Antioxidant and antibacterial studies of *Phoenix dactylifera* and its varieties

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**ABSTRACT**

The study investigated the comparative antioxidant ability of date fruit (*Phoenix dactylifera*) and date bark. Fardh, Khasab and Khalas are the three major date palm varieties grown in Sultanate of Oman. The antioxidants of these varieties were assessed using 2,2-diphenyl-1-picrylhydrazyl (DPPH), ferric reducing/antioxidant power (FRAP). The total phenolic content and total antioxidant activity of the bark extract and date fruit was quantified. The total phenolic content of bark extract obtained was 0.57±0.072 µg gallic acid equivalents (GAE)/10 g of dry weight (DW) and all the three varieties of dates ranged from 0.88-0.94 µg GAE/10 g of DW. The total antioxidant activity of bark extract obtained was 0.14±0.07 µg ascorbic acid equivalent (AAE)/10 g of DW, whereas the antioxidant activity of all the three varieties of dates ranged from 0.24-0.32 µg AAE/10 g of DW. The percent scavenging activity of bark extract and the three varieties of dates was 42% and ranged from 67-89%, respectively; observed from DPPH assay at different concentrations. Whereas in hydrogen peroxide percent inhibition of bark extract and three varieties was 60% and ranged from 26-39% at different concentrations, respectively. The reducing ability was observed in a decreasing order of Khalas>Fardh>Khasab>Bark extract. While the hydroxyl radical scavenging activity was observed in a decreasing order of Khalas>Bark extract>Fardh>Khasab at different wavelengths. Date fruit as well as bark extract exhibited an excellent antioxidant ability probably because they contain huge amount of phenolic content as well as a high capacity of electron donating groups that neutralize the free radicals. Comparison between bark extracts of the three varieties (Khasab, Khalas and Fardh) revealed that Khalas exhibited maximum antioxidant ability as well as the phenolic content.

**INTRODUCTION**

Antioxidants have always helped in preventing the damage done to cells by free radicals that are released during normal metabolic process of oxidation. These free radicals include reactive oxygen free radical species (ROS), reactive hydroxyl radicals (OH), the superoxide anion radical (O2•−) hydrogen peroxides (H2O2) and peroxyl (ROO•). The nitric oxide (NO) and peroxynitrite anion (ONOO•) are the nitrogen derived free radicals (Young and Woodside, 2001). Over production of ROS is due to exposure to pollutants, cigarette smoking, UV-rays, radiations and toxic chemicals, (Bagchi and Puri, 1998; Ebadi, 2001). It is also produced due to deep fried food, spicy food and physical stress. They cause depletion of immune system antioxidants, change in gene expression and induce abnormal proteins. Various human diseases such as atherosclerosis, arthritis, ischemia and reperfusion injury of many tissues, central nervous system injury, gastritis and AIDS (Alfadda and

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Sallam, 2012; Valko et al., 2007). They also protect unsaturated fats in the body from oxidation by peroxides and antioxidants that inhibit enzyme–catalysed oxidation includes substances that bind free oxygen such as ascorbic acid and substances that inactivate enzymes such as citric acid and sulfites (Lobo et al., 2010). Enzymatic antioxidants such as superoxide dismutase (SOD), catalase and glutathione peroxidase and non-enzymatic such as glutathione and vitamin E also plays an important role in preventing cell injuries (Starlin and Gopalakrishnan, 2013). The total antioxidant activity of vegetables is due to the combination of individual activities of the individual antioxidants present such as ascorbic acid, tocopherols, carotenoids and phenolic compounds and polyphenols have the greatest and most beneficial antioxidants (Kim et al., 2003). The antioxidant activity of polyphenols is mainly due to redox properties which are important in quenching oxygen or decomposing peroxides (Karou et al., 2005). Synthetic antioxidants such as butylated hydroxy anisole (BHA), butylated hydroxyl toluene (BHT), tertiary butylated hydroquinone and gallic acid esters are not used anymore as they have a negative health effect and they show low solubility and moderate antioxidant activity (Barlow, 1990). Due to this there is a huge demand of natural antioxidants in food industry for replacing the synthetic preservatives that are used to prevent fat rancidity or color loss (Halliwell, 2008). Traditionally used antioxidants from tea, wine, fruits, vegetables and spices are being exploited commercially either as nutritional supplements or antioxidant additives (Dai and Mumper, 2010; Odunkoya, 2005). Our body produces natural antioxidants when catalase and hydroperoxidase enzymes convert hydrogen peroxide and hydro peroxides to non-radical forms. Gallic acid and ascorbic acid are natural antioxidants found in various food sources. Several studies have shown that these posses a variety of pharmacological activities such as antioxidant, anti-inflammatory and anti-cancer activities (Chen et al., 2013; Barrita and Sanchez, 2013). Secondary metabolites from medicinal plants function as smaller molecular weight antioxidants but their known mechanism of actions is variable and so they depend on both the structure as well as environment (Halliwell, 2008).

