UJMR, Vol. 8 No. 1, June, 2023, pp. 61 - 65 *E-ISSN: 2814 – 1822; P-ISSN: 2616 – 0668*

https://doi.org/10.47430/ujmr.2381.007

Received: June 10, 2023 **Accepted:** June 20, 2023

Evaluation of the medicinal potentials of honey against *Pseudomonas aeruginosa* isolated from various forms of wound infections

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Abstract
The rise of multidrug resistant microbial species is a major public health problem worldwide. Complementary natural sources with antimicrobial potentials including plants and plant-based products such as honey, are currently receiving great attention. As such, this study aimed to evaluate the medicinal potentials of honey by testing its antibacterial efficacy against Pseudomonas aeruginosa isolates associated with wounds infection. Clinical isolates of Pseudomonas aeruginosa from infected wounds such as sepsis, bite, surgical, laceration and gunshots were obtained from Federal Teaching Hospital Katsina. The antibacterial activity of honey was assayed by Agar well diffusion technique using different concentrations of honey; 25%, 50%, 75%, and 100% (v/v). Minimum Inhibitory Concentration and Minimum Bactericidal Concentration of honey were also determined. The mean zone of inhibition of honey against the isolates range from 10.6 mm to 22.6 mm. Moreover, the inhibitory effects of honey at 100% (v/v) differ significantly compared to other concentrations. The Minimum Inhibitory Concentration of honey was determined to be 25% (v/v) while the Minimum Bactericidal Concentration was estimated to be 75% (v/v). Pseudomonas aeruginosa isolate from gunshot wound was more susceptible to honey and ciprofloxacin (control) compared to Pseudomonas aeruginosa from other wound types. This study's findings demonstrated honey's efficacy in treating wound infections. Natural products, such as honey, have enormous potential for combating antibiotic resistance. In vivo studies should be carried out to further study the impact of honey on biological systems.
Keywords: Antibacterial activity, Honey, Wounds infection, Pseudomonas aeruginosa

INTRODUCTION

There are more than 38 million chronic wound patients globally due to inadequacies in the wound-healing process, which has an enormous financial impact on healthcare systems (Olsson *et al.*, 2019). This might be the outcome of indiscriminate antibiotic usage, which promotes the establishment of multidrug-resistant bacterial strains and leading to high rate of morbidity and mortality in patients (Martinotti and Ranzatu, 2018).

Many antimicrobial treatments are turning ineffective in wound therapy due to the rise of multiple drug-resistant bacteria, making most wounds infections difficult to treat. As a result, alternative treatments such as the use of honey as a wound healing agent have been sought. Honey is both safe and cost-effective in the treatment of several types of wounds (Tashkandi, 2021). Because it is a natural substance, it rarely presents adverse effects when compared to the chemically synthesized drugs.

Honey has long been known to have antibacterial and wound-healing properties. The healing property of honey is a result of its antibacterial effect, ability to keep a wound moist, and its high viscosity which aids in the formation of a protective barrier that prevents infection. Furthermore, its mechanism of action might be linked to its low pH and high sugar content (hyperosmolarity), both of which are sufficient to limit microbial growth (Mandal and Mandal, 2011).

According to studies, honey may serve as an anti-inflammatory, shorten the time it takes for wounds to heal, deodorize them, and promote in-vitro cell multiplication and expansion (Proano *et al.*, 2021).

It has demonstrated broad spectrum of inhibitory activity against several Gram-positive and Gram-negative, aerobic and anaerobic microorganism (Halawani and Shohayeb, 2011). In addition to its broad spectrum of antibacterial action against wound-infecting germs, honey has been proven to be effective against antibiotic-resistant bacteria and to restore the potency of some medications against bacteria with previously acquired resistance. (Combarros-Fuertes *et al.*, 2020). Vallianou *et al.* (2014) described that use of honey enhance the healing of infected wounds that are resistant to standard therapy (such as antibiotics and antiseptics), it can improve healing of wounds infected with Methicillin resistance *Staphlycoccoccus aureus* or multidrug-resistant *Pseudomonas aeruginosa*. Antibacterial resistance has become a problem worldwide. Multidrug-resistant bacteria are becoming more prevalent, which emphasizes the necessity for developing alternative or additional antimicrobial methods (Combarros-Fuertes *et al.*, 2020). Hence, this study aimed to identify honey's antibacterial action against *Pseudomonas aeruginosa* isolates linked to wounds infection, providing a path for wound treatment.

MATERIALS AND METHODS

Ethical clearance

Ethical approval for the study was obtained from the Katsina State Ministry of Health Research and Ethical Review Committee (HREC-MOH/ADM/SUB/1152/I/701).

Collection of *Pseudomonas aeruginosa* isolates

The clinical isolates of *Pseudomonas aeruginosa* from infected wounds such as sepsis, bite, surgical, laceration and gunshots were obtained from the Microbiology laboratory of Federal Teaching Hospital Katsina, Katsina State.

