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Antimicrobial Efficacy of Wasp Honey Extracts against Multidrug-Resistant Bacteria

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ABSTRACT

This study investigates the antibacterial activity of ethanol extracts derived from wasp honey against eight multidrug-resistant (MDR) bacterial species. The tested isolates included *Acinetobacter baumannii*, *Burkholderia cepacia*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Streptococcus agalactiae*, *Staphylococcus aureus*, and *Enterococcus faecalis*; antibiotic susceptibility showed that 96% of isolates were resistant to tetracycline, followed by Ampicillin and Ciprofloxacin, each with 58% resistance. Notably, resistance to critical last-line antibiotics such as imipenem was 10%; colistin, amikacin and moxifloxacin were 8%; and tigecycline was 4%, indicating relatively limited resistance to these antibiotics. We used Soxhlet extraction to isolate phenolic chemicals: powder, Polyphenolic-rich oil, oil without polyphenols and polyphenol-only extracts, using ethanol as the solvent. We assessed antibacterial activity using both broth microdilution and well diffusion techniques. The microdilution assay revealed minimum inhibitory concentration (MIC) values between 2.5 and 10 mg/mL, indicating modest antibacterial efficacy against all examined multidrug-resistant organisms. A concentration of just 1.25 mg/mL demonstrated inhibitory effects against *Escherichia coli* and *Streptococcus agalactiae*, underscoring the extract's efficacy at reduced dosages. In the well diffusion assay, inhibitory zones ranged from 8 mm to 19 mm, with the largest zone against *Streptococcus agalactiae* (19 mm). The results show that wasp honey extracts are effective against both Gram-positive and Gram-negative bacteria with similar potency. This is the first study in the Kurdistan Region, Iraq, to identify and highlight the efficacy of wasp honey extracts as an innovative natural antibacterial agent.

Keywords: Paper wasp honey, Antimicrobial activity, Multidrug-Resistant Bacteria, *Pseudomonas spp.*, *Streptococcus agalactiae*

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مستخلصات عسل الدبور: نهج جديد لمكافحة البكتيريا المقاومة للأدوية المتعددة

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الملخص

يدرس هذا البحث الفعالية المضادة للبكتيريا لمستخلصات الإيثانول المُستخلصة من عسل الدبابير، مع التركيز على ثمانية أنواع من البكتيريا المقاومة للأدوية المتعددة. تشمل هذه الأنواع: *Acinetobacter baumannii*، و *Burkholderia cepacia*، و *Escherichia coli*، و *Klebsiella pneumoniae*، و *Pseudomonas aeruginosa*، و *Streptococcus agalactiae*، و *Staphylococcus aureus*، و *Enterococcus faecalis*. وقد أظهرت نتائج تحليل أنماط المضادات الحيوية المختبرة أن معظمها (96%) مقاوم للتتراسيكلين، يليه الأمبيسلين والسيبروفلوكساسين (58%) لكل منهما. والجدير بالذكر أن مقاومة المضادات الحيوية الأساسية من الخط الأخير، مثل إيميبينيم، كانت (10%)، وكل من كوليسيتين وأميكاسين وموكسيفلوكساسين (8%)، وتيجيسيكلين (4%)، ظلت محدودة نسبياً. استُخدم استخلاص سوكسليت لعزل المواد الكيميائية الفينولية، بما في ذلك المسحوق، والزيت الغني بالبوليفينول، والزيت الخالي من البوليفينول، ومستخلصات البوليفينول فقط مع الإيثانول كمنظف. قُيِّمت الفعالية المضادة للبكتيريا باستخدام كلٍّ من تقنيتي التخفيف الدقيق للمرق والانتشار البثري. كشف اختبار التخفيف الدقيق عن قيم تركيز مثبط أدنى (MIC) تتراوح بين 2.5 و 10 ملغم/مل، مما يشير إلى فعالية مضادة للبكتيريا متواضعة ضد جميع الكائنات الحية المقاومة للأدوية المتعددة التي تم فحصها. أظهر تركيز 1.25 ملغم/مل فقط تأثيرات مثبطة ضد الإشريكية القولونية والمكورات العقدية القاطعة للدر، مما يؤكد فعالية المستخلص بجرعات منخفضة. في اختبار الانتشار البثري، تراوحت المناطق المثبطة من 8 مم إلى 19 مم، مع تسجيل أوسع منطقة ضد العقدية القاطعة للدر (19 مم). تُظهر النتائج أن المستخلصات المشتقة من عسل الدبابير تُظهر فعالية ضد كل من البكتيريا موجبة الجرام وسالبة الجرام بقوة متشابهة. نتيجتنا هي أول دراسة في إقليم كردستان العراق وجدت وأكدت فعالية مستخلصات عسل الدبابير كعامل مضاد للبكتيريا طبيعي مبتكر لمعالجة الأمراض المقاومة للأدوية المتعددة.

