Dijlah Journal of Medical Sciences (DJMS) Vol. 1, No. 3, May, 2025, pp. 24-29
P-ISSN: 3078-3178, E-ISSN: 3078-8625, Paper ID: 04

The Effect of Honeybee Propolis on Pseudomonas aeruginosa Isolated from Patients with Tooth Decay

Milad Hameed[1*], Rana Jalal Shaker[2] and Thabet Mudheher Khalaf[1]
[1]Department of Biology, College of Education for Women, Tikrit University, Tikrit, Iraq
[2]Department of Biology, College of Pure Science, Tikrit University, Tikrit, Iraq

Article Info

Article history:

Received November, 23, 2024
Revised January, 18, 2025
Accepted February, 27, 2025

Keywords:

Pseudomonas Aeruginosa,
Propolis,
Honeybee,
Dental Caries,
Tooth Decay

ABSTRACT

This study aimed to evaluate the effectiveness of propolis in its ethanolic and aqueous extract forms against Pseudomonas aeruginosa, a bacterium associated with tooth decay. Propolis samples were sourced from local beekeepers in Salah al-Din province. The findings demonstrated significant inhibitory effects of both extracts on the growth of the bacterial strains, with the inhibition rates increasing proportionally with the concentration of the extracts. The ethanolic extract exhibited superior antibacterial activity compared to the aqueous extract, as evident in the inhibition zone measurements obtained through the agar diffusion method.

The results highlighted that Pseudomonas aeruginosa, a Gram-negative bacterium, was less sensitive to the extracts compared to Gram-positive bacteria. Among the tested concentrations, 2 ml of the ethanolic extract achieved the highest inhibition against the bacterial isolates. This study underscores the potential of ethanolic propolis extract as a natural antibacterial agent, particularly in combating pathogens involved in dental caries.

Corresponding Author:

* Milad Hameed
Department of Biology, College of Education for Women, Tikrit University, Tikrit, Iraq
milad.hamid23@st.tu.edu.iq

1- INTRODUCTION

Bankova [1] indicated that many plants produce gums and resinous substances at the site of wounds or around buds or new leaves. These substances protect against water intrusion and serve as a defense against bacterial, mold, yeast, fungal, and insect attacks.

Cushnie and Lamb [2] explained that honeybees often collect these substances, adding various secretions to them within the hive, resulting in a compound known as propolis.

Cross et al [3] indicated that tooth decay is a disease caused by bacteria in the mouth that affect the hard tissues of teeth, particularly in specific areas of the tooth. This bacterium results from weak decomposition or demineralization of the hydroxyapatite crystals, which are the primary component of tooth structure. The breakdown of these crystals leads to a disruption in the structural integrity of the tooth tissues.

Deng et al. [4] highlighted that decay affects the main structures of the tooth by producing bacteria that are present in the mouth, creating small, mineral-free cavities in the enamel. These cavities then expand into the dentin and pulp, causing various degrees of pain.

Hetrus and Ion [5] noted that decay initially appears as white spots without pain but rapidly progresses to larger cavities and discoloration, turning brown. When decay reaches the dentin, it causes pain, especially when consuming sugary, thermally stimulating, or acidic foods and drinks.

Koneman *et al* [6] described *Pseudomonas aeruginosa* as a Gram-negative, facultative anaerobic bacterium. It cannot grow in environmental conditions but can ferment lactose. It is non-motile and is naturally found in the gastrointestinal tract in 35% of cases and the throat in 6% of cases. It may also appear temporarily on the skin, with the percentage increasing among hospitalized patients.

2- MATERIAL AND METHOD

٢.1 Preparation of Culture Media

٢.1.1 Blood Agar

The medium was prepared according to the manufacturer's instructions. The medium was sterilized using an autoclave and then allowed to cool to a temperature of 45-50°C. Human blood was added at a concentration of 5%, while stirring slowly. The mixture was then poured into sterile Petri dishes [7].

٢.1.2 Muller-Hinton Agar

The medium was prepared by dissolving 38 g of the agar in 1 liter of distilled water, then sterilized in an autoclave at 121°C and 15 psi for 15 minutes. After sterilization, the medium was allowed to cool to 40-45°C, then poured into plates and stored in the refrigerator until use.

٢.1.3 Nutrient Agar

The medium was prepared by dissolving 28 g of the agar in 1 liter of distilled water, then sterilized in an autoclave at 121°C and 15 psi for 15 minutes. After sterilization, the medium was poured into plates and stored in the refrigerator for use as a growth and preservation medium.

