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Palm date (*Phoenix dactylifera*) seeds: A rich source of antioxidant and antibacterial activities

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Abstract: Two varieties of palm date seeds (PDS), Sukkari and Khalas, were examined for their antioxidant and antibacterial activities. Total phenol content was 2 014.37 ± 212.05 and 2 060.40 ± 176.0 mg GAE 100 g^{-1} dry weight (DW); and total flavonoid content was 83.98 ± 1.10 and 94.97 ± 1.36 mg QE 100 g^{-1} DW for Sukkari and Khalas, respectively. PDS extracts showed potential scavenging activity against ABTS, DDPH, and hydroxyl radical; the calculated IC_{50}s were 431.17 ± 12.45, 400 ± 10.87 and 680 ± 18.12 µg for Sukkari, and 476 ± 20.25, 302.24 ± 14.08 and 284.18 ± 11.62 µg for Khalas, respectively. PDS extracts did not show potential activity against superoxide anion. Both extracts showed a high percentage of reducing power as the calculated FRAP (ferric reducing antioxidant power) concentrations were 12.66 ± 1.08 and 16.14 ± 1.94 mmol ascorbic acid equivalent 100 g^{-1} for Sukkari and Khalas, respectively. *Bacillus subtilis* showed a remarkable sensitivity to PDS extracts; the inhibition zones were 21 ± 0.82 and 22 ± 0.67 mm for Sukkari and Khalas, respectively. PDS extracts possess good antioxidant and antibacterial activity, and therefore PDS could be effectively used as a natural source of antioxidants and to be detected against gram-positive bacteria.

Keywords: free radicals; gram-positive bacteria; phenolic acids; scavenging activity; total flavonoids; total phenols

Date palms, *Phoenix dactylifera*, are an important fruit crop in the Kingdom of Saudi Arabia. Annual production reached 754 761 tonnes (FAOSTAT 2017: Available at www.fao.org/faostat/en/#data/QC). There are more than 20 million fruit-producing date palm trees in the Kingdom which add a substantial value to the national economy (Abdulaziz 2011). Approximately 72 000 tonnes of palm date seeds (PDS) are disposed off annually in Saudi Arabia, which represents about 10% of all date palm fruits. PDS are the major waste product of the date industry, and they could offer potentially valuable material for the production of useful food ingredients. However, a very small fraction is used locally for animal feed (Al-Farsi & Lee 2011).

PDS contain substantial secondary metabolites that include polyphenolic compounds such as flavonoids, antioxidants, and phenolic acids, which can be utilized as a good source for functional foods (Al-Farsi & Lee 2011). Abdelaziz et al. (2015) demonstrated that the aqueous extract of PDS possesses a potential protective effect against diabetic complications in liver and kidney tissues of streptozotocin-induced diabetic rats. Another investigation conducted by Abiola et al. (2018) reported the antidiabetic effect of PDS extract on alloxan-induced

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diabetic rats. Saryono et al. (2017) reported that the levels of total cholesterol and LDL were significantly decreased in rats induced with high cholesterol diet, after their diet was supplemented with PDS extract.

Here, we studied the antioxidant potential and antibacterial activity of two varieties of PDS; the possibility of utilizing PDS in different industries was also a focus.

MATERIAL AND METHODS

Chemicals. All chemicals were purchased from Sigma-Aldrich (USA) unless stated otherwise. Trolox was obtained from Fluka Chemie AG (Buchs, Switzerland). Methanol and other chemicals were of analytical grade and were obtained from BDH (UK).

Material. Two varieties of palm date fruit, namely Khalas and Sukkari, were purchased from the local market in Riyadh. Samples were manually cleaned, and seeds were rinsed with plenty of water and kept in an electrical oven at 50 °C for two days until constant weight. The final weights after moisture evaporation were recorded. PDS samples were powdered using a heavy-duty miller.

Preparation of PDS extracts for antioxidant study. The method of Al-Farsi & Lee (2008b) was applied with some modifications. PDS powder (2 g) was extracted with 25 mL of acetone-water mixture (1 : 1 v/v) and maintained in a shaker at a low speed for 1 h. Filtrates were collected and residues obtained after the first centrifugation step were extracted with the same solution for 1 h and filtrates were collected as before. Supernatants were pooled and the solvent was evaporated using a rotary evaporator under vacuum (Buchi, Switzerland). Fifty mL of butanone-water mixture (1 : 1 v/v) was added and extracts were left overnight in an orbital rotary shaker at 60 rpm and 60 °C. Residues were dissolved in ultrapure water and the final volumes were recorded. A set of serial dilutions of 100, 500, and 2 000 was performed.

