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ANTIBACTERIAL EFFICACY OF MANUKA HONEY UMF 15+ AGAINST PSEUDOMONAS AERUGINOSA IN DIFFUSE OTITIS EXTERNA SECRETIONS

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ABSTRAK

Otitis eksterna (OE) adalah infeksi kulit liang telinga luar yang umumnya disebabkan oleh bakteri, terutama Pseudomonas aeruginosa dan Staphylococcus aureus. Pengobatan tradisional untuk OE biasanya melibatkan antibiotik topikal, tetapi penggunaan jangka panjang dapat menyebabkan resistensi antibiotik. Penelitian ini bertujuan untuk mengevaluasi efek antibakteri Madu Manuka (MM) UMF 15+ terhadap Pseudomonas aeruginosa pada pasien dengan OE difus menggunakan metode difusi cakram. Konsentrasi MM yang diuji adalah 10%, 30%, 60%, 80%, dan 100%, dengan kontrol negatif DMSO 1% dan kontrol positif Ofloksasin. Hasil penelitian menunjukkan bahwa Madu Manuka UMF 15+ pada konsentrasi 100% menghasilkan diameter zona hambat tertinggi yaitu 19,06 mm, sedangkan konsentrasi 80% menghasilkan zona hambat sebesar 17,82 mm. Uji statistik menggunakan ANOVA satu arah menunjukkan perbedaan yang signifikan antara kelompok kontrol negatif dan semua kelompok perlakuan MM ($p < 0,001$), serta antar konsentrasi MM ($p = < 0,001$ hingga 0,003). Penelitian ini menyimpulkan bahwa Madu Manuka UMF 15+ memiliki aktivitas antibakteri yang signifikan terhadap Pseudomonas aeruginosa dan dapat menjadi pengobatan alternatif untuk Otitis Eksterna. Dengan demikian, Madu Manuka menunjukkan potensi sebagai agen antibakteri alami yang dapat digunakan tanpa risiko resistensi, tidak seperti antibiotik konvensional.

Kata Kunci: Otitis Eksterna, Madu Manuka, Pseudomonas Aeruginosa, Antibakteri, Resistensi, UMF 15+, Difusi Diskus.

ABSTRACT

Otitis externa (OE) is an infection of the external ear canal skin commonly caused by bacteria, especially Pseudomonas aeruginosa and Staphylococcus aureus. Traditional treatment for OE typically involves topical antibiotics, but long-term use can lead to antibiotic resistance. This study aims to evaluate the antibacterial effect of Manuka Honey (MM) UMF 15+ on Pseudomonas aeruginosa in patients with diffuse OE using the disk diffusion method. The MM concentrations tested were 10%, 30%, 60%, 80%, and 100%, with a negative control of DMSO 1% and a positive control of Ofloxacin. The results showed that Manuka Honey UMF 15+ at a concentration of 100% produced the highest inhibition zone diameter of 19.06 mm, while the 80% concentration produced a zone of inhibition of 17.82 mm. Statistical testing using One-

way ANOVA showed significant differences between the negative control group and all MM treatment groups (p < 0.001), as well as between MM concentrations (p = <0.001 to 0.003). This study concludes that Manuka Honey UMF 15+ has significant antibacterial activity against Pseudomonas aeruginosa and can be an alternative treatment for Otitis Externa. Thus, Manuka Honey shows potential as a natural antibacterial agent that can be used without the risk of resistance, unlike conventional antibiotics.

Keywords: *Otitis Externa, Manuka Honey, Pseudomonas Aeruginosa, Antibacterial, Resistance, UMF 15+, Disk Diffusion.*

INTRODUCTION

Otitis externa is an inflammatory condition that results from a cutaneous and subcutaneous infection of the ear canal, potentially involving the tympanic membrane and the pinna. It is typically characterized by otalgia, with or without ear discharge, and affects approximately 5 to 20% of patients visiting ENT clinics. Bacterial infections are responsible for the majority (98%) of acute otitis externa cases in North America, with *Pseudomonas aeruginosa* (prevalence 20-60%) and *Staphylococcus aureus* (prevalence 10-70%) being the most common pathogens, often in polymicrobial associations (Luthra et al., 2020; Kim SK et al., 2022). In fact, over 90% of otitis externa cases are attributed to bacterial infections, with *Pseudomonas aeruginosa* being the most prevalent (22-62%), followed by *Staphylococcus aureus* (11-34%). Fungal infections account for approximately 10% of otitis externa cases, with *Aspergillus* (60-90%) and *Candida* species (10-40%) being the primary pathogens. Risk factors for fungal otitis externa include long-term antibiotic usage, immunosuppression, and diabetes mellitus (Wiegand et al., 2019).

The choice of treatment for otitis externa typically involves the use of topical antibiotics, which are selected based on the severity of the condition. Quinolone and aminoglycoside antibiotic ear drops are commonly prescribed. However, the local application of antibiotics not only eradicates pathogenic microbes but also disrupts the normal ear flora, potentially leading to the emergence of resistant microbes. Consequently, the combination of antibiotic ear drops with steroid ear drops may be administered, though this treatment should not exceed two weeks, as prolonged use may contribute to resistance (Kumar et al., 2020).

Recent studies, such as one conducted at the ENT clinic of BLU RSUP Prof. Dr. R. D. Kandou Manado, have highlighted the growing issue of antibiotic resistance in otitis externa patients, with *Clindamycin* and *Erythromycin* being particularly resistant, while *Levofloxacin* demonstrated the highest sensitivity. A similar study at Sao Paolo Hospital, Brazil, from February 1, 2010, to January 31, 2011, found otitis externa to be one of the most common infectious diseases treated in the ENT emergency unit, with a monthly incidence of 1,558 cases (Ibnu, Y.S., 2019).

