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Antimicrobial activity and antioxidant potential of the methanolic leaf extracts of three cultivars of date palm trees (Phoenix dactylifera) from Saudi Arabia

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Abstract
The date palm tree (Phoenix dactylifera) is one of the oldest cultivated plants ever known; it is considered the life tree for Arabs. However, there is no adequate information about the bioactivity of the leaves. In this study, the methanol extract of leaves demonstrated the presence of some compounds with potential biomedical properties for human, such as cardiac steroids, flavonoids, phenols/polyphenols, phytosterols, quinines, saponins, tannins and resin. The results of the antimicrobial test indicate that the methanol leaf extracts of the three date palm cultivars contained potential antibacterial agents, particularly against Gram-positive bacteria and no antifungal effect can be detected on Candida albicans. The most susceptible bacteria were Staphylococcus aureus, Staphylococcus epidermidis, Bacillus cereus and Enterococcus faecalis, respectively. The antioxidant activities of methanol extracts of different leaf cultivars were also investigated, using DPPH (2, 2-diphenyl-2-picrymhydrasyl hydrate) radical-scavenging activity assay, all extracts showed considerable antioxidant potential. The current investigation suggests that leaves of date palm can be used as a promising source of natural antibacterial and antioxidant drugs.

Keywords: Antimicrobial, antibacterial, antioxidant, phytochemical, leaf extract, date palm, Phoenix dactylifera

Introduction

The antimicrobial resistance is a growing global health threat, which threatens the achievements of modern medicine. Almost each newly launched antibiotic has been followed by the development of resistance to it [1]. Accordingly, the global failure in controlling the spread of antibiotics-resistant pathogens and the emergence of new bacterial infections has urged the international healthcare bodies and the scientific community to search for new antibacterial agents. One of the major candidates as an alternative source for antibacterial drugs is plants [2]. On the other hand, overproduction of oxidants in the human body could lead to development of many functional and chronic diseases such as cardiovascular diseases, diabetes mellitus, inflammations, obesity, neurodegenerative diseases and even cancer and hence plants could play a major role in the scavenging of these oxidants that produce various potent antioxidant agents [3]. Accordingly, Plants could be a major source of drugs in the future. Recently, about 42% of 25% top selling medications around the globe are derived from medicinal plants [4].

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The date palm tree Phoenix dactylifera Linn., which belongs to the family Arecaceae (Palmae) is a medium-sized tree, dioeciously, with pinnate leaves containing up to 150 leaflets with spines on the petiole. It is extensively cultivated for its fruit [5, 6], Date palm tree is native to the Middle East and North Africa, it is one of the oldest plants, it has been cultivating in the Mesopotamia (Iraq) since 7000 years ago; however, scientists believe that the utilization of the date Palm began thousands of years earlier [7]. The exact number of date palm cultivars is not available. However, it was mentioned that there are about 1500 cultivars of date palm, up to 450 female cultivars are reported in Iraq and about 55 major cultivars are recorded in Saudi Arabia [8]. Phoenix dactylifera had great religious and cultural significance for Arabs in particular and for the Islamic nation all over the globe in general, as date palm and its fruits are mentioned in Quran and Haddith. Muslims believe that dates cure several illnesses; it is the main food during Ramadan, the month of fasting for Muslims [9]. Almost all parts of the date palm tree are used; yet the major part used is its fruits. In the last decades, the interest in the fruits of the date palm is growing [6]. Regarding the medical uses, the date fruits are extensively studied and they are claimed to have anti-tumor agents, anti-inflammatory, anti-microbial, anti-oxidant, nephro-protective, hepato-protective, anti-diabetic and sex hormone modulator [10]. Although, the seeds of dates are less explored in biomedical research, they were found to have an antioxidant and anti-diabetic

activity [11]. Investigations on leaves of date palm are scant, it was reported that they have anti-diabetic activity [12]. The current study aimes to evaluate the antioxidant and antimicrobial efficacy of the methanolic leaf extracts of three famous cultivars (Sukkaria, Hillaliah and Hoshana) of date palm trees (Phoenix dactylifera) from Qassim region, Saudi Arabia.

Material and Methods

Collection of plant materials

Leaves of three different cultivars of date palm (Sukkaria, Hillaliah and Hoshana), were generously provided by Dr. Hamad Abdullah Al-Khweiter from his own farm from Onaizah city, Qassim, Saudi Arabia. Leaves were collected manually from the trees (Figure 1) and identified by the Department of Laboratory Sciences, College of Science and Arts at Al-Rass, Qassim University. Then, leaves were kept in a well-ventilated dark cupboard for up to 15 days at room temperature until dried.

