Online ISSN: 2588-3526

Journal of Wildlife and Biodiversity

Volume 9(3): 391-403 (2025) (http://www.wildlife-biodiversity.com/)

Research Article

Preliminary analysis of antibacterial activity of honey produced by various *Apis* species from Punjab, Pakistan

Memoona Yasmeen[1], Shehar Bano[1], Ghulam Muhayyaodin[1], Sidra Kanwal[2], Sana Amjad[1], Hassan Ashfaq[3], Hira Rafiq[1], Mudasar Hussain[1], Rufsha Sanaullah Cheema[1]

[1]Department of Wildlife and Ecology, University of Veterinary and Animal Sciences, Lahore, Pakistan

[2]Department of Zoology, Wildlife and Fisheries, University of Agriculture, Faisalabad Sub Campus Burewala, Pakistan

[3]Institute of Continuing Education & Extension, University of Veterinary and Animal Sciences, Lahore, Pakistan

*Email: memoona.yasmeen662@gmail.com

Received: 17 June 2025 / Revised: 19 September 2025 / Accepted: 30 September 2025/ Published online: 18 October 2025.

How to cite: Yasmeen, M., Bano, S.H., et al. (2025). Preliminary analysis of antibacterial activity of honey produced by various Apis species from Punjab, Pakistan, Journal of Wildlife and Biodiversity, 9(3), 391-403. DOI: https://doi.org/10.5281/zenodo.17386231

Abstract

Honey has been used as a natural remedy for centuries, mainly due to its antibacterial properties. The present study explored the antibacterial activity of honey from three different species of honeybees, including *Apis dorsata*, *Apis florea*, and *Apis mellifera* from Punjab, Pakistan. The honey samples were tested against common harmful bacteria such as *Staphylococcus aureus, Pseudomonas aeruginosa,* and *Escherichia coli.* Honey from *Apis dorsata* showed the strongest antibacterial effects, particularly against *E. coli*, with an inhibition zone of 39 mm at 100% concentration. *Apis florea* honey also exhibited strong inhibition, particularly against *P. aeruginosa*, with a maximum zone of 38 mm at 100% concentration. In contrast, *Apis mellifera* honey showed the weakest activity, with the highest inhibition zone of 18.8 mm against *S. aureus* at 100%. The study found that honey's antibacterial power increased with higher concentrations. At 50% concentration, honey from *Apis dorsata* inhibited *E. coli* by 17.5 mm, and *Apis florea* showed a 25 mm inhibition zone for *S. aureus*. The results suggest that honey from *Apis dorsata* and *Apis florea* could be a promising natural alternative for fighting bacterial infections, particularly those caused by antibiotic-resistant strains. Further research should focus on identifying active compounds like phenolics and flavonoids in these honeys, which may contribute to their antibacterial benefits.

Keywords: Antibacterial activity, *Pseudomonas aeruginosa*; *Staphylococcus aureus*, *Escherichia coli*

Introduction

Honey is economically significant throughout the world because of its nutritional properties. It is composed of important minor components like enzymes, free amino acids, minerals, and vitamins, organic and volatile compounds (Silva et al., 2013). Honey is used in food, cosmetics and pharmaceutical industries due to several nutrients that are very useful for humans (Sjamsiah et al., 2018). Honey consumed by the United States and China is about 153,000 and 123,000 tons per annum, respectively (Batt & Liu, 2012). The physicochemical parameters of honey play a significant role in order to determining its quality. Honey contains sugars like glucose and fructose which are energy suppliers as fructose/glucose and glucose/water ratios are considered the main elements in the quality of the honey (El Sohaimy et al., 2015). Honey is basically obtained from various bee species mainly by stingless bees belonging to the genus *Meliponin* and stinging bees of the genus *Apis* (Rao et al., 2016). About eight species of honeybees exist in the world i.e. *Apis mellifera*, *A. dorsata*, *A. florea*, *A. koschevnikovi*, *A. andreniformis*, *A. nuluensis*, *A. cerana* and *A. nigrocincta* (Powell et al., 2014). Four species of genus *Apis* exist in Pakistan including three endemic species i.e. *A. florea*, *A. dorsata* and *A. cerana* and one exotic species *A. mellifera* (Khan et al., 2020; Sajid et al., 2020; Shakeel et al., 2020). From *Apis* spp. *A. mellifera* is the extensively studied species and well known because of its medicinal properties (Machado et al., 2018). Honey also contains bioactive compounds that are obtained from plants and bees. Therefore, these compounds are associated with antimicrobial resistance having the ability to prevent and inhibit the growth of pathogens (Libonatti et al., 2014). The resistance of pathogenic microorganisms by antimycotics and antibiotics are being replaced by using plants and honey as therapeutic source (Mcloone et al., 2015). Honey is being used for medicinal purposes since primitive ages with zero bacterial resistance. After discovering its antibacterial properties in the year 1892 it was grown significantly for medicinal purposes (Kuropatnicki et al., 2018). During foraging activities honeybees visit different plants which lead to antimicrobial activities, biological as well as physical-chemical properties (Balkanska et al., 2020). The nectar which is the most important raw material used in the production of the honey is obtained by honeybee foraging activities. Nectar is produced by the particular tissues known as nectaries which are present in different parts of plants like leaves and flowers (Gotelli et al., 2016). Several wounds are involved to support the colonization of polymicrobial communities because several bacterial species have been identified from the wounds (Lasa & Solano, 2018). Despite the type of the wound *Staphylococcus aureus* is the most commonly isolated bacteria (Bessa et al., 2015). Similarly, some other commonly found bacteria are *Pseudomonas aeruginosa,* methicillin-resistant

