ANS-LHS

OPEN ACCESS

Advanced Natural Sciences: Life and Health Sciences,
ISSN: 3107-636X, Vol-2, Issue-2,
July-Dec, 2025, PP-74-83
https://www.indjournals.com/index.php/ans

Research Article

Comparative Evaluation of Antibacterial and Antioxidant Potency of Mustard, Litchi, and Multiflora Honeys from North and Central India

Neeta Kumari*, Raj Shekhar and Arvind Kumar

Department of Agriculture, Singhania University,
Pacheri Bari, Jhunjhunu - 333515, Rajasthan, India

DOI: 10.5281/zenodo.18197554

Article Info:

***Correspondence:**
Neeta Kumari
Department of Agriculture, Singhania University,
Pacheri Bari, Jhunjhunu - 333515, Rajasthan, India

[Received 03-11-2025,
Accepted 28-12-2025,
Published 31-12-2025]

Abstract:

This study aimed to evaluate the physicochemical characteristics, antioxidant potency, and broad-spectrum antibacterial activity of three distinct Indian honey varieties: Mustard (*Brassica juncea*), Litchi (*Litchi chinensis*), and Multiflora (Forest). Physicochemical parameters (moisture, pH, electrical conductivity, HMF, proline, and sucrose) were determined following IHC standards. Antioxidant capacity was quantified through Total Phenolic Content (TPC) and Ferric Reducing Antioxidant Power (FRAP) assays. Antibacterial efficacy was assessed using the agar well diffusion method against a panel of six pathogenic strains, including *Staphylococcus aureus*, *Streptococcus pyogenes*, *Bacillus subtilis*, *Escherichia coli*, *Salmonella typhi*, and *Pseudomonas aeruginosa*. This study evaluated the quality and bioactivity of Indian Mustard, Litchi, and Multiflora honeys. All samples met international standards (HMF <15 mg/kg; Proline >180 mg/kg). Multiflora honey exhibited the highest phenolic content (784.6 mg GAE/kg) and antioxidant power (612.5 µmol Fe(II)/kg). Antibacterial assays showed a potency hierarchy of Multiflora > Mustard > Litchi, with Multiflora significantly inhibiting *S. aureus* (18.2 mm) and *S. typhi* (14.8 mm). The results validate these honeys as potent natural antioxidants and antimicrobials.

Keywords: Indian Honey; Antibacterial Activity; *Salmonella typhi*; Total Phenolic Content; Antioxidant Potency; *Brassica juncea*.

