Comparison of the effectiveness of livestock honey without royal jelly, forest honey and propolis against *Pseudomonas aeruginosa*, *Acinetobacter baumanii* and *Staphylococcus aureus* in vitro

Amrin Amir Lubis[a] , Frank Bietra Buchari[b], Utama Abdi Tarigan[b],

R. Lia Kusumawati Iswara[c], Aznan Lelo[d]

[a]Department of Surgery, Faculty of Medicine, University of North Sumatra
[b]Division of Plastic Surgery, Department of Surgery, Faculty of Medicine, University of North Sumatra
[c]Department of Clinical Microbiology, Faculty of Medicine, University of North Sumatra
[d]Department of Pharmacology, Faculty of Medicine, University of North Sumatra

Abstract

Introduction: This study investigates the effectiveness of Nusantara pure hone , wild honey, and Manuka honey against common bacteria in burn wounds , highlighting the unique antimicrobial properties of each type .
Methods : Employing and in vitro experimental design , the research evaluates the antibacterial effectiveness of livestock honey without royal jelly , forest honey , and propolis against Pseudomonas aeruginosa , Acinetobacter baumannii , and Staphylococcus aureus . The study was conducted in a microbiology laboratories with adherence to ethical standards .
Results : The study did not observe any antibacterial effect from either livestock honey without royal jelly or forests honey . However , propolis showed an antibacterial effect , particularly significant against Pseudomonas aeruginosa at 100% concentration , indicating its potential as a superior antibacterial agent in this context .
Discussion : The findings suggest that while some forms of honey did not exhibit antibacterial properties under the study conditions , propolis demonstrated a significant effect , emphasis the potential for propolis in antimicrobial applications , especially against biofilm- forming bacteria .
Conclusion : Propolis exhibits a strong antibacterial effect against specific burn wounds pathogens , particularly Pseudomonas aeruginosa , underscoring its potential in burn wounds management

Keywords : Nusantara pure honey , wild honey , Manuka honey , propolis , antibacterial effectiveness , burn wounds , Pseudomonas aeruginosa , Acinetobacter baumannii , Staphylococcus aureus

1. Introduction

Indonesia, as part from the archipelago, has riches abundant nature , including production honey from bee local . Honey pure Indonesian, wild honey and honey effi own various contents and properties , each of which provides contribution unique in potential healing wound burn . Honey pure Indonesian, produced in Indonesia and surrounding areas , offers combination of natural sugar , antioxidants such as flavonoids, as well characteristic antimicrobial . Natural sugar give source energy that supports the healing process cell , meanwhile antioxidant can protect cells from damage radical free , speed up regeneration network . Wild honey , produced by wild bees in their natural habitat , perhaps own variation content depending on the wild plants that become source the nectar . Wild honey often linked with diversity compound bioactive , incl enzymes and compounds phytochemicals , which can strengthen potency antibacterial and anti-inflammatory in wounds burn . Temporary that , honey efi , or Manuka honey , specifically generated from Nectar Manuka

plants in Zealand New , have activity High antibacterial , esp _ caused by compounds methylglyoxal . This matter make it effective in oppose bacteria and microbes reason infection in the wound burn . By overall , third type honey This can role in support healing wound burn .(Diah et al., 2012; Sundoro et al., 2012)

Although There is a number of research that has been highlighting benefit honey on treatment wound burn , comparison direct between effectiveness honey pure Indonesian, wild honey and honey effi to growth bacteria most common in patients wound burn Still limited . Therefore _ that 's necessary done more research _ in -depth and systematic comparison For get more understanding _ Good about potential of each type honey in hinder growth bacteria in wounds burn . Study This aim For give contribution significant on knowledge scientific about role honey as agent antimicrobials in treatment wound burn . Besides that , research This expected can give more views _ Specific about comparison effectiveness honey pure Indonesian, wild honey and honey effi in hinder growth bacteria certain things often appears on the wound burn . Through study This is expected can developed approach maintenance wound burn more _ personalized and effective based on source Power nature in the archipelago. Research result This expected can give base strong scientific _ For guide practitioner medical in choose material optimal care for speed up healing and prevention infection in patients wound burn.

