Comparative Analysis of Antimicrobial Activity of Date Palm Leaf and Seed Extracts Against Foodborne Pathogenic Bacteria

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Abstract: Date palm by-products are a rich source of bioactive compounds used as antimicrobial agents against common foodborne pathogenic bacteria. which represent a promising option within the modern trend toward natural and safe solutions in food and pharmaceutical applications. The date palm (Phoenix dactylifera L.) seeds showed a significant advantage over leaves in their bioactive compounds. The total phenols reached 3856.67 ± 15.28 mg GAE/100 g in seeds, and 1623.33 ± 25.17 mg GAE/100 g in leaves. Similarly, total flavonoids reached 2310.00 ± 20.00 mg RE/100 g in seeds, and 1963.33 ± 90.74 mg RE/100 g in leaves. A low molecular weight protein/peptide fraction (<14.4 kDa) was also isolated from both leaves and seeds using gel filtration on Sephacryl S-200. The seed extracts exhibited the highest inhibition at 100 mg/mL, reaching 34.00 ± 1.00 mm and 38.00 ± 1.00 mm for the extract of the active compounds against Escherichia coli and Salmonella typhimurium, respectively, and 34.33 ± 0.58 mm and 35.00 ± 1.00 mm for the protein/peptide fraction against Staphylococcus aureus and Bacillus cereus, respectively. MIC/MBC results showed that the active compounds were more effective against Gram-positive bacteria (G^+) at 12.5/25 mg/mL, whereas the protein/peptide fraction was more active against Gram-negative bacteria (G^-) at the same 12.5/25 mg/mL. The synergistic effect also showed that combining the two extracts resulted in a synergistic effect against S. typhimurium, S. aureus, and B. cereus with FIC_{index} values of 0.457 ± 0.017, 0.475 ± 0.077, and 0.315 ± 0.021, respectively, compared to a combined effect against E. coli with a value of 0.587 ± 0.041 .

1 INTRODUCTION

The date palm (P. dactylifera L.) is one of the most important plants in arid and semi-arid regions, having long been linked to food security in the Middle East and North Africa [1]. As date-processing industries expand, by-products like leaves and seeds have increased. However, these are often discarded despite containing many bioactive compounds such as phenolics, flavonoids, tannins, fiber, and proteins or peptides, which can be extracted, depending on the processing or extraction method [2], [3]. Notably, recent studies confirm that date palm by-products are among the richest sources of bioactive phenolics. These show varying degrees of antibacterial activity, depending on the variety and extraction conditions [4]-[6]. Furthermore, studies also indicate that polyphenols can exert antimicrobial effects in several ways, including disrupting cell membranes, inhibiting specific biological pathways, and interfering with virulence factors. This enhances the potential to use palm byproduct extracts in food and pharmaceutical applications [7], [8].

Foodborne bacteria pose a significant challenge to food safety. Pathogens include S. typhimurium and certain strains of E. coli. Spoilage-associated bacteria, like Pseudomonas aeruginosa and B. cereus, affect sensory qualities [9]-[12]. Global interest in safe, natural food preservatives has grown in recent years. Green extracts are gaining attention for their safety, nutritional value, and applicability in the food industry [4]. These extracts are promising natural sources of bioactive compounds. They also include low-molecular-weight proteins/peptides with antibacterial effects, such as inhibition of cell wall and biofilm formation. This makes them part of a growing trend toward green solutions in food safety [5], [13]-[16]. In this context, this research aims to

extract phenolics, flavonoids, and proteins/peptides from date palm leaves and seeds using a green extraction method. It also evaluates their antibacterial activity against common foodborne pathogens, providing natural solutions for food safety and enhancing the economic value of neglected agricultural by-products.

