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Research Article

In Vitro Antioxidant Activities of Three Selected Dates from Tunisia (*Phoenix dactylifera* L.)

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Second-grade dates (*Phoenix dactylifera* L.), with hard texture, from three selected Tunisian cultivars (Allig, Deglet Nour, and Bejo) were analysed from their antioxidant activities using DPPH radical scavenging activity, FRAP assay, H_2O_2 scavenging activity, and metal chelating activity. Date extracts showed strong and concentration-dependant activity in all tested methods. The results showed that the best antioxidant activity was obtained in Allig, followed by Bejo and Deglet Nour. Total phenolics, total flavonoids, carotenoids, and tannins were determined spectrophotometrically in three date extracts. Results indicated that date contained significantly different amounts of these compounds. In fact, Allig presented the highest antioxidant compounds, followed by Bejo and Deglet Nour. Correlation analyses indicated a positive linear relationship between antioxidant activities and total phenolic and flavonoid content of date. This study demonstrates the potential antioxidant activity with Tunisian date, where we can use these natural extracts as food additives in replacement of synthetic compounds.

1. Introduction

Reactive free radicals, such as superoxide anion radical, hydroxyl radical, and hydrogen peroxide, have been implicated in the development of many diseases such as cancer, coronary heart disease, autoimmune disease, diabetes, sclerosis, atherosclerosis, cataracts, and chronic inflammation. The damage caused by free radicals is due to their ability to inactivate many cells like protein denaturation, lipid peroxidation, membrane destabilisation, and DNA mutation which may lead to cancer [1–5].

Antioxidants, which can link reactive free radicals, are supposed to play an important role in human health and prevent the rancidity and lipid oxidation in food systems [2, 6]. Different methods were used to evaluate the antioxidant properties such as radical scavenging activity, reducing properties, metal chelating activity, hydrogen peroxide scavenging activity, or activation of various antioxidant enzymes and inhibition of oxidases [2, 4, 7].

The consumption of fruit and vegetables is associated with many benefits like anticarcinogenic, antiinflammatory, antimicrobial, anti-mutagenic, antithrombotic, neuroprotective, and antibiotic activities as well as reduction of cardiovascular diseases and cholesterol [1, 2, 5, 8, 9].

The protection offered by fruits and vegetables has been attributed to the presence of dietary antioxidants. These beneficial compounds were represented by polyphenols, flavonoids, ascorbic acid, carotenoids, and tocopherols [1].

Therefore, in recent years, the interest in the natural antioxidants has increased considerably in the human diet and the pharmaceutical products. These natural compounds can replace synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) that may exhibit toxicity and require high manufacturing costs.

Palm date (*Phoenix dactylifera* L.) has been an important crop in arid and semiarid regions of the world. This fruit has always played an important role in the nutritional, economic and social lives of the people of these regions.

Tunisia is considered to be one of the date producing countries. The amount of dates produced annually is about 145000 tonnes [10]. From this production, 30% is lost during picking, storage, commercialization, and conditioning processes [11–13]. These important quantities of by-products are generally discarded or used in animal feeding because of too hard texture, contamination with fungus, and infestation by insect or simply due to their low quality [14].

Some studies have been carried out to use these by-products to develop new products such as metabolites or biomass production [13]. In Tunisia, date by-products are classified as second-grade dates. However, besides nutritional components (carbohydrates, aminoacid, proteins, dietary fibres, etc.), they represent a potential source of various biologically active compounds (total phenolic compounds, flavonoids, carotenoids, etc.) which have antioxidant activity [8, 13, 15].

Antioxidant activity of this fruit from different origins has been studied from Algeria [16], Kuwait [17], Oman [8, 18], Iran [1, 15], and Yemen [4]. These results showed that date palm fruit possesses antioxidant properties which can vary on their phenolic content. However, there is no study, so far, that has dealt with the antioxidant activity of dates from Tunisia.

In this paper, our interest has been to study antioxidant activity and the antioxidant compounds in Tunisian dates.