2. Methods

This study including in category study laboratory in vitro experiments For evaluate comparison effectiveness between honey cattle without royal jelly, honey forest and propolis. Study This carried out in the Laboratory Microbiology Faculty USU Medicine with get agreement from Commission Ethics USU Health Research . Implementation research and data collection is planned in month December 2023 later will followed with stage processing and analysis of collected data . Activity inspection growth bacteria as well as effect giving honey cattle without royal jelly, honey forest , and propolis will carried out in the Department Microbiology Faculty North Sumatra University of Medicine – Prof. Hospital. Dr. Chairuddin Panusunan Lubis University of North Sumatra. Study This involves the process of bacterial culture Pseudomonas aeruginosa, Acinetobacter baumannii , and Staphylococcus aureus on Muller Hinton Agar (MHA) petri dish media.

Analysis statistics descriptive used For evaluate profile data bacteria that have generated from culture. Temporary that , analysis inferential using the ANOVA test or , as alternative , Kruskal-Wallis, is used For test comparison effectiveness between honey cattle without royal jelly, honey forest , propolis and aquabides to growth bacteria in patients wound burnt at H. Adam Malik General Hospital, Medan. Research result analyzed in a way statistics with use device soft statistics , and differences considered significant in a way statistics If p value < 0.05

3. Results

The subjects of this study were diabetic foot patients treated at Haji Adam Malik Central General Hospital in Medan from 2019 to 2021, totaling 34 individuals who met the inclusion and exclusion criteria . In this study, the characteristics of the respondents can be observed in the following table :

Table 1. Diameter of inhibition zone on administration honey forest in bacteria *Methicillin-sensitive Staphylococcus aureus* (MSSA)

Treatment	Inhibition Zone Diameter Repetition (mm)			Average (mm)	p value
	I	II	III		
Concentration 25% MH	0	0	0	0	<0.001 [a]
Concentration 50% MH	0	0	0	0	
Concentration 75% MH	0	0	0	0	
100% MH concentration	0	0	0	0	
Gentamicin	24.5	25	25	24.8333333	
Aquabidest	0	0	0	0	

[a] Kruskal -Wallis
MH: Honey Forest

Table 1 shows there is no diameter of the inhibition zone formed upon administration honey forest in bacteria *Methicillin-sensitive Staphylococcus aureus* (MSSA). Inhibition zone diameter paste on the disc gentamicin with an average of 24.8334 mm.

Table 2. Diameter of inhibition zone on administration honey forest in bacteria *Methicillin-resistant Staphylococcus aureus* (MRSA)

Treatment	Inhibition Zone Diameter Repetition (mm)			Average (mm)	p value
	I	II	III		
Concentration 25% MH	0	0	0	0	<0.001 [a]
Concentration 50% MH	0	0	0	0	
Concentration 75% MH	0	0	0	0	
100% MH concentration	0	0	0	0	
Gentamicin	23.75	24	24	23,916	
Aquabidest	0	0	0	0	

[a] Kruskal -Wallis
MH: Honey Forest

Table 2 shows no diameter of the inhibition zone formed upon administration honey forest in bacteria *Methicillin-resistant Staphylococcus aureus* (MRSA). Inhibition zone diameter appears on the disc gentamicin with an average of 23,916 mm.

Table 3. Diameter of inhibition zone on administration honey forest in bacteria *Acinetobacter baumannii*

Treatment	Inhibition Zone Diameter Repetition (mm)			Average (mm)	p value
	I	II	III		
Concentration 25% MH	0	0	0	0	<0.001 [a]
Concentration 50% MH	0	0	0	0	
Concentration 75% MH	0	0	0	0	
100% MH concentration	0	0	0	0	
Gentamicin	18.6	19.75	19	19.1166667	
Aquabidest	0	0	0	0	

[a] Kruskal -Wallis
MH: Honey Forest

Table 3. shows no diameter of the inhibition zone formed upon administration honey forest in bacteria *Acenitobacter baumannii* . Inhibition zone diameter appears on the disc gentamicin with an average of 19.1166 mm.

