

Original Article

Antibacterial Activity of the Extracts of Dates and Their Leaves Against some Pathogenic Bacterial Isolates

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Abstract

Background: Many plant-derived natural products, including fruits, fruit skins, seeds, and barks, have been studied for their antibacterial properties. This study was prompted by the global increase in antibiotic-resistant bacterial strains, which pose a growing challenge to global health. Natural phytochemicals are being explored as potential alternatives to conventional antibiotics. This study evaluated the antibacterial activity of five types of dates (Ajwa, Maryam, Sagai, Safawi, and Amber) and date leaves against five clinical isolates, including *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Enterococcus* spp.

Methods: To this end, aqueous crude homogenates were prepared by homogenizing fresh samples and tested using the agar well diffusion method. Additionally, sun-dried samples were powdered and extracted using ethanol, methanol, and water. Antibacterial activity was assessed against multidrug-resistant (MDR) clinical isolates.

Results: Among twenty-eight antibiotics tested, *P. aeruginosa* showed resistance to ten, and *Enterococcus* spp. to eight. Ethanol and methanol extracts exhibited significantly higher antibacterial activity compared to aqueous crude homogenates and aqueous extracts, with methanol extracts being the most effective. Aqueous extracts demonstrated the least antibacterial potential. Among all tested samples, Amber extracts displayed the highest antibacterial activity, while the other dates represented moderate but comparable results.

Conclusion: The ability of the extracts to inhibit MDR clinical isolates suggests their promising potential as alternative agents for treating infections caused by resistant bacteria.

Keywords: Antibacterial activity, Resistance, Extracts, MIC, MBC

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Introduction

Date palm (*Phoenix dactylifera* L.) corresponds to the Arecaceae family, and it is considered a major fruit in the Arabian Peninsula (1,2). Nearly 20 types of dates have been discovered so far, and this fruit is now cultivated in many other countries besides the Arabian Peninsula. Some of these countries include Australia, the United States of America (specifically California and Texas), Mexico, and Southern Africa (1,3). Date palms are considered a nutrition-rich fruit that has also been documented in the Islamic religious book of the Holy Quran (3). It contains many different kinds of vitamins (including vitamins A, C, B1, and B2, and nicotinic acid), minerals (zinc, cadmium, magnesium, sodium, calcium, and potassium), saturated fatty acids (e.g., stearic acid and palmitic acid), unsaturated fatty acids (e.g., linoleic acid and oleic acid), fiber, sugars, and amino acids (4-6). Their high phytochemical content has been linked to various biological properties, including

antioxidant, antimicrobial, anti-inflammatory, antiviral, and anticancer effects (7-18).

Treating infectious diseases has become a key alarm in recent years due to the development of multidrug-resistant (MDR) pathogenic microorganisms. Hence, antibiotic therapies often do not work, resulting in high morbidity and mortality rates across the world. Natural therapeutics are being sought to use against such drug-resistant microbes. Plants, fruits, and leaves can contain several distinct phytochemicals that can help combat the resistant forms of microbes (19,20). The antibiotics or any other synthetic drugs take a long time to be completely cleared out from the system after consumption, and occasionally impart some adverse side effects. Natural products are free from such problems, and being inexpensive is an additional benefit (1). The phytochemical content, which is responsible for antimicrobial activity, varies depending on the handling, storage, and extraction method. Thus,



the ultimate antimicrobial effects may vary with these differences in processing (21).

Although the antimicrobial potential of date fruits has been reported, studies often focus on a single or a few varieties (e.g., Barhee, Sukri, and Rothana), and very few explore the leaves, which are typically agricultural waste (22). In this study, five commercially important and widely consumed date varieties—Ajwa, Mariam, Amber, Safawi, and Sagai—have been selected along with their leaves. These varieties have been chosen based on their popularity in Middle Eastern traditional medicine, their distinct phytochemical profiles, and limited prior data on their comparative antibacterial efficacy, especially against clinical MDR strains.

This study attempts to uncover the antibacterial activity of five different date palms, namely, Ajwa, Mariam, Amber, Safawi, and Sagai, and the leaves of date palm trees. To our knowledge, this is one of the first comparative studies evaluating both fruit and leaf extracts for their antimicrobial activity against selected clinical MDR bacterial isolates. Extracts prepared using ethanol, methanol, and water were utilized, in addition to the aqueous crude homogenate samples, to determine their antimicrobial potency. The minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) values are assessed following the verification of the antibacterial activity of the extracts against selected MDR bacterial strains obtained from clinical specimens.

Materials and Methods

Study Location and Sampling Procedures

Five pathogenic bacterial isolates (*Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Enterococcus* spp.) were chosen to determine their inhibition by the antibacterial activity of natural phytochemicals. The antibacterial activity assays were performed using clinical bacterial isolates obtained from our laboratory collection. Standard reference strains (e.g., ATCC strains) were not used due to their unavailability. Five different date samples (Amber, Safawi, Mariyam, Sagai, and Ajwa) and the leaves of the date tree were obtained from various markets across Dhaka, Bangladesh. The research was conducted in the microbiology laboratory at the Department of Microbiology, Stamford University, Bangladesh, between September and December 2020.

Antibiotic Susceptibility Testing of Pathogenic Isolates

A total of twenty-eight frequently prescribed antibiotics were selected to evaluate the sensitivity of the clinical isolates, including amoxicillin (25 µg), azithromycin (15 µg), meropenem (10 µg), ceftazidime (30 µg), ciprofloxacin (5 µg), gentamicin (10 µg), amikacin (30 µg), cefixime (30 µg), cefuroxime (30 µg), cephradine (30 µg), and nitrofurantoin (300 µg). The remaining antibiotics were vancomycin (30 µg), teicoplanin (30 µg),

clotrimazole (30 µg), piperacillin/tazobactam (30 µg), colistin (30 µg), doxycycline (30 µg), fusidic acid (10 µg), amoxiclav (30 µg), imipenem (10 µg), linezolid (30 µg), doripenem (10 µg), tigecycline (15 µg), clindamycin (10 µg), levofloxacin (5 µg), cefepime (30 µg), nalidixic acid (30 µg), and ceftriaxone (30 µg). Antibiotic susceptibility testing was performed using the Kirby-Bauer disc diffusion method (23). The zones of inhibition were measured, and bacterial isolates were categorized as sensitive or resistant according to the Clinical and Laboratory Standards Institute guidelines (24).

Processing of Samples

Upon arrival at the laboratory, the samples were thoroughly rinsed with tap water, followed by multiple washes with distilled water to remove salts, soil, and other contaminants. Equal weights (10 g) of raw fruits were used for all extractions to ensure standardization. For aqueous crude homogenate preparation, each 10 g sample was homogenized with 90 mL of 0.85% saline solution.

