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GREEN THERAPEUTICS FROM NATURE: DATE PALM (*PHOENIX DACTYLIFERA)* LEAVES A SUSTAINABLE SOURCE OF BIOACTIVE COMPOUNDS WITH POTENT ANTIBACTERIAL ACTIVITY

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ABSTRACT

Infectious diseases remain a major threat to global health, responsible for millions of deaths every year, and the disturbing rise of antimicrobial resistance is quickly diminishing the effectiveness of traditional antibiotics and posing an urgent challenge to modern medicine. Date palm (*Phoenix dactylifera*), is one of the oldest cultivated plants with high cultural and medicinal value in the Arab world, among the oldest cultivated plants. The present study was carried out to evaluate the phytochemical constituents and antimicrobial activity of the aqueous and methanolic leaf extracts of *P. dactylifera*. Phytochemical screening showed the presence of significant bioactive compounds like flavonoids, glycosides, phenols, saponins, tannins, terpenoids and steroids. The antibacterial potential of the extracts was determined by agar well diffusion method against four common strains of bacteria, Ciprofloxacin was used as standard control. Among the tested samples, the methanolic leaf extract demonstrated the strongest antibacterial activity against *Pseudomonas aeruginosa*, producing a zone of inhibition of 33 ± 0.2 mm. Lower inhibition zones were observed against *Staphylococcus aureus* (25.0 ± 0.2 mm) and *Escherichia coli* (24.0 ± 0.5 mm). The aqueous extract also exhibited notable antibacterial activity, particularly against *S. aureus*, with a zone of inhibition of 13 ± 0.3 mm. Phytochemical analysis of the methanolic extract confirmed the presence of flavonoids, glycosides, phenols, tannins, steroids, and saponins. Overall, the findings demonstrate the promising antimicrobial potential of both aqueous and methanolic extracts of *P. dactylifera* leaves against *E. coli*, *S. aureus*, *P. aeruginosa*, and *Bacillus subtilis*. The present study reveals the therapeutic potential of the date palm leaves as a natural source of anti-bacterial agents and their possible use in the development of value-added pharmaceutical products including the treatment of complex diseases like cancer.

KEYWORDS: Antibacterial activity, *Phoenix dactylifera,* inhibition zone, agar well diffusion assay, methanolic extract, aqueous extract, phytochemical, leaf extract.

1. INTRODUCTION

In developing countries, a large proportion of health problems are due to infectious diseases (Sashi et al., 2003). The excessive use of chemically synthesized antibiotics in recent years has resulted in the emergence of drug-resistant pathogens, thus decreasing the effectiveness of conventional therapeutic approaches and adversely affecting the human immune system. This alarming trend has intensified the global search for alternative, natural remedies to fight such conditions. Almost every new antibiotic has been met with the development of resistance to it (WHO, 2014). Therefore, the global failure to control the spread of antibiotics-resistant pathogens and emergence of new bacterial infections has forced the international healthcare bodies and scientific community to search for new antibacterial agents.

The search for plant-derived compounds in drug development has renewed interest, especially those with the capacity to overcome antimicrobial resistance (Bhardwaj V., 2025). Nature provides a vast resource of medicinal plants that are of utmost importance in the promotion of human health and survival (Prajapati et al., 2002). The greater interest in herbal medicine in the present era is highly relevant today as pharmaceutical industries are still isolating and synthesizing bioactive constituents from medicinal plants to develop new therapeutic agents. Accordingly, Plants could be a major source of drugs in future. Medicinal plants have been the source of around 42% of 25% top selling medications worldwide recently (Al- Daihan. S et al., 2012).

Antimicrobial compounds can be found in abundance in certain plants. A significant portion of newly developed antibiotics are derived from natural or semi-synthetic sources, and about 20% of the world's plants have undergone pharmacological or biological testing (Mothana and Lindequist, 2005). The general perception that "Green Medicine" is safer and more reliable than expensive synthetic medications that may have negative side effects is the main reason why many pharmaceutical corporations are interested in plant-derived medications. 80% of people worldwide currently utilize herbal medicine for some form of primary health care, according to a World Health Organization (WHO) report (Sujatha, 2005).