The antioxidant property of dates varies from different cultivars grown in different countries such as Algeria (Mansouri et al., 2005), Kuwait (Vayalil, 2002), Oman (Al-Farsi et al., 2005), USA (Vinson et al., 2005), Bahrain (Allaith, 2008) and Iran (Bilgari et al., 2008). Date fruits are grown in North-African, the Middle Eastern and the Asian countries are considered to be one of the most important agricultural commodities and also considered as the nutritious and staple fruit (Khan et al., 2008). They contain a major source of carbohydrates such as simple sugars like glucose and fructose (Al-Hooti et al., 1997; Myhara et al., 1999); and it also contains sucrose (Guizani et al., 2010). It also has a good source of dietary fiber and important minerals such as iron, potassium, selenium, calcium and vitamins such as vitamin C, B1, B2 and vitamin A. Riboflavin and niacin is also present but it has a low content of fat and protein. Besides nutritional value they also contain antioxidant activities and this is due to some bioactive compounds such as phenolics, flavonoids and vitamins such as C, E.

The aim of this study was to estimate the total phenolic and antioxidant activity in the bark extract as well as the three varieties of dates namely Khalas, Khasab and Fardh. These varieties are considered the premium quality and the most consumed in Sultanate of Oman.

**MATERIALS AND METHODS**

**Plant material**

The three varieties of dates namely Khalas, Khasab and Fardh were obtained from the local orchard in Muscat during the harvesting season in the year 2012. The varieties were confirmed from the available information about their characteristics and further from the experts of plants and stored at -40°C until used for experiments. Inside soft tissue of the date palm was taken to prepare the bark extract.

**Preparation of bark and date fruit extracts**

The preparation of bark extract was done as described by Al-Dalhan and Bhat (2012). About 10 g of bark powder was accurately weighed and 100 ml of 70% (v/v) aqueous acetone was added and placed in a flask. The flask was covered with aluminum foil and shaken for 24 h at 50°C on a platform shaker. The flask was removed from the shaker and polyphenol extract was separated from the solid plant material by filtering the mixture through a funnel equipped with Whatman No.1 filter paper. The filtered polyphenol extract was placed in an appropriate sized round bottom flask and was exposed to air in order to allow the evaporation of acetone. The aqueous polyphenol extract was stored at -20°C. Whereas the preparation of date extract is done as described by Singh et al. (2002); 10 g of edible part of date fruit was accurately weighed and 90 ml of water or methanol (99%) was added and placed in a flask. After being mixed by magnetic stirrer for half an hour, and then filtered with filter paper Whatman No. 41. The remaining materials were re-extracted three times.

**Total phenolic content**

The total phenolics content was determined and measured using Folin–Ciocalteau reagent (Subhashini et al., 2010). Absorbance was taken at 750 nm and the
concentration of bark extracts was compared with the dates extract against Gallic acid standards. The concentration of bark extract and dates was expressed as GAE. All the measurements were taken in duplicates.

**Total antioxidant activity**

Total antioxidant activity was measured using the method described by Tyagi et al. (2010). Absorbance was taken at 695 nm and aqueous extract of bark was compared with the date extracts against ascorbic acid standard. The concentration of bark extract and dates was expressed as AAE. All the measurements were taken in duplicates.

**Hydrogen peroxide scavenging activity**

Extracts of bark and date of various concentrations (2, 3, 4, 5 and 6 ml) were mixed with 0.3 ml of 4 Mm H2O2 solution prepared in phosphate buffer (0.1 M, pH 7.4) and incubated for 10 min. The absorbance was taken at 230 nm (Gülçin et al., 2003a). The absorbance was estimated based on the percentage of scavenging of hydrogen peroxide and was calculated using the following equation:

\[
\text{Scavenging effect (\%) = } \frac{[(\text{control absorbance-sample absorbance})]}{\text{(control absorbance)}} \times 100
\]

**DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity**

DPPH (2, 2-diphenyl-1-picrylhydrazyl) scavenging activity was assessed by the method of Bagul et al. (2003). Stock solution of DPPH was prepared by dissolving 6 mg DPPH with 25 ml methanol and then the absorbance of about 0.980 at 517 nm was obtained and the solution was stored at 20°C until needed. 3 ml of this stock solution was mixed with the extract solution which was taken in varying concentrations (0.1 to 0.9 ml). A positive control of 1 ml extract was also taken. The solution was shaken well and incubated in the dark for 15 min at room temperature. The absorbance was estimated based on the percentage of scavenging of DPPH and was calculated using the following equation:

\[
\text{Scavenging effect (\%) = } \frac{[(\text{control absorbance-sample absorbance})]}{\text{(control absorbance)}} \times 100.
\]

**Reducing power of bark extract**

Ferric reducing power of the extracts was evaluated by the methods of Bhalodia et al. (2013). Various concentrations of extract were mixed with 2.3 ml phosphate buffer (0.2 M, 6.6) and 2.5 ml of 1% potassium ferricyanide (K3[Fe(CN)6]). After incubation at 37°C for 20 min, 10% trichloroacetic acid (TCA) was added to the mixture and centrifuged for 10 min at 1000 rpm; the supernatant distilled water and 0.1% Ferric chloride (FeCl3). After standing for 10 min, the absorbance was measured at 700 nm.

**Hydroxyl radical scavenging activity by DPPH, Bark extract and Mannitol**

Different concentrations of DPPH, extract and mannitol were taken. 1 ml of DPPH was mixed with 1 ml bark extract while the other 1 ml of DPPH was mixed with 1 ml mannitol. The absorbance was taken from 380 to 700 nm. The values were compared.

**Antibacterial activity**

The antimicrobial activity was studied by disc diffusion method (Serban et al., 2011; Valgus et al., 2007). The strains used were Lactobacillus brevis, Salmonella typhii, Escherichia coli and Pseudomonas spp. The extracts were tested against each bacterial strain. All the strains were pre-cultured in nutrient broth overnight in rotary shaker at 37°C. The cell density was standardized spectrophotometrically. Then nutrient agar plates of the strains were prepared on which the paper discs of 5 mm, was impregnated with the extracts (50 µl) of bark and Khala, Fardh and Khasab were placed. The zone of inhibition was measured.

**RESULTS AND DISCUSSION**

**Total phenolic content**

The results of the total phenolic content shows that the maximum phenolic content was found in Khalas (0.94 µg ± 0.03 GAE/10 g of dry weight, DW) followed by Khasab (0.9 µg ± 0.12 GAE/10 g of DW) and Fardh (0.88 µg ± 0.03 GAE/10 g of DW). The least content was found in bark extract (0.57 µg ± 0.072 GAE/10 g of DW). The order can be given as Khalas>Khasab>Fardh>Bark extract (Figure 1). A slightly different pattern was observed by Singh et al. (2012) in which Fardh exhibited highest phenolic content. The observed low value of the phenolic component is attributed to the ripening and maturation level of dates (Myhara et al., 1999).

**Total antioxidant activity**

The results of the total antioxidant capacity showed that maximum antioxidant activity was found in Khalas (0.32 µg ± 0.05 AAE/10 g of DW) followed by Khasab (0.28 µg
±0.66 AAE/10 g of DW) and Fardh (0.24 ± 0.017 µg AAE/10 g of DW). The least content was found in bark extract (0.14 ± 0.07 µg AAE/10 g of DW). The antioxidant activity was observed in a decreasing order of Khalas>Khasab>Fardh>Bark extract (Figure 2). When compared with the edible stages of Saudi Arabian (Al-Humaid et al., 2010) and Iranian dates (Reza et al., 2010), it exhibited extensive antioxidant ability. This might be due to different extraction methods used. The phenolic compounds that consist of antioxidant activity is mainly due to redox properties that play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen and decomposing peroxides (Hassan et al., 2008).

**Hydrogen peroxide scavenging activity**

The maximum hydrogen peroxide scavenging activity was given by Bark extract (60%) followed by Fardh (39%) and the least was given by both Khalas and Khasab (26%), respectively (Figure 3). Hydrogen peroxide scavenging activity may be due to the phenolics as they donate electrons to H₂O₂ and hence they neutralize it to water. The scavenging capacity of the extract is due to their structural features of the active compounds that help in determining the electron donating abilities (Wettasinghe and Shahidi, 2000).