٢.2 Samples Collection

Six samples were collected from areas affected by tooth decay in patients at Tikrit Teaching Hospital in Salah al-Din province. The patients' ages ranged from 22 to 50 years, and both genders were included. The samples were collected between January 16, 2024, and placed directly in sterile tubes containing transport medium, specifically Brain-Heart Infusion Broth. The samples were then immediately transported to the laboratory for cultivation on enrichment and nutrient media. The plates were incubated for 24 hours at 37°C for subsequent diagnostic tests. A month later, four additional samples were collected from the same hospital, using the same method and age range, and similar tests were performed.

٢.4 Cultivation of Clinical Samples

The clinical samples were directly inoculated using the streaking method onto appropriate culture media for the growth of the bacteria under study. These media included MacConkey agar and Blood Agar. The plates were incubated at 37°C for 18-24 hours. The samples were then re-inoculated on fresh plates using the same media on which they initially grew. The isolated and purified colonies were transferred to slant nutrient agar, then incubated at 37°C for an hour before being stored at 4°C for diagnostic tests [8].

3- RESULTS AND DISCUSSION

The current study demonstrated that *Pseudomonas aeruginosa* exhibited sensitivity to the ethanolic extract of propolis, with the results showing that even low concentrations inhibited its growth, as shown in **Fig. 1**. The inhibition occurred at a concentration of 2 ml, which represents the minimum inhibitory concentration (MIC), with an inhibition zone diameter of 8 mm. At 4 ml, the inhibition zone was 12 mm; at 6 ml, it was 8 mm; and at 8 ml and 10 ml, the inhibition zones were both 6 mm. Therefore, the 4 ml concentration is considered bactericidal, as it produced the highest inhibition zone for the Gram-negative bacteria studied. The highest mean type of extract was recorded at 5.6B.

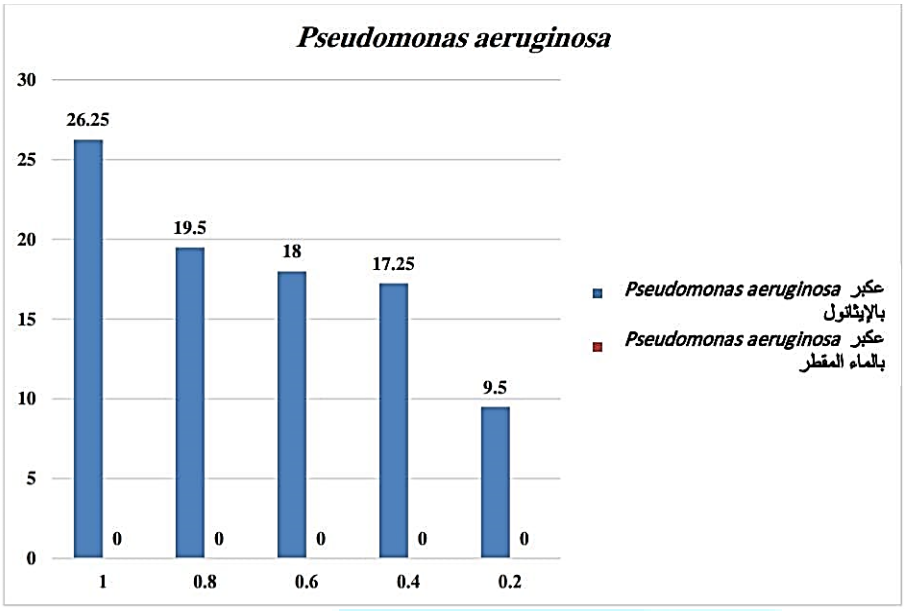

Fig. (1): Inhibitory Activity of Propolis on *Pseudomonas aeruginosa*.

The **Inhibitory Activity of Ethanolic Propolis Extract on *Pseudomonas aeruginosa*** is shown in **Fig. 2**, which highlights the varying inhibitory effects at different concentrations of ethanolic propolis extract.