Phoenix dactylifera Linn., a dioecious, medium-sized date palm tree with pinnate leaves that can have up to 150 leaflets and spines on the petiole, is a member of the Arecaceae (Palmae) family. The date palm tree, which is native to the Middle East and North Africa, is widely grown for its fruit (Anjum et al., 2012; Abed et al., 2015). It is one of the oldest plants and has been cultivated in Mesopotamia since 7000 years ago, although scientists think that date palm use dates back thousands of years (Jaradat, 2011). Although practically every component of the date palm tree is utilized, its fruits are the most important. The interest in date palm fruits has increased over the past few decades (Jaradat, 2011). Date fruits are said to have anti-tumor agents, anti-inflammatory, anti-microbial, anti-oxidant, nephro-protective, hepato-protective, anti-diabetic properties (Rahmani et al., 2014). There are few studies on date palm leaves, but it has been suggested that they contain anti-diabetic properties (Mard et al., 2010).

(a) (b)

Figure 1: (a) The date palm tree, (b) Leaves, of *P. dactylifera*.

The goal of the current study was to assess the characteristics of *P. dactylifera* leaves, a significant medicinal plant with a wide range of therapeutic applications. The work focuses on the phytochemical profiling and assessment of the antibacterial properties of *P. dactylifera* leaf extracts, both aqueous and methanolic. In order to meet the increasing need for efficient substitutes for synthetic antibiotics worldwide, the goal is to investigate natural sources for the creation of innovative antimicrobial agents.

2. MATERIAL AND METHODS

2.1 Collection and Preparation of Plant Material

In January 2026, fully grown *P. dactylifera* (date palm) leaves (fig. 1b) were collected from the premises of Environment Laboratory Ras Al Khaimah Municipality and Khuzam area, United Arab Emirates. To establish aseptic conditions, the collected plant leaves were first properly cleaned under running tap water to get rid of dust and surface impurities, and then they were rinsed with sterile distilled water. Sterile filter paper was used to eliminate any excess moisture. A laboratory-grade electric mixer was used to grind the leaves into a fine

powder after they had been thoroughly separated and air-dried for six hours at 45°C in a controlled drying oven (Bhardwaj, V. 2023). Until it was needed for extraction and analysis, the powdered material was kept at room temperature in sealed containers.

2.2 Preparation of Plant extracts

Ten grams of powdered *P. dactylifera* leaves were steeped in 100 milliliters in two different solvents ;aqueous (distilled water) and methanol, respectively, for the extraction procedure. To ensure uniform mixing and the best possible solubilization of bioactive chemicals, the plant material was soaked in a rotary shaker for an entire night during the aqueous extraction process. After that, the supernatant was gathered and filtered for additional examination.

According to Bhardwaj, V (2022), the continuous hot extraction method was used to prepare the methanolic extract utilizing the Soxhlet apparatus technology. For further phytochemical and antimicrobial examination, the extract was concentrated by evaporating the solvent under low pressure and kept in sterile containers at 4 °C.

2.3 Microbial Strains and Culture Conditions

In the present study, the bacterial strains used were *Bacillus subtilis* (ATCC 6633), *E. coli* (ATCC 8739), *Staphylococcus aureus* (ATCC 25923), *Pseudomonas aeruginosa* (ATCC 27853) obtained from the American Type Culture Collection (ATCC) to determine the antibacterial activity. The bacterial strains were procured from Liofilchem Srl Italy. Pure culture of bacteria was maintained at 4 °C on nutrient agar slants. For the experimental assays, subcultures were prepared and used in triplicates to ensure the reproducibility and reliability of results.

2.4 Methodology for the Detection of Antibacterial Activity

2.4.1 Inocula preparation

To create actively developing cultures, the bacterial isolates were first cultivated in 5 mL of sterile nutrient broth and incubated at 37 °C for 18 hours. These overnight cultures used as inocula for the antimicrobial tests that followed (Bhardwaj, V. 2021).

2.4.2 Agar well diffusion assay

According to Bhardwaj, V. (2025), the agar well diffusion method was used to evaluate the antibacterial activity of *P. dactylifera* leaf extracts (methanolic and aqueous). Mueller-Hinton agar plates were inoculated with standardized bacterial suspensions, and wells of 6 mm in diameter were punched with a volume of 30 µL of each extract. The zones of inhibition were measured in millimeters to assess the antibacterial efficiency after the plates were incubated for 24 hours at 37°C. To guarantee accuracy and repeatability, each assay was run in triplicate, and the mean data were noted. To avoid any contamination, the entire experiment was carried out under stringent aseptic conditions.

2.5 Phytochemical analysis

Qualitative phytochemical screening was conducted on the plant extracts to identify the presence of bioactive compounds using standard procedures.

