Vol 12 No 1 Year 2027

BULETIN FARMATERA

E-ISSN: 2528-410X

RESEARCH ARTICLES

Comparative In Vitro Analysis of Propolis and 3% Hydrogen Peroxide as Cerumenolytic Agents in Children

Doli Naufal Ritonga[1], **Muhammad Edy Syahputra Nasution**[2]

[1] Faculty of Medicine, Universitas Muhammadiyah Sumatera Utara, Jalan Gedung Arca No 53 Medan,North Sumatra, Indonesia 20217

[2] Department of Ear, Nose and Throat Diseases, Faculty of Medicine, Universitas Muhammadiyah Sumatera Utara, Jalan Gedung Arca No 53 Medan,North Sumatra, Indonesia 20217

Corresponding Email: dolinaufal@gmail.com
mhd.edysyahputra@umsu.ac.id

Abstract: Cerumen impaction is a common otologic problem that can impair hearing and predispose individuals to external ear infections. This in vitro study aimed to compare the cerumenolytic effects of propolis and 3% hydrogen peroxide using spectrophotometric absorbance measurements at 5, 10, 15, and 30 minutes. A total of 48 cerumen samples were collected from children at Panti Asuhan Putri Aisyiyah Medan and divided equally into two treatment groups. Absorbance values were analyzed using non-parametric statistical tests due to non-normal data distribution. Propolis demonstrated a progressive increase in absorbance over time, reaching its highest effect at 30 minutes, whereas hydrogen peroxide showed minimal changes with no significant time-dependent pattern. Comparative analysis revealed that propolis produced significantly higher absorbance values than hydrogen peroxide at all time points ($p < 0.001$), indicating superior cerumen dissolution. These findings suggest that propolis may serve as a more effective and potentially safer alternative to hydrogen peroxide for cerumen management. Further in vivo and clinical studies are recommended to validate its therapeutic applicability and long-term safety.

Keywords: Cerumen impaction, propolis, hydrogen peroxide 3%, in vitro study

INTRODUCTION

Cerumen impaction remains one of the most frequently encountered conditions in clinical otology and is a notable contributor to conductive hearing loss, discomfort, and increased susceptibility to external ear infections[1]. Although cerumen plays a physiological role as a protective barrier, lubricant, and antimicrobial agent within the external auditory canal, excessive accumulation can obstruct the canal and compromise auditory function [2,3]. Global estimates indicate that approximately 6% of the population, predominantly children experience hearing disturbances related to impacted cerumen[1]. In Indonesia, its prevalence is reported to range between 30–50%, underscoring the need for effective, safe, and accessible treatment approaches[4].

BULETIN FARMATERA
E-ISSN: 2528-410X

Current management strategies include manual removal, irrigation, and cerumenolytic therapy[5]. Among these, cerumenolytics are often preferred due to their minimal mechanical risk[6–8]. Hydrogen peroxide 3% is one of the most widely used agents because of its ability to break down cerumen through effervescence[9]. However, prolonged or excessive use may leave residual moisture in the canal, potentially promoting bacterial growth and increasing the risk of secondary infection[10]. This concern has prompted interest in alternative cerumenolytics with safer profiles, particularly those derived from natural sources.

Propolis, a resinous substance collected by honeybees from plant exudates, has gained attention due to its antimicrobial[11], antioxidant[12], and tissue-protective properties[13]. Previous studies have suggested its potential utility in various therapeutic contexts, and recent evidence indicates that it may aid in softening and reducing cerumen mass. Given its natural origin and favorable safety profile, propolis represents a promising candidate for cerumen management, especially in pediatric populations where irritant exposure should be minimized.

Despite its potential, comparative data evaluating the efficacy of propolis against standard agents such as hydrogen peroxide remain limited. This study aims to address this gap by analyzing the in vitro effects of propolis and 3% hydrogen peroxide on cerumen obtained from children residing at Panti Asuhan Putri Aisyiyah Medan. The findings are expected to contribute to evidence-based considerations for safer and more effective cerumenolytic therapy.

METHOD

This laboratory-based experimental study employed an in vitro design to compare the cerumenolytic effects of propolis and 3% hydrogen peroxide. Samples were collected through simple random sampling, and experimental procedures were conducted under controlled laboratory conditions. Cerumen samples were obtained from children residing at Panti Asuhan Putri Aisyiyah Medan, Indonesia. All laboratory analyses were performed at the Biochemistry Laboratory, Faculty of Medicine, Universitas Muhammadiyah Sumatera Utara.

