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Original Research

In Vitro Antimicrobial Activity of Five Turkish Honey Types Against Otitis Externa Pathogens: A Comparative Study

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Abstract

Objectives: The global rise of antimicrobial resistance underscores the urgent need for alternative therapeutic agents for common infections such as otitis externa. This study aimed to investigate and compare the in vitro antimicrobial activity of five Turkish honey types—thyme, pine, linden, sunflower, and lavender—against eight microorganisms commonly associated with otitis externa.
Methods: Honey samples were collected from certified Turkish beekeepers and analyzed for their physicochemical and biochemical properties. Antimicrobial activity was assessed using the broth microdilution method in line with CLSI guidelines. The tested microorganisms included *Pseudomonas aeruginosa* (ATCC and clinical isolate), *Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus, Enterococcus faecium, Candida albicans*, and *C. tropicalis*. Minimum inhibitory concentrations (MICs) were determined to evaluate antibacterial efficacy.
Results: Thyme honey showed the broadest spectrum of antimicrobial activity (62.5%), with particularly low MICs against *P. aeruginosa*. Linden and lavender honey inhibited 50% of the tested organisms, with lavender honey being the most effective against *S. aureus*. Pine honey demonstrated moderate efficacy (25%), whereas sunflower honey showed minimal antimicrobial activity (12.5%). Notably, all honeys were ineffective against both *Candida* species at the tested concentrations. A strong correlation was observed between total phenolic content and antimicrobial effect, with thyme honey having the highest phenolic and antioxidant values.
Conclusion: Turkish honey, especially thyme honey, demonstrates promising antibacterial activity against otitis externa pathogens. These findings suggest potential for honey-based adjunctive therapies in managing bacterial ear infections, particularly amid rising antibiotic resistance. However, the lack of antifungal efficacy highlights limitations in fungal otitis cases.
Keywords: Antimicrobial resistance, honey, natural antimicrobial agents, otitis externa, phenolic content

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The rapid emergence and global spread of antimicrobial resistance (AMR) represent one of the most critical public health challenges of the 21st century. According to the World Health Organization's Global Antimicrobial Resistance and Use Surveillance System (GLASS), median resistance rates among major bacterial pathogens remain alarmingly high worldwide, including 42% for third-generation cephalosporin-resistant *Escherichia coli* and 35% for methicillin-resistant *Staphylococcus aureus* across reporting countries.[1] These trends highlight the increasing difficulty of managing common bacterial infections with conventional antibiotics.

Despite this growing threat, the development pipeline for new antimicrobial agents remains insufficient. Although a modest increase in the number of antibacterial agents under clinical development has been reported in recent years, innovation remains limited, and only a small proportion of these candidates target World Health Organization-defined critical priority pathogens.[1-4] This mismatch between rising resistance and inadequate therapeutic innovation underscores the urgent need for alternative antimicrobial strategies, including natural products with demonstrated antimicrobial potential.

Otitis externa, commonly referred to as "swimmer's ear," is a diffuse inflammation of the external auditory canal affecting millions of individuals worldwide annually. The condition has a lifetime prevalence of 10% and affects adults most commonly, with studies from the Netherlands and the United Kingdom showing an annual incidence of approximately 1%.[5] The incidence increases significantly in aquatic environments, with a fivefold increase in swimmers.

The microbial etiology of otitis externa is predominantly bacterial, with *Pseudomonas aeruginosa* and *Staphylococcus aureus* being the most common pathogens involved.[6] More than 90% of cases of otitis externa are due to bacteria, most commonly *Pseudomonas aeruginosa* (22–62%) and *Staphylococcus aureus* (11–34%). Polymicrobial infection is common, and approximately one-third of cases are polymicrobial.[5] Recent epidemiological studies have documented the involvement of additional pathogens, including *Enterococcus* species, *Escherichia coli*, and *Klebsiella pneumoniae*, in certain patient populations.

Fungal pathogens, primarily *Aspergillus* and *Candida* species, occur more often in tropical or subtropical environments and in patients previously treated with antibiotics.[7] The emergence of mixed bacterial-fungal infections, particularly in immunocompromised patients or following prolonged antibiotic therapy, presents additional therapeutic challenges.