Total phenol determination. The Folin-Ciocalteu (0.1 mL) reagent was added to 0.5 mL of PDS extract and incubated at room temperature for 15 min. Sodium carbonate solution (2.5 mL, 7.5%) was added and the reaction was incubated at room temperature for 30 min. Absorbance was measured at 760 nm. Gallic acid (GA) (0–50 μg) was used as a standard McDonald et al. (2001). Total phenol content was expressed as GA equivalent (GAE).

Total flavonoid determination. The $AlCl_3$ method by Chang et al. (2002) was applied. The reaction mixture consisted of 1.0 mL of PDS extract, 1.0 mL of ethanol, 0.5 mL of 1.2% $AlCl_3$ and 0.5 mL of 120 mM potassium acetate. The mixture was incubated at room temperature for 30 min and the absorbance was read at 415 nm. Quercetin (0–50 μg) was used as a standard. Total flavonoid content was expressed as quercetin equivalent (QE).

ABTS radical cation scavenging activity. The ability of PDS extracts to decolorize the ABTS free radicals was assessed as described by Re et al. (1999) with minor modification. The radical solution was prepared through the reaction of 7 mM ABTS and 2.45 mM of potassium persulphate after incubation at room temperature in the dark for 16 h. It was then diluted with 80% ethanol to an absorbance of 0.7 at 734 nm. The solution (3.9 mL) was added to 0.1 m of PDS extract and mixed, left at room temperature for six min and the absorbance was read at 734 nm. A calibration curve of Trolox (0–15 μM) was constructed.

DPPH scavenging activity. DPPH in methanol (1.0 mL, 0.3 mM) was added to 1.0 mL of PDS extract, mixed, then 1.0 mL of methanol was added. Mixtures were incubated in the dark for ten min. The optical density was read at 517 nm using ascorbic acid as a standard (McCune & Johns 2002) and the IC_{50} was calculated.

Hydroxyl radical scavenging activity. The following solutions were added in this order: 0.1 mL of 2-deoxyribose (0.028 M in 0.02 M potassium phosphate buffer, pH 7.4), 0.5 mL of PDS extract, 0.2 mL (1 : 1 v/v) of 1.04 mM EDTA solution and 0.2 mM of $FeCl_3$ solution, 0.1 mL of 1.0 mM H_2O_2 solution, and 0.1 mL of 1.0 mM ascorbic acid. The mixture was incubated at 37 °C for one hour. One mL of 1% TBA and 1.0 mL of 2.8% TCA were added in this order, mixed, and incubated in a boiling water bath for 20 min. It was then cooled, and the absorbance was read at 532 nm (Kunchandy & Rao 1990). Calibration was performed using GA as a standard (100–1000 μg).

Superoxide anion radical scavenging activity. The following reagents were prepared in 16 mM Tris Cl^{-1} buffer pH 8.0 in this order: 0.5 mL of 0.3 mM NBT, 0.5 mL of 0.936 mM NADH solution, 1.0 mL of PDS extract and 0.5 mL of 16 mM Tris Cl^{-1} buffer pH 8.0. The reaction was initiated by adding 0.5 mL of 0.12 mM PMS solution and the mixture was incubated at room temperature for 5 min. The absorbance was read at 560 nm (Robak & Gryglewski 1988). Calibration was performed using GA as a standard (100–1000 μg).

Ferric reducing antioxidant power (FRAP). A mixture of 6.0 mL consisted of 1 mL of PDS extract, 2.5 mL of 0.2 M phosphate buffer pH 6.6, and 2.5 mL of 0.03 M

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potassium ferricyanide. It was incubated at 50 °C for 20 min, then 2.5 mL of 0.6 M TCA was added. After mixing, 2.5 mL of this solution was mixed with 2.5 mL distilled water, then 0.5 mL of 6 mM $FeCl_3$ solution was added, and the absorbance was read at 700 nm (Athukorala et al. 2006). Calibration was performed using ascorbic acid as a standard (20–200 µg).