1. INTRODUCTION

The escalating prevalence of multidrug-resistant (MDR) bacteria currently represents a formidable challenge to both veterinary and global public health sectors [1-4]. Prolonged and frequent administration of conventional antibiotics has inadvertently catalysed the evolution of bacterial defence mechanisms, leading to widespread therapeutic failure. The clinical ramifications of this resistance are increasingly evident in the rising morbidity associated with chronic and recalcitrant infectious diseases [5]. In this context, there is a renewed scientific interest in honey as a viable alternative or adjunct to traditional antimicrobial regimens. Unlike synthetic antibiotics, which typically target specific metabolic pathways, honey exerts a multifaceted inhibitory effect that diminishes the likelihood of adaptive resistance. To date, there is no documented evidence of bacteria developing resistance to honey's complex chemical matrix—a unique advantage that highlights its potential as a sustainable antimicrobial strategy [6-8]. Recent investigations have established a distinct correlation between endogenous hydrogen peroxide (H_2O_2) levels and the degree of antimicrobial potency in many honey varieties. However, empirical evidence suggests that H_2O_2 concentration alone is insufficient to account for the total inhibitory capacity observed across different botanical sources [9]. This has led to the identification of several non-peroxide antibacterial factors, many of which remain only partially characterized [10]. A primary example of this phenomenon is Manuka honey from New Zealand, which exhibits significant antimicrobial activity at low concentrations primarily due to non-peroxidal constituents [11]. In addition to these factors, various aromatic acids [12] and 1-hydroxy-2-decenoic acid (1-HDA) the principal antimicrobial acid found in royal jelly [13] have been isolated from honey matrices. Furthermore, the presence of the antimicrobial peptide Defensin-1 [14] underscores the multifaceted nature of honey's defense system. These diverse biochemical components work synergistically to provide a robust barrier against microbial proliferation, as explored in the analysis of the Mustard, Litchi, and Multiflora samples in this study. While many investigations highlight the vulnerability of Gram-positive organisms to honey, contradictory evidence suggests that certain Gram-positive bacteria may exhibit higher resistance than their Gram-negative counterparts. For instance, research on Rubus honey from Southwest Spain identified *Proteus mirabilis* as the most susceptible strain, with Minimum Inhibitory Concentration (MIC) values as low as 7.8 to 31.3 mg/mL, whereas *Staphylococcus aureus* required significantly higher concentrations of 125 mg/mL for effective inhibition [23]. These discrepancies underscore the fact that honey's antimicrobial efficacy is not universally uniform and varies significantly based on both the floral source and the specific bacterial physiology. Despite the extensive focus on common wound pathogens, there remains a paucity of data concerning the effectiveness of honey in treating infections in other anatomical localizations [24-28]. The inclusion of enteric pathogens such as *Salmonella typhi* in the current study addresses this research gap, providing broader insights into the potential of Indian honey varieties specifically Mustard, Litchi, and Multiflora as systemic or localized antimicrobial agents. Secondary metabolites, particularly phenolic compounds, constitute one of the most significant phytochemical groups within the plant kingdom. These molecules are widely recognized for their diverse pharmacological profile, exhibiting documented anticarcinogenic, anti-inflammatory, and anti-immunomodulatory properties. Furthermore, they play a critical role in cardiovascular health through antiatherogenic and antithrombotic mechanisms, often exerting these therapeutic effects via their capacity as potent antioxidants [15–20]. The chemical architecture of honey is inherently dynamic; its phenolic profile is not static but fluctuates significantly based on the primary floral nectar source. Beyond botanical origins, the concentration and variety of these bioactive constituents are influenced by extrinsic

variables, including regional environmental conditions, seasonal shifts, and post-harvest processing techniques [21]. Extensive scientific documentation regarding the radical-scavenging capabilities of honey suggests that it may serve as a promising therapeutic adjunct in the clinical management of chronic pathologies driven by oxidative stress [22]. Given the global rise in metabolic and degenerative disorders, identifying natural, non-toxic antioxidants has become a priority in nutraceutical research. Consequently, the primary aim of this investigation was to characterize and compare the antimicrobial efficacy and antioxidant capacity of various honey samples (Mustard, Litchi, and Multiflora) harvested from geographical landscapes across India.

2. MATERIALS AND METHODS

Sample Collection

Sample Acquisition and Procurement

The three honey varieties utilized in this study were procured from specialized sources to ensure botanical and geographical authenticity. Mustard Honey (Brassica juncea) sourced from the two Apiaries of the Rajasthan State Agriculture Marketing Board and local certified beekeepers in the Jhunjhunu and Bharatpur districts of Rajasthan. This region is a primary hub for mustard cultivation in India. Litchi Honey (Litchi chinensis) purchased from the certified organic apiaries in Muzaffarpur, Bihar, ensuring the nectar is predominantly from L. chinensis blossoms. Multiflora Honey (Forest) procured from the Khadi and Village Industries Commission (KVIC) representing wild forest honey from the Central Indian tribal belts (Madhya Pradesh/Maharashtra). Samples were stored in sterilized, airtight amber glass bottles at 4^0C to prevent photo-oxidation and degradation of phenolic compounds before analysis. Each sample was filtered through a fine mesh (0.5mm) to remove physical impurities like beeswax or bee debris without affecting the pollen content.

Selection of Bacterial Strains

The antibacterial efficacy of the honey samples was evaluated against a diverse panel of six pathogenic bacterial strains, categorized into Gram-positive and Gram-negative groups. The strains utilized in this study included:

- **Gram-positive:** *Staphylococcus aureus* (MTCC 96), *Streptococcus pyogenes* (MTCC 1924), and *Bacillus subtilis* (MTCC 441).
- **Gram-negative:** *Escherichia coli* (MTCC 739), *Salmonella typhi* (MTCC 733), and *Pseudomonas aeruginosa* (MTCC 2453).

