



Synergistic Effectiveness of Phoenix dactylifera Seed Extract with Some Antibiotics on *Staphylococcus aureus* Bacteria In-vitro

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Abstract

IN the community and hospital settings, antibiotic resistance poses a significant 21st-century challenge. As a result, diverse therapeutic methods are imperative. Medicinal plants, known since ancient times, are gaining increased attention for treating bacterial infections. This study aimed to evaluate the inhibitory effectiveness of Phoenix dactylifera seed extract, comparing it with antibiotics, and assessing its synergy with antibiotics against *S. aureus*. Various extract concentrations (10%, 25%, 50%, 75%, 100%) and antibiotics (Ampicillin, Ceftriaxone, Gentamicin, Piperacillin) were employed. 37 isolated bacteria samples were grown on Muller-Hinton agar, and the well diffusion method was applied. Results revealed Gentamicin as the most effective antibiotic, averaging 29. Phoenix dactylifera extract exhibited inhibitory effects on *S. aureus*, with the 100% concentration yielding the best results (average of 13.667). Synergism between Gentamicin and the 100% extract concentration surpassed individual antibiotics and varied extract concentrations, achieving an average of 33.667. The combined use of antibiotic, extract, and their synergy significantly reduced germ count. Evaluation of outcomes highlighted that the 100% extract plus HLG antagonist synergy produced the greatest bacterial elimination results. These findings support the concept that bacterial illnesses can be eradicated by employing antibiotics in concert with plant extracts. Future research on different bacteria should consider various extract concentrations.

Keywords: Synergistic activity, Phoenix dactylifera seed extract, *Staphylococcus aureus*.

Introduction

Due to the ease with which these plants can be used to make concentrates, extracts, dry pills, or tablets that contain substances that are both highly effective at eliminating infections and have a physiological effect that promotes rapid healing, interest in medicinal plants has recently increased in most parts of the world. Using medicinal plants has other advantages in addition to no negative effects [1]. The research [2] also demonstrated that plants have vital minerals including Na, K, Mg, and P, which improves the plant's ability to eradicate germs. The seeds of the Phoenix dactylifera are one of the significant medicinal plants that are used to cure a variety of ailments. Peroxidase is one of the redox enzymes that catalyses the conversion of hydrogen peroxide into water, which removes the damage that hydrogen peroxide causes to the organism's cells.

While it is still present. Seeds are thought of as a component of the plant and are a source of numerous active substances and enzymes, including peroxidase. Given that date pits are used to treat a variety of serious illnesses, including skin infections, and that studies have shown them to be anti-inflammatory, anti-carcinogenic, and anti-mutagenic, this suggests that it can interfere with a number of essential processes due to their release of free radicals [3], furthermore, they function Because it includes potent components that are shown to be antioxidants by science-flavonoids and phenolic compounds-it lowers the risk of cardiovascular illnesses. Proanthocyanidins, which are also present, aid in the prevention of diabetes, DNA damage, excessive cholesterol, digestive problems, and other illnesses [4].

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According to [5] microorganisms are a varied collection of living things that make up over 60% of the biomass on Earth. *S. aureus* bacteria are classified as staphylococci and can be either monogamous or dioecious [6]. For 24 hours, at 37°C, these bacteria are cultured on nutrient agar, blood agar, and milk agar, among other nutrient-rich culture medium [7]. These colonies have a round appearance with a diameter of two to three millimeters. They are shiny, smooth, opaque, and colored. Golden in hue, it can also be orange, yellow, or cream in tone. To isolate these bacteria from samples, selective media such as mannitol salt agar, broth medium with 10% table salt, or milk medium with 7% table salt can be used. Pathogenicity [8]. Numerous virulence factors, including enterotoxins, beta, delta, and beta toxins, yeast coagulase, and white cell killer, are present in *Staphylococcus aureus* [9]. Many illnesses are caused by *Staphylococcus aureus*. illnesses include burn infections, bacteremia, septicemia, osteomyelitis, skin pustules, boil ulcers, carbuncle, conjunctivitis, and infections from scrapes and wounds, among many more illnesses [10]. This investigation sought to determine the inhibitory efficacy of date seed extract fro, as well as how well it worked in conjunction with many antibiotics.