الكلمات المفتاحية / عسل الدبور الورقي، النشاط المضاد للميكروبات، البكتيريا المقاومة لعدة أدوية، جنس الزائفة، المكورات العقدية الغربية.

INTRODUCTION

The World Health Organization (WHO) and the United States Centers for Disease Control and Prevention (CDC) recognize antimicrobial resistance as a major global public health problem. The burden of untreatable illnesses is anticipated to escalate in the forthcoming decades, incurring substantial economic expenses, owing to the proliferation of multidrug-resistant (MDR) bacteria and the absence of a novel research and development pipeline for antibiotics ⁽¹⁾. Furthermore, emerging antibiotic-resistant bacterial

strains have become a major public health concern ⁽²⁾. This has necessitated the development of novel bactericidal materials. Consequently, innovative strategies are urgently needed to combat antibiotic resistance and develop alternative antimicrobial medicines to manage infections ^(3, 4). The rise of multidrug-resistant bacteria has prompted the investigation of alternative therapies, such as natural substances like honey ⁽⁵⁾. Wasps are often misunderstood insects, commonly associated with stings and aggression, but they play an important

role in nature. Some species of social wasps, like those found in the Kurdistan Region of Iraq, also produce a honey-like substance, much like bees and aphids. Although they do not store honey in large amounts, wasps can collect sugary secretions or nectar and transform them enzymatically. Recent research shows that wasp honey has distinct biochemical characteristics compared to bee and aphid honey. It contains higher levels of minerals such as magnesium, iron, aluminum, and even vanadium, which was found only in wasp honey. Honey's antimicrobial activity is widely attributed to several synergistic factors, including its acidic pH, high sugar content, hydrogen peroxide production, and bioactive compounds such as polyphenols and trace minerals. Among these, polyphenolic compounds, including flavonoids and phenolic acids, are particularly important, as they can disrupt microbial membranes, inhibit bacterial enzymes, and interfere with microbial metabolism ⁽⁶⁾. Given that the wasp honey collected from Choman in the Kurdistan Region is influenced by diverse wild flora and high-altitude environmental conditions, its unique chemical composition, especially its rich polyphenol and mineral content, may contribute to enhanced antimicrobial properties. These findings open new perspectives on wasps not just as aggressive insects, but as organisms capable of producing a unique and potentially valuable natural product ⁽⁷⁾. While bee honey's antibacterial effects are well known ⁽⁸⁾, wasp paper honey remains largely unstudied. Early findings show it contains unique oils and polyphenols. This study extracted various compounds, oil, polyphenol-rich oil, honey powder, and isolated polyphenols, from wasp honey and tested their antibacterial activity. The results offer new insight into wasp honey as a potential natural treatment for pathogenic and drug-resistant bacteria.

MATERIALS AND METHODS

2.1- Sample Collection:

We obtained 50 bacterial isolates from various clinical sources, including urine, sputum, and wound samples, at the Microbiology Laboratory of

Rizgary Hospital in Erbil. We collected samples over two months, from September to early November. All isolates were identified based on their cultural characteristics on MacConkey agar, Blood agar, and mannitol salt agar, as well as through microscopic examination using Gram staining, and some biochemical tests including catalase, oxidase, Voges–Proskauer (VP), methyl red (MR), citrate utilization, and growth on Simmons citrate agar ^(9, 10). Moreover, we confirmed the identification of bacterial isolates using an automated bacterial identification system, the VITEK® 2 compact system (BioMérieux) ^(11, 12). We identified isolates of various bacterial species, including *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Burkholderia cepacia*, *Acinetobacter baumannii*, *Staphylococcus aureus*, *Streptococcus agalactiae* and *Enterococcus faecalis*. Each bacterial isolate was preserved by transferring a pure colony of each type of bacterial isolate into 1.5 mL tubes containing BHI broth (80%) and glycerol (20%), and growth was observed after incubation for 24 hours at 37°C; the isolates were subsequently stored at -20°C ⁽¹³⁾ for future research use.

