Malays. Appl. Biol. (2019) 48(2): 103–108

TOTAL PHENOLIC CONTENT AND ANTIOXIDANT ACTIVITIES OF DATE FRUIT EXTRACTS

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Accepted 7 May 2019, Published online 31 May 2019

ABSTRACT

Dates (*Phoenix dactylifera* L.) are nutrient-rich fruit consumed worldwide in a raw form or processed value-added products such as paste, syrup and energy bars. Dates are considered as functional food or ingredient due to its nutrients which derived from carbohydrate, lipids, protein, vitamin, mineral and phytochemical compounds, contributing to their potential antioxidant activities. However, the antioxidant properties of dates found in the local market are not widely available in the scientific literature, thus merits investigation. The objective of this study is to determine the total phenol content (TPC) and antioxidant activities (DPPH scavenging activity, oxygen radical absorbance capacity (ORAC) and ferric reducing antioxidant power (FRAP)) of five dates varieties namely Khalas, Deglet Noor, Lulu, Khenaizi and Medjool. The results showed TPC, ORAC and FRAP values for Lulu and Khenaizi were significantly ($p<0.05$) higher than Khalas, Deglet Noor and Medjool. Meanwhile, Deglet Noor, Lulu and Khenaizi significantly ($p<0.05$) possessed greater DPPH scavenging activities as compared to Khalas and Medjool. There was a significant ($p<0.05$) correlation (r) between the TPC and ORAC ($r=0.952$) and FRAP ($r=0.987$) suggesting that phenolic compounds may contribute to the overall antioxidant activity of the dates.

Key words: Dates, total phenolic content, antioxidant activity

INTRODUCTION

Date fruit, *Phoenix dactylifera* L., is one of popular food among Muslims. Date fruits grow in the desert as it has a high tolerance towards salty and alkaline soil (Chao & Krueger, 2007). Date fruits go through four growth stages which are kimri, khalal, rutab and tamar. Date fruits in tamar stage are more acceptable commercially and it can be processed into date juice concentrated, fermented date products and date pastes (Chandrasekaran & Bahkali, 2013; Siddiq & Greiby, 2013). At this stage, the fruits have very low moisture content and therefore are ideal for long-term storage to be consumed offseason (Maria *et al*., 2014).

Dates fruits are high in nutrients and provide a good source of rapid energy to the human body. Date fruits contain 70–80% of carbohydrate which is easily absorbed by the human body (Al-Farsi, 2005a). Chao and Krueger (2007) stated that sucrose is hydrolysed to reducing sugar (such as glucose and fructose) during the growth of date fruits. Its fiber content is two times higher than other fruits such as apple and orange (Vayalil, 2012). Dates fruits are high in fibre, minerals and vitamins (Al-Farsi & Lee, 2008; Vayalil, 2012). Al-Farsi and Lee (2008) stated that 100 g of date fruits contain a moderate concentration of manganese, iron, phosphorus and calcium supplying more than 7% of the daily recommended dietary allowance.

Date fruit possesses antioxidant capacity capable in delaying or preventing oxidation from occurring in the food or human body. The antioxidant properties of date fruits vary depending on their content of phenolic components, vitamin C and flavonoids (Al-Turki *et al.*, 2010). The phenolic compound in fresh dates and dried dates fruits are 193.7 mg/100 g and 239.5 mg/100 g accordingly (Al-Farsi and Lee, 2008). According to the study done by Ismail *et al.* (2015), total flavonoid content

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in date fruits was in the range of 1.22 and 2.82 mg/100 g DW whereas vitamin C content was in the range of 0.051 and 0.541 μmol·g^{-1} FW.

Although it is known that the consumption of dates provide good nutrition which benefits human health, but the research on antioxidant properties of dates available and consumed in Malaysia are limited. Therefore, this study was carried out to determine the total phenolic content and *in vitro* antioxidant activities of five dates varieties (Khenaizi, Khalas, Medjool, Deglet Noor and Lulu) commonly consumed in Malaysia.

