

Comparative analysis of total phenolics, flavonoid content and antioxidant profile of different date varieties (*Phoenix dactylifera* L.) from Sultanate of Oman

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Abstract: The present study investigates the comparative antioxidant ability of the date fruits (DF's) (*Phoenix dactylifera*) of three major date palm varieties such as Fardh, Khasab and Khalas grown in the Sultanate of Oman at two edible maturation stages, namely Rutab (fresh) and Tamr (dried). Antioxidant ability was assessed by using 2, 2-azinobis (3-ethyl benzo thiazoline-6-sulphonic acid) radical cation (ABTS.+), 2,2-diphenyl-1-picrylhydrazyl (DPPH), Superoxide-radical scavenging (SRSA), ferric reducing/antioxidant power (FRAP assay), Total reducing power ability (TRPA) and Metal chelation assays. The total phenolic content (TPC) and total flavonoid content (TFC) of the DF's were also quantified. The total phenolic content (TPC) of all three varieties at Rutab and Tamr stages ranged from 81-178 and 194-234 mg gallic acid equivalents (GAE)/100 gm of DM and total flavanoid content (TFC) ranged from 19-66 and 25-34 mg catechin equivalents (CEQ)/100 gm of DM respectively. The % inhibition in ABTS assay of all three varieties of DF's at Rutab and Tamr stages ranges from 80- 92% and 84-92% respectively and the % inhibition of DF's at Rutab and Tamr stages ranges from 63-65% and 70-73 % respectively observed from DPPH assay. Whereas in SRSA, % inhibition of DF's at Rutab and Tamr stages ranges from 34-43% and 29-44% respectively. The % chelating effect of all three varieties of DF's at Rutab and Tamr stages was 93% and 80-87% respectively. The observed antioxidant ability of DF's may be due to abundant presence of phenolic contents and high electron donating ability to neutralize free radicals. Overall the fardh variety showed significant antioxidant ability and phenolic contents than other varieties at different stages.

Keywords: Antioxidant activity, date palm fruits, flavonoids, DPPH, ABTS, reducing power, superoxide anion

Introduction

In recent years, research efforts are under-way on the possibilities of utilization of natural source of bioactive compounds for the dietary management of certain chronic diseases such as diabetes, obesity, cardiovascular diseases, cancer etc. (Vadivel and Biesalski, 2011). There is increasing body of evidence that many of the today's diseases are caused by the oxidative stress which is the result of imbalance between formation and neutralization of reactive free radicals. These free radicals are continuously produced and neutralized in our body so as to maintain the constant internal environment i.e. redox state. These reactive free radicals are generated due to either endogenous sources for example by-products of normal metabolic processes for ATP production (in reducing molecular oxygen) or exogenous

sources like air pollution, cigarette smoking, UV radiation, high polyunsaturated fatty acid diet, trace metals in diet, absence of exercise (Williams and Jeffrey, 2000). Reactive free radicals include reactive oxygen species (ROS) like superoxide anion radical (O₂⁻), hydroxyl free radical (.OH), peroxy radical (ROO.) or reactive nitrogen species (RNS). The ROS or RNS cause oxidative damage of biological macromolecules such as lipid, protein and nucleic acid which plays pivotal role in the pathogenesis of various degenerative diseases like diabetes, Alzheimer's, Parkinson's, cardiovascular diseases including programmed cell death i.e. aging (Halliwell, 1999). All human cells can protect themselves through the antioxidant defense systems which include enzymatic antioxidants such as superoxide dismutase (SOD), catalase and glutathione peroxidase as well as non-enzymatic including glutathione, vitamins C

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and E plays an important role in scavenging oxidants thereby preventing cell injuries (Halliwell, 1994; Ames *et al.*, 1999). When this protective defense mechanism gets disrupted by different pathological conditions, there is a need of dietary antioxidants in order to control the oxidative stress. Recently, there has been increasing interest in the direction of use and development of ethno medicines having strong antioxidative effect but probably with low toxicities. Therefore, dietary intake of plant based antioxidants has been increased in recent decades due to its abundant source and least side effects. As plant based antioxidants from fruits, vegetables, spices played an important role in maintenance of human health by neutralizing the effect of the free radicals which are causative for many diseases (Shahidi, 1997; Silva *et al.*, 2007) without having any side effects (Nakayama *et al.*, 1993; Halliwell, 1997). Fruits and vegetables offered protection against oxidative stress due to the presence of bioactive components such as polyphenolics including flavonoids, vitamins C, E and carotenoid content (Shahidi and Naczki, 1995; Shahidi and Naczki, 2004; Zhang *et al.*, 2011). In particular flavonoids have been found to possess anti-inflammatory, antioxidant, antiallergic, neuro protective, antithrombotic and anti-carcinogenic effects (Sala *et al.*, 2003).

