in antibacterial activity between both honey samples were observed at 100%, 40%, and 20% concentrations, with p-values less than 0.05 ($p < 0.05$). This indicates that the two honey types exhibit markedly different inhibitory effects at these concentrations. However, at 80% and 60% concentrations, no significant difference was observed ($p > 0.05$), suggesting similar antibacterial activity at these intermediate levels.

Table II: Comparison of mean zone of inhibition for *Lactobacillus* and *Pseudomonas* using commercial honey.

Organism	Honey Concentration	Zone of Inhibition in mm (Mean±S.D)	P<value
<i>Lactobacillus</i>	100%	11±1	<0.5
	80%	11±0.70	0.05
	60%	10±1.22	<0.5
	40%	8±0.70	0.004
	20%	6.4±0.54	0.001
<i>P.aeruginosa</i>	100%	7±0.70	0.03
	80%	7±1.22	1
	60%	8±1	<0.5
	40%	9±0.70	0.17
	20%	6.4±0.54	0.05

For *Pseudomonas aeruginosa*, a significant difference ($p < 0.05$) in antibacterial activity between the two honey types was observed at 100%, 80%, and 60% concentrations. At 40% and 20%, the p-values exceeded 0.05, indicating no statistically significant variation between natural and commercial honey at lower concentrations.

Discussion

The inhibitory potential of honey against bacterial strains was recorded in this study. Natural honey used in this study demonstrated significant antibacterial activity at higher concentrations which is consistent with previously reported results on Sidr and Dharm honey varieties.¹⁴ Higher concentration of natural honey was more effective as compared to commercial honey against both *P. aeruginosa* and *Lactobacillus* strains. These results are consistent with previous study on Polyflora and Longan honey.¹⁵ The results also support the efficacy of natural honey as an alternative to standard antibiotics which will help in reducing antibiotic resistance and growing global health concerns. The zone of inhibition pattern obtained for natural honey aligns with results from previous research, particularly for Durian honey, whose antibacterial activity was identified using various concentrations.¹⁶

In case of commercial honey, the mean (ZOI) at 100% concentration was 11 mm for *Lactobacillus* and 7 mm for *P. aeruginosa*. These results agree with earlier research where commercial honey exhibited comparable antibacterial effects.¹⁷ Also, the antibacterial activity of commercial honey using different concentrations is according to the findings of Khalil et al., where they reported similar inhibition against *Staphylococcus aureus* using 50% concentration.¹⁸ Similarly, the 20% concentration of commercial honey in this study produced results with slight differences like those reported for 25% Dabur honey.¹⁹

This study indicates that natural honey exhibited the ability to inhibit plaque-forming bacteria, making it a potential natural

therapeutic agent for oral health as reported previously for Manuka honey.²⁰ As conventional sweeteners contribute to dental caries, honey serves as a natural sweetener providing protective effects and its role in caries prevention.²¹

The antimicrobial activity of honey against both Gram-positive and Gram-negative bacteria offers a safer alternative like chlorhexidine or xylitol, especially considering that chlorhexidine, while bactericidal, does not prevent caries formation. Also, while Saudi Sidr honey has been shown to exhibit antibacterial activity at 30% concentration, the Pakistani honey used in this study demonstrated inhibitory effects even at 20% concentration although to a lesser extent.²² Further research is need of the time to explore the antibacterial potential of honey against a broader range of dental plaque and caries-associated pathogens. Moreover, additional studies exploring different types of honey are required to fully establish their clinical efficacy and comparative effectiveness in oral healthcare.

Conclusions

This research demonstrates the potential of natural honey as a cost-effective, safe, and good alternative to conventional antibiotics for the prevention and management of dental plaque and dental caries. As we know about antimicrobial activity, the use of honey against dental plaque bacteria remains underexplored, and further research is necessary to identify its efficacy and mechanisms of action. Antibiotic resistance is a major threat to health globally while honey can represent a potential alternative or adjunct to conventional antibiotics. The application of honey in dental and medical therapies warrants further clinical investigation.

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