Plant extraction

The dried leaves from each cultivar of date palm were ground to a fine powder, the powdered materials were extracted by maceration in a hydro-alcoholic solvent by adding 50 g of the powder to 500ml of 80% methanol and soaked for a while, kept in a well-tighten dark container in incubator with frequent shaking (2-3 times a day) for up to one week. Then, the macerate was first filtered through four layers of muslin cloth and then the filtrate was filtered again using Whatman No. 1 filter paper. The filtrate was evaporated in an incubator at 45 oC for up to 10 days to get a dry crude methanol extract.

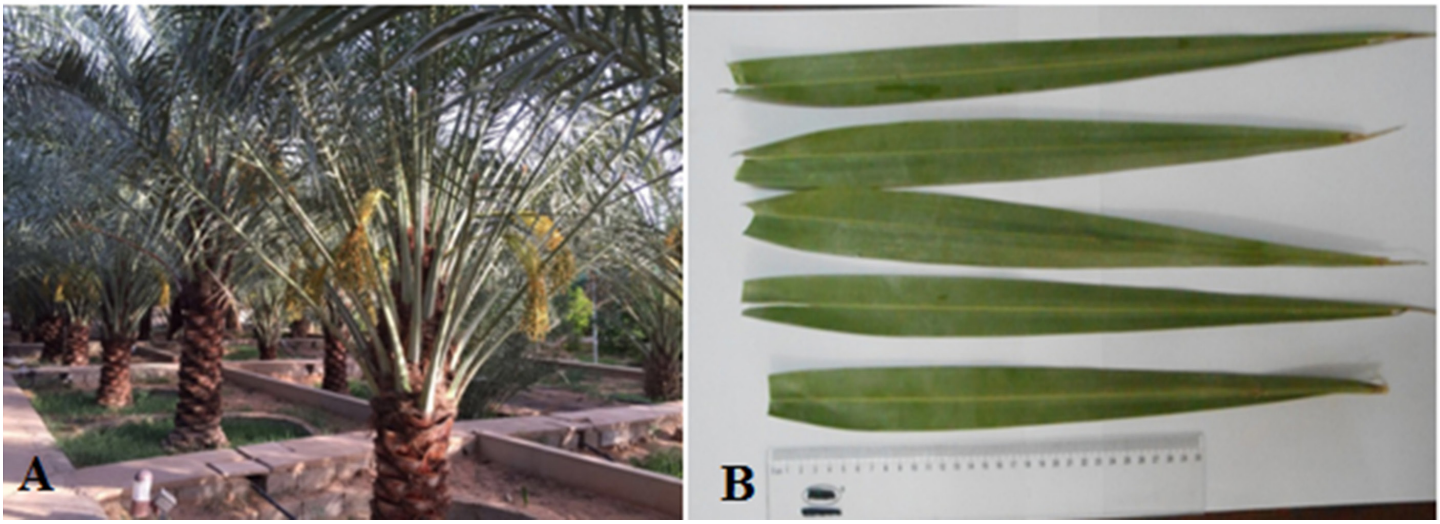

Figure 1. The cultivated date palm (Phoenix dactylifera), the trees (A) and leaves (B)

Microbial strains

Six referenced bacterial strains were obtained from were obtained from both of the Department of Pharmaceutics, Unaizah College of Pharmacy and the Department of Laboratory Sciences, College of Science and Arts at Al-Rass, Qassim University. They included different Gram-positive and Gram-negative bacteria (Staphylococcus aureus ATCC 25923, Staphylococcus epidermidis ATCC 12228, Enterococcus faecalis ATCC 29212, Bacillus cereus ATCC 10876, Klebsiella pneumoniae ATCC 700603 and Escherichia coli ATCC 35218) also in addition to a fungal strain Candida albicans (Clinical isolate) was used.

Preparation of Inoculums

Microbial cultures were identified microscopically and/or biochemically. Then, they sub-cultured in sterile bottles containing Nutrient Broth for bacteria or Sabouraud dextrose broth for fungi. Microorganisms were incubated at 37°C for up to 18 hours for bacteria and 24 hours for Candida albicans. Prior to the antimicrobial experiment, the working microbial samples were diluted using 0.9% sterile normal saline and adjusted to 0.5 McFarland standard.