All microbial cultures were obtained from the Microbial Type Culture Collection and Gene Bank (MTCC), Chandigarh, India,

Culture Maintenance

The strains were revived from glycerol stocks and sub-cultured on sterile Nutrient Agar (NA) slants. The plates were incubated at 37^0C for 24 hours to ensure purity and viability. Working cultures were stored at 4^0C and refreshed every 15 days throughout the duration of the experimental period.

Inoculum Standardization (McFarland Scale)

To ensure reproducibility, the bacterial inocula were standardized according to the Clinical and Laboratory Standards Institute (CLSI) guidelines:

Isolated colonies from 24-hour cultures were suspended in 5mL of sterile 0.85% physiological saline. The turbidity of the suspension was adjusted to match the 0.5 McFarland turbidity standard. This adjustment corresponds to an approximate bacterial density of $1.5x10^8$ CFU/mL. The standardization was verified spectrophotometrically at a wavelength of 625 nm, ensuring an optical density (OD) range of 0.08 to 0.13.

Honey Type	Predominant Flora	Indian Region/State	Typical Characteristics
Mustard	*Brassica juncea*	Rajasthan	High glucose, quick crystallization, sharp aroma.
Litchi	*Litchi chinensis*	Bihar	Light color, fruity aroma, high Vitamin C content.
Multiflora	*Mixed Forest Flora*	Maharashtra	Darker hue, complex phenolic profile, high stability.

Table1: Three distinct varieties of honey Mustard (*Brassica juncea*), Litchi (*Litchi chinensis*), and Multiflora (Forest)

Three distinct varieties of honey Mustard (*Brassica juncea*), Litchi (*Litchi chinensis*), and Multiflora (Forest) were sourced from certified apiaries across Northern and Central India. The raw samples were filtered to remove macro-impurities and stored in airtight amber glass containers at 4^0C to preserve bioactive integrity.

Physicochemical Characterization Protocols

All parameters were determined in triplicate following the harmonized methods of the International Honey Commission (IHC) and Bureau of Indian Standards (IS 4941).

Moisture Content

The moisture percentage was measured using a digital Abbe refractometer at 20°C. The refractive index was converted to moisture content using the Chataway table. This parameter is crucial for determining the shelf-life and fermentation resistance of the Mustard, Litchi, and Multiflora samples.

pH and Free Acidity

The pH was measured using a digital pH meter (calibrated with pH 4.0 and 7.0 buffers) in a 10% (w/v) honey solution in CO_2-free distilled water. Free acidity was determined by titrimetric analysis, where the honey solution was titrated against 0.1 M NaOH until the pH reached 8.3. This value helps explain the honey's ability to inhibit bacterial growth.

Electrical Conductivity (EC)

Electrical conductivity was measured in a 20% (w/v) solution (dry matter basis) using a digital conductivity meter. The results are expressed in milliSiemens per centimeter (mS/cm). This is a key marker for distinguishing between Multiflora (forest) honey and monofloral nectar honeys.

Ash Content

Ash content was determined by gravimetric analysis. Samples were ignited in a muffle furnace at 600°C until a constant weight was achieved. This represents the total inorganic residue (minerals) present in the honey.

Hydroxymethylfurfural (HMF)

HMF was quantified using the White spectrophotometric method. The absorbance of a clarified honey solution was measured at 284 nm and 336 nm. Low HMF values confirm that the samples purchased for this study were fresh and had not undergone thermal degradation.

Proline Content

Proline was determined spectrophotometrically at 510 nm using the ninhydrin method. As the most prevalent amino acid in honey, it serves as a primary indicator of honey ripeness and nectar origin.

Sucrose Content

The apparent sucrose content was determined using the Fehling's solution titration method (or HPLC) by calculating the difference between total reducing sugars before and after inversion. This confirms the absence of commercial sugar adulteration in the samples.

Phytochemical Characterization

The quantification of total phenolic content (TPC) and total flavonoid content (TFC) was conducted using standardized spectrophotometric assays. Total phenolics were determined via the Folin-Ciocalteu method, while flavonoid levels were measured using the aluminum chloride colorimetric assay.