For solvent extraction, the samples were chopped and air-dried at room temperature for 10 days until fully dehydrated. The dried samples were ground into fine powder and stored in airtight containers at room temperature until use. Equal amounts (5 g) of the powdered sample were extracted using different solvents (ethanol, methanol, and distilled water), each at a volume of 100 mL, under identical maceration conditions (72 hours at room temperature with occasional shaking). The extracts were filtered, concentrated under reduced pressure, and stored at 4 °C. The use of a fixed weight-to-volume ratio of 5 g of the powdered sample per 100 mL of the solvent follows commonly accepted protocols for crude plant extract preparation in phytochemical and antimicrobial studies (25). This method ensures consistent extraction conditions across all varieties and solvents. While concentration-based methods are standard for purified compounds, weight-to-volume ratios are preferred for comparative crude extract studies.

Although this study did not include phytochemical screening or standardization, the aim was to conduct a preliminary comparison of the antibacterial activity of the aqueous crude homogenates. Future studies will incorporate phytochemical profiling to help identify bioactive constituents responsible for observed effects.

Antibacterial Activity of Extracts (Crude, Ethanolic, Methanolic, and Aqueous)

Antibacterial activity was assessed using the agar well diffusion method. Positive control (Gentamicin 10 µg/disc) was included for comparison with the extracts, and an appropriate solvent blank (saline) was used as a negative control. The zones of inhibition were measured in mm to compare the efficacy of the extracts with standard antibiotics.

Bacterial suspensions were prepared by inoculating the isolates into normal saline and incubating them at

37 °C until the turbidity matched the McFarland standard (approximately 10^8 CFU/mL) (26). A bacterial lawn was then created on Mueller-Hinton agar plates using sterile cotton swabs for each bacterial strain separately. The wells were made in the agar, and 100 µL of crude, ethanolic, methanolic, and aqueous date extracts were introduced into the respective wells. The plates were incubated at 37 °C for 24 hours. After incubation, the zones of inhibition around the wells were measured in mm to evaluate antibacterial activity.

Sample sizes varied for each bacterial species, which are detailed in the figure legends ($n=2-5$). For instance, *K. pneumoniae* was represented by two isolates ($n=2$), which limits the statistical power for this species.

Positive and negative controls were included only in the agar well diffusion assays to confirm the responsiveness of bacterial isolates and validate the assay conditions. These controls ensured that observed zones of inhibition were attributable to antibacterial activity.

For MIC and MBC assays, controls were not included because these tests aimed specifically to quantify the potency of the crude plant extracts under investigation. Considering that the antibacterial activity of the extracts was already established and validated through the diffusion method with controls, the MIC and MBC determinations focused on measuring extract effectiveness without additional control antibiotics.

Determination of Minimal Inhibitory Concentration and Minimal Bactericidal Concentration

Sample extracts were diluted to concentrations of 500 mg/mL, 250 mg/mL, and 125 mg/mL using sterile nutrient broth. Overall, 0.2 mL of bacterial suspension was added to each dilution tube. The tubes were incubated at 37

°C for 24 hours, and the lowest concentration showing no visible bacterial growth was recorded as the MIC. Subsequently, loopful samples from the clear tubes were streaked onto fresh nutrient agar plates to determine the MBC, identified as the lowest extract concentration where no bacterial growth occurred.

All experiments were performed with biological replicates ($n=2-5$, specified in figure legends). The data are presented as means $\pm$ standard deviations (SD). Statistical analyses were conducted using RStudio.

To compare inhibition zones among different extracts and bacterial species, one-way analysis of variance (ANOVA) was performed, followed by Tukey's post-hoc test to identify significant pairwise differences. Where only two groups were compared, differences between extract types and bacterial strains were assessed for MIC and MBC values using ANOVA with appropriate post-hoc tests.

A *P* value of less than 0.05 was considered statistically significant. All statistical tests were two-tailed.

Results

Antibiotic Resistance Profile

Enterococcus spp. showed resistance to eight antibiotics, while *K. pneumoniae* exhibited intermediate susceptibility to three and resistance to one. *S. aureus* was resistant to three antibiotics. *P. aeruginosa* demonstrated the highest resistance, being resistant to 10 out of the 28 tested antibiotics. *E. coli* represented resistance to three antibiotics and intermediate resistance to one. Overall, the isolates were MDR, with *P. aeruginosa* and *Enterococcus* spp., representing the broadest resistance spectra, while the remaining species retained susceptibility to several antibiotics (Figure 1).

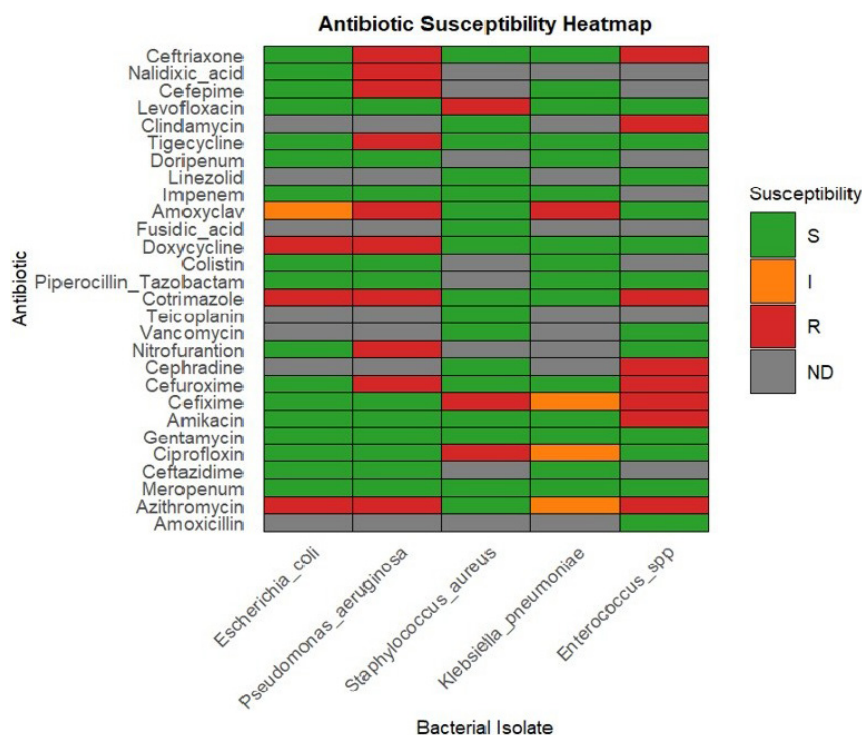


Figure 1. Antibiotic Susceptibility of Bacterial Isolates

Antimicrobial Activity – Aqueous Crude Homogenates

The antibacterial activity of aqueous crude homogenates was assessed by measuring the mean zone of inhibition (mm $\pm$ SD). For *E. coli*, Maryam and leaf extracts produced inhibition zones of 12.3 ± 2.08 mm and 12.0 ± 2.00 mm, respectively, compared to 10.3 ± 2.08 mm for Amber; these differences were not statistically significant ($P=0.486$). Similar non-significant differences ($P=0.502$) were observed for *S. aureus* across Amber (11.0 ± 1.41 mm), Maryam (10.8 ± 0.96 mm), Sagai (9.5 ± 1.91 mm), and leaf extracts (10.8 ± 1.50 mm). For *P. aeruginosa*, *K. pneumoniae*, and *Enterococcus* spp., only the leaf extract was tested, resulting in moderate inhibition; however, a statistical comparison was limited by small replicate numbers. Overall, no statistically significant differences were found among aqueous crude homogenates (Figure 2).

