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Nutritional Composition and *in vitro* Evaluation of the Antioxidant Properties of Various Dates Extracts (*Phoenix dactylifera* L.) from Libya

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Abstract: The aim of this study was to examine the nutritional content and antioxidant potential of three date fruit varieties (*Phoenix dactylifera* L.) native to Libya, namely, Bekraray, Deglet-nour and Khathori. The fruits were collected from three districts of Libya covering the northern, southern and eastern areas, respectively. The fruits were pitted and the flesh was extracted at 60°C for 6 h. The antioxidant activity of the extract was measured using 1-diphenyl-2-picrylhydrazyl (DPPH) method and iron (3) reduction (FRAP) assay. The Total Phenolic Content (TPC) of the date was measured using the Folin-Ciocalteau method. The results showed that the nutritional content of dates varied by source. The flesh of Bekraray dates contained high percentage of carbohydrates (76.97%), vitamin C (8.50 mg 100 g) and potassium (6043.2 mg kg^{-1}) whereas, Deglet-nour variety demonstrated the high percentage of total sugar (73.25%), vitamin A (10.50 μ/100 g) and vitamin E (12.98 mg kg^{-1}). On the other hand, Khathori variety showed high percentage of vitamin B2, magnesium, calcium, sodium and ferum with concentrations of 824.98, 660.74, 614.21, 485.86 and 20.29 mg kg^{-1}, respectively. The Bekraray dates exhibited a significantly high concentration of TPC ($p<0.05$) from those of the two other varieties. The antioxidant activity correlated positively with the TPC of the extracts. The Bekraray also showed high FRAP value and free radical scavenging activity (DPPH) among the studied date varieties and the values corresponded to 13.46±0.11 μmol (Fe)/g and 78.9%, respectively. These results suggest that Libyan dates varieties have a high nutritional value and possess beneficial antioxidant properties. Bekraray date was found to be superior than Deglet-nour and Khathori variety.

Key words: *Phoenix dactylifera*, antioxidant, dates palm

INTRODUCTION

Reactive Oxygen Species (ROS), such as the superoxide anion, hydroxyl radical and peroxyl radical, are biological products from reduction process of oxygen (Williams and

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Jeffrey, 2000) and are particularly active to damage chemical species (Jing *et al*., 2007). The ROS are highly reactive to the extent that they can oxidize biomolecules such as lipids, DNA, proteins and carbohydrates (Chao *et al*., 2006; Fridovich, 1978; Kinsella *et al*., 1993) and contribute to the onset of various diseases such as heart diseases, cancer, a weak immune system and aging (Feinman, 1988; Maharaj *et al*., 2006; Puntel *et al*., 2007). On the other hand, antioxidant is a molecule that capable to inhibit the oxidation of other molecules hence provides a protective effect against ROS activity. Antioxidant compounds can be classified according to their mechanism of action. Firstly, it decelerates the production of free radical species that are induced by transition metal reactions. Secondly, it inhibit series of free radical reactions and thirdly is through the combination of the two previous mechanisms (Lima *et al*., 2004). In this respect, phytochemicals from fruits and vegetables have been shown to possess several antioxidant properties, depending on their content of phenolic compounds, carotenoids, flavonoids and vitamins C and E (Javanmardi *et al*., 2003; Saura-Calixto and Goni, 2006).

Date palm (*Phoenix dactylifera* L.) is an important commercial harvest in the Middle East and North African countries (Al-Farsi *et al*., 2005). The fruit production contributes in the economic role and social life within these regions (Bastway *et al*., 2008) and considered as a vital component of the diet (Vayalil, 2002). Libya is regarded to be one of the date-producing countries. Dates are a good source of energy due to their high iron, potassium, calcium, sodium, iodine and sugar contents though their sugar content is less than the recommended daily intake. The recommended daily sugar intake established for adult men is 36 g while that for women is 20 g (Paja, 2010). In addition, it is rich in vitamins and low in fats and proteins (Vayalil, 2002; Al-Farsi *et al*., 2007). Beside its nutritional value, date is rich in phenolic and flavonoid compounds possessing antioxidant activity (Saura-Calixto and Goni, 2006; Biglari *et al*., 2008; Mansouri *et al*., 2005). The antioxidant property of date is attributed to the wide range of phenolic components including p-coumaric, ferulic and sinapic acids, flavonoids and procyanidins (Bastway *et al*., 2008). Consequently, its significant nutritional composition and potential health promotion activities, call for detailed report. This preliminary study was designed to investigate the nutritional composition of three Libyan native date varieties and to examine their antioxidant capacity. The scientific clarification from this study will provide information on dates as an alternative source of natural antioxidant that could improve health status.