2 MATERIALS AND METHODS

2.1 Sample Collection and Preparation

Leaves and seeds of the Iraqi Zahdi date palm (P. dactylifera L.) were washed with tap water, rinsed with deionized distilled water, and dried with tissue wipes. Leaves were cut into 1-2 cm pieces. The seeds were crushed into several sections using a ceramic mortar and pestle. Samples were dried in a preheated forced-air oven at 40 °C until the weight stabilized. After drying, samples were ground in a laboratory mill to obtain a fine powder (≤ 0.5 mm). The powder was stored in opaque, tightly sealed glass containers at 4 °C until extracting the active compounds and proteins/peptides [17].

2.2 Extraction of Bioactive Compounds

The hot water extraction method, consistent with the green extraction principle, was used to extract phenolic and flavonoid compounds from date palm leaf and seed powders [17] by mixing the sample with deionized distilled water at a 1:20 (w/v) ratio, and heating at 65 °C for 60 min with slow stirring on a hot plate magnetic stirrer. Then, the cells were centrifuged at 8000 × g for 15 min, and the supernatant was collected. A second extraction cycle was performed to improve the release of target compounds. Half the initial volume of deionized distilled water was added to the precipitate, and extraction and centrifugation were repeated under the same conditions. The two supernatants were combined, freeze-dried, and stored as a powder in airtight glass containers under refrigeration until use.

2.3 Protein Estimation

Protein concentration was estimated using the Bradford [18] method.

2.4 Total Phenolic Content (TPC)

The Folin-Ciocalteau colorimetric method [19] at 760 nm was used to estimate TPC (mg gallic acid equivalents/g) using a gallic acid standard curve.

2.5 Total Flavonoid Content (TFC)

The colorimetric assay method [19] at 510 nm was used to estimate TFC (mg of rutin equivalents/g). This estimation was based on a standard curve of rutin.

The final results of TPC and TFC were expressed in mg/100g after converting the calculated values from mg/g for clarity and better comparison.

2.6 Purification of Proteins/Peptides

A 10 mM ammonium bicarbonate buffer at pH 7.8 containing 0.1 M sodium chloride and 1 % PVPP was used as the extraction solution. This followed the green extraction approach for proteins/peptides from date palm leaf and seed powder at 4°C, as described by Sun et al. [20] (ammonium bicarbonate volatile buffer solution) and Alsoufi and Aziz [21] (extraction of proteins from plant tissues), by mixing the sample with the extraction solution at a 1:10 (w/v) ratio, then stirring at 10 rpm for 3 h, and centrifugation at 8000 × g for 15 min. Supernatant was collected, freeze-dried, and stored in a desiccator at 4 °C. Then, 3 mL (10 mg/mL) of the sample was loaded onto a Sephacryl S-200 column (1.5 × 60 cm) after complete dissolution and filtration (0.45 μm).

Purification of proteins/peptides was carried out as described by Alsoufi and Aziz [21] using the same buffer at a flow rate of 18 mL/h, with 3 mL collected per tube. Proteins/peptides were monitored by absorbance at 280 nm to follow the elution. Lysozyme (14.4 kDa) served as a standard for separating fractions with lower molecular weights. Lysozyme eluted from tube 36, with peak maximum at tube 39 and last detected at tube 42. Fractions from tubes 43 to 62 were pooled to represent the <14.4 kDa proteins/peptides. These fractions were freeze-dried and stored in a desiccator at 4 °C. Due to the limitation of Sephacryl S-200 for low-molecular-weight proteins, the separation was used as preparative fractionation for <14.4 kDa proteins/peptides. without aiming for precise molecular weight resolution.

2.7 Bacterial Strains

Bacterial strains (E. coli, S. typhimurium, S. aureus, and B. cereus) from the Department of Biology, College of Science, Mustansiriyah University, Baghdad, Iraq, were used to test the antibacterial activity of date palm leaf and seed extracts.

2.8 Bacterial Inoculum

Bacterial strains were reactivated, prepared a McFarland standard (approximately 10^8 CFU/mL), adjusted to 0.5, and cultured in Mueller-Hinton broth at 37 °C for 24 h, as described by Lima-Filho and Cordeiro [12].