In order to identify flavonoids, strong sulfuric acid was added after the extract was combined in a 1:5 ratio with ammonia solution. The presence of flavonoids was shown by the emergence of a yellow tint that fades with standing.

The extract was treated with strong sulfuric acid, glacial acetic acid, and a few drops of ferric chloride in order to identify glycosides. The presence of glycosides was verified by the creation of a brown ring at the contact.

Phenolic compounds were tested by adding a few drops of 0.5% neutral ferric chloride solution to the extract. Dark green coloration indicated a positive result.

Steroids were identified by dissolving the extract in chloroform and carefully layering it with concentrated sulfuric acid. A red upper layer and a yellow lower layer exhibiting green fluorescence were indicative of steroids.

To test saponins, the extract was combined with distilled water, boiled in a water bath, and vigorously shaken. Persistent froth formation confirmed the presence of saponins.

The presence of tannins was determined by boiling the extract with distilled water, cooling it, and adding 0.1% ferric chloride solution. The development of a brownish-green or blue-black color indicated the presence of tannins.

Terpenoids were tested by mixing the extract with chloroform and concentrated sulfuric acid. The appearance of a reddish-brown layer at the interface confirmed their presence.

3. RESULTS AND DISCUSSION

The current investigation sought to assess the antibacterial activity and phytochemical makeup of *P. dactylifera* leaf methanolic and aqueous extracts. Ten grams of each extract were made for every 100 millilitres of solvent. Four bacterial strains *Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa,* and *Staphylococcus aureus* were used to test the extract's antibacterial activity. The following

discussion of the agar well diffusion assay and phytochemical screening results highlights the plant's potential as a source of bioactive antimicrobial compounds.

3.1 Antibacterial Activity of *P. dactylifera* Leaf Extracts

The agar well diffusion method was used to assess the antibacterial activity of *P. dactylifera* leaf extracts. With a maximal inhibition zone of 33 ± 0.2 mm against *P.aeruginosa*, the methanolic extract demonstrated the most noteworthy efficacy among the tested samples. With a zone of inhibition against *Staphylococcus aureus* of 13 ± 0.6 mm, the aqueous extract had the strongest antibacterial activity. (Table 1).