2.2- Antibacterial Sensitivity Testing

We determined the antibiotic susceptibility of the bacterial isolates using the disk diffusion method, following Clinical and Laboratory Standards Institute (CLSI) guidelines. We standardized bacterial suspensions to 0.5 McFarland turbidity and evenly spread them onto Mueller-Hinton agar plates. We used 18 commercially prepared antibiotic discs to evaluate resistance patterns. The antibiotics tested included amikacin (10 µg), ampicillin (25 µg), ceftazidime (30 µg), ciprofloxacin (10 µg), gentamicin (10 µg), imipenem (10 µg), levofloxacin (30 µg), nitrofurantoin (100 µg), tetracycline (30 µg), trimethoprim (10 µg), piperacillin (10 µg), cefazolin (30 µg), cefoxitin (30 µg), ceftriaxone (30 µg), cefepime (30 µg), tigecycline (15 µg), colistin (5 µg), and moxifloxacin (10 µg). We placed the antibiotic discs on the agar surface and incubated

the plates at 37 °C for 18–24 hours. After incubation, we measured the diameters of the clear zones around the discs in millimeters. We interpreted these measurements using CLSI criteria to determine whether each bacterial strain was sensitive, intermediate, or resistant. All antibiotic discs used were sourced from Bioanalyse (Turkey) to ensure quality and consistency ⁽¹⁴⁾.

Wasp (*Parapolybia escalerae*) discovery and its honey

In recent years, interest in alternative natural products with antimicrobial activity has grown, particularly amid increasing antibiotic resistance. Among these, bee honey has been extensively studied for its broad-spectrum antimicrobial effects, attributed to its osmotic pressure, hydrogen peroxide content, low pH, and the presence of various phytochemicals ⁽⁷⁾. This perspective was expanded by comparing the biochemical properties of honeys produced by bees, aphids, and wasps in the Kurdistan Region of Iraq, suggesting that wasp honey may also hold untapped therapeutic potential. In parallel, ⁽¹⁵⁾ reported the first record of *Parapolybia escalerae* (Meade-Waldo, 1911) in Kurdistan, a paper wasp species of the subfamily Polistinae. The discovery included clear photographic documentation of the nest and adult wasp morphology (Figure 1A & B), contributing valuable entomological data to the region.



(A)



(B)

Figure 1. (A) The wasp from Kurdistan. (B) Its honey showing unique color and texture.

2.3 Soxhlet extraction

The Soxhlet extraction method was used to extract bioactive substances. Powdered honey was obtained by dehydrating raw honey under controlled conditions. For oil extraction, approximately 50 grams of powdered honey were placed in a thimble and extracted with 250 mL of ethanol in a Soxhlet apparatus for 6 to 8 hours, until the solvent in the siphon tube became colorless ⁽¹⁶⁾. The ethanol extract was then concentrated using a rotary evaporator under reduced pressure to obtain crude honey oil. A portion of the crude oil was further fractionated using 80% aqueous ethanol to increase polyphenol content. To manufacture the general polyphenol extract, 20 grams of powdered honey were extracted with 80% ethanol using Soxhlet extraction, and the solvent was subsequently evaporated to yield the concentrated polyphenol fraction. All extracts were preserved at 4°C in opaque containers until subsequent antibacterial evaluation. This successive extraction facilitated the isolation of various chemical constituents to assess their distinct antibacterial properties ⁽¹⁷⁾. Soxhlet

extraction was selected for its cost-effectiveness and extraction efficiency in isolating bioactive compounds from natural substances like honey. Compared to alternative techniques such as maceration, ultrasound-assisted extraction (UAE), or microwave-assisted extraction (MAE), Soxhlet offers a balanced approach—requiring relatively simple equipment while still achieving high extraction yields. Although newer methods may offer faster processing times, they often demand expensive instruments and specialized settings. In contrast, Soxhlet allows for continuous solvent recycling and ensures consistent extraction over extended periods, making it a practical and economical choice for laboratories with limited resources ⁽¹⁸⁾.