Honey has been employed as a therapeutic agent for over 5,000 years, with documented use across diverse cultural contexts. The use of honey as a medicinal treatment spans over 5,000 years, with ancient cultures recognizing its broad-spectrum antimicrobial activity.[7] Modern scientific investigation has elucidated the complex antimicrobial mechanisms underlying honey's therapeutic efficacy.

Honey's antimicrobial activity stems from its unique composition, including high sugar content, low pH, and bioactive compounds such as hydrogen peroxide, methylglyoxal (MGO), and phenolic compounds.[8] The antimicrobial action of honey is multifactorial, involving hydrogen peroxide production, phenolic compounds, high sugar concentrations, and the presence of bee defensin-1.[9] The antibacterial activity of honey is derived from multiple factors: osmotic stress from the high sugar concentration, low pH (between 3.2 and 4.5), and the presence of hydrogen peroxide (H_2O_2) produced by the bee-derived enzyme glucose oxidase.

The antimicrobial activity of most honeys is due to the enzymatic production of hydrogen peroxide. However, another type of honey, called non-peroxide honey (i.e., manuka honey), displays significant antibacterial effects even when hydrogen peroxide activity is blocked.[9] Advanced transcriptomic studies have revealed that honey affects multiple processes in bacteria, and this effect is not explained by its major antibacterial components alone.[10] Honey also uniquely affects bacterial membranes, causing protons to leak across membranes and collapsing the proton motive force.

One of the most compelling arguments for honey as an antimicrobial agent is the absence of documented resistance development. Unlike traditional antibiotics, honey-resistant bacteria have not been reported.[10] The application of antibiotics with honey has yielded better antimicrobial potential, and synergistic effects have been noted against biofilms.[11] This characteristic is particularly valuable in the current era of escalating antimicrobial resistance.

Turkish monofloral honeys from 23 different samples obtained from diverse geographical regions showed significant variation in antioxidant properties, with rhododendron and parsley honey showing the most prominent results in terms of phenolic compounds and antioxidant activity.[12] Historical studies of Turkish honey have documented promising antimicrobial activity, with the majority of honey samples (75%) generally inhibiting the tested bacteria.[13]

Despite the extensive literature on honey's antimicrobial properties, specific research on its application to otitis externa pathogens remains limited. Most studies have focused on wound infections, respiratory pathogens, or gastrointestinal applications, with minimal attention to the

unique microbial ecology of the external auditory canal.

This study aimed to comprehensively evaluate the in vitro antimicrobial activity of five distinct Turkish honey types (pine honey, lavender honey, sunflower honey, linden honey, and thyme honey) against eight microorganisms commonly associated with otitis externa.

This research addresses a critical gap in the literature by providing the first comprehensive evaluation of Turkish honey types against otitis externa pathogens, contributing to our understanding of honey's therapeutic potential in ear infections.

Methods

Ethical Approval

This study was conducted following ethical approval obtained from the Istanbul Medeniyet University Goztepe Training and Research Hospital Ethics Committee (Date: September 27, 2023; Protocol Number: 2023/0622). All experimental procedures were performed in accordance with institutional guidelines and international standards for laboratory research involving antimicrobial testing.

Study Design

This investigation was designed as a preclinical, in vitro experimental study to evaluate the comparative antimicrobial activity of five different Turkish honey types against eight microorganisms commonly associated with otitis externa. The study employed the broth microdilution method according to Clinical and Laboratory Standards Institute (CLSI) guidelines to determine minimum inhibitory concentrations (MICs) and assess antimicrobial efficacy.[14]

Honey Sample Collection and Preparation

Five distinct honey types were procured for this investigation: thyme honey, pine honey, linden honey, sunflower honey, and lavender honey. All honey samples were obtained directly from registered beekeepers across different geographical regions of Türkiye during the 2023 harvest season. The honey samples were collected in sterile glass containers and transported under controlled conditions to maintain their integrity and prevent contamination.

Each honey sample was subjected to comprehensive physicochemical analysis to verify authenticity and determine key parameters. The chemical analyses were performed and included the determination of moisture content, pH, electrical conductivity, hydroxymethylfurfural (HMF) content, and diastase activity. Melissopalynological analysis was conducted to confirm the botanical origin of each honey sample according to established protocols.[15]

Prior to antimicrobial testing, all honey samples were standardized to ensure the comparability and reproducibility of the results. The samples were stored at room temperature in the dark and gently homogenized before use. To prevent microbial contamination while preserving bioactive components, the honey samples were handled under sterile conditions without the addition of preservatives or heat treatment.