MATERIALS AND METHODS

The date fruits used in this study were Khenaizi, Khalas, Medjool, Deglet Noor and Lulu varieties. Khenaizi and Medjool were obtained from Palestine suppliers from while Khalas, Deglet and Lulu were supplied by Man'z Delight, Bangi, Selangor. 2,2-diphenyl-1-picrylhydrazyl (DPPH) ($C_{18}H_{12}N_5O_6$), sodium acetate trihydrate ($C_2H_3O_2Na.3H_2O$), 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ) ($C_{18}H_{12}N_6$), ˆ-ascorbic acid (sodium salt) ($C_6H_7O_6Na$), Folin-Ciocalteu reagent, sodium carbonate (Na_2CO_3), gallic acid ($C_7H_6O_5$), (±)-6-hydroxy-2,5,7,8-tetramethylchromane-2 carboxylic acid (Trolox) ($C_{18}H_{12}O_6$), methanol (CH_3OH), hydrochloric acid (HCl) and azobis 2-amidinopropane dihydrochloride (AAPH) were of analytical grade and supplied by Merck, Germany.

Extraction

The extraction method was done according to Biglari *et al*. (2008) with some modification. Date fruits (100 g) were cut into small pieces by using a knife and ground using a dry grinder. The small pieces of date fruits were extracted with 300 mL of methanol solution and water (4:1, v/v) at 20°C for 5 hours. Supernatants were collected, filtered and concentrated by rotary evaporator at 40°C for 3 hours. The methanolic dates extract was kept in an amber bottle and frozen until further analysis.

Determination of total phenolic content (TPC)

Total phenolic content (TPC) of dates extracts were determined using the Folin-Ciocalteu method described by Singleton *et al.* (1999) with slight modification. The date fruit extract (0.5 g) was dissolved in distilled water (10 mL). Extract solution (1 mL) was mixed with 5 mL of 0.2 N Folin-Ciocalteu reagent and 4 mL of 7.5% (w/v) sodium carbonate solution. Then, the mixture was incubated at 25°C for 2 hours in the dark. 200 μL of the sample was placed in 96-well plate and measured with UV-VIS Epoch (Biotech) at a wavelength of 765 nm. The total phenolic content was calculated with the following formula:

$$\text{T (mg GAE/100g extract)} = \frac{C \times V}{M}$$

Where ,
C = concentration of gallic acid from graph
V = volume of extract (mL)
M = weight of extract (g)

Determination of the radical-scavenging activity of DPPH using the conventional method

The DPPH activity of date fruit extracts was determined using the method described by Musa *et al.* (2013) with slight modification. The extract (0.5 g) was dissolved in 10 mL methanol solution. Sample extract (100 μL) was mixed with 200 μL DPPH methanolic solution. DPPH stock solution (control) and sample extract were placed in 96-well plate. The absorbance was measured after 30 minutes at a wavelength of 517 nm using UV spectrophotometer (UV 2450, Shimadzu, Japan). Percentage of DPPH activity was calculated as follows:

$$\text{DPPH Activity (\%)} = \frac{A_1 - A_2}{A_1} \times 100$$

where,
A_1 = reading of DPPH stock solution
A_2 = reading of sample extract

Determination of DPPH activity using dry reagent array (DPPH kit)

The DPPH radical scavenging activity of date extracts was determined using DPPH kit (Musa *et al*., 2003). Date extract (100 μL) was diluted with methanol (100 μL) and fill into the kit. The kit was closed and mixed at 25°C. The absorbance was measured after 30 minutes at a wavelength of 517 nm using UV spectrophotometer (UV 2450, Shimadzu, Japan).

Determination for oxygen radical absorbance capacity (ORAC)

Oxygen radical absorbance capacity (ORAC) was determined using Cao *et al.* (1993) method with slight modification. Dates extract (0.2 g/ml) was dissolved with distilled water. Trolox standard solution of (0.4–10 mM concentration) was prepared. Fluorescence solution (10 mM) was prepared by dissolving it in phosphate buffer at a ratio of 1:999. Fluorescence solution (150 μL) was filled into each well of the plate. Then, Trolox standard solution (25 μL), fluorescence solution

(25 µL) and sample (25 µL) were added onto wells containing 150 µL fluorescence solution. The plate was incubated in an incubator (THERMOstar) for 30 minutes. Fluorescence was recorded at a wavelength of 485 nm to 520 nm every minute using fluorescence reader (FLUOstar Omega, BMG LABTECH, Germany). After the third reading, 25 µL of 20 mM AAPH solution was added automatically and the concentration of fluorescence was recorded again. The effect of protection against antioxidant was measured by calculating the area under the curve of fluorescence decomposition (AUC) of the samples and was compared to control. The result was expressed in µM Trolox per g of the extract with the following formula:

$$\text{T (µM TE/g sample extract)} = \frac{C \times V}{M}$$

where,
C = concentration of Trolox from graph
V = volume of extract (mL)
M = weight of extract (g)

