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EFFICACY OF HONEY AGAINST CLINICAL ISOLATES OF STAPHYLOCOCCUS AUREUS AND PSEUDOMONAS AERUGINOSA

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ABSTRACT
Skin serves as the first line of defense of the body against invading microorganisms. When it becomes compromised due to a cut or a burn, infections may set into the wound. Honey produced by bees can serve as an alternative treatment to available antibiotics to which microorganisms are already becoming resistant to. This study was undertaken to evaluate the efficacy of honey sold in Samaru, Zaria against bacterial wound isolates. Proximate compositions of two honey samples were determined. Pure isolates of *P. aeruginosa* and *S. aureus* were challenged against a pool of the two honey samples that passed sterility test using agar well diffusion method. The MIC and MBC of the honey were determined using tube dilution method. The honey samples had a mean pH of 4.93, with composition of 76.23% carbohydrate, 0.16% ash, 2.23% lipid and 3.45% protein. The honey demonstrated its highest activity against *P. aeruginosa* (20.0 mm), than *S. aureus* (16.0 mm) at a concentration of 100%v/v. Activity of the honey reduced with reducing concentration, until no activity was recorded at a concentration of 25%v/v. The MIC of the honey was 25%v/v against *S. aureus* and 12.5%v/v against *P. aeruginosa.* However, the MBC of the honey against *S. aureus* and *P. aeruginosa* was 25%v/v each. This study confirms that honey sold in Zaria possess antibacterial activity against wound pathogens.

Keywords: Honey, Efficacy, *Staphylococcus aureus, Pseudomonas aeruginosa,* wounds.

INTRODUCTION

A wound is a breach on the skin that exposes the subcutaneous tissues. Wounds are prone to microbial colonization and proliferation (Bowler *et al.,* 2001). There is a rise of multidrug resistant bacterial species worldwide. Hence, alternative natural sources with antimicrobial potentials like the use of honey are currently receiving great attention (Mansur and Mukhtar, 2023). Honey is a natural sweet liquid substance produced by bees from nectar of flowers (Saranraj and Sivasakthi, 2018). Since ancient times, honey has been used for wound care. It has widely been used in the treatment of acute, chronic, traumatic and post-surgical wounds. It is also used against ulcers, burns, eye diseases, skin diseases, oro-pharygeal problems and necrosis necrotic areas. Hence, honey is a possible alternative to other antibacterial agents with a promising therapeutic potential in medical practice (Almasaudi, 2021).

Honey acts against most types of Gram-positive and Gram-negative bacteria (Mohaptra *et al.,* 2011). Different components of honey contribute to its antibacterial activity. These components include sugar, polyphenolic compounds, hydrogen peroxide, 1,2-dicarbonyl compounds and bee defensin-1; however, their concentrations depend on the source of nectar, type of bee and storage (Almasaudi, 2021).

Staphylococcus aureus (*S. aureus*) is a Gram-positive bacterium, measuring 0.5–0.7 µm in diameter and can occur singly, in pairs or form clusters. They are found on the skin, perianal area, inguinal area and the anterior nares (Severn *et al.,* 2023). Carrier rate of *S. aureus* among healthy adults is about 30% (Tong *et al.,* 2015). Prevalence of 20 to 80% is commonly reported in general population (Liaqat *et al.,* 2015; Sakr *et al.,* 2018). Intravenous drug users, as well as individuals with severe underlying illnesses, previous antimicrobial therapy or hospitalization are more at risk of bacterial infections. Burns can get infected due to contact with environmental surfaces or by airborne route (Tong *et al.*, 2015).

Pseudomonas aeruginosa (*P. aeruginosa*) is a Gram-negative bacterium and is non-fermentative. It has a wide distribution in nature and is capable of surviving in different surfaces and hospital environments (Moghnieh *et al.,* 2018; Rossi *et al.,* 2021). About 10-20% of nosocomial infections that include bacteraemia and sepsis, especially in intensive care units are caused by *P. aeruginosa*. It also causes cystic fibrosis, pneumonia, urinary tract infections, and burns/wounds infections (Percival *et al.*, 2015). Resistance of pathogenic bacteria to available antibiotics has increased (Yang *et al.,* 2020). Resistance to all broad-spectrum antibiotics by *P. aeruginosa* made it to be enlisted on first-priority critical pathogens of the World Health Organization (Petrovic *et al.*, (2024). Multidrug-resistant *P. aeruginosa* develops resistance by various mechanisms that may include use of efflux pumps, biofilm formation, production of β-lactamases and aminoglycoside modifying enzymes (El Zowalaty *et al.,* 2015).