Material and Methods

This study compared the inhibitory effects of Phoenix dactylifera seed extract with various antibiotics and looked at how well they worked in concert with resistance. It was carried out at the labs of the University of Anbar's College of Education for Girls. The seeds for Phoenix dactylifera were procured from the College of Agriculture at Anbar University. Five different concentrations of Phoenix dactylifera seeds were examined in this investigation. The percentages are 10%, 25%, 50%, 75%, 100%, and five distinct antibiotics: ceftriaxone, ampicillin, lincomycin, gentamicin, and piperacillin. Additionally, the synergy between these antibiotics is being studied.

The extraction of bioactive compounds from Phoenix dactylifera (date palm) seeds involved using a solvent extraction method, employing either ethanol as solvent [11]. This process included dissolving and extracting bioactive compounds from the seeds. A Soxhlet extractor was utilized as the extraction device. The Soxhlet extractor, comprising a condenser, a boiling flask, and a thimble, facilitated the continuous extraction of compounds from the

plant material using a solvent. The choice of the Soxhlet extraction method was based on its efficiency in extracting various compounds from seeds, including phenolic compounds and antioxidants [12].

Collection of samples

This study included collecting 50 samples from various clinical sources from Ramadi Teaching Hospital.

Diagnosis of samples

It was discovered that 37 samples were *S. aureus* after the sample was cultured on blood agar media, colouring it appropriately, looking at it under a microscope, analysing its phenotypic traits, doing biochemical tests, and looking at it with the Vitech 2 equipment.

Biochemical tests

Five types of antibiotics specific for *Staphylococcus aureus* bacteria were used, and the results were as shown in Table 1.

Statistical analysis:

A one-way fully randomised design (C.R.D.) was used to analyse the data, and the L.S.D. test was used to determine if there were any significant mean differences below the 0.05 threshold. The statistical application SPSS was utilised for data analysis, and the Excel programme was employed to present the findings [13].

Results and Discussion

Detecting the effect of Phoenix dactylifera seed extract on the growth of S. aureus bacteria:

By distributing 0.1 ml of bacterial suspension over Muller-Hinton agar medium, the susceptibility of bacteria to Phoenix dactylifera seed extract was examined. After that, the plates were kept at room temperature for fifteen minutes so that the inoculum could be absorbed. Next, using a sterile cork drill with a 5. mm diameter, holes (wells) were formed in the feeding medium that had been infected with bacteria. Then, 10 microliters of each concentration of the extract were transferred and placed within the hole using a micropipette. Simultaneously, control dishes were inserted into the hole and incubated for 18–24 minutes at 37°C using 10 microliters of sterile distilled water in lieu of the Phoenix dactylifera seed extract. An hour later, the diameter of the inhibition zone(the region around the hole where no bacterial

growth occurs) was measured using a ruler to determine the outcome.

There were no statistically significant differences between Ampicillin, which had a mean of (4.667) and a standard error of (0.333), and Ceftriaxone, which had a mean of [5] and a standard error of (0.33), as well as between Ceftriaxone and Piperacillin, which had a mean of [5] and standard error of (0.333). (0.577) There are no statistically significant differences between Gentamicin, which had a mean [29] and standard error (1), and Lincomycin, which had a mean (27.667) and standard error (1.202), but there are statistically significant differences between Gentamicin, which has the highest mean, and Ampicillin, which has the lowest mean compared to the other antibiotic types, and the LSD value was (2.562).

The maximum inhibitory efficiency in eradicating *S.aureus* bacteria is found in the highest concentration (100%) of Phoenix dactylifera seed extract. This is due to the extract's biological activity, antioxidant and anti-inflammatory properties, which operate to suppress *Staph. aureus* bacteria.