2.9 Stock Solution

A stock solution of date palm leaf and seed extracts was prepared using sterile deionized distilled water at a concentration of 100 mg/mL. The suspension was gently stirred with a magnetic stirrer until completely dissolved. Then filter through a 0.22 µm membrane filter. The filtrate was stored in sterile dark glass bottles at 4 °C and used within 24 h as described in Lima-Filho and Cordeiro [12].

2.10 Antibacterial Activity

The agar well diffusion method, as described by Lima-Filho and Cordeiro [12], was used to evaluate the antibacterial activity of date palm leaf and seed extracts at 100, 50, 25, and 12.5 mg/mL on Mueller-Hinton agar, which was incubated at 37 °C for 24 h. Gentamicin 10 µg/mL was used as a positive control, and sterile distilled water was used as the solvent for the extracts as a negative control for comparison with the results obtained from the plant extracts. All tests were carried out in triplicate in a sterile laminar airflow cabinet, and the average readings were used in the statistical analysis.

2.11 Minimum Inhibitory Concentration (MIC)

The MIC of the seeds' bioactive compound and protein/peptide was determined by a broth microdilution method in a 96-well microplate. Two serial dilutions of the extracts were prepared in Mueller-Hinton broth with deionized distilled water as the solvent, resulting in approximate final concentrations of 0.5-64 mg/mL per well. A fresh bacterial suspension was prepared from a 24 h culture adjusted to a McFarland standard of 0.5, and diluted in Mueller-Hinton broth to a final concentration of 5×10^5 CFU/mL per well. Then 100 µL of each extract and 100 µL of the bacterial suspension were added to each well. The plates were incubated at 37°C for 24 h, and the lowest extract concentration that showed no visible growth (absence of turbidity compared to the growth control) was recorded as the MIC following Balouiri et al. [11]. All tests were carried out in triplicate in a sterile laminar airflow cabinet.

2.12 Minimum Bactericidal Concentration (MBC)

The lowest extract concentration at which no colonies were observed (or at least 99.9% fewer colonies compared to the growth control) from the MIC result was recorded as the MBC, consistent with the definitions of MIC and MBC and the steps for dilution in broth and inoculation on solid media as described by Balouiri et al. [11].

2.13 Synergistic Effect

The synergistic activity of the seeds' bioactive compounds and proteins/peptides was tested using a checkerboard method in a 96-well microplate, based on the MIC result. The MIC values were determined individually under the same experimental conditions, using the same medium, inoculum density, and incubation conditions, to ensure the accuracy of the FICI index calculation. Graded binary dilutions of each extract in Mueller-Hinton broth were prepared within the same concentration range as the MIC test (0.5-64 mg/mL), with 100 µL added to each well. The concentrations of bioactive compounds (A) were arranged horizontally, and proteins/peptides vertically (B), with each well containing a different combination of the two extracts within this range. Then 100 µL of bacterial suspension was added to each well to achieve a final concentration of approximately 5×10^5 CFU/mL. The microplate was incubated at 37 °C for 24 h, and the MIC of each extract in the presence of the other was recorded.

The fractional inhibitory concentration index (FIC) of (A) and (B) was calculated by the following equations (1)-(3):

$$FIC_A = \frac{MIC_A\,(with\,B)}{MIC_A\,(alone)}. \quad (1)$$

$$FIC_B = \frac{MIC_B\,(with\,A)}{MIC_B\,(alone)}. \quad (2)$$

The overall effect index (FIC_{index}) was calculated as follows:

$$FIC_{index} = FIC_A + FIC_B. \quad (3)$$

The effect was considered synergistic when $FIC_{index} \leq 0.5$; non-synergistic (additive or neutral) when $0.5 < FIC_{index} \leq 4$; and antagonistic when $FIC_{index} > 4$, based on the methodological guidelines for synergistic testing of natural preparations [22]. All tests were carried out in triplicate in a sterile laminar airflow cabinet.

