



THE IMPACT OF THE EXTRACTING SOLVENT AND THE CULTIVAR ON THE DETERMINATION OF TOTAL PHENOLIC CONTENTS AND ANTI-RADICAL ACTIVITIES OF EXTRACTS FROM ROASTED DATE-SEEDS OF TWO DATE CULTIVARS CULTIVATED IN IRAQ

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Abstract

The objectives of the current study were to determine the total phenolic content (TPC) and the anti-radical activities of extracts prepared from roasted date-seeds of two date cultivars (Zahdi and Barhi) using different solvents. The TPC was measured using Folin–Ciocalteu method; while the antioxidant activity was measured using DPPH-radical scavenging assay. The results showed that the extracting had a significant role in the determination of the total amount of the TPC and the free radical scavenging activity of roasted date-seeds. Acidification of the water with hydrochloric acid (1% and 5%) resulted in a significant increase ($P < 0.001$) in both the TPC and anti-radical activity in comparison with the distilled water alone. Extracts prepared from the powdered roasted seeds of Zahdi cultivar had significantly higher ($P < 0.5$) TPC and free-radical scavenging activity than their counterparts from Barhi cultivar and in all solvents used. In the extracts from the powdered roasted seeds of both cultivars, positive correlation was found between the TPC and the anti-radical activity, indicating that the phenolic compounds are the main ingredients contributing to the free-radical scavenging activity of the two Iraqi date cultivars. In conclusion, the solvent used for the extraction and the cultivar of the date play a significant role in determining the TPC and free radical scavenging activity and that the roasted date-seeds powder could be a good potential source of bioactive compounds that have anti-radical capacities.

Key words : Date-pits (seeds), total phenolic contents, anti-radical activity.

Introduction

Date fruits play a significant role in the life of the people living in the Middle East and North Africa for a long time through the history. Hossain et al. (2014) stated in their review that “a naturally caffeine-free drink can be produced from roasted date-pits and this drink has been used in the Middle Eastern region for long time and it could be popular as an alternate to coffee drink”.

As a by-product from the date fruit processing industry, date seeds (pits) constitute about 10% of the fruit mass, which means that huge amounts are generated each year (Guizani *et al.*, 2014). According to FAO (2013), in 2011 the world production of dates was 7.5 million tonnes in 2011, which could generate approximately 750 thousand tonnes of seeds. In Iraq, for example, the production capacity was 662,447 metric tonnes in 2014

(Al-Alawi *et al.*, 2014).

Al-Farsi *et al.* (2007) reported that date seeds are good source of fibers, various bioactive phenolic compounds, carbohydrates and oil. Consequently, if the date seeds are utilized wisely via producing various value-added products, the income of the date production sector will be improved. More recently, Sirisena *et al.* (2017) stated that date seed extracts are rich in polyphenols and have shown various bioactivities.

Free radicals are produced in human body by many internal and external conditions and for a proper physiological function; the body needs a balance between the radicals and antioxidants and if this balance is disturbed for some reasons, a condition known as oxidative stress which triggers a number of human diseases such as cancer, heart diseases, Alzheimer’s and Parkinson’s disease (Singh *et al.*, 2004; Lobo *et al.*, 2010;

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Gorrini *et al.*, 2013; Forest *et al.*, 2014; Kim *et al.*, 2015; Moss and Ramji, 2016). In order to protect itself against the harmful effect of free radicals, the human body naturally generates a wide range of endogenous antioxidants that counteract the activity of the free radicals, but usually they are not enough to negate the side effects of free radicals (Nathan and Cunningham-Bussell, 2013). Consequently, the human body needs sources for exogenous antioxidants via food or other routes.

Although, synthetic antioxidants showed the ability to scavenge the free radicals, they have been reported to be with some side effects that affect adversely the human health (Lobo *et al.*, 2010). Accordingly, the need for natural nontoxic antioxidants is necessary. Consequently, the goal of the current present study was to determine the total contents of the polyphenolic compounds in extracts prepared from roasted date-seeds of two Iraqi date cultivars (Zahdi and Barhi) using various extracting solvents and to evaluate their free radical scavenging activity under *in vitro* conditions.

Materials and Methods

Source of dates and preparation of roasted date-seeds

The ripe fruits of two date cultivars (Zahdi and Barhi) were purchased from the local market of Baquba City, Diyala Province, Iraq and seeds (pits) were removed manually from the fruits and then washed with tap water and then spread them on tissue paper to get rid of the excess surface water. The date-seeds were dried at 50°C for 48 hours and then roasted. Roasted date-seeds were ground into powder by house coffee grinder and stored at -40°C until used for analysis.

Preparation of date-seeds extracts and determination of total phenolic contents (TPC)

The extracts were prepared as described previously (Molan *et al.*, 2017). Briefly, forty milligrams of roasted seeds powder have been weighed and put in 50-ml plastic centrifuge tubes and then 40 milliliters of the selected solvents [distilled water alone at room temperature, boiling distilled water and diluted hydrochloric acid (concentration: 36%) solutions (1% and 5% HCL with cold distilled water/ actual concentrations were 0.36 - 1.79%)] have been poured into each tube, mixed by vortexing for 5 minutes and then left overnight (stock solution; 10 mg per ml) at room temperature. After 24 hours, the tubes have been centrifuged at 1000 rpm for 5 minutes and the supernatant was used in the experiments.