The aim of this study was to evaluate the antioxidant activity of extracts from three date varieties cultivated in Tunisia using DPPH test, FRAP method, scavengers of H_2O_2, and metal chelating activities. Total phenolic, flavonoids, carotenoids, and tannins were evaluated and the correlation between antioxidant activities and total phenolic and flavonoid compounds has been reported.

2. Materials and Methods

2.1. Origin of Date Fruit. Second-grade dates (*phoenix dactylifera* L.), with texture defect (relatively hard) of the three cultivars in Tunisia, Deglet Nour, Allig, and Bejo, were obtained from Tozeur region (Tunisia). Dates were collected at "Tamr stage" (full ripeness). Ten kilograms from each variety were directly divided into bags of 500 grams and stored at −20°C prior to analysis.

2.2. Preparation of Extract. Prior to extraction processing, the date fruits were defrosted, cleaned, and pitted and the edible part of date was dried at room temperature before grinding it with a meat grinder (Moulinex, type Ne 401, france) to produce date paste.

The extraction of antioxidant compounds from all date cultivars was carried using acetone/H_2O (70 : 30, v/v) as described by Al-Farsi et al. [8], with slight modifications.

Two grams of sample was mixed for 2 h with 20 mL of solvent extract at room temperature and with continuous agitation.

The mixture was centrifuged at 5000 g for 15 min, and the supernatant was decanted. The pellets were extracted under identical conditions. Supernatants were combined and concentrated by a rotary evaporator at 40°C. The residues were dissolved in distillated water at different concentrations and used for the following experiments.

2.3. Determination of Antioxidant Components

2.3.1. Determination of Total Phenolic Contents. The polyphenols were determined by the Folin-Ciocalteu procedure according to Al-Farsi et al. [8].

200 μL of date extract (10 mg/mL) was mixed with 1.5 mL of Folin-Ciocalteu reagent (previously diluted 10-fold with distilled water) for 5 min at room temperature. 1.5 mL of aqueous sodium bicarbonate (60 g/L) was added, and the mixture was vortexed and allowed to stand at room temperature. After 90 min, the absorbance was measured at 725 nm. The total phenol concentration was expressed as mg of gallic acid equivalent (mg GAE) per 100 g of extract.

2.3.2. Determination of Total Flavonoids. Total flavonoid content was determined using colorimetric method described by Zhishen et al. [19].

1 mL of date extract (10 mg/mL) was diluted with 4 mL of distilled water. Then, 0.3 mL of 5% $NaNO_2$ was added. After 5 min, 0.3 mL of $AlCl_3$ (10%) was added and allowed to stand for 1 min. Then, 2 mL of NaOH (4%) was added and the mixture was diluted with 2.4 mL of distilled water. The solution was mixed and the absorbance was read at 510 nm after 15 min.

The total flavonoid content was calculated on the basis of the standard curve for catechin solutions and expressed as catechin equivalents.

2.3.3. Determination of Tannin Compounds. The total tannin content was estimated according to a literature procedure [20] by the Folin-Ciocalteu method, after removal of tannins by their adsorption on insoluble matrix (polyvinylpolypyrrolidone, PVPP).

In brief, 1 mL of extract was added to 100 mg of PVPP. After 15 min at 4°C, the mixture was vortexed and centrifuged for 10 min at 1500 g. Aliquots of supernatant (200 μL) were transferred into test tubes and nonabsorbed phenolics were determined by Folin-Ciocalteu procedure. Calculated values were subtracted from total polyphenols contents and total tannin contents were expressed as mg gallic acid equivalents (GAE) per 100 g extract.

2.3.4. Determination of Total Carotenoids. Total carotenoids were measured according to the method of Al-Farsi et al. [8] with slight modifications. In brief, 2 g of date was extracted in 25 mL of acetone/ethanol (1 : 1, v/v) containing 200 mg/mL butylated hydroxytoluene (BHT). After extraction, the homogenate was centrifuged at 1500 g for 15 min at 4°C. The supernatant was collected and the residue was reextracted using the same method until exhaustion of colour. Finally, the combined supernatants were made up to 100 mL with the extraction solvent. Absorbance was measured at 470 nm using a spectrophotometer (SHIMADZU, UV mini 1240, Japan). Total carotenoids were calculated

using the following equation and expressed as milligrams per 100 g of fresh weight:

$$\text{Total carotenoids} = \frac{\left(\text{Ab} \times V \times 10^6\right)}{\left(A^{1\%} \times 100\, G\right)}. \quad (1)$$

Ab is the absorbance at 470 nm, V is the total volume of extract, $A^{1\%}$ is the extinction coefficient for a 1% mixture of carotenoids at 2500, and G is the weight of sample (g).