In addition, [14, 2] concurred that Phoenix dactylifera seed extract had a beneficial impact against Gram-positive bacteria strains.

The extract and the antibiotic were used in four synergistic concentrations, with 100% of the extract being utilised since it is the best concentration, and it was used synergistically with four different antibiotics. The inhibitory efficiency of the synergism between the plant extract and the antibiotics is shown in Table (3), and the statistical analysis findings demonstrate that there are significant differences. With an arithmetic mean of 33.667 and a standard error of 0.882, the 100% concentration of the anti-HLG (gentamicin) produced the greatest statistically significant difference when compared to the other concentrations (100%+AM), (100%+LE), and (100%+PC). The corresponding arithmetic mean values were 10.333, (11.667), and (10), respectively, with standard errors of (0.333),

(0.333), and (0.577), and the LSD value below the 5% level ranged from (1.912).

In comparison to the other concentrations, the 100% concentration of the HLG antibiotic exhibited the best inhibitory efficiency due to the synergistic activity of the plant extract and the antibiotics in removing *Staphylococcus aureus* germs. Additionally, the synergy outperformed the extract and the antibiotics in terms of efficacy.

Plant extracts block the production of some necessary proteins or the development of the microscopic organism's cell wall, which explains the mechanism of their suppression.

Phoenix dactylifera seed extract works well because it contains antibacterial agents such as proanthocyanidins, alkaloids, steroids, flavonoids, vitamins, and cinnamic acids [15]. Moreover, the anti-HLG can stop the development of bacteria by attaching itself to the cell wall and preventing it from growing. It breaks down or alters the function of cell membranes, impairs the control of permeability, and inhibits certain enzymes that play a crucial metabolic role in growth and reproduction [16] these results support the use of synergism between Phoenix dactylifera seed extract and anti-HLG to treat bacterial infections.

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The authors declare that the present study has no financial issues to disclose.

Conflict of interest

None

Authors contributions

Iman Raeed Azzam and Wafa Talaa Radif: Practical work and Manuscript writing.

TABLE 1. The biochemical tests for *Staphylococcus aureus* bacteria

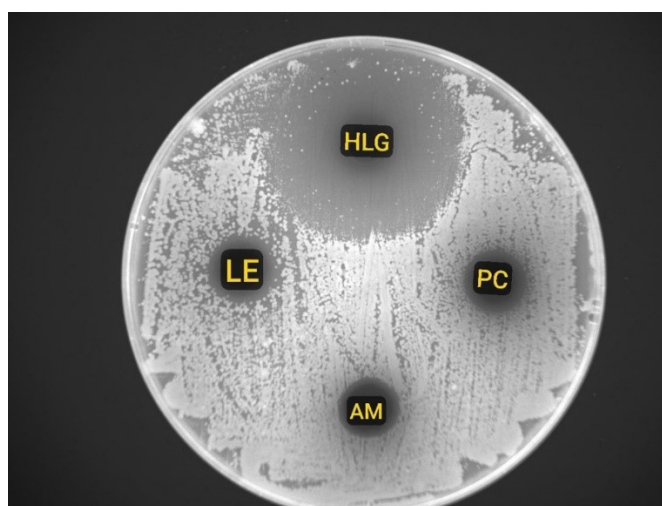
No.	Biochemical tests:	<i>Staphylococcus aureus</i>
1	Mannitol salt fermentation	+
2	Catalase test	+
3	Oxidase test	+
4	Coagulase test	+
5	Urease test	+

TABLE 2. Shows the effectiveness of antibiotics against isolates of *S.aureus* bacteria

Treatment	Character 1
	Zones of inhibition (mm)
Ampicillin	4.667 ±0.333
Ceftriaxone	5.000 ±0.577
Gentamicin	29.000 ±1.000
Lincomycin	27.667 ±1.202
Piperacillin	5.000. ±0.577
LSD 5%	2.562
C.V.	9.746

