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Antioxidant Activity and Total Phenolic Content of Date Palm (*Phoenix dactylifera* L.) Fruits from Taif Governorate, Saudi Arabia.

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ABSTRACT

Date palm (*Phoenix dactylifera* L.) fruit is well known for its nutritional and health benefits in Middle East countries. The present study has been carried out to evaluate antioxidant activity and total phenolic content of three date varieties known as Sefri, Sari and Ruzeiz, from Taif Governorate, Saudi Arabia. The samples, at their tamr stage of ripening, were extracted separately with five solvents of decreasing polarity; 20% and 50% aqueous ethanol, absolute ethanol, acetone and ethyl acetate using incubator orbital shaker. Their antioxidant activity was evaluated by 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay. The results were expressed as efficient concentration (EC_{50}) and ascorbic acid equivalent antioxidant capacity (AEAC). The total phenols were quantified by Folin-Ciocalteu assay as tannic acid equivalent (TAE). The highest antioxidant activity was shown by Sari 50% aqueous ethanolic extract with EC_{50} 9.23±0.09 mg/mL and AEAC 61.38±2.05 mg AE/100 g fresh weight of dates. Highest phenolic content was shown by the aqueous alcoholic extracts in the range 1744.90±37.82-1165.49±120.02 mg TAE/100 g fresh weight of dates. Statistical analyses revealed strong positive, significant correlation ($r=0.89$, $P<0.0014$) between antioxidant activity and total phenolic content of alcoholic extracts. Strong negative, significant correlation ($r=-0.87$, $P<0.0022$) had been found between EC_{50} and AEAC.

Keywords: Date palm fruits, *Phoenix dactylifera* L., free radical scavenging activity, ascorbic acid equivalent antioxidant capacity, total phenolic content, tannic acid equivalent.

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INTRODUCTION

Date palm (*Pheonix dactylifera* L.) of the family Arecaceae is of great socio-economic importance in Saudi Arabia. Saudi Arabia is one of the leading date producing countries with about 450 cultivars known [1]. The estimated annual production of dates in Saudi Arabia is 1.05 million tons as reported by Food and Agricultural Organization (FAOSATA, 2012) [2]. Many areas in Saudi Arabia are famous for its own cultivars like Ajwa and Ambara in Madinah, Sukkary in Qassim and khalas in Al-Hassa [1]. Date palm fruits are characterized by high nutritional value. They contain sugars (70% of the pulp), mainly glucose, sucrose and fructose, fibers and less amount of proteins and fats [3]. They also contain vitamins like riboflavin, thiamine, biotin, folic and ascorbic acid that are essential for the human body [3]. They are rich in minerals such as iron, calcium, cobalt, copper, fluorine, magnesium, manganese, potassium, phosphorus, sodium, copper, sulfur, boron, selenium and zinc [3,4]. Several Studies on date palm fruits of different origins, cultivars and ripening stages have shown that they possess antioxidant properties such as the date fruits from Bahrain [5], Tunisia [6,7], Algeria [8,9] and Iran [10,11]. Total phenols and antioxidant activity of the aqueous and alcoholic extracts of three date varieties, Khalas, Sukkari and Ajwa, from Saudi Arabia was evaluated by Saleh and co-workers [12]. Also date fruit seeds from seven samples of Saudi Arabia origin known as Soukari, Soulag, Barhi, Khulas, Roziaz, Soughi and Monaif were evaluated for energy value, crude oil content, crude protein, total phenolic and antioxidant activity [13]. The presence of phenolic compounds which possess an extremely high antioxidant capacity was also reported by many authors in date palm fruits. They contain anthocyanins, phenolic acids such as protocaechuic, p-hydroxy benzoic, vanillic, syringic, caffeic, coumaric, ferulic, hydroxy benzoic, cinnamic acid and flavonoids [14-17].

Antioxidants are vital substances, which possess the ability to protect the body from damages caused by free radical-induced oxidative stress [18]. Free radicals are produced in the body during drug metabolism, exposure to ionizing radiation, UV light and pollution [19]. There are also a number of reports in the literature confirming that excessive production of reactive oxygen and nitrogen species (ROS and RNS) lead to many diseased states like aging, inflammation, cancerous mutation, cell death, diabetes, hypertension, atherogenesis, Alzheimers, Parkinsons and cardiovascular disease [14,19,20]. Antioxidants scavenge the free radicals, in other words, present of antioxidants can inhibit or delay the oxidation of other molecule by inhibits the initiation or propagation of oxidizing chain reactions by free radicals [21].

Recently, more research studies are focus on the development of new drugs and antioxidants based on different products of plant species. Many methods are used to evaluate the antioxidant activity of plant extracts, among them are the scavenging of 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical, the trolox equivalent antioxidant capacity assay (TEAC), superoxide radicals scavenging, hydrogen peroxide scavenging (H_2O_2), hypochlorous acid scavenging (HOCl), hydroxyl radical scavenging, peroxyl radical scavenging and the scavenging of the radical cation 2,2-azinobis-(3-ethylbenzothiazoline-6-sulphonate) (ABTS) as reviewed by Sanchez-Moreno [22].

To the best of our knowledge there is no report on antioxidant capacity of date palm fruit varieties grown in Taif Governorate, Saudi Arabia. Therefore, this study was carried out to assess the antioxidant activity of three date palm fruit varieties from Taif Governorate, using 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical assay. An attempt was made to relate the antioxidant activity with total phenolic content, which was evaluated by the spectrophotometric Folin-Ciocalteu assay.

MATERIALS AND METHODS

Plant Material

Three varieties of date palm (*Phoenix dactylifera* L.) fruits were collected from a date palm farm in Alelaba, Turabah, Taif Governorate, Saudi Arabia during August 2015. The varieties were identified by experience date farmer and merchants to be Sefri, Sari and Ruzeiz. The samples were in Tamr stage of ripening. They were characterized by light to deep brown colour as shown in Figure 1. After collection, the samples were thoroughly washed with distilled water followed by surface drying on the bench at room temperature for 24 hrs. Then they were wrapped with aluminum foil and kept in food plastic bags in the refrigerator freezer until analysis.