Both *S. aureus* and *P. aeruginosa* are highly prevalent pathogens in chronic wound infections like leg ulcers, burns and necrotising fasciitis (Yung *et al.,* 2021). This study was carried out to assess the antibacterial efficacy of honey against clinical isolates of *S. aureus* and *P. aeruginosa* isolated from wound infections.

MATERIALS AND METHODS

Clinical isolates of *S. aureus* and *P. aeruginosa* from wound infections were obtained from A.B.U Medical Center, Zaria and reconfirmed by cultural and biochemical tests. Cultures of the isolates (after 24 h) were standardized to obtain turbidity that was equivalent to 0.5 McFarland standard. Two different honey samples were obtained from different vendors in Samaru, Zaria. The honey samples were subjected to sterility test by plating aliquots on Nutrient agar at 37^oC for 24h. After passing the sterility test (as no microbial growth was recorded), the honey samples were subjected to proximate analysis. The two samples were pooled together and thoroughly mixed. The test isolates were challenged with various concentrations (at 25%, 50%, 75% and

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100% v/v) of the sterile honey to determine its antibacterial efficacy using agar well diffusion method on sterile Mŭller-Hinton Agar plates. Zones of inhibition were measured to the nearest whole number in millimeter (mm). Ciprofloxacin (5µg) was used as a positive control. The minimum inhibitory concentration (MIC) was determined using tube serial dilution to obtained concentrations of 50%, 25%, 12.5% and 6.25% v/v, and seeded with 0.5mL of standardized inocula. The MIC was determined to be the lowest honey concentration that did not show any evidence of visible turbidity. Aliquots (0.5mL) from all the tubes were plated out on sterile Mŭller-Hinton Agar plates and incubated at 37°C for 24h in order to determine the Minimum bactericidal concentration (MBC) of the honey. The MBC was determined to be the lowest concentration that did not show evidence of bacterial growth (Almasaudi *et al.*, 2017; Mansur and Mukhtar, 2023).

RESULTS

The honey samples had a mean pH of 4.93, with composition of76.23% carbohydrate, 0.16% ash, 2.23% lipid and 3.45% protein (Table 1). The honey at a concentration of 100%v/v demonstrated the highest activity against *P. aeruginosa* with zone of inhibition of 20.0 mm, than *S. aureus* (16.0 mm) (Table 2). The activity of the honey was observed to reduce with reducing concentration, until no activity was recorded at a concentration of 25%v/v. The minimum inhibitory concentration (MIC) of the honey against *S. aureus* was at 25%v/v, while the MIC against *P. aeruginosa* was at 12.5%v/v (Table 3). The minimum bactericidal concentration (MBC) of the honey against *S. aureus* and *P. aeruginosa* was at 25%v/v each (Table 4).

Table 1: Proximate Composition of Honey Samples obtained in Samaru, Zaria

Honey Sample	% Moisture	% Ash	% Lipid	% Protein	% Carbohydrate	pH
A	18.21	0.15	2.21	3.45	75.98	4.93
B	17.66	0.16	2.25	3.45	76.48	4.70
Average	17.94	0.16	2.23	3.45	76.23	4.82

Table 2: Antibacterial Activity of Honey against *S. aureus* and *P. aeruginosa*

Organisms	Zone of Inhibition (mm)				
	25%v/v	50%v/v	75%v/v	100%v/v	Ciprofloxacin (5µg)
S. aureus	NA	8.0	14.0	16.0	26
P. aeruginosa	NA	13.0	17.0	20.0	24

Key: NA = No activity

Table 3: Minimum Inhibitory Concentration (MIC) of Honey against Clinical Isolates

Concentration of honey (%v/v)	*S. aureus* Growth pattern	*P. aeruginosa* Growth pattern
50	-	-
25	-*	-
12.5	+	-*
6.25	+	+
NC	-	-
PC	+	+

Keys: + = Turbidity, - = No turbidity, * = MIC,
NC = Negative control (honey + sterile distilled water 0.5mL each)
PC = Positive control (inoculum + sterile distilled water 0.5mL each)

Table 4: Minimum Bactericidal Concentration (MBC) of Honey against the Clinical Isolates

Concentration of honey (%v/v)	*S. aureus* Growth pattern	*P. aeruginosa* Growth pattern
50	-	-
25	-*	-*
12.5	+	+
6.25	+	+
NC	-	-
PC	+	+

