

## Original Research Article

### Preliminary screening for antibacterial properties of the male flowers of *Phoenix dactylifera*

Emad M. Abdallah\*

\*Department of Chemistry, College of Science & Arts at Al-Rass, Qassim University, P.O. 53, Saudi Arabia

#### Authors' contributions

The sole author designed, analyzed and interpreted, prepared and approved the manuscript.

#### ABSTRACT

**Objective:** The aim of this study was to evaluate the antibacterial potential of the male flowers of *Phoenix dactylifera* (date palm tree) against five Gram-positive and five Gram-negative bacteria.

**Methods:** Male flowers were collected and extracted by maceration using 80% methanol and the antibacterial activity was determined using cup-plate diffusion test, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) tests.

**Results:** the methanol extract of male flowers of *Phoenix dactylifera* showed varying degrees of antibacterial activity against tested bacterial strains, the most susceptible gram-positive bacteria were *Bacillus cereus* and *Streptococcus pneumonia* which recorded  $12.2 \pm 0.3$  and  $9.0 \pm 0.0$  mm zone of inhibition (ZI), MIC values were 50 and 100 mg/ml, MBC values were 200 and <200 mg/ml, respectively. The most susceptible gram-negative bacteria were *Proteus vulgaris*, *Klebsiella pneumonia* and *Pseudomonas aeruginosa* which recorded  $10.0 \pm 0.0$ ,  $9.7 \pm 0.3$  and  $9.0 \pm 0.0$  mm ZI, MIC values were 100 and MBC values were 200, respectively. Based on MBC/MIC ratio, the extract has some degree of bactericidal effect. However, the results were not competitive with the standard drug (Chloramphenicol).

**Conclusion:** As a result, the tested methanol extract of male flowers of date palm tree exhibited some degree of antibacterial activity with a bactericidal property. More future studies such as fractionation process are required to isolate and investigate its bioactive compounds.

**Keywords:** Antibacterial activity, agar-well diffusion, MIC, MBC, *Phoenix dactylifera*

#### 1. INTRODUCTION

In recent years, the search for antibacterial drugs from plants has been accelerated. On the other side, the traditional healers have used botanical treatments against infectious diseases for centuries, while Western medicine has used none of them [1]. Nowadays, bacterial pathogens have developed resistance to almost

all conventional antibiotics which could lead to increasing the rate of morbidity and mortality from infectious diseases all over the globe [2]. The date palm tree (*Phoenix dactylifera* L.) is a perennial woody tree, belongs to family Arecaceae, which has more than 200 genera and 3000 species, date palm is able to endure high temperature, so it grows well in the desert and semi-desert areas [3]. *Phoenix dactylifera* (*P. dactylifera*) is an indigenous tree in the Arabian Peninsula and is cultivated in Iraq since 7000 years ago. It is also common in North Africa and some parts in the world, with a great cultural and religious significance for Arabs and Muslims around the world, as it is the main food during the fasting month for Muslims “Ramadan” [4]. In traditional medicine, different parts of genus *Phoenix* are widely used for the treatment of different ailments which include fever, inflammation, liver disorders, abdominal troubles, nervous disorders memory disturbances, paralysis, cystitis, gonorrhoea, oedema and also as a detersive and an astringent [5]. The male flowers of *P. dactylifera* were used in ancient Egypt to enhance and promote male and female fertility [6]. In scientific investigations, some studies have been conducted on the genus *Phoenix*, although these investigations were very meagre. Fresh aqueous extract of *P. dactylifera* fruit exhibited dose-dependent anti-oxidant and anti-mutagenic properties [7]. Leaves of *P. paludosa* were reported as a potential antioxidant, analgesic and antidiarrheal agent [8]. The extract of the ground pollen grains collected from the male flowers of *P. dactylifera* contains estrogenic compounds and estrone, which were found to be a gonad-stimulating agent and improved male infertility in adult male rats [9]. Information on the antibacterial activity of the plant extracts of male flowers of *P. dactylifera* grown in Qassim region, Saudi Arabia is very scant. Therefore, this study was designed to investigate the antibacterial potency of the methanol extract of male flowers of *P. dactylifera* against different referenced Gram-positive and Gram-negative bacteria.

