Antioxidant and Antibacterial Activities of some Saudi Arabia Palm Date Cultivars' Pits

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ABSTRACT

The disposal of date seeds is a major problem facing date manufacturers. Date pits were found to have many bioactive components and functional properties. Four cultivars namely, Barhi, Manifi, Khulas and Shishi, were used in the present study to compare the total phenols content, scavenge free radicals ability, and antibacterial activity of their pits extracts. Antioxidants activity against diphenyl picryl hydrazyl (DPPH) for ethanol extract of palm date pits ranged from 86.73% in Barhi to 90.25% in Manifi. Lipid peroxidation inhibition test was conducted using egg yolk as fat media. The results showed that the inhibition in lipid peroxidation ranged between 42.03% in Khulas to 60.39% in Manifi pits extract. All cultivars' extracts of date pits showed antibacterial effect against *Staph. Saprophyticus* compared with Chloramphenicol that was found to be ineffective against *Staph. Saprophyticus* in this study. Insignificant differences were found between the commercial antibiotic Sulfadimidin, Barhi and Khulas extract against *Salmonella enteric*. This study indicated that palm date pits extracts from these cultivars have the ability to scavenge free radicals, diminish lipid oxidation, and inhibit bacterial growth.

Key Words: Antimicrobial, Lipid peroxidation, Palm date, Seeds.

INTRODUCTION

Palm date (*Phoenix dactylifera L.*) has long been established as cultural heritage in the Arabian Gulf. It is related to many social and religious habits as well as economical activities. Saudi Arabia produced approximately 550000 tons of palm date in 2011, resulting in about 55000 tons of seeds (Chandrasekaran and Bahkali, 2013). Saudi manufacturers produce diversity of date processed products like table dates, de-pitted dates, date syrup (Dibes), date vinegar, filled dates, date paste and date jam and discard tons of pits daily (Chandrasekaran and Bahkali, 2013). Palm date fruit is composed of a flesh (85-90%) and pits (10-15% of fruit weight). Date pits contain 0.9–1.8% ash, 3.1–7.1% moisture, 5.0–13.2% fat, 2.3–6.4% protein and the main component was dietary fiber (22.5–80.2%). (Al-Farsi *et al*., 2005). In addition, seeds were found to contain high levels of phenolics (3102-4430 mg Gallic acid/ 100 g (Ardekani *et al*., 2010). Metoui *et al*. (2017) analyzed 11 varieties collected at freshly ripen stage "tamr" for their main chemical composition, antioxidant and antibacterial activity. They found that sugar content ranged between 1.20g/ and 3.80g/100g and the phenolic content ranged between 5.224g and 9.532g/100g. The higher antioxidant activity was 55.47% of DPPH radical scavenging activity. Ammar *et al*. (2009) identified some flavonoids from palm date pits such as luteolin, isoquercetrin, genistein, and apigenin. Palm date fruits and pits extracts showed many health properties as antiulcer, anticancer, hepatoprotective, antihyperlipidemic, and nephroprotective in rats (Al-Qarawi *et al*., 2005, Ishurda and John, 2005, Ahmed *et al*., 2008). Date pits paste was found effective in treating ague (Morton, 1987). Some bioactive components create potential health aids of date pits that may increase their incorporation into new functional foods (Almana and Mahmoud, 1994). The date pits possess anti-aging effect and diminish skin wrinkles (Hamada *et al*., 2002). Saddiq and Bawazir (2010)

studied the influence of palm date pits as antibacterial agent against *Escherichia coli* and *Klebsiella pneumonia*. They found that date pits are more effective in inhibiting *Escherichia coli* and *Klebsiella pneumonia* than conventional antibiotics, they suggested a strategy to minimize the side effect of antibiotics. Application of date by-products, particularly pits, as functional food or drug replacement would have direct implication in improving date cultivation and industry as well as increasing the national income.

The aim of the present study was to assess four palm date cultivars pits for their chemical composition, phenols content, antioxidant capacity, inhibition of lipid peroxidation, and antibacterial activity against some selected pathogenic bacteria compared to control standard antibiotics.

MATERIALS AND METHODS

Date samples

Four cultivars, Shishi, Manifi, Khulas and Barhi were collected at the ripeness phase from local markets from Al-Ahsa, Saudi Arabia. The date pits were removed manually from 3 kg of date fruit. The pits were washed then dried at 60 °C. Date pits were powdered in a heavy-duty grinder (Thomas Wiley Laboratory Mill, Model 4, Arthur H. Thomas Co., Philadelphia, PA, USA) and stored at -20 °C until extraction.

Extraction

Pits powder (100 g) was mixed with 500 mL of ethanol for 12 hours at 26 °C for extraction. Vacuum filtration was used to filter the extract using Whatman No.42 filter paper. Ethanol was removed using rotary evaporator under vacuum at 30°C (IKA rotary evaporators, Model 724102, Staufen, Germany). The extracts (very thick syrup) were stored at -20 °C for analysis.