2.4. Antibacterial activity Assays of wasp honey extracts

2.4.1- Minimum inhibition concentration (MIC) assay.

The antibacterial efficacy of the honey extract was evaluated using the broth microdilution method in 96-well microtiter plates (Figure 2), in accordance with standards established by the Clinical and Laboratory Standards Institute (CLSI). Each well was administered 80 μ L of Mueller–Hinton broth (MHB) and 100 μ L of a 10 mg/mL paper wasp honey solution. Subsequent serial dilutions were executed to generate concentrations of 10, 5, 2.5, 1.25, 0.625, and 0.3125 mg/mL. Subsequently, 20 μ L of bacterial suspension, calibrated to about 1×10^6 CFU/mL, was introduced to each well. Positive controls (broth containing bacterial inoculum) and negative controls (broth containing only extract) were included to validate the assay. Following 24 hours of incubation at 37°C, bacterial proliferation was quantified by measuring the optical density at 600 nm (OD600) with a microplate reader ⁽¹⁴⁾.



Figure .2: Representative 96-well microtiter plate illustrating the determination of Minimum Inhibitory Concentration (MIC) for tested extracts against selected microbial strains. Serial dilutions were performed across wells, with observable turbidity or colorimetric changes indicating bacterial growth, and clear wells representing inhibitory concentrations.

2.4.2- Agar well diffusion assay

The antibacterial efficacy of the extracts was assessed utilizing the agar well diffusion technique. In summary, we introduced 0.1 mL of each of the eight bacterial isolates into sterile test tubes and cultured them overnight. We prepared Mueller-Hinton agar, autoclaved it, and poured it into sterile Petri dishes. After solidification, we uniformly distributed bacterial suspensions across the agar surface using sterile cotton brushes. We then formed wells approximately 6 mm in diameter using a sterile cork borer. Each well contained 50 μ L of the paper wasp honey extract at a concentration of 100 mg/mL. All plates were incubated at 37°C for 24 hours, and the resulting inhibition zone diameters were measured to evaluate antibacterial activity. ⁽⁹⁾.

RESULTS AND DISCUSSION

Initial Gram staining of the 50 clinical isolates showed a clear predominance of Gram-negative organisms (84%; n=42), while 16% (n=8) were Gram-positive. This distribution reflects the typical microbial pattern observed in many clinical settings, where Gram-negative bacteria are often associated with more severe and resistant infections. Biochemical characterization using oxidase, catalase, methyl red, Voges-Proskauer, and Simmons citrate tests further supported identification. Table 1 presents a detailed account of these findings. We identified all 50 bacterial isolates in this study with high accuracy using the VITEK® automated identification system. This system uses advanced biochemical profiling to provide rapid, precise species-level identification of clinical and environmental microorganisms. Its implementation ensured standardization and minimized human error, increasing the reliability of the identification process; the VITEK® system produced identification results that highlighted the diversity and classification of the isolates. Including this technology provides a methodological advantage, supporting the accuracy and reproducibility of the microbiological data (19).

Table 1: Identification of isolates according to some phenotypic characteristics

isolates	Catalase	Oxidase	Voges-Proskauer (VP)	Methyl Red (MR)	Citrate Test	Gram Stain-Shape
<i>E.coli</i>	+ Ve	- Ve	- Ve	+ Ve	- Ve	-Rod
<i>K.pneumonia</i>	+ Ve	- Ve	+ Ve	- Ve	+ Ve	-Rod
<i>P.aeruginosa</i>	+ Ve	+ Ve	- Ve	- Ve	+ Ve	-Rod
<i>Br.cepacia</i>	+ Ve	+ Ve	- Ve	- Ve	+ Ve	-Rod
<i>A. baumannii</i>	+ Ve	- Ve	- Ve	- Ve	+ Ve	-Rod
<i>S. aureus</i>	+ Ve	- Ve	-	-	- Ve	+cocci
<i>S. agalactiae</i>	- Ve	- Ve	- Ve	- Ve	- Ve	+cocci
<i>E. faecalis</i>	- Ve	- Ve	- Ve	- Ve	- Ve	+cocci