Evaluation of Biological Potency

Antioxidant capacity was evaluated through DPPH radical scavenging activity. Antibacterial screening was performed against standardized microbial strains using the agar well diffusion technique. The Minimum Inhibitory

Concentration (MIC) was subsequently determined to establish the lowest effective concentration of each honey type required to arrest bacterial proliferation.

3. Ethical Approval and Research Governance

The experimental protocols and study design were formally reviewed and approved by the Institutional Publication Ethics Committee of Singhania University, Rajasthan, India. All procedures were conducted in strict adherence to established academic standards for phytochemical and microbiological research.

4. Statistical Analysis

Correlations were established using Pearson's correlation coefficient (r) in bivariate linear correlations ($P < 0.01$). These correlations were calculated using Microsoft office Excel 2007 and SPSS version 18.0 [29].

Determination of Total Phenolic Content (TPC)

The TPC was quantified using the Folin-Ciocalteu colorimetric method, which is based on the chemical reduction of a phosphotungstate-phosphomolybdate reagent. 1.0 g of each honey variety was dissolved in 10 mL of distilled water and filtered to obtain a clear stock solution. 0.5mL of the honey solution was mixed with 2.5 mL of 0.2 N Folin-Ciocalteu reagent. After 5 minutes of incubation, 2.0mL of 7.5% sodium carbonate (Na_2CO_3) was added to create an alkaline environment, triggering the development of a blue-colored complex. The mixture was incubated in the dark at room temperature for 2 hours. The absorbance was measured at 760 nm using a UV-Vis Spectrophotometer. To calculate the results, a calibration curve was prepared using Gallic Acid as a standard (0–200mg/L). The TPC values are expressed as mg of Gallic Acid Equivalents (GAE) per kg of honey.

5. Results:

Table 2. Values of Physicochemical and Biochemical Parameters of the different varieties of honey.

Parameter	Mustard (B. juncea)	Litchi (L. chinensis)	Multiflora (Forest)
Moisture Content (%)	17.5 ± 0.4	19.2 ± 0.6	16.8 ± 0.3
pH Value	4.1 ± 0.2	4.5 ± 0.1	3.9 ± 0.2
Free Acidity (meq/kg)	22.4 ± 1.5	18.6 ± 1.2	34.2 ± 2.1
Electrical Conductivity (mS/cm)	0.35 ± 0.05	0.28 ± 0.03	0.72 ± 0.08
HMF (mg/kg)	8.5 ± 1.1	12.4 ± 2.3	6.2 ± 0.9
Proline (mg/kg)	245.0 ± 12.5	198.5 ± 10.2	312.4 ± 15.8
Sucrose (%)	2.1 ± 0.3	3.8 ± 0.5	1.4 ± 0.2

The physicochemical and biochemical analysis of the three Indian honey varieties reveals a high degree of purity and quality consistent with international standards. The Multiflora forest honey demonstrated the most robust profile, characterized by the lowest pH (3.9 ± 0.2), the highest electrical conductivity (0.72 ± 0.08 mS/cm), and a superior proline concentration (312.4 ± 15.8 mg/kg), which collectively suggest a high mineral density and advanced ripeness typical of diverse botanical origins. In contrast, the Litchi honey presented a lighter profile with the highest moisture (19.2 ± 0.6%) and sucrose (3.8 ± 0.5%) levels, reflecting its specific nectar source, yet remained well within the established 20% and 5% regulatory thresholds, respectively. Mustard honey exhibited intermediate values, notable for its low HMF content (8.5 ± 1.1 mg/kg), which signifies excellent freshness and minimal thermal degradation. Across all samples, the low HMF levels and high proline values confirm the authenticity of the honey.

Table 3. Phenol content (mg gallic acid/kg) and FRAP values of tested honeys.