Table 4. Diameter of inhibition zone on administration honey forest in bacteria *Pseudomonas aeruginosa*

Treatment	Inhibition Zone Diameter Repetition (mm)			Average (mm)	p value
	I	II	III		
Concentration 25% MH	0	0	0	0	<0.001 [a]
Concentration 50% MH	0	0	0	0	
Concentration 75% MH	0	0	0	0	
100% MH concentration	10	11	10	10.3333333	
Gentamicin	23	23.5	23	23.1666667	
Aquabidest	0	0	0	0	

[a] Kruskal -Wallis
MH: Honey Forest

Table 4 shows the diameter of the inhibition zone formed upon administration honey forest with concentration of 100%, with an average inhibitory zone diameter of 10,334 mm in bacteria *Pseudomonas aeruginosa.* Diameter of the inhibition zone appears on the disc gentamicin with an average of 23.1667 mm.

Table 5. Diameter of inhibition zone on administration honey cattle without *Royal Jelly* on bacteria *Methicillin-sensitive Staphylococcus aureus* (MSSA)

Treatment	Inhibition Zone Diameter Repetition (mm)			Average (mm)	p value
	I	II	III		
Concentration 25% MTRJ	0	0	0	0	<0.001 [a]
50% MTRJ concentration	0	0	0	0	
Concentration 75% MTRJ	0	0	0	0	
100% MTRJ concentration	0	0	0	0	
Gentamicin	25	25	24.5	24.8333333	
Aquabidest	0	0	0	0	

[a] Kruskal -Wallis
MTRJ: Honey Cattle No Royal Jelly

Table 5 shows no diameter of the inhibition zone formed upon administration honey forest in bacteria MSSA . Inhibition zone diameter appears on the disc gentamicin with an average of 24.8334 mm.

Table 6. Diameter of inhibition zone on administration honey cattle without *Royal Jelly* on bacteria *Methicillin-resistant Staphylococcus aureus* (MRSA)

Treatment	Inhibition Zone Diameter Repetition (mm)			Average (mm)	p value
	I	II	III		
Concentration 25% MTRJ	0	0	0	0	<0.001 [a]
50% MTRJ concentration	0	0	0	0	
Concentration 75% MTRJ	0	0	0	0	
100% MTRJ concentration	0	0	0	0	
Gentamicin	23.75	24	23.5	23.75	
Aquabidest	0	0	0	0	

[a] Kruskal -Wallis
MTRJ: Honey Cattle No Royal Jelly

Table 6 shows no diameter of the inhibition zone formed upon administration honey forest in bacteria MRSA . Inhibition zone diameter appears on the disc gentamicin with an average of 23.5 mm.

Table 7 Diameter of inhibition zone on administration honey cattle without *Royal Jelly* on bacteria *Acinetobacter baumannii*

Treatment	Inhibition Zone Diameter Repetition (mm)			Average (mm)	p value
	I	II	III		
Concentration 25% MTRJ	0	0	0	0	<0.001 [a]
50% MTRJ concentration	0	0	0	0	
Concentration 75% MTRJ	0	0	0	0	
100% MTRJ concentration	0	0	0	0	
Gentamicin	20	20	19	19.6666667	
Aquabidest	0	0	0	0	

[a] Kruskal -Wallis

MTRJ: Honey Cattle No Royal Jelly

Table 7. shows no diameter of the inhibition zone formed upon administration honey forest in bacteria *Acenitobacter baumannii* . Inhibition zone diameter appears on the disc gentamicin with an average of 19,667 mm.

Table 8. Diameter of inhibition zone on administration honey cattle without *Royal Jelly* on bacteria *Pseudomonas aeruginosa*

Treatment	Inhibition Zone Diameter Repetition (mm)			Average (mm)	p value
	I	II	III		
Concentration 25% MTRJ	0	0	0	0	<0.001 [a]
50% MTRJ concentration	0	0	0	0	
Concentration 75% MTRJ	0	0	0	0	
100% MTRJ concentration	12	11	11	11.3333333	
Gentamicin	23	23.75	23.5	23.4166667	
Aquabidest	0	0	0	0	

[a] Kruskal -Wallis

MTRJ: Honey Cattle No Royal Jelly

Table 8 shows the diameter of the inhibition zone formed upon administration honey forest with concentration of 100%, with an average inhibitory zone diameter of 11,334 mm in bacteria *Pseudomonas aeruginosa.* Inhibition zone diameter paste on the disc gentamicin with an average of 23,416 mm.