Not done +Ve = Positive - Ve = Negative

Antibiotic susceptibility profiling of the 50 clinical isolates revealed a high burden of multidrug resistance. Of the 50 isolates, 64% (n=32) were resistant to three or more antibiotic classes and were classified as multidrug-resistant (MDR), while 36% (n=18) remained sensitive. Resistance rates varied by antibiotic class, with the highest against Tetracycline (98%), followed by ampicillin (58%),

ciprofloxacin (58%), cefazolin (40%), and levofloxacin (40%), as shown in Figure 3. These alarming resistance levels reflect the growing challenge in treating common infections with conventional antibiotics. Based on these data, we selected eight MDR isolates with pronounced resistance profiles for further evaluation against alternative treatments. This resistance pattern highlights an urgent need to explore new antimicrobial solutions.

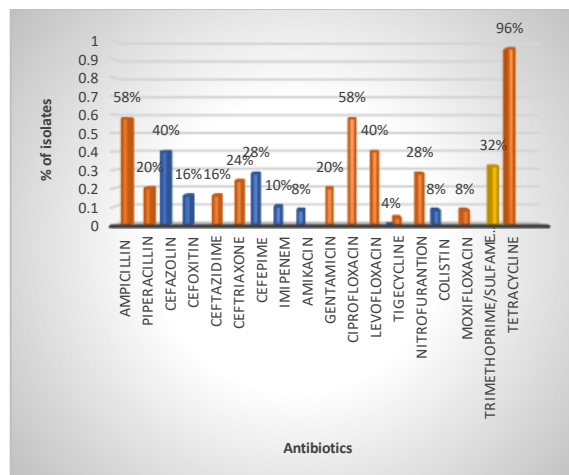


Figure 3: Antibiotic resistance profile of bacterial isolates. The figure illustrates the percentage of resistance among tested isolates against 18 antibiotics. Antibiotics are grouped by class: β -lactams (Ampicillin, Piperacillin, cefazolin, cefoxitin, ceftazidime, ceftriaxone, cefepime, imipenem), aminoglycosides (amikacin, gentamicin), fluoroquinolones (ciprofloxacin, levofloxacin, moxifloxacin), glycylicycline (tigecycline), nitrofurantoin (nitrofurantoin), polymyxin (colistin), sulfonamides (trimethoprim/Sulfamethoxazole), and tetracyclines (tetracycline). Resistance was particularly high against tetracycline (96%) and ciprofloxacin (58%). The antibacterial activity of the tested extracts varied across eight multidrug-resistant bacterial strains, with inhibition zones ranging from 8 mm to 19 mm (Table 2). The powder extract demonstrated the highest activity, especially against *Streptococcus agalactiae* (19 mm zone), highlighting its potent effect on Gram-positive bacteria. The polyphenol-rich oil exhibited consistent antibacterial properties against multiple strains, including *Staphylococcus aureus* and *Klebsiella pneumoniae*, with inhibition zones between 8 and 11 mm. Notably, it maintained a

stable, robust inhibition zone of 10 mm against *Burkholderia cepacia* over 48 hours despite increased bacterial inoculum, reflecting sustained antimicrobial effects (Figure 4).

The antibacterial mechanisms of polyphenols involve multiple pathways, such as disruption of bacterial membranes, inhibition of quorum sensing and biofilm formation, and interference with bacterial metabolic enzymes and genetic material, contributing to their broad-spectrum activity against both Gram-positive and Gram-negative bacteria. (20). The observed weaker activity against Gram-negative strains relates to their complex outer membrane and intrinsic multidrug resistance features. (21).

The extracts' demonstrated stability and activity against resistant strains, notably *B. cepacia*, a pathogen of critical clinical concern, underscore their potential as natural antibacterial agents. These findings align with recent research highlighting the promise of plant-derived, polyphenol-rich extracts as effective antimicrobials that can overcome some resistance mechanisms and contribute to developing alternative or adjunct therapies for multidrug-resistant infections. (20, 22, 23).