Solvent-Based Extract Efficacy

Ethanol, methanol, and aqueous extracts were further evaluated for antibacterial potency. The ethanol extract of Amber inhibited *E. coli* with a mean zone of 26.33 ± 3.05 mm, which was the largest inhibition zone observed among ethanol extracts for this species. *P. aeruginosa* was most inhibited by the methanol extract of Safawi, producing a zone of 12.0 ± 2.83 mm. Aqueous extracts consistently showed low or no inhibition zones (<10 mm). For *S. aureus*, the ethanol extract of Sagai displayed the greatest inhibition zone (13.75 ± 1.26 mm). *K. pneumoniae* was most susceptible to the Safawi methanol extract, with an inhibition zone of 28.0 ± 2.83 mm. *Enterococcus* spp. exhibited inhibition zones ranging from 29.0 ± 1.41 mm

to 33.0 ± 4.24 mm for ethanol and methanol extracts. No inhibition was recorded for negative controls, validating experimental controls. In general, ethanol extracts, particularly Amber and Sagai, were most effective against *E. coli* and *S. aureus*, while methanol extracts—especially Safawi—were effective against *P. aeruginosa* and *K. pneumoniae* (Table 1, Figure 3). Biological replicates ranged from 2 to 5, depending on species, with *K. pneumoniae* represented by only two isolates ($n=2$), limiting statistical power for this species.

Antibacterial Activity of Various Date Extracts Against Five Bacterial Species

Bar plots represent the mean zone of inhibition (mm) $\pm$ SD for each extract type. The extracts from six types of date samples (Ajwa, Amber, Safawi, Maryam, Sagai, and leaf) were tested using three solvents (ethanol, methanol, and aqueous). The antibacterial effect was assessed against *E. coli*, *P. aeruginosa*, *S. aureus*, *K. pneumoniae*, and *Enterococcus* spp. Error bars indicate the SD from biological replicates ($n=2-5$). Each bacterial species is color-coded, and legends are arranged below the plot for clarity.

Minimum Inhibitory Concentration

Extract concentrations were evaluated to determine the MIC, defined as the lowest concentration at which no visible turbidity was observed ($OD_{600} \leq 0.1$ or no turbidity visible by eye). For ethanol extracts, the visible inhibition of *P. aeruginosa* and *K. pneumoniae* occurred at $500 \mu\text{g/mL}$ for Amber, and similarly for Ajwa (*P. aeruginosa*, *S.*

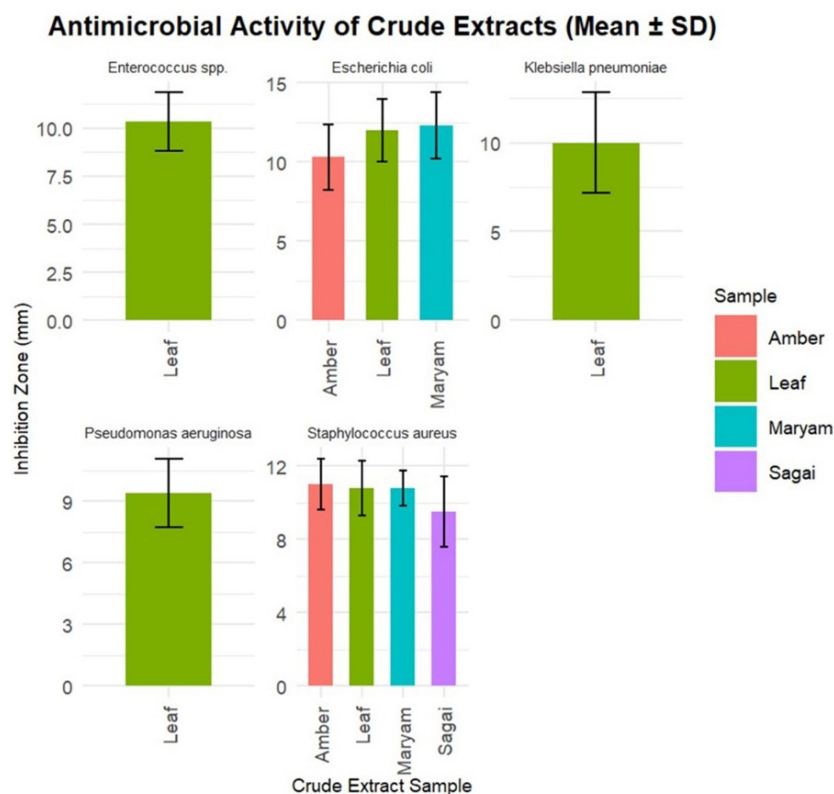


Figure 2. Antibacterial Activity of Crude Extracts

Table 1. Antibacterial Effects of Ethanol, Methanol, and Aqueous Extracts (100 µL) From Date Fruit and Leaf Samples Against Pathogenic Bacteria (Zone of Inhibition in mm, Mean±SD)

Samples/ Isolates	Amber			Safawi			Maryam			Sagai			Ajwa			Leaf			Positive Control	Negative Control
	Ethanol	Methanol	Aqueous	Ethanol	Methanol	Aqueous	Ethanol	Methanol	Aqueous	Ethanol	Methanol	Aqueous	Ethanol	Methanol	Aqueous	Ethanol	Methanol	Aqueous		
<i>Escherichia coli</i> (n=3)	26.33±3.05	10.00±2.00	7.67±0.58	9.67±2.08	10.00±1.00	7.67±1.53	11.33±2.08	9.67±1.53	0	12.00±2.00	9.00±1.00	7.33±1.53	9.33±1.53	13.00±2.65	8.33±0.58	9.33±2.52	11.00±3.00	6.33±1.53	21.00±3.61	0
<i>Pseudomonas aeruginosa</i> (n=5)	10.40±1.97	10.60±2.45	3.80±2.17	9.00±2.94	3.6±3.51	0	10.60±2.07	12.0±2.83	6.67±1.53	9±1	10±2.16	7±1.41	11.4±2.19	5.4±4.98	2.8±3.9	7.8±4.49	2.6±3.72	0	20.8±2.28	0
<i>Staphylococcus aureus</i> (n=4)	12±2.94	11.25±1.5	3.25±3.77	10±1.63	9.75±2.06	3±3.56	11.75±2.75	11.25±1.89	8±1.63	13.75±1.26	10.25±2.87	9.25±0.95	9.75±2.36	9.75±2.06	7.5±1.29	10.75±0.95	11.25±2.22	3.5±4.12	24.25±3.30	0
<i>Klebsiella pneumoniae</i> (n=2)	12±2.83	28±2.83	8.5±2.12	13±1.41	12.5±3.54	7.5±2.12	13±1.41	0	0	13±2.83	0	0	14±1.41	16±1.41	8.5±2.12	9±1.41	28.5±2.12	9.5±2.12	29±4.24	0
<i>Enterococcus</i> <i>spp.</i> (n=2)	10.5±2.12	29±1.41	7±1.41	13.5±2.12	32.5±3.54	11±1.41	9±1.41	13.5±2.12	8.5±2.12	13.5±2.12	29±1.41	11.5±2.12	16±1.41	16.5±2.12	9.5±2.12	12±2.83	9.5±0.71	5.5±2.12	33±4.24	0

Note. *** Ethanol-ethanol solvent-based extract, methanol-methanol solvent-based extract, aqueous-water-based extract.