The rising prevalence of antibiotic-resistant bacterial infections presents an escalating challenge in the treatment of otitis externa, prompting interest in alternative therapeutic agents. One such candidate is Manuka Honey (MM), which has garnered attention due to its distinct and effective antibacterial mechanisms. MM's ability to disrupt bacterial metabolism and membrane potential, particularly against *S. aureus* and *E. coli*, positions it as a potential alternative or adjunct therapy for multidrug-resistant (MDR) bacterial infections (Girma, Seo, & She, 2019). Additionally, MM has been shown to exhibit broad-spectrum antibacterial properties, with previous studies demonstrating its efficacy against a variety of pathogens, including those responsible for ear infections such as *Haemophilus influenza*.

Given the increasing concerns surrounding antibiotic resistance in otitis externa, this study aims to explore alternative therapeutic options such as Manuka Honey (MM), which offers antibacterial properties without the risk of resistance development. MM has been used historically for its antimicrobial, anti-inflammatory, and wound-healing properties, which make it a promising treatment for otitis externa. Recent research, including a case report of chronic otitis externa caused by antibiotic-resistant *Pseudomonas aeruginosa*, showed successful treatment using honey-based ear drops (Ibnu, Y.S., 2019).

Manuka Honey (MM), produced from the nectar of the *Leptospermum scoparium* plant native to New Zealand, has particularly high antibacterial activity, earning recognition by the FDA in 2008 for its use in wound care. The Unique Manuka Factor (UMF) scale, which measures the antibacterial potency of MM, has shown that Manuka Honey with a UMF of 20+ exhibits antibacterial effects comparable to a 20% phenol solution (Karawasa et al., 2020). Furthermore, Manuka Honey has demonstrated effective bactericidal activity against *Methicillin-resistant Staphylococcus aureus* (MRSA) and other resistant bacteria (Almasaudi et al., 2016).

The therapeutic potential of MM in otitis externa is further supported by its success in veterinary medicine, where it has been used to treat ear infections in animals, suggesting its applicability in human medicine. Its antibacterial, anti-inflammatory, and wound-healing properties make MM a promising candidate for treating otitis externa without the risk of contributing to antimicrobial resistance. However, while MM has been shown to be effective, concerns regarding its safety, particularly its potential ototoxicity, must be carefully considered. Previous studies have shown that while higher concentrations of MM may be ototoxic, lower concentrations appear to be safe for ear application (Aron M et al., 2012, 2015). This study seeks to assess the antibacterial activity of Manuka Honey (MM) UMF 15+ in various concentrations, evaluating its safety and efficacy as a therapeutic alternative for otitis externa.

LITERATURE REVIEW

Anatomy of the Ear

The human ear is divided into three main parts, each with distinct structures and functions. These parts are the outer ear, middle ear, and inner ear or labyrinth (Dhingra, 2018).

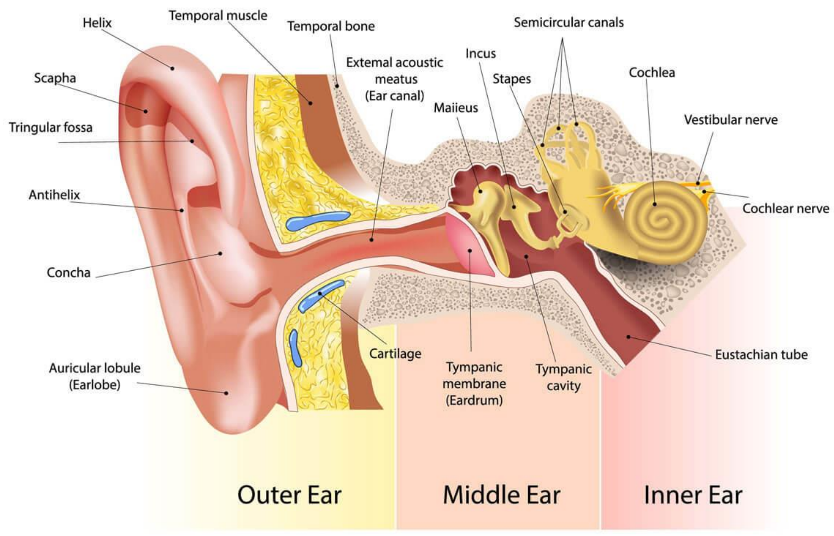

Figure 1. Anatomy of the outer, middle and inner ear (Dewsnup, 2022)

Embryology of the Outer Ear

The outer ear, or auricle, develops from the mesenchymal proliferation of the first and second pharyngeal arches by the end of the fourth week of embryonic development. Six protrusions, known as auricular hillocks, form around the external auditory meatus and eventually fuse to shape the auricle. The first three auricular hillocks arise from the first pharyngeal arch and form the tragus, helix, and cymba concha, while the fourth to sixth auricular hillocks arise from the second pharyngeal arch and form the concha, antihelix, and antitragus (Helwany et al., 2023).

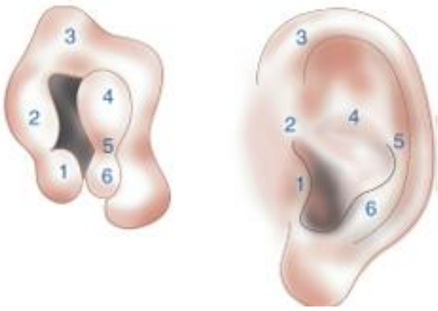

Figure 2. Embryology of the auricle (Dhingra, 2018).