2.4. Evaluation of Antioxidant Activities

2.4.1. DPPH Radical Scavenging Activity. The antiradical activity of date sample, based on the scavenging activity of the stable free radical, DPPH (2,2-diphenyl-2-picrylhydrazyl), was estimated according to the method of Bertoncelj et al. [9].

Date extracts were dissolved in water at concentrations from 5 to 50 mg/mL. 0.1 mL of each solution was mixed with 1.9 mL of a solution of DPPH in absolute ethanol (130 μM) and 1 mL of acetate buffer solution (100 mM, pH 5.5).

Absorbance at 517 nm was determined after 90 min at room temperature in the dark.

The percentage inhibition activity was calculated from $[(A_0 - A_1)/A_0] * 100$. A_0 is the absorbance of the control, and A_1 is the absorbance of the extract.

2.4.2. FRAP Assay. Ferric reducing antioxidant power (FRAP) was determined in the extracts according to Bertoncelj et al. [9].

The principle of this method is based on the ability of the extract to reduce a ferric 2,4,6-tripyridyl-s-triazine complex (Fe^{3+}-TPTZ) to its ferrous colored form (Fe^{2+}-TPTZ) in the presence of antioxidants.

The FRAP reagent contained 2.5 mL of a 10 mM TPTZ solution in 40 mM HCl plus 2.5 mL of 20 mM $FeCl_3$ and 25 mL of 0.3 M acetate buffer, pH 3.6, and was prepared freshly and warmed at 37°C. 200 μL of sample (10 mg/mL) was mixed with 1.8 mL of FRAP reagent. After incubation for 10 min at 37°C, the absorbance was measured spectrophotometrically at 593 nm against a blank solution containing distilled water. A calibration curve is prepared using an aqueous solution of $FeSO_4$ as standard.

2.4.3. Scavenging Activity of Hydrogen Peroxide. The scavenging activity of date extracts on hydrogen peroxide was determined according to the method of Kumaran and Karunakaran [21].

A solution of hydrogen peroxide (2 mmol/L) was prepared in phosphate buffer (pH 7.4).

1.2 mL of each solution at different concentrations from 2 to 50 mg/mL was added to 1.2 mL of hydrogen peroxide solution. A blank solution was prepared in the same way but without H_2O_2.

The absorbance of hydrogen peroxide was measured spectrophotometrically at 230 nm after incubation during 10 min.

The percentage inhibition activity was calculated using the following formula:

$$\%\text{scavenging activity} = \left[\frac{(A_0 - A_1)}{A_0}\right] * 100, \quad (2)$$

where A_0 is the absorbance of the control and A_1 is the absorbance of the extract.

2.4.4. Metal Chelating Activity. The metal chelating capacity was determined by the method of Lee et al. [22].

Briefly, 1 mL of date extracts with different concentrations (10–150 mg/mL) was mixed with 3.7 mL of methanol, and 0.1 mL of a solution of 2 mmol/L $FeCl_2$ was added. After that, the reaction was mixed with 0.2 mL of 5 mmol/L ferrozine and the mixture was shaken and left standing for 10 min at room temperature.

Then, absorbance of solution was measured spectrophotometrically by measuring the formation of ferrous ion-ferrozine complex at 562 nm.

The metal chelating activity was calculated from $[(A_0 - A_1)/A_0] * 100$, where A_0 is the absorbance of the control and A_1 is the absorbance of the extract.

2.5. Statistical Analysis. All values are the means of three independent replications. Data are presented as mean ± standard deviation ($X \pm$ SD). Ducan's test and one-way analysis of variance (ANOVA) were used to compare results with $\alpha = 5\%$ (SPSS program, Windows 11.0).