Preparation of PDS extracts for antibacterial study. PDS powder was soaked in methanol (1 : 10 w/v) for 3 days (Alade & Irobi 1993). The mixture was agitated every 24 h using a shaking incubator at room temperature. The extract was centrifuged at 5 000 rpm for 10 min and the supernatant was collected. Methanol was evaporated using a rotary evaporator under vacuum at 45°C. The dry extract was resuspended in 2 mL of sterile distilled water and stored at –20 °C.

Bacterial strains and cultivation conditions. The antibacterial effect of PDS was examined against 6 bacterial species: Gram-positive bacteria: *Staphylococcus aureus* (clinical isolate), *Bacillus subtilis* NCTC 10400, *Enterococcus faecalis* ATCC 29212, and gram-negative bacteria: *Escherichia coli* NCTC 10418, *Salmonella typhimurium* ATCC 13311, and *Pseudomonas aeruginosa*. The tested bacterial strains were cultured on Brain Heart Infusion Agar (BHIA) at 37 °C for 24 h before use.

PDS antibacterial activity analysis. The antibacterial activity of PDS was measured using the agar well diffusion method (Singh et al. 2015).

Antibacterial screening of phenolic acids. Eight standard phenolic acids were tested for their antibacterial activities. Each standard phenolic acid (50 mg) was dissolved in 1.0 mL of solvent mixture containing 400 µL DMSO and 600 µL 25% acetone in water (Elisha et al. 2017). The antibacterial activity was measured using the agar well diffusion method. A pure solvent mixture was used as negative control.

Determination of minimum inhibitory concentration (MIC) of PDS extracts. The MIC was determined as the lowest concentration of each extract that inhibits the visible growth of the tested bacteria.

Statistical analysis. All experiments were carried out with three samples of PDS and each entire assay was carried out in triplicate. Mean and standard deviation were calculated using MS Excel 2010.

RESULTS AND DISCUSSION

Antioxidant activity of PDS. Total phenolics and total flavonoids of PDS extracts are shown in Table 1. The total phenolic content was 2 014.37 ± 212.05 and 2 060.40 ± 176.0 mg GAE 100 g^{-1} DW while the total flavonoid content was 83.98 ± 1.10 and 94.97 ± 1.36 mg QE 100 g^{-1} DW for Sukkari and Khalas, respectively. The total phenolic content of PDS found here is in agreement with Mistrello et al. (2014), who studied the total phenolic content in seeds of three date varieties of the UK market and reported a range between 2 058 and 2 984 mg 100 g^{-1}. A review report conducted by Al-Farsi& Lee (2008a) showed that PDS has average phenolics of 3 942 mg 100 g^{-1}.

Table 1. Total phenolic and total flavonoid content of palm date seeds (PDS)

Variety	Total phenolics	Total flavonoids
	(mg 100 g^{-1} DW)	
Sukkari	2 014.37 ± 212.05	83.98 ± 1.10
Khalas	2 060.40 ± 176.0	94.97 ± 1.36

PDS extracts showed good scavenging activity against the ABTS radical cation (Figure 1). The IC_{50} of Trolox standard was determined to be 11.29 µM, however, when each extract of 100 dilution was used, the IC_{50} was 403.59 ± 12.45 µg for Sukkari and 400 ± 10.87 µg for Khalas. It seems that different factors affect PDS scavenging activity of ABTS including species, age, as well as extraction procedure. Salomon-Torres et al. (2019) reported that PDS grown in Mexico showed an IC_{50} of 238 mg against ABTS. Djaoudene et al. (2019), who studied PDS cultivars