2.14 Statistical Analysis

Variance analysis (ANOVA according to the CRD) was used to analyze the data. Means were compared using the LSD test at $p \leq 0.05$, as described by Gomez and Gomez [23], using SPSS (version 25).

3 RESULTS AND DISCUSSION

3.1 Bioactive Compounds

The results in (Table 1) present the mean ± standard deviation ($n = 3$) for TPC and TFC in date palm leaf and seed extracts, along with statistical significance (LSD grouping, $p \leq 0.05$), p-value (ANOVA), and coefficient of variation (CV %).

The results show that extracts from date palm leaves and seeds are rich in TPC and TFC. This finding is consistent with the literature on the high concentrations of these compounds in palm by-products [2]. As shown in the table, the seeds recorded higher values than the leaves for both TPC and TFC, and these differences were highly significant for TPC ($p = 2.01 \times 10^{-8}$) and significant for TFC ($p = 0.00295$). This is important because many indicators of antioxidant activity are more strongly associated with TPC [24]. Furthermore, these compounds contribute to antimicrobial effects by affecting membrane permeability [1], [5], [7]. The superiority of the seeds leads to the ability to store these compounds, which play defensive roles and protect their components from oxidation and microbial damage [1], [5]. In contrast, leaves are more exposed to environmental changes and post-harvest oxidation processes. Differences in the tissue matrix between seeds and leaves may also affect the efficiency of releasing associated compounds during extraction, making the observed differences logical within a botanical and chemical context [1]. In parallel, the results, as indicated by the CV % values, confirm the high analytical reliability of the replicates. These values were very low in the seeds for both parameters. Conversely, total leaf flavonoids exhibited higher relative dispersion than the other measurements [17]. Preparation and extraction methods may preserve or lose some active compounds, directly affecting the final measured values [24]. Therefore, the remarkable superiority of the seeds, particularly in total phenols, supports their adoption as a more promising source for subsequent bioactivity tests. Nevertheless, TPC and TFC content indices are widely accepted tools in preliminary (screening) studies to assess the biological potential of plant extracts, and are closely related to antimicrobial activities. However, it should be emphasized that the current estimate is limited to TPC and TFC, a general quantitative indicator that does not reveal the qualitative composition or detailed chemical signature that may explain differences in bioactivity between compounds, even when the total content is similar. Therefore, in subsequent studies, advanced qualitative/quantitative analysis of TPC and TFC, including HPLC or other advanced techniques, is recommended to identify the predominant compounds and more accurately correlate them with efficacy results [17].

3.2 Proteins/Peptides

Proteins/peptides with molecular weights below 14.4 kDa were isolated from date palm leaves and seeds by gel filtration, using lysozyme (14.4 kDa) as a standard to define the cut-off point and guide pooling of fractions eluting after lysozyme. Purification of leaf and seed extract on Sephacryl S-200 (Fig. 1) produced multiple peaks in both extracts after the lysozyme peak. Fractions were combined from 43 to 62 for each sample (leaves and seeds) separately and freeze-dried as powder for subsequent examination.

The gel filtration results on Sephacryl S-200 confirm the success of a functionally targeted purification approach for the low-molecular-weight protein/peptide fraction [13], using lysozyme (14.4 kDa) as a practical cutoff [21].

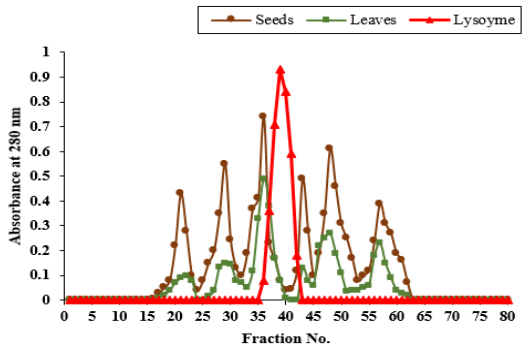

Figure 1: Gel filtration profiles of Proteins/peptides from date palm (P. dactylifera L.) leaves and seed extracts on Sephacryl S-200.