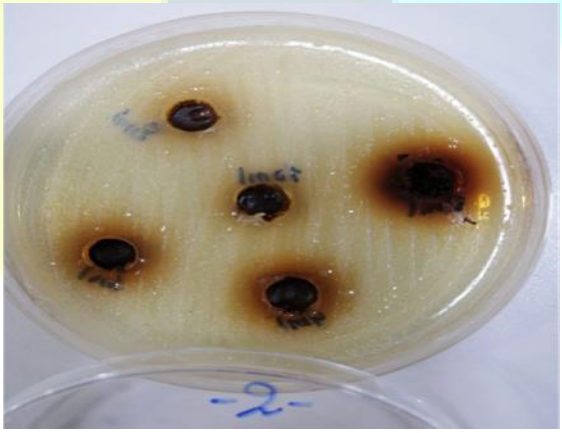

Fig. (2): Inhibitory Activity of Ethanolic Propolis Extract on Pseudomonas aeruginosa

These findings differ from those of [9], who found that Turkish propolis extract had no inhibitory activity against bacteria. Additionally, Hossain [10] reported that propolis extracts from Brazil and Korea had no antibacterial effects, despite being derived from different geographic regions. This may be due to differences in the plant source from which the propolis was collected, the season in which it was harvested, the type of hive, and the type of bees involved, as noted by [11]. However, the results of this study agree with those of [12], who showed that green propolis extract from Brosimum gaudichaudii, containing chlorogenic acid derived from cinnamaldehyde and apigenin, exhibited antibacterial activity.

The study also evaluated the inhibitory activity of the aqueous propolis extract on Pseudomonas aeruginosa, which showed a lower inhibitory effect compared to the ethanolic extract. The inhibition zones for the aqueous extract were smaller across all tested concentrations, as shown in Fig. 3, indicating a reduced antibacterial activity. This suggests that the ethanolic extract is more effective in inhibiting the growth of Pseudomonas aeruginosa than the aqueous extract.

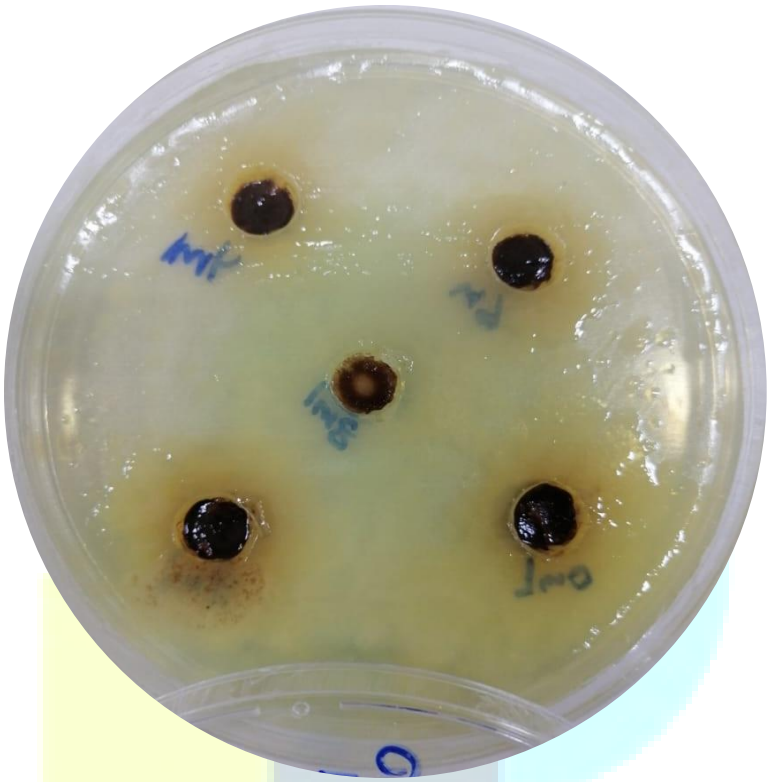

Fig. (3): Inhibitory Activity of Aqueous Propolis Extract on *Pseudomonas aeruginos*.

5- CONCLUSION

From this study, it was concluded that:

1. Pseudomonas aeruginosa is a Gram-negative bacterium and is less sensitive than the Gram-positive species.
2. Both aqueous and alcoholic extracts are effective in inhibiting Pseudomonas aeruginosa bacteria.
3. *The alcoholic extract is more effective than the aqueous extract in inhibiting Pseudomonas aeruginosa bacteria.*

REFERENCES

[1] Bankova, V. V. (2005). Chemical diversity of propolis and the problem of standardization. *Journal of Ethnopharmacology, 100*(1–2), 114–117. https://doi.org/10.1016/j.jep.2005.05.004

[2] Cushnie, A. S., & Lamb, A. J. (2005). Antimicrobial activity of flavonoids. *International Journal of Antimicrobial Agents, 26*(5), 343–356. https://doi.org/10.1016/j.ijantimicag.2005.09.002