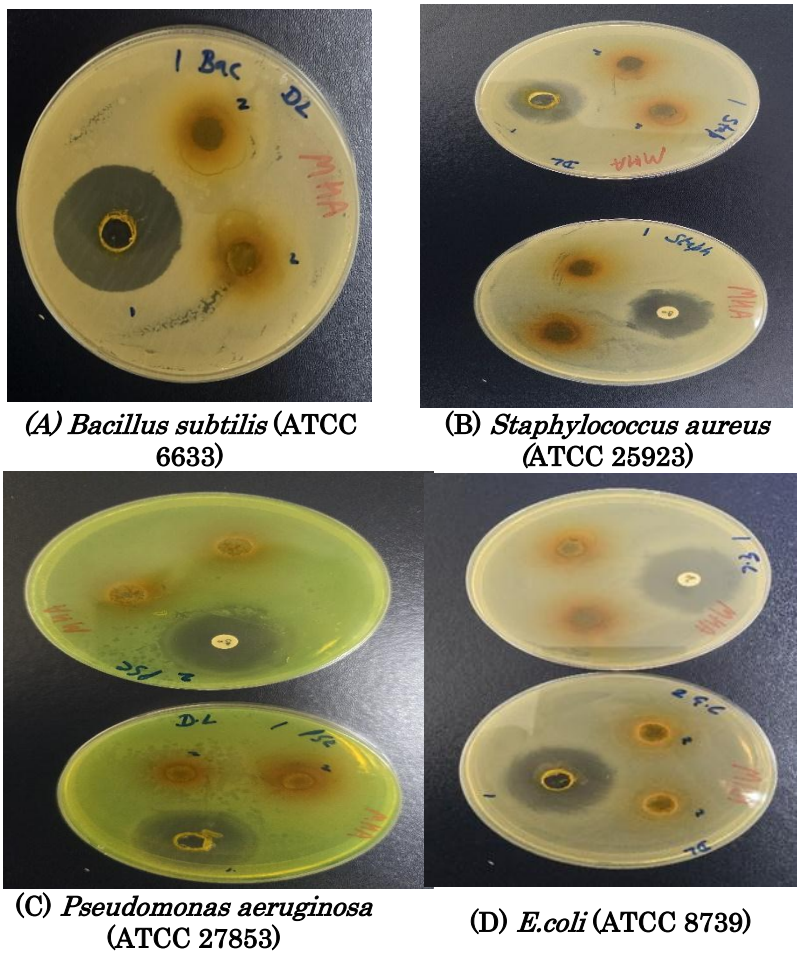
With a zone of 25±0.2 mm against *S. aureus* (figure 2B) and 24±0.5 mm against *Escherichia coli* (figure 2D), the methanolic extract had the least inhibitory effect. These findings imply that different bacterial strains have varying susceptibility to the kind of solvent utilized during the extraction procedure. The *Staphylococcus aureus* results were intriguing since it was more sensitive to the date palm leaf extract than it was to the antibiotic ciprofloxacin. There is a dearth of information on date palm leaves' antibacterial effectiveness. Al-daihan et al. (2012) reported that date palm leaves exhibit varying degrees of antibacterial activity against various Gram-positive and Gram-negative bacteria when extracted in water, acetone, and methanol. In a similar way, it was reported that the ethanol extract of date palm leaves exhibited strong antibacterial activity against *Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae, Proteus sp., Enterobacter sp., Staphylococcus aureus, and Streptococcus pneumoniae* (Abdul, M et al., 2013). According to Perveen et al. (2012), date palm leaf acetone and methanol extracts had strong antibacterial activity against *Escherichia coli, Pseudomonas aeruginosa, Shigella flexeneri, Staphylococcus aureus, Streptococcus pyogenes, and Bacillus subtilis*. The phytochemical components of date palm leaves are generally responsible for their antibacterial action in our study. According to Al-daihan et al. (2012), the main groups of antibacterial chemicals found in plants are phenolic compounds, alkaloids, flavonoids, and tannins. The results are consistent with earlier research, which found that aqueous extracts have less antibacterial action than methanol (Sooad et al., 2012). Sooad et al. (2012) reported that leaf extract had the best activity against *E. coli* in response to methanol extract (13.5 ± 0.33 mm), which is nearly identical to our results. The current investigation demonstrates that date palm leaf extracts were efficient for bacterial growth inhibitors. When it came to all test microorganisms, the methanol extracts outperformed the aqueous extracts. This could be because water extracted fewer chemicals than methanol, which was able to extract a greater variety of chemical components from plant material (Cowan, 1999). These findings support earlier research showing that methanol is a more reliable solvent for extracting antimicrobial compounds from medicinal plants than other solvents like water and hexane (Ahmad et al., 1998; Eloff, 1998; Lin et al., 1999, Karaman et al., 2003).

Table 1: Zone of inhibition (mm) of methanolic and aqueous extracts of *Phoenix dactylifera* leaves against test bacterial strains and ciprofloxacin as control.

SNo.	Microorganisms	Zone of Inhibition (mm) Methanol extract (ME)	Zone of Inhibition (mm) Aqueous extract (Aq E)	Zone of Inhibition (mm) Ciprofloxacin as control
1	Bacillus subtilis (ATCC 6633)	30 ± 0.0	10 ± 0.5	23 ± 0.5
2	E.coli (ATCC 8739)	24 ± 0.5	12 ± 0.8	18 ± 0.2
3	Pseudomonas aeruginosa (ATCC 27853)	33 ± 0.2	10 ± 0.3	22 ± 0.5
4	Staphylococcus aureus (ATCC 25923)	25 ± 0.2	13 ± 0.6	27 ± 0.3

Note: Values represent the mean ± standard deviation of triplicate experiments.

Similar results were reported by Emad et al. (2017), who found that the three date palm cultivars' methanol leaf extracts contained possible antibacterial agents, especially against bacteria, according to the antimicrobial test results. A visual comparison of the antibacterial activity of the methanolic and aqueous extracts of *Phoenix dactylifera* leaves against the tested bacterial strains is shown in Figure 2.



(A) *Bacillus subtilis* (ATCC 6633)

(B) *Staphylococcus aureus* (ATCC 25923)

(C) *Pseudomonas aeruginosa* (ATCC 27853)

(D) *E.coli* (ATCC 8739)

Figure 2: The zone of inhibition created by methanolic and aqueous extracts against *Bacillus subtilis* is shown in Figure 2(A). (B) illustrates the difference in the zone of inhibition between methanolic and aqueous extracts against *Staphylococcus aureus*; (C) shows the zone of inhibition that both extracts produce against *Pseudomonas. aeruginosa* (D) displaying the difference in the zone of inhibition between methanolic and aqueous extracts against *E. coli*. The control is ciprofloxacin.