Reconfirmation of the bacterial isolates

The isolates were re-confirmed at Department of Microbiology Laboratory of Umaru Musa Yar'adua University, Katsina. The isolates were inoculated into nutrients broth for 18 hours and then culture on plates of Nutrient agar and incubated for 24 hours. Standard microbiological procedures comprising of cultural and morphological identification of the isolates, Gram staining and a battery of biochemical test (as catalase, coagulase, citrate utilization, acid production from carbohydrates, Methyl Red, urease, motility, oxidase, and indole tests) were used to identify and confirm the test isolates (Wang, 2012 and Maleki *et al.*, 2013).

Collection and preparation of honey sample

Determination of the Antibacterial activity of honey against the test isolates

A suspension of the bacterial isolate in nutrient broth was prepared and compared to match the turbidity of 0.5 McFarland standards. The antibacterial activity of honey was tested u sing the agar-well diffusion method on Mueller-Hinton agar plates. A bacterial lawn was made on the plates and a sterile cork-borer was used to create wells, and various honey co ncentrations (25, 50, 75, and 100% v/v) were pl aced in each well. Ciprofloxacin (5µg) was used as the control in this in-vitro experiment.

The plates were incubated for 24 hours at 37 °C . After the incubation period, zones of inhibition were measured in millimeter (Ali *et al.*, 2017).

Determination of Minimum Inhibitory Concentration (MIC) of honey

The MIC of the honey was determined using nutrient broth. The following honey concentrations were prepared serially; 100 v/v, 75 v/v, 50v/v and 25 v/v. A test tube containing only broth serves as negative control. The test tubes were filled with 0.5 mL of the standardized of the test bacterial isolates, and they were then incubated at 37 °C for 24 hours. After incubation, the test tubes were checked for turbidity (Abouzekry *et al.*, 2020).

Determination of Minimum Bactericidal Concentration (MBC) of honey

From the tubes that were not turbid from the MIC above, 0.1 mL was aseptically transferred on to the surface of Mueller-Hinton agar plates and incubated for 24 hours, at 37°C. After the incubation period, the plates were observed for growth. MBC was defined as the least concentration at which growth on Mueller-Hinton agar plates did not occurred (Abouzekry *et al.*, 2020).

Data Analysis

Data were taken in triplicate and mean zone of inhibition and standard deviations were calculated.

RESULTS

Result of cultural, microscopic and biochemical characterization of the isolates confirmed the presence of *Pseudomonas aeruginosa* isolates from the various wound samples as present in table 1.

Table 2 present the antibacterial activity of honey against the *Pseudomonas aeruginosa* isolates. It shows the mean zones of inhibition which indicates the antibacterial activity of honey against *Pseudomonas aeruginosa*

associated with wounds. The mean zone of inhibition varies with concentration, at 25% (v/v), the mean zone of inhibitions ranged from 10.6 - 13.0mm while at 50% (v/v), the mean zone of inhibitions ranged from 12.0 - 16.0mm. At 75% (v/v), the mean zone of inhibitions ranged from 16.0 - 19.0mm and at 100% (v/v). While for the control (Ciprofloxacin), the mean zone of inhibitions range 19.0 - 26.0mm

Table 3 present the Minimum Inhibitory Concentration of the honey against the isolates. The MIC of honey against isolates P1, P2, and P4 was 25% while the MIC against isolates P3 and P5 was 75%.

Table 4 present the result of Minimum Bactericidal Concentration of honey against the isolates. The MBC of honey for isolates P2 and P4 was 75% while that of isolates P1, P3, and P5 was 100%.

Table 1: Reconfirmation of *Pseudomonas aeruginosa* isolates collected from Federal Teaching Hospital, Katsina

			Biochemical characterizations									
Isolates	**Cultural characteristics**	**Gram staining & microscopic**	**Cat**	**Coa**	**Cit**	**AP**	**Ure**	**MR**	**Mot**	**Oxi**	**Ind**	**Inference**
P1	Greenish fluorescent colonies with slimy surface	Gram -ve Rod shaped	+	-	+	-	-	-	+	+	-	*Pseudomonas aeruginosa*
P2	Greenish fluorescent colonies with slimy surface	Gram -ve Rod shaped	+	-	+	-	-	-	+	+	-	*Pseudomonas aeruginosa*
P3	Greenish fluorescent colonies with slimy surface	Gram -ve Rod shaped	+	-	+	-	-	-	+	+	-	*Pseudomonas aeruginosa*
P4	Greenish fluorescent colonies with slimy surface	Gram -ve Rod shaped	+	-	+	-	-	-	+	+	-	*Pseudomonas aeruginosa*
P5	Greenish fluorescent colonies with slimy surface	Gram -ve Rod shaped	+	-	+	-	-	-	+	+	-	*Pseudomonas aeruginosa*

Keys: P1 to P5 *Pseudomonas aeruginosa* isolates from sepsis, bite, laceration, surgical and gunshot wounds respectively.

