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Antioxidant and anti-hemolytic activities of phenolic constituents of six moroccan date fruit (*Phoenix dactylifera L.*) syrups

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ABSTRACT

Date fruits are traditionally used to prepare a wide range of products such as vinegar, jam and syrup named locally "*Tahlawt*"moreover, their direct consumption. The aim of this study is to investigate the antioxidant and anti-hemolytic activities of six syrups prepared traditionally form six different date fruit cultivars grown in southeast Morocco. Significant difference($P < 0.05$) was established among analysed syrups. The highest phenolic (6.70 g GAE /100g DW) and flavonoid content (932.82 mg RE /100g DW) was found in *Jihl* syrups which possessed the highest antioxidant activity based on FRAP (9.55 mmol TE/100g DW), ABTS (8.27 mmol TE /100 g DW), $DPPH_{IC50}$(381.99 µg/mL) and exhibited the highest membrane protective effect (317.70 min). *Tamaajount* syrup contains the lowest phenolic (3.72g GAE /100g DW) and flavonoid content (528.19 mg RE /100g DW) and presented the lowest antioxidant activity based on FRAP (5.30 mmol TE/100g DW), ABTS (4.68 mmol TE /100 g DW) and $DPPH_{IC50}$ (1.095 mg/mL) as well as the lowestmembrane protective effect (226.44 min). The results obtained suggest that date fruit syrups could be considered as a functional food or functional food ingredient because of their highphenolic compounds, which act as antioxidants and membrane stabilizer.

KEYWORDS

Syrups;
Antioxidant;
Antihemolytic;
Phenolic content.

INTRODUCTION

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are constantly generated in the human body and play dual role as both beneûcial species at moderate concentrations and deleterious species when there is animbalance between these species and antioxidant known as oxidative stress[1]. The oxidative stress has been implicated in over one hundred human disease conditions, such as cancer, cardiovascular disease, aging and neurodegenerative diseases[2]. Hence, antioxidant supplementation is one plausible strategy to maintain redox homeostasis by directly quenching excessive ROS and pro-

tecting or reinforcing endogenous antioxidative defense systems against oxidative stress[3]. In recent decades, a great deal of attention is being paid to a healthier diet, increasing the intake of fruit and vegetables. This is mainly justified because these foods are an important source of bioactive compounds, which prevent chronic ailments through combating oxidative stress[4].

Morocco occupies the 12 th in the world and 9 th Arab-largest producer of dates with an annual production above 113,000 tons[5].40% of this production is constituted from low-quality cultivars[6]. These important quantities of low quality dates are sold at low prices or integrated in animal feed, because of too hard texture. Although they still considered as a good source of sugars, minerals and other substances[7]. The oasis populations have developed a methods for processing this low-quality dates in various products more profitable, and more appreciated by consumers such as syrup locally known as "*Tahlawt*".

The aim of this research was to evaluate the phenolics content, antioxidant potential and antihemolytic activity of six Moroccan date fruit syrups. Hopefully, this study will provide sufficient experimental evidence of antioxidant activity and potential for further development and utilization of these syrups.

EXPERIMENTAL

Preparation of date fruit syrups

Six Moroccan date fruits varieties (Bouslikhen, Bousthammi, Iklan, Jihl, Lhafs and Tamaajount,) at low quality (hard texture or poorly conserved because of high content on water). The date fruits were pitted, crushed and cut to small pieces with a sharp knife. The result date fruit pieces was mixed with water at 1:3 ratio and left overnight to facilitate the extraction. The result mixture was boilled for about 2 hours than filtered through a cloth and water is again added to the presscake and the mixture is boiled again. This extraction is repeated 3 times, than the collected juice is concentrated by boiling to increase the Brix.

Preparation of rich polyphenol extracts

The rich Phenolic compounds extract was prepared according to the method of[8] with slight modiûcations. Briefly, 30 g of date fruit syrup was extracted with 150 ml acetone–water (4:1, v/v), at 35°C for 12 h using an orbital shaker-incubator. The mixture was then filtered and the filtrate was concentrated under reduced pressure at 40°C until the total evaporation of solvent, using a rotary evaporator. The results acetonic crude extract were kept at -20°C in dark glass bottles until use. The extract was dissolved in the water in known dilution to determine phenolic, flavonoids and condensed tannins content and their antioxidant capacity was evaluated using the same dilution.

