Journal of Taibah University Medical Sciences (2015) 10(3), 346–352

Taibah University

Journal of Taibah University Medical Sciences

www.sciencedirect.com

Original Article

Quantification of phenolic compounds, evaluation of physicochemical properties and antioxidant activity of four date (*Phoenix dactylifera* L.) varieties of Oman

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Received 27 October 2014; revised 3 December 2014; accepted 16 December 2014; Available online 7 February 2015

الملخص

أهداف البحث: تهدف هذه الدراسة إلى تقييم نشاط مضادات الأكسدة وقياس المركبات الفينولية بواسطة الطرق التحليلية اللونية السائلة عالية الأداء لأصناف النخيل المزروعة في عُمان.

طرق البحث: تم تجميع الفاكهة الناضجة المجففة بالشمس (مرحلة التمر) لأصناف البونارنجة، والقصب، والفارد، والخلاص من مناطق الدخيلية والشارقية، في عُمان في بداية موسم الحصاد ٢٠١٣م. تم فحص نشاطات مضادات الأكسدة لفاكهة التمر بطريقة الكسح الجذري الحر باستخدام ١،١ دايفينايل-٢ بيكريلهيدرازيل. كما استخدمت طريقة فولين-سيوكالتيو اللونية والطريقة اللونية السائلة عالية الأداء لتحديد محتوى إجمالي الفينول وقياس الأحماض الفينولية، على التوالي من المستخلص المائي الكحولي لفاكهة التمر.

النتائج: أظهر التحليل الأولي لفاكهة التمر وجود الأحماض الفينولية مثل حمض الغال، وحمض الفانيليك، وحمض الكافيك، وحمض ب-كوماريك، وحمض السيرينجك بنسب مختلفة. وتراوح إجمالي محتوى الفينول للأصناف الأربعة من ٢٤،٣٢ مجم إلى ٨٤،٣٥مجم معادل لحمض كافيك/١٠٠ جم من الوزن الطازج. وأظهرت جميع الأصناف نشاط الكسح الحر المتطرف بنسبة كبيرة معتمدة على التركيز (٧٨،٢٨-٦٢،٧٠٪).

الاستنتاجات: تؤكد هذه الدراسة أن التمور العمانية هي مصدر غني للمركبات الفينولية وتمتلك خصائص جيدة مضادة للأكسدة. حمض الغال هو حمض الفينول السائد في جميع أصناف النخيل.

الكلمات المفتاحية: مضادات الأكسدة؛ نخيل التمر؛ إجمالي الفينول؛ طريقة اللوني السائل عالية الأداء

Abstract

Objectives: This study aimed to evaluate the antioxidant activity and to quantify the phenolic compounds by colorimetric and High Performance Liquid Chromatography (HPLC) analytical methods of four dates palm varieties cultivated in Oman.

Methods: The sun-dried mature fruits (Tamer stage) of Bunarinja, Khasab, Fard and Khalas date cultivars were collected from Al-Dakhiliya and Al-Sharqiya regions, Oman at the beginning of the 2013 harvest season. The antioxidant activities of date fruits were investigated by 1,1 diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging method. Folin-Ciocalteau colorimetric and HPLC methods were used to determine the total phenolic content (TPC) and to quantify the phenolic acids, respectively in the hydroalcoholic date fruit extracts.

Results: A preliminary HPLC analysis of the date fruits showed the presence of phenolic acids like Gallic acid, Vanillic acid, Caffeic acid, p-Coumaric and Syringic acid in different proportions. The TPC of all four varieties ranged from 32.24 mg to 35.84 mg Caffeic acid equivalents/100 g of fresh weight. All varieties exhibited significant free radical scavenging activity (28.78–70.62%) in a concentration dependent manner.

Conclusion: The present study confirms that Omani dates are a rich source of phenolic compounds and posses good antioxidant properties. HPLC also revealed the Gallic acid as the predominant phenolic acid in all date cultivars.

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Peer review under responsibility of Taibah University.