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Figure 1: Varieties of date palm fruits: [a]Sefri, [b]Sari, [c]Ruzeiz

Chemicals

2,2-Diphenyl-1-picrylhydrazyl free radical ($DPPH^{\bullet}$) and tannic acid were purchased from Sigma-Aldrich, Germany. Folin & Ciocalteu's phenol reagent, Fehling B reagent, ammonia solution, mercuric chloride, potassium iodide and ferric chloride were obtained from Loba Chemie, India. Fehling A reagent was obtained from Panreac Quimica, Spain. Benedict's reagent was purchased from Com-india-lab, India. L-(+)-Ascorbic acid was purchased from Scharlab S. L., Spain. Anhydrous copper sulphate was purchased from Scharlau Chemie S. A., Spain. Anhydrous sodium carbonate was purchased from PRS Panreac, Spain. The solvents; absolute ethanol, methanol, acetone, ethyl acetate and chloroform, were of analytical grade purchased from different sources.

Moisture Content

Moisture content (%) of the date varieties under study was carried out using electric oven (EO) (WTC binder, B28, Tuttlingen/Germany) and domestic microwave oven (MWO) (Elekta, EMO-306ss). Known weight of dates was carried out after removing the seeds and dividing into small pieces (average size: 0.5x0.5x0.3 mm). The EO temperature was adjusted in the range of 60-65ºC. The weight loss was recorded at different intervals of time until 24 hrs. The MWO power was adjusted to 270 W and the weight loss was recorded every 1 min for a period of 42 min until the weight did not decrease significantly with increasing drying time.

Preparation of Date Palm Fruits Extract

A weight of 20 g of dates was taken after removing the seeds and dividing the pulp to small pieces (av. size: 0.5x0.5x0.3mm) into 250 mL conical flask covered with aluminum foil and extracted separately with 150 mL of 20% aqueous ethanol (20% aq EtOH), 50% aqueous ethanol (50% aq EtOH), absolute ethanol (abs EtOH), Acetone and ethyl acetate (EtOAc) for 48 hrs at 40ºC with shaking rate at 120 rpm using incubated orbital shaker (MaxQ 4450 benchtop orbital shaker, Model 4334, Thermo Scientific, USA). The aq EtOH and abs EtOH extracts were centrifuged at 5000 rpm for 2 min using a centrifuge (Hettich, EBA20, Tuttlingen/Germany). The supernatant liquid was dropped out and the residue was washed with more solvent and centrifuged again. The supernatant liquids were combined and the volume of each extract was adjusted to 200 mL by the same solvent. The acetone and EtOAc extracts were separated by decantation and the residue was washed with more solvent and separated. The supernatants were combined and the solvent was evaporated to obtain a crude extract. All the resulted extracts were kept in refrigerator before analysis.

Phytochemical Analysis

The date palm fruits extracts were analyzed for phytochemical constituents following standard methods previously reported in literature [23-25].

Test for Sugars

Fehling's Test

To 0.5 mL of extract, equal amount of Fehling's reagent A and B was added. The mixture was heated in water bath. Formation of brick red precipitate indicates the presence of sugars.

Benedict's Test

To 0.5 mL of extract, few drops of Benedict's reagent were added. The mixture was boiled in water bath. Formation of orange-red precipitate indicates the presence of sugars.

Test for Phenols

To a fraction of the extract few drops of 10% aqueous ferric chloride were added. Appearance of green or blue colour indicates the presence of phenolic compounds.

Test for Flavonoids

To 0.5 ml of the extract, 2 mL of dilute ammonia solution was added followed by addition of concentrated sulphuric acid. Yellow colouration indicates the presence of flavonoids.

Test for Coumarins

To a fraction of extract, 10% sodium hydroxide was added. Formation of yellow colour indicates the presence of coumarins.

Test for Saponins

The extract was dissolves in distilled water and vigorously shaken. Formation of stable forth indicates the presence of saponins.

Test for Carotenoids

1 mL of chloroform was added to fraction of extract and well shaken followed by addition of few drops of concentrated sulphuric acid. A blue colouration at the interface indicates the presence of carotenoids.

Test for Terpenoids

1 mL of chloroform was added to a fraction of extract followed by addition of few drops of concentrated sulphuric acid. A reddish brown colour at the interface indicates the presence of terpenoids.

Test for Proteins

To a fraction of the extract, 10% sodium hydroxide was added followed by addition of few drops of 0.1% Copper (II) sulphate. A violet or pink colour indicates the presence of proteins.

Test for Alkaloids

Wagner's reagent

To a fraction of extract, few drops of Wagner's reagent (2 g of iodine and 6 g of potassium iodide in 100 mL distilled water). Formation of reddish brown precipitate indicates the presence of alkaloids.

Mayer's reagent

To a fraction of extract, few drops of Mayer's reagent (1.36 g of mercuric chloride and 5 g of potassium iodide in 100 mL distilled water). Formation of creamish precipitate indicates the presence of alkaloids.

Determination of Antioxidant Activity

Free Radical Scavenging Assay

Free radical scavenging activity (RSA) of the date palm fruits varieties was evaluated using 2,2-diphenyl-1-picrylhydrazyl free radical (DPPH$^{\bullet}$) as already described by Brand-Williams et al. [26] and Christine Stanly et al. [27] with slight modifications. In brief, series of extracts dilutions with the same extracting solvent were prepared (acetone and EtOAc extracts were dissolved in methanol). 1 mL of each solution was mixed with 1 mL of DPPH$^{\bullet}$ methanolic solution (0.1 mM) in a test tube. The mixture was well shaken, wrapped with aluminum foil and left to stand for 30 min at room temperature. Absorbance of the remaining DPPH$^{\bullet}$ was measured at 517 nm using UV-Visible Spectrophotometer (UV-2400PC, Unicom-Optics). Mixture of extracting solvent and methanol (1:1) was taken as blank, while a mixture of 1 mL extracting solvent or methanol and 1 mL of DPPH$^{\bullet}$ was used as control. L-(+)-ascorbic acid was used as reference antioxidant. The measurements were done in triplicate. The radical scavenging activity was expressed as percentage of inhibition (%I) and calculated using the following formula:

$$Inhibition\ percent\ (\%I) = \frac{Abs_{control} - Abs_{sample}}{Abs_{control}} \times 100$$

Where Abs_{sample} is the absorbance of remaining DPPH$^{\bullet}$ when the extract was added and $Abs_{control}$ is the absorbance of remaining DPPH$^{\bullet}$ when only extracting solvent or methanol was added.