Date fruits (DF) (*Phoenix dactylifera L.*) are one of the important agricultural commodities in North-African, the Middle Eastern, and Asian countries. DF's are well known as a staple nutritious food and source of wealth for many years (Khan *et al.*, 2008). It is composed of a fleshy pericarp and seed (Besbes *et al.*, 2004; Erskine *et al.*, 2004). DF's are cultivated on about 2.9 million acres of land in 35 countries worldwide. Edible dates pass through four distinct stages of ripening termed in arabic – Kimri, Khalal, Rutab and Tamr which represent, respectively, the immature green, the mature full colored, the soft brown and the hard raisin-like stages of development (Ahmed and Ahmed, 1995). The DF's are consumed either as fresh fruits at Khalal and Rutab stages (short shelf life), or at Tamr stage (good storability), or in the form of processed products such as date paste, syrup or powder which are used as ingredients in cookies or cake manufacturing. DF's are largely consumed at Tamr stage due to their good storability and availability all over the season.

DF's are considered as major source of carbohydrates which mainly include simple sugars like glucose and fructose (Ahmed and Ahmed, 1995; Al-Hooti *et al.*, 1997; Myhara *et al.*, 1999) and sucrose (Guizani *et al.*, 2010). DF's are good source of dietary fiber and some important minerals example e.g., iron,

potassium, selenium, calcium and vitamins example vitamin C, B1, B2, A, riboflavin and niacin but it is a low in fat and protein content (Sawaya *et al.*, 1983; Myhara *et al.*, 1999). Apart from nutritional value, date fruits are found to possess antioxidant activities due to the presence of some bioactive compounds like phenolics including flavanoids, vitamins C, E etc. It has been reported that property of date fruits from different cultivars grown in different countries such as Algeria (Mansouri *et al.*, 2005), Kuwait (Vayalil, 2002), Oman (Al-Farsi *et al.*, 2005b), USA (Vinson *et al.*, 2005), Bahrain (Allaith, 2008) and the Iran (Biglari *et al.*, 2008).

Though DF's has been reported as potent antioxidant but their antioxidant ability can be varied at different cultivars and at different stages of development and it might be due to their altered phenolic content. Therefore, present study was carried out to evaluate and compare the antioxidant activity of the DF's of three major date palm varieties grown in the Sultanate of Oman at two edible maturation stages, namely Rutab (fresh) and Tamr (dried). The results obtained from mono antioxidant assay system might not be enabling us to reach the concrete conclusion. Moreover each type of free radical produced under physiological condition can manifest their detrimental effect in different way. So as to conclude DF's are potent antioxidant, it is mandatory to reveal their ability in different in vitro antioxidant activity procedure where reactions mimic as in vivo system. To the best of our knowledge there is no such data are available for DF's especially cultivated in Sultanate of Oman. Results of this study reveals that DF's has potent antioxidant ability and it is proven by testing with different antioxidant assay system and it can be recommended to include in balanced normal diet.

Materials and Methods

Plant material

DF's were purchased from local market at the time of harvesting in the year 2010 and were stored at -40°C until used for the experiments.

Extraction

Extraction of polyphenols was done as described previously (Biglari *et al.*, 2008). Briefly, the edible part of DF's (100 g) was pitted, crushed and cut to small pieces with a sharp knife and dried at 45°C until it reaches the constant dry weight. Then DF's were blended for 3 min with a blender (Braun, turbo, 500 W, Spain). The DF's was then extracted with 300 ml methanol–water (4:1, v/v), at room temperature (20°C

for 24 h using a magnetic stirrer). The extracts were then filtered and centrifuged at 6000 RCF, for 30 min at 3°C and the supernatant were concentrated under reduced pressure at 40°C for 3 to 4 h using a rotary evaporator to obtain the DF's methanol crude extract. The crude extract was kept in dark glass bottles at -40°C until used. The storage conditions (time and temperature) were similar for all cultivars. The % of yield calculated from the following equation.