3. Results and Discussion

3.1. Antioxidant Compounds

3.1.1. Total Phenolic Compounds. The amount of total phenolic contents was determined in the different extracts of three date varieties in Tunisia using the Folin-Ciocalteu method.

As shown in Table 1, there were significant differences ($P < 0.05$) among the different varieties of date. The result, expressed as gallic acid equivalent, ranged from 240.38 mg GAE/100 g extract to 505.49 mg GAE/100 g extract (Table 1).

The highest phenolic compound was obtained in Allig variety and the lowest was found in Deglet Nour variety.

These results are comparable to those obtained by previous studies despite using different phenolic acid standards for quantification. In fact, Al-Farsi et al. [8] reported that the total phenolic content ranged from 134 to 280 mg of ferulic acid equivalents (FAE)/100 g in fresh date varieties in Oman. Besides, the total phenolic compounds of two date varieties studied by Wu et al. [23] were 576 and 661 mg GAE/100 g. These results are comparable with those found in the present study.

However, Mansouri et al. [16] studied the phenolic profiles of seven different varieties of Algerien date. They found that phenolic content varied between 2.49 and 8.36 of GAE/100 g fresh weight. These levels are much lower compared to those found in this study. On the other hand, Biglari et al. [1] reported that total phenolic compounds

Table 1: Content of total phenolic, total flavonoid, total carotenoid, and tannins in different date extracts.

Sample	Phenolic content[a]	Total flavonoid[b]	Total carotenoid[c]	Tannins[a]
Allig	505.49 ± 3.36[a]	213.76 ± 1.52[a]	8.14 ± 0.16[b]	390.20 ± 4.22[a]
Deglet Nour	240.38 ± 1.12[b]	58.92 ± 2.13[b]	4.02 ± 0.45[c]	142.67 ± 2.42[b]
Bejo	391.94 ± 5.18[c]	150.11 ± 0.66[c]	12.41 ± 0.59[a]	285.80 ± 6.31[c]

Data are expressed as means ± SD ($n = 3$). Means, in the same column, with different letters are significantly different ($P < 0.05$).
[a]Milligrams of gallic acid equivalent per 100 g extract. [b]Milligrams of catechin equivalent per 100 g extract. [c]Milligrams per 100 g fresh weight.

varied between 2.89 and 141.35 mg GAE/100 g dry weight of Iranian date.

These results strongly suggest that date fruit may contain a higher level of phenolic compounds among fresh and dried fruits. These compounds are commonly found in plants and they have been reported to have a strong antioxidant activity. So, phenolic compounds are supposed to have multiple biological effects, such as anti-inflammatory, antimicrobial, antimutagenic activities. These constituents can also protect from coronary heart disease and cancer [9, 20, 21, 24].

The total phenolic content could be regarded as an important indication of antioxidant properties. This activity is mainly due to their redox properties; they can scavenge reactive oxygen species, neutralize free radicals, and decompose peroxides [3, 21].

The discordance in total phenolic content of date between different studies is due to variety, growing condition, maturity, season, agronomical differences, fertilizer, soil type, genomics, moisture content, climate, storage conditions, methods of extraction, and standards used [1, 13, 15, 18, 25–27].

This large variability can also be explained by the methods and the choice of solvent and the differences in extractability and solubility of phenolic compounds in different solvent. However, a mixed polarity solvent could extract more phenolic compounds, such that addition of water to 50% in acetone can increase extraction of condensed tannins and total phenolic content [2, 28].

3.1.2. Total Flavonoids. Total flavonoid content was determined by aluminium chloride methods and expressed as mg catechin/100 g extract.

From the results summarized in Table 1, significant differences ($P < 0.05$) between all types of date were observed. The results obtained showed that the total flavonoid content of three varieties of date varied considerably from 58.92 mg catechin/100 g extract to 213.76 mg catechin/100 g. Allig variety contained the highest amount of flavonoids (213.76 mg catechin/100 g) followed by Bejo and Deglet Nour varieties (150.11 mg catechin/100 g and 58.92 mg catechin/100 g, resp.).

