JOBS
مجلة العلوم الأساسية
Journal of Basic Science
Print -ISSN 2306-5249
Online-ISSN 2791-3279
العدد الثالث والأربعون
٢٠٢٦م /١٤٤٧هـ
العدد الثالث والأربعون
(574)- (555)

تأثير المستخلص الكحولي لغلاف النورة الزهرية لنخيل التمر والمشخص بتقنية الطيف الكتلي على حيوية بكتيريا *Proteus mirabilis*

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المستخلص:

أظهر تحليل GC–MS للمستخلص الكحولي لقينوة نخيل التمر وجود أربعة مركبات رئيسة، تشمل أحماض دهنية مشبعة وغير مشبعة Decanoic acid، Octadecenoic acid، مركب أميني متعدد الوظائف Propane–1,2,3–triamine، وإستر عطري طويل السلسلة ١،٤–Benzenedicarboxylic acid، جميعها مرتبطة بالنشاط البيولوجي للمستخلص مثل المضاد للبكتيريا والخصائص المضادة للأكسدة. وأظهرت نتائج دراسة التأثير على Proteus mirabilis أن مستخلص قينوة نخيل يمتلك قدرة واضحة على تثبيط نمو البكتيريا، مع زيادة مناطق التثبيط بارتفاع التركيز. اذ سجل المستخلص فعالية عالية ، و بلغ أقصى تثبيط ، لمستخلص قينوة نخيل التمر إلى ١٧,٠٠ ملم عند ٢٠٠ ملغم/مل .

وربما يُعزى النشاط المضاد للبكتيريا إلى احتواء المستخلصات على مركبات نشطة حيويًا مثل الفينولات، الفلافونويدات، والتربينات، التي يمكنها التفاعل مع الجدار الخلوي للبكتيريا وتعطيل

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وظائفها الداخلية. كما يعزز استخدام الكحول في الاستخلاص من فعالية المستخلصات عبر استخراج المركبات الفعالة بشكل أكبر. تشير النتائج إلى أهمية تحديد الجرعة المثلى لتحقيق أقصى تأثير مضاد للبكتيريا، ويدعم هذا النشاط الحيوي إمكانية استخدام مستخلصات نخيل التمر كمضادات ميكروبية طبيعية.

الكلمات المفتاحية : *Phoenix dactylifera* ،الاستخلاص ، التحليل بالكروماتوغرافيا الغازية–مطيافية الكتلة (GC–MS) ، *Proteus mirabilis* .

Effect of the alcoholic extract of the inflorescence sheath of the date palm, characterized by mass spectrometry, on bacterial viability of *Proteus mirabilis* bacteria

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Abstract

GC-MS analysis of the alcoholic extract of *Phoenix dactylifera* inflorescence revealed four major compounds: decanoic acid, octadecenoic acid, a multifunctional amino acid compound propane-1,2,3-triamine, and a long-chain aromatic ester 1,4-benzenedicarboxylic acid. These compounds are all associated with the extract's biological activity, including its antibacterial and antioxidant properties. The results of the study on Proteus mirabilis showed that the date palm kernel extract possesses a clear ability to inhibit bacterial growth, with the areas of inhibition increasing with

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increasing concentration. The extract exhibited high activity, reaching a maximum inhibition of 17.00 mm at 200 mg/ml.

The antibacterial activity may be attributed to the presence of bioactive compounds in the extract, such as phenols, flavonoids, and terpenes, which can interact with the bacterial cell wall and disrupt its internal functions. The use of alcohol in the extraction process also enhances the extract's effectiveness by extracting a greater number of active compounds. These findings highlight the importance of determining the optimal dosage to achieve maximum antibacterial effect, and this bioactivity supports the potential use of date palm extracts as natural antimicrobials.