Determination of ferric reducing antioxidant power (FRAP)

Ferric reducing antioxidant power (FRAP) of the date extracts were determined using Benzie and Strain (1996) method with slight modification. Dates extracts (0.5 g) were dissolved in distilled water (10–40 mL). Date fruits extract (200 µl) at 0.1 g/mL reacted with 1.5 mL of FRAP solution incubated for 30 minutes in the dark. The extract (200 µL) was placed in a 96-well plate and analysed at 593 nm using UV spectrophotometer (UV 2450, Shimadzu, Japan). A standard curve of 0.2, 0.4, 0.6, 0.8 and 1.0 mM Trolox concentration was obtained. The reducing power of ferric was determined using the following formula:

$$\text{Total reducing power of ferric (mM TE/100 g sample extract)} = \frac{C \times V}{M}$$

where,
C = concentration of Trolox from graph
V = volume of extract (mL)
M = weight of extract (g)

Statistical analysis

Statistical analysis was carried out using Minitab version 2017 software. All analyses were performed in triplicate. Analysis of variance (ANOVA) was used to analyse data from total phenolic content and *in vitro* antioxidant (DPPH, ORAC and FRAP) assays. Fisher test was used to determine the significant differences between samples and correlation value was obtained from Pearson correlation. All data are presented as mean values ± standard deviation.

RESULTS AND DISCUSSION

Total phenolic of date extracts

Total phenolic contents of date extracts were evaluated using the diluted Folin-Ciocalteu reagent. The presence of phenolics compounds changed the yellow colour of the reagent to blue colour. The colour changes are proportional to the concentration of antioxidant compound. Table 1 showed Khenaizi and Lulu extracts had the highest phenolic content ($p<0.05$) in comparison to Khalas, Medjool and Deglet Noor extracts. Vinson *et al.* (2005) explained that the phenolic content increased in dried date fruits due to the decomposition of tannin by heat and enzymes releasing phenolic compounds during the drying process. However, the TPC of Khalas, Medjool and Deglet Noor extracts were different from the study reported by Al-Farsi *et al.* (2005a). The TPC value reported for Khalas and Medjool was 572 mg GAE/100 g while Deglet Noor was between 150 mg GAE/100 g to 670 mg GAE/100 g (Al-Farsi *et al.*, 2005a). This is possibly due to factors such as harvesting procedure and post-harvest treatment, growing environment, type of fertilizer used and the loss of active component during extraction (Al-Farsi & Lee, 2008).

***In vitro* antioxidant activities of date extracts**

The DPPH radical scavenging assay measures the ability of the antioxidant compound to reduce the purple chromogen radical (DPPH•) into a non-radical pale yellow hydrazine form (DPPH-H) (Brand-Williams *et al.*, 1995). Lulu, Deglet Noor and Khenaizi extracts had higher ($p<0.05$) DPPH scavenging activities than Medjool and Khalas extracts (Table 1). The high DPPH activity reflects the greater ability of the date extracts to function as an antioxidant by scavenging the free radicals. Rock *et al.* (2009) showed that Medjool has 44% scavenging activity, however, the reported value was lower than the present study. This possibly due to scavenging properties of the foods, electron or hydrogen donating ability, the solubility of the sample in the solvent and pH of the reaction (Sharma & Bhat, 2009).

The principle underlying the determination of DPPH radical scavenging activity using the DPPH test kit is similar to the conventional method. The trend obtained from DPPH kit was in accordance with those obtained conventionally except for Medjool. This is supported by the statistical analysis indicating that there were no significant

Table 1. Total phenolic content and antioxidant activities of Khalas, Deglet Noor, Lulu, Khenaizi and Medjool dates extracts