3.2 Phytochemical Analysis of *Phoenix dactylifera* Leaves

The current study examined the presence of important phytochemicals, such as steroids, saponins, tannins, terpenoids, flavonoids, glycosides, and phenolic compounds, in methanolic extracts of *Phoenix dactylifera* leaves. All of the secondary metabolites that were evaluated were found in the methanolic leaf extract, as shown in Figure 3 and Table 2. The plant's medicinal potential is known to be influenced by these bioactive components, especially its antibacterial and antioxidant qualities.

Table 2: Phytochemicals in methanolic crude extracts of *Phoenix dactylifera* leaves.

SNo.	Phytochemicals	*Phoenix dactylifera* leaves methanolic extract
1	Flavonoids	+
2	Glycosides	+
3	Phenol	+
4	Saponins	+
5	Tannins	+
6	Terpenoids	-
7	Steroids	+

The results are in line with those published by Emad et al. (2017), who also found that the methanolic extracts of *Phoenix dactylifera* leaves included important phytoconstituents like flavonoids, saponins, alkaloids, and tannins. The pharmacological significance of *Phoenix dactylifera* is strengthened by the presence of such a diverse range of phytochemicals, which also supports the plant's traditional usage in herbal medicine. A prior investigation on date palm leaves found flavonoids,

phenols, terpenoids, alkaloids, and tannins (Al-Dawah et al., 2013), which is somewhat consistent with our results.

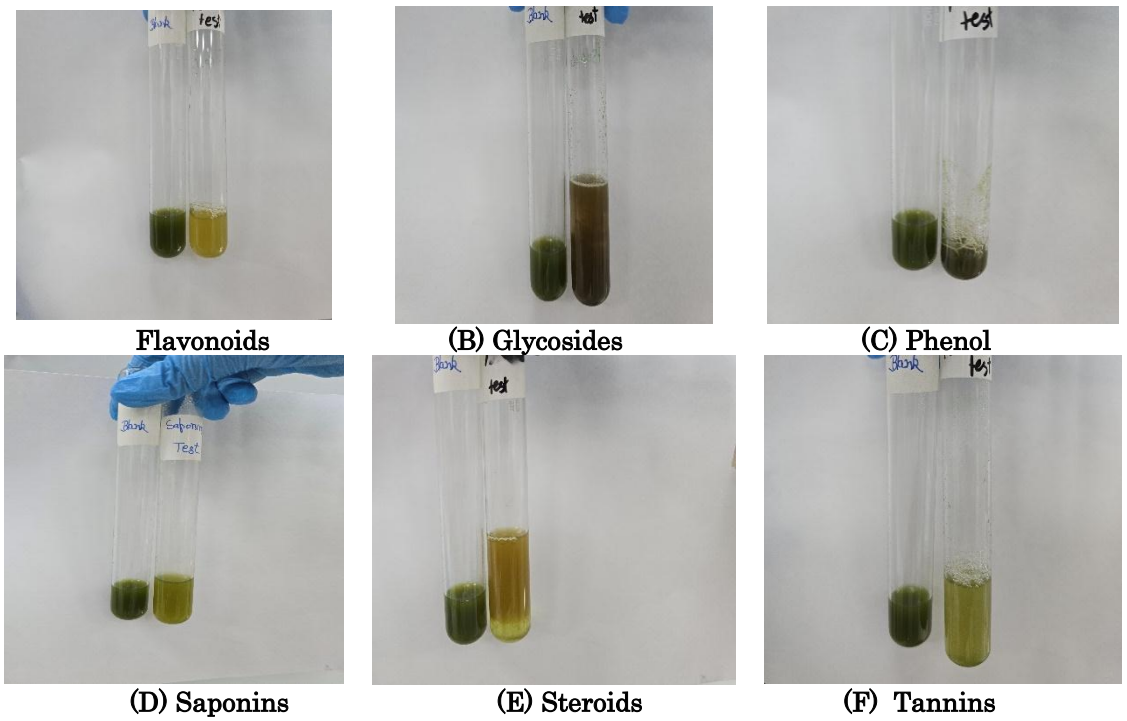


Figure 3: Showing the confirmation of Phytochemicals present in crude extract of *Phoenix dactylifera* leaves Figure 3 (A)Flavonoids (B) Glycosides (C) Phenol (D) Saponins (E) Steroids (F) Tannins.