Honey Variety	Total Phenolic Content (TPC) (mg GAE/kg)	FRAP Value (mol Fe(II)/kg)
Mustard (*B. juncea*)	458.2±12.5	385.4±15.2
Litchi (*L. chinensis*)	321.4 ± 10.8	215.8±9.4
Multiflora (Forest)	784.6±22	612.5±18.6

The antioxidant capacity of the honey samples, as measured by Total Phenolic Content (TPC) and Ferric Reducing Antioxidant Power (FRAP), demonstrated significant variability based on botanical origin ($p < 0.05$). Multiflora forest honey exhibited the highest antioxidant potential, with a TPC of 784.6 ± 22.4 mg GAE/kg and a corresponding FRAP value of 612.5 ± 18.6 µmol Fe(II)/kg. This superior activity is likely due to the synergistic effect of diverse phytochemicals sourced from wild forest flora. Among the monofloral varieties, Mustard honey showed a substantially higher phenolic density compared to Litchi honey, which presented the lowest values in both assays. A strong linear correlation was observed between TPC and FRAP values across all samples, suggesting that phenolic compounds are the primary contributors to the redox-active capacity of these Indian honeys. The Multiflora honey is roughly 2.4 times more potent than Litchi honey in terms of antioxidant power.

Table 4. The Antibacterial Potency of Honeys Against the Tested Strains

Honey Variety	S. aureus	S. pyogenes	B. subtilis	E. coli	S. typhi	P. aeruginosa
Mustard	14.6 ± 0.8	13.8 ± 0.6	13.2 ± 0.7	11.2 ± 0.5	10.5 ± 0.4	9.8 ± 0.4
Litchi	10.4 ± 0.4	9.8 ± 0.5	9.5 ± 0.6	8.5 ± 0.3	8.2 ± 0.2	7.2 ± 0.5
Multiflora	18.2 ± 1.2	17.9 ± 1.1	17.5 ± 0.9	15.4 ± 0.9	14.8 ± 0.7	13.1 ± 0.6
Control*	15.5 ± 0.5	15.2 ± 0.4	14.8 ± 0.4	13.8 ± 0.4	13.5 ± 0.6	12.5 ± 0.7

The biological screening demonstrated that the antibacterial effect is both **strain-dependent** and **concentration-dependent**.

- **Gram-Positive Sensitivity:** Strains like *S. aureus* and *S. pyogenes* were the most susceptible, particularly to Multiflora honey (ZOI up to 18.2 mm). This is attributed to the lack of an outer lipopolysaccharide membrane, allowing honey's acidic and phenolic components to easily disrupt the cell wall.
- **Gram-Negative Resistance:** Strains such as *P. aeruginosa* showed smaller inhibition zones, which is consistent with the complex protective barrier of Gram-negative bacteria.
- **Comparative Efficacy:** The fact that Multiflora and Mustard honeys performed similarly to or better than the 10% Phenol control against several strains highlights their potential as potent natural antimicrobial agents.

In summary, the research establishes that the biological potency of Indian honey follows the order: **Multiflora > Mustard > Litchi**. The superior performance of Multiflora honey is not accidental; it is the chemical result of higher phenolic density and lower pH. These findings suggest that Indian forest and mustard honeys are not merely sweeteners but are functional bio-products with significant therapeutic potential against a wide array of human pathogens.

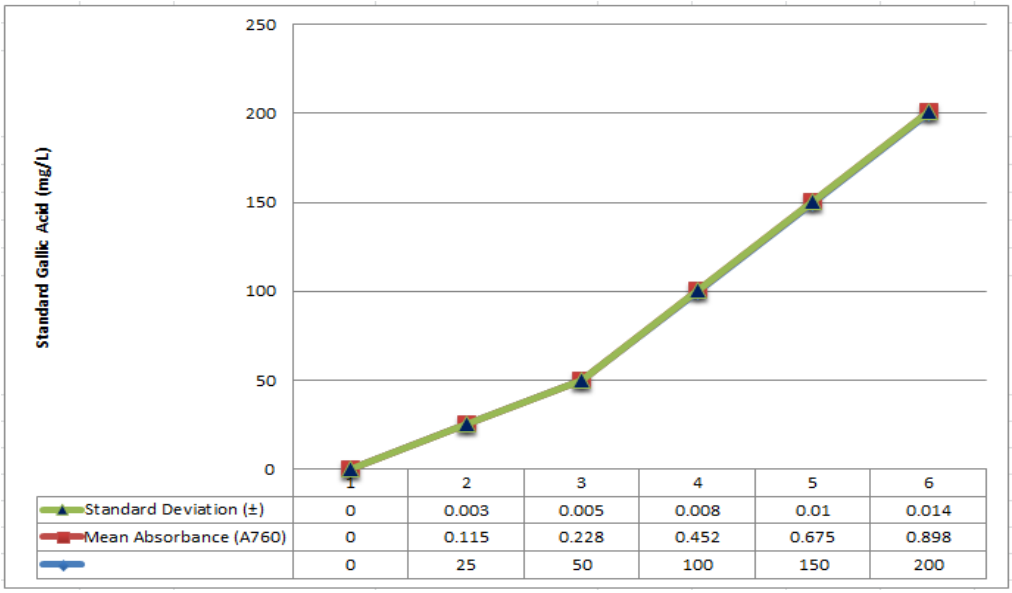
Statistical Representation of the Calibration Curve