ELSEVIER Production and hosting by Elsevier

 http://dx.doi.org/10.1016/j.jtumed.2014.12.006

Keywords: Antioxidant; Date palm; HPLC; *Phoenix dactylifera* L.; Total phenol

Introduction

Phoenix dactylifera L. commonly known as date palm is one of the oldest and most popular fruit trees in the hot arid regions of the world, particularly in the Gulf countries of the Middle East.[1] Date fruits are a good source of essential nutrients, including sugars, proteins, fibers, trace elements, etc., and form an important part of the daily diet.[2] They are also popular in other parts of the world due to their delicious taste, nutritional value and health benefits. Although ripe and mature sun dried dates are consumed throughout the year, but during the holy month of Ramadan their consumption increases many folds as most of the Muslims break their fast with dates.[3] It is considered to be an important subsistence crop in dry and semi dry regions of the world due to its socioeconomic and traditional importance.[4,5]

Based on the traditional Arabic practice and accepted international terminology, dates are classified into five stages viz. Hababouk (first stage which lasts for 4–5 weeks post fertilization), Kimri (green stage, lasts for 9–14 weeks), Khalal (color stage), Rutab (soft ripe stage) and Tamar (full ripe stage).[6] Dates are available in different shape and size. The ripe dates are; oval-cylindrical in shape, usually 3–7 cm long, 2–3 cm diameter and bright red to bright yellow in color depending on the variety. Dates contain a seed (pit) which is about 2–2.5 cm long and 6–8 mm thick and is approximately 13–15% of date's weight.[7] However, fruit quality is influenced by many factors such as size, color, texture, cleanliness, freedom from defects and the effects of decay-causing pathogens.[8,9]

Dry dates contain approximately 70% of carbohydrates in the form of simple sugars that makes them high energy food. In addition to the macromolecules and other essential micronutrients, phytochemicals like flavonoids, carotenoids, phenolic acids, sterols, procyanidins, and anthocyanins are also present in the dates.[6] Because of these antioxidant phytoconstituents, dates are used in traditional system of medicine for the treatment of hypertension, atherosclerosis, microbial infections, constipation, diabetes and cancer.[10] Consumption of dates on a regular basis is even considered to be beneficial in increasing sexual stamina, reducing sterility caused by various sexual disorders, decreasing fatigue and sluggishness in anemic patients.[11,12]

P. dactylifera L. is the main crop in Oman and represents 82% of all fruit crops grown in the country.[13] Oman produces about 270,000 metric tons of date fruits annually and is world's eighth largest producer.[14] Apart from boosting the economy, dates are an integral part of Omani diet and are consumed either fresh or sun-dried along with Arabic tea or coffee. But a high degree of biodiversity has been reported among various date varieties grown in different parts of the Sultanate.[13] The antioxidant activity of medicinal plants is attributed to their phenolic content. Ripe date fruits also contain numerous phenolic phytoconstituents like p-Coumaric, Ferulic and Sinapic acids and some Cinnamic acid derivatives[15] but their composition can vary from cultivar to cultivar depending on soil conditions and agronomic practices.[16] Previously published data have shown a strong correlation between the antioxidant activity and the total phenolic contents of date fruits.[17]

It is, therefore, of great interest to determine the total phenolic content (TPC), quantify the phenolic acids, compare and correlate the antioxidant activity with phenolic content of four commonly consumed date varieties viz. Bunarinja, Khasab, Fard and Khalas native to Sultanate of Oman.

Materials and Methods

Chemicals and reagents

Gallic acid, Caffeic acid, p-Coumaric acid, Vanillic acid, Syringic acid (Figure 1), DPPH, Sodium carbonate, Folin–Ciocalteu's reagent and methanol were purchased from Sigma–Aldrich, USA. All other chemicals and reagent used were obtained locally and were of high purity.

Sample collection

Four different varieties of *P. dactylifera* L. namely Faradh, Khasab, Bunarinja and Khasab were purchased from the local market of Al- Dakhilya and Al-Sharqiya regions of Oman at the beginning of the 2013 harvest season. Palm dates fruits of uniform size, free of physical damage and injury from insects and fungal infections were selected and used for all experiments. Samples were washed with tap water and the pits were removed by hand for preparation of 50% hydro-alcoholic extract.

Preparation of extract

50g of each date fruit pulp was mixed with 200 mL of the 50% ethanol. The extraction was carried out at 80 °C for about 60 min, and then the extract was filtered through muslin cloth to get 150 mL of extract. This was centrifuged at 4000 × g for 30 min. Double filtration was done to get a clear 100 mL of supernatant from each sample and then concentrated by heating on a water bath to obtain a viscous syrupy mass. The extract was kept in dark glass bottles and stored at −18 °C until further use.

