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Exploring the Antibacterial Activity of Oils Extracted from *Phoenix dactylifera* (Ajwa and Breem) Seeds Against Multidrug Resistance Bacterial Isolates

 Roand muqdad Abdullah Ismail¹ , Payman Akram Hamasaeed²
^{1,2}Department of Biology, College of Education, Salahaddin University, Erbil, Kurdistan, Iraq

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ABSTRACT

Medicinal herbs have historically been used to treat infections, and current research focuses on isolating active plant compounds because of increasing antibiotic resistance and side effects. This study investigates the antimicrobial properties of polyphenol-depleted oil and polyphenol-rich extracts obtained from the seeds of two *Phoenix dactylifera* (date palm) varieties, Ajwa and Breem. We tested their activity against multidrug-resistant (MDR) *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Burkholderia cepacia*, *Acinetobacter baumannii*, *Staphylococcus aureus*, *Streptococcus agalactiae*, and *Enterococcus faecalis* isolates. Antibiotic susceptibility testing showed that most isolates (96%) were resistant to tetracycline, followed by ampicillin and ciprofloxacin (58%). Notably, resistance to critical last-line antibiotics such as imipenem was 10%; colistin, amikacin and moxifloxacin were 8%; and tigecycline was 4%, indicating that resistance remained relatively limited. Antibacterial efficacy of extracted oils of *Phoenix dactylifera* (date palm) varieties Ajwa and Breem seeds was assessed using both the Minimum inhibitory concentration (MIC) and well diffusion assay. MIC results indicated strong inhibitory potential, ranging from 5 to 10 mg/ml. The well diffusion assay exhibited weaker activity, with inhibition zones reaching up to 13 mm. These results highlight the potential of date seed oil and its polyphenolic components as antimicrobial agents. The study shows that *Phoenix dactylifera* extracts have promising antimicrobial effects against MDR bacteria. To improve effectiveness, delivery methods such as nanoemulsions or carriers may be needed to enhance the oil's dispersion and bioavailability.

Keywords: Barhi date, Ajwa date, Seed oil, Antimicrobial activity, Multidrug-resistant bacteria

Name: Roand Muqdad Abdullah Ismail E-mail: Roand.Abdullah@su.edu.krd



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استكشاف النشاط المضاد للبكتيريا لزيت بذور التمر (العجوة والبريم) ضد العزلات البكتيرية المقاومة للأدوية المتعدد

روند مقداد عبدالله اسماعيل¹، پیمان اکرم حمه سعيد²

^{1,2} قسم علم الأحياء، كلية التربية، جامعة صلاح الدين، أربيل، كردستان، العراق

الملخص

استُخدمت الأعشاب الطبية تاريخيًا لعلاج العدوى، وتُركز الأبحاث الحالية على عزل المركبات النباتية النشطة بسبب تزايد مقاومة المضادات الحيوية وآثارها الجانبية. في هذه الدراسة تم استكشاف الخصائص المضادة للميكروبات لزيت بذور العجوة والبريم (الخالي من البوليغينولات) والمستخلصات الغنية بالبوليفينول، والمستخلصة من نخيل التمر *Phoenix dactylifera*.. واختبار نشاطها ضد العزلات البكتيرية المقاومة للأدوية. تشمل هذه الأنواع: *Burkholderia*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Escherichia coli*, *Acinetobacter baumannii*, *Staphylococcus aureus*, *Streptococcus agalactiae*, و *Enterococcus faecalis*. أظهرت نتائج تحليل مقاومة المضادات الحيوية أن معظم العزلات (96%) كانت مقاومة للتراسيكلين، تلاه الأمبيسلين والسيفروفلوكساسين بنسبة (58%) لكل منهما. جدير بالذكر أن مقاومة المضادات الحيوية ذات الخط الأخير مثل الإيميبينيم كانت (10%)، بينما كانت المقاومة لكل من الكوليسيتين والأميكاسين والموكسيفلوكساسين (8%)، وتيجيسيكلين (4%)، مما يدل على محدودية المقاومة لهذه العقاقير الحيوية. تم تقييم الفعالية المضادة للبكتيريا لزيت بذور نخيل التمر من صنف العجوة والبريم باستخدام الحد الأدنى من التركيز المثبط (MIC) واختبار حفر الانتشار. أظهرت نتائج MIC قدرة مثبطة قوية تتراوح بين 5 إلى 10 ملغم/مل، بينما أظهر اختبار حفر الانتشار نشاطاً أقل مع مناطق تثبيط وصلت إلى 13 مم. بينما أظهر اختبار الانتشار في الأطباق نشاطاً أضعف، حيث بلغت مناطق التثبيط حتى 13 ملم. تؤكد هذه النتائج على إمكانات زيت بذور التمر ومكوناته البوليفينولية كمضادات ميكروبية. وتشير الدراسة إلى أن مستخلصات التمر تُظهر تأثيرات واعدة ضد البكتيريا متعددة المقاومة للأدوية. ولزيادة فعاليتها، قد يكون من الضروري استخدام طرق توصيل مثل المستحلبات النانوية أو النواقل لتعزيز انتشار الزيت وتوافره الحيوي.