These findings support the existence of several phytochemical groups of therapeutic value, especially those with antibacterial, antioxidant, and anti-inflammatory properties. The methanolic extract's better solvent capacity for both polar and non-polar bioactive chemicals may be the reason for its increased potency.

The current investigation demonstrates the promising antibacterial activity of leaf extracts from *Phoenix dactylifera*, especially the methanol extract, against strains of both Gram-positive and Gram-negative bacteria. Because of its polarity, methanol is frequently employed for phytochemical extraction; however, the success of aqueous extraction in this work may indicate that certain chemicals, including glycosides and tannins, are more soluble or active in water.

The plant's rich phytochemical profile, which includes flavonoids, glycosides, phenolics, saponins, tannins, and steroids all of which are known to have antimicrobial potential through a variety of mechanisms is credited with the observed antibacterial benefits.

It is commonly known that flavonoids and phenolic chemicals have antibacterial properties through interfering with nucleic acid production, chelating metal ions necessary for microbial metabolism, and rupturing bacterial cell membranes. Saponins have the power to make cell membranes more permeable, which can cause vital intracellular components to seep out (Bhardwaj, V., 2025). Tannins can bind to proteins and enzymes, rendering microbial adhesins and pathogenicity-related enzymes inactive. It is thought that steroids damage the integrity of microbial cell membranes by interfering with the lipid layer, which causes cell lysis.

The bacterial strains investigated have important medicinal implications. Skin infections, pneumonia, and bloodstream infections are frequently caused by *Staphylococcus aureus*, which is also frequently linked to antibiotic resistance, especially methicillin-resistant *S. aureus* (MRSA). Skin, blood, lungs, GI tract, and other bodily regions can all be impacted by *Pseudomonas aeruginosa*. While *Bacillus subtilis* is a model for Gram-positive bacteria and can cause opportunistic infections, *Escherichia coli* is a major cause of foodborne diseases and urinary tract infections. The hunt for new antimicrobial drugs, especially from natural sources, has become necessary due to the rising rise of drug-resistant forms of these infections.

From a medicinal perspective, the findings encourage the possible use of extracts from *Phoenix dactylifera*

in the creation of complementary or alternative antimicrobial therapies. The current study concludes that *Phoenix dactylifera* leaf extracts have antibacterial potential, with methanolic extracts showing greater effectiveness. The traditional usage of *P. dactylifera* in herbal therapy has a scientific foundation thanks to these findings, which also call for more research into possible pharmaceutical development, particularly in light of the rising antibiotic resistance.

4. CONCLUSION

In the current study, aqueous and methanolic leaf extracts of *Phoenix dactylifera*, often known as date palm, were subjected to phytochemical and antimicrobial studies. The methanolic extract was more effective in phytochemical screening, identifying the presence of important secondary metabolites like glycosides, flavonoids, phenols, tannins, steroids, and saponins, and it showed superior antibacterial activity across all tested microbial strains between the two solvent systems.

These results corroborate the traditional therapeutic usage of *Phoenix dactylifera* leaves and highlight their excellent antibacterial potential. Furthermore, *P.dactylifera* is a promising option for the creation of plant-based antibacterial medicines due to its proven broad-spectrum activity. This study emphasizes the use of date palm leaves in green synthesis techniques and provides an affordable, sustainable, and environmentally beneficial substitute for treating newly emerging and reemerging infectious illnesses. The study supports *Phoenix dactylifera* therapeutic potential for upcoming biomedical and pharmaceutical applications, adding to the growing field of medicinal plant research.

5. ABBREVIATIONS

SD, standard deviation; **ATCC**, American Type Culture Collection; **E**, Extract; **h**, hours; **C**, ciprofloxacin; ***P. dactylifera***, *Phoenix dactylifera.*

6. Ethics approval and consent to participate

Not applicable.

7. CONSENT FOR PUBLICATION

Not applicable.

8. AVAILABILITY OF DATA AND MATERIALS

The relevant data and materials are available in the present study.

9. COMPETING INTERESTS

The authors declare that they have no competing interests. All procedures followed were in accordance with the ethical standards (institutional and national).

10. FUNDING

Not applicable.

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12. AUTHORS' CONTRIBUTIONS

VB performed all the experiments. VB analysed the data and wrote the manuscript.

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