+ = Positive - = Negative

Cat = Catalase Coa = Coagulase Cit = Citrate

AP = Acid production Ure = Urease MR = Methyl red

Mot = Motility Oxi = Oxidase Ind = Indole

Table 2: Antibacterial activity of honey against *Pseudomonas aeruginosa* isolates recovered from different types of wounds infection

Isolates codes	**Concentrations of honey (%v/v)**				**Control (Ciprofloxacin)**
	25%	**50%**	**75%**	**100%**	
	Mean zone of inhibition(mm) / Standard deviation (mm)				
P1	12.6 ± 2.52	14.0 ± 4.00	16.0 ± 4.00	19.0 ± 2.00	25
P2	12.0 ± 3.00	15.6 ± 1.53	18.0 ± 2.00	21.0 ±3.00	23
P3	10.6 ± 1.53	12.0 ± 2.00	16.0 ± 2.00	17.6 ± 0.53	19
P4	11.0 ± 3.00	15.0 ± 4.00	18.0 ± 4.00	21.6 ± 3.51	21
P5	13.0 ± 6.00	16.0 ± 3.00	19.0 ± 1.53	22.6 ± 2.52	26

Keys:

P1= *Pseudomonas aeruginosa* isolates from sepsis wound, P2= *Pseudomonas aeruginosa* isolates from bite wound, P3= *Pseudomonas aeruginosa* isolates from laceration wound, P4= *Pseudomonas aeruginosa* isolates from surgical wound and P5= *Pseudomonas aeruginosa* isolates from gunshot wound

Table 3: Minimum Inhibitory Concentration of honey against *Pseudomonas aeruginosa* isolates from wounds infection

Isolates	Concentrations of honey (v/v)			
	25%	50%	75%	100%
P1	-**	-	-	-
P2	-**	-	-	-
P3	+	+	-**	-
P4	-**	-	-	-
P5	+	+	-**	-

Keys:
+ = bacterial growth; - = no bacterial growth -**= MIC

Table 4: Minimum Bactericidal Concentration of honey against *Pseudomonas aeruginosa* isolates from wounds infection

Isolates	Concentrations of honey (v/v)			
	25%	50%	75%	100%
P1	+	+	+	-**
P2	+	+	-**	-
P3	+	+	-**	-
P4	+	+	-**	-
P5	+	+	+	-**

Keys:
+ = bacterial growth; - = no bacterial growth; -**= MBC

DISCUSSION

This study presents the antibacterial efficacy of honey against isolates of *P. aeruginosa* from wounds infection. The test organisms were susceptible to honey as shown by the varying degree of zone of inhibition as well as the MIC and MBC. The findings of this investigation agreed with those of Shenoy, *et al.* (2012) who discovered that honey inhibited the growth of *Pseudomonas sp.* and with Bucekova *et al.* (2019), who revealed that honey exhibited good antibacterial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

According to the findings, the zone of inhibition increased with increasing honey concentration, implying that a rise in honey concentration increases the antibacterial efficacy of the honey. It can be seen that zone of inhibitions produced by honey at 100% (v/v) concentration differ significantly (P> 0.05) compared to the other concentrations in this study. This implies that, the antibacterial efficacy of honey is better achieved when it is undiluted.

Interestingly, the susceptibility to honey and ciprofloxacin exhibited by the *Pseudomonas aeruginosa* isolates from the different wound types differ. For instance, lower zones of inhibition (10.6 ± 1.53 - 17.6 ± 0.53 mm) were observed in *Pseudomonas aeruginosa* isolate from laceration wound and the same for the control (ciprofloxacin) implying it is more resistant compared to the *Pseudomonas aeruginosa* isolate from other wound samples, on the other side, there was higher zones of inhibition (13.0 ± 6.00 - 22.6 ± 2.52 mm) observed against *Pseudomonas aeruginosa* isolate from gunshot wound and the same observation in the control. Implying that, *Pseudomonas aeruginosa* isolate from gunshot wound is more susceptible to honey and the ciprofloxacin compared to *Pseudomonas aeruginosa* from other wound types.

The Minimum Inhibitory Concentration of honey was observed to be 25% (v/v) while the Minimum Bactericidal Concentration was 75% (v/v). This indicates that honey has bacteriostatic and bactericidal activity against the *P. aeruginosa* isolates. However, this is in contrast to the findings of Shenoy *et al.* (2012), in a study on honey of Indian origin, reported 20% as the Minimum Inhibitory Concentration which inhibits the growth of *P. aeruginosa* isolates. Shenoy *et al.* (2012) further describes that, honeys from different countries and regions may have wide variations in their antimicrobial activity. Our findings are consistent with previous study that found honey to have antibacterial activity at concentrations ranging from less than 3% to 50% and even higher concentrations (Wilkinson and Cavanagh, 2005).

The reason for the observed antibacterial effect of honey against the test bacteria might be attributed to honey's bioactive compounds. According to Tashkandi (2021), the natural characteristics of honey, as well as its active ingredients, are critical for wound healing. Natural honey contains around 82% water,

including carbohydrates, proteins, hytochemicals, antioxidants, and minerals.

CONCLUSION

Findings from the study revealed that honey sample from central market Katsina was found to have antibacterial activity against *Pseudomonas aeruginosa* isolates associated with wounds infection collected from Federal Teaching Hospital Katsina. This research demonstrated honey as a potent alternative to antibiotics or complementary therapy for treating various forms of infected wounds.

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