Digital literacy has evolved into a critical competency in the modern era, encompassing the ability to access, evaluate, and utilize digital technologies effectively. Policymakers and educational institutions need to develop strategies that promote digital literacy as a collective effort, reshaping organizations to meet digital demands (Bravo et al., 2021; Marín & Castañeda, 2023; Whiteside et al., 2022). The rapid development of digital platforms has reshaped the way individuals interact with information, making digital literacy a prerequisite for participation in contemporary knowledge economies. Digital literacy is not merely about technical proficiency but also involves cognitive and socio-emotional skills required to navigate the digital landscape securely and ethically (Eshet-Alkali & Amichai-Hamburger, 2004; Jeffrey et al., 2011; Mayisela, 2018). The concept of digital literacy is multidimensional, comprising elements such as information literacy, media literacy, technological literacy, and computational thinking. Libraries, as knowledge hubs, play a crucial role in fostering digital literacy by providing access to digital resources, training programs, and community engagement initiatives (LaPierre & Kitzie, 2019; Sharikov, 2016; Tomczyk & Eger, 2020). However, the implementation of digital literacy programs in special autonomous regions presents unique challenges that must be addressed through tailored strategies and policies.

Manuka Honey

Manuka Honey (MM) is a monofloral honey obtained from the Manuka tree (Leptospermum scoparium), which belongs to the Myrtaceae family and grows as a shrub or small tree, predominantly across New Zealand and Eastern Australia. Manuka honey is highly prized for its non-peroxide antibacterial properties, primarily due to the exclusive presence of methylglyoxal (MGO). Additionally, Manuka honey is rich in both macro and microelements, including sugars, free amino acids, proteins, enzymes, essential minerals, vitamins, and various secondary metabolites (such as flavonoids, phenolic acids, and 1,2-dicarbonyl compounds). The presence of these beneficial phytochemicals is directly linked to its health benefits, which include wound healing, anticancer effects, antioxidant properties, and anti-inflammatory effects (El-Senduny et al., 2021).

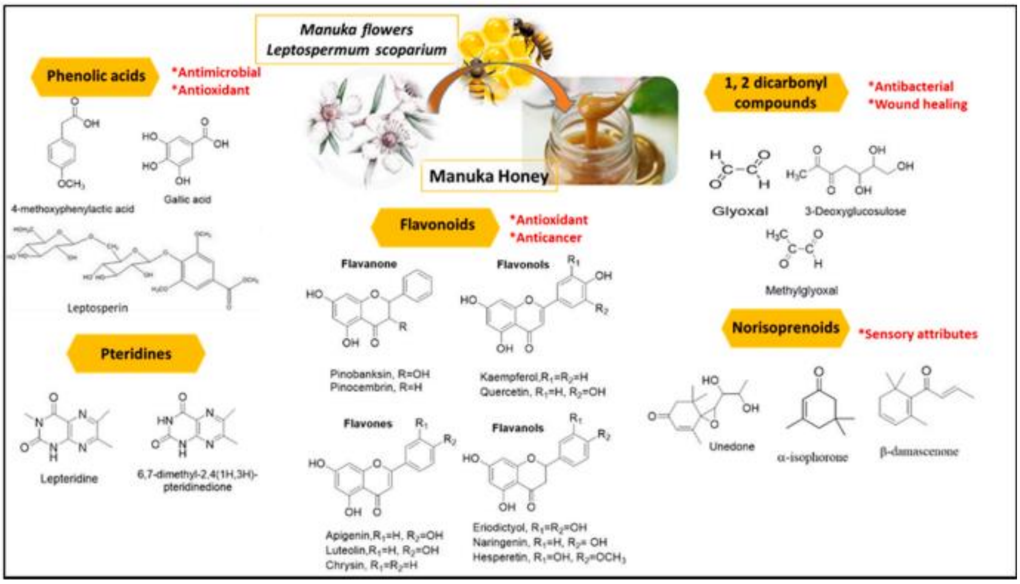

Figure 3. The main ingredients of manuka honey (El-Senduny, et al. 2021).

Manuka Honey Antibacterial Activity

The antibacterial properties of Manuka Honey (MM) are primarily attributed to methylglyoxal (MGO), with the concentration of MGO being measured as the 'Unique Manuka Factor' (UMF). The antibacterial activity correlates with the UMF level, with higher UMF values indicating stronger antibacterial effects against both Gram-positive and Gram-negative bacteria, including drug-resistant strains. UMF is based on MGO content, with UMF 5+ containing ≥ 83 mg/kg MGO, UMF 10+ having ≥ 263 mg/kg, and UMF 15+ containing ≥ 514 mg/kg of MGO.

UMF is a quality grading system used to assess the potency, authenticity, shelf life, and freshness of Manuka honey, ensuring it meets specific standards. The MGO concentration, along with other compounds such as Leptosperin and DHA, contributes to the honey's antibacterial activity. Higher DHA levels indicate longer shelf life for MGO. Manuka honey's antibacterial activity results from the synergy between MGO and other components, such as phenolic compounds and flavonoids, which also have antioxidant, anti-inflammatory, and antibacterial properties.

MGO has shown selective toxicity against bacteria, including *E. coli*, *S. aureus*, and *P. aeruginosa*, and is effective against antibiotic-resistant strains like MRSA. The antimicrobial effectiveness of MGO is influenced by its concentration, with higher concentrations being more effective in killing bacteria. The therapeutic benefits of Manuka honey extend to wound healing, as it promotes tissue regeneration, reduces inflammation, and exhibits antibacterial effects, thanks to its low pH, dehydrating ability, and phytochemicals.