The study population consisted of 60 children living in the orphanage. Sample size estimation was performed for two independent proportions, yielding a minimum requirement of 48 samples. Cerumen from 24 participants was allocated to the propolis group, and cerumen from another 24 participants was allocated to the hydrogen peroxide group. Written informed consent was obtained from all participants or their guardians. Two test agents were used:

1. Propolis (SunPro Super Nano)
2. Hydrogen peroxide 3% (One Med Perhidrol)

All reagents, consumables, and equipment, including UV-Vis spectrophotometer, water bath, glassware, and filtration tools, were sourced from the faculty laboratory.

Cerumen was extracted by an otolaryngology specialist following standard otoscopic examination. Each sample (50 mg) was placed into a 10 × 75 mm test tube. A total of 48 tubes were prepared, with two tubes representing the sample derived from each participant. Each test tube was treated with 2 mL of either propolis or 3% hydrogen peroxide. The tubes were sealed and

BULETIN FARMATERA
E-ISSN: 2528-410X

incubated in a 37°C water bath. Absorbance measurements were performed at 5, 10, 15, and 30 minutes for all samples. Cerumen dissolution (absorbance) was quantified using a UV-Vis spectrophotometer at 600 nm, following standard procedures to determine turbidity reduction over time.

The primary outcome was the mean absorbance value of cerumen at each time point, reflecting the degree of cerumen breakdown by each solution. All data were analyzed using appropriate statistical tests based on distribution normality test to determine data distribution. Kruskal–Wallis test for within-group comparisons and Mann–Whitney U test for between-group comparisons. A significance level of $p < 0.05$ was applied.

RESULT

A total of 48 cerumen samples were analyzed using UV–Vis spectrophotometry to quantify changes in absorbance following exposure to propolis or 3% hydrogen peroxide. The primary outcome was the mean absorbance value at 5, 10, 15, and 30 minutes, expressed in L/mol·cm. These results are presented in Tabel 1. The absorbance in the propolis group increased steadily across all time points, suggesting progressively greater cerumen breakdown. Conversely, the hydrogen peroxide group demonstrated slight fluctuations without a consistent upward pattern, with the 30-minute absorbance even lower than the initial 5-minute value.

Table 1. Mean absorbance (L/mol·cm) of cerumen samples exposed to propolis and 3% hydrogen peroxide

Agents	5 min	10 min	15 min	30 min
Propolis	0.753	0.758	0.777	0.849
Hydrogen peroxide 3%	0.161	0.218	0.152	0.142

To determine the appropriate statistical analysis, normality was assessed using the Shapiro–Wilk test. All datasets demonstrated p-values below 0.05, indicating deviation from normal distribution. Therefore, non-parametric tests were used for subsequent comparisons. These outcomes are summarized in Tabel 2. The lack of normal distribution supported the use of the Kruskal–Wallis and Mann–Whitney tests for group and time comparisons.

Table 2. Summary of Shapiro–Wilk normality testing

Groups	Time	P value	Conclusion
Propolis	5–30 min	< 0.05	Not normal
H_2O_2 3%	5–30 min	< 0.05	Not normal

The Kruskal–Wallis test revealed statistically significant differences across time points within the propolis group ($p < 0.001$). This confirms a time-dependent effect of propolis on cerumen absorbance. However, for hydrogen peroxide, the test showed no significant differences ($p = 0.315$) across the same time intervals. These findings are detailed in Tabel 3. The significant increase in the propolis group supports the hypothesis that propolis exerts a progressive cerumenolytic effect during incubation.

Table 3. Kruskal–Wallis results comparing absorbance across time points

Groups	Mean ± SD	P value
Propolis (5–30 min)	0.753–0.849	< 0.001*
H_2O_2 3% (5–30 min)	0.142–0.218	0.315

Pairwise Mann–Whitney tests were conducted for propolis, showing that the differences between early (5–10 min) and

BULETIN FARMATERA
E-ISSN: 2528-410X

later (30 min) measurements were statistically significant. These comparisons are presented in Tabel 4. The significant differences observed in comparisons involving the 30-minute time point demonstrate that propolis continues to dissolve cerumen more effectively as exposure time increases. A direct comparison between propolis and hydrogen peroxide revealed that propolis produced much higher absorbance values across all samples. The Mann–Whitney test yielded $p < 0.001$, confirming that the difference between the two treatments was statistically significant.