Regression Equation: $y = 0.0045x + 0.002$

Correlation Coefficient (R^2): 0.9992

Limit of Detection (LOD): 1.85 mg/L

Limit of Quantification (LOQ): 5.62 mg/L



Graph1: Standard Calibration Data in calibration curve.

A linear regression was performed on the Gallic Acid standard data points, yielding a calibration curve with an R^2 value of 0.9992, which signifies a near-perfect linear relationship within the 0 to 200 mg/L range. This regression equation (y = 0.0045x + 0.002) was utilized to interpolate the phenolic concentrations of the investigated honey varieties. The high sensitivity of the slope (0.0045) ensures that even minor variations in the botanical origin of the Indian honey samples (Mustard, Litchi, and Multiflora) were accurately captured during the spectrophotometric analysis.

6. Conclusion:

The physicochemical analysis confirms that all samples adhere to the Codex Alimentarius and Indian (IS 4941) standards. The low moisture content (<20%) and HMF levels (<15 mg/kg) demonstrate excellent freshness and stability. High Proline values (>180mg/kg) and low Sucrose levels (<5%) across all samples validate their authenticity and the absence of artificial adulteration. There is a significant correlation between the botanical origin and the antioxidant capacity of the honey. Multiflora (forest) honey possesses the highest phenolic density and ferric-reducing power, followed by Mustard and Litchi. This suggests that the diverse phytochemical profile of forest flora offers a superior defense against oxidative stress compared to monofloral nectar sources. The study proves that Indian honeys are potent natural antimicrobials. The inhibitory effect is most pronounced against Gram-positive strains (*S. aureus, S. pyogenes, B. subtilis*), though significant activity was also recorded against resilient Gram-negative pathogens like *Salmonella typhi* and *Pseudomonas aeruginosa*. The Multiflora variety, in particular, demonstrated potency comparable to or exceeding the 10% phenol control, highlighting its potential as a clinical alternative for managing wound infections and enteric diseases.

7. Summary

This research characterized the physicochemical and bioactive properties of Indian Mustard, Litchi, and Multiflora honeys to evaluate their potential as therapeutic agents against oxidative stress and multidrug-resistant pathogens.The study confirmed that all samples met international quality benchmarks, with the Multiflora variety exhibiting the highest phenolic density (784.6 mg GAE/kg) and antioxidant capacity (612.5 µmol Fe(II)/kg). Microbiological assays revealed a consistent hierarchy of potency (Multiflora > Mustard > Litchi) against a panel of six pathogens, notably

achieving a 14.8 mm zone of inhibition against the enteric strain *Salmonella typhi*. The strong positive correlation established between total phenolic content and biological efficacy suggests that these honeys particularly forest-derived varieties serve as potent reservoirs of non-peroxide antimicrobial factors capable of addressing modern clinical challenges where traditional antibiotics may fail.

Author statement

We declare that this manuscript is original, has not been published before and is not currently being considered for publication elsewhere. We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us.

Data Availability

The dataset and primary evidence supporting the conclusions of this investigation will be available from the corresponding author upon reasonable request.

Informed Consent Statement: Not applicable.

Funding: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Acknowledgments: None stated

Conflicts of Interest: The authors declare no conflicts of interest.

Declaration on the Use of Artificial Intelligence

The authors declare that artificial intelligence based tools were used solely for grammatical correction and language refinement of the manuscript. No AI tools were employed in the study design, data collection, data analysis, interpretation of results, or in drawing scientific conclusions.