Extract	TPC (mg GAE/100 g sample)	[1]DPPH (%)	[2]DPPH (%) kit	ORAC (μM TE/g sample)	FRAP (mM TE/100 g sample)
Khalas	866.95 ± 50.10[b]	57.25 ± 2.54[c]	55.88 ± 3.20[b]	21,244.45 ± 4012.17[b]	7.88 ± 1.59[b]
Deglet Noor	26.47 ± 15.59[b]	73.41 ± 4.94[a]	71.63 ± 8.10[ab]	29,815.69 ± 3945.15[b]	9.10 ± 0.86[b]
Lulu	26,935.35 ± 691.46[a]	75.98 ± 0.89[a]	70.49 ± 4.46[ab]	43,157.12 ± 5879.31[a]	16.05 ± 1.17[a]
Khenaizi	27,106.76 ± 2336.57[a]	71.74 ± 2.41[a]	70.33 ± 16.27[ab]	46,389.27 ± 7087.57[a]	12.02 ± 1.75[a]
Medjool	53.15 ± 18.82[b]	63.93 ± 4.07[b]	73.28 ± 5.69[a]	23,811.47 ± 4825.13[b]	7.36 ± 1.08[b]

[1]DPPH activity determined by the conventional method.
[2]DPPH activity determined using DPPH test kit.
[ab]Within each assay (TPC, DPPH, ORAC and FRAP), mean values (± standard deviation) bearing different superscripts are significantly different, $p<0.05$.

Table 2. Pearson correlation (r) for total phenolic content (TPC) and antioxidant activities (DPPH, ORAC and FRAP) of date extracts

Pearson correlation (r)	TPC	[1]DPPH	[2]DPPH	ORAC	FRAP
TPC	1.000	0.623	0.247	0.952*	0.987*
[1]DPPH		1.000	0.718	0.794	0.676
[2]DPPH			1.000	0.434	0.270
ORAC				1.000	0.937*
FRAP					1.000

[1]DPPH activity determined by the conventional method.
[2]DPPH activity determined using DPPH test kit.
Symbol (*) indicated the significant correlation ($p<0.05$) between the *in vitro* antioxidant assays used to evaluate the activities of date extracts.

differences ($p>0.05$) between DPPH activity obtained using conventional and rapid test methods (Table 2).

Khenaizi and Lulu extracts had a higher ORAC value ($p<0.05$) when compared to Deglet Noor, Medjool and Khalas extracts. It is believed that high phenolic content may potentially inhibit lipid oxidation (Ahmad & Abdullah, 2013). The reported ORAC values in Deglet Noor was 12,983 μM TE/g, Medjool was 7957 μM TE/g and Khalas was 4180 μM TE/g (Wu *et al.*, 2004; Al-Farsi *et al.*, 2005b). ORAC values for Deglet Noor, Medjool and Khalas dates were different from the reported previous study possibly due to the difference in the growing environment of date fruits (Al-Farsi & Lee, 2008).

Lulu and Khenaizi extracts had higher ($p<0.05$) FRAP values than Khalas, Deglet Noor and Medjool extracts. Lulu and Khenaizi had high FRAP values presumably due to a high level of phenolic content. For Medjool dates, the FRAP value was different from studies reported by Bouhlali *et al.* (2015) which was 1.56 mM TE/100 g sample. Generally, the variations in antioxidant properties of different date cultivars probably due to the variation in agro-climatic conditions, variety as well as the country of origin (Al-Jasass *et al.*, 2015).

Correlation between *in vitro* assays

Table 2 shows the high correlation ($p<0.05$) between *in vitro* assays i.e. TPC and ORAC, TPC and FRAP as well as ORAC and FRAP. The high phenolic content in date fruit extracts (Table 1) may contribute to the antioxidant activities (Vayalil, 2012). The high correlation ($p<0.05$) between TPC and ORAC methods possibly due to the capacity of the phenolic component to rapidly scavenge peroxyl radicals thus inhibiting lipid oxidation (Ahmad & Abdullah, 2013). Meanwhile, significant correlation ($p<0.05$) between TPC and FRAP methods may be derived from the ability of phenolic compounds to break free radical chains by donating hydrogen atom (Vayalil, 2012). However, the correlation values between methods that did not show perfect correlation ($r=1.00$) indicated that the antioxidant activity of date fruits may also be derived from other components such as vitamin, flavonoid and carotenoids (Mohamed & Al-Okbi, 2004).

CONCLUSION

Generally, all dates varieties exhibited antioxidant activities *in vitro* but Lulu and Khenaizi possessed greater antioxidant power as compared to others (Khalas, Deglet Noor and Medjool). It can be concluded that the phenolic compounds may contribute to the overall antioxidant activity of the date fruits.

ACKNOWLEDGEMENT

This study was funded by NMRC Universiti Islam Malaysia research grant.

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