Staphylococcus aureus (MRSA), *Escherichia coli*, Enterococci, *Klebsiella pneumoniae*, *Streptococcus* spp., *Proteus* spp., and *Acinetobacter* spp. (Rai et al., 2017). It has been examined that wounds of different types have influenced on different pathogens such as *P. aeruginosa* and MRSA are commonly found pathogens that are linked with burn infection (Dawra et al., 2017). Antibiotics are usually used to treat the wound despite knowing its etiology which might be treated orally or in the form of topical preparation in order to decrease the infection of the wound. Several wound pathogens have been resistant to commercial antibiotics as resistance of the wound pathogens towards antibacterial agents has been investigated (Mohammed et al., 2017). It has been examined that not all kind of honey exhibit same level of antibacterial activity because several extrinsic and intrinsic factors are involved such as site of honey collection, sources of the flower, phytochemicals, osmotic effect and level of hydrogen peroxide matters (Elbanna et al., 2014; Matzen et al., 2018). Several pathogenic bacteria of animals and plants exist in water. Similarly, various pathogens can be transmitted from animal feces towards plants because of insects, fertilizers and through the irrigation water. Pathogenic bacteria of plants like *Pseudomonas syringae* and *Erwinia amylovora* can be transmitted through the honeybees during pollination (Pattemore et al., 2014). This study was planned to analyze antimicrobial properties of honey from various Apis species.

Material and methods

Sample collection

Honey samples were obtained from natural hives from the species of *Apis* in Punjab, Pakistan. The collection was performed according to the occurrence of peak blooming with the aim to include as wide spectrum of pollen sources as possible, that probably gives the honey the diversity of antimicrobial properties. The honey was extracted using sterile equipment in an effort to reduce contamination and ensure purity. Both honey samples were directly obtained from the honeycomb of the active hives. Samples were collected in aseptic conditions to avoid exposure to external contaminants, which may potentially affect sample quality. The samples of honey were then transferred to the Postgraduate Lab Department of Wildlife and Ecology, UVAS, Ravi Campus, for further processing. During transportation, the samples were kept in insulated containers to prevent temperature fluctuations that could alter the honey's properties. The samples were stored at room temperature in a dark place to further preserve honey properties before they were used in the experiments. The physical characteristics, including color and moisture content, were analyzed following the official methods outlined by the AOAC International (Horwitz & Latimer, 2000).

Microorganism testing

Three pathogenic bacterial species were selected for testing the antibacterial activity of the honey samples. These species included *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli*, which were procured from the Department of Zoology, University of the Punjab, Lahore. These bacterial strains were chosen for their relevance in both clinical and environmental contexts.

Determination of Minimum Inhibitory Concentration (MIC)

The antibacterial activity of the honey samples was assessed using the agar dilution method (Cooper et al., 2002). Stock solutions of honey were prepared by dissolving the honey in sterile deionized water. A series of concentrations (25%, 50%, 75%, and 100% v/v) was prepared from the stock solution. To prepare the agar plates, an adequate volume of deionized water and 10 mL of each honey stock solution were added to 10 mL of either tryptic soy agar (for *E. coli* and *S. aureus*) or nutrient agar (for *P. aeruginosa*) that had been preheated to 50°C. The mixtures were then transferred to sterile petri dishes and thoroughly mixed to ensure an even distribution of the honey concentration. The plates were allowed to cool and dry for 15 minutes at 37°C before use.

Inoculation and incubation

The bacterial cultures were prepared by inoculating *P. aeruginosa* in nutrient broth and both *E. coli* and *S. aureus* in tryptic soy broth. All cultures were incubated at 37 °C for 24 hours to achieve optimal growth. Following incubation, bacterial suspensions were adjusted to a concentration of 1×10^8 CFU/mL. For each honey concentration, 0.5 µL of the bacterial culture was applied onto the surface of the agar plates containing the honey solution, using a sterile micropipette. The inoculated plates were then incubated at 37°C for 24 hours to allow bacterial growth. The antibacterial activity was evaluated by measuring the zones of inhibition (if any) around the honey spots. All trials were conducted in triplicate for each honey concentration to ensure the reproducibility and reliability of the results.