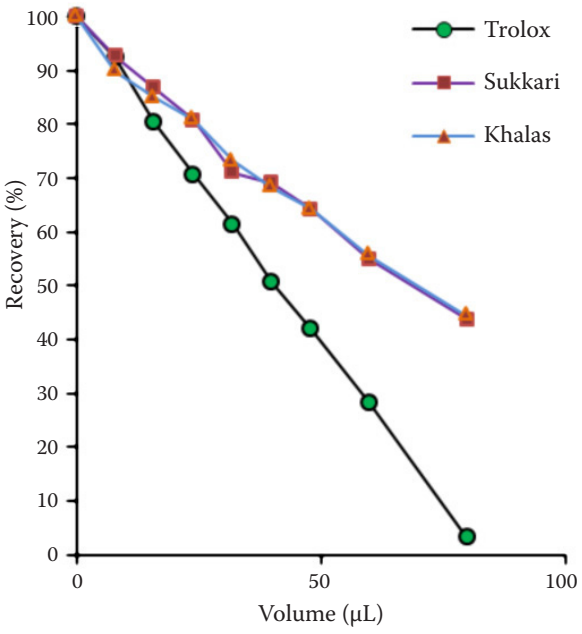

Figure 1. Palm date seed (PDS) extracts scavenging activity against ABTS

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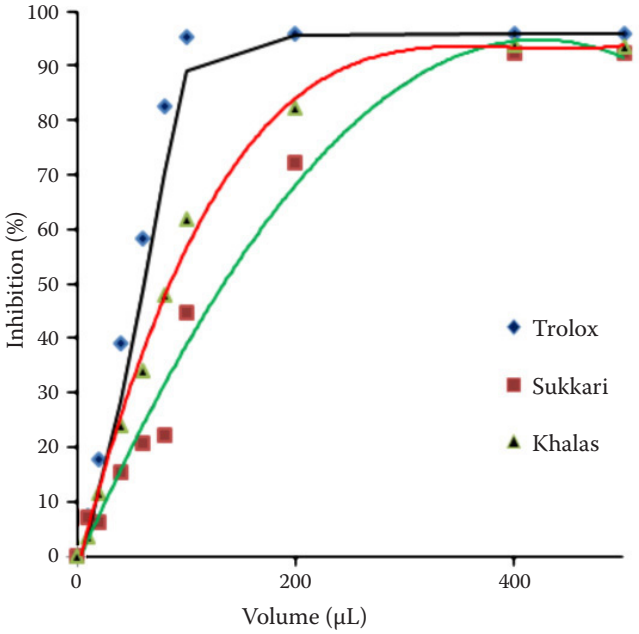

Figure 2. Palm date seed (PDS) extracts scavenging activity against DPPH

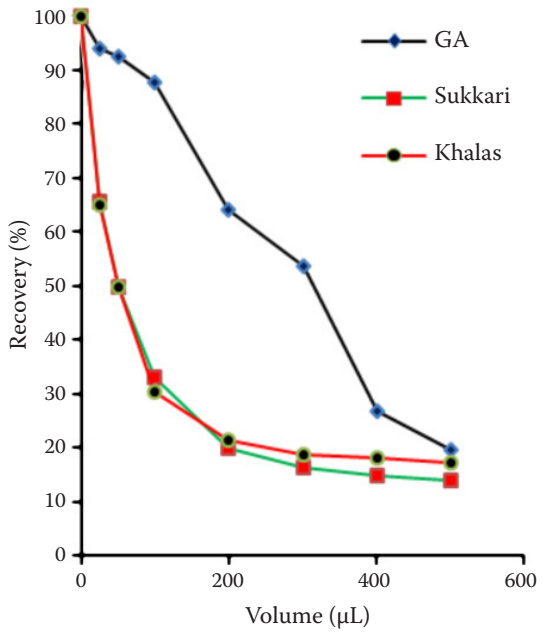

Figure 3. Palm date seed (PDS) extracts scavenging activity against hydroxyl radical

grown in the south of Algeria, reported an IC_{50} of 13.89 µg mL^{-1}. Extracts of Sukkari and Khalas PDS also showed a considerable scavenging activity against DPPH as compared with standard ascorbic acid. The standard ascorbic acid showed an IC_{50} of 19.45 µM (10.3 µg) against DPPH, whereas the calculated IC_{50} for Sukkari and Khalas PDS extracts required to scavenge DPPH were 680 ± 18.12 µg and 476 ± 20.25 µg, respectively (Figure 2). Our findings are comparable with the report of Bouhlali et al. (2017), who studied three different varieties of date seeds grown in southeastern Morocco and reported IC_{50} ranging from 112 to 166 µg mL^{-1}. Our findings that PDS exerted a strong scavenging activity against ABTS and DPPH is in full agreement with the report of Barakat et al. (2020), as they reported that PDS showed an IC_{50s} of 0.060 ± 0.05 and 2.20 ± 0.15 µg ml^{-1} against ABTS and DPPH, respectively.