Table2: Antibacterial Activity of Paper wasp honey Extracts against Gram-Positive and gram-negative ⁽⁹⁾ Multidrug-Resistant Isolates Using the Well Diffusion Method.

Extraction Type	<i>S. aureus</i>	<i>S. agalactiae</i>	<i>E. faecalis</i>	<i>E. coli</i>	<i>K. pneumoniae</i>	<i>P. aeruginosa</i>	<i>B. cepacia</i>	<i>A. baumannii</i>
Powder	10 mm	19 mm	8 mm	Weak	11 mm	Weak	Weak	Weak
Polyphenol-rich oil	10 mm	10 mm	9 mm	8 mm	11 mm	8 mm	10 mm Fig. 2	10mm
Oil without polyphenol	8 mm	9 mm	14 mm	9 mm	8 mm	Weak	9 mm	Weak
Polyphenol	14 mm	Weak	13 mm	Weak	9 mm	Weak	Weak	Weak

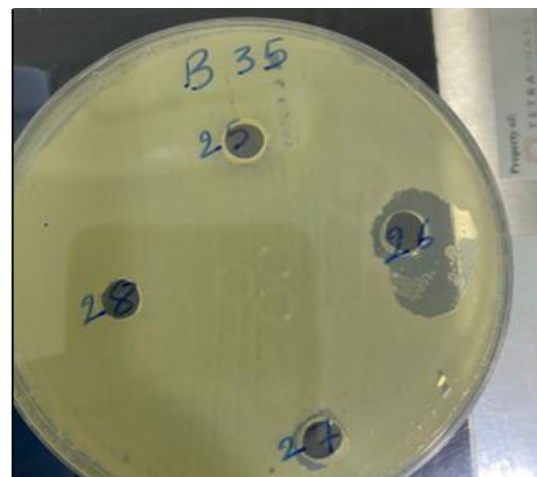


Figure 4: The oil-rich polyphenolic extract labeled as sample 26 demonstrated consistently high antibacterial efficacy against *Burkholderia cepacia*, a multidrug-resistant Gram-negative pathogen known for its clinical relevance and intrinsic resistance mechanisms. Even at higher bacterial concentrations, the extract maintained a stable 10 mm inhibition zone after 48 hours of incubation, indicating a robust, long-lasting antimicrobial effect. This stability across varying bacterial loads strongly suggests that the extract not only inhibits bacterial growth effectively but also resists degradation over time.

Table 3 summarizes the Minimum Inhibitory Concentration (MIC) values for paper wasp honey extracts against eight multidrug-resistant Gram-positive and Gram-negative bacterial strains. The powder extract exhibited the highest antibacterial potency, with MIC values as low as 1.25 mg/mL against *Streptococcus agalactiae* and *Escherichia coli*, and MICs ranging from 2.5 to 10 mg/mL for other tested strains. This potent activity mirrors the strong inhibition zones observed in the well diffusion assay, confirming the powder's broad-spectrum effectiveness.

The polyphenol-rich oil extract also demonstrated notable antibacterial activity, with MICs between 2.5 and 10 mg/mL against all tested isolates. In particular, it maintained inhibitory action against *Burkholderia cepacia* at 2.5 mg/mL and *Acinetobacter baumannii* at 5 mg/mL, suggesting strong efficacy against resistant Gram-negative pathogens.

In comparison, the oil without polyphenols showed comparatively reduced activity, with MIC values

mostly at 5 to 10 mg/mL, indicating polyphenols' critical role in the antibacterial properties of the extracts. Moreover, isolated polyphenols alone showed limited inhibitory effect, with activity against *Enterococcus faecalis* and *Acinetobacter baumannii* only at 10 mg/mL, suggesting that synergy with other compounds is necessary for maximal antibacterial efficacy.

Polyphenols may partially volatilize or degrade during MIC assay procedures such as broth microdilution. This evaporation can reduce effective concentrations in wells, potentially underestimating the antimicrobial effects of isolated polyphenols. Supporting this, MIC values for the oil without polyphenols, measured using an ELISA microplate reader, showed limited but measurable inhibition, primarily at higher concentrations and against Gram-positive bacteria such as *S. agalactiae* and *S. aureus*.