The amount of total phenolic content (TPC) in the

extracts was determined according to the Folin-Ciocalteu procedure as used by Molan *et al.* (2016b) with some modifications. The TPC of the extract was expressed as mg gallic acid equivalent (GAE) per gram of powdered roasted seeds. The values were expressed as mean of three replicates \pm standard error.

Scavenging of diphenyl-picrylhydrazyl (DPPH) radicals

This assay was performed using a previously described method (Molan *et al.*, 2016b) with some minor modifications.

The antiradical activity was calculated as a percentage of DPPH decolouration relative to a negative control using the following equation :

$$\text{Antiradical activity (\%)} = \frac{\text{absorbance of control incubation} - \text{absorbance of the extract}}{\text{absorbance of control incubation}} \times 100.$$

Statistical analysis

The results were expressed as mean \pm SEM of three measurements and analyzed using SAS version 9.2 for windows. One way analysis of variance followed by Tukey's posttest was used to test for any significant difference between the different treatments. Pearson's correlation and regression analysis were carried out for analyzing the data obtained from different extracts and to determine the correlation between TPC and free radical scavenging activity.

Results and Discussion

The findings of the current study indicate that the extracting solvent and the date cultivar have a significant role in determining the amounts of polyphenolic compounds (fig. 1) in the extracts prepared from the roasted date-seeds powder and their ability to scavenge the free radicals (fig. 2). Some previous studies on date-seeds (Al-Farsi *et al.*, 2007; Molan *et al.*, 2017) have shown that the extracting solvents and the date cultivars play a strong and significant role in estimating the total polyphenolic compounds (TPC) and the antioxidant activities. In addition, Guizani *et al.* (2014) reported that the TPC extracted from date-pits powder using different solvents at different temperatures varied significantly and that the maximum TPC were obtained in the extracts using acetone as a solvent, at all temperatures used. The authors concluded that the temperature and solvent had a significant effect on the extraction of polyphenols. Furthermore, it has been found that the composition of date phenolic compounds varies from cultivar to cultivar depending on soil conditions and agronomic practice, for example, the nutritional quality of date fruits alters among

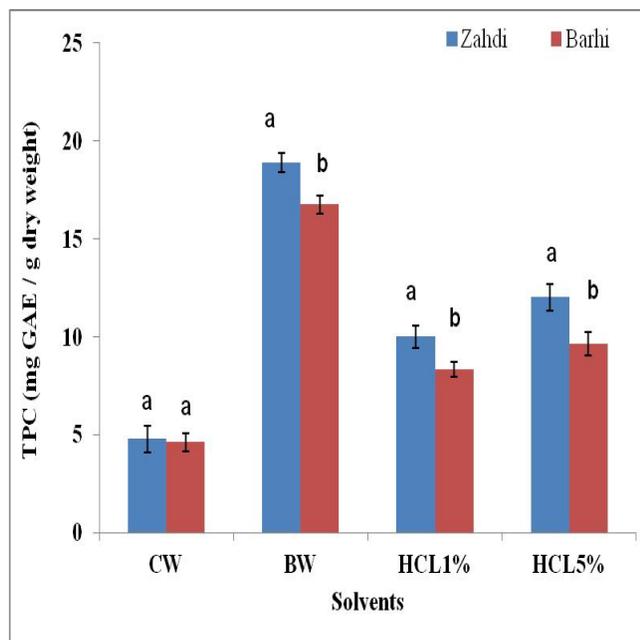


Fig. 1 : Comparison between the two date cultivars (Zahdi and Barhi) regarding the total phenolic contents (TPC) of roasted seeds. Different superscript letters indicate significant differences between the two cultivars in each solvent.

Table 1 : Correlation (R^2 values) between the total phenolic content (TPC) and anti-radical activity of extracts prepared from roasted seeds of two Iraqi date (*Phoenix dactylifera* L.) cultivars (Zahdi and Barhi).

| Solvents | Correlation coefficient (R^2) | |
|----------------------------|-----------------------------------|--------|
| | Zahdi | Barhi |
| Cold distilled water only* | 0.5004 | 0.622 |
| Boiling distilled water | 0.7317 | 0.5521 |
| 1% HCL aqueous solution | 0.9406 | 0.6521 |
| 5% HCL aqueous solution | 0.7808 | 0.774 |

*Distilled water at room temperature (22-24 °C).

varieties grown in Algeria (Saleh *et al.*, 2011), Oman (Al-Farsi *et al.*, 2005; Chaira *et al.*, 2009), Bahrain (Allaith, 2008) and Sudan (Mohamed *et al.*, 2014).

It has been found that the solubility of polyphenols depends on the type of solvents with varied polarity. Previous researchers also reported that higher extraction yields of polyphenols were obtained with increasing polarity of the solvent (Cheung *et al.*, 2000; Al-Rawahi *et al.*, 2013). Different types of polyphenols present in the date-pits could exhibit different polarities, thus affected the amounts of polyphenols in the extracts.