1. INTRODUCTION

Antimicrobial resistance (AMR) has become one of the most pressing global public health threats, posing a serious risk to both human and animal lives. The rapid rise and spread of multidrug-resistant (MDR) bacteria represent a formidable challenge in the 21st century, particularly as these organisms often include opportunistic pathogens found in commensal microbiota. These bacteria are responsible for a substantial proportion of hospital- and community-acquired infections, yet their resistance to multiple antibiotic classes renders conventional treatments increasingly ineffective, leading to elevated rates of morbidity and mortality

(1-5). In response to this growing crisis, interest has surged in exploring bioactive compounds from natural sources, mostly plants, for their potential antimicrobial properties (6). Among these, *Phoenix dactylifera* L. (date palm) has gained attention for its rich pharmacological profile. Date seeds, in particular, have demonstrated notable therapeutic potential and have historically been used in traditional medicine, including prophetic traditions that highlight the health benefits of dates during the holy month of Ramadan (7). Recent studies have confirmed that date seed oil possesses anti-inflammatory, antioxidant, and even anticancer

efficacy, further validating its value in modern biomedical research ^(8, 9). In this study, we aimed to evaluate the antimicrobial efficacy of polyphenols-depleted and polyphenolic-rich oil extracts derived from *Phoenix dactylifera* seeds to assess their potential as natural alternatives against MDR bacterial strains.

2. MATERIALS AND METHODS

We isolated 50 Pathogenic bacteria in the Microbiology Laboratory at Rizgary Hospital, Erbil, over two months, from September to early November.

2.1: Sample Collection: Bacterial Isolation and Identification

We obtained 50 pathogenic bacteria from different clinical sources, including urine, sputum, and wound samples. All isolates were identified according to cultural characteristics on MacConkey agar, blood agar and Mannitol salt agar; microscopic examination by Gram stain; 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 preserved at -20°C ⁽¹³⁾ for research continuity.

2.2: Antibacterial Sensitivity Testing

The antimicrobial susceptibility of bacterial isolates was evaluated using the disk diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) guidelines. Standardized bacterial suspensions were prepared by adjusting cultures to

0.5 McFarland turbidity and inoculated uniformly onto Mueller-Hinton agar plates. A total of 18 commercial antibiotic disks were used to assess the resistance profiles of the isolates. These included amikacin (AMK, 10 µg), ampicillin (AMP, 25 µg), ceftazidime (CAZ, 30 µg) ciprofloxacin (CIP, 10 µg), gentamicin (CN, 10 µg), imipenem (IPM, 10 µg), levofloxacin (LVX, 30 µg), nitrofurantoin (NIT, 100 µg), tetracycline (TE, 30 µg), trimethoprim (TMP, 10 µg), piperacillin (PRL, 10 µg), cefazolin (CZ, 30 µg), cefoxitin (FOX, 30 µg), ceftriaxone (CRO, 30 µg), cefepime (FEP, 30 µg), tigecycline (TGC, 15 µg), colistin (CST, 5 µg), and moxifloxacin (MXF, 10 µg). The disks were carefully placed on the surface of the inoculated plates, which were then incubated at 37 °C for 18–24 hours. Following incubation, the diameters of the inhibition zones were measured in millimeters. The results were interpreted according to CLSI breakpoints to classify each isolate as sensitive, intermediate, or resistant. All antibiotic disks used in this study were obtained from Bioanalyse (Turkey), ensuring consistency and quality in the testing process ⁽¹⁴⁾.