Table 9. Diameter of the inhibition zone when administering propolis to bacteria *Methicillin-sensitive Staphylococcus aureus* (MSSA)

Treatment	Inhibition Zone Diameter Repetition (mm)			Average (mm)	p value
	I	II	III		
Concentration 25% P	0	0	0	0	<0.001 [a]
50% P concentration	0	0	0	0	
Concentration 75% P	0	0	0	0	
100% P concentration	0	0	0	0	
Gentamicin	25	25	24.5	24.8333333	
Aquabidest	0	0	0	0	

[a] Kruskal -Wallis

Q: Propolis

Table 9. shows no diameter of the inhibition zone formed when administering propolis. Inhibition zone diameter paste on the disc gentamicin with an average of 24,833 mm.

Table 10. Diameter of the inhibition zone when administering propolis to bacteria *Methicillin-resistant Staphylococcus aureus* (MRSA)

Treatment	Inhibition Zone Diameter Repetition (mm)			Average (mm)	p value
	I	II	III		
Concentration 25% P	0	0	0	0	<0.001 [a]
50% P concentration	0	0	0	0	
Concentration 75% P	0	0	0	0	
100% P concentration	0	0	0	0	
Gentamicin	23.75	24	23.5	23.75	
Aquabidest	0	0	0	0	

[a] Kruskal -Wallis

Q: Propolis

Table 10. shows no diameter of the inhibition zone formed when administering propolis with MRSA. Inhibition zone diameter paste on the disc gentamicin with an average of 23.75 mm.

Table 11. Diameter of the inhibition zone when administering propolis to bacteria *Acinetobacter baumannii*

Treatment	Inhibition Zone Diameter Repetition (mm)			Average (mm)	p value
	I	II	III		
Concentration 25% P	0	0	0	0	<0.001 [a]
50% P concentration	0	0	0	0	
Concentration 75% P	0	0	0	0	
100% P concentration	0	0	0	0	
Gentamicin	20	20	19	19.6666667	
Aquabidest	0	0	0	0	

[a] Kruskal -Wallis
Q: Propolis

Table 11 shows no diameter of the inhibition zone formed when administering propolis with *Acenitobacter baumannii* . Inhibition zone diameter paste on the disc gentamicin with an average of 19,667 mm.

Table 12. Diameter of the inhibition zone when administering propolis to bacteria *Pseudomonas aeruginosa*

Treatment	Inhibition Zone Diameter Repetition (mm)			Average (mm)	p value
	I	II	III		
Concentration 25% P	0	0	0	0	<0.001 [a]
50% P concentration	0	0	0	0	
Concentration 75% P	0	0	0	0	
100% P concentration	12	11	11	11.3333333	
Gentamicin	23	23.75	23.5	23.4166667	
Aquabidest	0	0	0	0	

[a] Kruskal -Wallis
Q: Propolis

Table 12 shows the diameter of the inhibition zone formed when administering 10% concentration of propolis with *Pseudomonas aeruginosa* with an inhibition zone diameter of 11,337 mm and. diameter of the inhibition zone paste on the disc gentamicin with an average of 23.41667 mm.

4. Discussion

Propolis is produced of the genus Leptospermum, shows activity unique antibacterial because its not depending on the content hydrogen peroxide. This activity its antibacterial not influenced by activity enzyme catalase in network wound. Activity antibacterial mediated by methylgloxal, a the compound formed through

conversion spontaneous from compound precursor , that is dihydroxyacetone. Methylgloxal is molecule sized water soluble and this is what becomes reason why this is effective oppose bacteria in biofilms. On wounds chronic, colonization generally occurs on the surface wound, forming a protective biofilm bacteria from system immunity host body. Bacteria in biofilms is also likely resistant to antimicrobial good given _ in a way topical nor systemic.(Gunawan, 2017)