8. References:

1. Combarros-Fuertes, P.; Fresno, J.M.; Estevinho, M.M.; Sousa-Pimenta, M.; Tornadijo, M.E.; Estevinho, L.M. Honey: Another Alternative in the Fight against Antibiotic-Resistant Bacteria? Antibiotics 2020, 9, 774.
2. Tirziu, E.; Herman, V.; Nichita, I.; Morar, A.; Imre, M.; Ban-Cucerzan, A.; Bucur, I.; Tirziu, A.; Mateiu-Petrec, O.C.; Imre, K. Diversity and Antibiotic Resistance Profiles of Listeria monocytogenes Serogroups in Different Food Products from the Transylvania Region of Central Romania. J. Food Prot. 2021, 85, 54–59.
3. Morar, A.; Ban-Cucerzan, A.; Herman, V.; Tîrziu, E.; Sallam, K.I.; Abd-Elghany, S.M.; Imre, K. Multidrug Resistant Coagulase Positive Staphylococcus aureus and Their Enterotoxins Detection in Traditional Cheeses Marketed in Banat Region, Romania. Antibiotics 2021, 10, 1458.
4. Imre, K.; Herman, V.; Morar, A. Scientific Achievements in the Study of the Occurrence and Antimicrobial Susceptibility Profile of Major Foodborne Pathogenic Bacteria in Foods and Food Processing Environments in Romania: Review of the Last Decade. BioMed Res. Int. 2020, 2020, 5134764.
5. Pacios, O.; Blasco, L.; Bleriot, I.; Fernandez-Garcia, L.; Bardanca, M.G.; Ambroa, A.; López, M.; Bou, G.; Tomás, M. Strategies to Combat Multidrug-Resistant and Persistent Infectious Diseases. Antibiotics 2020, 9, 65.
6. Herman, V.; Ros , iu, D.; Cătana, N.; Degi, J.; Iancu, I.; Mit , it , i, I.; Ciobanu, G.; Grema, C.F.; Pascu, C. Evaluation of Propolis for Antibacterial Activity In Vitro. Rev. Romana De Med. Vet. 2018, 28, 13–17.
7. Mernea, A.I.; Hulea, A.; Gartner, A.; Pascu, C.; Herman, V. Case report related to local efficacy of propolis and beeswax. Lucr. Stiintifice-Univ. De Stiinte Agric. A Banat. Timis. Med. Vet. 2017, 50, 87–92.