Figure 3 shows the hydroxyl radical scavenging activity of PDS extracts, when 50 µL of the 100 dilution of each extract was used; it showed a very high scavenging activity as the percentage of inhibition reached 50.13% and 50.27% for Sukkari and Khalas, respectively. The calculated IC_{50} were 302.24 ± 14.08 and 284.18 ± 11.62 µg for Sukkari and Khalas respectively. The standard GA showed an IC_{50} of 648 µg. The recorded IC_{50} of PDS extracts were less than fifty percent required by the standard GA to reach $IC_{50.}$ Our findings revealed that PDS extracts are potentially scavenging the hydroxyl radical. Vayalil (2002) reported that the date fruit extract has an IC_{50} of 2.2 mg mL^{-1} against the hydroxyl radical.

In contrast to the previous findings, PDS extracts did not show a potential activity against superoxide anion. The percentage of inhibition reached only 19.83% and 25.0% for Sukkari and Khalas, respectively, when 1000 µL of the 100 dilution of both extracts was used; however, the standard GA at the same volume (1 000 µL equivalent to 1000 µg) recorded 74.16% inhibition (Figure 4). The calculated IC_{50} were 15.28 ± 2.37 and 11.43 ± 2.06 mg for Sukkari and Khalas, respectively, whereas the standard GA recorded 385.21 µg. Research conducted by Vayalil (2002) reported that when the riboflavin photoreduction method was used, the date fruit extract showed an IC_{50} of 0.8 mg mL^{-1} against superoxide radical.

Both 100-fold diluted extracts of Sukkari and Khalas showed a powerful reducing power. The mean of the calculated FRAP concentration was 12.66 ± 1.08 and 16.14 ± 1.94 mmol ascorbic acid equivalent 100 g^{-1} for Sukkari and Khala, respectively. A report conducted by Mohamed et al. (2014), who studied six palm date fruit varieties grown in Sudan, showed that FRAP activity ranged from 2.82 to 27.5 mmol 100 g^{-1}.

Antibacterial activity of PDS. The high inhibitory activity of extracts was detected more clearly in gram-positive strains rather than in gram-negative ones (Figure 6). The order of the high sensitivity of gram-positive

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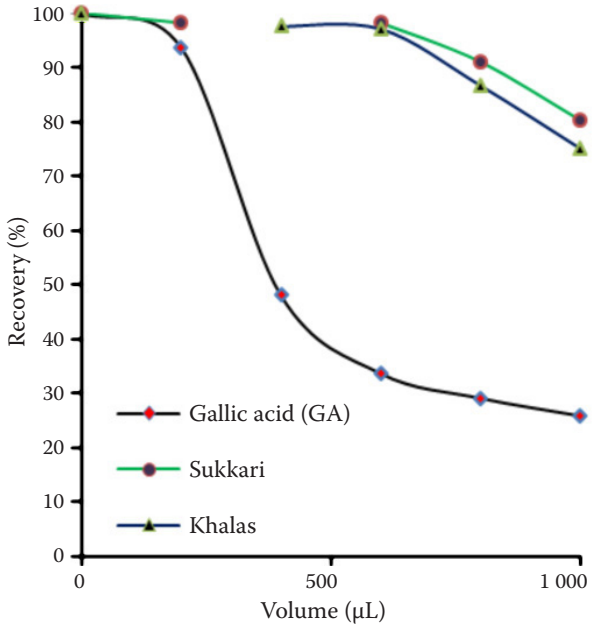

Figure 4. Palm date seed (PDS) extracts scavenging activity against superoxide anion

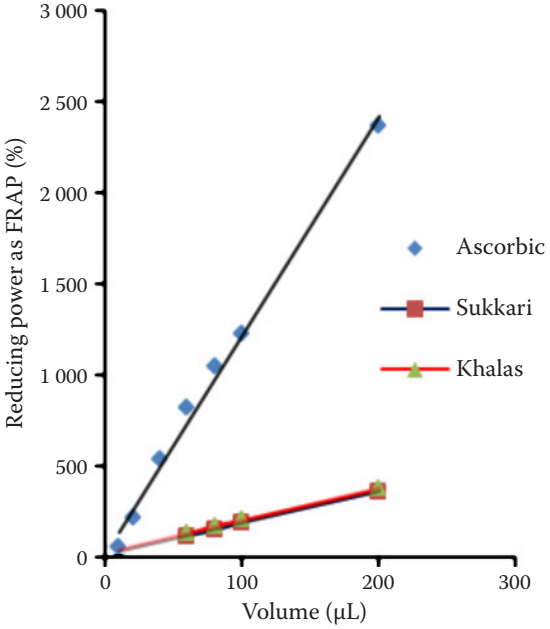

Figure 5. Palm date seed (PDS) extracts reducing power activity (as FRAP)