Statistical analysis

Data from the antibacterial assays were analyzed using one-way analysis of variance (ANOVA) to determine significant differences between the antibacterial activity of honey samples from different Apis species. Post-hoc comparisons were performed using Tukey's test. All statistical analyses were conducted using SPSS version 25.0 (IBM, USA), and a p-value of <0.05 was considered statistically significant.

Results

A total of 30 honey samples were collected, with 10 samples from each Apis species from selected sites in Punjab, Pakistan. The samples were sourced from three different Apis species, including *Apis dorsata*, *Apis florea,* and *Apis mellifera*. These species forage on a variety of local floral sources to ensure diversity in the nectar composition. These samples were carefully harvested from active hives during the peak blooming season and immediately stored in sterile plastic containers to maintain their purity. The honey samples were first diluted with sterile deionized water to prepare stock solutions at concentrations of 25%, 50%, 75%, and 100% (v/v). The dilution was done under sterile conditions to avoid microbial contamination, and the samples were filtered using a sterile 0.22 µm membrane filter to remove any solid particles or impurities. The diluted honey samples were then kept in sterile containers at room temperature in dark place until further use in antibacterial testing**.**

Physical characteristics of honey samples

The honey samples collected from various Apis species showed notable differences in their physical characteristics. Table 1 summarizes the results for the color and moisture content of each honey sample.

Apis mellifera honey was dark amber in color, while *Apis florea* honey appeared light amber, and *Apis dorsata* honey was amber in color. The color variation was attributed to the floral source from which the nectar was collected. Honey color is often influenced by the type of nectar and environmental factors such as climate and altitude.

Moisture content is an important factor affecting honey's stability and preservation. In this study, the moisture content of honey from *Apis florea* was 18.5%, which was slightly lower than that of *Apis dorsata* (19.3%) and *Apis mellifera* (20.2%). These variations in moisture content are consistent with findings from previous studies that show different species of honeybees produce honey with distinct moisture levels, which in turn affects its shelf life and antibacterial properties.

Table 1. Physical characteristics of honey samples collected from various *Apis* species.

Apis Species	**Color**	**Moisture Content (%)**
Apis dorsata	Amber	19.3
Apis florea	Light amber	18.5
Apis mellifera	Dark amber	20.2

Antibacterial activity of honey

The antibacterial activity of honey was tested against three pathogenic bacteria: *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. The effectiveness of each honey sample varied significantly based on both the *Apis* species and the type of bacteria. The data on the antibacterial activity are presented in Table 2.

***Escherichia coli* inhibition**

Honey from *Apis dorsata* exhibited the strongest antibacterial effect against *E. coli* (19.62 ± 1.02 mm), followed by *Apis cerana* (18.50 ± 5.44 mm). However, honey from *Apis mellifera* showed significantly lower inhibition (8.12 ± 6.32 mm). The difference in activity could be attributed to the inherent chemical composition of the honey, such as the presence of phenolic compounds, which are known to exhibit antimicrobial properties.

***Staphylococcus aureus* inhibition**

Honey from *Apis florea* was the most effective against *S. aureus*, with an inhibition zone of 23.75 ± 8.53 mm. This was significantly higher than the inhibition observed for *Apis mellifera* (11.82 ± 5.81 mm) and *Apis dorsata* (12.25 ± 1.15 mm). The greater inhibitory effect of *Apis florea* could be linked to specific phytochemicals present in the nectar of plants visited by this species.

***Pseudomonas aeruginosa* inhibition**

Honey from *Apis florea* also showed the highest antibacterial activity against *P. aeruginosa*, with an inhibition zone of 26.50 ± 9.46 mm. Honey from *Apis dorsata* (14.75 ± 1.59 mm) and *Apis mellifera* (7.92 ± 6.86 mm) exhibited lower inhibition. This suggests that *Apis florea* honey may have higher levels of bioactive compounds that are particularly effective against Gram-negative bacteria like *P. aeruginosa*. The data analysis revealed that honey from *Apis florea* was generally the most effective against both Gram-positive (*S. aureus*) and Gram-negative (*P. aeruginosa*) bacteria. Honey from *Apis dorsata* was particularly effective against *E. coli*, while honey from *Apis mellifera* exhibited the least antibacterial activity across all bacteria tested.

Table 2. Antibacterial effect of honey from various *Apis* species against selected pathogenic bacteria. Means with different superscripts in a row are statistically different at $p<0.05$.