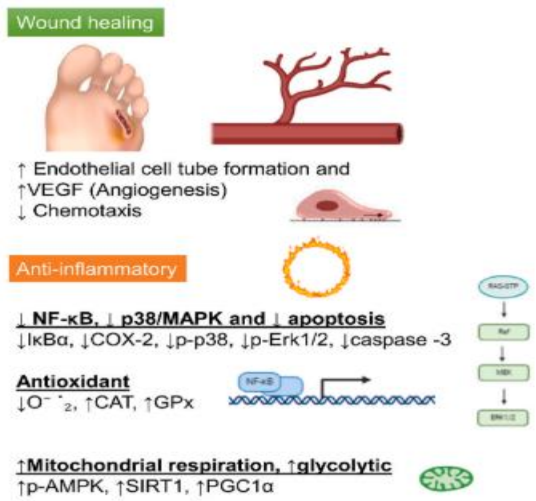

Figure 3. Wound healing and anti-inflammatory properties of manuka honey (El-Senduny, et al. 2021).

RESEARCH METHOD

Type and Design of Research

This type of research is True Experimental Laboratories with a post test with control group design model which aims to test the activity of UMF 15+ manuka honey against Pseudomonas.

Population and Sample

The study uses Pseudomonas aeruginosa bacteria from ear secretions of patients at the ENT clinic of RS Prof. Dr. Chairuddin P. Lubis. The sample size was calculated using Federer's formula, resulting in a minimum of 4 repetitions per treatment group. With 7 treatment groups, the total sample size is 28 bacterial cultures. Samples were obtained using a non-probability consecutive sampling technique between November 2024 and February 2025.

Sample Criteria

1. **Inclusion Criteria**
   - Ear discharge from patients with diffuse otitis externa (OE) collected via ear canal swab.
   - Patients willing to participate in the study.
   - Patients who have not received antibiotic therapy prior to sample collection.

2. **Exclusion Criteria**
   - Damaged samples that cannot be analyzed.
   - Presence of tympanic membrane perforation.
   - Bacteria that do not grow during bacterial culture.

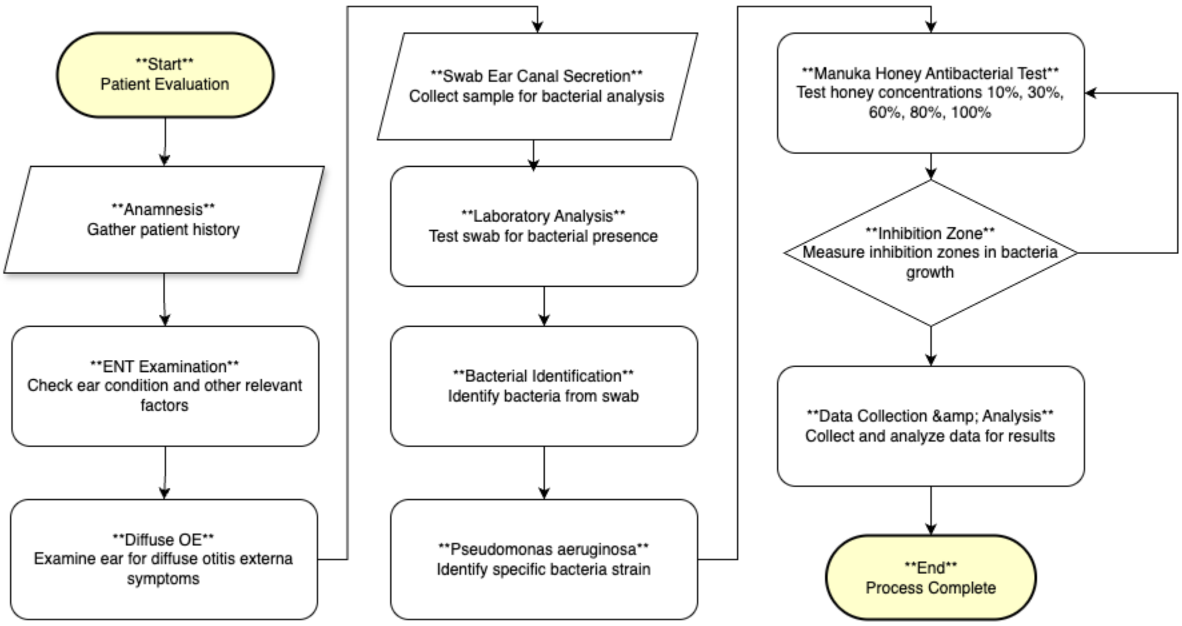

Figure. 4 Research flowchart

3. Data Processing and Analysis

All collected data will be analyzed statistically. The data will be presented in tabular form to determine the effective concentration in inhibiting the growth of *Pseudomonas aeruginosa* in Otitis Externa, using an ANOVA test if the data follows a normal distribution, and a Kruskal-Wallis test if the data does not follow a normal distribution.

RESULT AND DISCUSSION

A. Inhibition Zone Diameter Based on Treatment Groups for the Growth of Pseudomonas aeruginosa Bacteria as the Cause of Otitis Externa

Table 4.1 presents the results of the measurement of the inhibition zone diameter of each treatment group for the growth of Pseudomonas aeruginosa bacteria which is the cause of otitis externa. The data displayed includes the mean value, standard deviation, median, minimum value, and maximum value of each treatment group.