The presence of multiple peaks beyond the lysozyme peak in the leaf and seed curves indicates that the fraction below 14.4 kDa is not a single compound but a mixture of small proteins and peptides with varying molecular weights, which may differ in biological activity [13], [15]. These peaks may be attributed to natural autolytic proteolysis and variations in amino acid ratios, which influence peak shape and elution positions [14], [15]. The difference in peak patterns between leaves and seeds (often more pronounced peaks in seeds) supports the seeds serve as a repository for compounds and proteins/peptides associated with storage and protection functions [5], [6], whereas leaves are more associated with structural and enzymatic proteins [1]. From a functional perspective, focusing on the <14.4 kDa fraction is logical because many short-chain bioactive peptides exhibit antimicrobial activity through membrane-related mechanisms (altering permeability or disrupting membrane integrity) [15]. These mechanisms are more commonly associated with small peptides than with large proteins [14]. Furthermore, the abundance of palm phenols (especially from seeds) and the growing discussion of microbial resistance/response mechanisms to plant compounds underscore the importance of accurately separating and identifying fractions before linking a specific fraction to a specific activity pattern in subsequent results [4], [7], [9], [16].

3.3 Antibacterial Activity

The results of the agar well diffusion test for leaf extracts Table 1 and seed extracts Table 2 showed clear differences in inhibition zone diameters among the extracts, depending on the plant source, extract type, and concentration. Statistical analysis examined the effects of extract type, concentration, and their interaction. A direct comparison between the leaves and seeds at the highest concentration (100 mg/mL) was performed Table 3 to illustrate differences in inhibition efficiency and to support the selection of the most effective plant source for conducting the minimum inhibitory concentration (MIC) and minimum lethal concentration (MBC) tests.

The results of the agar diffusion test Tables 1 and 2 showed significant differences in inhibition diameters across plant sample source, extract type, and concentration, with significant interactions among the factors. Although this test is a suitable method for detecting antibacterial activity, the zone diameter is influenced by physical and chemical factors that govern compound diffusion within the agar, such as solubility, polarity, molecular weight, and interactions with medium components [11], [12]. When comparing leaves and seeds, seed extracts generally show larger inhibition diameters, especially at medium and high concentrations, as directly illustrated in Table 3. This superiority can be attributed to the biological role of date palm seeds as a concentrated defense reserve that protects the plant embryo. Consequently, they are rich in phenolic compounds, tannins, and other bioactive compounds with antibacterial and antioxidant properties, compared with other plant parts [1], [5], [6], [8], [24]. Mechanistically, phenolic compounds can disrupt bacterial cell membrane permeability, inactivate essential proteins and enzymes, and alter intracellular oxidative balance, mechanisms known to contribute to bacterial growth inhibition [7], [9], [16]. Furthermore, differences in bacterial responses are linked to cell wall structure. In G^-, the outer membrane provides an additional barrier to certain compounds, whereas G^+ are more susceptible to effects associated with cell wall or membrane disruption [10], [11]. Regarding protein/peptide fractions (<15 kDa), antibacterial activity is often linked to the ability of these peptides to bind to the cell membrane and disrupt its structure or to target internal cellular components, with efficacy depending on net charge, secondary structure, and membrane-binding capacity [13]-[15]. Therefore, the overall superiority of seed may be attributed to their containing more potent or higher concentrations of defensive peptides than leaves [6], [13], [15]. Given the overall significant superiority of the seed extracts in the agar diffusion assay Tables 1-3, particularly at higher concentrations, they were selected as the most active plant source for further, more precise quantitative microbiological evaluation using the MIC and MBC test [11], [12].

3.4 MIC and MBC

The results in Table 4 reflect a selective pattern related to cell membrane structure; the extract of the active compounds was more effective against G^+ (S. aureus and B. cereus), with MIC values of 12.5 mg/mL and MBC values of 25 mg/mL, compared with G^- (E. coli and S. typhimurium), with MIC values of 25 mg/mL and MBC values of 50 mg/mL.