Physical properties

10 dates from each variety were randomly selected and were individually weighed using an analytical balance. The date pit was removed and it was re-weighed again. The average weight of date fruit, fruit pulp, date pit, fruit flesh percentage, total no of fruits/kg and pulp/pit ratio were calculated.

Other physical properties investigated include size, color, sugar migration and consistency of flesh. Photos were taken using canon 5D Mark III to report the appearance. The sugar migration indicates the quality of the date.

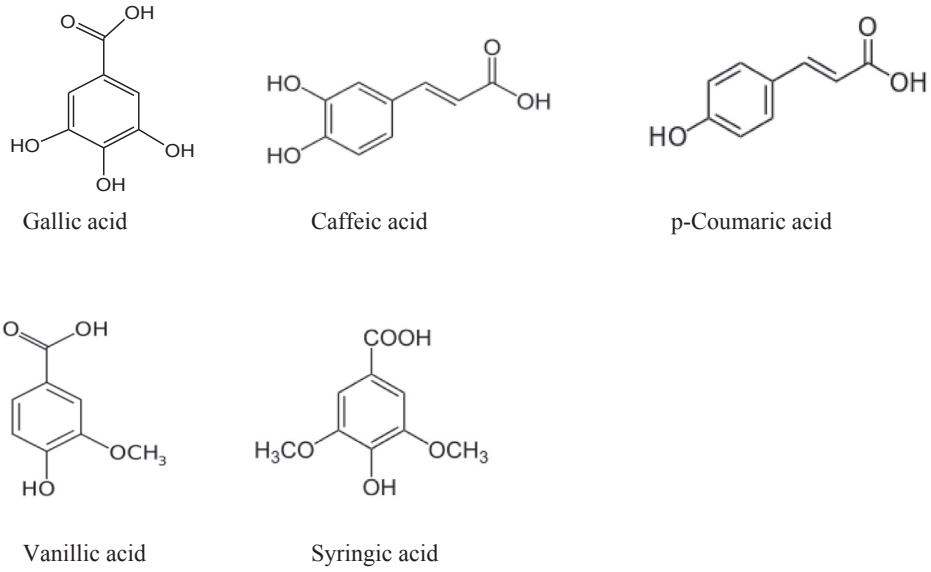

Figure 1: Chemical structure of phenolic acids.

Determination of moisture content

5 g date pulp was accurately weighed and dried to a constant mass in Memmert Cooled Vacuum Oven at 70 °C and 415 mbar for 30 min. The sample was reweighed after 30 min and moisture content was calculated and expressed as percentage moisture content of the fresh weight.

$$\%\,\text{Moisture Content} = \frac{(\text{Initial Weight} - \text{Oven Dry Weight})}{\text{Initial Weight}} \times 100$$

Determination of total phenols by (Folin-Ciocalteau reagent) colorimetric method

The total phenolic content (TPC) was measured according to the reported method of Al-Farsi et al.[3] using a UV-Visible spectrophotometer (UV Analyst- CT 8200) and Folin Ciocalteau reagent. The calibration curve of standard reference Caffeic acid was constructed using concentration in the range of 4–64 μg/mL. The TPC were calculated using linear regression equation obtained from the standard plot of Caffeic acid. The content of total phenolic compounds was calculated as mean ± SD (n = 3) and expressed as mg of Caffeic acid equivalent (CAE) in 100 gm fresh weight.

Determination of antioxidant activity

The free radical scavenging activity of different concentration of date extracts (0.5–10 μg/mL) was determined using 1,1-diphenyl-2-picrylhydrazyl (DPPH) as per the reported method.[18] The IC_{50} value (concentration of sample required to scavenge 50% of the DPPH free radical) was determined from the curve of percent inhibition plotted against the respective concentration.

Chromatographic determination of individual phenolic acids

HPLC analysis to quantify the individual phenolic acids was carried on Waters 2690 HPLC Separations modules, Waters 2996 photodiode array detector, Waters Nova-Pak C18 Cartridge reverse phase column (spherical silica particle size 4 μm, 3.9 mm i.d. × 150 mm length.) at 40 °C. Mobile phase consisted of 0.1% (v/v) formic acid in water (eluent A) and acetonitrile (eluent B). Gradient system was used with a total run time of 90 min and flow rate was adjusted to 0.5 mL/min. The injection volume was 10 μL. Photodiode array absorption spectrum was scanned in the range of 200–500 nm. Standard solutions of Gallic acid, Vanillic acid, Caffeic acid, Syringic acid and p-Coumaric acid (Figure 1) were prepared in methanol. Calibration standard samples were prepared at a concentration of 10 ppm from stock. Concentrations of the phenolic compounds in samples were determined by Waters HPLC application pack.