Phytochemical analysis

The methanolic leaf extracts of date palm were qualitatively screened for some bioactive phytochemical compounds through some colorimetric qualitative tests. The extracts were examined for the presence or absence of alkaloids, carboxylic acid, cardiac steroids, glycosides, coumarins, emodins, flavonoid, leucoanthocyanins, phenol/polyphenols, phlobatannin, phytosterols, quinones, resin, saponins, tannin, terpenoids and volatile oils. Methods for phytochemical analysis were followed as mentioned in [13].

Antimicrobial assay

The antimicrobial assay was performed using paper disc diffusion method [14], with minor modification. Using a sterile cotton swab, the fresh adjusted microbial cultures were swabbed on the surface of sterile Mueller-Hinton agar plates for bacterial strains or sabouraud dextrose agar for Candida albicans and allowed for 5 minutes. The tested dry extracts were re-constituted in 10% DMSO (Dimethyl sulfoxide) to make 500 and 250 mg/ml, respectively. 6 mm Sterile filter paper discs (Whatman No.1) were impregnated in these extracts; So each disc trap approximately 10 and 5 mg/disc, respectively. These saturated discs were placed on the surface of the inoculated plates. Gentamicin (10 µg/disc) was used as positive control, while sterile paper discs saturated with 10% DMSO was used as negative control. The plates were then incubated overnight at 37°C. The microbial growth was determined by measuring the diameter of the zone of inhibition in mm using a transparent ruler. Each extract was analyzed twice and the mean and standard error of means were calculated.

Antioxidant assay

The antioxidant activity was evaluated by the DPPH radical scavenging assay. The radical scavenging activity of the methanol leaf extracts of Phoenix dactylifera against 2,2-diphenyl-1-picrylhydrazyl (DPPH) was determined with minor modification following the method mentioned in [15]. Briefly, 2 ml methanolic DPPH solution (30 mg/L) of leaves from each variety of Phoenix dactylifera were mixed with 20 µl of the working sample extract (10 mg/1 mL). Samples were incubated inside an incubator at room temperature for 30 minutes and then the absorbance of the solution at 516 nm was measured using spectrophotometer at 516 nm. The percent scavenging of DPPH was calculated according to the following formula:

$$\text{Radical Scavenging Activity} = (\text{AControl} - \text{ASample}) / \text{AControl} \times 100$$

Where: AControl and ASample are the absorbance values at 516 nm of the control and the sample extracts of Phoenix dactylifera, respectively.

Statistical analysis

Data were expressed as Mean ± Standard error of means, The statistical software used was SPSS (v.16.0).

Results and discussion

In the present study, the phytochemical screening showed positive results for cardiac steroids, flavonoids, phenol/polyphenols, phytosterols, quinines, saponins, tannins and resin (Table 1).

Table 1. Screening of some chemical compounds from the methanol leaf extracts of three date palm cultivars

	Methanolic leaf extract		
	Sukkaria	Hilalia	Hoshana
1. Alkaloids	-	-	-
2. Carboxylic acid	-	-	-
3. Cardiac steroids	+	+	+
4. Coumarins	-	-	-
5. Emodins	-	-	-
6. Flavonoid	+	+	+
7. Glycosides	-	-	-
8. Leucoanthocyanins	-	-	-
9. Phenol/Polyphenols	+	+	+
10. Phlobatannin	-	-	-
11. Phytosterols	+	+	+
12. Quinones	+	+	+
13. Resin	+	+	+
14. Saponins	+	+	+
15. Tannin	+	+	+
16. Terpenoids	-	-	-
17. Volatile oil	-	-	-

* + = Preesent, - = Absent

The phytochemical examinations were carried out in order to understand the nature of the bioactive compounds of date-palm leaf extracts. It was observed also there were no differences in the phytochemical constituents of the three cultivars of date palm's leaves at the qualitative assessment, perhaps some variations appeared at the quantitative level, which requires further future studies. Our findings are partially in agreement with a previous study from Iraq on the leaves of date palm, which reported the presence of flavonoids, phenols, trepenoids, alkaloids and tannins [16].

Another study from Saudi Arabia showed that leaves of date palm have alkaloids, steroids, tannins but no saponins or flavonoids [4]. This is due to the influence of the environmental conditions.