Ascorbic Acid Equivalent Antioxidant Capacity assay

DPPH free radical scavenging activity was evaluated as ascorbic acid equivalent antioxidant capacity (AEAC) following a modified method reported in literature [28-30]. To 1 mL aliquots of ascorbic acid in methanol (0.5-8 µg/mL) or known dilutions of date palm fruits extracts 1 mL of DPPH$^{\bullet}$ methanolic solution (0.1 mM) was added. The mixture was well shaken and incubated for 30 min at room temperature. The absorbance of the remaining DPPH$^{\bullet}$ was measured at 517 nm. Pure methanol was taken as blank and a mixture of 1 mL methanol and 1 mL DPPH$^{\bullet}$ solution was taken as control. The measurements were done in triplicate. The result was expressed as mg ascorbic acid equivalent (AE)/100 g fresh weight (FW) of date palm fruits by using an equation that was obtained from the standard ascorbic acid curve.

Determination of Total Phenolic Content

Total phenolic content (TPC) of date varieties extracts was measured using Folin-Ciocalteu's colorimetric assay as described by Christine Stanly and co-workers [27] with tannic acid as standard phenolic compound [31, 32]. A volume of 100 µL of the date palm fruits extracts or tannic acid standard (in the range of 25-350 µg/mL) was mixed with 400 µL distilled water in a test tube followed by addition of 100 µL of Folin-Ciocalteu's phenol reagent (2N). The solution was well mixed and allowed to stand for 6 min at room temperature. Then 1 mL of 7% sodium carbonate solution was added and the mixture was diluted to 2.4 mL with distilled water. Absorbance of the colour developed for 90 min was recorded at 760 nm using UV-Visible Spectrophotometer (UV-2400PC, Unicom-Optics). The measurements were repeated three times. TPC was calculated by using an equation that was obtained from the standard tannic acid curve and expressed as tannic acid equivalent (TAE)/100 g fresh weight (FW) of date palm fruits.

Kinetic Evaluation

Kinetics of the reaction between DPPH$^{\bullet}$ and the abs EtOH extracts of date palm fruits were carried out using Spectrophotometer (UV-2400PC, Unicom-Optics) at 517 nm light absorbance. For this 1 mL of the DPPH$^{\bullet}$ (50 µg/mL) in methanol is added to 500 µL of sample extract in methanol in a disposable cuvette, the volume was completed to 2 mL with methanol. The control was prepared by adding 1 mL of DPPH$^{\bullet}$ in methanol to 1 mL of methanol. The drop in absorbance was followed immediately after the mixing by recording the reading each one or two seconds for the first two min, then each five min until reaching platue (approximately 30 min), absorbance of DPPH$^{\bullet}$ was converted to remaining concentration using molar extinction coefficient from Beer-Lambert plot obtained by measuring the UV-Visible absorbance at 517 nm of serial dilutions (100-6.25 µM) of DPPH$^{\bullet}$ solution in methanol. Pure methanol was used as blank.

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Statistical Analysis

Statistical data analyses were carried out using MS Excel 2010. All data presented in this study are means of three replicates along with standard deviations. Pearson's correlation coefficient was calculated where necessary for the presented data.

RESULTS AND DISCUSION

Moisture Content

The moisture content of date samples was shown in Figure 2 as determined using EO and MWO. The EO temperature was adjusted in the range of 60-65ºC as higher temperatures above 65ºC involve visual and quality degradation of foods [33,34]. The moisture content was calculated as percent of fresh weight (FW) of dates. Highest moisture contents 18.75 and 18.28% were shown for Ruzeiz in both EO and MWO drying method respectively, while Sari showed the lowest moisture content as 7.81 and 10.26%. Dates ripen in four stages, named by their Arabic denominations; kimri (unripe), khalal (full-size, crunchy), rutab (ripe, soft) and tamr (ripe, reduced moisture) [10]. The date goes from one extreme of moisture content of 85% at early Kimiri stage to 50–60% for Khalal, about 35–40% for Rutab, and about 20% for Tamr [10,35]. Therefore the date samples under study were classified as Tamr with moisture content of 7.81-18.75%.

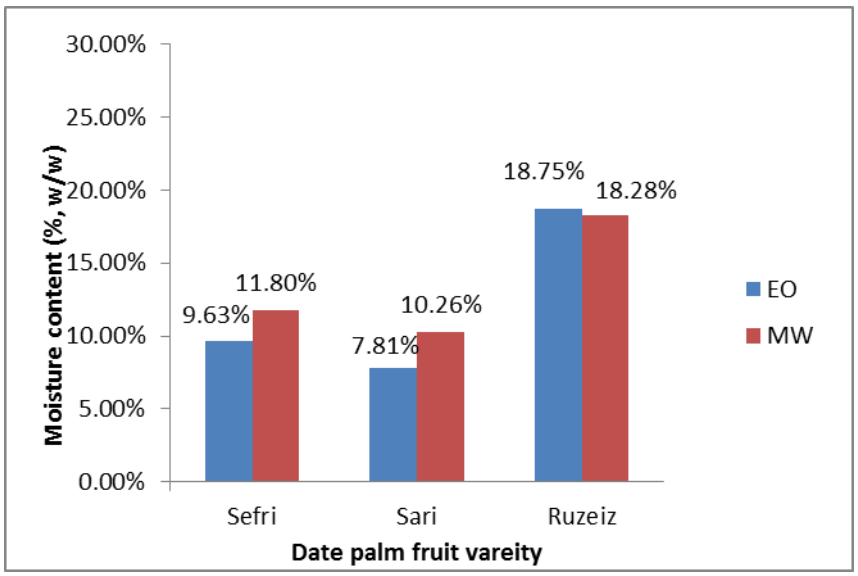

Figure 2: Moisture content of date varieties (% w/w, FW)

Extracts of Date Palm Fruits

The dates varieties under study were extracted separately with solvents with decreasing polarity as follows: 20% aq EtOH, 50% aq EtOH, abs EtOH, acetone and EtOAc. The higher yields were obtained by the aq EtOH solvents followed by the abs EtOH and very low yields were obtained by acetone followed EtOAc as shown in Table 1. The high polar constituents of date, mainly sugars (71.2–81.4% dry weight) [36], account for the high yield given by aqueous alcoholic solvents.