Bacterial Species	***Apis Species***		
	A. dorsata	*A. florea*	*A. mellifera*
Escherichia coli	19.62± 1.02^{a}	18.50±5.44^{a}	8.12±6.32^{a}
Staphylococcus aureus	12.25± 1.15^{a}	23.75±8.53^{a}	11.82±5.81^{a}
Pseudomonas aeruginosa	14.75± 1.59^{ab}	26.50± 9.46^{a}	7.92±6.86^{b}

The honey of *A. dorsata* showing the mean zone of inhibition against three bacterial species is presented in Figure 1. The findings revealed the highest inhibition of *E. coli* at the concentration of 100% and showing the zone of inhibition of 39 mm. All the bacterial species were inhibited at a concentration of 25% of honey. At the 50% concentration of the honey, the inhibition zone of the *E. coli* was recorded at 17.5 mm, *P. aeruginosa* was recorded at 8mm, while *S. aureus* showed no inhibition. Similarly, at the concentration of 75% of the honey inhibition zones of the *E. coli*, *P. aeruginosa,* and *S. aureus* were 22, 17, and 24 mm, respectively. Growth inhibition order for the concerned bacterial species at 100% concentration of honey was as follows: *E. coli* > *P. aeruginosa* > *S. aureus*. The maximum inhibitory zone was recorded for *E. coli* (39 mm). *P. aeruginosa* showed a 34 mm inhibition zone, while in *S. aureus* 25 mm inhibition zone was recorded.

The honey samples of *A. florea* showing the zone of inhibition against three bacterial species are presented in Fig. 2. The results depicted that at a concentration of 25% of honey, *P. aeruginosa* had a maximum inhibition of 25 mm, while *S. aureus* and *E. coli* showed zones of inhibition of 20 and 17 mm. At the 50% concentration of the honey the inhibition zone of the *S. aureus* was recorded at 25 mm, *E. coli* at 20 mm and *P. aeruginosa* was zone of inhibition was 15 mm. Similarly, at the concentration of 75% of the honey inhibition zone of the *P. aeruginosa*, *S. aureus* and *E. coli* were 28, 15 and 12 mm, respectively. While on the 100% concentration of the honey, the overall order of growth inhibition for the concerned bacterial species was recorded as: *P. aeruginosa* > *S. aureus* >*E. coli*. The maximum inhibitory zone was recorded for *P. aeruginosa* (38 mm). S. aureus showed a 35 mm inhibition zone, while in *E. coli* 25 mm inhibition zone was recorded.

The honey samples of *A. mellifera* showed the zone of inhibition against three bacterial species (Fig. 3). The findings of this study showed that at a concentration of 25% of honey mean zone of inhibition against *S. aureus* was 5 mm, while the growth of *E. coli* and *P. aeruginosa* was not inhibited. At the 50% concentration of the honey, the inhibition zone of the *S. aureus* was recorded at 10 mm, *E. coli* at 7 mm, and *P. aeruginosa* at 5 mm, respectively. Similarly, at the concentration of 75% of the inhibition zone of *S. aureus* was recorded at 13.5 mm, *P. aeruginosa* at 11 mm, and *E. coli* was recorded 10 mm, respectively. While on the 100% concentration of the honey, the overall order of growth inhibition for the concerned bacterial species was recorded as: *S. aureus* > *P. aeruginosa* > *E. coli*. The maximum inhibitory zone was recorded for *S. aureus* (18.8 mm). *P. aeruginosa* showed a 15.7 mm inhibition zone, while in *E. coli* 15 mm inhibition zone was recorded.