Data analysis

The results are expressed as mean ± SD. The obtained data were statistically analyzed by SPSS (Ver. 19) using one way of analysis of variance (ANOVA) and significance of difference between means of tested parameters was carried out using Turkey Post hoc test. A p-value less than 0.05 were considered statistically significant.

Results

Physical characteristics and % moisture content

The physical characteristic data of four Omani date varieties presented in Table 1 revealed that significant differences ($p < 0.05$) exist between varieties for almost all the physical parameters studied. Total number of fruits/kg in date varieties range from 139 to 215. Khasab and Khalas dates were found to contain the highest and lowest

Table 1: Physical characteristic of date fruits.

Date variety	Fruits No/Kg	Average weight in g (mean ± SD)			Flesh/pit ratio	Fruit flesh %
		Fruit	Flesh	Pit		
Bunarinja	147[a]	6.836[a] ± 0.7	6.25[a] ± 0.80	0.576[a] ± 0.17	10.85	91.43
Fard	172[b]	5.824[a,c] ± 0.87	5.347[a] ± 0.81	0.477[a,b] ± 0.10	11.21	91.81
Khalas	215[c]	4.663[a] ± 0.97	3.979[b] ± 0.91	0.684[a] ± 0.12	5.81	85.33
Khasab	139[a]	7.226[b] ± 0.78	6.628[c] ± 0.67	0.599[a,c] ± 0.13	11.06	91.72

Means within a column with no common letter differ significantly ($p < 0.05$).

number of fruits/kg respectively, therefore, mean weights of fruit and flesh were also highest (7.226 g and 6.628 g) for Khasab date. Similarly Khalas date had the lowest fruit and flesh weights (4.663 g and 3.979 g) but similar relationship was not observed between the two cultivars for average pit weights. Also no statistically significant difference in mean pit weights was found unlike a number of fruits, fruit and flesh weight respectively. It was expected that Khalas being lighter and smaller in size (215 fruits/kg) will have the lowest pit weight (0.684 g) but contrary to this Fard had the lowest pit weight (0.477 g). It was interesting to note that no significant difference was found in mean pit weights of Bunarinja, Fard, Khasab and Khalas but Khasab and Fard differed significantly. The flesh and pit weight (3.979 g and 0.576 g) of Bunarinja date, the lowest quality variety among all, was very similar to Khasab date (6.628 g and 0.599 g respectively), a superior variety.

The percentage of fruit flesh in date varieties ranged from 85.33 to 91.81% and Khalas date was found to have the lowest edible portion (85.33%) with respect to other three varieties. The highest fruit flesh was found in Fard (91.81%). Conversely, the waste part i.e. seed or pit constituted approximately 8.5% in Bunarinja, Fard and Khasab while in Khalas it was 14.66%. Consequently, the flesh/pit ratio varied from 5.81 to 11.21. It could be noted from Table 1, Khalas had the lowest ratio (5.81) and Fard exhibited the highest flesh/pit ratio (11.21) followed by Khasab (11.06) and Bunarinja (10.85).

As it can be observed from Table 2, that all date varieties differ in their physical appearance such as color, dimensions and % moisture content. Bunarinja, the inferior quality of date was brown to black in color and showed clear migration of sugar to flesh. However, the two superior varieties, Khalas and Khasab did not show any sugar migration and were of golden brown and black color respectively. The flesh of Bunarinja and Khalas date fruit was soft in touch while it was a bit hard for Fard and Khasab. Fard was dark brown in color and showed little sugar migration to the flesh. Khalas had the lowest length and width while Khasab had the highest. This variation in physical properties and composition of date varieties occurs due to genetic variations, environmental and growth conditions.

The percentage moisture content of date fruits determined by oven drying method was found to be within the permissible limits. The moisture content ranged from 2.948 to 5.008%. The highest content was observed in Khasab and the lowest content in Fard respectively (Table 2).