Keywords: *Phoenix dactylifera* ,GC –Mass , extract , *Proteus mirabilis.*

1- Introduction

The date palm (*Phoenix dactylifera* L.) is one of the oldest cultivated plants in human civilization. Historical evidence suggests that its original habitat dates back to southern Iraq approximately 6,000 years ago. From there, its cultivation spread to vast areas due to its high adaptability to arid and semi-arid conditions and its abundant fruit production. Over time, date palm cultivation expanded to include most countries in the Middle East, North Africa, Southern Europe, and the Arabian Deserts. Later, it was introduced to new regions such as Australia, India, Pakistan, Mexico, South Africa, South America, and the United States [1]. Dates are currently cultivated in more than 30 countries, with Egypt, Saudi Arabia, and Algeria being among the top producers (Soomro *et al.*, 2023).

Date seeds are receiving increasing scientific attention due to their high content of carbohydrates, oils, dietary fiber, and protein, as well as their richness in bioactive phenolic compounds. Studies have shown that its chemical composition makes it an important substance in the food, cosmetics and medical supplement industries, as well as its potential role as a therapeutic nutritional agent for chronic diseases (AlKhoori *et al.*, 2022).

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Recent studies have also indicated that date palm fruits contain high levels of sugars (44–88%), fats (0.2–0.5%), protein (2.3–5.6%), and approximately fifteen types of minerals and vitamins, in addition to a significant amount of fiber (6.4–11.5%) (Nwaedh *et al*., 2024)

Chemical studies have shown that date palm kernels contain high levels of fatty acids. GC–MS analysis revealed oleic acid and lauric acid, and pollen grains contain significant amounts of palmitic, linoleic, and linolenic acids (Al-Jaber, 2017 and Allehaib).

Herbal medicine is one of the most important branches of complementary medicine due to the role of medicinal plants in promoting human health and preventing disease. Modern scientific evidence indicates that many plant extracts possess therapeutic efficacy exceeding that of conventional drugs, due to their richness in active compounds such as alkaloids, tannins, flavonoids, volatile oils, glycosides, and saponins, which exhibit clear antimicrobial effects' (Tlhapi *et al*., 2024). Plants are a major source of more than 50% of modern clinical drugs (Jain *et al*., 2019; Singh *et al*., 2021).

Although numerous studies have addressed the antimicrobial effects of date palm extracts, research on the male flowers as a source of antibacterial compounds remains limited. One local study demonstrated the clear efficacy of date palm pollen extract against bacterial species such as *Escherichia coli* and *Staphylococcus aureus* .

(Habib *et al*., 2023).

Proteus mirabilis, a member of the Enterobacteriaceae family, is a leading cause of catheter-associated urinary tract infections (CAUTIs) due to its rapid motility and its ability to produce urease and form antibiotic-resistant crystalline biofilms (Armbruster *et al*., 2018; Wasfi *et al*., 2020). Furthermore, P. mirabilis can cause various infections, including meningitis, osteomyelitis, and nosocomial infections of the urinary tract, wounds, and respiratory system. In certain cases, it can lead to bacteremia, particularly in the elderly (Bahashwan & El Shafey, 2013). This is attributed to its multiple

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pathogenicity factors, most notably the production of urease, biofilm formation, and the production of cytotoxic HpmA toxin (Fusco *et al*., 2017; Veisi *et al*., 2025).

Therefore, the need to search for natural sources with inhibitory activity against Proteus mirabilis becomes evident, especially given the increasing challenges of antibiotic resistance. This study aimed to achieve the following objectives.

1- To identify the chemical compounds in the crude extract of date palm Spathe using GC-MS technology.
2- To evaluate the inhibitory activity of the alcoholic extract of date palm Spathe against Proteus mirabilis bacteria under controlled laboratory conditions.

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Materials and Methods

Collection and Classification of the Plants Used in the Study

Samples of the spathe (*Phoenix dactylifera*) date palm cultivar 'Ghani al-Akhdar', grown in Mosul, northern Iraq, were collected and classified in the classification laboratory of the Department of Life Sciences, College of Education for Women, University of Mosul.

The samples were then dried away from sunlight and moisture for four days, with continuous stirring. Afterward, they were ground using a special electric grinder and stored in sterile containers, protected from light and moisture, until use.