$$\% \text{ extractive value (yield \%)} = \frac{\text{Weight of dry extract}}{\text{Weight taken for extraction}} \times 100$$

Total phenolic content

Total phenolics of DF's extracts were measured by Singleton and Rossi (1965) method with minor modifications. Briefly, the Folin-Ciocalteu reagent was mixed with serial dilutions of DF's extract and gallic acid standards and incubated for 5 min at room temperature. Then, 1.9 M sodium carbonate was added and reaction mixture was again incubated for 2 h. The absorbance at 765 nm was obtained and compared with that from gallic acid standards. The concentration of phenolics in DF's extracts was expressed as gallic acid equivalents (GAE). All the measurements were taken in triplicate.

Total flavonoids

Flavonoids were determined by the method of Kim et al. (2003). Distilled water (4 ml) was added to 1 ml of DF's extract. Then, 5% sodium nitrite solution (0.3 ml) was added, followed by 10% aluminum chloride solution (0.3 ml). Test tubes were incubated at ambient temperature for 5 min, and then 2 ml of 1M sodium hydroxide were added to the mixture and then the volume of reaction mixture was made up to 10 ml with distilled water. The mixture was thoroughly vortexed and the absorbance of the pink colour developed was determined at 510 nm. A calibration curve was prepared with catechin and the results were expressed as mg catechin equivalents (CEQ)/100 gm of DM. All the measurements were taken in triplicate and the mean values were calculated.

Total antioxidant activity (AA)

Total Antioxidant activity (AA) was measured as described earlier (Re et al., 1999; Cai et al., 2004). The ABTS radical cation (ABTS.+) solution was pre-generated by mixing 7 mM ABTS and 2.45 mM potassium persulphate, and incubating for 16 h in the dark at room temperature. The ABTS.+ solution was then diluted with 100% methanol to obtain an absorbance of 0.700 ± 0.005 at 734 nm. Methanolic stock solution of different date extracts were prepared to obtain concentration of 50 mg/ml.

Then serial dilutions of different date extracts were prepared from stock solution and the volume was adjusted to 1 ml using methanol. ABTS.+ solution (2 ml; absorbance of 0.700 ± 0.005) was added to different concentration of the test sample and mixed vigorously. The reaction mixture was allowed to stand at 23°C for 6 min and the absorbance at 734 nm was recorded immediately. A standard curve was obtained by using Trolox standard solution at various concentrations (ranging from 0 to 15 mM) in 80% methanol. The percentage inhibition by DF's was calculated using equation:

$$\% \text{ ABTS Inhibition} = \frac{[(\text{Abscontrol} - \text{Abssample})]}{\text{Abscontrol} \times 100}$$

DPPH radical scavenging activity

The ability of the extract to scavenge stable DPPH radicals was assessed as described by Gyamfi et al. (1999). Methanolic stock solution of different date extracts were prepared to obtain concentration of 50 mg/ml. Then serial dilutions of different extracts were prepared from stock solution and the volume was adjusted to 1 ml using methanol. Then, different concentrations of DF's extract was mixed with 450 µl PBS (10 mmol/l, pH 7.4) and 1.0 ml methanolic DPPH (0.1 mM) solution and incubated for 30 min at room temperature and absorbance was recorded at 517 nm. Ascorbic acid and BHT were used as a positive control. All the measurements were taken in triplicate and the mean values were calculated. The percentage inhibition by DF's was calculated using equation:

$$\% \text{ DPPH Inhibition} = \frac{[(\text{Abscontrol} - \text{Abssample})]}{\text{Abscontrol} \times 100}$$

Superoxide-radical scavenging assay (SRSA)

The superoxide scavenging ability of the extract was assessed by the method of Nishikimi et al. (1972). Briefly, stock solution of different DF's extract was prepared to a concentration of 100 mg/ml. Then serial dilutions of stock solution (SS) of DF's extract were prepared. Different amount of SS were taken and the total volume made up to 400 µl using phosphate buffer (0.1M pH 7.4). The reaction mixture containing 400 µl of different serial dilutions, 1 ml of PMS (60 µM), 1 ml of NADH (677µM) and 100 µl of NBT (144 µM) in phosphate buffer (0.1M pH 7.4), was incubated at room temperature for 5 min. The color was read using spectrophotometer at 560 nm against a blank. All the measurements were taken in triplicate and the mean values were calculated and SRSA was calculated using the following equation:
 $\% \text{ SRSA} = [1 - \text{Abssample} / \text{Abscontrol}] \times 100$