## **2. MATERIALS AND METHODS**

### **2.1 Plant material**

The male flowers of *Phoenix dactylifera* (Figure 1), were collected from date palm trees (*P. dactylifera* L.) which grown in Qassim district in Saudi Arabia, during the summer season in August 2017. The plant

parts have been verified and authenticated in the Department of Laboratory Sciences at the College of Sciences and Arts, Qassim University. Plant materials were dried in shade and ground into a fine powder using a blender.



**Figure 1. Male Flowers of *Phoenix dactylifera*.**

## **2.2 Preparation of methanol extract**

50 grams of the male flowers of *P. dactylifera* powder was macerated in 500 ml of 80% methanol (400 ml MeOH/100 H<sub>2</sub>O) using a well tighten dark container made of glass and kept in an incubator at 37 °C for 3 days. After incubation, the mixture was centrifuged at 5000 r.p.m. for 15 minutes and filtered through a filter paper (Whatman, number -1). The filtrate was evaporated until it turned into a semi-solid mushy crude extract, which was then dried in at 45°C for 48 hours and then kept in a refrigerator until used in the antibacterial testing.

## **2.3 Microorganisms**

A diverse referenced bacterial strains were used in this screening, including five gram-positive bacteria, namely *Staphylococcus epidermidis* (ATCC 12228), *Bacillus cereus* (ATCC 10876), *Staphylococcus aureus* (ATCC 29213), *Streptococcus pneumonia* (ATCC 49619) and *Staphylococcus saprophyticus* (ATCC 43867) , and five gram-negative bacteria, namely *Shigella flexneri* (ATCC 12022), *Escherichia coli* (ATCC 25922), *Klebsiella pneumonia* (ATCC 27736), *Proteus vulgaris* (ATCC 6380) and *Pseudomonas aeruginosa* (ATCC 9027).

#### **2.4 Cup-plate diffusion test**

The antibacterial efficacy of the methanol extract of male flowers of *P. dactylifera* was evaluated by means of the cup-plate diffusion test [10]. Into a sterile Petri-dish, 20 ml of molten Mueller-Hinton agar (45°C) was poured and left to solidify at ambient temperature, then put for 15 minutes in the fridge in upside down position at 4 °C to get rid any water droplets. 100 µl of the bacterial suspension adjusted to McFarland standard, containing approximately 10<sup>8</sup> CFU/ml was loaded on the plate and spread using a sterile cotton swab. Thereafter, three holes were made on the agar plate using a sterile cork borer (size 6 mm), two wells were filled with 50 µl of the methanolic extract (concentration 400 mg/ml) which was reconstituted before in 10%DMSO and one well was filled with 50 µl of chloramphenicol (concentration 5 mg/ml) to serve as a positive control. The previous experimental test showed that 10% DMSO did not show any inhibitory effect on the bacterial growth. The cultured plates were then incubated at 37°C for 24 hours, the mean zone of inhibition against the test organism was calculated from two replicates.

#### **2.5 Minimum inhibitory concentration**

Minimum inhibitory concentration (MIC) was estimated using the broth dilution method [11]. Briefly, the crude extract was re-constituted to make concentration 200 mg/ml (lower than the concentration used in the cup-plate diffusion test) and only the tested bacteria, which showed significant sensitivity to the extract with the cup-plat, test were subjected to the MIC test. 1 ml of the extract (200 mg/ml) was added

to 1 ml of Mueller-Hinton broth, and subsequently transferred to a group of sterile tubes containing one ml of Mueller-Hinton medium in a two-fold serial dilutions to make a tube containing descending concentrations of extracts (200, 100, 50, 25, 12.5, 6.25 and 3.123 mg/ml). In addition, 1 ml of antibiotic (Chloramphenicol 5mg/ml) was poured to one broth tube to serve as a positive control, and 1 ml of methanol was poured to another broth tube to serve as a negative control. Then, 1 ml from the suspension of the chosen bacteria was transferred using Eppendorf pipette and added to all tubes, separately. These tubes were then incubated at 37°C for 24 hours. the MIC value was the tube with the lowest dilution with no detectable growth.