Phytochemical Analysis

Preliminary phytochemical screening results were shown in Table 1. Sugars were almost present in all extracts with very low concentrations in acetone and EtOAc extract as tested by Benedict's and Fehling's reagent. Although ferric chloride test was negative for the presence of phenols, considerable quantity of phenols was given by Folin-Ciocalteu spectrophotometric assay in aqueous and absolute alcoholic extracts of the dates (Table 2).

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Table 1: Yield % and preliminary phytochemical analysis of the date palm fruits varieties extract from Taif Governorate, Saudi Arabia

Date variety	Extracting solvent	Yield (%)	Sugars (Fehling's)	Sugars (Benedict's)	Phenols	Flavonoids	Coumarins	Saponins	Carotenoids	Terpenoids	Proteins	Alkaloids (Wagner's)	Alkaloids (Mayer's)
Sefri	20% aq EtOH	68.12	++++	++++	-	+	+	+	-	-	-	-	-
	50% aq EtOH	73.00	++++	++++	-	+	+	-	-	-	-	-	-
	abs EtOH	15.62	++	++	-	+	+	-	-	-	-	-	-
	Acetone	2.15	+	+	-	+	-	-	-	-	-	-	-
	EtOAc	0.32	+	+	-	+	-	-	-	-	-	-	-
Sari	20% aq EtOH	72.02	++++	++++	-	+	+	+	-	-	-	-	-
	50% aq EtOH	64.62	++++	++++	-	+	+	-	-	-	-	-	-
	abs EtOH	15.41	++	++	-	+	+	-	-	-	-	-	-
	Acetone	1.60	+	+	-	+		-	-	-	-	-	-
	EtOAc	0.23	+	+	-	+	-	-	-	-	-	-	-
Ruzeiz	20% aq EtOH	65.56	++++	++++	-	+	+	+	-	-	-	-	-
	50% aq EtOH	63.86	++++	++++	-	+	+	-	-	-	-	-	-
	abs EtOH	13.43	++	++	-	+	+	-	-	-	-	-	-
	Acetone	2.21	+	+	-	+		-	-	-	-	-	-
	EtOAc	0.25	+	+	-	+	-	-	-	-	-	-	-

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Table 2: DPPH free radical scavenging activity (EC_{50}), ascorbic acid equivalent antioxidant capacity (AEAC) and total phenolic content (TPC) of date varieties from Taif Governorate, Saudi Arabia (based on fresh weight, FW)

Date variety	Extracting solvent	DPPH$^{\bullet}$, EC_{50} (mg/mL) FW [b]	AEAC (mg AE/100 g) FW [c]	TPC (mg TAE/100 g) FW [d]
Sefri	20% aq EtOH	14.11±0.24	39.74±0.41	1343.92±34.09
	50% aq EtOH	13.73±0.15	35.85±2.08	1165.49±120.02
	Abs EtOH	80.63±0.40	6.13±0.19	560.59±70.71
	Acetone	> 200	1.33±0.06	16.47±8.32
	EtOAc	> 200	0.87±0.15	< 10
Sari	20% aq EtOH	9.98±0.16	39.25±0.56	1560.59±41.18
	50% aq EtOH	9.23±0.09	61.38±2.05	1744.90±37.82
	Abs EtOH	74.35±0.55	7.36±0.19	513.53±66.55
	Acetone	> 400	0.81±0.06	37.06±4.16
	EtOAc	> 400	0.67±0.04	< 10
Ruzeiz	20% aq EtOH	15.73±0.09	24.09±0.25	1196.86±130.22
	50% aq EtOH	17.14±0.09	29.19±0.19	1259.61±29.46
	Abs EtOH	81.11±0.26	6.39±0.21	287.06±54.07
	Acetone	150.92±1.53	1.63±0.01	90.98±22.08
	EtOAc	> 400	0.37±0.01	< 10
Ascorbic acid [a]	-	4.60×10^{-3}±0.02	-	-

Results are mean (n = 3) ±SD.
[a] Positive control.
[b] Efficient concentration, EC_{50} (mg/mL): mg/mL fresh weight (FW) of sample needed to decrease the initial DPPH$^{\bullet}$ concentration by 50%.
[c] Antioxidant activity calculated as mg equivalent of ascorbic acid equivalent (AE)/100 g of fresh weight of sample.
[d] Total phenolic content calculated as mg tannic acid equivalent (TAE)/100 g of fresh weight (FW) of sample.

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DPPH Free Radical Scavenging Activity

DPPH$^{\bullet}$ scavenging assay is fast and commonly used test to evaluate the radical scavenging activity of plant extracts. DPPH$^{\bullet}$ is stable free radical, has a deep violet colour in solution, and it becomes colourless or pale yellow when reacted with an antioxidant. The discoloration of DPPH$^{\bullet}$ solution usually depends on antioxidant capacity and concentration. Antioxidant activity of extract is evaluated by spectrophotometric measurement at 517 nm of absorbance of DPPH$^{\bullet}$ after it reacted with different concentrations of extract. The results were reported either as inhibition (%I) or efficient concentration (EC_{50}). EC_{50} is defined as the concentration of extract needed to decrease the initial DPPH$^{\bullet}$ concentration by 50%. Potent antioxidants had higher %I and lower EC_{50}. Based on the data obtained in this study, the date palm fruits extracts showed a concentration-dependent antioxidant activity (Figures 3-5). High antioxidant activity (%I) was shown by the 50% aq EtOH extracts of all the three date samples (>80%) followed by the 20% aq EtOH (>70%) and abs EtOH (≈30%) (see Figure 6). Acetone and EtOAc extracts showed low antioxidant activity (<10%) due to low yield that was obtained (Table 1).