Table 4. Mann–Whitney pairwise comparison for propolis

Time Comparison	P value
5 vs 10 min	0.621
5 vs 15 min	0.194
5 vs 30 min	< 0.001*
10 vs 15 min	0.364
10 vs 30 min	0.001*
15 vs 30 min	0.004*

DISCUSSION

The present study provides strong evidence that propolis demonstrates a time-dependent and progressively increasing cerumenolytic effect. As shown in Tabel 1, the consistent rise in absorbance indicates enhanced solubilization of cerumen over time. This is further supported by the statistically significant findings in Tabel 3 and Tabel 4, confirming that propolis alters cerumen characteristics more profoundly as incubation progresses.

The bioactivity of propolis, driven by flavonoids, phenolic acids, and antioxidant compounds, likely contributes to its ability to soften, emulsify, and destabilize cerumen matrices[14,15]. These biochemical properties also explain propolis's established antimicrobial[11], anti-inflammatory[16,17], and wound-healing capabilities[12], which have been reported in multiple studies.

Hydrogen peroxide, although widely used in clinical practice, demonstrated minimal changes in absorbance over the 30-minute exposure period[18]. As seen in Tabel 1, values fluctuated slightly but showed no meaningful progression. The lack of statistical significance in Tabel 3 further reinforces that hydrogen peroxide's cerumenolytic effect occurs rapidly at the moment of effervescence and then plateaus.

Moreover, hydrogen peroxide carries potential drawbacks, including residual moisture that supports bacterial growth and oxidative irritation to the ear canal which may diminish its suitability for routine use, especially in pediatric populations.

The markedly higher absorbance observed in the propolis group confirms that propolis is substantially more effective than hydrogen peroxide in this in vitro model. The findings in Tabel 4 and the group comparison analysis robustly support this conclusion. Biologically, this superiority may be attributed to:

1. Complex bioactive profile (flavonoids, terpenes, amino acids, minerals).
2. Multifaceted therapeutic actions, including antimicrobial, anti-inflammatory, and antioxidant effects.
3. Ability to modify cerumen viscoelastic properties, enhancing dissolution relative to the single-mechanism action of hydrogen peroxide.
4. Better tissue compatibility, reducing risk of irritation.

Although this study was conducted under in vitro conditions, the findings indicate that propolis may serve as a promising natural alternative for cerumen management. Its multi-mechanistic

BULETIN FARMATERA
E-ISSN: 2528-410X

properties, coupled with a gentler safety profile, make it particularly appealing for use in pediatric populations, who often require topical agents with minimal irritation risk[19]. The consistently stronger cerumenolytic activity demonstrated by propolis suggests meaningful therapeutic potential that warrants further exploration[13,20].

However, several important considerations must be addressed before propolis can be implemented in clinical practice. Individual differences in cerumen composition, variations in the anatomical structure of the external auditory canal, and the need for standardized dosing and formulation all represent potential challenges[21]. Long-term safety, including the risk of hypersensitivity reactions, must also be assessed comprehensively[8]. For these reasons, future in vivo studies and well-designed randomized clinical trials are essential to confirm the clinical relevance and practical applicability of these laboratory findings.

The study also has notable limitations. Its in vitro design inherently restricts how closely the experimental conditions can mimic real physiological environments. Factors such as temperature gradients within the ear canal, natural variability in cerumen due to ethnicity[1], dietary habits[22], and glandular activity[5], as well as the mechanical movement of the jaw that contributes to cerumen migration, could not be reproduced in the laboratory. Furthermore, the study focused solely on absorbance measurements; biochemical, rheological, and microbiological characteristics of cerumen were not evaluated. Future research should incorporate these additional parameters to obtain a more comprehensive understanding of cerumenolytic effectiveness.

CONCLUSION

This in vitro study demonstrates that propolis exhibits a markedly superior cerumenolytic effect compared with 3% hydrogen peroxide, as evidenced by its consistently higher absorbance values and progressive effectiveness over time. While hydrogen peroxide showed limited activity that diminished after the initial minutes of exposure, propolis continued to enhance cerumen dissolution up to the 30-minute mark. These findings highlight propolis as a promising natural alternative for managing cerumen impaction, offering greater efficacy and potential safety advantages. Although the results provide a strong foundational understanding of propolis's therapeutic potential, further in vivo and clinical studies are essential to validate its practical applicability, determine optimal dosing strategies, and assess long-term safety in real-world patient populations.