2.3: Soxhlet Extraction of Polyphenol-Free and Polyphenol-Rich Oils

Using the Soxhlet method, we extracted two types of oil from *Phoenix dactylifera* seeds: one regular (polyphenol-depleted oil) and one rich in polyphenols. The seeds came from two well-known date varieties: Breem from Iraq and Ajwa from Saudi Arabia. First, we thoroughly cleaned the seeds and pulp, then dried them at a steady temperature of 50–60°C until their weight no longer changed. Once dried, we ground them into a fine powder to make the extraction process more efficient. For the regular oil, we weighed about 50 grams of the powder, added 250 mL of Absolute ethanol, placed it in a cellulose thimble, and inserted it into the Soxhlet extractor ⁽¹⁵⁾. We used absolute ethanol as the solvent, and the extraction ran for 6- 8 hours at 40–60°C. We then used a rotary evaporator to remove the ethanol, leaving the oil. We stored it in dark glass bottles at 4°C to protect it from light and keep

it stable until we needed it. To extract oil containing polyphenols, we followed a similar process but used 80% ethanol instead, since it's more polar and better at pulling out both fats and phenolic compounds. This extraction took longer, about 6 to 10 hours. After removing the solvent, the polyphenol-rich oil was stored at 4°C for future analysis ⁽¹⁶⁾.

2.4: Antibacterial activity assay of date seed oil extracts

2.4.1: Minimum inhibition concentration (MIC) assay.

According to ⁽¹⁷⁾, the microdilution method was used in a 96-well plate to evaluate the antibacterial activity of date seed extracts. In summary, we prepared stock solutions of date seed oils from the two date varieties at 10 mg/mL. We prepared subsequent concentrations of 5 mg/mL, 2.5 mg/mL, 1.25 mg/mL, 0.625 mg/mL, and 0.3125 mg/mL by serial dilution. Subsequently, 80 µL of Mueller–Hinton broth (MHB) and the seed oil extracts at the different concentrations (10, 5, 2.5, 1.25, 0.625, and 0.3125 mg/mL) were added to the wells of a 96-well microtiter plate. Finally, add 20 µL of the bacterial suspension prepared for each isolate to each well. A positive control (broth containing only the bacterial inoculum) and a negative control (broth containing only the extract) were placed in the control wells. The plates were incubated for 24 hours at 37°C. After incubation, a microplate reader was used to measure the optical density at 600 nm (OD₆₀₀) to assess bacterial growth. The lowest extract concentration at which no discernible bacterial growth occurred was known as the minimum inhibitory concentration, or MIC ⁽¹⁴⁾.

2.4.2: Agar well diffusion assay

The antibacterial efficacy of the extracts was assessed using the agar well diffusion technique. In summary, 0.1 mL of each of the eight bacterial isolates was introduced into sterile test tubes and cultured overnight. Mueller-Hinton agar was formulated, sterilized using autoclaving, and then dispensed into sterile Petri dishes. Following solidification, bacterial suspensions were uniformly distributed across the agar surface utilizing sterile

cotton brushes. Wells of approximately 6 mm in diameter were subsequently formed using a sterile cork borer. Each well contained 50 µL of the date extracts at a concentration of 100 mg/ml. All plates were incubated at 37°C for 24 hours, and the diameters of the resultant inhibition zones were measured to evaluate antibacterial activity ⁽⁹⁾.

3. RESULTS AND DISCUSSION

We identified 50 bacterial isolates using Gram staining, biochemical testing, and confirmation via the Vitek system, as summarized in Table 1. Most isolates (86%, n=43) were Gram-negative, highlighting the predominance of these pathogens in the samples studied, while Gram-positive bacteria comprised the remaining 14% (n=7) (Figure 1).