### **2.6 Minimum bactericidal concentration**

The Minimum bactericidal concentration (MBC) was performed as cited in Doughari [12] with minor modifications. In brief, after the MIC test, 100 µl from the MIC tubes that showed no visible growth was poured on the surface of Petri-dishes containing Mueller-Hinton agar. 100 µl from the MIC tube containing chloramphenicol and 100 µl from the MIC tube containing 10% DMSO were also poured to serve as the positive control and negative control, respectively. The inoculated Petri-dishes were then incubated for upto 18 hours at 37 °C and inspected for bacterial growth. The Petri-dishes that showed no visible growth were considered as the MBC for the plant extract.

### **2.7 Statistical analysis**

The independent-Sample T-test was used to compare between the means of the extract and antibiotic and One-way ANOVA was used to compare the sensitivity of different microorganisms to the extract. SPSS version 15 was employed in this investigation.

## **3. RESULTS**

As shown in (Tables 1 and 2), the methanol extract of male flowers of *P. dactylifera* revealed average antibacterial activity against tested bacterial strains when compared with the standard antibiotic

(Chloramphenicol), the T-test analysis explained that the methanol extract of the male flowers of *P. dactylifera* is non-significant when compared with the chloramphenicol. However, (Table 1) and (Table 2) showed some bacterial strains with significant sensitivity at ( $p < 0.05$ ) using ANOVA. The extract revealed significant antibacterial effect against some gram-positive bacteria, namely *Bacillus cereus* and *Streptococcus pneumonia* which recorded  $12.2 \pm 0.3$  and  $9.0 \pm 0.0$  mm zone of inhibition (ZI), respectively. The extract exhibited also significant antibacterial activity against some gram-positive bacteria, namely *Proteus vulgaris*, *Klebsiella pneumonia* and *Pseudomonas aeruginosa* which recorded  $10.0 \pm 0.0$ ,  $9.7 \pm 0.3$  and  $9.0 \pm 0.0$  mm ZI, respectively. The results of the MIC and MBC, which are shown in (Table 3), demonstrated that, the methanol extract of male flowers of *P. dactylifera* has a bactericidal effect against most of those bacterial strains which were showed significant sensitivity, as indicated by MBC/MIC ratio. The MIC values ranged between 50 and 100 mg/mL, while the MBC values were 200 mg/mL or above, the MBC/MIC ratio was between 2 and 4.

#### 4. DISCUSSION

Overall, the methanol extract of the male flowers of *P. dactylifera* that was tested against 5 gram-positive and 5 gram-negative bacteria, exhibited varying degrees of susceptibility. Interestingly, 2 gram-positive and 3 gram-negative bacteria were significantly inhibited. In general, studies on male flowers of *P. dactylifera* are scant. Accordingly, the results of the current study are remarkable; although, the extract was not competitor to the standard antibiotic, putting in consideration that the tested standard antibiotic is in a pure form while the extract of the male flowers of *P. dactylifera* is in a crude form that consists of numerous compounds. It is believed that this antibacterial activity could be attributed to presence of some bioactive phytochemical compounds of the male flowers of *P. dactylifera*. Fawkeya and Ateya [13] reported that male flowers of *Phoenix dactylifera* are rich in some bioactive phytochemical constituents such as estradiol, cholesterol, estrone, esteriol, diosgenin, beta-sitosterol, and different flavonoids.