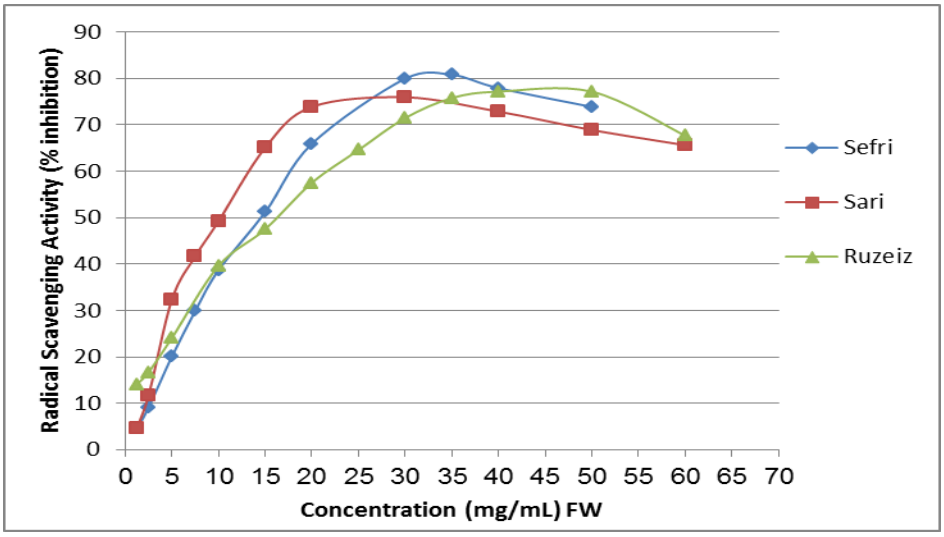

Figure 3: Scavenging effect of 20% aq EtOH extracts on DPPH free radical

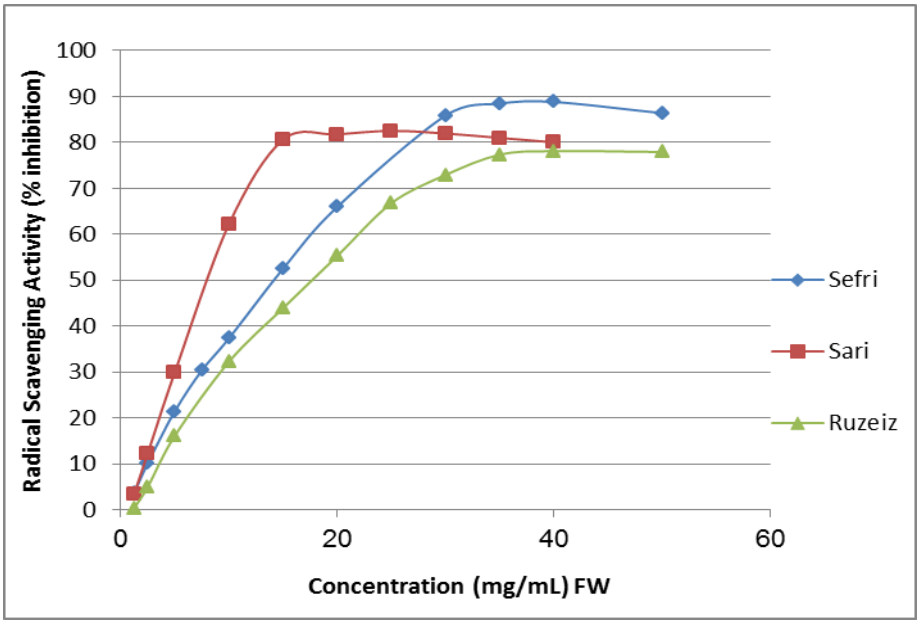

Figure 4: Scavenging effect of 50% aq EtOH extracts on DPPH free radical

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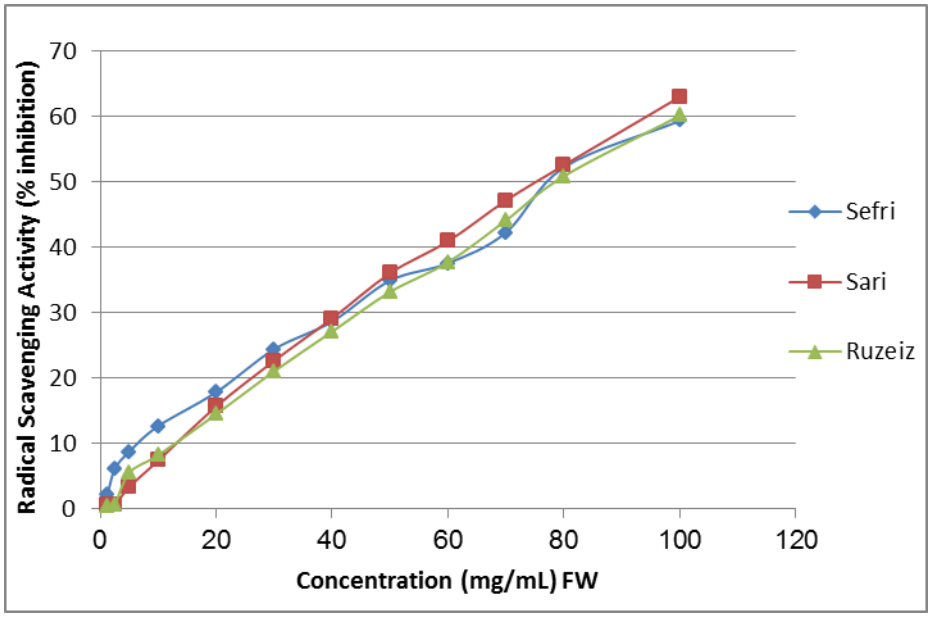