Total phenolic content

The total content of phenolic compounds in date cultivars is expressed as mg of Caffeic acid equivalent in 100 g fresh weight and is presented in Table 3. An equation of the line ($y = 0.0133x - 0.0133$, $R^2 = 0.9925$) obtained from the constructed plot of standard Caffeic acid (Figure 2) was used to calculate the TPC of date varieties. All cultivars showed little variation in phenolic content (32.24–35.84 mg CAE/100 g), however Khasab date (35.84 mg) had the highest amount of phenolic compounds followed by Bunarinja (34.90 mg) Fard (34.58 mg) and Khalas (32.24 mg). ANOVA followed by Turkey Post hoc test revealed significant differences ($p < 0.05$) in phenolic content between Khalas and other date varieties (Table 3).

In vitro antioxidant activity

The antioxidant activity of four date varieties at four different concentrations (0.5–10 μg/mL) was investigated by commonly used DPPH radical scavenging method. The scavenging effect of hydroalcoholic date extracts on the DPPH is expressed as % inhibition. All date varieties showed dose dependent scavenging activity of DPPH. No statistically significant difference in antioxidant activities was observed among the four varieties at concentrations of 2.5 and 10 μg/ml. The highest percentage inhibition of DPPH radicals was shown by Khalas date (70.62%) followed by

Table 2: Physical appearance and % moisture content of date fruits.

Date variety	Color	Dimensions (cm)		Sugar migration to flesh	Consistency of flesh	% Moisture content
		Length	Width			
Bunarinja	Brown to black	2.60	1.90	Yes	Soft/attached	3.408
Fard	Dark brown	3.30	2.10	Little	Hard/strewing	2.948
Khalas	Golden brown	2.50	1.80	No	Soft/attached	4.005
Khasab	Black	3.30	2.50	No	Hard/strewing	5.008

Table 3: Total phenolic content in four date cultivars.

Date variety	Total phenolics (mg of Caffeic acid equivalent/100 gm fresh wt)
Bunarinja	34.90 ± 0.15^{a}
Fard	34.58 ± 0.20^{a}
Khasab	35.84 ± 0.04^{a}
Khalas	32.24 ± 0.21^{b}

Total phenol as Caffeic acid equivalent (CAE) is expressed as Mean ± SD, n = 3; Means within a column with no common letter differ significantly ($p < 0.05$).

Bunarinja, Khasab and Fard. The IC_{50} value (in ug/mL) of the dates was found to in the order Bunarinja > Fard > Khasab > Khalas (Table 4). It was interesting to note that IC_{50} value of Bunarinja (0.875 ug/mL) was approximately half of Fard (1.717 ug/mL) and three times lower than Khasab (2.436 ug/mL) or Khalas (2.461 ug/mL) dates.

$$\%\,\text{inhibition} = \text{Absorbance(control)} - \text{Absorbance(test)}/\text{Absorbance(control)} \times 100$$

Individual phenolic acid content by liquid chromatography (HPLC)

The content of individual standard phenolic acids viz. Gallic acid, p-Coumaric acid, Caffeic acid, Vanillic acid and Syringic acid in date varieties were quantified by RP-HPLC method and are presented in Table 5. The retention time of the standard acids at 280 nm ranged from 4.026 to 17.578 min (Figure 3) and was in the following order Syringic acid > Vanillic acid > Caffeic acid > p-Coumaric acid > Gallic acid. It was observed that Gallic acid and Syringic acid were present in all the four varieties in different concentrations but Gallic acid was found to be the predominant phenolic acid of dates. The concentration of Gallic acid was noted to be highest in Bunarinja and lowest in Khasab (19.14 and 7 mg/100 gm of date flesh respectively). Syringic acid content of Khasab and Burnainja was almost similar (0.35 and 0.37 mg/100 gm of date flesh) while it was 0.49 mg/100 gm of date flesh in Fard. Caffeic acid and Vanillic acid were present in three varieties each and were absent in Bunarinja and Khasab respectively. However, p-Coumaric acid was detected in only one variety i.e. Khasab at 280 nm with a retention time of 6.285 min. The total content of individual phenolic acids was greatest in Bunarinja because of its high content of Gallic acid. Though Khasab is a premium quality date which also showed the presence of four phenolic acids including p-Coumaric acid but its total phenolic content was found to be the lowest (Table 5).