Keys: + = Growth; - = No Growth; * = MBC

DISCUSSION

Honey is characteristically acidic with pH between 3.2- 4.5, which is low enough to be inhibitory to several bacterial pathogens (Olaitan *et al.,* 2007). However, the honey samples obtained from Samaru, Zaria for this study had a slightly higher pH and moisture content than those reported by Almasaudi *et al.* (2017). Honey obtained from Samaru in Zaria demonstrated antibacterial activity against *S. aureus* and *P. aeruginosa*, but its efficacy against *P. aeruginosa* was higher than against *S. aureus*. This might be due to emergence of resistance by *S. aureus* to antibiotics. Findings from this study agrees with the report of Abd-El *et al.* (2007), who showed that honey has a greater inhibitory effects on Gram-negative bacteria (*P. aeruginosa, Enterobacter spp*. and *Klebsiella*) when compared to Gram positive bacteria. More than 70 species of microorganisms have been found to be susceptible to honey (Bowler (2001). Our findings of mean zone of inhibition of 13 to 20 mm across the various honey concentrations are comparable to reported 10.6 to 22.6 mm by Mansur and Mukhtar (2023). Though this study did not record any antibacterial activity of the honey at 25% v/v against the clinical isolates, its highest activity at 100% was similarly reported by Mudenda *et al.* (2023).

Some types of honey are more potent than others, but all of them contain the same antibacterial substance (hydrogen peroxide, (H2O2), except for Manuka honey (Romário-Silva et al., 2022). However, there are demonstrable efficacy of honey against P. aeruginosa (Yang et al., 2020) and S. aureus (Cremers et al., 2020). Bioactive compounds in honey exhibit variable levels of antibacterial effects that depend on the bacterial species (Petrovic et al., (2024).

Present study shows that the two isolates were all susceptible to the honey. It was observed that the zone of inhibition against the isolates increased as the concentration of the honey was increased. This might be due to increase in concentration of inhibitory substances present in the honey. The honey required a higher minimum inhibitory concentration against *P. aeruginosa* than *S. aureus*, but its bactericidal concentration against the two bacteria was the same. Efficacy of different types of honey against *S. aureus* is dependent on the type of honey and the concentration used (Almasaudi *et al.*, 2017). Honey has a complex composition, where the individual components act either individually or in synergistic manner to prevent antibiotic resistance. Hence, no

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antibacterial resistance to honey that has been reported yet (Cooper *et al.* 2010; Almasaudi *et al.*, 2017).

Conclusion

Staphylococcus aureus and *Pseudomonas aeruginosa* were identified from wound infection and challenged with various concentration of honey. Finding of this study indicated that the honey was more active against *S. aureus* (16.0 mm) and *P. aeruginosa* (20.0 mm) at a concentration of 100%v/v). The MIC of the honey was 25%v/v against *S. aureus* and 12.5%v/v *against P. aeruginosa.* The MBC against the two wound isolates was 25%v/v for each.

REFERENCES

Abd-El, A.M., El-Hadidy, N.B., El-Mashad, N.B. and El-Sebaie, A.H. (2007). Antimicrobial effect of bee honey in comparison to antibiotics on organisms isolated from infected burns. *Journal of Euro-Mediterranean Council for Burns and Fire Disasters*, **20**(2):83-88.

Almasaudi, S. (2021). The antibacterial activities of honey. *Saudi Journal of Biological Sciences*, **28**(2021):2188-2196. https://doi.org/10.1016/j.sjbs.2020.10.017

Almasaudi, S.B., Al-Nahari, A.A.M., Abd El-Ghany, S.M., Barbour, E., Al Muhayawi, S.M., Al-Jaouni, S., Azhar, E., Qari M., Qari, Y.A. and Harakeh, S. (2017). Antimicrobial effect of different types of honey on *Staphylococcus aureus*. *Saudi Journal of Biological Sciences*, **24**(2017):1155-1261. http://dx.doi.org/10.1016/j.sjbs.2016.08.007

Bowler, P.G., Duerdan, B.I. and Amstrong, D.G. (2001). Wound Microbiology and Associated Apparatus to Ward Management*. Journal of Clinical Microbiology Reviewed,* **14**:244-269.

Cooper, R.A., Jenkins, L., Henriques, A.F., Duggan, R.S. and Burton, N. F. (2010). Absence of bacterial resistance to medical-grade manuka honey. *European Journal of Clinical Microbiology and Infectious Diseases*, **29**(10):1237–1241. http://dx.doi.org/10.1007/s10096-010-0992-1.

Cremers, N., Belas, A., Santos Costa, S., Couto, I., de Rooster, H. and Pomba, C. (2020). *In vitro* antimicrobial efficacy of two medical grade honey formulations against common high-risk Meticillin-resistant Staphylococci and *Pseudomonas* Spp. pathogens. *Veterinary. Dermatology,* **31**(2):90-96.

Liaqat, F., Sheikh, A.A., Nazir, J., Hussain, T., Rabbani, M., Shaheen, A.Y., and Muhammad, J. (2015). Isolation identification and control of vancomycin resistant *Staphylococcus aureus*. *Pakistan Journal of Pharmaceutical Sciences*, **28**(3):997-1004.