The total flavonoid content determined in this study confirms previous results reported by Al-Mamary et al. [4] on different types of date cultivated in Yemen. They showed that the total flavonoid content ranged from 170 to 290 as quercetin equivalent/100 g palm date, Tamr.

On the other hand, these results are significantly higher than the values reported for different types of Iranien dates. However, Biglari et al. [1] reported that the total flavonoid content varied from 1.62 to 81.79 mg catechin equivalents/100 g dry weight of sample.

The difference between total flavonoid content in this study and other types of dates could be due to different factors such as the solubility and the extractability of the flavonoids in solvent, use of different analytical methods, and use of different standards [28].

In general, flavonoids are one of the most important phenolics which contribute to the antioxidant activity. These compounds possess many chemical and biological activities such as radical scavenging properties [1, 21].

It is possible that other phenolic compounds could also contribute to the antioxidant properties of these types of dates.

3.1.3. Determination of Tannins. The determination of tannin contents in acetone/H_2O extracts from three varieties of dates, Allig, Bejo, and Deglet Nour, was estimated using the Folin-Ciocalteu method and the results were expressed as gallic acid equivalents.

As displayed in Table 1, a significant difference ($P < 0.05$) between all types of date extracts was observed. In fact, we can conclude that Allig, Bejo, and Deglet Nour were rich in tannins, but we found that the best compounds were obtained in Allig extract.

So, this variety contained a significantly higher amount of tannins (390.20 mg GAE/100 g extract) than Bejo and Deglet Nour (285.80 mg GAE/100 g and 142.67 mg GAE/100 g, resp.).

These results are similar to those obtained in phenolic contents and suggest that tannins play an important role in the total phenolic compounds of date fruit.

3.1.4. Total Carotenoid Content. The total carotenoid content in the different varieties of date was presented in Table 1. Of the varieties studied, significant differences ($P < 0.05$) between all types of date were observed. Bejo had the highest amount of carotenoids (12.41 mg/100 g) followed by Allig (8.14 mg/100 g) and Deglet Nour (4.02 mg/100 g).

These levels are higher compared to those found in previous studies. In fact, Al-Farsi et al. [8] mentioned that carotenoids ranged from 1.31 to 3.03 mg/100 g in three varieties of fresh date grown in Oman (Fard, Khasab, and Khalas). This variation is probably due to the existing differences between the variety, growing condition, maturity, storage, and analysis conditions.

By comparison with other fruits, dates can be considered a good source of carotenoids. Moreover, Al-Farsi et al. [8] showed that carotenoid content in eight fruits studied

Table 2: Comparison of antioxidant properties of date extracts.

Samples	DPPH IC_{50} (mg/mL)	Metal chelating activity IC_{50} (mg/mL)	H_2O_2 IC_{50} (mg/mL)	FRAP ([$FeSO_4$] mmol/100 g extract)
Allig	16.70 ± 0.07^a	85.19 ± 0.19^a	10.51 ± 0.06^a	4.98 ± 0.19^a
Deglet Nour	46.79 ± 1.48^b	91.71 ± 1.40^b	20.44 ± 0.18^b	1.96 ± 0.03^b
Bejo	25.90 ± 0.21^c	90.72 ± 0.46^b	12.50 ± 0.05^c	3.24 ± 0.02^c

Each value is expressed as mean ± SD ($n = 3$).
Means, in the same column, with different letters are significantly different ($P < 0.05$).

Table 3: Correlation between total phenolic contents (TPC) and antioxidant activities of date extracts.

Correlation	R^2
TPC versus DPPH	0.97
TPC versus FRAP	0.96
TPC versus H_2O_2	0.94
TPC versus metal chelating activity	0.74

Table 4: Correlation between total flavonoid contents (TFC) and antioxidant activities of date extracts.