Figure 5: Scavenging effect of abs EtOH extracts on DPPH free radical

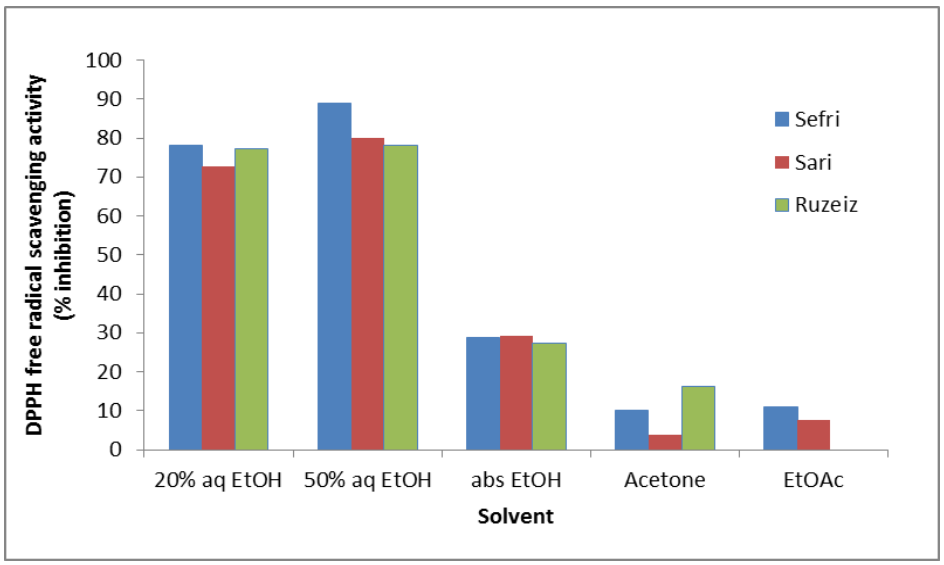

Figure 6: DPPH free radical scavenging activity (% I) of date varieties at concentration of 40 mg/mL fresh weight

The EC_{50} was calculated from equation that was obtained from the second order polynomial line using MS Excel 2010 software. The line regression (R^2 > 0.99) for all alcoholic extracts and slightly less for acetone and EtOAc extract. The calculated EC_{50} was shown in Table 2. Low EC_{50} value indicates the high antioxidant activity of the sample under study. The higher free radical scavenging activity was shown by the aq EtOH extracts with EC_{50} in the range of 9.23±0.09 – 17.14±0.09 mg/mL FW. The Abs EtOH extracts showed moderate activity in the range of 74.35±0.55-81.11±0.26 mg/mL FW, while very low activities were shown by acetone and EtOAc extracts.

Ascorbic Acid Equivalent Antioxidant Capacity

Ascorbic acid is well known for its antioxidant activity [37,38]. It is commonly used as positive control in antioxidant activity evaluation studies. In this study the antioxidant capacity of the date palm fruits towards $DPPH^{\bullet}$ was determined in comparison to antioxidant capacity of ascorbic acid. The antioxidant capacity of ascorbic acid determined by $DPPH^{\bullet}$ scavenging assay, showed a dose response of first order for the used concentration range (0.5-8 µg/mL in methanol). MS Excel 2010 was use to draw the graph of ascorbic acid

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concentration (µg/mL) against UV-Visible absorbance of the remaining DPPH$^{\bullet}$ as a difference from the control. A linear correlation (y = 0.0608x + 0.0027) with high regression (R^2 = 0.9985) was obtained (Figure 7). The absorbance of known dilutions of the date extracts which had antioxidant capacity towards DPPH$^{\bullet}$ within the range of the curve was recorded by the same method. AEAC was calculated from above equation and expressed as mg ascorbic acid equivalent (AE) per 100 g FW of date palm fruits as shown in Table 2. The higher the AEAC value the more potent is the extract as antioxidant. In this study higher AEAC value in the range 24.09±0.25 – 61.38±2.05 mg AE/100 g FW was shown by aq EtOH extracts of the three date varieties, followed by the abs EtOH extracts in the range 6.13±0.19 – 7.36±0.19 mg AE/100 g FW. Low AEAC values were resulted for acetone and EtOAc extracts for all date samples. When correlated the calculated AEAC with EC_{50}, lower EC_{50} value indicates higher antioxidant activity. The calculated values of AEAC support this statement. Statistical analysis showed a strong negative correlation (Pearson's coefficient, r = -0.87) between the EC_{50} and AEAC of ethanolic date extracts which is highly significant (p <0.0022). As shown in Table 2 for example the 50% aq EtOH extract of Sari date variety showed the highest AEAC value as 61.38±2.05 mg AE/100 g FW in agreement with its lowest EC_{50} value of 9.23±0.09 mg/mL FW. Therefore, it can be concluded that the AEAC assay is also quite reliable for evaluation of antioxidant activity of date palm fruits.