Table 4.1 Inhibition Zone Diameter Based on Treatment Groups for the Growth of Pseudomonas aeruginosa Bacteria as the Cause of Otitis Externa

Group	n	Mean ± SD	Median (Min – Max)
Control (-)	5	0	0
MM UMF 15+ 10% (P1)	5	8.56 ± 1.42	8.3 (6.8 – 10.4)
MM UMF 15+ 30% (P2)	5	13.74 ± 0.8	13.9 (12.7 – 14.7)
MM UMF 15+ 60% (P3)	5	15.82 ± 0.86	15.9 (14.6 – 16.8)
MM UMF 15+ 80% (P4)	5	17.82 ± 1.79	17.8 (15.5 – 19.8)
MM UMF 15+ 100% (P5)	5	19.06 ± 1.32	19.2 (17.0 – 20.5)

In the treatment group given honey, the highest inhibition zone diameter was observed in the *Manuka UMF 15+ 100%* group, with an average diameter of 19.06 mm (SD = 1.32 mm). The second highest inhibition zone diameter was shown by the treatment group given *Manuka UMF 15+ 80%* honey, with an average of 17.82 mm (SD = 1.79 mm).

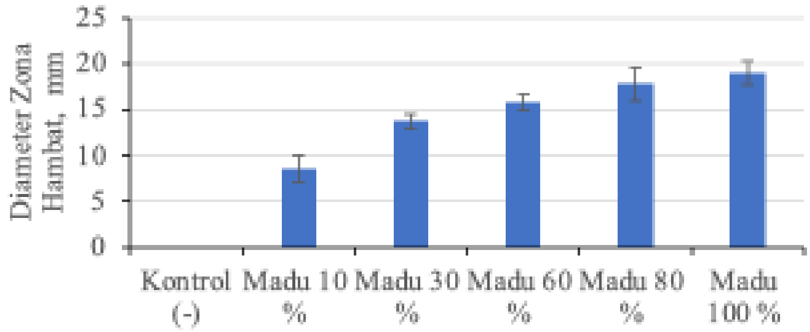

Figure 4.1 Error Bar Diagram of Inhibition Zone Diameter Based on Treatment Groups against *Pseudomonas aeruginosa* Growth as a Cause of Otitis Externa.

B. Difference in Inhibition Zone Diameter Based on Treatment Groups against *Pseudomonas aeruginosa* Growth as a Cause of Otitis Externa.

Table 4.2 presents the results of the analysis of the difference in inhibition zone diameters based on treatment groups using the One-Way ANOVA test.

Table 4.2 Difference in Inhibition Zone Diameter Based on Treatment against *Pseudomonas aeruginosa* Growth as a Cause of Otitis Externa

Group	p
Control (-)	<0,001*
MM UMF 15+ 10 % (P1)	
MM UMF 15+ 30 % (P2)	
MM UMF 15+ 60 % (P3)	
MM UMF 15+ 80 % (P4)	
MM UMF 15+ 100 % (P5)	

* *Oneway Anova*

Using the One-Way ANOVA test, it was shown that there is a significant difference in the inhibition zone diameters based on the treatment groups ($p < 0.001$).

C. **Post-hoc Test of the Difference in Inhibition Zone Diameter Based on Treatment Groups against *Pseudomonas aeruginosa* Growth as a Cause of Otitis Externa.**

Table 4.3 presents the results of the post-hoc test analysis (continuation) of the difference in inhibition zone diameters based on treatment groups using the One-Way ANOVA test.

Table 4.3 Difference in Inhibition Zone Diameter Based on Treatment against *Pseudomonas aeruginosa* Growth as a Cause of Otitis Externa

Group	*Posthoc**					
	K 1+	P1	P2	P3	P4	P5
Control (-)	0,003	<0,001	<0,001	<0,001	<0,001	<0,001
Honey 10 % (P1)			0,003	0,005	0,001	<0,001
Honey 30 % (P2)				0,061	0,062	0,002
Honey 60 % (P3)					0,645	0,038
Honey 80 % (P4)						0,987

* *Tamhane*

Table 4.3 shows the results of the inhibition zone diameter test for *Pseudomonas aeruginosa* growth after treatment with honey at concentrations of 10% (P1), 30% (P2), 60% (P3), and 80% (P4), as well as the negative control (K1+). The post-hoc analysis using the Tamhane test revealed significant differences between the negative control and all honey treatments ($p < 0.001$ to 0.003), indicating that honey at all concentrations significantly inhibited bacterial growth compared to the negative control.

Comparisons between honey concentrations also showed significant differences. For example, 10% honey (P1) differed significantly from 30% honey (P2) ($p = 0.003$), 60% honey (P3) ($p = 0.005$), and 80% honey (P4) ($p = 0.001$). However, the difference between 30% honey and 60% honey was not significant ($p = 0.061$), nor was the difference between 60% and 80%

honey (p = 0.987), suggesting that increasing the honey concentration above 30% does not always result in a significant increase in inhibition.

This study demonstrates that Manuka Honey UMF 15+ has a significant antibacterial effect against the growth of *Pseudomonas aeruginosa*, the causative agent of otitis externa. The highest inhibition zone was observed in the group treated with 100% honey (19.06 mm) and 80% honey (17.82 mm). Statistical analysis using One-Way ANOVA and post-hoc Tamhane tests revealed significant differences between the negative control and all honey treatments, indicating that honey in all concentrations was able to inhibit bacterial growth significantly.

Manuka Honey UMF 15+ is more efficient in inhibiting *Pseudomonas aeruginosa* growth compared to the antibiotic treatment with ofloxacin in the disc diffusion method. The inhibition zone diameters for 80% and 100% honey were larger than the zone produced by ofloxacin, suggesting that honey has an antibacterial potential equal to or even greater than that of antibiotics.