Ferric reducing/antioxidant power assay (FRAP)

The Antioxidant activity of DF's extract was determined using a method of Ferric reducing/antioxidant power (FRAP) assay of Benzie and Strain (1999) with minor modifications. Briefly, the FRAP reagent was prepared by mixing 0.1M acetate buffer at pH 3.6 (100 ml), 10 mM tripydyltriazine (TPTZ) solution in 40 mM HCl (10 ml) and 20 mM Ferric chloride solution (FeCl₃) (10 ml). The FRAP reagent was prepared fresh daily and was warmed to 37°C in a water bath prior to use. Stock solutions of DF's extracts were prepared (50 mg/ml). Freshly prepared FRAP reagent (2 ml) were mixed with serial dilutions of different DF's extracts, prepared by using stock solution to the final volume 1 ml using methanol. Then, reaction mixtures were incubated at 37°C for 4 min. Absorbance was recorded at 593 nm with a reference to a reagent blank (containing FRAP reagent and diluents except sample/standard). The standard curve was constructed using Ascorbic acid (µM). All the measurements were taken in triplicate.

Metal chelating assay

The metal chelating ability of the DF's extract was determined according to the method of Decker and Welch (1990) with minor modification. Briefly, stock solutions (100 mg/ml) of different DF's extract were prepared. Then serial dilutions from the stock were taken and total volume was made up to 3 ml with methanol. 120 µl of 2 mM FeCl₂ was then added and the solution was activated by the addition of 240 µl of 5mM Ferrozine solution. After vortex, reaction mixture was incubated for 15 min at room temperature under shaking conditions. Then, its chelating activity was spectrophotometrically measured at 562 nm. All the measurements were taken in triplicate and the mean values were calculated. The ability of the extract to chelate ferrous ion was calculated using the following equation:

$$\% \text{ chelating effect} = [1 - \text{Abs}_{\text{sample}} / \text{Abs}_{\text{control}}] \times 100$$

Statistical analysis

Data are presented as means ± standard deviation. Two way analysis of variance (ANOVA) was performed using SPSS software to analyze the statistical significance. The values $p < 0.001$, $p < 0.05$ considered as significant.

Results and Discussion

Total phenolic content

Results of the total phenolic content showed that maximum phenolic content was found in Fardh-Tamr

(235 mg GAE/100 gm of DM) which was followed by Khalas-Tamr (231 mg GAE/100 gm of DM) and Khasab-Tamr (194 mg GAE/100 gm of DM). Whereas, in case of Rutab stage, higher phenolics was found in Fardh-Rutab (178 mg GAE/100 gm of DM) which was followed by Khasab-Rutab (116 mg GAE/100 gm of DM) and Khalas-Rutab (81 mg GAE/100 gm of DM) (Table 1). From the results it is evident that DF's are rich in phenolics which were shown in both Rutab and Tamr stages. But the total phenolics at the Rutab stage of development were lower as compared to the Tamr stage. The total phenolics content reported by Al-Farsi *et al.* (2005) ranges between 167 mg to 343 mg GAE/100 gm fresh weight of Omani dates which resembles our observation. Total phenolic content of three date fruit varieties observed the same pattern which was observed by Al-Farsi *et al.* (2005a). Therefore, the order of TPC of DF's was Fardh-T > Khalas-T > Khasab-T > Fardh-R > Khasab-R > Khalas-R. Generally, drying method is regarded as unfavorable due to the possibility of decomposition of phenolic compounds either enzymatically by polyphenol oxidase and glycosidase or by thermal degradation (Shahidi and Naczek, 2004). Contrastingly in this study, phenolic content of DF's varieties has been increased after drying which may be due to the degradation of tannins by heat and maturation enzymes during the drying process, which could considerably interfere with phenolic contents (Maillard and Berset, 1995). It is concomitant with Myhara *et al.* (1999) where Tannins, phenolic compounds were high in the initial stages of development than the matured Tamr stage DF's.