International classification of date palm:

The Swedish botanist Linnaeus classified the plant kingdom “ ”.according to the following botanical divisions

(Division)	(Tracheophyta)
(Sub division)	(Anchiospermeae)
(Class)	(Monocotylendoneae)
(order)	Palmae (Arecacae)
(Family)	Palmaceae (Arecaceae)
(Genus)	(Phoenix)
(Speciece)	(Dactylifera)

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Preparation of the Alcoholic Extract of Date Palm Spathe Using a Soxhlet Apparatus .

The extraction process was carried out based on the boiling point of each solvent. Initially, 45 grams of finely ground date palm quinoa powder were placed in the extraction chamber of the Soxhlet apparatus. Then, 500 ml of petroleum ether was added to extract the oils, and the extraction process continued for 7 hours daily until the solvent in the apparatus became colorless. Subsequently, 500 ml of 70% ethanol was added to extract the active compounds, and the process continued until the solvent became colorless .

The extract was then concentrated using a Rotary Vacuum Evaporator (RVE) at 40°C and placed in sealed, opaque glass bottles, which were stored in a refrigerator until use. (Al-Jaghifi, 2022 and Abdullah, 2021).

Characterization of the Alcoholic Extract of Date Palm Clusters by GC-MS

GC-MS analysis was performed at the Environmental and Water Research Laboratories/Department of Environmental Research/University of

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Technology using an Agilent 5977A instrument equipped with an HP-5MS column (30 m × 0.25 mm) and an electron ionization mode at 70 eV. Helium was used as the carrier gas at a rate of 1 mL/min. A 0.5 µL sample was injected at a 10:1 ratio. The injector temperature was 250°C and the ion source temperature was 280°C. Mass scanning was performed within the range of 45–450 Da. The total analysis time was 60 minutes.

Preparation of Alcoholic Extract Concentrations of Date Palm spathe :

At concentrations of 25, 50, 75, 100, 125, 150, 175, and 200 mg/ml, the alcoholic extracts were prepared by dissolving them in DMSO. The solutions were then autoclaved at 62°C for 10–15 minutes.

Bacterial Isolates

The Gram-negative Proteus mirabilis isolates used in this study were obtained from the Life Sciences Research Laboratory, College of Science, University of Mosul. These isolates were isolated from clinical samples and identified using the Vitek-2 system. They were used in the current study to evaluate the inhibitory effect of the alcoholic extract.

Preparation of Mueller-Hinton Medium :

The ready-to-use Mueller-Hinton agar medium was prepared according to the manufacturer's instructions, NEOGEN, as indicated on the package. This involved dissolving 38 grams of Mueller-Hinton agar powder in 1 liter of distilled water, adjusting the pH to 7.3, and sterilizing the mixture in an autoclave at 121°C and 15 psi for 15 minutes. The sterile medium was then poured into sterile dishes for use .

Sensitivity Testing Method: The inhibitory activity of the alcoholic extracts of date palm sap was investigated against Proteus mirabilis bacteria using the agar well diffusion method. 5 mm diameter holes were made in Mueller-

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٢٠٢٦م /١٤٤٧هـ

Hinton agar dishes, and the surface of the medium was inoculated with a bacterial suspension prepared from 14–16 hour cultures. Subsequently, 0.1 cm^3 of the diluted bacterial suspension was homogeneously distributed onto the agar surface using a sterile cotton swab .

The plates were incubated at 37°C for 30 minutes to allow absorption. The wells were then filled with 10 µL of extract concentrations (25, 50, 75, 100, 125, 150, 175, and 200 mg/mL), and the plates were incubated again at 37°C for 24 hours. Areas of inhibition were measured and recorded in millimeters (mm). (Parez & Bezerque, 1990)

Results and Discussion

GC-MS Mass Spectrometry Analysis Results of the Alcoholic Extract of Date Palm Spathe :