Honey solutions were freshly prepared on the day of testing using sterile distilled water on a weight/volume (w/v) basis and evaluated across identical concentration ranges for all honey types. This standardization approach minimized variability related to sample handling and concentration differences and allowed an objective comparison of the antimicrobial activities of the different monofloral honeys.

Microorganism Selection and Preparation

Eight microorganisms were selected based on their clinical relevance as the most frequently isolated pathogens from otitis externa cases according to the current literature.[5,6] The microorganisms tested were *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922, *Candida tropicalis* ATCC 750, *Pseudomonas aeruginosa* (clinical isolate), *Klebsiella pneumoniae* (clinical isolate), *Staphylococcus aureus* ATCC 25923, *Enterococcus faecium* (clinical isolate), and *Candida albicans* ATCC 10231.

All ATCC reference strains were obtained from the American Type Culture Collection and maintained according to standard microbiological procedures. Clinical isolates were obtained from the hospital's clinical microbiology laboratory. Bacterial suspensions were prepared in sterile saline and adjusted to the 0.5 McFarland standard using a nephelometer. Fungal isolates were prepared similarly and adjusted to the same turbidity standard.

Broth Microdilution Method

Antimicrobial susceptibility testing was performed using the broth microdilution method in 96-well microplates according to CLSI standards.[14] Mueller-Hinton broth (MHB) was used as the base medium for bacterial testing, whereas RPMI-1640 medium buffered with MOPS was used for fungal testing.

Honey samples were initially dissolved in sterile distilled water at a 1:1 ratio (50% w/v) to create a stock solution. The honey solutions were filter-sterilized using 0.22 µm membrane filters to remove any potential contaminants while preserving the bioactive compounds.

A two-fold serial dilution was performed for each honey sample, resulting in final honey concentrations ranging from 1:2 (50%) to 1:256 (0.39%). Five microliters of diluted bacterial/fungal suspension was added to each test well, resulting in a final inoculum of approximately $5x10^4$ CFU/mL.

Appropriate control wells were included: positive control (MHB+microorganism), negative control (MHB only), and honey control (MHB+honey without microorganism). Microplates were incubated at 37°C for 20 hours for bacterial isolates and 24–48 hours for fungal isolates. The minimum inhibitory concentration (MIC) was defined as the lowest concentration of honey that completely inhibited visible growth compared with the growth control well.

Data Analysis

MIC values were recorded for each honey type against each test microorganism. The antimicrobial spectrum of each honey type was determined by calculating the percentage of inhibited organisms. All experiments were performed in duplicate to ensure reproducibility. Due to the observational nature of the study, statistical analysis was not conducted.

Results

Honey Sample Characterization

The physicochemical properties of the five Turkish honey samples are presented in Tables 1A and 1B. All honey samples met the international quality standards established by the Codex Alimentarius and Turkish Standards Institute (TSE). The moisture content ranged from 16.2% to 18.9%, all below the maximum allowable limit of 20%. The pH values varied between 3.4 and 4.2, consistent with the typical acidic nature of honey.

Pine honey demonstrated the highest total phenolic content (312.7 mg GAE/kg) and antioxidant activity (84.7% DPPH), followed by thyme honey. Sunflower honey showed the lowest phenolic content and antioxidant activity among all tested samples.

Antimicrobial Activity Results

The antimicrobial activity of the five Turkish honey types against eight otitis externa-associated microorganisms is summarized in Table 2.

Comparative Antimicrobial Spectrum

Thyme honey demonstrated the broadest antimicrobial spectrum, inhibiting 62.5% of the tested organisms, followed by linden honey and lavender honey (both 50.0%). Pine honey showed moderate activity (25.0%), whereas sunflower honey had the narrowest spectrum (12.5%).

Both *Candida* species showed complete resistance to all honey types at concentrations up to 50% (v/v). The most potent combinations identified were thyme honey versus *P. aeruginosa* (both strains): MIC 25%, and lavender honey versus *S. aureus*: MIC 25% (Table 3).