Flavonoids are a large group of natural compounds and hundreds of studies reported that they have antibacterial activity and some of these studies showed up to six-fold stronger antibacterial activities than standard antibacterial drugs [14]. Accordingly, it is recommended to study the antibacterial capacity of the flavonoids contents of the male flowers of *P.dactylifera*. Interestingly, flavonoids of some medicinal plants have been used for centuries against some human diseases, they revealed different mode of actions against bacterial cells, such as inhibit DNA synthesis and cytoplasmic membrane function [15]. In agreement to our assumption, Bentradi et al. [16] have evaluated the antibacterial activity of the pollens (from flowers) of *P. dactylifera*, the organic fraction that was rich in flavonoids exhibited high antibacterial activity. Moreover, the methanol extract of pollen was reported to contain six types of phenolic compounds that showed active antibacterial effects [17]. The antibacterial activity of the other parts of *P. dactylifera* were also investigated, Al-daihan and Bhat [18] published that, among different parts of date palm investigated for antibacterial properties (leaf, fruit, seed and tree bark), fruits showed the highest inhibitory activity against different Gram-positive and Gram-negative bacteria. Also, Bentradi et al. [19] Studied the lipophilic fractions from seeds and pollen of *P. dactylifera*, some of these fractions recorded significant antibacterial activity against some bacterial pathogens.

**Table 1. Antibacterial properties of male flowers of *Phoenix dactylifera* against Gram-positive bacteria.**

Tested	Zone of Inhibition (mm)				
	Bc	Se	Ss	Sa	Sp
Methanol extract (400mg/ml)	12.2±0.3*	7.0±0.0	8.0±0.0	7.0±0.0	9.0±0.0*
Chloramphenicol	36.0	35.0	33.0	36.0	33.0

(5mg/ml)

Se=*Staphylococcus epidermidis* ATCC 12228, Bc=*Bacillus cereus* ATCC 10876, Ss=*Staphylococcus saprophyticus* ATCC 43867, Sp=*Streptococcus pneumoniae* ATCC 49619, Sa=*Staphylococcus aureus* ATCC 29213, zone diameter equals 6 mm= no inhibition. \* =Significant (p<0.05).

**Table 2. Antibacterial properties of male flowers of *Phoenix dactylifera* against Gram-negative bacteria.**

Tested	Zone of Inhibition (mm)				
	Ec	Pv	Kp	Pa	Sf
Methanol extract (400mg/ml)	8.5±0.5	10.0±0.0*	9.7±0.3*	9.0±0.0*	6.7±0.3
Chloramphenicol (5mg/ml)	30.0	28.0	27.0	22.0	26.0

Pv=*Proteus vulgaris* ATCC 6380, Ec=*Escherichia coli* ATCC 25922, Kp=*Klebsiella pneumoniae* ATCC 27736, Sf=*Shigella flexneri* ATCC 12022, Pa=*Pseudomonas aeruginosa* ATCC 9027, zone diameter equal 6 mm= no inhibition. \* =Significant (p<0.05).

**Table 3. MIC and MBC values (mg/ml) of male flowers of *Phoenix dactylifera* methanol extract.**

Microorganism	MIC	MBC	MBC/MIC
Bc	50	200	4
Sp	100	<200	NA



<b>Pv</b>	100	200	2
<b>Kp</b>	100	200	2
<b>Pa</b>	100	200	2

Bc=*Bacillus cereus* ATCC® 10876™, Pa=*Pseudomonas aeruginosa* ATCC® 9027™, Sp=*Streptococcus pneumonia* ATCC® 49619™, Pv=*Proteus vulgaris* ATCC® 6380™, Kp=*Klebsiella pneumonia* ATCC® 27736™, NA=Not applicable.

## 5. CONCLUSION

In conclusion, date palm (*P. dactylifera*) is a famous tree with numerous traditional therapeutic values. Different parts of this plant were profusely investigated but little is known about the bioactive potential of their flowers. This study has shown that methanol extract of the male flowers of date palm possesses some degree of antibacterial activity against some Gram-positive and Gram-negative bacteria. The crude extract requires further future studies in order to determine its chemical constituents and separate the potential antibacterial agents from the crude, which could reveal antibacterial activity higher than the crude methanol extract. Such studies are important for drug discovery.

## COMPETING INTERESTS

The author has declared that no competing interests exist.

## FUNDING

No funding to declare.

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