MATERIALS AND METHODS

Chemicals and Reagents

Butylated hydroxytoluene (BHT), ferric chloride, hydrochloric acid, ferrous sulphate, acetic acid, sodium acetate and gallic acid were purchased from Sigma Chemical Co. (USA). Folin-Ciocalteu and sodium carbonate were from Merck (Germany). The DPPH (2,2-diphenyl-1-picryhydrazyl radical) and 2,4,6-tris(2-pyridyl)-1,3,5-triazine (TPTZ) were from Fluka (Switzerland). All Chemicals and reagents used were of analytical grade.

Preparation of *Phoenix dactylifera*-L. Pericarp Flesh Extract

Fresh, matured date fruits were purchased from markets at three different districts selected in the North, South and East of Libya. Three date varieties namely Bekraray, Deglet-nour and Khathori, were selected for the experiment and were standardized in terms of the size (7-10 g per fruit), colour (light brown) and ripening stage. The pericarp was separated from the seed and minced. A 10% aqueous pulp extract was prepared by soaking

100 g of the fresh pulp (equivalent to 10 date pods) in 1000 mL of distilled water and mixed thoroughly. The mixture was incubated in a shaking water bath at temperature and time setting: 60°C for 6 h. Once filtered, the filtrates were freeze-dried and kept at -80°C until use.

Proximate and Mineral Analysis

Total ash, moisture, crude protein, fat and carbohydrate contents of the dates were determined in accordance to Association of Analitical Communities (AOAC) test methods (AOAC, 1995). The proximate analyses were carried out in triplicate and results are reported in percentage. The minerals, including both macro- and micro-elements, were determined by scanning electron microscopy attached with energy dispersive X-ray (SEM-EDX) equipment (Japan).

1,1-diphenyl-2-picrylhydrazyl (DPPH) Test

The DPPH test was conducted following the method established by Yen and Hsieh (1998). A 0.45 mM of DPPH was prepared by adding 17.74 mg of DPPH to 100 mL of absolute ethanol. A 1.0 mL volume of the 0.45 mM DPPH was added to 0.5 mL of the samples each (P. dactylifera extract, vitamin C, BHT and control). The 0.5 mL aliquot of the samples was prepared by adding 5 mg each of the crude extract, vitamin C and BHT to 1.0 mL of absolute ethanol. The mixture was kept in the dark at room temperature for 30 min. The absorbance (OD) of the free radical scavenging activity was measured by spectrophotometer at the wavelength of 517 nm. The percentage of inhibition (IP) was determined using the formula:

$$\text{Percentage of inhibition (\%)} = \left[\left(\frac{OD_{control}-OD_{sample}}{OD_{control}}\right)\right]\times 100\%$$

Ferric Reducing/Antioxidant Power (FRAP) Assay

The ferric reducing capacity of the sample was assessed using the method adapted from (Benzie and Strain, 1996). The FRAP reagent, made-up of 2.5 mL of 10 mM of 2,4,6-trispyridyl triazine (TPTZ) solution in 40 mM hydrochloric acid (HCl); 2.5 mL of 20 mM ferric chloride ($FeCl_3$); and 25 mL of 0.25 M acetate buffer (pH 3.6), was freshly prepared. A 100 μL volume of sample (studied extract) was added to 300 μL distilled water followed by addition of 3 mL of FRAP reagent. The absorbance was read at 593 nm after 4 min of incubation at room temperature against a blank. The standard curve was constructed using ferrous sulphate standard solutions over the linearity range 0.2-1.0 mmol L^{-1}. The antioxidant activity of the samples was determined from the standard curve of ferrous sulphate using their measured absorbance values. The results were expressed in mmoles per litter (mmol L^{-1}). Subsequently, the antioxidant activities of the samples were compared with standard BHT and vitamin C.