Correlation	R^2
TFC versus DPPH	0.98
TFC versus FRAP	0.96
TFC versus H_2O_2	0.95
TFC versus metal chelating activity	0.72

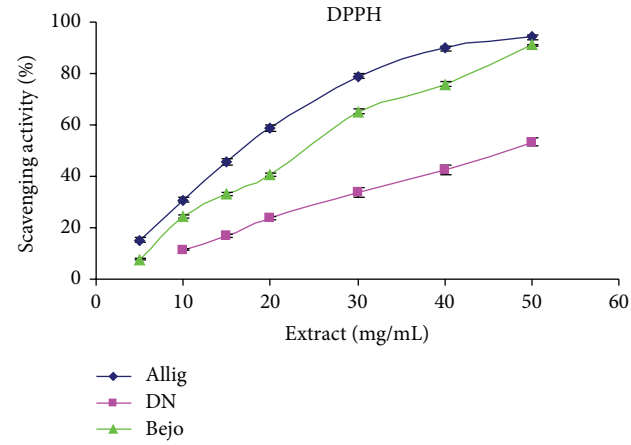

Figure 1: Effect of different concentrations on DPPH scavenging activity in different date extracts: Allig, Bejo and Deglet Nour (DN). Values are means of three replications ±SD.

ranged from 0.02 mg/100 g of fresh weight in strawberries to 2.26 mg/100 g in mandarins.

3.2. Antioxidant Activity. Antioxidant activity of three date varieties, based on DPPH, ferric reducing antioxidant power (FRAP), hydrogen peroxide scavenging activity, and metal chelating activity, was evaluated in this study.

3.2.1. DPPH Radical Scavenging Activity. DPPH is a stable organic free radical with a maximum absorption at 517 nm. The free radical scavenging activity is a commonly used method to evaluate the antioxidant activity in vitro. This method is based on the ability of antioxidant to scavenge the DPPH radical that can donate an electron or hydrogen atom.

As shown in Figure 1, it is noticed that the different extracts of date exhibited a potential free radical scavenging activity. In fact, high DPPH radical scavenging activity was observed in the following order: Allig > Bejo > Deglet Nour. These results suggest that scavenging abilities of different extracts of date against DPPH radical were concentration-dependant. So, by increasing the concentration of different extracts of date, their DPPH radical scavenging ability increases.

At the dose of 20 mg/mL, the DPPH radical scavenging activity is in the following order: Allig (58.77%), Bejo (40.78%), and Deglet Nour (23.98%).

DPPH is usually expressed as IC_{50}, the amount of antioxidant necessary for decreasing the initial concentration of DPPH by 50%. When the IC_{50} value of the sample was lower, the antioxidant activity was higher [9].

Table 2 presents the IC_{50} values of different date extracts. Significant differences ($P < 0.05$) were observed between their DPPH IC_{50} values. The highest antiradical activity was observed in the Allig extract and the lowest activity in Deglet Nour. The IC_{50} values of Allig, Bejo, and Deglet Nour were 16.70, 25.90, and 46.79 mg/mL, respectively.

The correlation between the free radical scavenging activity and total phenolic contents has been studied, and a positive correlation between them was observed ($R^2 = 0.97$) (Table 3). The high correlation coefficient reported that free radical scavenging activity may be attributed to their phenolic compounds.

Other studies showed good correlation between total phenolic contents and free radical scavenging activity [9].

The free radical scavenging activity of phenolic compounds is generally due to their redox properties, their ability to give a hydrogen using DPPH, and a single oxygen quencher. On the other hand, this activity is dependent not only on the concentration of phenolic compounds but also on the degree of hydroxylation and polymerisation [4, 7, 29–31].

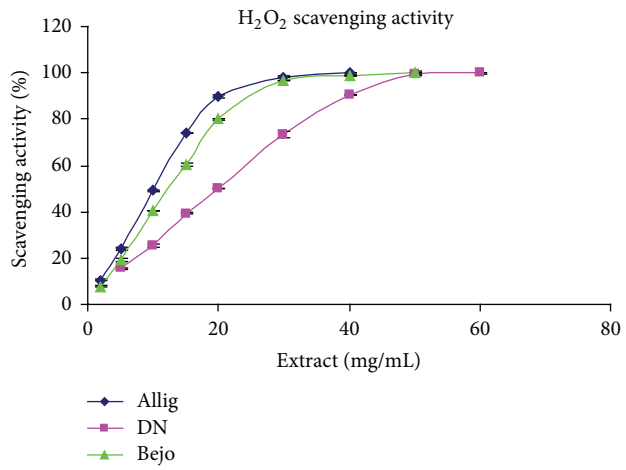

FIGURE 2: Effect of different concentrations on H_2O_2 scavenging activity in different date extracts: Allig, Bejo, and Deglet Nour (DN). Values are means of three replications ±SD.