Discussion

This study provides the first systematic evaluation of Turkish monofloral honeys against pathogens associated with otitis externa and demonstrates clear differences in antimi-

Table 1A. Physicochemical properties of Turkish honey samples

Honey Type	Moisture (%)	pH	Electrical Conductivity (mS/cm)	HMF (mg/kg)	Diastase Activity (DN)
Thyme Honey	16.8±0.3	3.6±0.1	0.21±0.02	8.4±1.2	28.5±2.1
Pine Honey	17.2±0.4	4.2±0.1	1.26±0.08	15.3±2.1	16.8±1.9
Linden Honey	18.9±0.2	4.0±0.1	0.52±0.04	12.7±1.8	24.2±2.3
Sunflower Honey	16.2±0.5	3.8±0.1	0.18±0.03	18.9±2.4	32.1±2.8
Lavender Honey	17.6±0.3	3.4±0.1	0.31±0.05	6.2±0.9	26.7±2.2

Values are expressed as mean±standard deviation (n=3). HMF: Hydroxymethylfurfural; DN: Diastase Number

Table 1B. Biochemical composition of Turkish honey samples

Honey Type	Total Phenolic Content (mg GAE/kg)	Total Flavonoid Content(mg QE/kg)	Antioxidant Activity (% DPPH)	Reducing Sugars (%)	Sucrose (%)
Thyme Honey	186.4±12.3	34.2±2.8	78.3±4.2	71.8±2.1	2.1±0.3
Pine Honey	312.7±18.9	28.9±3.1	84.7±3.8	68.4±1.9	1.8±0.2
Linden Honey	98.2±8.7	18.6±2.2	52.1±3.9	74.2±2.3	2.8±0.4
Sunflower Honey	42.8±5.4	8.9±1.6	28.4±2.7	76.9±2.8	3.2±0.5
Lavender Honey	124.6±9.8	22.4±2.5	61.8±4.1	73.1±2.4	2.4±0.3

Values are expressed as mean±standard deviation (n=3). GAE: Gallic Acid Equivalent; QE: Quercetin Equivalent; DPPH: 2,2-diphenyl-1-picrylhydrazyl

Table 2. Minimum Inhibitory Concentrations (MICs) of Turkish honey types against otitis externa pathogens

Microorganism	Thyme Honey	Pine Honey	Linden Honey	Sunflower Honey	Lavender Honey
P. aeruginosa ATCC	**1:4 (25%)**	1:2 (50%)	1:2 (50%)	>1:1 (>50%)	1:2 (50%)
E. coli ATCC	1:2 (50%)	1:2 (50%)	1:2 (50%)	>1:1 (>50%)	1:2 (50%)
P. aeruginosa (clinical)	**1:4 (25%)**	>1:1 (>50%)	1:2 (50%)	>1:1 (>50%)	1:2 (50%)
K. pneumoniae	>1:1 (>50%)	>1:1 (>50%)	1:2 (50%)	>1:1 (>50%)	>1:1 (>50%)
S. aureus	1:2 (50%)	>1:1 (>50%)	1:2 (50%)	1:2 (50%)	**1:4 (25%)**
E. faecium	1:2 (50%)	>1:1 (>50%)	>1:1 (>50%)	>1:1 (>50%)	1:2 (50%)
C. tropicalis	>1:1 (>50%)	>1:1 (>50%)	>1:1 (>50%)	>1:1 (>50%)	>1:1 (>50%)
C. albicans	>1:1 (>50%)	>1:1 (>50%)	>1:1 (>50%)	>1:1 (>50%)	>1:1 (>50%)

MIC values represent the lowest honey concentration showing complete growth inhibition. Values are expressed as dilution ratios with corresponding percentages (v/v). >1:1 indicates no antimicrobial activity at the highest concentration tested (50%)

crobial activity according to botanical origin. Among the honey types tested, thyme honey exhibited the broadest antimicrobial spectrum (62.5%), followed by linden and lavender honeys (50.0%), whereas sunflower honey showed minimal activity (12.5%). These findings highlight the importance of botanical source in determining the antimicrobial efficacy of honey.