Additionally, Manuka Honey UMF 15+ shows strong antibacterial activity against various pathogens, including multi-drug-resistant strains. The high methylglyoxal content in the honey plays a crucial role in its antimicrobial activity by disrupting bacterial cell walls and inhibiting growth. It also demonstrates significant antibiofilm activity, which is valuable for combating biofilm-related infections, such as those caused by *Pseudomonas aeruginosa*.

This study provides strong evidence that Manuka Honey, especially at higher concentrations, can be used as a natural alternative for treating bacterial infections, including those caused by *Pseudomonas aeruginosa*. Furthermore, Manuka Honey shows potential for use as an adjunctive therapy that may help reduce antibiotic resistance spread and enhance the effectiveness of treatment against resistant bacterial infections.

CONCLUSION

Based on the results of the research, the following conclusions can be drawn:

1. This study found significant differences in the inhibition zones between MM UMF 15+ 60%, 80%, and 100% and the negative control DMSO 1% (p = <0.001).
2. There were significant differences in inhibition zones between the negative control and all honey treatments (p < 0.001 to 0.003), indicating that honey at all concentrations is

able to inhibit bacterial growth significantly compared to the negative control (DMSO 1%).

3. Based on the antibiotic resistance of ofloxacin against *Pseudomonas aeruginosa* according to CLSI standards, it can be stated that the antibacterial effect of MM UMF 15+ 80% and 100% is as potent as ofloxacin antibiotic therapy in the disc diffusion method, being comparable to ofloxacin.

REFERENCES

Almasaudi SB, Al-Nahari AAM, El-Ghani ESMA, barbour E, Muhayawi SMA, Al-Jaouni S, Azhar E, Qari M, Qari YA, Harakeh S,. 2016. Antimicrobial effect of different types of honey on Staphylococcus aureus., Faculty of Medicine, King Abdulaziz University, Saudi Arabia., Saudi Journal of Biological Sciences., www.sciencedirect.com

Al-mutalib, L.A.A. and Zgair, A.K. (2023) 'Sub-inhibitory doses of Ofloxacin reduce adhesion and biofilm formation of Pseudomonas aeruginosa to biotic and abiotic surfaces.', Pharmaceutical Sciences Asia, 50(3).

Alkafaween, M.A., Kafaween, H. and Al-Groom, R.M. (2022) 'A comparative study of antibacterial activity of citrus and Jabali Honeys with Manuka Honey', Applied Environmental Biotechnology, 7(1), pp. 28–37.

Alshahni MM, Alshahni RZ, Fujisaki R, Tamura T, Shimizu Y, Yamanishi C, Makimura K., 2021., A Case of Topical Ofloxacin-Induced Otomycosis and Literature Review., Original Article., https://doi.org/10.1007/s11046-021-00581-x Received: 25 April 2021 / Accepted: 30 July 2021

Ankley, L.M. et al. (2020) 'Manuka honey chelates iron and impacts iron regulation in key bacterial pathogens.', Journal of Applied Microbiology, 128(4), pp. 1015–1024. Available at: https://doi.org/10.1111/JAM.14534.

Aron M, Akinpelu OV, Gasbarrino K, Daniel SJ., 2015. Ear safety of 4% manuka honey in a chinchilla animal model. Eur Arch Otorhinolaryngol ;272:537-2.

Aron M, Victoria Akinpelu O, Dorion D, Daniel S (2012) Otologic safety of manuka honey. J Otolaryngol Head Neck Surg 41(suppl 1):S21–S30

Bashir, I. et al. (2024) ‘Exploring the potent antimicrobial activity of manuka honey as against clinically isolated multi-drug resistant and ceftriaxone-resistant Salmonella Typhi in septicemia patients: a promising therapeutic option’.

Bouzo, D. et al. (2020) ‘Characterizing the mechanism of action of an ancient antimicrobial, Manuka honey, against Pseudomonas aeruginosa using modern transcriptomics’, MSystems, 5(3), pp. 10–1128.

Bukhari, M.A. et al. (2024) ‘Biological studies of the activity of Manuka honey against Carbapenem-resistant Enterobacterales (CRE) bacteria’, Saudi Medical Journal, 45(9), pp. 876–887. Available at: https://doi.org/10.15537/smj.2024.45.9.20240153.

Blasini YM., Sharman T., [Updated 2023 july 31].Otitis Externa. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2025 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK556055/

Bustamante J.,Tran T., Rodriguez CR., (2025). Otitides: Acute and Chronic Otitis Media and Externa. Primary Care : Clinics in Office Practice. Volume 52, Issue 1, March 2025, Pages 1-14. Available from: https://doi.org/10.1016/j.pop.2024.09.003

Carney AS, 2018., Otitis externa and otomycosis, Collage of medicine and public health. Scott-Brown's Otorhinolaryngology and Head and Neck Surgery, Publishing; 2018, 953-958 p.

Chevalier S., Bouffartigues E., Bodilis J., 2017., Review article; Structure, function and regulation of Pseudomonas aeruginosa., Laboratory of Microbiology Signals and Microenvironment LMSM EA 4312, University of Rouen, Normandy University, 27000 Evreux, France and 2IUEM, Laboratoire de Biotechnologie et Chimie Marines EA 3884, Universit´ e de Bretagne-Sud (UEB), 56321 Lorient, France; doi: 10.1093/femsre/fux020

Clinical and Labolatory Standars Institute (CLSI). 2023. Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Third Informational Supplement. CLSI document M100-S23. CLSI, Wayne, PA.