Table (1) shows the results of the GC-MS analysis of the alcoholic extract of the date palm kernel, revealing four major compounds identified based on mass spectra and probability values. These compounds include fatty acids, aromatic compounds, and amino acids. Decanoic acid, which appeared at a retention time of 17.350 minutes with the formula C10H20O2, a mass of 172, and a probability of 75%, is a saturated fatty acid common in plants and oils. It possesses antibacterial and antifungal activity and contributes to enhanced antioxidant properties. Its concentration in the alcoholic extract indicates the alcohol's ability to extract fatty acids, confirming the richness of the flower kernel in these compounds, which are linked to the extract's biological activity. A study by Mancini (2015)confirmed the presence of this acid in date palm kernel oil. Octadecenoic acid (O6) was detected at a retention time of 19.192 minutes with the formula C18H34O2, a mass of 282, and a probability of 83%. This unsaturated fatty acid possesses anti-inflammatory properties and significant antimicrobial activity. It also strengthens the immune system and reduces oxidative stress, giving the extract high pharmacological efficacy compared to saturated fatty acids. A study confirmed the presence of octadecenoic acid in Cassia fistula (yellow cassia tree(Kadhim *et al.*, 2016).

JOBS
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٢٠٢٦م /١٤٤٧هـ

Propane-1,2,3-triamine appeared at a retention time of 21.125 minutes with the formula C3H11N3, a mass of 89, and a probability of 76%. It is a multifunctional amine compound that may indicate the presence of biologically active nitrogenous compounds. It is characterized by its ability to inhibit bacteria by interacting with enzymes or changing the surface charge of cells. It may also contribute to antioxidant activity. Its presence, despite its rarity, reflects the diversity of compounds in the extract.

The analysis confirmed the presence of 1,4-Benzenedicarboxylic acid at a retention time of 4.183 minutes, with the formula C24H38O4, a mass of 390, and a probability of 79%. The compound is likely a long-chain ester derived from terephthalic acid. These esters are characterized by antioxidant properties and the ability to inhibit certain microbial enzymes. In sesame (Sesamum prostratum), gas chromatography-mass spectrometry (GC-MS) analysis also revealed the presence of benzenedicarboxylic acid (Dhanaraj *et al*., 2025)For more details, see the figures.

JOBS
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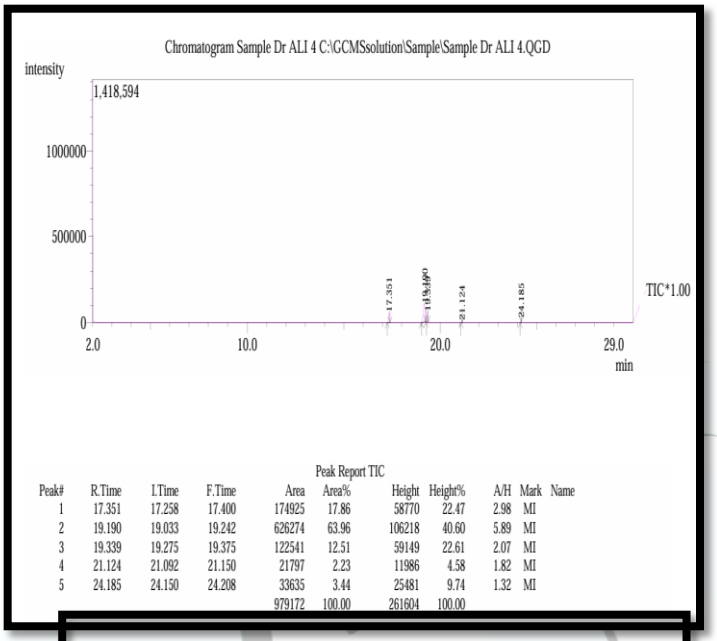
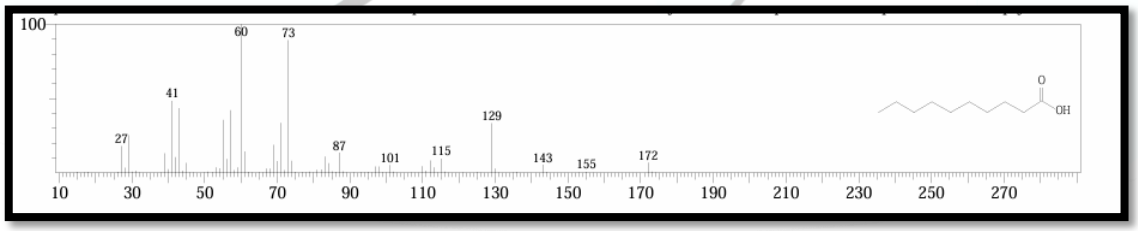
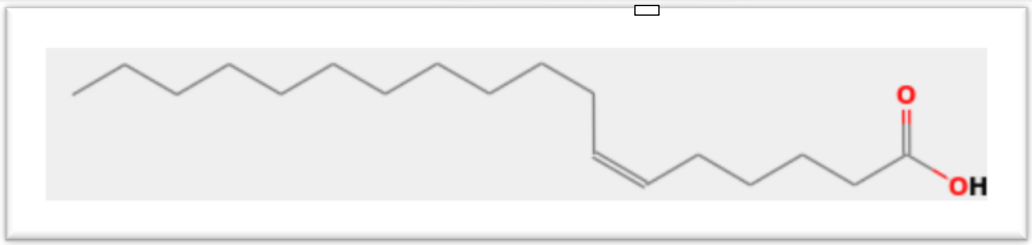