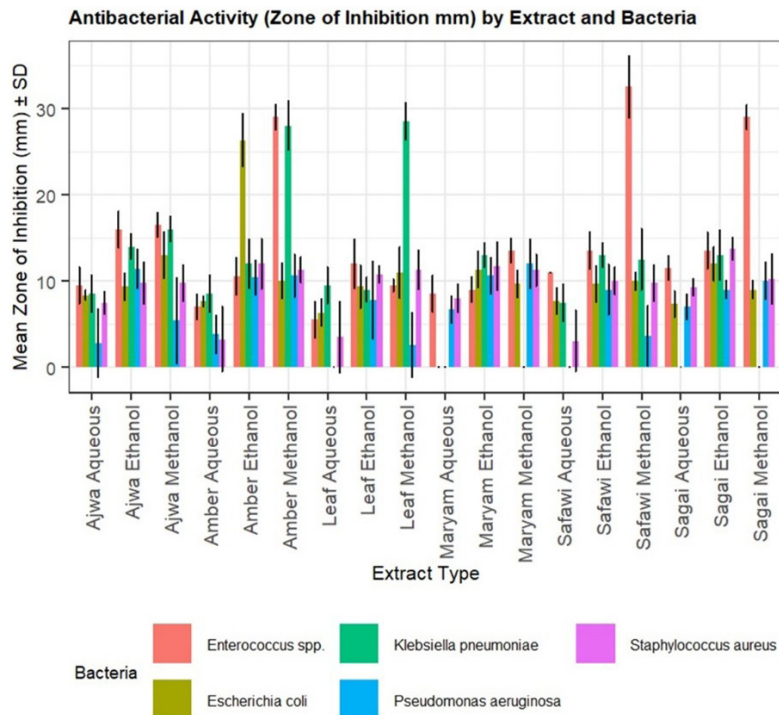


Figure 3. Antibacterial Activity of Different Extracts Against Different Bacteria

aureus, and *Enterococcus* spp.). Leaf extracts also inhibited *S. aureus* and *Enterococcus* spp. at this concentration. The Sagai and Maryam ethanol extracts inhibited bacterial growth as early as 250 µg/mL (Figure 4). Methanol

extracts showed greater potency, with MICs as low as 125 µg/mL for Maryam, Safawi, and Ajwa against *E. coli*. The same MIC was noted for Sagai (*K. pneumoniae*, *P. aeruginosa*, and *S. aureus*), Maryam (*P. aeruginosa*), and

Ajwa (*K. pneumoniae*). Notably, *P. aeruginosa* required a higher MIC (1000 µg/mL) for the Safawi methanol extract (Figure 5). Aqueous extracts generally displayed higher MICs (≥ 375 µg/mL) or no inhibition at concentrations up to 1000 µg/mL, particularly for *P. aeruginosa* with the Safawi extract (Figure 6). Sample sizes for MIC assays are specified in figure legends; *K. pneumoniae* data were

limited ($n = 2$), which may affect statistical interpretation.

Quantitative MIC assessment confirmed the lowest MIC (187.5 µg/mL) for ethanol extracts of Maryam (*E. coli*, *P. aeruginosa*, and *Enterococcus* spp.) and Sagai (*E. coli*, *K. pneumoniae*, and *Enterococcus* spp.). Comparable MICs were recorded for methanol extracts of Maryam (*E. coli* and *P. aeruginosa*), Sagai (all tested bacteria except

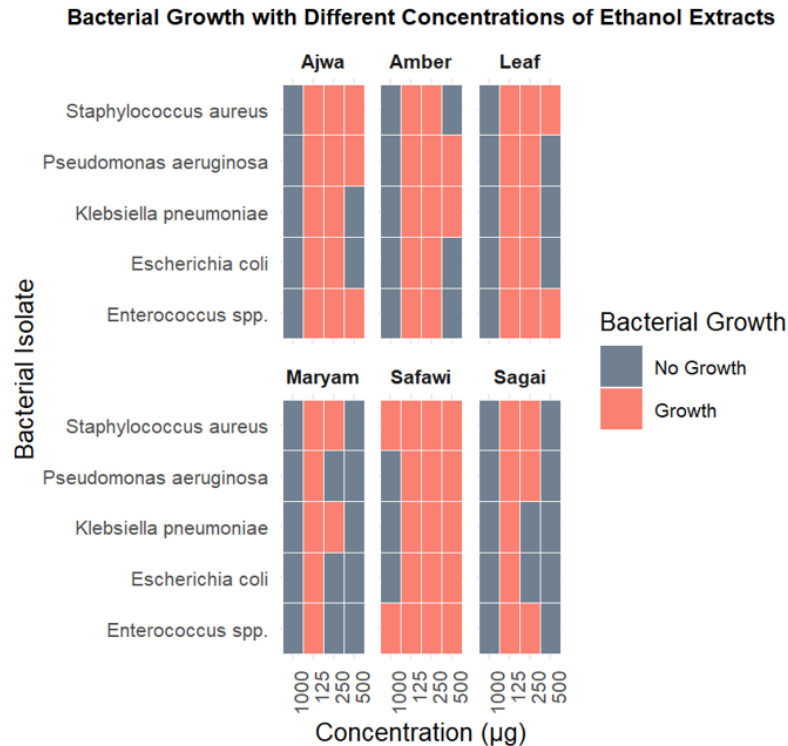


Figure 4. Bacterial Growth with Different Concentrations of Ethanol Extracts of Date Fruits and Leaves