Total flavonoids

Results of total flavonoids content (TFC) showed that TFC of DF's varied considerably from 19-66 mg in terms of catechin equivalents/100 gm of DW of sample. In general, higher flavonoids values were associated with Rutab stage which indicates that the drying process may have a destructive effect on these compounds. For the tamr stage maximum flavonoids were found in Fardh-Tamr (34 mg CEQ/100 gm of DW) which was followed by Khasab-Tamr (27 CEQ/100 gm of DW) and Khalas-Tamr (25 mg CEQ/gm of DW). In case of Rutab stage, similar trend has been observed i.e. maximum TFC was found in Fardh-Rutab (66 mg CEQ/100 gm of DW) followed by Khasab-Tamr (46 mg CEQ/100 gm of DW) and Khalas-Rutab (19 mg catechin equivalents/100 gm of DW) (Table 1). Therefore, the order of TFC of DF's was Fardh-R > Khasab-R > Khalas-R > Fardh-T > Khasab-T > Khalas-T. Interestingly, a significant

Table 1. Total phenolic and flavonoid contents of fardh, khasab and khalas dates fruits extracts at Rutab (fresh) and Tamr (dried) stages

Particulars	Fardh -Rutab	Khasab-Rutab	Khalas- Rutab	Fardh-Tamr	Khasab-Tamr	Khalas- Tamr
TPC (mg of GAE/100 gm of DW)	17±0.19*	116±0.72	81±0.55	235±0.28*	194±0.43	231±0.38
TFC (mg of CEQ/100 gm of DW)	66±0.2*	46±0.4	19±0.3	34±0.6*	27±0.7	25±0.1

Values are presented as mean ± SD (n=3)
TPC- Total phenolic content; TFC – Total flavonoid content; GAE – Gallic acid equivalents; CEQ- Catechin equivalents.
* indicates the level of significance at p<0.05 compare to other varieties.

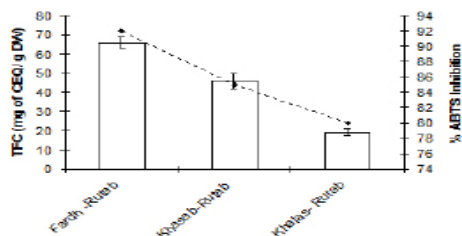


Figure 1. Relationship between ABTS radical scavenging and total flavonoid content of Fardh, Khasab and Khalas dates fruits extracts at Rutab (fresh) and Tamr (dried) stages

TFC – Total flavonoid content
CEQ- Catechin equivalents

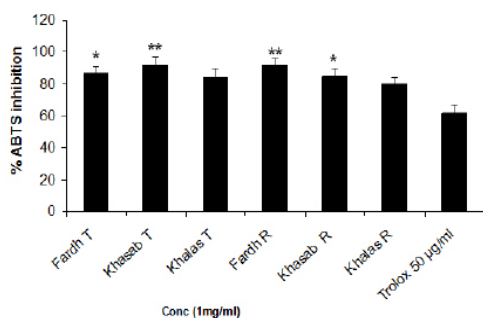


Figure 2. Trolox antioxidant capacity of Fardh, Khasab and Khalas dates fruits extracts at Rutab (fresh) and Tamr (dried) stages. The values are presented as mean ± SD. * indicates the significance level at p < 0.05, ** indicates the significance level at p < 0.001 compare with other varieties

correlation (r=0.99) has been observed between TFC and trolox antioxidant ability of the extracts at rutub stages but not with tamr stages (Figure 1). This could be due to stage specific accumulation of flavonoids but the mechanism is unknown.

Total antioxidant activity

In the present study, the total antioxidant activity of the DF's extract was evaluated by ABTS radical decolorization assay which was measured spectrophotometrically at 734 nm. The results were expressed as a percentage of inhibition based on absorbance. Maximum total antioxidant activity was shown by Khasab-Tamr (92%) followed by Fardh-Tamr (87%) and Khalas-Tamr (84%). Whereas, higher antioxidant capacity in case of Rutab stages of development was shown by Fardh-Rutab (92%)