Discussion

The physical properties of dates vary greatly from one cultivar to another mainly due to genetic variations and different environmental and growth conditions. In Oman, qualities of dates are characterized according to their physical sensory characteristics, usage and prices.[19] Khalas, Khasab and Burnarinja are considered as premium, moderate and inferior quality dates respectively because of their difference in pricing and sensory characteristics. Fard is generally used for processing purposes in industry. In general, good quality date varieties have high flesh % and fruit/pit ratio. Though Khalas is a premium quality date but it showed the lowest total pulp and a flesh/pit ratio. The fruit/pit ratio for all varieties was found to be lower than the 'Khudari variety of Kingdom of Saudi Arabia and higher than the most of the commonly used Tunisian and Algerian dates.[20–22] Another commonly used criterion to judge the quality of date is to observe the sugar migration on the surface of date. Khalas and Khasab being the better quality dates showed no sugar migration on their surfaces. Low moisture content is desirable in dried dates because it makes the date extremely resistant to fungal spoilage. The % moisture content in all four varieties was different from those reported by Al Farsi et al.[19] and were significantly lower than the commonly used Tunisian and Moroccan cultivars.[22,23] This difference could probably be due to seasonal variations and storage conditions in cultivars.

The total phenolic content of dates expressed as Caffeic acid equivalent ranged from 32.24 to 35.84 mg/100g. The slight variation between samples could be due to date variety or because of difference in cultivation time, harvesting time, sun drying time, etc. The phenolic content of Tamar or sun-dried dates is usually higher due to degradation of tannins by

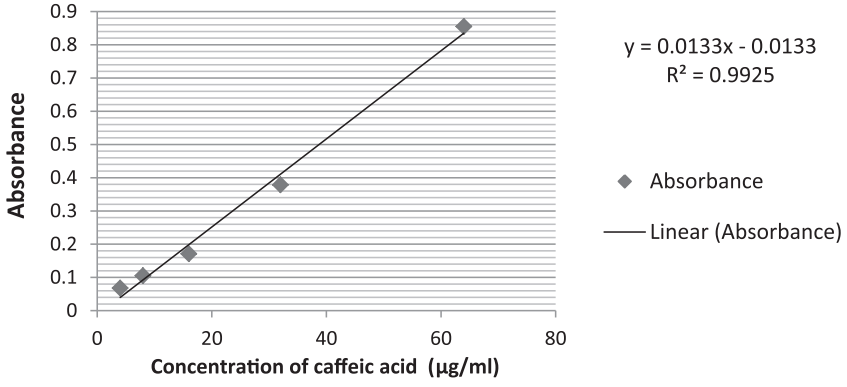

Figure 2: Standard curve of Caffeic acid.

Table 4: Percentage inhibition of DPPH free radical by date extracts.

Concentration µg/ml	Bunarinja	Fard	Khasab	Khalas
0.5	$43.03^{a} \pm 0.6$	$40.06^{a} \pm 0.47$	$32.94^{b} \pm 1.1$	$26.71^{b} \pm 2.6$
1.0	$52.3^{a} \pm 0.38$	$43.03^{b} \pm 2.1$	$46.29^{a,b} \pm 0.9$	$28.78^{c} \pm 3.1$
2.5	$55.49^{a} \pm 1.6$	$56.97^{a} \pm 1.3$	$51.63^{a} \pm 2.1$	$51.34^{a} \pm 1.1$
10	$64.68^{a} \pm 1.3$	$58.49^{a} \pm 0.33$	$62.61^{a} \pm 0.7$	$70.62^{a} \pm 2.9$
IC_{50}	0.875	1.717	2.436	2.461

Means within a row with no common letter differ significantly ($p < 0.05$).

Table 5: Contents of individual phenolic acids (mg/100 g of date flesh) determined by HPLC.

Date variety	Retention time (min)	Bunarinja	Faradh	Khasab	Khalas
Gallic acid	4.026	19.14	15.50	7.00	13.05
p-Coumaric acid	6.285	–	–	1.67	–
Caffeic acid	9.786	–	1.75	0.34	1.32
Vanillic acid	14.452	0.23	0.27	–	0.18
Syringic acid	17.578	0.37	0.49	0.35	0.25