Peak Report TIC

Peak#	R.Time	I.Time	F.Time	Area	Area%	Height	Height%	A/H	Mark	Name
1	17.351	17.258	17.400	174925	17.86	58770	22.47	2.98	MI	
2	19.190	19.033	19.242	626274	63.96	106218	40.60	5.89	MI	
3	19.339	19.275	19.375	122541	12.51	59149	22.61	2.07	MI	
4	21.124	21.092	21.150	21797	2.23	11986	4.58	1.82	MI	
5	24.185	24.150	24.208	33635	3.44	25481	9.74	1.32	MI	
				979172	100.00	261604	100.00			

Decanoic acid





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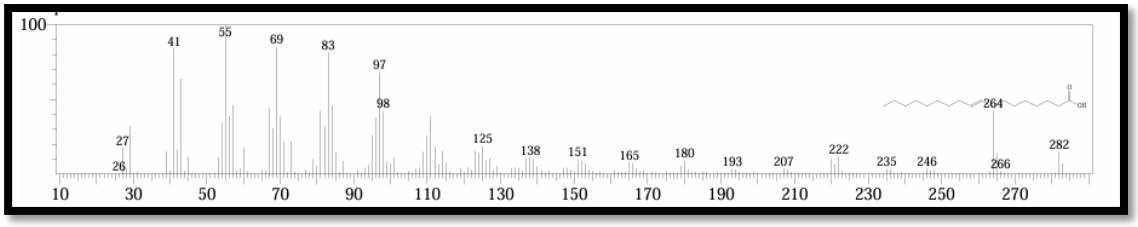
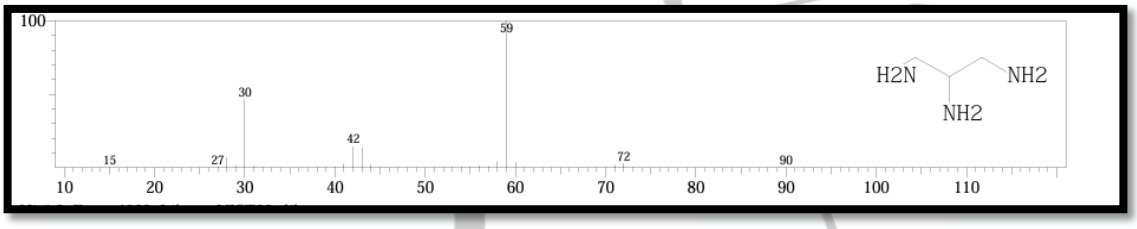
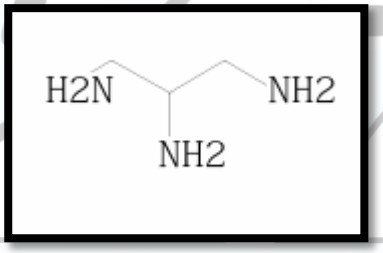