Chemical analysis

Moisture, fat, and protein content were determined using standard protocol (AOAC, 2000). The data were expressed on the dry weight basis.

Determination of total phenols content

The method reported by Boyer and Hai Liu (2004) was used to determine total content of phenols in samples. One ml of extract was mixed with 5 ml of 10 % Folin-Ciocalteu reagent in distilled water and 4 ml of 7.5 % sodium carbonate solution. The samples were maintained at room temperature for 30 min, the absorbance was measured at 765 nm (UV-VIS spectrophotometer, Apel, Japan). The calibration curve was constructed within the concentration range 0.075−0.6 mg/ml of gallic acid. Means were calculated from three parallel analyses as gallic acid equivalents in g/100 g of dry plant material using the following equation:

$C = a \times \gamma \times (V/m) \times 100$,

C: total phenols g/100g as gallic acid; a: dilution factor; γ: concentration obtained from calibration curve (mg/ml); V: volume of extract (ml); m: weight of sample (g).

Free radical scavenging capacity

The free radical scavenging capacity of date pits extract against DPPH (1,1-diphenyl-2 picryl hydrazyl) was estimated according to Zhang and Hamauzu (2004). One ml extract was mixed with 1 ml of 0.4 mmol l^{-1} methanolic solution containing DPPH radicals. The mixture was left in the dark for 30 min and the absorbance was measured at 516 nm (UV-VIS spectrophotometer, Apel, Japan).

Lipid peroxidation inhibition

To estimate the lipid peroxidation induced in egg yolk (fat source) by FeSO4, Thiobarbituric acid reactive substances (TBARS) method was conducted (Ohkowa *et al*., 1979). Dare pits extract (1 ml) was added to egg homogenate (6 ml, 10% v/v) and the volume was completed to 15 ml with distilled water. Fifty microliter of FeSO4 (1g/100ml) was added and the mixtures were incubated for 30 min. Then 1.5 ml of 20% acetic acid (pH = 3.5) and 1.5 ml of thiobarbituric acid (0.8% w/v) and 0.5 ml

trichloro-acetic acid (20%) were added. The mixtures were stirred and boiled for 60 min then cooled to room temperature. Butanol (5 ml) was mixed, vortexed, and left for 10 min. The absorbance of butanol layer was read at 532 nm (UV-VIS spectrophotometer, Apel, Japan).

Inhibition of lipid peroxidation (%) = (1-E /C) x 100

Where, C is the absorbance value of control and E is the absorbance value of samples.

Bacterial strains

American Type Culture Collection of pathogenic bacteria strains, *Staph. saprophyticus* (ATCC 15305) and *Listeria monocytogenes* (ATCC 7644) as G+ and *Salmonella enteric* (ATCC 13076) and *Escherichia coli* (ATCC 25922) as G- were used in the present work to estimate the antibacterial activities of ethanol extract of date pits.

Antibacterial activity

The date pits extracts were examined against *Staph. saprophyticus*, *Listeria monocytogenes, Salmonella enteric* and *Escherichia coli* by agar well diffusion method (Khan *et al*., 2011). Nutrients broth agar (Oxoid, Hampshire, UK), exactly 10 ml, was inoculated with the selected bacteria and incubated for 24h at 37°C. To obtain uniform inoculums, sterile cotton swabs were dipped in the bacterial suspension and uniformly lined over the entire surface of the agar. Four wells were made in each plate. Each filtered extract (50 µl) was tipped in individual well. Sulfadimidin (100 mg/ml) and chloramphenicol (25 mg/ml) were used as controls. All plates were incubated for 24 h at 37°C .The antibacterial activity of the date pits extract was explained as inhibition zone diameters surrounding the wells (mm).

Minimum inhibitory concentration (MIC)

The lowest concentration (mg/ml) of the extract resulting in no growth of bacteria was examined. The agar dilution method of Clinical and Laboratory Standard Institute, CLSL, (2006) was used to accomplish the MIC. Mueller-Hinton Agar, (MHA, CM0337, Oxoid) was used for susceptibility testing against *Staph. saprophyticus*. The media (MHA) were added to test tubes containing different concentrations (0–10 mg/ml) of tested extracts in aseptic condition. Saline solution as control was used under the same conditions. The content of each tube was gently mixed and poured in Petri plates. After hardening, the agar media were spotted with 5µl (10^4 cfu) of the tested bacteria. After the spots were dried, the plates were inverted and incubated for 12–24h at 30 °C.

Statistical analysis.

SAS software program, version 6.11 was used to define the significance of differences between date cultivars pits at significance levels of $P \leq 0.05$ using Duncan's test.