Table 1: Identification of isolates according to some phenotypic characteristics

Biochemical tests	<i>E. coli</i>	<i>K. pneumoniae</i>	<i>P. aeruginosa</i>	<i>B. cepacia</i>	<i>A. baumannii</i>	<i>S. aureus</i>	<i>S. agalactiae</i>	<i>E. faecalis</i>
Catalase	+ Ve	+ Ve	+ Ve	+ Ve	+ Ve	+ Ve	- Ve	- Ve
Oxidase	- Ve	- Ve	+ Ve	+ Ve	- Ve	- Ve	- Ve	- Ve
Voges-Proskauer (VP)	- Ve	+ Ve	- Ve	- Ve	- Ve		- Ve	- Ve
Methyl Red (MR)	+ Ve	- Ve	- Ve	- Ve	- Ve		- Ve	- Ve
Citrate Test	- Ve	+ Ve	+ Ve	+ Ve	+ Ve	- Ve	- Ve	- Ve
Gram Stain – Shape	Rod	Rod	Rod	Rod	Rod	Cocci	Cocci	Cocci

+Ve = Positive - Ve = Negative

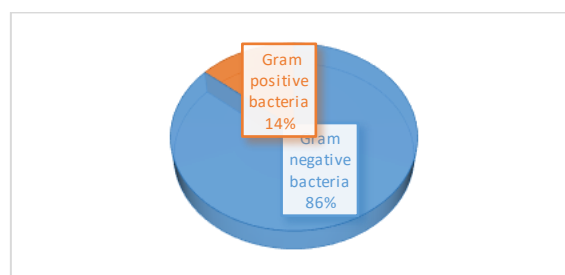


Fig. 1: Classification of bacterial isolates as Gram-positive or Gram-negative (n = 50).

In the present study, Gram-negative bacteria accounted for 86% of all bacterial isolates, demonstrating their predominance in urinary tract infections (UTIs). This is consistent with previous findings from Duhok Province, where ⁽¹⁸⁾ reported that 84.9% of uropathogens isolated from female UTI patients were Gram-negative, with *Escherichia coli* being the most common isolate (58.5%). Although the Mosul-based study by ⁽¹⁹⁾ focused on antibiotic resistance patterns in *E. coli*, it also

supported the high occurrence of Gram-negative uropathogens. These findings collectively emphasize the consistent predominance of Gram-negative bacteria, especially members of the Enterobacterales family.

3.1: The prevalence of pathogenic bacteria isolated from clinical samples

3.1.1: Bacterial Isolates from Urine Samples

total of 33 clinical urine isolates were analyzed for bacterial identification. *Escherichia coli* was the most frequently isolated organism, accounting for 60% (n = 20) of all samples. This was followed by *Klebsiella pneumoniae* (12%, n = 4), *Pseudomonas aeruginosa* and *Burkholderia cepacia* (9% each, n = 3), *Streptococcus agalactiae* (6%, n = 2), and *Enterococcus faecalis* (3%, n = 1). These findings show a clear predominance of Gram-negative bacteria (90.9%) in the urine samples compared with Gram-positive isolates (9.1%), as illustrated in Figure 2.

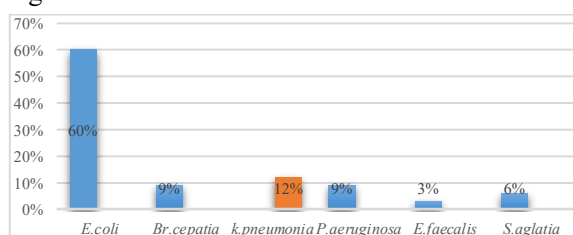


Fig. 2: Distribution of bacterial isolates from clinical urine samples (n = 33).

In the present study, *Escherichia coli* was the most prevalent isolate in urine samples, accounting for 60% of the total (n = 20/33), followed by *Klebsiella pneumoniae* (12%), *Pseudomonas aeruginosa* and *Burkholderia cepacia* (each 9%), *Streptococcus agalactiae* (6%), and *Enterococcus faecalis*. These findings align with global epidemiological data, where *E. coli* remains the primary uropathogen because of its virulence factors and ability to colonize the urinary tract (20,21). Our findings closely align with a study conducted in Karbala, Iraq, which reported *E. coli* as the most frequent pathogen in UTIs (42%) and *K. pneumoniae* at 20% (22). Similarly, a study conducted in Mosul city characterized multidrug-resistant *Escherichia coli* and *Klebsiella pneumoniae* isolates from women with urinary tract infections, confirming the clinical

significance and prevalence of these pathogens in Iraq (23). Research from Rizgary Hospital in Erbil confirmed that *E. coli* and Gram-negative bacilli were the leading isolates from urine samples (24).

The notable presence of *P. aeruginosa* and *B. cepacia* (each 9%) in this study reflects the emergence of opportunistic and nosocomial pathogens in urinary infections, especially among immunocompromised individuals. Although Gram-positive bacteria such as *S. agalactiae* and *E. faecalis* showed lower prevalence, their identification reinforces their ongoing clinical relevance in complicated or recurrent UTIs.