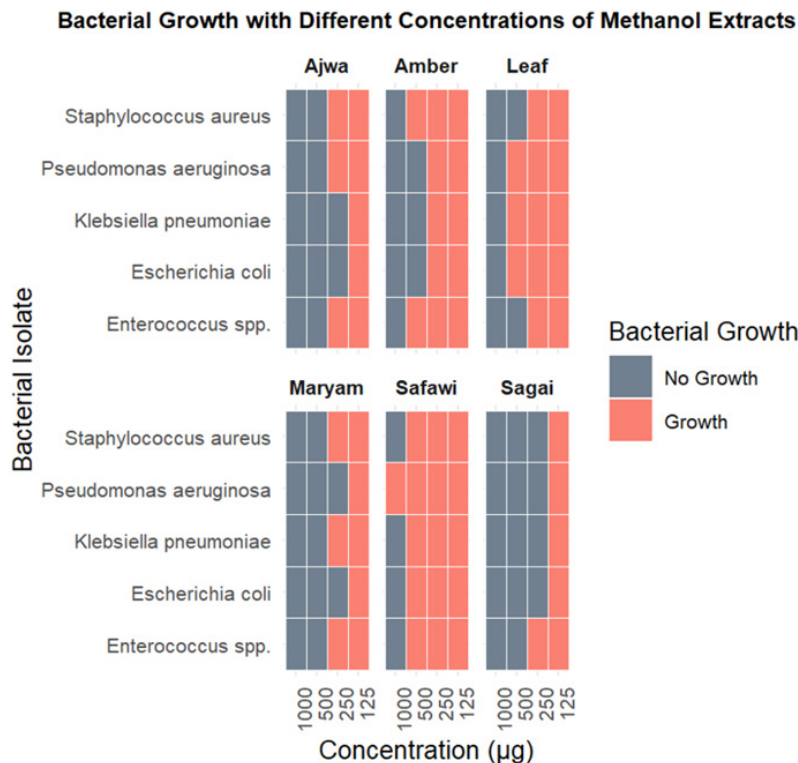


Figure 5. Bacterial Growth with Different Concentrations of Methanol Extracts of Date Fruits and Leaves

Bacterial Growth on Different Concentrations of Aqueous Extracts

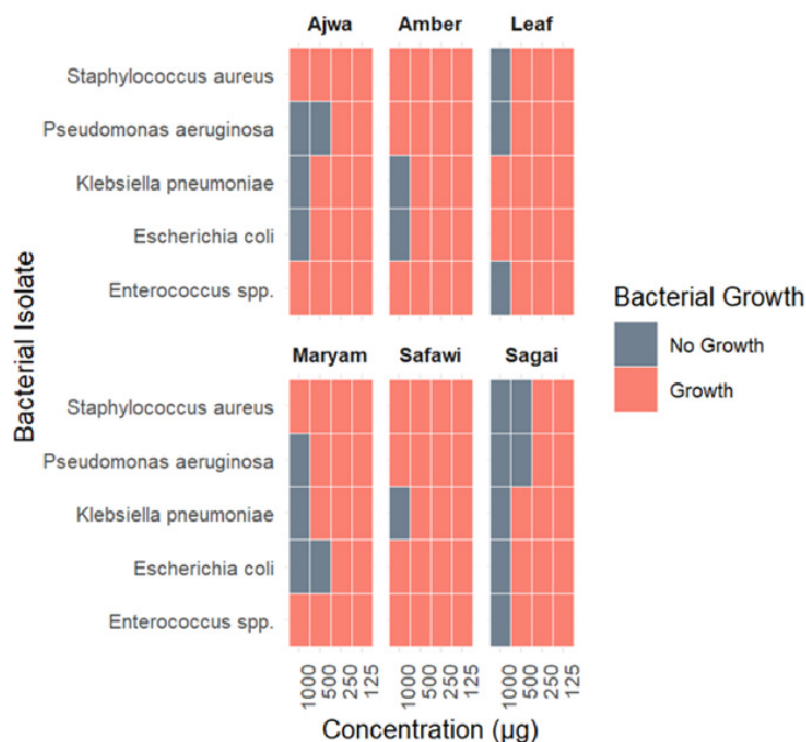


Figure 6. Bacterial Growth with Different Concentrations of Aqueous Extracts of Date Fruits and Leaves

Enterococcus spp.), and Ajwa (*E. coli* and *K. pneumoniae*). In contrast, aqueous extracts generally had MIC values ≥ 375 $\mu\text{g/mL}$ or no detectable inhibition at tested concentrations (Table 2, Figure 7).

Statistical analysis revealed significant differences in MIC values among extract types and bacterial species ($P < 0.001$), supporting the superior antibacterial efficacy of ethanol and methanol extracts compared to aqueous extracts. *K. pneumoniae* demonstrated no significant difference between methanol and aqueous extracts ($P > 0.05$).

Minimum Bactericidal Concentration

Bactericidal activity, measured by MBC, was detected only for ethanol and methanol extracts; aqueous extracts did not exhibit bactericidal effects at the tested concentrations. The ethanol extract of Maryam showed the lowest MBC compared to *P. aeruginosa* at 350 ± 111.8 $\mu\text{g/mL}$. Significant differences ($P < 0.05$) in MBC values were observed for *P. aeruginosa* between Maryam versus Ajwa, Sagai versus Ajwa, and Maryam versus leaf extracts. For *E. coli*, *S. aureus*, *K. pneumoniae*, and *Enterococcus* spp., no significant differences in MBC were found across extracts. Maryam, Sagai, and Amber extracts consistently demonstrated lower MBC values, indicating stronger bactericidal activity (Table 3, Figure 8).

Discussion

To contextualize the novelty of our study, we compiled a comparative summary of previous research on the antimicrobial activity of *P. dactylifera* (Table 4). Most

prior studies focused on fruit or seed extracts, used basic diffusion methods without MIC/MBC quantification, tested a limited range of bacterial species, rarely included multiple varieties, and did not assess leaf extracts or MDR clinical isolates.

In contrast, our study is the first to evaluate five named varieties of *P. dactylifera* along with their leaf extracts, using aqueous crude homogenates and solvent-based extractions against the MDR clinical isolates of *S. aureus*, *E. coli*, *K. pneumoniae*, *P. aeruginosa*, and *Enterococcus* spp., reporting quantitative MIC and MBC values. These pathogens cause serious infections and exhibit increasing resistance due to factors such as unregulated antibiotic use (43–50).

Our antimicrobial susceptibility tests confirmed high resistance, particularly in *P. aeruginosa* and *Enterococcus* spp. Ethanol and methanol extracts demonstrated stronger antibacterial activity than aqueous extracts, likely due to the improved extraction of phenolics and flavonoids. Among varieties, Maryam, Sagai, and Amber showed potent activity, with MICs as low as 187.5 $\mu\text{g/mL}$ and significant bactericidal effects—especially Maryam ethanol extract against *P. aeruginosa* (MBC 350 ± 111.8 μL). The Safawi methanol extract was most effective against *K. pneumoniae*. Statistical analyses highlighted the influence of solvent and variety on efficacy.