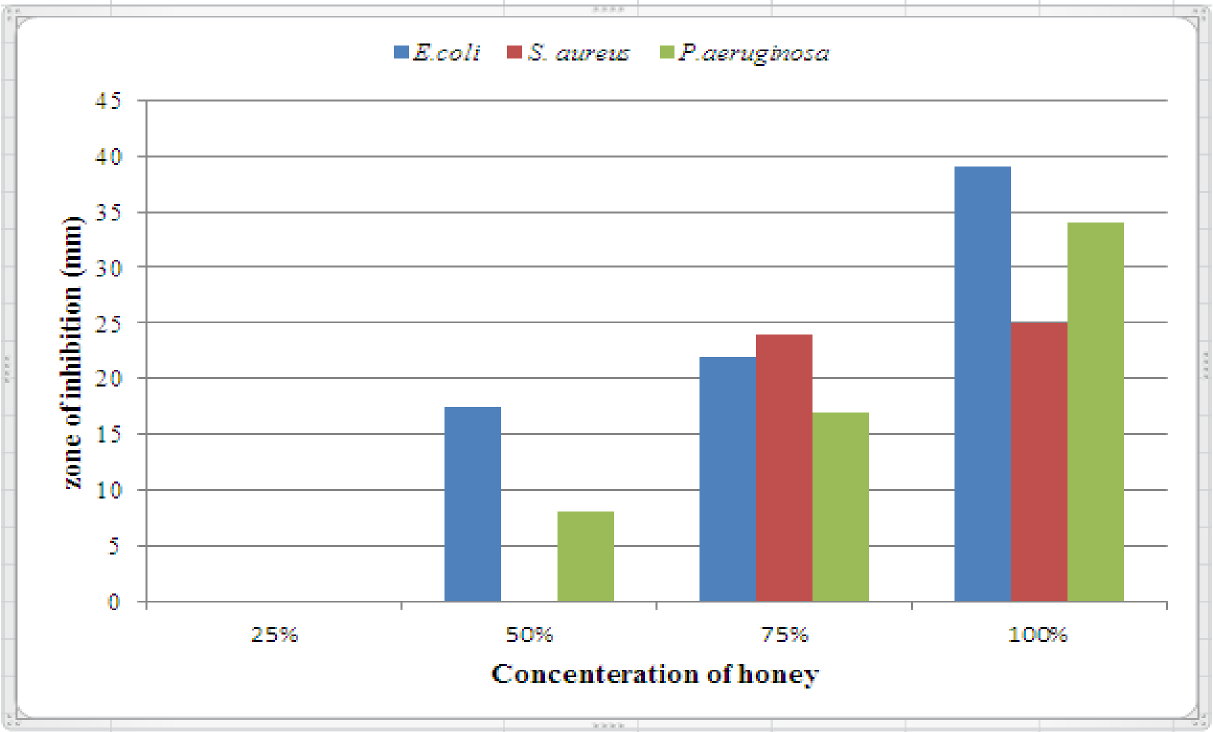

Figure 1. Antibacterial activity of *A. dorsata* honey at different concentrations against selected pathogenic bacteria.

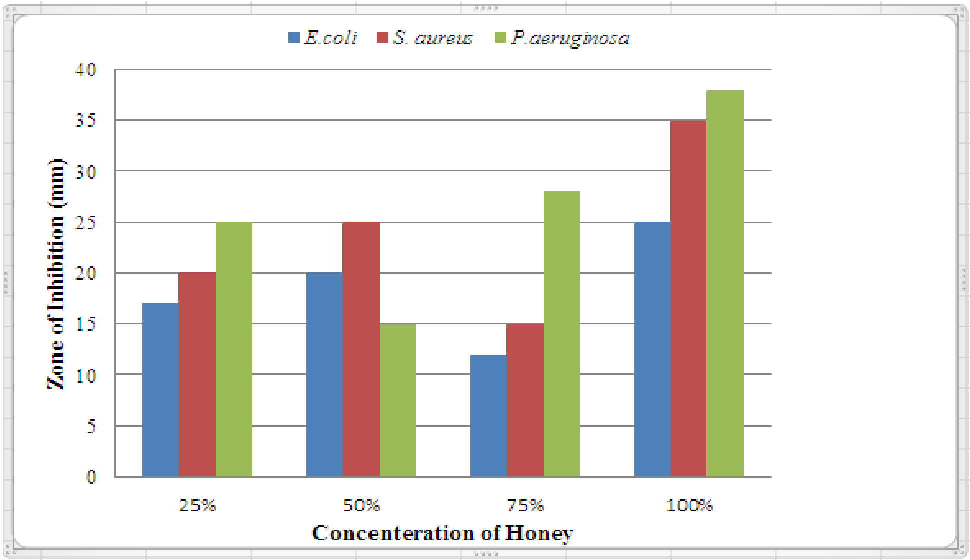

Figure 2. Antibacterial activity of *A. florea* honey at different concentrations against selected pathogenic bacteria.

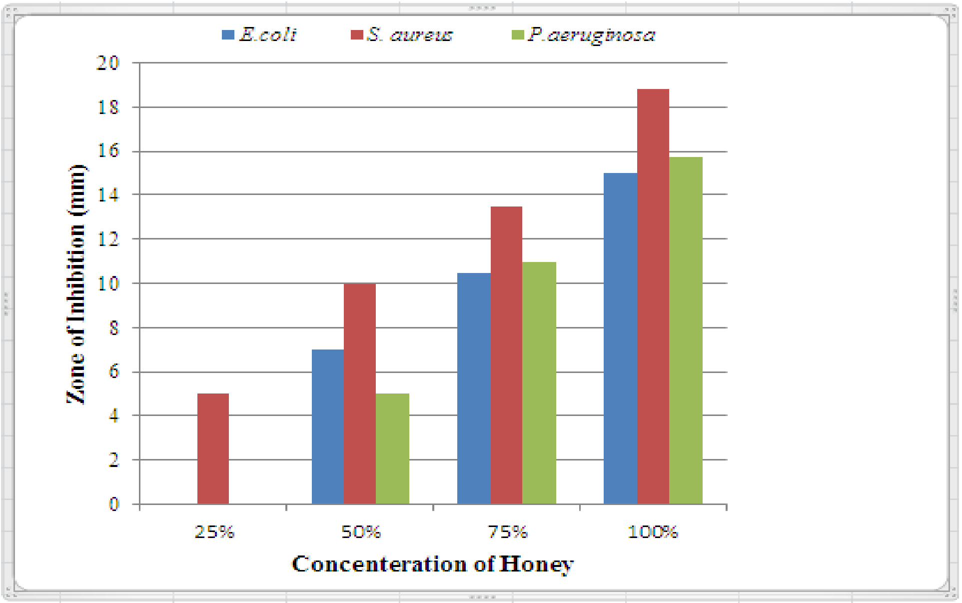

Figure 3. Antibacterial activity of *A. mellifera* honey at different concentrations against selected pathogenic bacteria.