3.1.2: Bacterial Isolates from Wound Samples

Among the eight bacterial isolates obtained from wound samples, *Burkholderia cepacia* was most prevalent (38%, 3/8), followed by *Pseudomonas aeruginosa* (25%, 2/8), and *Escherichia coli*, *Klebsiella pneumoniae*, and *Staphylococcus aureus* each at 13% (1/8 each) (Figure 3). These findings highlight the significant role of Gram-negative bacilli in wound infections.

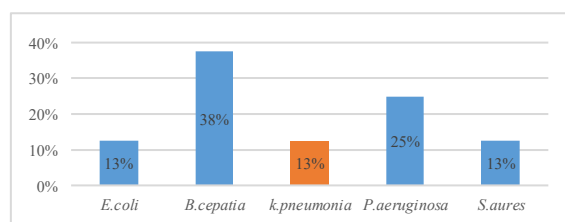


Fig. 3: Distribution of bacterial isolates from clinical wound samples (n = 8).

The predominance of *B. cepacia* is particularly notable. Although data on wound-specific isolates is limited, *B. cepacia* has been increasingly identified in various clinical settings across Iraq, including hospital-acquired infections, signaling its emerging clinical relevance (25). *P. aeruginosa*'s substantial presence (25%) aligns with its well-documented role in chronic and nosocomial wound infections globally and within Iraq.

A study from Duhok indicates significant antimicrobial resistance among *P. aeruginosa* isolates in burn and wound cases (26). The detection of enteric bacteria, notably *Escherichia coli* and *Klebsiella pneumoniae*, in wound and burn specimens likely reflects contamination sources or

underlying patient-related risk factors. For instance, in a study from Misan City, Iraq, *E. coli* and *K. pneumoniae* were present in 7.6% and 6.6% of burn wound isolates, respectively (27). The consistent presence of *S. aureus* (13%) parallels regional studies highlighting its frequent isolation from wound and burn samples and its status as a prevalent multidrug-resistant, biofilm-forming pathogen (28).

3.1.3: Bacterial Isolates from Sputum Samples

We recovered 9 bacterial isolates from sputum specimens. *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Klebsiella pneumoniae* were the most frequently detected species, each accounting for 22.2% of the total isolates ($n = 2$) (Figure 4). *Enterococcus faecalis*, *Escherichia coli*, and *Acinetobacter baumannii* each accounted for 11.1% ($n = 1$) of the isolates. These results indicate a mixed profile of Gram-positive and Gram-negative respiratory pathogens.

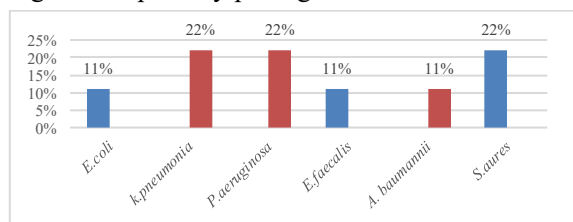


Fig. 4: Distribution of bacterial isolates recovered from clinical sputum samples ($n = 9$)

In the present study, *Klebsiella pneumoniae* was isolated from sputum specimens at a notable prevalence consistent with previous reports from Al-Najaf City, where 31.9% of sputum samples from patients with community-acquired pneumonia yielded *K. pneumoniae*. Of these, 25.2% produced extended-spectrum β -lactamases (ESBL), showing resistance to most β -lactams but remaining fully susceptible to imipenem and norfloxacin (29). Our data show *Pseudomonas aeruginosa* and *Staphylococcus aureus*, each accounting for 22% of sputum isolates, reflecting their well-documented roles in respiratory infections. The prevalence of *S. aureus* aligns with the 3.6–4.1% range reported from Zakho and Duhok hospitals (30), indicating a consistent presence across regions.

Escherichia coli and *Acinetobacter baumannii* were each isolated in 11% of sputum samples,

highlighting the polymicrobial nature of lower respiratory tract infections and the diversity of bacterial pathogens involved in this Iraqi population. These findings emphasize the necessity for continued surveillance and antimicrobial stewardship to guide effective management of respiratory infections.