Research result This Enough contrast Because No found effect antibacterial from honey without royal jelly or honey forest However found effect antibacterial from propolis. Ani *et al* 's research in 2018 showed that All propolis samples show effect antibacterial moderate to Gram- positive microorganisms with MIC range between 0.08 mg/mL to 2.5 mg/ mL. Besides that , extract Propolis ethanol shows activity moderate to Gram negative bacteria with an MIC between 0.6 mg/mL to 5 mg/ mL. Besides that , extract Propolis ethanol shows activity antifungal moderate (MIC value between 0.6-2.5 mg/mL). Results obtained from kinetic tests time killing and dilution tests plaid combination two drug between extract propolis ethanol and antibiotics like vancomycin , oxacillin , and levofloxacin show synergistic interaction especially to pathogen resistant microbes to drugs , including MRSA and *Vancomycin-resistant enterococci* . (Ani *et al* , 2018)

Study Milah et al in 2016 aims determine influence concentration antibacterial propolis against growth S. pyogenes bacteria in vitro and determine mark *Minimum Inhibitory Concentration* (MIC). Propolis is diluted so that obtained concentrations of 100%, 50%, 25%, and 12.5%. Antibacterial test in research This use method diffusion with four repetitions. Research result show Propolis can be given 100%, 50%, 25% and 12.5% forming the average diameter of the inhibition zone consecutive namely 19.76 mm, 10.9 mm, 5.97 mm and 3.3 mm. Conclusion from study This is In vitro propolis concentration has an effect to growth S. pyogenes bacteria . The more tall propolis concentration then the more strong Power resistor the bacteria . Propolis has characteristic antibacterial Because contain flavonoid compounds that work with bother permeability cell bacteria . Concentration minimum inhibition of propolis for Streptococcus pyogenes bacteria is 12.5%. Influence concentration This No so seen in research This . (Milah *et al* , 2016)

This study find effect antibacterial MTRJ and MH against *Pseudomonas aeruginosa* . Study from Kaligis et al in 2020 showed that second honey forest and honey black show characteristic antibacterial . MIC in honey forest against S. aureus, E. coli, and P. aeruginosa were 12.5%, 12.5%, and 25% (v/v). MIC in honey black against S. aureus, E. coli, and P. aeruginosa were 25%, 12.5%, and 25% (v/v). (Kaligis *et al* , 2020). Weakness from study This is design study *in vitro* . Study clinical trials in animals required For evaluate more Far efficacy from MTRJ, MH and propolis especially the effect when simultaneously with physiological organ systems body . Study This is the most basic research However important For determine effectiveness from substance in hinder growth bacteria

5. Conclusion

100% concentration of MRTJ and MH has effect antibacterial against Pseudomonas aeruginosa whereas concentrations of 50%, 75% and 100% have effect antibacterial against Pseudomonas aeruginosa.

References

Nurazmi , A., Rijai , L., & Rahmawati , D. (2016). POTENTIAL OF WILD AND FARM BEES HONEY AS A MEDICINE FOR IN VIVO BURNS. In *Proceedings of the 3rd National Pharmacy Seminar* (pp. 9–14). Faculty Mulawarman University Pharmacy .

Oryan , A., Alemzadeh , E., & Moshiri, A. (2016). Biological properties and therapeutic activities of honey in wound healing: A narrative review and meta-analysis. *Journal of Tissue Viability* , *25* (2), 98–118. https://doi.org/10.1016/j.jtv.2015.12.002

Prasetyo , AT, & Herihadi , E. (2013). The Application of Moist Exposed Burn Ointment (MEBO) and Binahong Leaves in Treating Partial Thickness Burn : A Case Report. *Journal Plastic Reconstruction* .

Pelczar , MJ and Chan, ECS, 2005, " Fundamentals Microbiology 1", Transfer languages : Hadioetomo , RS, Imas , T., Tjitrosomo , SS and Numbers, SL, UI Press, Jakarta.

Proceedings of the American Burn Association 49th Annual Meeting. (2017). *Journal of Burn Care & Research* , *38* , S1–S49. https://doi.org/10.1097/BCR.0000000000000510

Release of the National Health Survey: Summary of Results, Australia 2001. (2002). *New South Wales Public Health Bulletin* , *13* (12), 241. https://doi.org/10.1071/NB02091

Renda , A. (2020). *Effectiveness Gargle Solution Honey Forest Against Gingivitis Scores in Muhammadiyah 2 Gamping Middle School Students* . Yogyakarta Ministry of Health Health Polytechnic .