Correlation between flavonoids for each variety and free radical scavenging activity was calculated. A highly significant influence was also exhibited with $R^2 = 0.98$ (Table 4). It is apparent that flavonoids were important phenolic compounds of the date contributing to the antioxidant activity. This is in agreement with the results reported by Mansouri et al. [16] and Biglari et al. [1, 15]. However, Harris and Brannan [28] mentioned that there is no positive correlation between flavonoid levels and radical scavenging activity.

In fact, the free radical scavenging activity of flavonoids is generally due to the number and arrangement of the hydroxyl groups of flavonoids [4].

3.2.2. FRAP Assay. For determination of antioxidant activity, we used the FRAP assay (ferric reducing antioxidant power). This test has been widely used to determine the antioxidant capacity of different extracts. The method is based on the ability of the sample to reduce the ferric iron (Fe^{3+}) to ferrous iron (Fe^{2+}) in the presence of antioxidants [1, 9, 20, 27].

The averages of antioxidant activity based on FRAP assay are given in Table 2. There was a significant difference ($P < 0.05$) between all types of dates.

The FRAP activity of different extracts is in the following order: Allig (4.98 mmol [$FeSO_4$]/100 g extract), Bejo (3.24 mmol [$FeSO_4$]/100 g extract), and Deglet Nour (1.96 mmol [$FeSO_4$]/100 g extract). This large variability can be explained by the influence of different varieties.

These results are in agreement with the results determined by DPPH scavenging activities which showed that the highest antioxidant activity was obtained in Allig extract and the lowest activity in Deglet Nour extract.

A positive linear correlation between the total antioxidant activity, determined by the FRAP method, phenolic contents, and flavonoids was observed ($R^2 = 0.96$ (Table 3) and $R^2 = 0.96$ (Table 4), resp.).

The strong positive relationship indicates that phenolic and flavonoid contents were important compounds contributing to the antioxidant activities of date extract, but it is also possible that other phenolic compounds could contribute to the antioxidant capacities of date. These results are in agreement with other previous studies which presented a strong correlation between reducing power determined by FRAP assay and phenolic and flavonoid contents [1, 9, 20].

The reducing capacities are generally due to the ability of natural extract to reduce the cations by breaking the free radical chain by donating a hydrogen atom.

3.2.3. H_2O_2 Scavenging Activity. The scavenging ability of different date extracts with hydrogen peroxide is shown in Figure 2. It is noticed that all the date extracts exhibited a potential hydroxyl radical scavenging activity and this activity increases with an increasing concentration. These results revealed that the highest hydroxyl radical scavenging activity was in the Allig date extract. In fact, at the dose of 20 mg/mL, the scavenging effect of date extract with the H_2O_2 radical is as follows: Allig (89.99%); Bejo (80.14%), and Deglet Nour (50.32%).

On the other hand, Table 2 summarized the IC_{50} values from all date extracts and showed that significant differences ($P < 0.05$) were observed between them. So, the IC_{50} values from Allig, Bejo, and Deglet Nour were 10.51, 12.50, and 20.44 mg/mL, resp.).

Concerning H_2O_2 scavenging activity, a strong correlation was established with the phenolic contents of different extracts ($R^2 = 0.94$; Table 3) and the flavonoid contents ($R^2 = 0.95$; Table 4). These results suggest that phenol and flavonoid contents could be the probable contributors to their antioxidant activities and may probably be involved in removing the H_2O_2.

The present study is in agreement with many authors who have observed a direct correlation between hydroxyl radical activity and total phenolic and flavonoid contents [2, 6].

The quenching hydroxyl radical activity of different date extracts can be explained by the prevention of the propagation of lipid peroxidation process and the reduction of chain reaction due to the presence of phenolic compounds. However, hydrogen peroxide is dangerous because it can form the hydroxyl radical. This reactive free radical may be the origin of the toxic effects, the cytotoxicity of the mammalian and bacterial cells [2, 4, 6, 21]. Therefore, it is important to remove the H_2O_2 for antioxidant activity in cells or food systems.