3.2: Sensitivity test of bacterial isolates

Antibiotic susceptibility testing of 50 isolates revealed substantial levels of multidrug resistance. Tetracycline demonstrated the highest resistance rate at 96% of isolates, followed by Ampicillin and ciprofloxacin, each with 58% resistance isolates cefazolin (40%), levofloxacin (40%), trimethoprim/sulfamethoxazole (32%), nitrofurantoin (28%), cefepime (28%), and ceftriaxone (24%) each of piperacillin and gentamicin were (20%), (20%), also for each of ceftazidime and ceftazidime were (16%) of isolates. Notably, resistance to critical last-line antibiotics such as imipenem was (10%), and resistance to colistin, Amikacin, and moxifloxacin was (8%) among isolates, remaining relatively limited. Tigecycline showed the lowest resistance rate (4%). The relatively low resistance rates observed for critical last-line antibiotics such as imipenem, colistin, amikacin, and tigecycline may be attributed to their restricted and judicious use in clinical practice. These antibiotics are often reserved for treating severe or multidrug-resistant infections, limiting the selective pressure for resistance development. Additionally, strict antibiotic stewardship programs and controlled prescribing practices in the studied healthcare settings may have helped preserve these agents' efficacy. However, continued surveillance is essential to monitor potential emergence of resistance. Figure 5 summarizes these findings. Among the tested isolates, 64% ($n=32$) exhibited multidrug-resistant (MDR) profiles, while 36% ($n=18$) were classified as sensitive. This highlights the serious threat posed by MDR isolates and the urgent need for alternative therapeutic options to manage resistant infections.

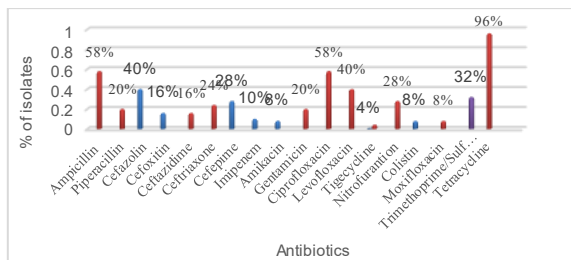


Fig. 5: Antibiotic resistance profile of bacterial isolates

A notable finding in the present study is the high proportion of multidrug-resistant (MDR) bacteria, accounting for 64% of the isolates, which raises significant concerns about empirical treatment. This rate is higher than that reported by (24), who examined bacterial pathogens isolated from urine specimens at Rizgary Hospital in Erbil and documented significant resistance to commonly used antibiotics such as ampicillin and tetracycline, highlighting the growing challenge of antimicrobial resistance in the region. However, the MDR prevalence observed in this study is comparable to findings from Mosul, where (19) reported 100% resistance to ampicillin and resistance exceeding 60% to several antibiotics among urinary isolates.

3.3: Antibacterial activity of *Phoenix dactylifera* against isolates

3.3.1: Minimum Inhibitory Concentration (MIC) of Seed Oil Extracts

The antibacterial potential of seed oils extracted from two *Phoenix dactylifera* cultivars, Ajwa and Breem, was quantitatively evaluated against multidrug-resistant Gram-negative and Gram-positive bacterial isolates using Minimum Inhibitory Concentration (MIC) assays (Tables 2 and 3). Remarkably, the polyphenolic-rich oil extracted from Ajwa seeds exhibited the most potent antimicrobial activity, consistently achieving the lowest MIC values of 5 mg/mL across all tested bacterial species. This uniform efficacy against both Gram-negative and Gram-positive isolates underscores its broad-spectrum antimicrobial potential.

In contrast, the Breem seed oils demonstrated a more variable inhibitory profile. While its polyphenol-rich oil matched Ajwa's potency for certain species such as *Escherichia coli* and

Acinetobacter baumannii (MIC = 5 mg/mL), it showed diminished activity against others, including *Pseudomonas aeruginosa* and *Burkholderia cepacia*, where MIC values increased to 10 mg/mL. The polyphenol-depleted oils from both cultivars uniformly required higher concentrations (10 mg/mL in most cases) to inhibit bacterial growth, reinforcing the critical role of polyphenolic compounds in antimicrobial efficacy. These findings collectively indicate that Ajwa's polyphenolic-rich seed oil (Table 2) is a superior candidate for natural antimicrobial development. The distinct variations in MIC values between Ajwa and Barhi (Table 3) further highlight how cultivar-specific phytochemical profiles influence antibacterial potency. This insight offers promising avenues for optimizing date seed oil extracts based on their polyphenolic content to combat multidrug-resistant bacterial infections effectively.