RESULTS AND DISCUSSION

Chemical analysis

Moisture content ranged from7.57 to 10.11% in Manifi and Barhi cultivars pits, respectively (Table 1). Oil content ranged from 3.45 to 7.98% in Manifi and Barhi cultivars, respectively. Al-Shahib and Marshall (2003) stated that oil percentage of date pits varied between among different cultivars. Meanwhile, protein content ranged from 3.30 to 6.45% in Barhi and Manifi cultivars, respectively. These data are in comparable range as obtained in previous studies (Akasha *et al*., 2012, Al-Farsi *et al*., 2005 and Ammar *et al*.,2009). Phenolic compounds ranged from 740 mg/100g in Khulas to 1200mg/100g in Manifi (Table 1). These results in agreement with the findings of Mahdy and Habiba, (2009), but lower than phenolics found by Al-Farsi *et al*. (2007) which ranged from 3102– 4430 mg gallic acid equivalents/ 100 g.

Table 1. Chemical analysis of some date kernel cultivars on dry weight basis

Cultivars	Moisture (%)	Protein (%)	Oil (%)	Phenols (mg/100g)
Shishi	9.33±0.02^{ab}	5.05±0.01^{ab}	6.66±0.04^{a}	940±0.01^{ab}
Manifi	7.57±0.00^{bc}	6.45±0.07^{a}	3.45±0.03^{b}	1200±0.02^{a}
Barhi	11.58±0.09^{a}	3.30±0.09^{c}	7.98±0.02^{a}	781±0.01^{b}
Khulas	10.11±0.01^{a}	4.44±0.05^{bc}	3.50±0.07^{b}	740±0.01^{bc}

Means with different letters are significantly different according to Duncan multiple range test at P≤ 0.05.

Antioxidant activity of date pits

Antioxidant activity of ethanol extract was examined by inhibition of 1,1-diphenyl-2-picrylhydrazyl (DPPH). DPPH is a stable nitrogen-centered free radical that yields a violet color in alcohol solution. All tested cultivars showed high antioxidant activity with DPPH assay ranged between 86.73 and 90.25% in Barhi and Manifi cultivars, respectively (Table 2). Antioxidant activity was significantly higher in Manifi and Shishi than Barhi and Khulas. The antioxidant activity may be due to the phenolic components. The obtained values are lower than antioxidant activities of Tunisian varieties that ranged between 55.47% and 33.12 % (Metoui *et al.,* 2017). However, the findings of some other studies (Al-daihan, and Bhat, 2012; Ardekani *et al*., 2010) are in line with our results (Table 2).

Anti-Lipid peroxidation assay

The inhibitory effect of date pits extract on TBARS formation in egg yolk homogenate induced by $FeSO_4$ is shown in Table 2. All date pits extract reduced lipid peroxide formation. Manifi pits extract showed the Most significant inhibition (60.39% inhibition) towards TBARS formation than Barhi and Khulas. No significant difference between Shishi and Manifi cultivars as anti-lipid oxidation agents was recorded. The lipid peroxidation inhibiting effect may be due the high content of total phenols that may contribute to the antioxidative action directly (Ramakrishnan *et al.,* 2010).

Table 2. Free radical scavenging capacity and inhibition of lipid peroxidation by date seed cultivars

Cultivars	Antioxidants capacity (%)	Inhibition of lipid peroxidation (%)
Shishi	90.06±0.91^{a}	51.77±0.91^{ab}
Manifi	90.25±1.01^{a}	60.39±1.08^{a}
Barhi	86.73±1.02^{b}	50.44±0.46^{b}
Khulas	87.04± 0.31^{b}	42.03±1.02^{b}

Means with different letters are significantly different according to Duncan multiple range test at P≤ 0.05.

Antibacterial activity of date pits

Antibacterial activity of ethanol extract of pits obtained from four cultivars of palm date was verified against four selected bacteria strains. Ethanol extract of pits showed antibacterial activity against *Listeria monocytogenes, Staph. saprophyticus,* and *Salmonella enteric* (Table 3). The most sensitive bacteria to the pits extract was *Staph. saprophyticus*, whereas *E. coli* was found to be more resistant to pits extract of all tested cultivars. The zone of inhibition against *Salmonella enterica* ranged between 20.0 mm in Manifi to 26.0 mm in Barhi cultivar. No significant difference was detected between the inhibition zone of Barhi cultivar and the antibiotic sulfadimidin (100 mg/ml) that was used as a standard control. The zone of inhibition against *Listeria monocytogenes* ranged from 12 mm in Khulas to 17 mm in Manifi. *Staph. saprophyticus* was the most sensitive pathogen against all tested cultivars whereas this strain was the most resistant to the chloramphenicol (25 mg/ml) (Table 3).