Damodharan N, Verma RK, Angrup A, Bakshi J, Panda NK, 2020., Manuka Honey versus Antibiotic Ear Drops in Healing of Post-Operative Mastoid Cavity: A Prospective Randomized Trial., Published online: February 10, 2020., Korean J Otorhinolaryngol-Head Neck Surg. 2020; 63(5): 199-205., https://doi.org/10.3342/kjorl-hns.2019.00430/

De Nava, ASL., Lasrado S., [Updated 2023 August 14]. Physiology, Ear. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2025 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK540992/

Dewsnup (2022). Anatomy of the Ear and Understanding Hearing. Happyearshearing.com. https://www.happyearshearing.com/anatomy-of the-ear/

Dhingra P, Dhingra S. 2018 Diseases of Ear, Nose and Throat & Head and Neck Surgery. 7th Edition. Disease of ear, nose and throat & head and neck surgery. Elsevier; 2018. 2–12 p.

Dhingra, P.L. and Dhingra, S., 2017. Diseases of Ear, Nose and Throat-EBook. Elsevier Health Sciences.

Doherty JK., Kung RW., 2023. Diseases of the External Ear . In : Bailey's Head & Neck Surgery Otolaryngology 6th Edition Vol 1., ISBN: 978-1-9751-6266-5., p 6799-6812

Ellis J., Lis ADL., Rosen CE., Simpson MTW., Beyea MM., Beyea JA., 2023., Approach to otitis externa : Clinical review., Vol 70 october 2024.

El-Senduny F.F, Hegazi, N.M, Elghani, G.E.A, Farag, M.A., 2021., Manuka honey, a unique mono-floral honey. A comprehensive review of its bioactives, metabolism, action mechanisms, and therapeutic merits., journal homepage: www.elsevier.com/locate/fbio/

Feng, Y. (2023) 'Antibacterial Properties of Manuka Honey and the Role of Methylglyoxal', Journal of Student Research, 12(4).

Girma, A., Seo, W. and She, R.C., 2019. Antibacterial activity of varying UMF-graded Manuka honeys. PloS one, 14(10), p.e0224495.

Greenwood D. 1995. Antibiotics Susceptibility (Sensitivity) Test, Antimicrobial and Chemotheraphy. United State of America: Mc Graw Hill Company

Guedes, G.M. de M. et al. (2023) 'Antibiofilm activity of promethazine, deferiprone, and Manuka honey in an ex vivo wound model', Letters in Applied Microbiology, 76(10), p. ovad119.

Hajioff, D. and MacKeith, S., 2019. Otitis externa. BMJ clinicalevidence. Henatsch. Honey. maastricht university.

Henatsch D,Nabuurs, CH, de Goor RMV, Petra F. Wolffs PF,Stokroos RJ., 2019., Treatment of Recurrent Eczematous External Otitis with Honey Eardrops: A Proof-of-Concept Study., Otolaryngology– Head and Neck Surgery 2017, Vol. 157(4) 696–699., American

Academy of Otolaryngology—Head and Neck Surgery Foundation., Reprints and permission: sagepub.com/journalsPermissions.nav DOI: 10.1177/0194599817718782 http://otojournal.org

Helwany M., Arbor T.C., Tadi P. . [Updated 2023 August 08]. Embryology, Ear. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2023 August-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK557588/

Hossain ML, Lim LY, Hammer K, Hettiarachchi, Locher C., 2023., Article : Monitoring the Release of Methylglyoxal (MGO) from Honey and Honey-Based Formulations., 28, 2858., https://doi.org/10.3390/molecules28062858., Academic Editor: Soraia I. Falcão

Hudzicki, J., 2016. Kirby-Bauer Disk Diffusion Susceptibility Test Protocol Author Information. American Society For Microbiology, December 2009, 1–13. https://www.asm.org/Protocols/Kirby-Bauer-Disk-Diffusion-Susceptibility-Test-Pro.

Ibnu, Y.S., 2019. Potensi Madu sebagai Terapi Topikal Otitis Eksterna. Jurnal Ilmiah Kedokteran Wijaya Kusuma, 8(2), pp.7-22.

Karawasa K, Takakura M, Kato S, Akatsuka M, Kato M., 2020., Simple and Rapid Evaluation of the Unique Manuka Factor in Manuka Honey Using Fluorescence Fingerprints and Principal Component Analysis., Division of Bioanalytical Chemistry, School of Pharmacy, Showa University; 1–5–8 Hatanodai, Shinagawa-ku, Tokyo 142–8555, Japan. Chem. Pharm. Bull. 68, 762–765., Vol. 68, No. 8

Kello, E. et al. (2023) 'The Effect of Antibiotic Treatment and Gene Expression of Mex B Efflux Transporters on the Resistance in Pseudomonas Aeruginosa Biofilms', Applied Microbiology, 3(3), pp. 709–721.

Kim, S.K., Han, S.J., Hong, S.J., 2022. Microbiome of Acute Otitis Externa. J.Clin. Med.,11, 7074. http://Doi.org/10.3390/jcm11237074

Kumar, A., Mittal, S., Tyagi, A.K., Romesh, H., Varshney, S. and Malhotra, M., 2020. Efficacy of medical grade manuka honey in acute otitis externa: A pilot study. Indian Journal of Otology, 26(3), p.151.

Linstorm C. 2014. Disease of external ear. In: Johnson J, ed. Bailey's head and neck surgery otolaryngology. 5th ed. Philadelphia: Lippincot Co; p. 2333-40

Lu, J. et al. (2019) 'Honey can inhibit and eliminate biofilms produced by Pseudomonas aeruginosa', Scientific reports, 9(1), p. 18160.