٦ -Octadecenoic acid



Propane-1,2,3-



Propane-1,2,3-

JOBS
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Journal of Basic Science
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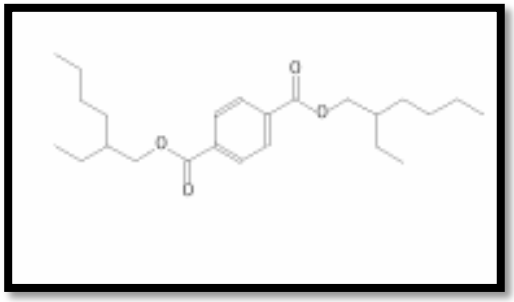
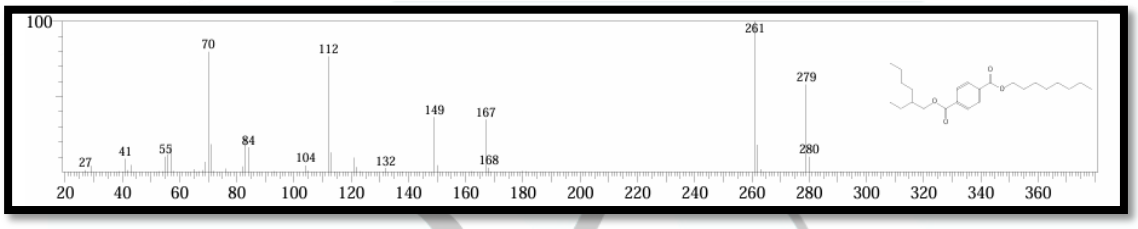


Benzenedicarboxylic



Benzenedicarboxylic acid١٠٤ -

Table (1): Results of GC-MS analysis of the alcoholic extract of date palm spathe

Compound Name	RT min	Formula	MW	probability
Decanoic acid	17.350	C10H20O2	172	%٧٥
Octadecenoic acid	19.192	C18H34O2	282	%٨٣
Propane-1,2,3-triamine	21.125	C3H11N3	٨٩	%٧٦
1,4-Benzenedicarboxylic acid	4.183٢	C24H38O4	٣٩٠	%٧٩

JOBS
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Journal of Basic Science
Print -ISSN 2306-5249
Online-ISSN 2791-3279
العدد الثالث والأربعون
٢٠٢٦م /١٤٤٧هـ

Effect of Alcoholic Extract of Date Palm spathe on the Viability of Proteus mirabilis

Table (2), Figure (1-2), shows the effect of date palm spathe extract on the growth of the bacterial isolate Proteus mirabilis at different concentrations (25–200 mg/ml). The results showed that the extract had a clear ability to inhibit bacterial growth, with a general increase in the zone of inhibition. With increasing concentration, the inhibitory effect of the date palm spathe extract started at 50 mg/ml (12.00 mm^3) and gradually increased to reach a maximum value at 200 mg/ml (17.00 mm^3), with an average of 13.25 mm^3. This aligns with the findings of (Gomaa *et al*. 2024) , whose study, evaluating the antimicrobial and anti-biofilm properties of *Phoenix dactylifera* seed extract in vitro, found that the PDPE extract exhibited varying antimicrobial activity against different microorganisms, with the highest effect against *Staphylococcus aureus*, followed by lower effects against *Salmonella* and *Shigella sonnei*. Furthermore, these results are consistent with those of Bentrad *et al*. (2017) , who reported that pollen extract exhibited strong inhibition against *Escherichia coli*, *Staphylococcus aureus*, MRSA, and *Enterococcus faecalis*. This is attributed to the abundant oleic acid (36.69%) and lauric acid (20.49%) present in date seeds. In contrast, pollen contains significant amounts of palmitic acid (22.27%), linoleic acid (33.4%), and linolenic acid (17.05%).

The antibacterial activity of the extract can be explained by the plant's chemical composition, as it contains bioactive compounds such as phenols, flavonoids, and terpenes, known for their ability to damage the bacterial cell wall and disrupt internal cellular processes. The variation in efficacy between concentrations may be attributed to the different concentrations of these compounds in each part of the plant, in addition to the effect of the extraction solvent, as alcohol enhanced the extraction of the active compounds.