Discussion

The current study provided an evaluation of the antibacterial properties of honey collected from three different species of *Apis,* namely *Apis dorsata*, *Apis florea,* and *Apis mellifera*. The results showed variable antibacterial effect among the three kinds of honey towards the selected pathogenic bacteria, such as *Staphylococcus aureus*, *Pseudomonas aeruginosa,* and *Escherichia coli*, with increasing effectiveness at higher concentrations.

The honey samples from *A. dorsata* showed the highest positive antibacterial efficacy; their inhibition zone against *E. coli* reached 39 mm at the maximum concentration (100%). This finding is consistent with the previous studies of Mundo et al. (2004) and Khalil et al. (2014, who reported that *A. dorsata* honey had greater antibacterial activity as compared to other honey bee species. The phenolic and flavonoid contents are generally higher in *A. dorsata* honey, which explains the darkness of the honey colour and the higher antibacterial efficacy (Tuksitha et al., 2018). It is supported by our physico-chemical analysis and observation of the intermediate amber color of *A. dorsata* honey and significantly higher moisture content land Moreover, beehive products obtained from *A. florea* exerted significantly higher antibiotic activity specifically for *P. aeruginosa* together with *S. aureus.*

Interestingly, even at 25% an inhibition could be measured, which points to a strong antibacterial composition. This is consistent with the results shown by Kulkarni et al. (2014) and Chauhan et al., (2010), who claimed that *A. florea* honey has considerable antibacterial activity and that the medicinal potential of honey is based on bioactive plant-derived compounds that exist in nectar during foraging. Our data also supports this notion, where *A. florea* honey exhibited the apparent inhibition zone (38 mm) against *P. aeruginosa*, a Gram-negative, antibiotic-resistant bacterium. On the other hand, *A. mellifera* honey showed relatively milder antibacterial effects with the lowest inhibition zones at each concentration, i.e., at 25% and 50% none. Although *A. mellifera* is the primary honeybee species in beekeeping and its honey is sold commercially around the world, it has been shown in multiple studies to possess lower antibacterial activity than many wild or less domesticated species.

El Sohaimy et al. (2015) and Machado et al. (2018) additionally reported wide variabilities in *A. mellifera* honey bioactivity that could be attributed to floral sources and geographical localities, possibilities which may further account for the lowered effectiveness seen in this study. The results are also consistent with previous reports from Cooper et al. (2002) and Irish et al. (2006). According to them, it is usually noticed that Gram-positive bacteria, such as *S. aureus*, are more sensitive than Gram-negative bacteria like *E. coli* and *P. aeruginosa* to the antimicrobial action of honey. But in the current study, *E. coli* was more susceptible to *A. dorsata* honey, indicating that honey composition appears to be a greater determinant of susceptibility than species classification alone. This corroborates the notion introduced by Pimentel et al. (2013) provides evidence that the antibacterial activity of honey is dependent on various factors, such as its enzymatic component (e.g., glucose oxidase), acidity, hydrogen peroxide content, and floral sources. The dose-dependent antibacterial activity of the honey types retained in the current investigation has broad literature support.

Khalil et al. (2014) showed that the osmotic effect is increased at high concentrations of honey, and the higher the dilution of honey, the lower the inhibition of bacterial metabolism and availability of antimicrobial constituents. The results of our study follow this trend, as inhibition zones increased steadily with higher concentrations, and 100% honey showed the highest antibacterial effect for all three *Apis* species. The first example mentioned earlier is actually seasonal and within the kind of flowers bees forage from. The nectar is influenced by seasonal changes, affecting the phytochemical profile of the honey (Matzen et al., 2018). The higher potency of *A. dorsata* and *A. florea* honeys in this study may be associated with their nectar foraging on a wide variety of wild flora in the Punjab region during the flowering season. Overall, this study emphasizes the natural potential of honey as an alternative to conventional antibiotics, especially the honey from *A. dorsata* and *A. florea*, against common multidrug-resistant bacterial infections like the one caused by *P. aeruginosa*. These results are in line with worldwide exploration of antibiotics complementary therapy against bacterial resistance (Al-Waili et al., 2012; Lu et al., 2014).