3.2.4. Metal Chelating Activity. The metal chelating capacity is based on chelating of Fe^{2+} ions by the ferrozine reagent.

The presence of Fe^{2+}-ferrozine complex was measured by the reduction of formation of red-coloured complex [21].

In the present study, the chelating power from the three date extracts is shown in Figure 3. The results obtained indicated a concentration-dependant antioxidant capacity. The percentage of metal scavenging capacity of 50 mg/mL of Allig, Bejo and Deglet Nour was found to be 35.84%, 34.68%, and 30.81%, respectively.

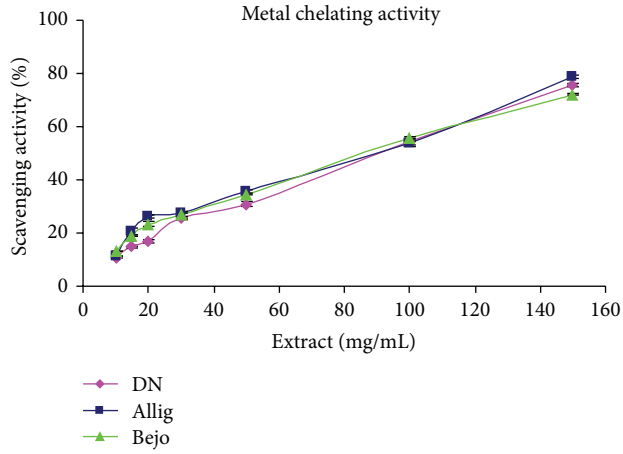

Figure 3: Effect of different concentrations on metal chelating activity in different date extracts: Allig, Bejo, and Deglet Nour (DN). Values are means of three replications ±SD.

These results were lower than that obtained by the previous activities. This is in agreement with the results obtained by Al-Mamary et al. [4] who showed that the palm date syrups have a low to intermediate iron binding capacity.

Results expressed as IC_{50} values are summarized in Table 2. Allig possessed the lowest IC_{50} value (85.19 mg/mL), but there is no significant difference between IC_{50} values from Bejo and Deglet Nour ($P < 0.05$) (90.72 mg/mL, 91.71 mg/mL, resp.).

In addition, the correlation of total phenolic contents and total flavonoids with metal chelating activity of various date extracts has been studied. Positive correlation factors were observed between chelating power and phenolic compounds ($R^2 = 0.74$; Table 3) and flavonoids ($R^2 = 0.72$; Table 4). These results suggest that metal chelating activity may be related to the presence of phenolic compounds. In fact, the metal chelating agents are effective as secondary antioxidants; they can inactivate metal ions (Fe^{2+}) and reduce the redox potential.

However, iron is essential for many activities like respiration, but it is capable of generating free radicals contributing to lipid peroxidation, protein modification, and DNA damage [4, 21].

4. Conclusions

The present study reported that Tunisian date varieties can be a good source of natural antioxidant. The antioxidant activities were evaluated using different methods, such as free radical scavenging activity, FRAP assay, H_2O_2 scavenging activity, and metal chelating activity. However, on the bases of the used methods, the antioxidant efficiency of date extracts can be arranged as follows: Allig > Bejo > Deglet Nour. This arrangement could be due to the difference in the phenolic, flavonoid, carotenoid, and tannin contents. A strong correlation was observed between antioxidant activities and phenolic and flavonoid contents.

These results signify that dates fruits can be a good source of natural antioxidant, which can play an important role in reducing oxidative stress and protecting of the human health from dangerous diseases, including cancer and liver and cardiovascular diseases.

Due to the importance of these scientific results, this work can be extended in vitro by evaluating the stabilization of edible oil with natural antioxidant and in vivo to evaluate the oxidative properties.

Conflict of Interests

According to this manuscript titled "In vitro antioxidant activities of three selected dates from Tunisia (*Phoenix dactylifera* L.)", there is no conflit of interests regarding the publication of this paper.

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