Rio. (2012). Comparison Effect Antibacterial Original Sikabu Honey with Honey Lubuk Minturun against Escherichia Coli and Staphylococcus Aureus In Vitro. *Andalas Health Journal* , *1* (2).

Rüttermann , M., Maier- Hasselmann , A., Nink -Grebe, B., & Burckhardt, M. (2013). Local Treatment of Chronic Wounds. In Patients With Peripheral Vascular Disease, Chronic Venous Insufficiency, and Diabetes. *Deutsch Arzteblatt International* . https://doi.org/10.3238/arztebl.2013.0025

Saqa , M. (2010). *Treatment with honey* . Al- Kautsar Library .

Shupp , J.W., Nasabzadeh , T.J., Rosenthal, D.S., Jordan, M.H., Fidler, P., & Jeng , J.C. (2010). A Review of the Local Pathophysiologic Bases of Burn Wound Progression. *Journal of Burn Care & Research* , *31* (6), 849–873. https://doi.org/10.1097/BCR.0b013e3181f93571

Singh, V., Devgan, L., Bhat, S., & Milner, S. M. (2007). The Pathogenesis of Burn Wound Conversion. *Annals of Plastic Surgery* , *59* (1), 109–115. https://doi.org/10.1097/01.sap.0000252065.90759.e6

Smolle , C., Cambiaso -Daniel, J., Forbes, A.A., Wurzer , P., Hundeshagen , G., Branski , L.K., Huss, F., & Kamolz , L.-P. (2017). Recent trends in burn epidemiology worldwide: A systematic review. *Burns* , *43* (2), 249–257. https://doi.org/10.1016/j.burns.2016.08.013

Sulistyorini , CA (2006). *Inventory plant feed bee honey apis cerana on the plantation tea Mount Mas Bogor* . IPB.

Sundoro , A., Nadia, K., Nur, A., Sudjatmiko , G., & Tedjo , A. (2012). Comparison of Physical–Chemical Characteristics and Antibacterial Effect Between Manuka Honey and Local Honey. *Journal Plastic Reconstruction* , 341–347.

Suranto , A. (2007). *Therapy honey* . Spreader Plus.

EMSB. (2016). *Emergency Management of Severe Burns (EMSB) by The Australian and New Zealand Burn Association* (18th ed.). ANZBA.

Weinstein, R. A., & Mayhall , C. G. (2003). The Epidemiology of Burn Wound Infections: Then and Now. *Clinical Infectious Diseases* , *37* (4), 543–550. https://doi.org/10.1086/376993

Wesson, HKH, Bachani , A.M., Mtambeka , P., Schulman, D., Mavengere , C., Stevens, K.A., Millar, A.J.W., Hyder , A.A., & van As, A.B. (2013). Pediatric burn injuries in South Africa: A 15-year analysis of hospital data. *Injury* , *44* (11), 1477–1482. https://doi.org/10.1016/j.injury.2012.12.017

Williams, C. (2011). Assessment and management of pediatric burn injuries. *Nursing Standards* , *25* (25), 60–68. https://doi.org/10.7748/ns2011.02.25.25.60.c8349

Williams, FN, Herndon, DN, Hawkins, HK, Lee, JO, Cox, RA, Kulp , GA, Finnerty, CC, Chinkes , DL, & Jeschke , MG (2009). The leading causes of death after burn injury in a single pediatric burn center . *Critical Care* , *13* (6), R183. https://doi.org/10.1186/cc8170

Zachariah, J.R., Rao, A.L., Prabha, R., Gupta, A.K., Paul, M.K., & Lamba, S. (2012). Post burn pruritus—A review of current treatment options. *Burns* , *38* (5), 621–629. https://doi.org/10.1016/j.burns.2011.12.003

Zarrilli , R., Crispino , M., Bagattini , M., Barretta, E., Di Popolo , A., Triassi , M., & Villari , P. (2004). Molecular Epidemiology of Sequential Outbreaks of *Acinetobacter baumannii* in an Intensive Care Unit Shows the Emergence of Carbapenem Resistance. *Journal of Clinical Microbiology* , *42* (3), 946–953. https://doi.org/10.1128/JCM.42.3.946-953.2004