Conclusion

The current experiment investigated the antibacterial activity of honey of three different species of *Apis* genus: *Apis mellifera*, *Apis dorsata,* and *Apis florea*. Results indicated that *honey from Apis dorsata and Apis florea differed more in antibacterial activity,* with stronger potency against *Pseudomonas aeruginosa* and *Staphylococcus aureus*. *Apis mellifera* honey exhibited comparatively lower antibacterial activity than other species. Honey exhibited its antibacterial potency in a dose-dependent manner, wherein the potency increased with increasing concentrations. These concentration-dependent antibacterial effects of honey are crucial in attaining the desired antibacterial effects. These results confirm that honey, particularly from specific Apis species, holds considerable potential as a natural antimicrobial agent, especially for combating multidrug-resistant pathogens.

The antibacterial potential demonstrated in the current study is promising, and future work should focus on identifying and quantifying the active components (i.e., phenolic compounds and flavonoids responsible for the activity) in honey. Knowledge of these compounds will aid in maximising the medicinal potential of honey. Additional clinical trials in humans, especially in wound healing and infection management, are also needed to evaluate the activity of honey, especially that of local *Apis florea* and *Apis dorsata* origins, against clinical pathogens and explore its therapeutic potential in the management of multidrug-resistant infections. Moreover, the production and medicinal use of honey, particularly in a healthcare setting, require standardized guidelines for both efficacy and safety measures.

References

Al-Waili, N., Salom, K., Al-Ghamdi, A., & Ansari, M. J. (2012). Antibiotic, pesticide, and microbial contaminants of honey: human health hazards. The scientific world Journal, 2012(1), 930849.

Balkanska, R., Stefanova, K., & Stoikova–Grigorova, R. (2020). Main honey botanical components and techniques for identification: a review. Journal of Apicultural Research, 59(5), 852-861.

Batt, P. J., & Liu, A. (2012). Consumer behaviour towards honey products in Western Australia. British Food Journal, 114(2), 285-297.

Bessa, L. J., Fazii, P., Di Giulio, M., & Cellini, L. (2015). Bacterial isolates from infected wounds and their antibiotic susceptibility pattern: some remarks about wound infection. International wound journal, 12(1), 47-52.

Chauhan, A., Pandey, V., Chacko, K. M., & Khandal, R. K. (2010). Antibacterial activity of raw and processed honey. Electronic journal of biology, 5(3), 58-66.

Cooper, R. A., Molan, P. C., & Harding, K. G. (2002). The sensitivity to honey of Gram-positive cocci of clinical significance isolated from wounds. Journal of Applied microbiology, 93(5), 857-863.

Dawra, R., Sharma, R., Bachhiwal, R., & Vyas, A. (2017). High incidence of multidrug resistant *Pseudomonas aeruginosa* isolated from infected burn wounds in a tertiary hospital. International Journal of Current Microbiology and Applied Sciences, 6(2), 1134-9.

El Sohaimy, S. A., Masry, S. H. D., & Shehata, M. G. (2015). Physicochemical characteristics of honey from different origins. Annals of Agricultural Sciences, 60(2), 279-287.

Elbanna, K., Attalla, K., Elbadry, M., Abdeltawab, A., Gamal-Eldin, H., & Ramadan, M. F. (2014). Impact of floral sources and processing on the antimicrobial activities of different unifloral honeys. Asian Pacific Journal of Tropical Disease, 4(3), 194-200.

Gotelli, M. M., Galati, B. G., & Medan, D. (2016). Morphological and ultrastructural studies of floral nectaries in Rhamnaceaea, b. The Journal of the Torrey Botanical Society, 144(1), 63-73.

Horwitz, W. I. L. L. I. A. M., & Latimer, G. (2000). International A: official methods of analysis of the AOAC international. The Association: Arlington, VA, USA.

Irish, J., Carter, D. A., Shokohi, T., & Blair, S. E. (2006). Honey has an antifungal effect against Candida species. Medical Mycology, 44(3), 289-291.

Khalil, A. T., Khan, I., Ahmad, K., Khan, Y. A., Khan, J., & Shinwari, Z. K. (2014). Antibacterial activity of honey in north-west Pakistan against select human pathogens. Journal of Traditional Chinese Medicine, 34(1), 86-89.

Khan, I., Akhtar, N., & Khan, S. A. (2020). Palynological investigation of some selected species of family Fabaceae from Pakistan, using light and scanning electron microscopy techniques. Microscopy research and technique, 83(3), 223-231.

Kulkarni, S. V., Pawankar, S., & Kulkarni, M. (2014). Antibacterial activity of *Apis florea* honey. International Journal of Research in Biosciences, Agriculture & Technology, 2(2), 263-268.

Kuropatnicki, A. K., Kłósek, M., & Kucharzewski, M. (2018). Honey as medicine: historical perspectives. Journal of Apicultural Research, 57(1), 113-118.

Lasa, I., & Solano, C. (2018). Polymicrobial infections: Do bacteria behave differently depending on their neighbours?. Virulence, 9(1), 895-897.

Libonatti C, Soledad V, Marina B. 2014. Antibacterial activity of honey: A review of honey around the world. Journal of Microbiology and Antimicrobials**.** 6(3): 51-56.