This result is commonly attributed to the absence of an outer membrane in G^+ [11]. In contrast, the protein/peptide (<15 kDa) fraction showed higher activity against G^- (MIC = 12.5 mg/mL; MBC = 25 mg/mL) than against G^+ (MIC = 25 mg/mL; MBC = 50 mg/mL). This is consistent with much of the literature reporting the effect of the proteins/peptides

on the cell wall membrane [14], [15]. Studies in this field indicate that MIC/MBC varies significantly depending on the plant part, extraction conditions, and phenolic content. A study of an Egyptian date palm seed extract reported MIC (0.25-1 mg/mL) and MBC (0.5-1 mg/mL) for several bacterial species, with variations among species [9]. At the level of individual compounds, the isolation of gallic acid from date pits and its demonstration of complete inhibition of S. aureus viability at 0.2 mg/mL within 6 h illustrate how the richness of a particular part in specific compounds can lower the MIC or bring the MBC closer to the MIC [6]. Furthermore, comparative reviews and studies indicate that variations in the phenolic profile between varieties and origins directly affect potency, and that G^+ are often more sensitive than G^- in aqueous extracts [24], [4], [8].

3.5 Synergistic Effect

The results of the checkerboard test showed that the relationship between the extract of the bioactive compounds (A) and the protein/peptide fraction (B) was characterized by synergistic or collaborative effects depending on the bacterial strain, as shown in Table 5 through the MIC values and FIC_{index} values (mean ± standard deviation) of three independent replicates.

The checkerboard test ($FIC_{index} \leq 0.5$) showed a synergistic interaction between bioactive compound (A) and protein/peptide (B) against three bacterial strains. Phenolic compounds are known to destabilize cell membranes, affect membrane proteins and bio-enzymes, and disrupt intracellular oxidative balance [7], [16], [22]. In contrast, antimicrobial peptides can directly interact with or penetrate membrane components, causing structural damage that leads to loss of cell integrity [14], [15].

Therefore, the observed decrease in FIC_{index} values against S. aureus and B. cereus may be attributed to enhanced membrane permeability mediated by phenolic compounds, which facilitates the delivery of peptides to their intracellular targets [5], [6], [8], (Table 6). Furthermore, studies on the extraction of phenolic compounds from date palm byproducts support the possibility of obtaining bioavailable concentrations that can interfere with microbial agents [3], [4], [24]. In contrast, the limited effect against E. coli can be explained by the nature of G^- [7]. Overall, the results are promising for a strategy to enhance antimicrobial activity, particularly against G^+, with potential applications in bio-preservation or functional food systems, especially considering the biological value of palm derivatives as a multi-source of active compounds [1], [2].

Table 1: TPC and TFC in date palm (P. dactylifera L.) leaf and seed extracts.

Parameter	Leaves (mean ± SD)	CV (%)	Seeds (mean ± SD)	CV (%)	p-value (ANOVA)
TPC (mg GAE/100 g)	1623.33 ± 25.17b	1.55	3856.67 ± 15.28a	0.40	2.01×10^{-8}
TFC (mg RE/100 g)	1963.33 ± 90.74b	4.62	2310.00 ± 20.00a	0.87	0.00295

Note. Values are reported as mean ± standard deviation (SD) (n = 3). ANOVA was conducted within a completely randomized design (CRD) framework, and means were compared using the least significant difference (LSD) test at $p \leq 0.05$. Values with different letters in the same row differ significantly. Coefficient of variation (CV) (%) = (SD/Mean) × 100. GAE: gallic acid equivalents. RE: rutin equivalents.

Table 2: Effect of concentration on antibacterial activity of bioactive compounds and protein/peptides of palm (P. dactylifera L.) leaves.