Determination of Total Phenolic Content

Determination of total phenolic content was based on the method described by Velioglu *et al.* (1998) with some modification. Briefly, a 2.0 mL aliquot of extracts each was mixed with 1 mL of 1 N Folin-Ciocalteu reagent in 10 mL volumetric flasks. After 5 min, a 4 mL volume of saturated sodium carbonate solution was added. The volume was then made up to 10 mL with distilled water and mixed thoroughly. The absorbance readings of the reaction mixtures were measured at 760 nm against a blank after 2 h of reaction. Gallic Acid (GA) was used to construct a standard curve (0-50 mg L^{-1}). The results are expressed as milligram gallic acid equivalents (GAE) per g fresh weight.

Statistical Analysis

All experiments were conducted in triplicate and statistical analysis was done using the Statistical Package Social Sciences (SPSS) version 16 programme. Results were expressed as Mean±SD. A value of $p<0.05$ was used to denote statistical significance.

RESULTS AND DISCUSSION

Proximate Analysis and Chemical Composition

The average chemical composition of date pericarp is illustrated in Table 1, there was no significant difference in carbohydrate content between dates from each area; however, Bekraray date variety demonstrated a higher protein content (2.78%) ($p<0.05$) than the other two counterparts (1.91 and 1.86%, respectively). With reference to the ash and moisture contents, there were no significant differences between the three examined varieties were found, however Khathori dates possessed higher vitamin B2, magnesium, calcium and sodium contents (824.98, 660.74, 614.21 and 485.86 mg kg^{-1}, respectively) than the other two varieties ($p<0.05$). On the other hand, Bekraray demonstrated higher vitamin C and potassium contents, whereas Deglet-nour dates showed higher vitamin E content (12.98 mg kg^{-1}) than Bekraray and Khathori (6.25 and 6.68 mg kg^{-1}, respectively). Present results also demonstrated that both Bekraray and Deglet-nour date varieties contain high soluble dietary fibers (6.33 and 7.24%, respectively) than in the Khathori variety (3.46%). Dietary fiber is a term denoting components of a variety of plants that are resistant to digestion by the human gastrointestinal enzymes (Eastwood and Passmore, 1983). Many studies have reported on the beneficial effect of soluble fibers in lowering the total and LDL cholesterol levels (Glore *et al*., 1994; Kris-Etherton *et al*., 1988; Trowell and Burkitt, 1981; Truswell, 1995).

Total Phenolic Content

The total phenolic content in the extracts was assessed according to the colorimetric Folin-Ciocalteu method with gallic acid as a standard compound ($R^2 = 0.994$, $y = 4.55x + 0.272$). The phenolic content in date samples was in the range from 51.67±0.12 to 71.62±0.10 mg/100 g, as shown in Fig. 1. Bekraray date demonstrated the highest phenolic content (71.62±0.10 mg/100 g) ($p<0.05$). Moreover, this study showed that date fruit having

Table 1: Proximate and mineral contents of dates samples from three local varieties

Constituents	Bekraray	Deglet-nour	Khathori
Carbohydrate (%w/w)	76.97	76.73	74.56
Protein (%w/w)	2.78	1.91	1.86
Fat (%w/w)	0.00	0.02	0.05
Ash (%w/w)	1.88	1.93	1.61
Moisture (%w/w)	18.37	19.41	21.92
Total sugar (%w/w)	59.61	73.25	52.06
Vitamin B2 (mg kg^{-1} sample)	776.60	699.83	824.98
Vitamin A (µ/100 g sample)	9.83	10.50	3.28
Vitamin C (mg/100 g sample)	8.50	5.94	6.40
Vitamin E (mg kg^{-1} sample)	6.25	12.98	6.68
Insoluble dietary fiber (%w/w)	0.20	0.00	0.41
Soluble dietary fiber (%w/w)	6.33	7.24	3.46
Potassium (mg kg^{-1} sample)	6043.20	5994.20	5325.70
Magnesium (mg kg^{-1} sample)	518.86	603.17	660.74
Calcium (mg kg^{-1} sample)	222.78	423.49	614.21
Sodium (mg kg^{-1} sample)	57.41	56.94	485.86
Ferum (mg kg^{-1} sample)	7.50	13.41	20.29
Lead (mg kg^{-1} sample)	0.04	0.02	0.05