Table 2: Minimum inhibitory concentration (MIC) of oils extracted from *Phoenix dactylifera* (Ajwa seed) against multidrug-resistant Gram-negative and Gram-positive bacterial isolates

Type of Oil	Seed Type	<i>E. coli</i>	<i>K. pneumoniae</i>	<i>P. aeruginosa</i>	<i>B. cepacia</i>	<i>A. baumannii</i>	<i>S. aureus</i>	<i>S. agalactiae</i>	<i>E. faecalis</i>
MIC mg/mL									
Polyphenol-depleted oil	Ajwa Seed	10	10	10	10	10	10	10	10
Polyphenolic-rich oil	Ajwa Seed	5	5	5	5	5	5	5	5

Table 3: Minimum inhibitory concentration (MIC) of oils extracted from *Phoenix dactylifera* (Breem seed) against multidrug-resistant Gram-negative and Gram-positive bacterial isolates

Type of Oil	Seed Type	<i>E. coli</i>	<i>K. pneumoniae</i>	<i>P. aeruginosa</i>	<i>B. cepacia</i>	<i>A. baumannii</i>	<i>S. aureus</i>	<i>S. agalactiae</i>	<i>E. faecalis</i>
MIC mg/mL									
Polyphenol-depleted oil	Breem Seed	10	10	5	10	5	10	10	10
Polyphenolic-rich oil	Breem Seed	5	5	10	10	5	10	5	5

3.3.2: Well Diffusion Method of Seed Oil Extracts

The antibacterial activity of seed oils extracted from *Phoenix dactylifera* cultivars Ajwa and Breem was further evaluated using the well diffusion method

against eight multidrug-resistant bacterial isolates, as summarized in Tables 4 and 5. Both polyphenol-depleted and polyphenol-rich oils produced measurable inhibition zones across the tested species, confirming their antimicrobial potential.

Acinetobacter baumannii showed the largest inhibition zone (13 mm) in Figure 6 when treated with Breem's polyphenol-depleted oil, highlighting particularly strong susceptibility. In general, inhibition zones ranged from 8 to 10 mm for most isolates treated with Ajwa seed oils, indicating moderate but consistent activity across Gram-negative and Gram-positive bacteria.

Conversely, Breem's polyphenolic-rich oil showed variable efficacy, with weaker inhibition observed for several isolates, particularly *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, and some Gram-positive species. This suggests that while the polyphenolic content is critical for antimicrobial action, factors such as oil diffusion in agar may influence the observed activity. Indeed, the hydrophobic nature of the oils may limit diffusion within the agar medium, potentially underestimating their true antibacterial potency when assessed via well diffusion assays. Overall, these results corroborate the promising antibacterial properties of date seed oils, especially those derived from Ajwa seeds, while also emphasizing the need for complementary methods to assess their antimicrobial efficacy accurately.

Table 4: Inhibitory zone diameters of oils extracted from *Phoenix dactylifera* (Ajwa seed) against multidrug-resistant Gram-negative and Gram-positive bacterial isolates

Type of oils	Seed Type	<i>E. coli</i>	<i>K. pneumoniae</i>	<i>P. aeruginosa</i>	<i>Br. cepacia</i>	<i>A. baumannii</i>	<i>S. aureus</i>	<i>Strep. agalactiae</i>	<i>E. faecalis</i>
Inhibition zone diameters (mm)									
Polyphenol-depleted oil	Ajwa Seed	9	10	10	8	8	8	8	9
Polyphenolic-rich oil	Ajwa Seed	9	9	9	10	9	8	8	9

Table 5: Inhibitory zone diameters of oils extracted from *Phoenix dactylifera* (Breem seed) against multidrug-resistant Gram-negative and Gram-positive bacterial isolates

Type of oils	Seed Type	<i>E. coli</i>	<i>K. pneumoniae</i>	<i>P. aeruginosa</i>	<i>Br. cepacia</i>	<i>A. baumannii</i>	<i>S. aureus</i>	<i>Strep. agalactiae</i>	<i>E. faecalis</i>
Inhibition zone diameters (mm)									
Polyphenol-depleted oil	Breem Seed	10	8	9	8	13	10	10	8
Polyphenolic-rich oil	Breem Seed	9	8	Weak inhibition.	8	Weak inhibition.	Weak inhibition.	Weak inhibition.	8