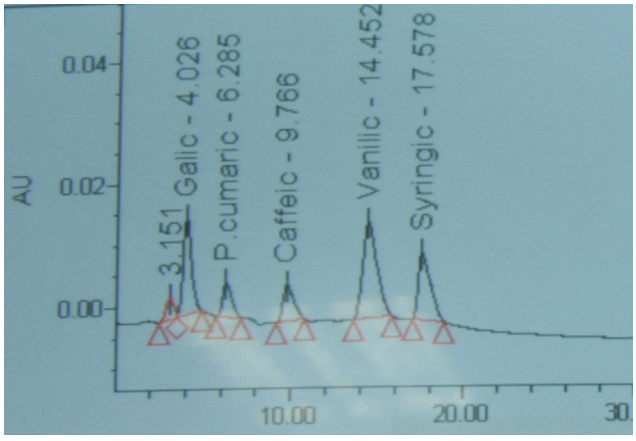

Figure 3: HPLC chromatogram of standard phenolic acids mixture of concentration 10 ppm at 280 nm.

temperature and maturation enzymes during drying.[18] Total phenolic content as Caffeic acid equivalent of Khalas, Khasab and Fard in the present study is found to be much lower than the previous study which used Ferulic acid or Gallic acid as standard phenolic acids for quantification.[3,24] However, phenolic content of studied date cultivars was higher than the Algerian dates which varied from 2.49 to 8.36 of GAE/100 g fresh weight.[18] Phenolic compounds are reported to possess diverse pharmacological actions; therefore, the beneficial biological effects of date could be attributed to their high phenolic content.[25]

1,1 diphenyl picrylhydrazine (DPPH) is a stable organic free radical whose reduction capability is measured at 517 nm absorbance. All dates significantly reduced the concentration of DPPH free radical in a concentration dependent manner. This is in agreement with the reported results which also showed concentration dependant activity.[26] However, similar to the finding of Khanavi et al., [2009][27], no correlation was observed in the TPC and antioxidant activity of the date extracts. This clearly indicates that phenols are not the only phytochemicals responsible for antioxidant activity in date, there might be some other non phenolic constituents present that are exhibiting antioxidant activity in dates.

Five commonly found phenolic acids viz. Gallic acid, p-Coumaric acid, Caffeic acid, Vanillic acid and Syringic acid in date cultivars were quantified with the help of HPLC. Reverse phase-liquid chromatography is a commonly used chromatographic technique for qualitative and quantitative analyses of phytochemicals especially phenolic compounds. It is also considered to be the most convenient method due to high sensitivity and resolution as compared to classical methods.[28] We found Gallic acid and Syringic acid to be the major and minor phenolic acids present in all the four varieties while p-Coumaric acid is detected only in Khasab variety. In contrast, Al-Farsi et al.[3] reported that Ferulic acid was the major phenolic acid in date cultivars from Oman. Gallic acid content of Fardh and Khalas as reported by Farsi et al.,[3] was much lower than our study. Also they did not detect Gallic acid in Khasab variety and Syringic acid in Khasab and Khalas cultivars. Caffeic acid content of Ajwa, Sukkari and Khalas varieties grown in Kingdom of Saudi Arabia was also reported to be lower than the Omani varieties.[1] The content of Gallic acid in Omani date compared to Algerian date was also found to be much higher.[2] The variation could be because of a difference in cultivation time, harvesting time, sun drying time, geographical conditions and other environmental factors related to date cultivars.

Conclusion

The presented data in this study confirm that Omani date fruits can be considered a rich source of antioxidant; this property is generally associated with the presence of phenols such as Gallic acid. HPLC also confirmed that Gallic acid is the predominant phenolic acid in all the four Omani cultivars. The quality of dates doesn't determine the antioxidant power of the date variety. We found that lower quality dates are just as good as the premium quality dates in exhibiting antioxidant activity or in total phenolic content. Therefore, we conclude that Omani dates have the potential to be used as nutraceutical or as an alternative source of natural antioxidant.

Conflict of interest

Authors declare no conflict of interest.

Authors' contribution

S.S. Al-Harthy and A. Mavazhe: Carried out the literature survey, designed the research protocol under the guidance of and in conjunction with the other authors. Performed the experiment with help and supervision of other authors. H Al-Mahroqi: Helped in HPLC analysis, Data analysis and review of manuscript. S.A. Khan: Intellectual input, supervision designing and preparation of research protocol, data analysis, manuscript preparation. Edited and finally reviewed the manuscript.

Acknowledgment

The authors are grateful to Dean, Associate Dean and Pharmacy Head of Oman Medical College for providing infrastructure and necessary research facilities.

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