strains against PDS extracts was *S. aureus*, *B. subtilis*, and *E. faecalis*. Interestingly, both extracts of Sukkari and Khalas PDS showed a high sensitivity against *B. subtilis* (21 ± 0.82 and 22 ± 0.67 mm, respectively) when compared to the positive control (13 ± 0.03 mm) (Figure 6, Table 2). Sukkari and Khalas PDS extracts showed less sensitive activity against gram-negative strains. Although Khalas extracts showed high activity against gram-negative bacterial strains, however, *E. coli* and *P. aeruginosa* showed resistant activity against Sukkari PDS extracts (Clinical and Laboratory Standards Institute, 2007: Performance standards for antimicrobial susceptibility testing; Seventeenth informational supplement, M100–S17). A recent report published by Radfar et al. (2019) studied the antibacterial effect of four different varieties of PDS referred

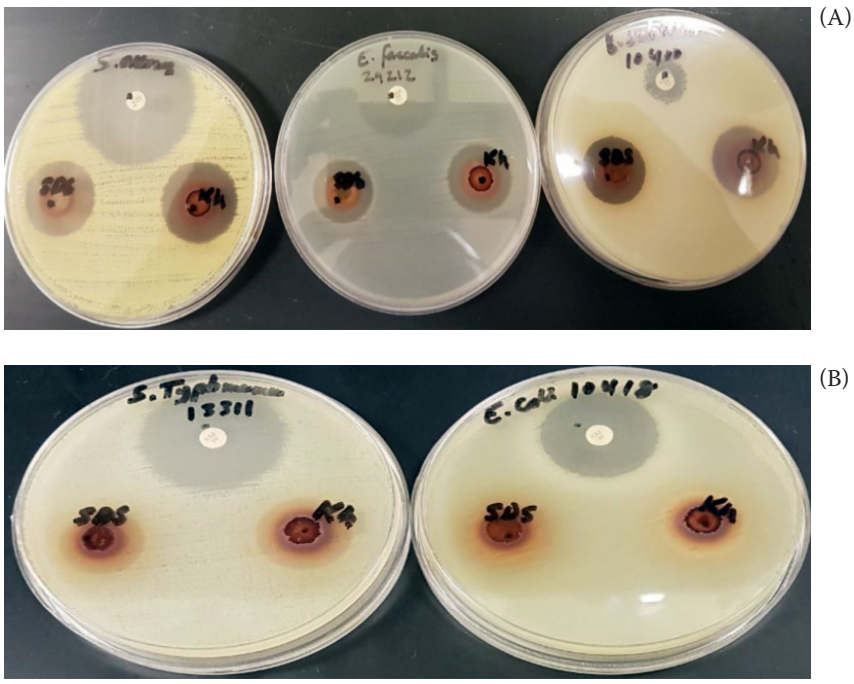

Figure 6. Palm date seed (PDS) extracts antibacterial activity

(A) against gram positive bacteria; (B) against gram negative bacteria; Kh – Khalas; SDS – Sukkari

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Table 2. The antibacterial activity of palm date seeds (PDS)

Tested bacteria species	Inhibition zone (mm)		Positive control* (mm)
	Khalas	Sukkari	
Staphylococcus aureus	28 ± 1.07 (S)	25 ± 0.96 (S)	40 ± 0.08
Bacillus subtilis	22 ± 0.67 (S)	21 ± 0.82 (S)	13 ± 0.03
Enterococcus faecalis	21 ± 0.87 (S)	20 ± 0.61 (S)	25 ± 0.12
Escherichia coli	19 ± 0.73 (S)	14 ± 0.45 (R)	22 ± 0.24
Salmonella typhimurium	19 ± 0.81 (S)	20 ± 1.05 (S)	33 ± 0.16
Pseudomonas aeruginosa	18 ± 0.54 (S)	13 ± 0.38 (R)	20 ± 0.07