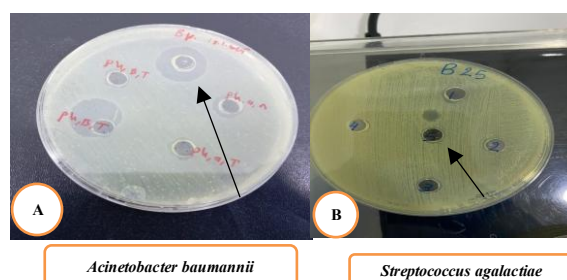


Fig. 6: Well diffusion assay shows (A) *Acinetobacter baumannii* and (B) *Streptococcus agalactiae* of Breem seed oil

The results show that most seed oil extracts have potential antibacterial activity against the isolated bacteria. A study by ⁽³¹⁾ on Zahidi date palm seed oil reported MIC values ranging from 10 to 42 mg/ml against non-resistant strains such as *Staphylococcus aureus* and *Escherichia coli*; in contrast, the present study demonstrated significantly lower MIC values (5–10 mg/ml) against multidrug-resistant (MDR) bacteria, highlighting the superior antimicrobial potential of Ajwa and Breem date oil extracts. On the other hand, a study by ⁽³²⁾ investigated the effect of a methanolic seed extract against several *E. coli* and reported a MIC value ranging between 10 and 19.9 mg/ml. However, another study ⁽¹⁴⁾ showed stronger MIC values against certain bacterial strains (10–15 µl/ml). An extensive review of the literature indicates a notable scarcity of studies investigating the antimicrobial effect of date seed oil using the broth dilution method.

These findings support the potential of Ajwa and Breem date seed oils as promising natural antimicrobial agents. In our current study, the most notable antibacterial effect was observed against *Acinetobacter baumannii*, an MDR strain, with an

inhibition zone diameter of 13 mm. Other isolates displayed inhibition zones ranging from 8 to 10 mm. These findings are consistent with ⁽²⁰⁾, where ethanolic extracts produced inhibition zones of 12 mm, while aqueous extracts reached only 8 mm, highlighting ethanol's superior extraction efficiency for bioactive compounds. This variation likely reflects the solubility of active phytochemicals in ethanol compared with water. Conversely, ⁽⁹⁾ documented significantly stronger inhibition zones exceeding 17 mm against *P. aeruginosa*. Inconsistencies in inhibition zones across studies may stem from differences in extraction techniques, solvent types, and bacterial strain variability ⁽¹⁴⁾. Ethanol, a commonly used solvent in phytochemical research, is favored for its low toxicity and widespread use in industries including food, cosmetics, and pharmaceuticals ⁽⁹⁾. The observed antimicrobial effects are likely attributed to the rich phytochemical profile of the extracts, particularly phenolics, flavonoids, and tannins, which are known to disrupt microbial membranes, inhibit enzymes, and interfere with nucleic acid synthesis ^(14, 33, 34). The oil extracts showed poor diffusion in the agar, which may have contributed to the smaller inhibition zones recorded with the well diffusion method. Interestingly, when the extracts were placed directly on the surface, the inhibition zones were much larger, indicating that direct contact can strengthen their antimicrobial effect. This limited diffusion in the agar wells is likely linked to the oil's hydrophobic nature, which makes it harder for it to spread through the medium ⁽³⁵⁾. This underscores the need to explore alternative delivery systems, such as nanoemulsions or encapsulation technologies, to enhance dispersion and bioavailability.

Furthermore, the specific phytochemicals responsible for the antibacterial activity remain unidentified. Therefore, future studies should focus on comprehensive phytochemical analyses, in vivo testing to assess therapeutic safety and efficacy, and the potential synergistic effects of combining date

seed extracts with conventional antibiotics to enhance their action against MDR pathogens.

4. CONCLUSION

Our study highlights the potential of *Phoenix dactylifera* seed polyphenol-depleted oil and polyphenol-rich oil as effective antibacterial agents. Incorporating them into antimicrobial strategies could help address the global challenge of antibiotic resistance, underscoring the need for continued research and development in this field.

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