Mansur, S. and Mukhtar, G.L. (2023). Evaluation of the medicinal potentials of honey against *Pseudomonas aeruginosa* isolated from various forms of wound infections. *UMYU Journal of Microbiology Research*, **8**(1):61-65. https://doi.org/10.47430/ujmr.2381.007

Moghnieh, R.A., Kanafani, Z.A., Tabaja, H.Z., Sharara, S.L., Awad, L.S. and Kanj, S.S. (2018). Epidemiology of common resistant bacterial pathogens in the countries of the Arab League. *The lancet Infectious Diseases*, **18**(12):e379-e394.

Mohaptra, D., Thakur, V. and Brar, S. (2011). Antimicrobial efficacy of raw and process honey. *Biotechnology Research International Journal,* **4**:6.

Mudenda, S., Hikaambo, C.N., Chabalenge, B., Mfune, R.L., Mufwambi, W., Ngazimbi, M., Matafwali S. and Daka V. (2023). Antibacterial activities of honey against *Escherichia coli* and *Staphylococcus aureus*: A potential treatment for bacterial infections and alternative to antibiotics. *Journal of Pharmacognosy and Phytochemistry*, **12**(2):6-13. https://doi.org/10.22271/phyto.2023.v12.i3a.14655

Olaitan, P.B., Adeleke, O.E. and Ola, I.O. (2007). Honey: a reservoir for microorganism and an inhibitory agent for microbes. *Journal of African Health Sciences*, **7**(3):159-165.

Percival, S. L., Suleman, L., Vuotto, C. and Donelli, G. (2015). Healthcare-associated infections, medical devices and biofilms: risk, tolerance and control. *Journal of Medical Microbiology*, **64**(4):323-334.

Petrovic, N., Lucic, M., Radovanovic, B. and Martic, N. (2024). Comparing antipseudomonal effects of honey samples with different geographical and botanical origin. *Global Pediatrics*, **7**(2024):100149. https://doi.org/10.1016/j.gpeds.2024.100149

Romário-Silva, D., Alencar, S.M., Bueno-Silva, B., Sardi, J.d.C.O., Franchin, M., Carvalho, R.D.P.d., Ferreira, T.E.d.S.A. and Rosalen, P.L. (2022). Antimicrobial activity of honey against oral microorganisms: current reality, methodological challenges and solutions. *Microorganisms,* **10**:2325. https://doi.org/10.3390/microorganisms10122325

Rossi, E., La Rosa, R., Bartell, J. A., Marvig, R. L., Haagensen, J. A., Sommer, L. M., ... & Johansen, H. K. (2021). *Pseudomonas aeruginosa* adaptation and evolution in patients with cystic fibrosis. *Nature Reviews Microbiology*, **19**(5):331-342.

Sakr, A., Brégeon, F., Mège, J.L., Rolain, J.M. and Blin, O. (2018). *Staphylococcus aureus* nasal colonization: an update on mechanisms, epidemiology, risk factors, and subsequent infections. *Frontiers in Microbiology*, **9**:2419.

Saranraj, P. and Sivasakthi, S. (2018). Comprehensive review on honey: biochemical and medicinal properties. *Journal of Academia and Industrial Research*, **6** (10):165–181.

Severn, M. M. and Horswill, A. R. (2023). *Staphylococcus epidermidis* and its dual lifestyle in skin health and infection. *Nature Reviews Microbiology*, **21**(2):97-111.

Tong, S.Y., Davis, J.S., Eichenberger, E., Holland, T.L. and Fowler Jr, V.G. (2015). *Staphylococcus aureus* infections: epidemiology, pathophysiology, clinical manifestations, and management. *Clinical Microbiology Reviews*, **28**(3):603-661.

Yang, C., Mavelli, G.V., Nacharaju, P., Li, K.; Cleare, L.G., Nosanchuk, J.D., Friedman, J.M. and Abuzeid, W.M. (2020). Novel nitric oxide– generating platform using Manuka honey as an anti biofilm strategy in chronic rhinosinusitis. *International Forum Allergy Rhinology*, **10**(2): 223–232. https://doi.org/10.1002/alr.22472

Yang, X., Lai, Y., Li, C., Yang, J., Jia, M. and Sheng, J. (2020). Molecular epidemiology of *Pseudomonas aeruginosa* isolated from lower respiratory tract of ICU patients. *Brazilian Journal of Biology*, **81**:351-360.

Yung, D.B.Y., Sircombe, K.J. and Pletzer, D. (2021). Friends or enemies? The complicated relationship between *Pseudomonas aeruginosa* and *Staphylococcus aureus*. *Molecular Microbiology*, **116**(1):1-15.