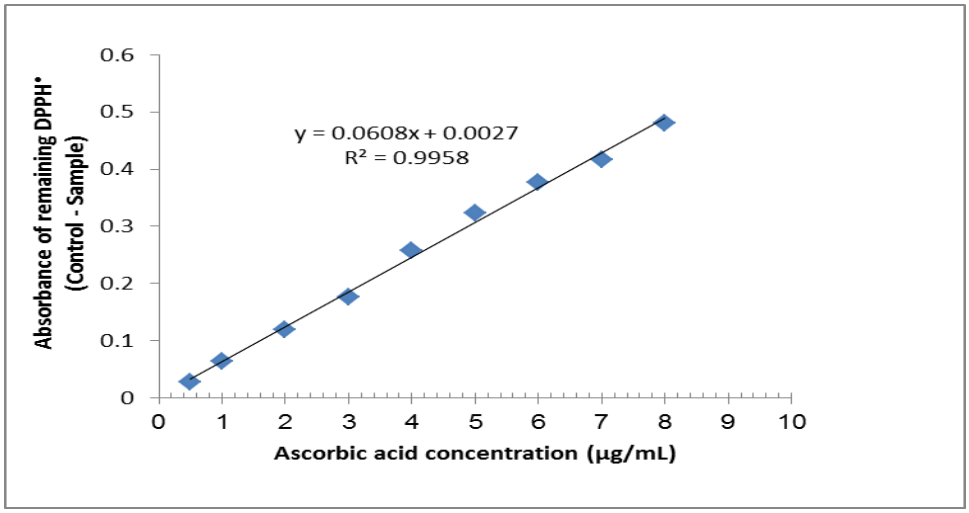

Figure 7: UV-Visible standard curve of ascorbic acid – DPPH free radical

Total Phenolic Content

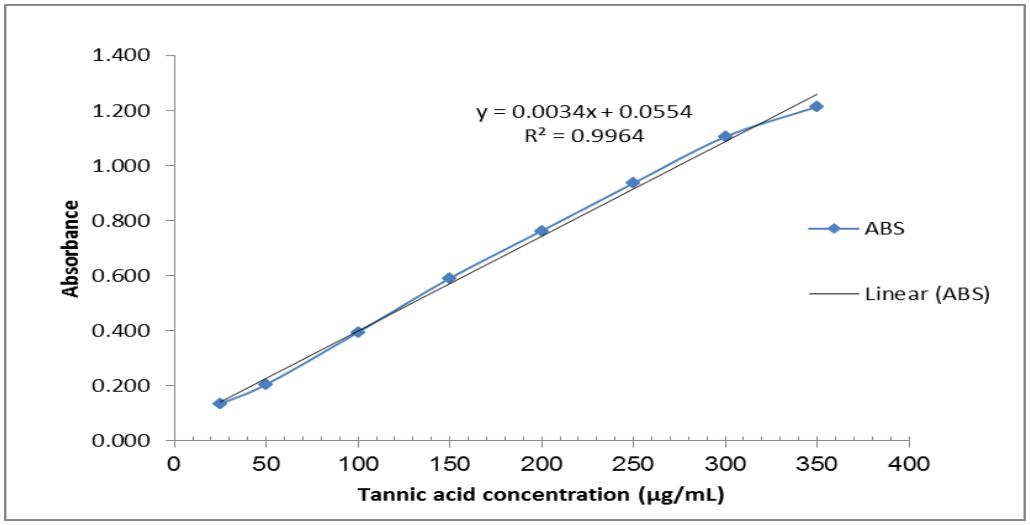

Figure 8: Standard curve of Folin-Ciocalteu – Tannic acid

Phenolic compounds are widely distributed in the plant kingdom. They are considered from the most abundant secondary metabolites and well known for their antioxidant potency [39,40]. Folin – Ciocalteu's

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Spectrophotometric assay is a reliable method and widely used for quantification of plant phenolic compounds [41]. Total phenolic content of date fruits extracts was quantified by Folin-Ciocalteu assay at 760 nm. The results were presented as mg TAE per 100 g FW from standard tannic acid curve (Figure 8, R^2 = 0.9964). As shown in Table 2 higher TPC was shown by aqueous alcoholic extracts with higher amount for Sari variety 50% aq EtOH equal to 1744.90±37.82 mg TAE/100 g FW, followed by Sari 20% aq EtOH, Safri 20% aq EtOH, Ruzeiz 50% EtOH, Ruzeiz 20% EtOH and Sefri 50% EtOH. Moderate TPC value was shown for abs EtOH extracts of the three samples between 560.59±70.71 - 287.06±54.07 mg TAE/100 g FW. Low TPC was shown by the three acetone extracts which less than 100 and very low amount was found for EtOAc which is less than 10 mg TAE/100 g FW.

The Correlation Between Antioxidant Activity and Total Phenolic Content

The positive correlation between antioxidant activity and total phenolic content of several date varieties of different origin had been reported by many authors [5,8,10,42]. The correlation between antioxidant activity and total phenolic content of dates varieties under study was shown in Figure 9. The Results showed a positive correlation coefficient between DPPH free radical assay and the TPC of date extracts (r=0.89) which is highly significant (p<0.0014). The coefficient R^2 = 0.7895 showed that the regression line is well represent the data association between DPPH free radical assay and TPC. The significant and linear relationship existed between the antioxidant activity and TPC of date extracts indicated that the phenolic compounds are highly contributed to antioxidant activity of the samples.

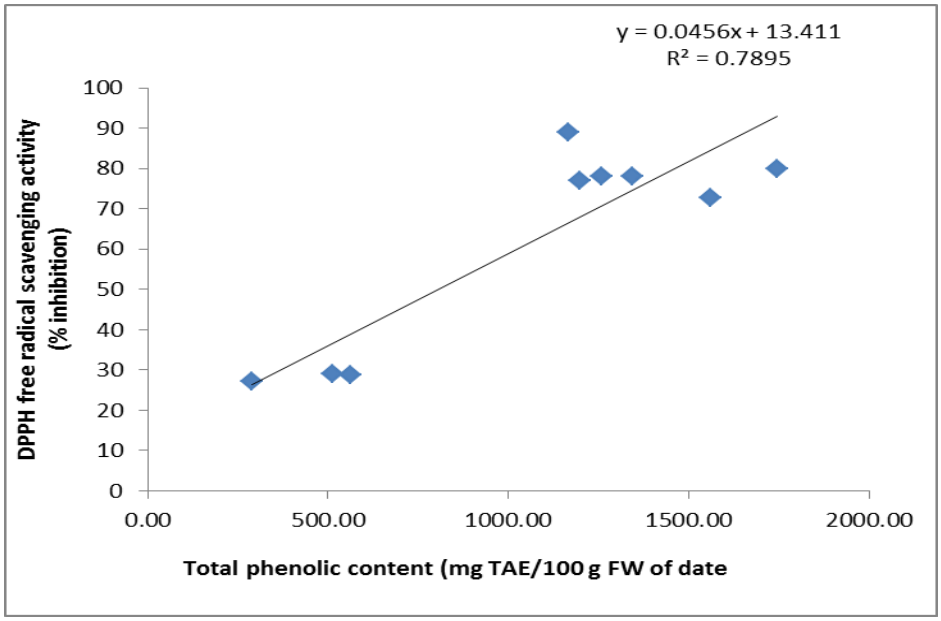

Figure 9: The correlation between total phenolic content and DPPH free radical scavenging activity of dates ethanolic extracts