*Ampicillin-sulbactam or ceftazidime; (S) – sensitive; (R) – resistant

Table 3. The antibacterial activity of some standard phenolic acids

Phenolic acids	Strains					
	S. aureus	*E. faecalis*	*B. subtilis*	*E. coli*	*S. typhimurium*	*P. aeruginosa*
CO	23 ± 0.18	19 ± 0.16	16 ± 0.13	15 ± 0.13	14 ± 0.16	13 ± 0.14
SG	21 ± 0.22	20 ± 0.36	16 ± 0.22	15 ± 0.18	15 ± 0.11	14 ± 0.17
CG	16 ± 0.09	14 ± 0.07	11 ± 0.12	*	*	*
SP	19 ± 0.11	14 ± 0.08	12 ± 0.06	*	*	*
V	24 ± 0.17	20 ± 0.15	18 ± 0.22	17 ± 0.17	16 ± 0.07	15 ± 0.11
CF	18 ± 0.14	15 ± 0.09	16 ± 0.17	14 ± 0.05	15 ± 0.12	14 ± 0.04
FA	30 ± 0.21	22 ± 0.11	18 ± 0.14	17 ± 0.11	15 ± 0.08	14 ± 0.10
HBA	27 ± 0.17	23 ± 0.14	20 ± 0.09	18 ± 0.16	19 ± 0.21	17 ± 0.11
Cont.(–)	*	*	*	*	*	*
Cont.(+)	39 ± 0.08	26 ± 0.12	12 ± 0.04	23 ± 0.10	32 ± 0.12	21 ± 0.04

CO – coumaric; SG – syringic; CG – chlorogenic; SP – sinapic; V – vanillic; CF – caffeic; FA – ferulic; HBA – ρ-hydroxybenzoic acid; Cont.(–): 40% DMSO + 15.3% acetone; Cont.(+): ampicillin-sulbactam or ceftazidime; *no inhibition zone; *S. aureus – Staphylococcus aureus*; *B. subtilis – Bacillus subtilis*; *E. faecalis – Enterococcus faecalis*; *E. coli – Escherichia coli*; *S. typhimurium – Salmonella typhimurium*; *P. aeruginosa – Pseudomonas aeruginosa*

to their inhibitory effect against *S. aureus* but it showed no inhibition against *E. coli*.

Table 3 summarizes the antibacterial activity of some standard phenolic acids: coumaric, syringic, chlorogenic, sinapic, vanillic, caffeic, ferulic, and hydroxybenzoic acids. Our results showed that most of these acids exhibited higher antibacterial activity against gram-positive strains than against gram-negative strains. However, chlorogenic acid and sinapic acid exhibited low activity against gram-positive strains and no activity against gram-negative strains. The standard phenolic acids showed similar antibacterial activity against *B. subtilis* compared to the positive control. The observed antibacterial activity of PDS extracts is consistent with the results obtained from standard phenolic acids (Table 3), and therefore the antibacterial effect of PDS extracts could be attributed to their phenolic acid content. Borges et al. (2013) reported the antibacterial activity of GA and ferulic acid against pathogenic bacteria. The antibacterial activity of p-hydroxycinnamic, syringic, ferulic, p-hydroxybenzoic, protocatechuic, gentisic, isovanillic and caffeic acids was reported by Fernandez et al. (1996). Another two investigations carried out by Kozyra et al. (2015; 2017) confirmed the antibacterial activity of phenolic acids.

The MIC value of Sukkari PDS extracts ranged from 0.25 to 1.5 mg mL^{-1}, whereas that of Khalas PDS extracts ranged from 0.5 to 2.5 mg mL^{-1} (Table 4). Several researchers attributed the antibacterial effect of certain

Table 4. Minimum inhibitory concentration (MIC) of palm date seeds (PDS)

Bacteria	MIC of methanol extracts (mg mL^{-1})	
	Khalas	Sukkari
S. aureus	0.50 ± 0.06	0.25 ± 0.02
B. subtilis	0.75 ± 0.05	0.50 ± 0.04
E. coli	2.50 ± 0.12	1.50 ± 0.08

For the strain abbreviations see Table 3

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plant extracts to their phenolic acid content (Fernandez et al. 1996; Naz *et al*. 2006; de Camargo et al. 2017).

CONCLUSION

Our findings clearly showed that PDS extracts possess a powerful antioxidant potential and potentially scavenged different sources of free radicals. PDS extracts also exerted good antibacterial activity, especially against gram-positive bacteria. Therefore our findings suggest that PDS could be effectively used as a natural source of antioxidants and as an antibiotic against gram-positive bacteria.

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