Data pertaining to the antimicrobial potential of methanol leaf extracts of three date palm cultivars (Sukkaria, Hillaliah and Hoshana), are exhibited in Table 2, Figure 2 and Figure 3. It was noticeable that leaves from all varieties have varying degrees of antibacterial potential, while no antifungal activity against Candida albicans has been recorded (Table 2). The Gram-positives were the most susceptible bacteria toward the date palm leaf extracts, while the gram-negatives revealed less or no antibacterial susceptibility (Figure 3). In respect of Gram-positive bacteria, Staphylococcus aureus was highly susceptible to the leaf extract of date palm cultivars, particularly Hoshana, compared to gentamicin; followed by Staphylococcus epidermidis, Bacillus cereus and Enterococcus faecalis, respectively. The results of Enterococcus faecalis were interesting since its sensitivity towards the leaves extract of date palm was higher than the antibiotic gentamicin. On the other side, with respect to Gram-negative bacteria, Klebsiella pneumoniae showed no susceptibility against all extracts, while Escherichia coli revealed some degree of susceptibility against Hillaliah extract only (Table 2 and Figure 3). Data regarding the antibacterial activity of date palm's leaves are scanty. However, it was published that aqueous, acetone and methanol extracts of the leaves of date palm have varied degrees of antibacterial activities against different Gram-positive and Gram-negative bacteria [4]. Similarly, it was cited that the ethanol extract of date palm's leaves showed good antibacterial activity against Staphylococcus aureus, Streptococcus pneumoniae, Enterobacter sp., Salmonella sp., Pseudomonas aeruginosa Escherichia coli, Klebsiella pneumoniae, and Proteus sp. [17]. It was also stated that acetone and methanol extracts from date palm's leaves exhibited good antibacterial activity against Escherichia coli, Pseudomonas aeruginosa, Shigella flexeneri, Staphylococcus aureus, Streptococcus pyogenes and Bacillus subtilis [18]. In general, the antibacterial activity of date palm's leaves in our study is attributed to its phytochemical constituents. Phenolic compounds, alkaloids, flavonoids, and tannins have been reported as the major classes of antibacterial compounds in plants [4].

The results of antioxidant activity using DPPH radical scavenging method are presented in (Table 3), Hillalia methanol extract recorded the highest value (93.2%) at concentration 100mg/ml, Hoshana and Sukkaria recorded 88.9% at concentration 100mg/ml, compared to the standard (Ascorbic acid) which recorded 68.7% at concentration 100µg/ml. The current results appeared to be as potent as the ascorbic acid (Vitamin C) when putting into consideration that the plant extracts are in a form of crude and not pure. DPPH free radical scavenging method is widely used to detect the antioxidant activity of plants [15]. These considerable antioxidant activities are related to the presence of phenolic

compounds, flavonoids and tannins. It is believed that the free radical scavenging activity of some medicinal plants is related to the phenolic compounds which contain phenols, flavonoids and tannins [19,20]. Our antioxidant results are in harmony with previous studies, which reported that the leaves of date palm have high antioxidant activity [21,22]. The antioxidant potency of the date palm leaf extracts could be attributed to the richness of these leaves in phytochemicals particularly phenols, the habitat, the environmental conditions, the biochemical and physiological processes of the plant [23].

Table 2 . The antimicrobial activity of the methanol leaf extracts of three date palm cultivars*

Tested compound	**Mean zone of growth inhibition in mm**						
	Gram-positive bacteria				**Gram-negative bacteria**		**Fungi**
	Sa	Se	Ef	Bc	Ec	Kp	Ca
Sukkaria	19.5	18.5	15.5	15.0	6.0	6.0	6.0
10 mg/disc	±0.5	±0.5	±1.5	±1.0	±0.0	±0.0	±0.0
Sukkaria	16.0	17.0	15.5	14.0	6.0	6.0	6.0
5 mg/disc	±1.0	±2.0	±0.5	±0.0	±0.0	±0.0	±0.0
Hillaliah	17.5	15.5	16.5	17.5	10.5	6.0	6.0
10 mg/disc	±0.5	±0.5	±1.5	±2.5	±1.5	±0.0	±0.0
Hillaliah	14.0	14.5	14.0	14.5	8.5	6.0	6.0
5 mg/disc	±1.0	±0.5	±1.0	±0.5	±2.5	±0.0	±0.0
Hoshana	21.0	18.5	14.0	16.5	6.0	6.0	6.0
10 mg/disc	±1.0	±0.5	±0.0	±0.5	±0.0	±0.0	±0.0
Hoshana	16.5	16.5	14.0	15.0	6.0	6.0	6.0
5 mg/disc	±0.5	±0.5	±1.0	±0.0	±0.0	±0.0	±0.0
Gentamicin	22.0 ±1.0	27.0	7.0	23.0	21.5	11.0	N/A
(10 µg/disc)		±2.0	±1.0	±1.0	±1.5	±1.0	
Clotrimazole	N/A	N/A	N/A	N/A	N/A	N/A	35.0±1.0
10 mg/ml							
10% DMSO	6.0±0.0	6.0±0.0	6.0±0.0	6.0±0.0	6.0±0.0	6.0±0.0	6.0±0.0