Libonatti, C., Varela, S., & Basualdo, M. (2014). Antibacterial activity of honey: A review of honey around the world. Journal of Microbiology and Antimicrobials, 6(3), 51-56.

Lu, J., Turnbull, L., Burke, C. M., Liu, M., Carter, D. A., Schlothauer, R. C., & Harry, E. J. (2014). Manuka-type honeys can eradicate biofilms produced by *Staphylococcus aureus* strains with different biofilm-forming abilities. Peer J, 2, e326.

Machado De-Melo, A. A., Almeida-Muradian, L. B. D., Sancho, M. T., & Pascual-Maté, A. (2018). Composition and properties of *Apis mellifera* honey: A review. Journal of apicultural research, 57(1), 5-37.

Matzen, R. D., Zinck Leth-Espensen, J., Jansson, T., Nielsen, D. S., Lund, M. N., & Matzen, S. (2018). The antibacterial effect in vitro of honey derived from various Danish flora. Dermatology Research and Practice, 2018(1), 7021713.

McLoone, P., Warnock, M., & Fyfe, L. (2016). Honey: A realistic antimicrobial for disorders of the skin. Journal of Microbiology, Immunology and Infection, 49(2), 161-167.

Mohammed, A., Seid, M. E., Gebrecherkos, T., Tiruneh, M., & Moges, F. (2017). Bacterial isolates and their antimicrobial susceptibility patterns of wound infections among inpatients and outpatients attending the University of Gondar Referral Hospital, Northwest Ethiopia. International journal of microbiology, 2017(1), 8953829.

Mundo, M. A., Padilla-Zakour, O. I., & Worobo, R. W. (2004). Growth inhibition of foodborne pathogens and food spoilage organisms by select raw honeys. International Journal of Food Microbiology, 97(1), 1-8.

Pattemore, D. E., Goodwin, R. M., McBrydie, H. M., Hoyte, S. M., & Vanneste, J. L. (2014). Evidence of the role of honey bees (*Apis mellifera*) as vectors of the bacterial plant pathogen *Pseudomonas syringae*. Australasian Plant Pathology, 43(5), 571-575.

Pimentel, R. B. D. Q., da Costa, C. A., Albuquerque, P. M., & Junior, S. D. (2013). Antimicrobial activity and rutin identification of honey produced by the stingless bee *Melipona compressipes manaosensis* and commercial honey. BMC Complementary and Alternative Medicine, 13, 1-14.

Powell, J. E., Martinson, V. G., Urban-Mead, K., & Moran, N. A. (2014). Routes of acquisition of the gut microbiota of the honey bee *Apis mellifera*. Applied and environmental microbiology, 80(23), 7378-7387.

Rai, S., Yadav, U. N., Pant, N. D., Yakha, J. K., Tripathi, P. P., Poudel, A., & Lekhak, B. (2017). Bacteriological profile and antimicrobial susceptibility patterns of bacteria isolated from pus/wound swab samples from children attending a tertiary care hospital in Kathmandu, Nepal. International Journal of Microbiology, 2017(1), 2529085.

Rao, P. V., Krishnan, K. T., Salleh, N., & Gan, S. H. (2016). Biological and therapeutic effects of honey produced by honey bees and stingless bees: a comparative review. Revista Brasileira de Farmacognosia, 26, 657-664.

Sajid, Z. N., Aziz, M. A., Bodlah, I., Rana, R. M., Ghramh, H. A., & Khan, K. A. (2020). Efficacy assessment of soft and hard acaricides against Varroa destructor mite infesting honey bee (*Apis mellifera*) colonies, through sugar roll method. Saudi Journal of Biological Sciences, 27(1), 53-59.

Shakeel, M., Ahmad, S., Ali, H., Al-Kahtani, S. N., Ghramh, H. A., & Khan, K. A. (2020). Seasonal impact and comparative analysis of hypopharyngeal glands in worker and forager honey bees of two different species: *Apis mellifera* and *A. cerana*. Fresenius Environ. Bull, 29(10), 9024-9030.

Silva, I. A. A., da Silva, T. M. S., Camara, C. A., Queiroz, N., Magnani, M., de Novais, J. S., ... & de Souza, A. G. (2013). Phenolic profile, antioxidant activity and palynological analysis of stingless bee honey from Amazonas, Northern Brazil. Food Chemistry, 141(4), 3552-3558.

Sjamsiah, S., Sikanna, R., & Saleh, A. (2018). Penentuan Sifat Fisikokimia Madu Hutan (*Apis dorsata*) Sulawesi Selatan. Al-Kimia, 6(2), 191-199.

Tuksitha, L., Chen, Y. L. S., Chen, Y. L., Wong, K. Y., & Peng, C. C. (2018). Antioxidant and antibacterial capacity of stingless bee honey from Borneo (Sarawak). Journal of Asia-Pacific Entomology, 21(2), 563-570.