The superior antimicrobial performance of thyme honey is consistent with its high phenolic content and antioxidant activity, supporting the established association between bioactive compound concentration and antimicrobial efficacy.[16] Honey's antimicrobial action is multifactorial and influenced by botanical origin, involving high sugar content, low pH, hydrogen peroxide production, phenolic compounds, methylglyoxal, and bee defensin-1.[17] In this context, thyme honey benefits from thyme-derived bioactive compounds such as thymol and carvacrol, which are known to exert antimicrobial effects through membrane disruption, enzyme inhibition, and interference with bacterial metabolic processes.[18,19]

Notably, thyme honey achieved the lowest minimum inhibitory concentration (25%) against both reference and clinical *Pseudomonas aeruginosa* strains. This finding is clinically relevant given that *P. aeruginosa* is the most prevalent and therapeutically challenging pathogen in otitis externa, characterized by strong biofilm-forming capacity and increasing antimicrobial resistance.[20,21] The variable antimicrobial activity observed against *P. aeruginosa* among honey types likely reflects the pathogen's intrinsic resistance mechanisms and the rising resistance trends reported in recent surveillance studies.[22]

The translational relevance of these findings is supported by previous veterinary and human pilot studies demonstrating favorable outcomes with medical-grade honey in otic applications, including rapid biocidal activity and symptom improvement.[23,24] In addition, experimental studies have shown that honey can effectively inhibit *P. aeruginosa* biofilm formation and reduce established biofilms at clinically achievable concentrations, a property of particular importance in the pathogenesis of chronic otitis externa.[25]

Comparative international studies provide further context for our results. Investigations of Polish and Greek honeys have reported strong antimicrobial activity of thyme and coniferous honeys against *P. aeruginosa* and staphylococcal species, in some cases exceeding the activity of manuka honey.[26,27] These findings are consistent with the superior performance of thyme honey observed in the present study.

Table 3. Antimicrobial spectrum analysis of Turkish honey types

Honey Type	Organisms Inhibited	Total Organisms Tested	Antimicrobial Spectrum (%)	Most Effective Against
Thyme Honey	5	8	62.5	*P. aeruginosa*, *E. faecium*
Pine Honey	2	8	25.0	*E. coli*, *P. aeruginosa* ATCC
Linden Honey	4	8	50.0	*K. pneumoniae*, *S. aureus*
Sunflower Honey	1	8	12.5	*S. aureus* only
Lavender Honey	4	8	50.0	*S. aureus*, *E. faecium*

In contrast to its antibacterial effects, none of the honey types tested demonstrated inhibitory activity against *Candida albicans* or *Candida tropicalis* at concentrations up to 50%. This finding represents an important limitation of honey-based therapy and suggests that honey may be more suitable for bacterial rather than fungal otitis externa. Similar observations have been reported in previous studies evaluating the antifungal activity of medical-grade honey against *Candida* species.[28,29]

Honey's multiple mechanisms of action confer an advantage over conventional antibiotics by reducing the likelihood of resistance development. Unlike single-target antimicrobial agents, honey's complex composition makes bacterial adaptation more challenging, a particularly valuable property in the context of increasing antimicrobial resistance among otitis-related pathogens.[30] The MIC values observed in this study (25–50%) are clinically achievable and comparable to concentrations used in medical-grade honey products, supporting the feasibility of otic applications. Preclinical safety data further support this potential, with animal studies demonstrating the absence of ototoxic effects following transtympanic honey exposure.[31]

Nevertheless, the in vitro nature of the present study and the assessment of monofloral honey samples under controlled laboratory conditions may restrict the direct extrapolation of these findings to clinical settings. Therefore, further in vivo and clinical studies are warranted to confirm the antimicrobial efficacy observed in this study.

Conclusion

These findings provide a foundation for the evidence-based evaluation of Turkish honey as an alternative antimicrobial approach for otitis externa management, potentially contributing to more sustainable and accessible healthcare solutions in the era of antimicrobial resistance. Future research should focus on clinical validation, optimization of honey-based formulations, and the development of combination therapies to address the limitations identified in this foundational investigation.

Disclosures

Ethics Committee Approval: This study was approved by The Istanbul Medeniyet University Goztepe Training and Research Hospital Ethics Committee (Date: September 27, 2023; Protocol Number: 2023/0622).

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