[3] Cross, E. L., Beall, C. J., Kutsch, S. R., et al. (2012). Beyond *Streptococcus mutans*: Dental caries onset to multiple species by 16S rRNA community analysis. *PLoS ONE, 7*(11), e47722. https://doi.org/10.1371/journal.pone.0047722

[4] Deng, D. M., Liu, M. J., Ten Cate, J. M., & Crielaard, W. (2007). The VicRK system of *Streptococcus mutans* responds to oxidative stress. *Journal of Dental Research, 86*(7), 606–610. https://doi.org/10.1177/154405910708600705

[5] Hetrus, V., & Ion, I. R. (2013). Dental plaque—Classification, formation, and identification. *International Journal of Medical Dentistry, 3*(2), 139–143.

[6] Koneman, E. W., Procop, G. W., Schreckenberger, P. C., Wood, G. L., Wic, Allen, S. D., & Janda, W. M. (2006). *Color atlas and textbook of diagnostic microbiology* (6th ed.). Lippincott Williams & Wilkins.

[7] Cheesbrough, M. (2006). *District laboratory practice in tropical countries* (2nd ed.). USA.

[8] Forbes, B. A., Sahm, D. F., & Weissfeld, A. S. (2007). *Bailey & Scott's diagnostic microbiology* (12th ed.). Elsevier.

[9] Kahraman, H. A., Tutun, H., Kaya, M. M., et al. (2022). Ethanolic extract of Turkish bee pollen and propolis: Phenolic composition, antiradical, antiproliferative, and antibacterial activities. *Biotechnology & Biotechnological Equipment, 36*(1). https://doi.org/10.1080/13102818.2022.2045217

[10] Hossain, R., Quispe, C., et al. (2022). Propolis: An update on its chemistry and pharmacological applications. *Chinese Medicine, 17*, Article 100. https://doi.org/10.1186/s13020-022-00651-2

[11] Ramon-Sierra, J., Peraza-Lopez, E., Rodriguez-Borges, R., Yam-Puc, A., Modera-Santana, T., & Ortiz-Vázquez, E. (2019). Partial characterization of ethanolic extract of *Melipona beecheii* propolis and in vitro evaluation of its antifungal activity. *Revista Brasileira de Farmacognosia, 29*(3), 319–324. https://doi.org/10.1016/j.bjp.2019.04.002

[12] Przybytek, I., & Karpiński, T. M. (2019). Antibacterial properties of propolis. *Molecules, 24*(11), 2047. https://doi.org/10.3390/molecules24112047

Dijlah Journal of Medical Sciences (DJMS) Vol. 1, No. 3, May, 2025, pp. 24-29
P-ISSN: 3078-3178, E-ISSN: 3078-8625, Paper ID: 04

تأثير عكبر نحل العسل على بكتريا المكورات العقدية Pseudomonas aeruginosa المعزولة من المصابين بتسوس الاسنان

الخلاصة

هدفت هذه الدراسة إلى تقييم فعالية العكبر (*Propolis*) في شكليه المستخلص الكحولي (الإيثانولي) والمائي ضد المكورات العقدية *Pseudomonas aeruginosa*، وهي بكتيريا مرتبطة بتسوس الأسنان. تم الحصول على عينات العكبر من نحالي محافظة صلاح الدين. أظهرت النتائج تأثيرات تثبيطية كبيرة لكلا المستخلصين على نمو السلالات البكتيرية قيد الدراسة، حيث ازدادت معدلات التثبيط مع زيادة تركيز المستخلصات. وأظهر المستخلص الكحولي نشاطاً مضاداً للبكتيريا أعلى مقارنةً بالمستخلص المائي، كما يتضح من قياسات مناطق التثبيط باستخدام طريقة الانتشار في الوسط الصلب.

أوضحت النتائج أن *Pseudomonas aeruginosa*، وهي بكتيريا سالبة الگرام، كانت أقل حساسية تجاه المستخلصات مقارنةً بالبكتيريا موجبة الگرام. من بين التراكيز المختبرة، حقق تركيز ٢ مل من المستخلص الكحولي أعلى تثبيط ضد العزلات البكتيرية. تؤكد هذه الدراسة إمكانات المستخلص الكحولي للعكبر كمضاد طبيعي للبكتيريا، خاصةً في مكافحة الممرضات المرتبطة بتسوس الأسنان.