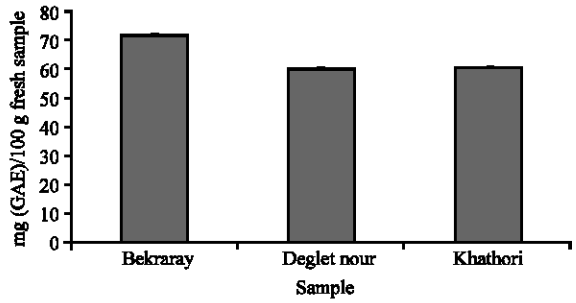

Fig. 1: Total phenolic content of Bekraray, Deglet nour and Bekraray date fruit varieties. Data were expressed as Mean±SD

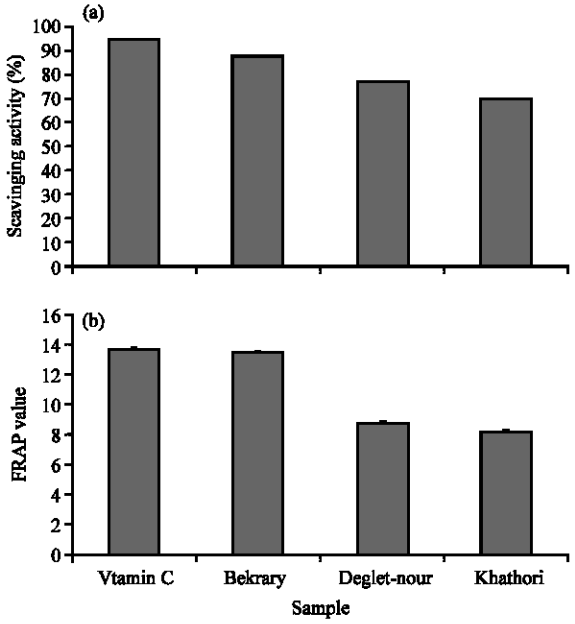

Fig. 2: (a)Antioxidative activity of Bekraray, Deglet nour and Khathori dates varieties indicated with scavenging activity of DPPH radicals. Data were expressed as Mean±SD ($p<0.05$) and (b) reducing power of Bekraray, Deglet nour and Khathori dates varieties. Data were expressed as Mean±SD ($p< 0.05$)

a high phenolic content comparable with some fruits and vegetables, such as tamarind seed (19.21±0.29 mg/100 g), tamarind pulp (2.14±0.05 mg/100 g) (Khairunnuur *et al.*, 2009), apples (48±1 mg/100 g) and tomato (30±1 mg/100 g) (Proteggente *et al.*, 2002).

Free Radical Scavenging Activity and Reducing Power

Figure 2a and b show free radical scavenging activity of dates extracts detected by 1, l-diphenyl-2-picrylhydrazyl (DPPH). In this study, vitamin C presents the greatest DPPH radical scavenging activity at 94.69%. Date flesh from Bekraray variety demonstrated a strong free radical scavenging activity 87.66%. These results were higher than those found by Chaira *et al.* (2007). Meanwhile, the reducing power of vitamin C was 13.73 mmol L^{-1}. The

reducing power of Bekraray variety was 13.46 mmol L^{-1} and was significantly higher than other samples varieties ($p<0.05$). There was no significant difference between Bekraray date extract with vitamin C. This data demonstrates a strong association between the free radical scavenging ability and total phenolic content of the date samples. The correlation coefficient between TPC and FRAP values ($R^2 = 0.99$) also demonstrate a strong positive relationship ($p<0.05$) between antioxidant activity and TPC.

CONCLUSION

Dates fruit contains certain nutrients and minerals as shown in the proximate analysis. The antioxidant activity of dates extract might be attributed to its high phenolic content that possesses effective hydrogen-donating ability and scavenge free radicals. The results suggest that dates could be used as an easily accessible source of natural antioxidants and a good source of dietary fiber.

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