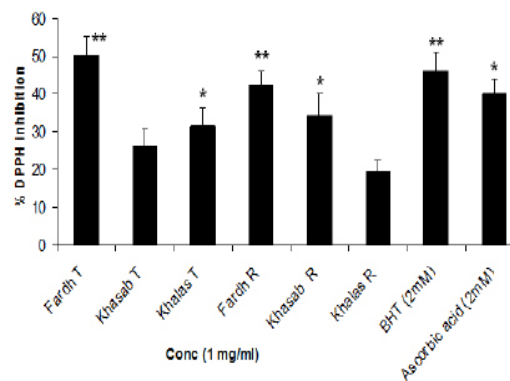


Figure 3. DPPH radical scavenging activity of Fardh, Khasab and Khalas dates fruits extracts at Rutab (fresh) and Tamr (dried) stages. The values are presented as mean ± SD. * indicates the significance level at p < 0.05, ** indicates the significance level at p < 0.001 compare with other varieties

followed by Khasab-Rutab (85%) and Khalas-Rutab (56%) (Figure 2). Trolox was used as the standard for the antioxidant activity measurement. Interaction with the extract or standard trolox suppressed the absorbance of the ABTS.+ radical cation. The results reveal that extracts considerably neutralize the radical ion. Similarly at edible stages Saudi Arabian (Al-Humaid *et al.*, 2010) and Iranian (Mohammad Reza *et al.*, 2010) dates are reported for their extensive antioxidant ability. The most effective antioxidants in this respect are reported to be the presence of phenolics and flavanoids which eventually facilitate the scavenging of free radicals by donating electron.

DPPH assay

DPPH is a stable free radical method is a rapid and sensitive way to survey the antioxidant activity. Neutralizing effect of different concentrations DF's extracts on DPPH free radical has been investigated. The degree of its discoloration is attributed to hydrogen donating ability of test compounds. The results were expressed as a percentage inhibition based on absorbance. Higher DPPH free radical scavenging activity was shown by Fardh-Tamr (72.7%) which is followed by Khalas-Tamr (72.1%) and Khasab-Tamr (69.5%). While, in Rutab stages, highest inhibition was shown by Fardh-Rutab (65%) which was followed by Khalas-Rutab (63.4%) and then Khasab-Rutab (62.5%) (Figure 3). The scavenging property of DF's extract can be attributed due to presence of hydroxyl groups which can donate the

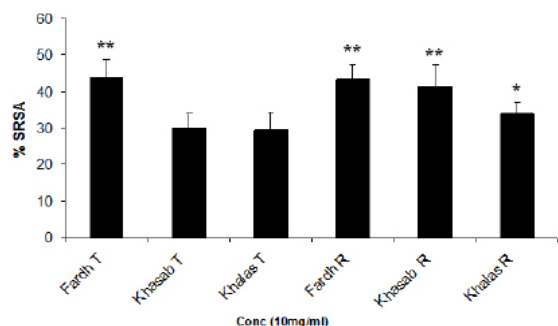


Figure 4. Superoxide anion scavenging activity of Fardh, Khasab and Khalas dates fruits extracts at Rutab (fresh) and Tamr (dried) stages

The values are presented as mean \pm SD. * indicates the significance level at $p < 0.05$, ** indicates the significance level at $p < 0.001$ compare with other varieties

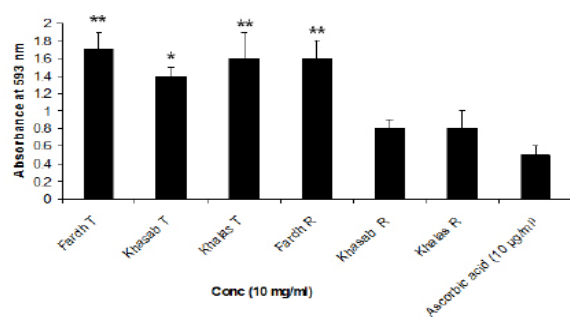


Figure 5. Reducing power ability of Fardh, Khasab and Khalas dates fruits extracts at Rutab (fresh) and Tamr (dried) stages

The values are presented as mean \pm SD. * indicates the significance level at $p < 0.05$, ** indicates the significance level at $p < 0.001$ compare with other varieties

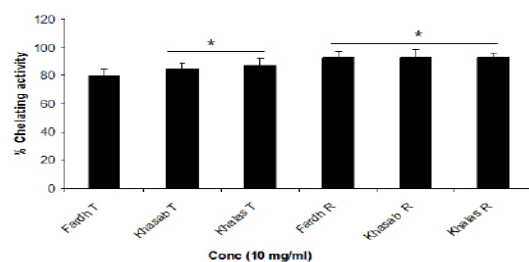


Figure 6. Metal chelating activity of Fardh, Khasab and Khalas dates fruits extracts at Rutab (fresh) and Tamr (dried) stages

The values are presented as mean \pm SD. * indicates the significance level at $p < 0.05$, compare with other varieties

electron and neutralize the existing free radical in the reaction mixture. Free radicals are often generated as byproducts of biological reactions or from exogenous factors. The involvements of free radicals in the pathogenesis of a large number of diseases are well documented (Williams and Jeffrey, 2000). A potent scavenger of free radicals may serve as a possible preventative intervention for the diseases.