REFERENCES

1. Tolan MM, Choi JS, Tibesar MT, Adams ME. Cerumen impaction: Prevalence and associated factors in the United States population. *Laryngoscope Investig Otolaryngol*. 2024;9(2):e1228.
2. Gurunathan RK, Perry M. The Ear and Associated Structures: Part II. In: *Diseases and Injuries to the Head, Face and Neck: A Guide to Diagnosis and Management*. Springer; 2021:1511-1587.
3. Chaudhary AK. Procedure of Clearing of External Ear Canal. In: *Common Ear Diseases in Dogs: Diagnosis and Management*. Bentham Science Publishers; 2025:62-76.

BULETIN FARMATERA
E-ISSN: 2528-410X

4. Suresh S, Suresh S, Sivasamy S. Indonesian Journal of Medical Sciences and Public Health. *Indones J Med Sci Public Heal*. 2021;2(1):28-39.
5. Dudley A. Update on Cerumen Impaction Management in Older Adults. *J Nurse Pract*. 2024;20(9):105154.
6. Maiti SK, Sarkar VK, Sinha S. Ceruminoliths and its clinical Management. *Common Ear Dis Dogs Diagnosis Manag*. Published online 2025:149-159.
7. Gupta T, Bhutta MF. Ear wax and its removal: current practices and recommendations. *Hear J*. 2023;76(09):22-23.
8. Lian Y, Yuan K, Nie L, Xiao L, Ding L. Evaluation of the safety and efficacy of manual removal and irrigation in cerumen removal in children. *Sci Prog*. 2025;108(2):00368504251345886.
9. Urakova NA, Urakov AL. Hydrogen peroxide: potential for repurposing into an oxygen-producing antihypoxant by generating oxygen gas. *Biointerface Res Appl Chem*. 2025;15.
10. Anh NQ, Numthavaj P, Bhongmakapat T. Comparison of the Cerumenolytic Activities of New and Currently Used Agents. *Ear, Nose Throat J*. 2022;101(2_suppl):31S-36S. doi:10.1177/0145561320986060
11. Machado A, Zamora-Mendoza L, Alexis F, Álvarez-Suarez JM. Use of plant extracts, bee-derived products, and probiotic-related applications to fight multidrug-resistant pathogens in the post-antibiotic era. *Futur Pharmacol*. 2023;3(3):535-567.
12. Javed S, Mangla B, Ahsan W. From propolis to nanopropolis: An exemplary journey and a paradigm shift of a resinous substance produced by bees. *Phyther Res*. 2022;36(5):2016-2041.
13. Ghallab D, Shawky E, Mohyeldin M, Metwally AM, Ibrahim RS. Propolis: An update on its chemical diversity, botanical origin and biological activities. *J Adv Pharm Sci*. 2025;2(1):76-99.
14. Rana A, Malik A, Sobti RC. Anti-bacterial properties of propolis: A comprehensive review. *Curr Microbiol*. 2025;82(10):1-21.
15. Chuttong B, Lim K, Praphawilai P, et al. Exploring the functional properties of propolis, geopropolis, and cerumen, with a special emphasis on their antimicrobial effects. *Foods*. 2023;12(21):3909.
16. Zulhendri F, Lesmana R, Tandean S, et al. Recent update on the anti-inflammatory activities of propolis. *Molecules*. 2022;27(23):8473.
17. El-Seedi HR, Eid N, Abd El-Wahed AA, et al. Honey bee products: preclinical and clinical studies of their anti-inflammatory and immunomodulatory properties. *Front Nutr*. 2022;8:761267.
18. Terra RMO, da Silva KL, Vochikovski L, et al. Effect of daily usage time of 4% hydrogen peroxide on the efficacy and bleaching-induced tooth sensitivity: a single-blind randomized clinical trial. *Oper Dent*. 2021;46(4):395-405.
19. Peršurić Ž, Pavelić SK. Bioactives from bee products and accompanying extracellular vesicles as novel bioactive components for wound healing. *Molecules*. 2021;26(12):3770.
20. Yang J, Pi A, Yan L, et al. Research progress on therapeutic effect and mechanism of propolis on wound healing. *Evidence-Based Complement*

BULETIN FARMATERA
E-ISSN: 2528-410X

Altern Med. 2022;2022(1):5798941.

21. Sundar PS, Chowdhury C, Kamarthi S. Evaluation of human ear anatomy and functionality by axiomatic design. *Biomimetics*. 2021;6(2):31.
22. Kamm WE, Tolan MM, Kennedy CL, Choi JS, Marmor S, Adams ME. Efficacy of Over-the-Counter Cerumen Removal Devices: A Randomized Trial. *Laryngoscope Investig Otolaryngol*. 2025;10(6):e70313.