Our findings are consistent with those of earlier studies, demonstrating the antibacterial properties of date extracts (51) and further advancing this understanding by including MDR pathogens and leaf-derived extracts. The

Table 2. Minimum Inhibitory Concentration of Different Extracts (Measured as Means $\pm$ SD) Against Five Pathogens (Concentrations in $\mu\text{g/mL}$)

Samples/ Isolates	Amber			Safawi			Maryam			Sagai			Ajwa			Leaf		
	Ethanol	Methanol	Aqueous	Ethanol	Methanol	Aqueous	Ethanol	Methanol	Aqueous	Ethanol	Methanol	Aqueous	Ethanol	Methanol	Aqueous	Ethanol	Methanol	Aqueous
<i>Escherichia coli</i> (n=3)	375 $\pm$ 0	375 $\pm$ 0	750 $\pm$ 0	750 $\pm$ 0	750 $\pm$ 0	0	187.5 $\pm$ 0	187.5 $\pm$ 0	375 $\pm$ 0	187.5	187.5 $\pm$ 0	750 $\pm$ 0	375 $\pm$ 0	187.5 $\pm$ 0	750 $\pm$ 0	375 $\pm$ 0	750 $\pm$ 0	0
Significance		***			***			***			***			***			***	
<i>Pseudomonas aeruginosa</i> (n=5)	750 $\pm$ 0	375	0	750 $\pm$ 0	0	0	187.5 $\pm$ 0	187.5 $\pm$ 0	750	$\pm$ 0375	187.5 $\pm$ 0	375 $\pm$ 0	750 $\pm$ 0	375 $\pm$ 0	375 $\pm$ 0	375 $\pm$ 0	750 $\pm$ 0	750 $\pm$ 0
Significance		***			***			***			***			***			***	
<i>Staphylococcus aureus</i> (n=4)	375 $\pm$ 0	750 $\pm$ 0	0	0	750 $\pm$ 0	0	375 $\pm$ 0	375	$\pm$ 00	375 $\pm$ 0	187.5 $\pm$ 0	375 $\pm$ 0	750 $\pm$ 0	375 $\pm$ 0	0	750 $\pm$ 0	375 $\pm$ 0	750 $\pm$ 0
Significance		***			***			***			***			***			***	
<i>Klebsiella pneumoniae</i> (n=2)	750 $\pm$ 0	375 $\pm$ 0	750 $\pm$ 0	750 $\pm$ 0	750 $\pm$ 0	750 $\pm$ 0	375 $\pm$ 0	375 $\pm$ 0	750 $\pm$ 0	187.5 $\pm$ 0	187.5 $\pm$ 0	750 $\pm$ 0	375 $\pm$ 0	187.5 $\pm$ 0	750 $\pm$ 0	375 $\pm$ 0	750 $\pm$ 0	0
Significance		***			***			***			***			ns			***	
<i>Enterococcus</i> spp. (n=2)	375 $\pm$ 0	750 $\pm$ 0	0	0	750	0	187.5	375 $\pm$ 0	0	375 $\pm$ 0	375 $\pm$ 0	750 $\pm$ 0	750 $\pm$ 0	375 $\pm$ 0	0	750 $\pm$ 0	375 $\pm$ 0	750 $\pm$ 0
Significance		***			***			***			***			0.465 (ns)			***	

Note. MIC: The lowest concentration of extract inhibiting growth + highest concentration that allows growth. *** $P < 0.001$.

MIC of Extracts on Bacteria by Sample

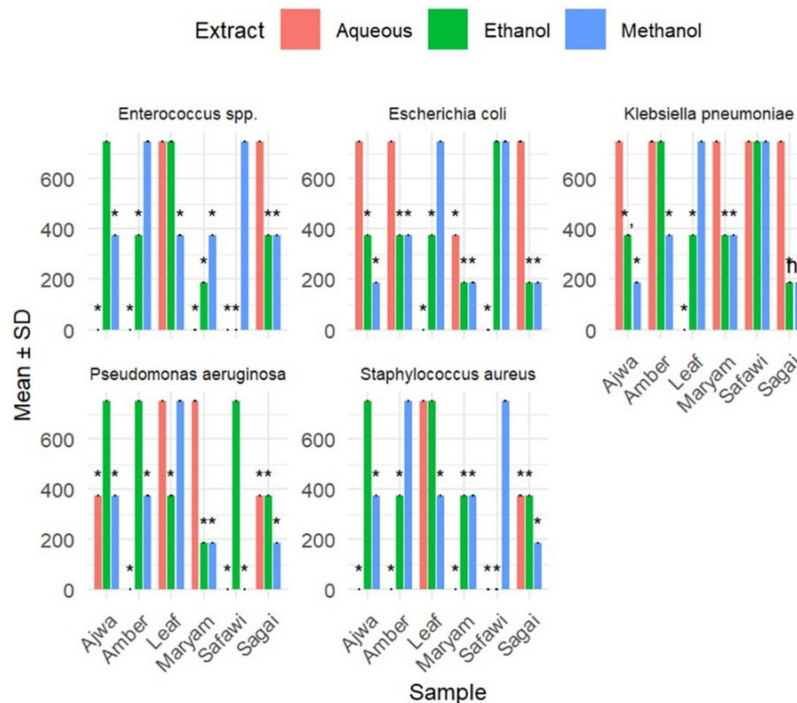


Figure 7. MIC of Different Extracts on Bacteria. Note. MIC: Minimal inhibitory concentration

observed bactericidal concentrations indicate therapeutic promise for certain varieties.

Varietal differences in antibacterial activity are likely

due to differences in phytochemical composition, which are influenced by cultivar genetics, ripening stage, and geographical factors, such as climate, soil, and water

Table 3. Determination of Minimal Bactericidal Concentration of Extracts (Concentrations in μL)

Samples/ Isolates	Amber			Safawi			Maryam			Sagai			Ajwa			Leaf		
	Ethanol	Methanol	Aquaous	Ethanol	Methanol	Aquaous	Ethanol	Methanol	Aquaous	Ethanol	Methanol	Aquaous	Ethanol	Methanol	Aquaous	Ethanol	Methanol	Aquaous
<i>Escherichia coli</i> ($n=3$)	1000 $\pm$ 0	-	-	-	-	-	666.7 $\pm$ 288.7	666.7 $\pm$ 288.7	-	1000 $\pm$ 0	666.7 $\pm$ 288.7	-	-	666.7 $\pm$ 288.7	-	-	-	-
Significance	Amber vs. Maryam: 0.1876, Amber vs. Sagai: 1.0000, Amber vs. Ajwa: 0.1876, Maryam vs. Sagai: 0.1876, Maryam vs. Ajwa: 1.0000, Sagai vs. Ajwa: 0.1876																	
<i>Pseudomonas aeruginosa</i> ($n=5$)	-	-	-	-	-	-	350 $\pm$ 111.8	700 $\pm$ 258.2	-	600 $\pm$ 244.9	1000 $\pm$ 0	-	-	-	-	700 $\pm$ 258.2	-	-
Significance	Maryam vs. Ajwa: Significant (0.0129), Sagai vs. Ajwa: Significant (0.0339), Maryam vs. Leaf: Significant (0.0413), Maryam vs. Sagai: 0.0558, Ajwa vs. Leaf: 0.1011, Sagai vs. Leaf: 0.6005																	
<i>Staphylococcus aureus</i> ($n=4$)	-	-	-	-	-	-	1000 $\pm$ 0	1000 $\pm$ 0	-	-	750 $\pm$ 288.7	-	-	-	-	1000 $\pm$ 0	750 $\pm$ 288.7	-
Significance	Maryam vs. Ajwa: 0.1814, Maryam vs. Leaf: 0.1814, Ajwa vs. Leaf: 1.0000																	
<i>Klebsiella pneumoniae</i> ($n=2$)	-	-	-	-	-	-	1000 $\pm$ 0	1000 $\pm$ 0	-	-	500 $\pm$ 0	-	-	750 $\pm$ 353.6	-	-	-	-
Significance	Maryam vs. Ajwa: 0.6171																	
<i>Enterococcus</i> spp. ($n=2$)	-	-	-	-	-	-	750 $\pm$ 353.6	1000 $\pm$ 0	-	-	-	-	-	-	-	-	-	-
Significance	Insufficient data for the test																	