Superoxide-radical scavenging assay

Superoxide anion plays an important role in plant tissues and is involved in the formation of

other cell-damaging free radicals (Duan *et al.*, 2007). Approximately 1-3% of the oxygen is converted to superoxide radical anion $O_2^{\cdot-}$. Superoxide dismutase (SOD) can dismutate O_2 *in vivo*. SOD can be destroyed in the stomach after the food intake and therefore couldn't be absorbed in the intestine. Superoxide anions are generated by the *in vitro* PMS/NADH system and monitored by the reduction of NBT. Figure 4 shows the effects of scavenging superoxide anions by the DF's extracts at Tamr and Rutab stages respectively. Maximum superoxide inhibition was shown by Fardh-Tamr (44%) which was followed by Khasab-Tamr (30%) and Khalas-Tamr (29.4%). Whereas, maximum inhibition was observed in Fardh-Rutab (43.3%) which was followed by Khasab-Rutab (41.4%) and Khalas-Rutab (34%) (Figure 4). Fardh-Tamr exhibited considerable superoxide anion scavenging activity as compared to Khasab-Tamr and Khalas-Tamr. It has been reported that, the increasing order in the superoxide anion scavenging activity was due to presence of hydroxyl group of the phenolics which may contributes to its superoxide anion scavenging activity by their electron donation (Bravo, 1998). Similarly, in this study DF's extract might scavenge the superoxide anion in a similar way since they are rich in phenolics containing more hydroxyl groups.

FRAP Assay

FRAP assay is based on the measurement of the ability of the substance to reduce Fe^{3+} to Fe^{2+} ion and it reveals the electron donating potential of tested compounds. Therefore we used this *in vitro* assay system to assess the ability of DF's extracts. Fe^{2+} ion is measured spectrophotometrically through the determination of its coloured complex with 2, 4, 6-Tris (2- pyridyl)-s-triazine (TPTZ) at 593 nm. Figure 5 shows reducing ability of DF's extract at different concentrations. From the results it can be ranked the reducing ability DF's extract at different stages. It is in the order of Fardh-R=Khasab-R>Fardh-T>Khalas-R=Khalas-T>Khasab-T. Since the antioxidant activity of a substance is usually correlated directly to its reducing capacity, these results directly evidenced the iron reducing capability of DF's extract.

Metal chelating assay

DF's extract at Tamr and Rutab stage indicates that both extracts harboring chelating Ferrozine can make complexes with ferrous ions. In the presence of chelating agents, purple colored complex formation is interrupted and as a result, there is decrease in purple color of the complex. Thus, the chelating effect of the coexisting chelator can be determined by measuring

the rate of color reduction. The formation of the ferrozine-Fe²⁺ complex is interrupted in the presence of different concentrations property. Maximum metal chelating effect was shown by Khalas-Tamr (87%) which was followed by Fardh, Khasab and Khalas varieties (93%) (Figure 6). From the results it can be inferred that metal chelating activity of Rutab stages fruits was higher than Tamr stage. This could be due to more accumulation of flavonoids at Rutab stages of DF's.

Conclusion

The antioxidant activities (AA) of Omani dates were determined by using different in vitro antioxidant assay system. Further total phenolic content and flavanoid contents were also analysed. Over all Fardh variety (both Tamr and Rutab stage) showed maximum antioxidant capability in all tested assays and concurrently these varieties showed higher phenolic contents. From the results it can be concluded that inclusion of fardh dates in normal balanced diet could alleviate number of oxidative stress induced illness. But before reaching definite conclusion the molecular aspect of fardh dates ingredients should be studied in detail.

Acknowledgements

The authors would like to acknowledge the financial assistance provided by the Sultan Qaboos University, Oman. Technical support was given by Ms. Resmi Nithin is gratefully acknowledged. "Conflict of Interest-None".

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