TABLE 3. Inhibitory effectiveness of the synergism between the plant extract and antibiotics

Treatment	Character 1
	Zones of inhibition (mm)
100%+ AM	10.333 ±0.333
100% + LE	11.667 ±0.333
100% + PC	10.000 ±0.577
100% + HLG	33.667 ±0.882
LSD 5%	1.912
C.V.	6.091

Fig. 1. The inhibitory effectiveness of the synergy between *Phoenix dactylifera* seed extract and the antibiotic Gentamicine (HLG) on *Staphylococcus aureus* bacteria

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الفعالية التأزيرية لمستخلص بذور *Phoenix dactylifera* مع بعض المضادات الحيوية على بكتيريا المكورات العنقودية الذهبية في المختبر

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في بيئة المجتمع أو المستشفى، تعتبر مقاومة الأدوية للمضادات الحيوية إحدى مشاكل القرن الناشئة، لذلك أصبح استخدام الطرق العلاجية المختلفة ضرورياً، حيث تم اكتشاف النباتات الطبية منذ القدم، وتزايد الاهتمام بها لاستخدامها في علاج الالتهابات البكتيرية، وذلك بتحويلها إلى مستخلصات أو مركبات أو حبوب جافة أو أقراص، وذلك لاحتوائها على العديد من المواد الفعالة. هدفت هذه الدراسة إلى تقييم الفعالية التثبيطية لمستخلص بذور *Phoenix dactylifera* ومقارنتها مع بعض المضادات الحيوية ومقارنتها. بتأزر المستخلص النباتي مع المضادات الحيوية على بكتيريا *S. aureus* المسببة للعديد من الالتهابات، حيث تم استخدام تراكيز مختلفة من المستخلص (10%، 25%، 50%، 75%، 100%) والمضادات الحيوية (أمبيسلين، سيفترياكسون، Gentamicin, Piperacillin) والتأزر بين أفضل تركيز للمستخلص في القضاء على البكتيريا وهو 100% مع عدد من المضادات الحيوية يتم استخدام 37 عينة بكتيرية معزولة ومحددة. تمت زراعة هذه العينات على وسط أجار Muller-Hinton، واستخدمت طريقة الانتشار الجيد، وتمت مقارنة النتائج بين المستخلص والمولد المضاد والمؤازر. أظهرت النتائج أن المضاد الحيوي الجنتاميسين أعطى أعلى فعالية تثبيطية في القضاء على البكتيريا. بكتريا بمتوسط 29. كما أن لمستخلص *Phoenix dactylifera* تأثير تثبيطي لبكتيريا *S. aureus*، وأعطى تركيز 100 أفضل نتيجة بمتوسط (13.667) في القضاء على البكتيريا. أظهرت النتائج أن التأزر بين المضاد الحيوي الجنتاميسين والمستخلص بتركيز 100% أعطى أفضل نتيجة في القضاء على البكتيريا مقارنة بالمضاد الحيوي والمستخلص بتركيزه المختلفة وبمتوسط حسابي 33.667.

إن الجمع بين المضاد الحيوي والمستخلص وتأزرهم يقلل بشكل كبير من كمية الجرثيم. عندما تم تقييم نتائج المستخلص والمضادات الحيوية والتأزر، أنتج المستخلص 100% بالإضافة إلى تأزر مضادات HLG أفضل النتائج من حيث القضاء على البكتيريا. وبالتالي فإن هذه النتائج تضيف مصداقية على فكرة أنه يمكن القضاء على الأمراض البكتيرية باستخدام المضادات الحيوية بالتنسيق مع البكتيريا. ننصح بأن الأبحاث المستقبلية على أنواع مختلفة من البكتيريا تستخدم تركيزات مختلفة من المستخلص.

الكلمات الدالة: النشاط التأزيري، مستخلص بذور *Phoenix dactylifera*، المكورات العنقودية الذهبية.