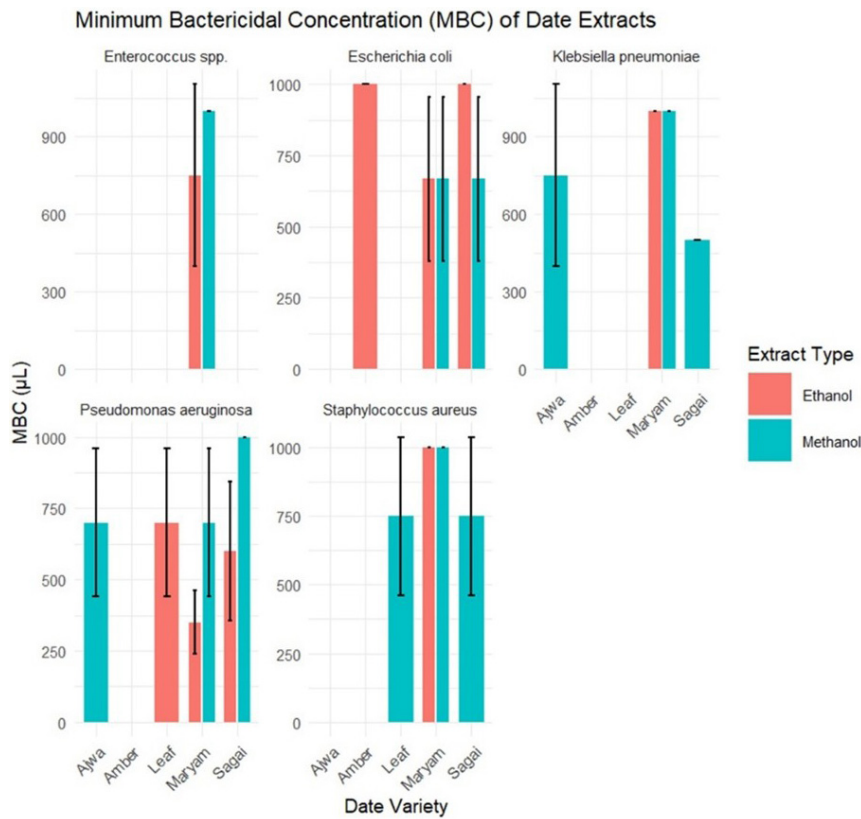


Figure 8. MBC of Extracts Against Bacteria. Note. MBC: Minimal bactericidal concentration

Table 4. Summary of Previous Studies on the Antimicrobial Activity of *Phoenix dactylifera* Compared to the Present Study

Study (Author, Year)	Plant Part Used	Extract Type/ Method	Microorganisms Tested	MDR Strains	MIC/MBC Reported	Variety Comparison	Notable Gaps/ Limitations
Al-Daihan et al, 2012 (27)	Fruit	Disc diffusion	<i>Staphylococcus aureus</i> , <i>Streptococcus pyogenes</i> , <i>Escherichia coli</i> , and <i>Pseudomonas aeruginosa</i>	No	No	×	Only fruit; no MIC/ MBC
Garba et al, 2013 (28)	Leaf	Disc diffusion	<i>Escherichia coli</i> , <i>Moraxella morganii</i> , <i>Proteus mirabilis</i> , and <i>Yersinia enterocolitica</i>	No	Yes (12.5–100 µg/mL)	×	Limited to leaf only
Parveen et al, 2012 (29)	Leaf, Pit	Agar well and MIC	<i>Bacillus subtilis</i> , <i>Escherichia coli</i> , <i>Enterococcus faecalis</i> , <i>Pseudomonas aeruginosa</i> , <i>Shigella flexneri</i> , <i>Staphylococcus aureus</i> , and <i>Streptococcus pyogenes</i>	No	Yes	✓ (3 varieties)	No MDR strains; unspecified variety details
Ayachi et al, 2012 (30)	Fruit	Disc–agar diffusion	<i>Salmonella typhi</i> and <i>Escherichia coli</i>	No	No	×	Few strains; only fruit
Yassein et al, 2012 (31)	Seed	Agar well diffusion	<i>Escherichia coli</i> , <i>Klebsiella pneumoniae</i> , <i>Proteus vulgaris</i> , and <i>Pseudomonas aeruginosa</i>	No	No	×	No MIC; inactive against <i>K. pneumoniae</i>
Bhat et al, 2012 (32)	Fruit	Disc diffusion and MIC	<i>Staphylococcus aureus</i> , <i>Streptococcus pyogenes</i> , <i>Bacillus subtilis</i> , <i>Escherichia coli</i> , and <i>Pseudomonas aeruginosa</i>	No	Yes	×	Did not compare varieties
Shakibaie et al, 2011 (33)	Seed	MIC/MBC	<i>Staphylococcus aureus</i> , <i>Escherichia coli</i> , <i>Bacillus cereus</i> , <i>Salmonella dysenteriae</i> , <i>Salmonella typhi</i> , <i>Klebsiella pneumoniae</i> , <i>Serratia marcescens</i> , and <i>Candida albicans</i>	No	Yes (5–40 mg/ mL)	×	<i>K. pneumoniae</i> , <i>S. marcescens</i> , <i>C. albicans</i> inactive
Bolin et al, 1972 (34)	Fruit	Storage extract	<i>Pseudomonas aeruginosa</i> , <i>Enterococcus faecalis</i> , <i>Diphtheroid coryneform bacteria</i> , <i>Proteus vulgaris</i> , and <i>Escherichia coli</i>	No	No	×	Old study; moisture-controlled whole fruit
Sallal et al, 2013 (35)	Fruit	Agar well	<i>Staphylococcus aureus</i>	No	No	×	One strain only
Mahmood et al, 2012 (36)	Fruit	Disc diffusion	<i>Escherichia coli</i> , <i>Staphylococcus aureus</i> , and <i>Pseudomonas aeruginosa</i>	No	No	×	Basic diffusion; no MIC
Al-Seeni et al, 2012 (37)	Fruit	Disc–agar diffusion	<i>Pseudomonas aeruginosa</i> , <i>Escherichia coli</i> , <i>Shigella</i> , <i>Klebsiella pneumoniae</i> , <i>Bacillus subtilis</i> , <i>Staphylococcus aureus</i> , and <i>Micrococcus</i>	No	No	×	No quantification of potency
Selim et al, 2014 (38)	Fruit	Disc diffusion	<i>Pseudomonas aeruginosa</i>	No	No	×	Single organism tested
Amiour et al, 2014 (39)	Fruit	Disc diffusion	<i>Staphylococcus aureus</i> , <i>Pseudomonas aeruginosa</i> , <i>Klebsiella pneumoniae</i> , <i>Escherichia coli</i> , <i>Bacillus subtilis</i> , and <i>Staphylococcus spp.</i>	No	No	×	No MIC/MBC
Abu-Elteen et al, 2000 (40)	Fruit	Antifungal and MIC	<i>Candida albicans</i>	No	Yes	×	Only fungal strains tested
Sallal et al, 1996 (41)	Fruit	MIC and germ tube	<i>Candida albicans</i>	No	Yes	×	Fungal only
Sharideh et al, 1998 (42)	Fruit	MIC, morphology	<i>Candida albicans</i>	No	Yes	×	Fungal only
Current study (2025)	Fruit + Leaf	Ethanol, methanol, water, and crude	<i>Staphylococcus aureus</i> , <i>Klebsiella pneumoniae</i> , <i>Escherichia coli</i> , and <i>Pseudomonas aeruginosa</i> (MDR clinical isolates)	✓	✓	✓ (5 varieties)	First to compare 5 named varieties + leaf extracts against MDR clinical isolates