Lucente F.E, Linstrom C.J, 2014. Disease of the external ear. In: Johnson JT, Rosen CA, Newlands S, Amin M, Branstetter B, Casselbrant M, et al., eds. Bailey's head and neck surgery otolaryngology, 5th edition, Vol 2, Philadelphia: Lippincott Williams & Wilkins, pp. 2333-57.

Luthra H, Sharma V, Jindal N., 2020., Microbiological Profile and Antibiotic Sensitivity of 100 Cases of Otitis Externa., Original Article., Indian J Otolaryngol Head Neck Surg https://doi.org/10.1007/s12070-020-02151-x

Mandal MD, Mandal S, 2011. Honey: its medicinal property and antibacterial activity, Asian Pac J Trop. Biomed 1(2):154-60

Qin S., Xiao W.,Zhou C., Pu Q., Lan L., Liang H., Songf X., 2022., REVIEW ARTICLE; Pseudomonas aeruginosa: pathogenesis, virulence factors, antibiotic resistance, interaction with host, technology advances and emerging therapeutics., https://doi.org? 10.1038/ s41392-022-01056-1

Rabie BSc E, Serem MSc JC, Oberholzer PhD HM, Gaspar PhD ARM, Bester PhD MJ., 2016: How methylglyoxal kills bacteria: An ultrastructural study, Ultrastructural Pathology, DOI: 10.3109/01913123.2016.1154914., http://dx.doi.org/10.3109/01913123.2016.1154914

Roberts, A.E.L. et al. (2019) 'Anti-pseudomonad activity of manuka honey and antibiotics in a specialized ex vivo model simulating cystic fibrosis lung infection', Frontiers in microbiology, 10, p. 869.

Roberts, A.E.L., Maddocks, S.E. and Cooper, R.A. (2012) 'Manuka honey is bactericidal against Pseudomonas aeruginosa and results in differential expression of oprF and algD', Microbiology, 158(12), pp. 3005–3013.

Sánchez López de Nava A, Lasrado S. 2022., Physiology, Ear. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK540992/

Shabbir, U. et al. (2023) 'Effect of honey use with Seriphidium chitralense podlech on growth and biofilm formation of Pseudomonas aeruginosa', Kuwait Journal of Science, 50(4), pp. 703–708.

Siddiqui M, Giasuddin M, Chowdhury S, Islam M, Chowdhury E. 2015. Comparative effectiveness of Dimethyl Sulphoxide (DMSO) and Glycerol as cryprotective agent in preserving Vero cells. Bangladesh Veterinatian. 4(1) : 34—40.

Sievers, J.T.O. et al. (2022) 'Comparing Manuka and other medical honeys as adjunct to antibiotic therapy against facultative anaerobes', Sains Malaysiana [Preprint].

Sihotang, T.S.U., Widodo, A.D.W. and Endraswari, P.D. (2022) 'Effect of Ciprofloxacin, Levofloxacin, and Ofloxacin onPseudomonas aeruginosa: A case control study with time kill curve analysis', Annals of Medicine and Surgery, 82.

Sinha, S. et al. (2023) 'Benefits of Manuka Honey in the Management of Infectious Diseases: Recent Advances and Prospects', Mini-reviews in Medicinal Chemistry, 23. Available at: https://doi.org/10.2174/1389557523666230605120717.

Smiyanov EV., 2023., Inflammatory and non-inflammatory diseases of the outer ear. Ministry of Education and Science of Ukraine Ministry of Health of Ukraine Sumy State University. 2023. – 120 p.

Suarez JMA, Gasparrini M, Hernandez TYF, Mazzoni L, Giamperi F, 2014. The composition and biological acivity of honey: a focus on manuka honey, Foods 3:420-32

T. B., S., Srivastava, T. and Chandra, I. (2023) 'Hype or healing: unveiling the truth behind manuka honey?', International Journal of Otorhinolaryngology and Head and Neck Surgery, 9(11 SE-Case Series), pp. 872–878. Available at: https://doi.org/10.18203/issn.2454-5929.ijohns20233217.

Unique Manuka Factor Honey Association, 2024. Unique Manuka Factor : " The Golden Standard in Manuka Honey"., Unique Mānuka Factor (UMF) Grading System Explained | UMF™

Watkinson,J.C.and Clarke, R.W. eds.,2018. Scott-Brown's Otorhinolaryngology and Head and Neck Surgery: 3 volume set. CRC Press.

Werner A, Laccourreye O, 2011. Honey in otorhinolaryngology: when, why and how?, European Annals of Otorhinolaryngology, Head and Neck disease 128:133-7

Wiegand, S., Berner, R., Schneider, A., Lundershausen, E., Dietz, A., 2019. Otitis Externa: Investigation and Evidence-Based Treatment., Dtsch Arztebl Int 116, 224–234.

Yulianti., 2017., Uji Efektifitas Madu Sebagai Antibakteri Terhadap Pertumbuhan Staphylococcus Aureus dan Pseudomonas Aeruginosae Dengan Metode Disk Diffusion., Fakultas Kedokteran UIN Syarif Hidayatullah Jakarta., Jurnal Profesi Medika., Vol.11, No. 1

Zaidi, M., Nurrohmah, N., & Kudriah. 2021. Madu dalam Al-Qur'an (Studi Penafsiran QS. an-Nahl: 68-69). Al Muhafidz: Jurnal Ilmu Al-Qur'an Dan Tafsir, 1(2), 121–135.