Bacterial strains	Type of leaf extract	Inhibition zone (mm)			
		Concentration (mg/mL)			
		12.5	25	50	100
E. coli	bioactive compounds extract	6.67 ± 0.58 f	13.67 ± 0.58 d	20.33 ± 0.58 b	23.33 ± 1.53 a
	protein/peptides fraction(<15 kDa)	0.00 ± 0.00 g	11.33 ± 0.58 e	15.67 ± 0.58 c	20.00 ± 1.00 b
S. typhimurium	bioactive compounds extract	7.00 ± 1.00 e	12.67 ± 0.58 d	19.33 ± 0.58 b	24.33 ± 1.53 a
	protein/peptides fraction(<15 kDa)	0.00 ± 0.00 f	12.00 ± 1.00 d	15.33 ± 1.53 c	21.00 ± 1.00 b
S. aureus	bioactive compounds extract	0.00 ± 0.00 f	10.33 ± 0.58 d	15.00 ± 1.00 c	19.67 ± 1.53 b
	protein/peptides fraction(<15 kDa)	5.67 ± 0.58 e	11.67 ± 0.58 d	19.00 ± 1.00 b	22.00 ± 1.00 a
B. cereus	bioactive compounds extract	0.00 ± 0.00 e	11.00 ± 1.00 c	17.33 ± 0.58 b	20.00 ± 1.00 b
	protein/peptides fraction(<15 kDa)	8.00 ± 1.00 d	14.00 ± 1.00 c	18.00 ± 1.00 b	27.00 ± 4.36 a

Note. Values represent the mean ± standard deviation (n = 3). A two-way ANOVA was performed to examine the effects of extract type, concentration, and their interaction for each bacterial strain. Means were compared using the least significant difference (LSD) test at $p \leq 0.05$. Different letters within each strain indicate significant differences among treatments.

Table 3: Effect of concentration on antimicrobial activity of bioactive compounds and protein/peptides of palm (P. dactylifera L.) seeds.

Bacterial strains	Type of leaf extract	Inhibition zone (mm)			
		Concentration (mg/mL)			
		12.5	25	50	100
E. coli	bioactive compounds extract	11.33 ± 0.58 e	16.00 ± 1.00 d	25.33 ± 1.15 b	34.00 ± 1.00 a
	protein/peptides fraction (<15 kDa)	0.00 ± 0.00 f	11.00 ± 1.00 e	19.33 ± 0.58 c	25.00 ± 1.00 b
S. typhimurium	bioactive compounds extract	12.00 ± 1.00 e	16.33 ± 0.58 d	28.67 ± 0.58 b	38.00 ± 1.00 a
	protein/peptides fraction (<15 kDa)	0.00 ± 0.00 f	11.00 ± 1.00 e	18.33 ± 0.58 c	29.33 ± 0.58 b
S. aureus	bioactive compounds extract	0.00 ± 0.00 g	14.33 ± 0.58 e	20.00 ± 1.00 c	28.00 ± 1.00 b
	protein/peptides fraction (<15 kDa)	10.00 ± 1.00 f	17.33 ± 0.58 d	27.00 ± 1.00 b	34.33 ± 0.58 a
B. cereus	bioactive compounds extract	0.00 ± 0.00 g	12.67 ± 0.58 e	20.00 ± 1.00 c	25.67 ± 0.58 b
	protein/peptides fraction (<15 kDa)	8.67 ± 0.58 f	16.00 ± 1.00 d	21.00 ± 1.00 c	35.00 ± 1.00 a

Note. Values represent the mean ± standard deviation ($n = 3$). A two-way ANOVA was performed to examine the effects of extract type, concentration, and their interaction for each bacterial strain. Means were compared using the least significant difference (LSD) test at $p \leq 0.05$. Different letters within each strain indicate significant differences among treatments.

Table 4: Direct comparison between date palm (P. dactylifera L.) leaves and seeds in inhibition diameter (mm) at a concentration of 100 mg/mL.