Note. MDR: Multidrug-resistant; MIC: Minimal inhibitory concentration; MBC: Minimal bactericidal concentration.

availability (52). Prior research identified flavonoids, tannins, phenolic acids, and polyphenols in date fruits and leaves, compounds known to disrupt bacterial membranes, inhibit nucleic acid synthesis, and interfere with metabolic enzymes (53,54). Due to equipment limitations, it was impossible to conduct phytochemical profiling in this study, but the presence of these bioactives is well documented in the literature (52–54).

The enhanced activity of ethanol and methanol extracts over aqueous ones can be attributed to the higher solubility of phenolic compounds in organic solvents (55). This is in line with the findings of previous research, showing that organic solvents improve the extraction of active constituents, such as flavonoids and phenolic acids (55).

Compounds such as quercetin and catechin—abundant in dates—are known to damage bacterial membranes and

promote the formation of reactive oxygen species, which play a critical role in killing Gram-negative bacteria (56,57). These compounds also inhibit bacterial DNA replication and protein synthesis (57).

Tannins, also present in date varieties, may exert additional effects by precipitating microbial proteins, inactivating enzymes, and reducing virulence, including adherence and biofilm formation (58).

The difference in antimicrobial efficacy across varieties further suggests the role of specific phytochemical profiles, which vary by variety and region (59). For example, differences in flavonoid or tannin content between Amber and Safawi could account for their varied MIC/MBC values.

Moreover, potential synergistic effects among different phytochemicals—such as phenolic–flavonoid or tannin–flavonoid interactions—may enhance antibacterial efficacy, especially against MDR bacteria (60). This warrants further investigation through fractionation and bioassay-guided studies.

The demonstrated antibacterial activity of *P. dactylifera* extracts, particularly those derived using ethanol and methanol, suggests potential for therapeutic application, especially in the context of MDR infections. Given their observed efficacy against skin-associated and wound-associated pathogens, such as *S. aureus* and *P. aeruginosa*, these extracts may be explored for topical use in the form of ointments or wound dressings (61). However, systemic application would require further investigation into the extracts' toxicity, bioavailability, and pharmacokinetics (62). The natural origin of these compounds offers appeal for developing plant-based adjuncts or alternatives to conventional antibiotics, particularly in resource-limited settings where resistance is prevalent and access to advanced treatments is restricted.

In summary, ethanol and methanol extracts of *P. dactylifera* varieties exhibit promising antibacterial and bactericidal activity against MDR pathogens, likely driven by phenolic compounds and influenced by varietal phytochemistry. Future research should include phytochemical analyses (e.g., thin-layer chromatography, high-performance liquid chromatography, and UV-Vis spectroscopy) to identify active constituents and clarify their mechanisms. Understanding synergistic interactions and varietal bioactivity differences will be critical in developing date-based antimicrobial agents.

Limitations of the Study

A critical limitation of this study was the absence of detailed phytochemical profiling techniques, such as thin-layer chromatography, high-performance liquid chromatography, or UV-Vis spectroscopic analyses, which were not accessible in our laboratory during this research. This absence significantly limited our ability to identify and quantify the specific bioactive compounds responsible for the observed antibacterial activity. Consequently, attributing the antibacterial effects to

phenolics, flavonoids, tannins, or other phytochemicals remains speculative and unconfirmed.

Another limitation was the reliance on clinical isolates rather than ATCC reference strains, which may impact the reproducibility of our results. The unavailability of ATCC reference strains in our laboratory was a constraint for this study. Future work should incorporate these reference strains to validate the antibacterial activity and ensure greater reproducibility of the findings.

While our primary objective was to establish whether extracts from dates and their leaves exhibit antibacterial activity against pathogenic isolates, we recognize that mechanistic insights into which compounds drive this activity are essential to fully understand and validate these effects. Therefore, the lack of phytochemical characterization and the absence of ATCC strains represent significant gaps in the current work.

It is strongly recommended that future research prioritize comprehensive phytochemical analyses to isolate, identify, and quantify the key antimicrobial constituents. Such analyses will not only confirm the roles of phenolic and flavonoid compounds but also help elucidate their mechanisms of action, particularly against MDR bacterial strains. Similarly, the inclusion of ATCC reference strains in future studies will enhance reproducibility and the robustness of antibacterial efficacy results.

Conclusion

Natural products are promising alternatives in the search for antibacterial agents effective against antibiotic-resistant bacteria. Numerous plants, fruits, leaves, and barks have demonstrated potential in infection treatment. Dates and their leaves, in particular, demonstrated antibacterial activity against several MDR clinical isolates. The next step involves identifying the specific phytochemicals responsible, which could pave the way for developing these compounds as therapeutic agents.

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Authors' Contribution

Conceptualization: Tasnia Ahmed.

Data curation: Navid Safa Nova.

Formal analysis: Tasnia Ahmed, Navid Safa Nova.

Investigation: Navid Safa Nova.

Methodology: Tasnia Ahmed.

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Resources: Tasnia Ahmed.

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Validation: Navid Safa Nova.

Visualization: Tasnia Ahmed.

Writing—original draft: Navid Safa Nova, Tasnia Ahmed.

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Competing Interests

The authors declare that they have no conflict of interests.

Ethical Approval

Ethical approval was not required for this study, as no human participants or personal data were involved. The bacterial isolates used in this study were archived strains originally preserved in the teaching laboratory of the Department of Microbiology for undergraduate practical classes and student thesis projects. These isolates were utilized solely for laboratory-based antimicrobial testing, with no access to clinical records or patient-identifiable information. Their use complies with the institutional policy for educational and research use of anonymized microbial cultures.

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