Kinetic Evaluation

The kinetics of the reaction between DPPH$^{\bullet}$ and the abs EtOH extracts of the date varieties was shown in Figure 10. The concentration of the remaining DPPH$^{\bullet}$ was calculated using the molar extinction coefficient (1.07×10^4) obtained from Beer-Lambert Plot (y = 0.0107x, R^2 = 0.9979) as shown in Figure 11. From the graph different kinetics by different samples was shown. The three samples showed same kinetics in the initial of the graph, while Ruzeiz sample showed the fastest kinetics at the end of the graph. This finding can't be correlated to the sugar or flavanoids contents from Table 1, as the sugar, flavanoids and coumarin contents were almost the same for the three samples, but taking into consideration that the evaluation procedures for the above contents have a limitation regarding the detection limits; therefore the difference in kinetics can be accounted for the slight differences in the contents of the samples and even for the differences in the mineral contents, which can be considered as catalysts for DPPH$^{\bullet}$ reaction.

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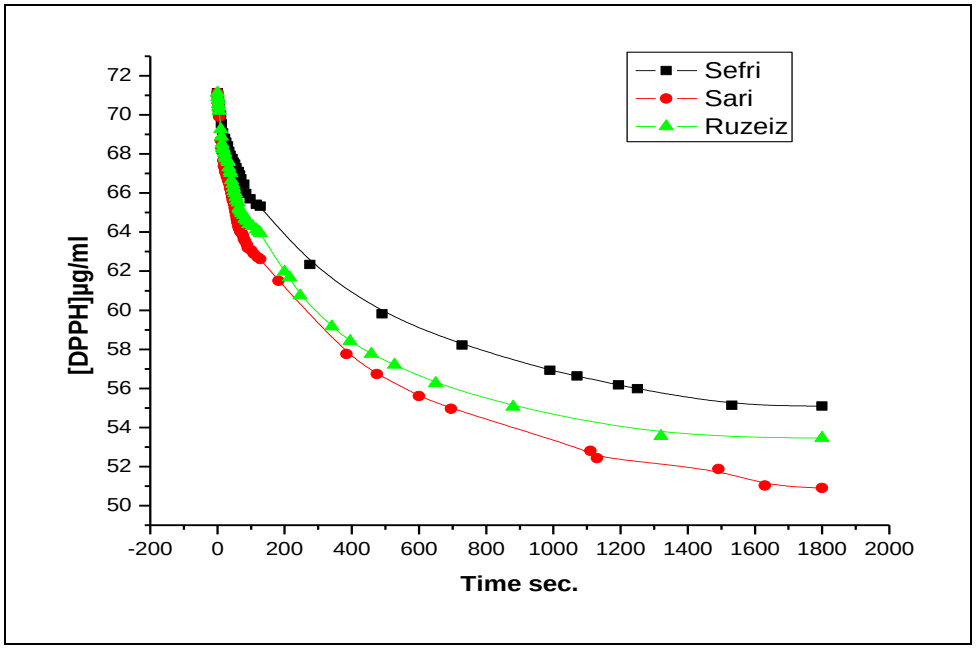

Figure 10: Drop in kinetics of the abs EtOH extract of date varieties (remaining DPPH$^{\bullet}$ concentration is calculated from absorbance using molar extinction coefficient value of 1.07x10^{4})

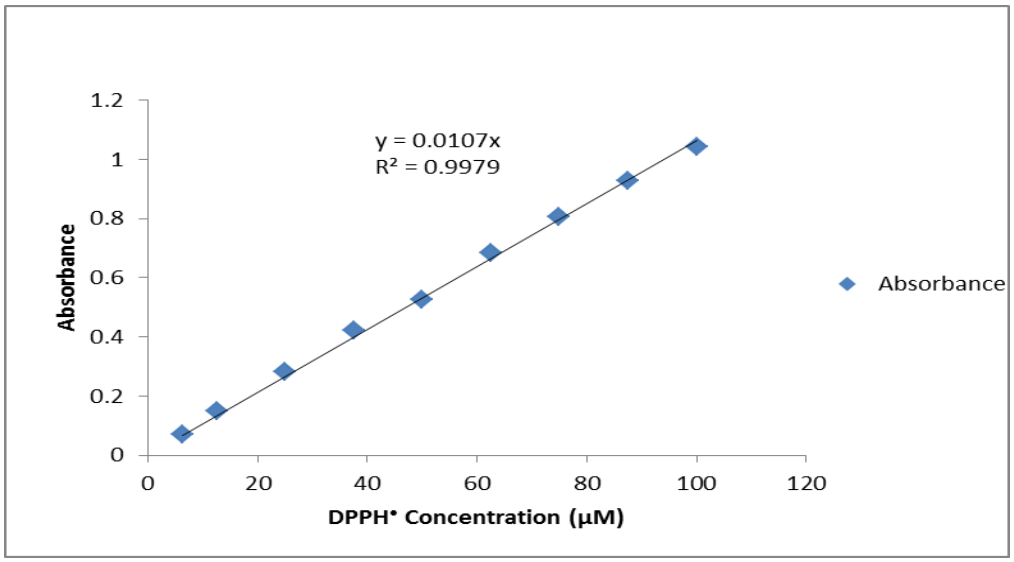

Figure 11: DPPH free radical UV-Visible spectrometry standard curve (Methanol, 517 nm)

CONCLUSION

The findings of this study indicate that the dates varieties herein studied possess antioxidant activity as evaluated by DPPH free radical scavenging assay and contained considerable amount of natural phenolic compounds which are strongly contributed to their antioxidant activity. The results indicate that the date palm fruits, in addition to their known nutritional value, are potent source of natural antioxidants which have numerous health benefits.

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