Bacterial strains	Type of leaf extract	Mean ± SD		p-value (t-test)
		leaves	seeds	
E. coli	bioactive compounds extract	23.33 ± 1.53	34.00 ± 1.00	0.00054
	protein/peptides fraction (<15 kDa)	20.00 ± 1.00	25.00 ± 1.00	0.0036
S. typhimurium	bioactive compounds extract	24.33 ± 1.53	38.00 ± 1.00	0.0002
	protein/peptides fraction (<15 kDa)	21.00 ± 1.00	29.33 ± 0.58	0.00024
S. aureus	bioactive compounds extract	19.67 ± 1.53	28.00 ± 1.00	0.00138
	protein/peptides fraction (<15 kDa)	22.00 ± 1.00	34.33 ± 0.58	0.00005
B. cereus	bioactive compounds extract	20.00 ± 1.00	25.67 ± 0.58	0.00105
	protein/peptides fraction (<15 kDa)	27.00 ± 4.36	35.00 ± 1.00	0.03628

Table 5: MIC and MBC values for date palm (P. dactylifera L.) seed bioactive compounds extract and proteins/peptides fraction (<15 kDa) against bacterial strains.

Bacterial strains	Bioactive compounds extract		Proteins/peptides fraction (<15 kDa)	
	MIC (mg/mL)	MBC (mg /mL)	MIC (mg /mL)	MBC (mg /mL)
E. coli	25	50	12.50	25
S. typhimurium	25	50	12.50	25
S. aureus	12.50	25	25	50
B. cereus	12.50	25	25	50

Note. MIC: Minimum Inhibitory Concentration; MBC: Minimum Bactericidal Concentration. The measurements were performed in three independent replicates ($n = 3$), and the values were highly consistent in all replicates, so they were presented as a single value.

Table 6: The MIC and the FIC_{index} for date palm seeds (P. dactylifera L.) bioactive compounds and the protein/peptide.

Bacterial strains	MIC of Extract A (mg/mL)	MIC of Fraction B (mg/mL)	FICI (Mean ± SD)	Interaction Type
E. coli	8	16	0.587 ± 0.041	Additive
S. typhimurium	8	16	0.457 ± 0.017	Synergistic
S. aureus	16	8	0.475 ± 0.077	Synergistic
B. cereus	16	8	0.315 ± 0.021	Synergistic

Note. A: Bioactive compound extract, B: Protein/peptide fraction; MIC values were determined by microdilution in 96-well plates, and the reaction was assessed using a checkerboard pattern; Values represent the mean ± standard deviation of three independent replicates; Synergism was considered when the FIC_{index} value was ≤ 0.5, and an Additive was considered when it was > 0.5-1; All MIC values are expressed in mg/mL.

4 CONCLUSIONS

This study demonstrated that date palm (*Phoenix dactylifera* L.) seeds represent a more promising source of antibacterial bioactive compounds and low-molecular-weight proteins/peptides than leaves. Seed-derived extracts consistently exhibited stronger antibacterial activity in agar diffusion assays, while MIC, MBC, and checkerboard analyses confirmed their enhanced antimicrobial potential and synergistic interactions.

A differential antibacterial pattern was observed between the two fractions. Bioactive compounds were more effective against Gram-positive bacteria, whereas the protein/peptide fraction exhibited greater activity against Gram-negative bacteria, indicating that these fractions may act through complementary mechanisms. Their synergistic interaction, particularly against foodborne pathogens, suggests that combining phenolic compounds with antimicrobial peptides may improve antibacterial efficacy while reducing the concentration required for each component.

These findings highlight the potential of date palm seed by-products as sustainable sources of natural antimicrobial agents for food preservation and functional food applications, thereby contributing to the valorization of agricultural waste and supporting environmentally friendly preservation strategies.

Nevertheless, this study was limited to in vitro antibacterial evaluation. Further investigations should focus on identifying the individual bioactive compounds and peptides responsible for the observed activity using advanced analytical techniques (e.g., HPLC and LC-MS/MS), elucidating their mechanisms of action, assessing their toxicity and stability, and validating their effectiveness in real food systems before industrial application.

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