**DPPH radical scavenging activity**

The results were expressed as percentage inhibition based on absorbance. Highest DPPH free radical scavenging activity was observed in Fardh (88%) followed by Khalas (87%), Khasab (67%) and least was found in bark extract (42%) measured (Figure 4). Similar pattern of DPPH scavenging activity was observed by Singh et al. (2012). The antioxidant effect on DPPH radical scavenging activity is because of their hydrogen donating ability as DPPH is considered as a free radical. Being a free radical it accepts an electron or hydrogen radical and becomes a stable diamagnetic molecule Soares et al. (1997). The reducing capacity of the DPPH radical is assessed by the decrease in its absorbance at 517 nm induced by antioxidants. Stable DPPH radical in ethanol at 517 nm shows the maximum absorption. It was found that their scavenging percentage is lesser compared to those found in this study. The possible explanation of this difference can be attributed to the
Figure 2. Total antioxidant activity of bark extract, Khalas, Fardh, Khasab dates fruit extracts. The value are presented as mean±SD (n=3) and indicates the significance level at p<0.05 compared with other varieties.

Figure 3. Hydrogen peroxide scavenging activity of bark extract, Khalas, Fardh, Khasab dates fruit extracts. The value are presented as mean±SD (n=3) and indicates the significance level at p<0.05 compared with other varieties.
Reducing power of bark extract

In the ferric reducing activity power, the samples containing antioxidants reduced Fe$^{3+}$/ferric cyanide complex to ferrous form by donating electrons. It donates a hydrogen atom to break the free radical chain and this indicates that reducing properties exert antioxidant action (Gordan, 1990). Absorbance increasing at 700 nm indicates increase of reducing ability. In this assay, the color of the test solution changes from yellow to green and blue. This indicates the reducing power of the specimen. The reducing ability was observed in a decreasing order of Khalas>Fardh>Khasab>Bark extract. Fardh had the maximum reducing ability according to one report (Singh et al., 2012). The antioxidant activity of the substance is directly correlated to the reducing capacity and hence these results are directly evidenced of iron reducing capacity.

Hydroxyl radical scavenging activity by DPPH, bark extract and mannitol

The hydroxyl radical is considered as a highly free radical and is capable of damaging almost every molecule of biological system. It has the capacity to join the nucleotides in DNA and cause strand breakage, which eventually causes carcinogenesis, mutagenesis and cytotoxicity (Babu et al., 2001). The radicals attack the sugar or the base of the DNA and generate large number of products. The hydroxyl radical scavenging activity is directly related to its antioxidant capacity. The hydroxyl radical scavenging activity was observed in a decreasing order of Khalas>Bark extract>Fardh>Khasab. Khalas can be considered as a good scavenger of active oxygen species and hence helps in reducing the rate of chain reaction (Figure 5).

Antibacterial activity

When the antibacterial studies were performed with four bacterial strains only bark extract showed antibacterial activity against *L. brevis*, *E. coli* and *Pseudomonas* spp. However, bark extract too was not resistant to *S. typhii*. Bark extract showed maximum activity against *L. brevis* followed by *Pseudomonas* spp. and least with *E. coli*. The fruit extracts did not exhibit any antibacterial activity (Table 1). Other researchers have found that the small sucrose derivatives could not inhibit the growth of *E. coli*. The resistance is attributed to the cytolysin lipopolysaccharide and membrane lipids which could screen out the fatty acid and prevent transport in the cell membrane (Ferrer et al., 2005). Other report also support the resistance of gram negative bacteria to the inhibitory effects of the sugar esters because of membrane structure and difference in cell wall (Ouattara et al., 1997). Similar reports are available for the antibacterial
activity of the date palm pit aqueous extracts with *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Klebsiella pneumoniae* and *E. coli* (Yassein, 2012). However, Egyptian date fruit extracts show antibacterial activity with some specific strains such as *E. coli* 0-143, *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATC6633 (El Sohaimy et al., 2015).

**Conclusion**

The maximum phenolic content was found in Khalas followed by Khasab and Fardh. The least content was found in bark extract. The maximum antioxidant activity was found in Khalas followed by Khasab and Fardh. The least content was found in bark extract. Highest DPPH free radical scavenging activity was given by Fardh followed by Khalas, Khasab and least was found in bark extract. The reducing ability was given in the order of Khalas>Fardh>Khasab>Bark extract. The maximum hydrogen peroxide scavenging activity was given by Bark extract followed by Fardh and the least was given by both Khalas Khasab (26%). The hydroxyl radical scavenging activity was observed in a decreasing order of Khalas>Bark extract>Fardh>Khasab at different wavelengths.

Khalas showed maximum antioxidant capability. The data in this study confirms that date fruit has higher levels of antioxidants and phenols when compared with bark extract. Moreover, antibacterial activity was observed in bark extract and not in fruit extracts. This opens new directions in the use of bark extract for medicinal purposes and use of fruit varieties of dates as antioxidant source.

**REFERENCES**


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