*Disc diameter=6.0 mm, 6.0±0.0= No activity (the disc diameter), Zone of inhibition is the mean of three replicates ±standard deviation, N/A=Not applicable, Sa=-Staphylococcus aureus ATCC 25923, Se=Staphylococcus epidermidis ATCC 12228, Ef=Enterococcus faecalis ATCC 29212, Bc=Bacillus cereus ATCC 10876, Ec=Escherichia coli ATCC 35218, Kp=Klebsiella pneumoniae ATCC 700603, An=Aspergillus niger ATCC 6275 and Ca=Candida albicans (Clinical isolate). DMSO= Dimethyl sulfoxide.

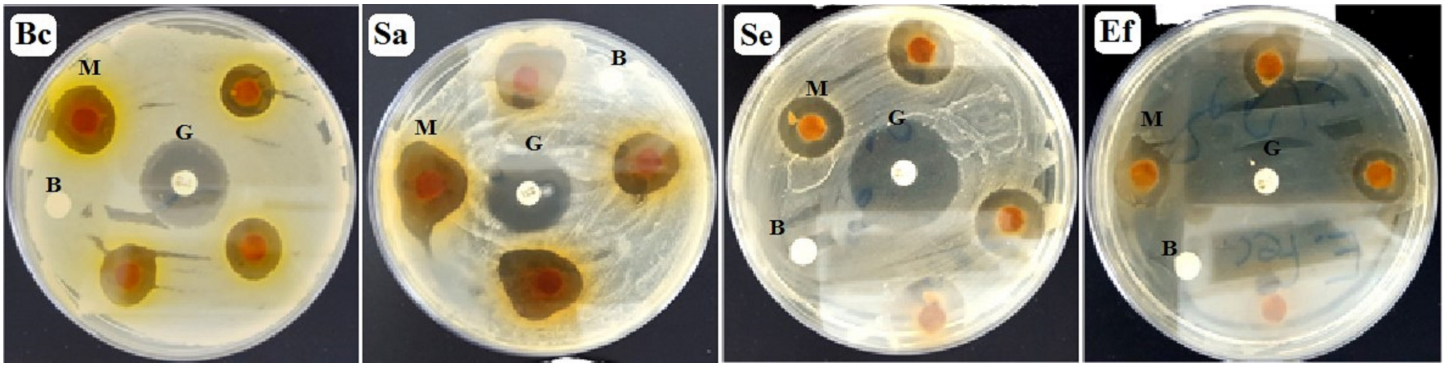

Figure 2. Representative photos showing potent activity of the methanolic leaf extract of Phoenix dactylifera against some bacteria*

*Bc=Bacillus cereus, Sa=Staphylococcus aureus, Se=Staphylococcus epidermidis, Ef=Enterococcus faecalis, G=Gentamicin, M=Methanol extract, B=negative control (10% DMSO).