Table 3. The inhibition zone of date pits extract against selected pathogenic bacteria

Cultivars	Diameter of inhibition zone (mm)			
	coli.E	*Salmonella enteric*	*Listeria monocytogenes*	*Staph. saprophyticus*
Shishi	ND	19.0 ± 0.0^{c}	16.5 ± 0.12^{c}	15.5 ± 0.71^{d}
Manifi	ND	20.0 ± 1.4^{c}	17.5 ± 0.17^{c}	15.5 ± 0.58^{d}
Barhi	ND	26.0 ± 0.0^{b}	13.5 ± 0.17^{d}	21.0 ± 1.30^{c}
Khulas	ND	23.5 ± 1.5^{bc}	13.0 ± 0.0^{d}	27.0 ± 0.53^{b}
Chloramphenicol	44.0±1^{a}	48.0 ± 0.0^{a}	60.0 ± 0.0^{a}	ND
Sulfadimidin	30.0±1^{b}	26.0 ± 0.0^{b}	30.0 ± 0.0^{b}	38.0 ± 0.0^{a}

Means with different letters are significantly different according to Duncan multiple range test at P≤ 0.05
Values are mean inhibition zone (mm) ± S.D
ND: Not Detected

The MIC for *Staph. saprophyticus* was found to be 3.75, 2.50, 1.2 and 1.2 mg/ml for Shishi , Manifi, Barhi, and Khulas seed extracts, respectively (Table 4). Barhi and Khulas seed extracts were most effective at lower concentration (1.2 mg/ml). The present results are agree with Perveen *et al*. (2012) who revealed that pits extracts from three cultivars of palm date (Barhi, Sukri, and Rothana) showed good antibacterial activity against some pathogenic bacteria responsible for range of contaminations, that may be due to various bioactive components present in palm date pits. The results also are in line with Bentrad *et al.* (2017) who found that organic extracts of date seeds and pollen showed an antibacterial behavior against gram-positive and gram-negative bacteria. Ammar *et al*. (2009) reported that acetone and ethanol extracts obtained from date pits from El Dakhla oases, Egypt, inhibited the growth of some pathogenic bacteria.

Table 4. Minimal inhibitory concentration of pits extract against *Staph. saprophyticus*

Cultivars	MIC (mg/ml)
Shishi	3.75 ±0.92^{a}
Manifi	2.50 ± 0.22^{b}
Barhi	1.20 ± 0.56^{c}
Khulas	1.20 ± 87^{c}

Means with different letters are significantly different according to Duncan multiple range test at P≤ 0.05

This study concluded that selected date cultivars pits showed antioxidant and antibacterial properties and could be used as natural economic source in food preservation and against some diseases. Manifi pits extract was found to possess higher antioxidant activity and anti-lipid peroxidation inhibition than Barhi and Khulas. Barhi and Khulas extracts showed antibacterial activity against *Salmonella enteric* and *Staph. Saprophyticus* but not against *Listeria monocytogenes.*

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النشاط المضاد للأكسدة والمضاد للبكتريا في بذور بعض أصناف تمر النخيل في المملكة العربية السعودية

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الملخص

تعد بذور تمر النخيل واحدة من المشاكل الرئيسة في الصناعات الغذائية، وثبت أنها تحتوي على مركبات حيوية مهمة. في هذه الدراسة تم استخدام بعض الأصناف (بارحي ومنيفي وخلاص وشيشي) لمقارنة محتوى البذور من الفينولات والقدرة على كبح جماح الشقوق الحرة والتأثير المضاد للبكتريا. تراوحت النسب المئوية لنشاط مضادات الأكسدة في الأصناف ضد مركب داي فينيل بكريل هيدرازيل بين 86.73 % في صنف بارحي و 90.25 % في صنف منيفي. تم اختبار القدرة على تثبيط أكسدة الدهون باستخدام دهون صفار البيض للأصناف، وأوضحت النتائج أن النسبة المئوية لتثبيط أكسدة دهون البيض تراوحت من 42.03 % في صنف خلاص إلى 60.39 % في صنف منيفي. أظهرت جميع مستخلصات بذور التمر تأثيرًا مضادًا لبكتريا ستافيلوكوكاس سابروفتيكس مقارنة بالمضاد الحيوي كلورومفينكول الذي لم يُظهر أي تأثير ضدها في هذا البحث. لم يكن هناك فرق معنوي بين تأثير المضاد الحيوي سلفاديدين ومستخلص بذور صنف البارحي ضد بكتريا السالمونيلا. خلصت هذه الدراسة إلى أن مستخلص بذور التمر من مختلف الأصناف له القدرة على كبح جماح الشقوق الحرة وتقليل أكسدة الدهون وتثبيط نشاط بعض أنواع البكتريا الممرضة.

الكلمات المفتاحية: البذور، تمر النخيل، مضادات الأكسدة، مضادات البكتريا.