It is important to determine the optimal dosage to achieve maximum antibacterial effect before any potential practical or clinical application. This

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العدد الثالث والأربعون
٢٠٢٦م /١٤٤٧هـ

effect may be due to the high capacity of these extracts to damage the bacterial cell wall and internal structure, and it is possible that alcohol facilitated the extraction of active compounds such as phenols, flavonoids, and terpenes, known for their antimicrobial activity

(Al Tamimi *et al*., 2021).

This aligns with Al-Luwaizi's (2019) assertion that the inhibitory effect is due to the plant extracts being rich in active compounds containing hydroxyl groups. It works by inhibiting the action of enzymes through oxidizing groups, which react with the sulfahydryl group or with proteins and cause them to denature or decompose, or by converting phenolic compounds into quinone compounds, which are characterized by their ability to closely bind to neutral amino acids, thus losing their effectiveness. Also, the alkaloids that are attracted by solvents are characterized by their medical and therapeutic efficiency through their association with the DNA of microbes, which is the reason that makes them have a high inhibitory ability towards many types of Gram-negative and Gram-positive bacteria.

JOBS
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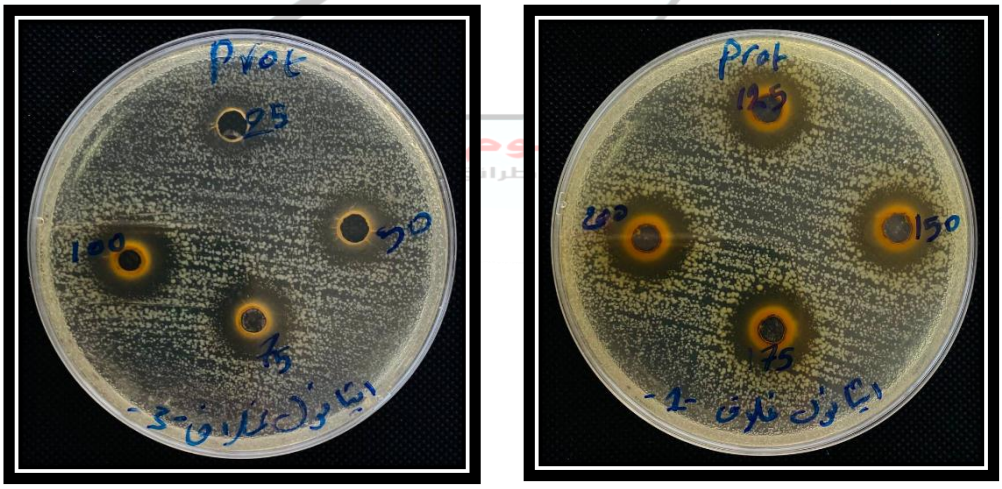
Table (2): Shows the effect of the alcoholic extract of date palm spathe on the viability of *Proteus mirabilis* bacteria

Date palm spathe extract	Concentrations
0.00 e	25%
12.00 d	50%
14.00 bcd	75%
15.67 bcd	100%
16.00 ab	%125
15.00 abc	150%
16.33 ab	%175
17.00 a	%200
13.25 a	**average**



Photos (1) illustrates the effect of the alcoholic extract of palm spathe on the bacteria *Proteus mirabilis*

JOBS
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٢٠٢٦م /١٤٤٧هـ

Conclusion :

The results of this study show that alcoholic extracts of the date palm spathe (*Phoenix dactylifera*) are rich in bioactive compounds, including fatty acids, aromatic compounds, amino acids, phenols, flavonoids, and terpenes, which contribute to antibacterial and antioxidant activity. Laboratory experiments demonstrated the clear efficacy of this extract against Proteus mirabilis, indicating the importance of the chemical composition of the plant part and the extraction solvent in determining biological activity. These results are also consistent with previous studies on the antimicrobial activity of extracts from the seeds and other plant parts of the date palm. The observation that maximum bacterial inhibition was achieved at a specific concentration (200 mg/ml) suggests the need to determine the optimal dosage to achieve maximum efficacy, thus opening the door to the use of this extract.

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٢٠٢٦م /١٤٤٧هـ

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Journal of Basic Science
Print -ISSN 2306-5249
Online-ISSN 2791-3279
العدد الثالث والأربعون
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