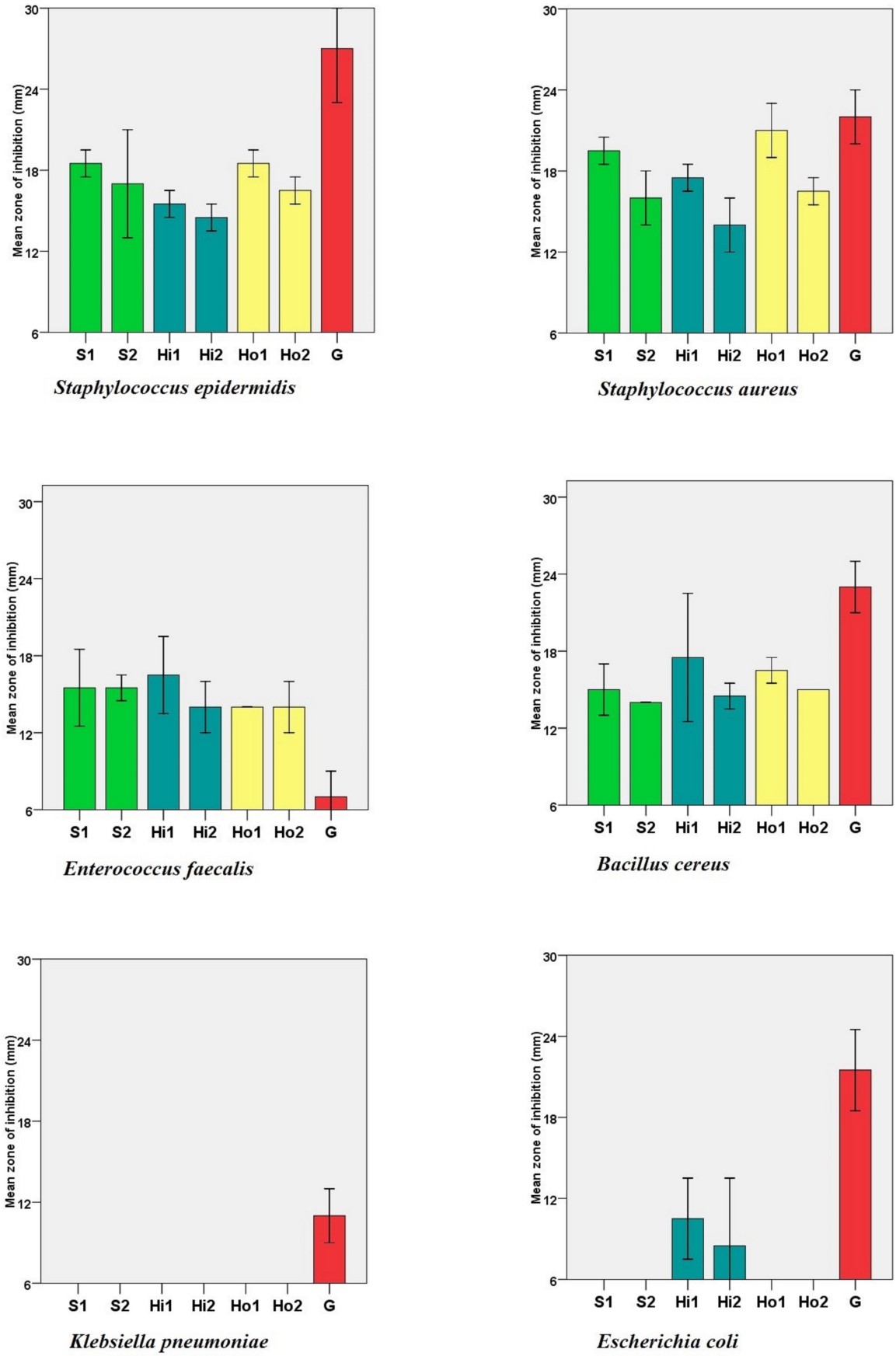

Figure 3. Sensitivity of bacterial strains toward three cultivars of methanolic leaf extract of Phoenix dactylifera compared to antibiotic*

*S1= Sukkaria (10 mg/disc), S2= Sukkaria (5 mg/disc), Hi1= Hillaliah (10 mg/disc), Hi2=Hillaliah (5 mg/disc), Ho1= Hoshana (10 mg/disc), Ho2= Hoshana (5 mg/disc), G= Gentamicin (10 µg/disc)

Table 3. DPPH scavenging activity of Methanolic leaf extracts of three cultivars of date palm compared to ascorbic acid

% Inhibition	Concentration	Compound
88.94737	10mg/mL	Sukkaria extract
93.26316	10mg/mL	Hillalia extract
88.94737	10mg/mL	Hoshana extract
68.73684	100µg/ml	Ascorbic acid

Conclusion

In conclusion, the results of our study demonstrated potential antibacterial activity by date palm leaves from three cultivars against various pathogens responsible for wide variety of infections, particularly the Gram-positive bacteria, which could be a good candidate for new natural antibacterial drugs competitor to current antibiotics. In addition, the high antioxidant activity detected in all the tested cultivars makes the leaves of date palm a promising source of natural nutraceuticals with possible medical applications to reduce oxidative stress and provide varied health benefits. Furthermore, leaves of palm trees is recommended to be consumed as dietary supplement -and suggested for antibacterial and antioxidant drug industry after safety approval and further biochemical, toxicological pharmacological and clinical studies.

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Competing of interest
The authors declare that, they have no competing interests.

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