It seems that the present study is the first to deal with roasted date-seeds and the results showed clearly that roasting of the date-seeds increases the TPC

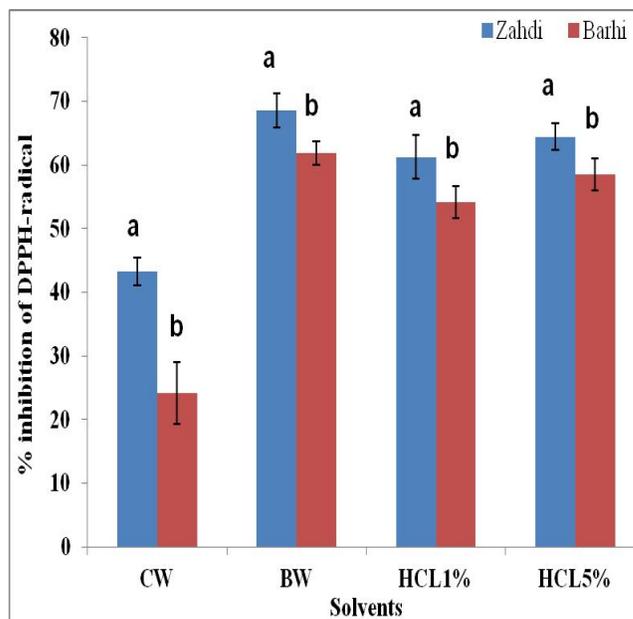


Fig. 2 : Comparison between the two date cultivars (Zahdi and Barhi) regarding the capacity of the extracts prepared from roasted seeds to inhibit the activity of the synthetic DPPH free radical. Different superscript letters indicate significant differences between the two cultivars in each solvent.

significantly in comparison with oven-dried seeds. Recently, Molan *et al.* (2017) reported that the extracts prepared from the oven-dried (unroasted) seeds of Zehdi cultivar contain 0.747-1.632 mg Gallic acid equivalent (GAE)/ g powdered dried seeds and this content depends mainly on the extracting solvent and the selected date cultivar. In comparison with the results of Molan *et al.* (2017), the roasted Zahdi date-seeds contain significantly more TPC as they varied from 4.79 to 18.9 mg GAE/ g roasted powdered seeds.

We came across one study which dealt with freeze-dried date-seeds and the authors reported that freeze-drying increased the TPC significantly in comparison with dried seeds as the polyphenol contents in freeze-dried date-pits varied from 21-62 mg gallic acid equivalents (GAE)/g freeze-dried date-pits when acetone/water, ethanol/water, methanol/water and water alone were used as solvents for extraction (Guizani *et al.*, 2014).

Messaoudi *et al.* (2013) determined the total phenolic contents and the antioxidant activity of date-pits of seven Algerian date palm fruit varieties and reported that the total phenolic content of the methanolic extracts ranged from 27.2 to 38.5 mg of caffeic acid equivalents CAE/ 100 g fresh weight, while the ethyl acetate extracts gave contents ranging from 22.8 to 42.6 mg CAE/100 g fresh weight. The antiradical and the hydroxyl scavenging activities of the methanolic extracts were much higher

than those of the ethyl acetate extracts.

The results of the present study showed that the extracts prepared from the roasted and powdered Zahdi date pits had significantly higher ($P < 0.05$) TPC and anti-radical activity than those prepared from the pits of Barhi cultivar in all solvents used. Similarly, Molan *et al.* (2017) reported that the extracts prepared from the entire pomace, flesh and seeds of Zahdi cultivar had significantly higher ($P < 0.5$) TPC and anti-radical activity than their counterparts from Khestawi cultivar in all solvents used.

Extracts prepared from roasted date-seeds using boiling water contain significantly ($P < 0.05$) more TPC than their counterparts prepared in distilled water alone at room temperature. Guizani *et al.* (2014) stated that the TPC extracted from date-seeds powder at 60°C were higher than that extracted at 22°C and the authors concluded that the extracting temperature had an important role in the extraction of phenolic compounds.

A strong positive correlation (table 1) has been found between the phenolic contents and the anti-radical activity of the extracts prepared from roasted date-seeds powder using four different solvents which confirms the findings of the previous studies conducted on the date-seeds from different countries and this correlation depends on the solvent and the selected date cultivar used (Kchaou *et al.*, 2013; Molan and Mahdy, 2016; Molan *et al.*, 2012; 2016a,b, 2017). This correlation indicates that the polyphenols are the main components contributing to the free-radical scavenging activities.

Conclusion

The extracting solvent and the date cultivar have an important role in the determination of the amounts of phenolic compounds and free radical scavenging activities. In addition, the roasting process increases the amounts of phenolic compounds and hence, the roasted seeds could be considered a good inexpensive source of natural antioxidants and could be used as alternatives for the synthetic antioxidants in pharmaceutical and food formulations. Furthermore, acidification of distilled water with hydrochloric acid significantly increased the efficiency of water as extracting solvent for the phenolic compounds. More research is needed to study the mode of action of roasting process in increasing the amounts of extractable phenolic compounds.

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