These findings, along with the well diffusion data, suggest that the antibacterial activity of paper wasp honey extracts arises from the combined, synergistic effects of polyphenols and other bioactive constituents.

Table 3: Minimum inhibitory concentration (MIC) of paper wasp honey extracts against multidrug-resistant Gram-positive and Gram-negative bacterial isolates.

Extraction Type	<i>S. aureus</i>	<i>S. agalactiae</i>	<i>E. faecalis</i>	<i>E. coli</i>	<i>K. pneumoniae</i>	<i>P. aeruginosa</i>	<i>B. cepacia</i>	<i>A. baumannii</i>
	MIC mg/mL							
Powder	5	1.25	10	1.25	2.5	2.5	2.5	2.5
Polyphenol-rich oil	10	5	5	2.5	2.5	2.5	2.5	5
Oil without polyphenol	5	5	5	10	5	5	10	5
Polyphenol	No effect	No effect	10	No effect	No effect	No effect	No effect	10

To the best of our knowledge, this is the first study to examine the antimicrobial activity of wasp-derived honey against multidrug-resistant (MDR) bacteria. The extract showed promising effects across all eight tested MDR species, with inhibition zones reaching up to 19 mm in the well diffusion assay, and an effective concentration of 1.25 mg/ml for the powder extract. A study on comparison between honey produced by bees, aphids and wasps

in Kurdistan Region - Iraq based on biochemical parameters found that the physical and biochemical properties of honey produced by wasps were similar to those of honey produced by bees, with differences in HMF, pH, Total acidity, Water % Reducing sugars %, Sucrose % Electrical conductance and Diastase number for Bee honey was 0.9, 4.0, 10.0, 15.4, 76, 1.7, 436.0 and 21.0 respectively, while for Wasp honey was 15.3, 6.7, 2.0, 9.0, 30, 28.0, 245.0 and 9.0. However, wasp honey contains high concentrations of Al, Mg, Fe, Mn, V, Ti, and Sr ⁽⁷⁾. Interestingly, the antimicrobial activity observed in wasp-derived honey may be partly attributed to its elemental composition. Unlike typical bee honey, a study by ⁽⁷⁾ suggests that wasp honey contains trace amounts of vanadium, a transition metal known for its antibacterial and antifungal properties. This raises the possibility that vanadium contributes to the observed inhibitory effects, either directly or by enhancing other bioactive compounds, since vanadium is associated with metal-based agents ⁽²⁴⁾. Because this investigation is novel, direct comparison with previously published data is not currently possible, as no similar studies on wasp honey have been reported. Nevertheless, the broad-spectrum activity observed across both Gram-positive and Gram-negative bacteria highlights the potential of wasp honey as a natural antimicrobial agent. While this study provides valuable insights into the antimicrobial potential of paper wasp-derived honey, several limitations warrant acknowledgment. First, due to the novelty of the subject, comparative analysis with existing literature was limited. The investigation was restricted to in vitro assays, which, although informative, do not fully reflect the complexity of biological systems; thus, further in vivo studies are required to validate the observed effects and assess safety. Future studies incorporating a broader range of extraction methods and dosage levels may reveal greater or more nuanced antimicrobial potential. Furthermore, this study did not comprehensively analyze the chemical composition of the honey samples, leaving the specific bioactive compounds

responsible for the antimicrobial activity to be identified in future work. The study did not include statistical analysis, including p-values for comparisons of MIC values and inhibition zone diameters among different extracts, which limits the quantitative strength of the findings. Despite these limitations, this research represents a valuable first step toward understanding the antimicrobial properties of wasp honey. It highlights its potential as a natural antimicrobial agent worthy of further investigation.

CONCLUSION

This study demonstrates, for the first time, the potential antimicrobial activity of paper wasp honey against a range of multidrug-resistant clinical isolates. The extract showed measurable inhibitory effects against both Gram-positive and Gram-negative bacteria. This natural product's ability to affect pathogens resistant to conventional antibiotics suggests its promise as an alternative or complementary therapeutic agent. Although susceptibility varied, the broad-spectrum activity warrants further investigation into the active components of wasp honey and their mechanism of action. These findings provide novel insights into antimicrobial research and open opportunities to develop natural, paper wasp honey-based treatments against resistant infections.

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