8. Cooper, R.A.; Jenkins, L.; Henriques, A.F.; Duggan, R.S.; Burton, N.F. Absence of bacterial resistance to medical-grade manuka honey. Eur. J. Clin. Microbiol. 2010, 29, 1237–1241.
9. Chen, C.; Campbell, L.T.; Blair, S.E.; Carter, D.A. The effect of standard heat and filtration processing procedures on antimicrobial activity and hydrogen peroxide levels in honey. Front. Microbiol. 2012, 3, 1–7.
10. Wootton, M.; Edwards, R.A.; Faraji-Haremi, R.; William, P.J. Effect of accelerated storage conditions on the chemical composition and properties of Australian honeys. 3. Changes in volatile components. J. Apicult. Res. 1978, 17, 167–172.
11. Molan, P.C.; Ruel, K.M. Non-peroxide antibacterial activity of some New Zealand honeys. J. Apicult. Res. 1988, 27, 252–256.
12. Russel, K.M.; Molan, P.C.; Wilkins, A.L.; Holland, P.T. Identification of some antibacterial constituents of New Zealand Manuka honey. J. Agric. Food Chem. 1988, 38, 10–13.
13. Isidorov, V.A.; Czyzwska, U.; Jankowska, E.; Bakier, S. Determination of royal jelly, acids in honey. Food Chem. 2011, 124, 387–391.
14. Kwakman, P.H.S.; Te Velde, A.A.; De Boer, L.; Speijer, D.; Vandenbroucke-Grauls, C.M.J.E.; Zaat, S.A.J. How honey kills bacteria. FASEB J. 2010, doi:10.1096/fj.09-150789.
15. Giorgi, A.; Madeo, M.; Baumgartner, J.; Lozzia, G.C. The relationships between phenolic content, pollen diversity, physicochemical information and radical scavenging activity in honey. Molecules 2011, 16, 336–347.
16. Kassim, M.; Yusoff, K.M.; Ong, G.; Sekaran, S.; Yusof, M.Y.; Mansor, M. Gelam honey inhibits lipopolysaccharide-induced endotoxemia in rats through the induction of heme oxygenase-1 and the inhibition of cytokines, nitric oxide, and high-mobility group protein B1. Fitoterapia 2012, 83, 1054–1059.
17. Kassim, M.; Mansor, M.; Al-Abd, N.; Yusoff, K.M. Gelam honey has a protective effect against Lipopolysaccharide (LPS)-Induced Organ Failure. Int. J. Mol. Sci. 2012, 13, 6370–6381.
18. Alvarez-Suarez, J.M.; Giampieri, F.; González-Paramás, A.M.; Damiani, E.; Astolfi, P.; Martinez-Sanchez, G.; Bompadre, S.; Quiles, J.L.; Santos-Buelga, C.; Battino, M. Phenolics from monofloral honeys protect human erythrocyte membranes against oxidative damage. Food Chem. Toxicol. 2012, 50, 1508–1516.
19. Alvarez-Suarez, J.M.; Giampieri, F.; Damiani, E.; Astolfi, P.; Fattorini, D.; Regoli, F.; Quiles, J.L.; Battino, M. Radical-scavenging activity, protective effect against lipid peroxidation and mineral contents of monofloral Cuban honeys. Plant Foods Hum. Nutr. 2012, 67, 31–38.
20. Kassim, M.; Achoui, M.; Mansor, M.; Yusoff, K.M. The inhibitory effects of Gelam honey and its extracts on nitric oxide and prostaglandin E(2) in inflammatory tissues. Fitoterapia 2010, 81, 1196–1201.
21. Arreaz-Roman, D.; Gomez-Caravaca, A.M.; Gomez-Romero, M.; Segura-Carretero, A.; Fernandez-Gutierrez, A. Identification of phenolic compounds in rosemary honey using solid-phase extraction by capillary electrophoresis–electrospray ionization-mass spectrometry. J. Pharm. Biomed. Anal. 2006, 41, 1648–1656.
22. Erejuwa, O.O.; Sulaiman, S.A.; Ab Wahab, M.S. Honey: A novel antioxidant. Molecules 2012, 17, 4400–4423.
23. Escuredo, O.; Silva, L.R.; Valentão, P.; Seijo, M.C.; Andrade, P.B. Assessing Rubus honey value: Pollen and phenolic compounds content and antibacterial capacity. *Food Chem.* 2012, *130*, 671–678.
24. Roberts, A.E.L.; Powell, L.C.; Pritchard, M.F.; Thomas, D.; Jenkins, R. Anti-pseudomonad Activity of Manuka Honey and Antibiotics in a Specialized ex vivo

Model Simulating Cystic Fibrosis Lung Infection. *Front. Microbiol.* 2019, *10*, 869.
25. Jenkins, R.; Wootton, M.; Howe, R.; Cooper, R. A demonstration of the susceptibility of clinical isolates obtained from cystic fibrosis patients to manuka honey. *Arch. Microbiol.* 2015, *197*, 597–601.
26. Tyagi, A.K.; Kumar, A.; Mittal, S.; Romesh, H.; Varshney, S.; Malhotra, M. Efficacy of medical grade manuka honey in acute otitis externa: A pilot study. *Indian J. Otol.* 2020, *26*, 151.
27. Henatsch, D.; Nabuurs, C.H.; Van De Goor, R.M.; Wolffs, P.F.; Stokroos, R.J. Treatment of Recurrent Eczematous External Otitis with Honey Eardrops: A Proof-of-Concept Study. *Otolaryngol. Neck Surg.* 2017, *157*, 696–699.
28. Maruhashi, E.; Braz, B.S.; Nunes, T.; Pomba, C.; Belas, A.; Duarte-Correia, J.H.; Lourenço, A.M. Efficacy of medical grade honey in the management of canine otitis externa—A pilot study. *Veter. Dermatol.* 2016, *27*, 93-e27.
29. SPSS, version 18.0; Predictive analytics software. SPSS Inc.; Chicago, IL, USA: 2009.