Measurement of total phenolics compounds

The total phenolic contents in date fruit syrups were determined according to the method described by the International Organization for Standardization[9]. Brieûy, 100 µL of the extract was added to 500 µL of a 1/10 dilution of Folin–Ciocalteau reagent in water, then 400µL sodium carbonate solution (7.5% w/v) was added. The mixture was left for 60 min at room temperature and the absorbance was measured at 765 nm. The calibration curve was prepared using gallic acid. The total phenolic compounds were expressed as gallic acid equivalent in mg/100 g dry weight (DW) of date fruit syrup.

Measurement of flavonoid content

The total flavonoid content of date fruit syrups was determined by the method of[10]. One mL of date syrup extract was mixed with 4mL of distilled water. Then 0.3mL sodium nitrite solution (5%) was added, followed by 0.3 ml aluminum chloride solution (10%). Test tubes were incubated for 5 min at room temperature, and then 2 mL of sodium hydroxide (1M) were added to the mixture and then the final volume was made up to 10 mL with distilled water. The mixture was thoroughly vortexed and the absorbance was determined at 510 nm. Measurements were calibrated to a standard curve of prepared Rutin solution and the results were expressed as mg Rutin equivalents (RE)/100 of dry weight (DW) of date fruit syrup.

Measurement of total condensed tannins

Total condensed tannins were determined using method described by Heimler et al[11]. 400µL of the date fruit syrup extract was mixed with 3mL of Methanolic solution of vanillin (4%) and 1,5mL of concentrated hydrochloric acid. The mixture was incubated at room temperature for 15 and the absorbance was determined at 500nm. A calibration curve of catechin (0-300µg/mL) was prepared, and the results were expressed as mg CE (catechin equivalents)/100g of date fruit syrup.

ABTS radical scavenging assay

The ABTS radical scavenging was measured using the method of[12]. The ABTS radical cations (ABTS+) were produced by reacting aqueous solution of ABTS (7mM) with aqueous solution of potassium persulphate (2.45mM). The mixture was allowed to stand in the dark at room temperature for 12-16 hours before use, then diluted with distilled water to obtain an absorbance of 0.700 ± 0.005 at 734 nm. 30µL of the sample added to 3 mL of the ABTS radical solution were allowed at room temperature for 6 min and the absorbance at 734 nm was recorded immediately. A standard curve was obtained by using aqueous solution of Trolox. The total antioxidants were expressed as mmol of Trolox equivalents per 100 g of dry weight (DW) of date fruit syrup.

Ferric reducing antioxidant power assay

The ferric reducing activity of date fruits syrup extract was estimated based on the method of[13]. The FRAP reagent was prepared by mixing 50mL of acetate buffer (0.3M) at pH 3.6, 5mL tripydyltriazine (TPTZ) solution 10mM prepared in HCl (40 mM) and 5mL of Ferric chloride solution ($FeCl_3$) (20mM). 2 mL of the freshly prepared FRAP reagent was added to the 10µL of syrup extract. Then the absorbance was measured at 593 nm against the blank after 10 minutes at room temperature. The standard curve was constructed using Trolox. The result was expressed as Trolox equivalent in mmol /100 g of dry weight (DW) of date syrup.

DPPH radical scavenging activity

Scavenging radical activity of date fruit syrups against stable DPPH was assessed as describedby[14] method with slight modifications. The reaction mixture contained 100 µL of syrup extract at different concentration and 1, 9 mL of methanolic DPPH (0,3mM). The mixture was incubated at room temperature for 20 min and the absorbance was determined at 517 nm. The IC_{50} (concentration providing 50% inhibition) values were calculated from the plotted graph of scavenging activity against the concentrations of the samples.

The protective effect date fruit syrups against AAPH induced erythrocyte oxidative hemolysis

The anti-hemolytic activity induced by a peroxyl radical initiator, AAPH was measured according to the method established by Blache and Prost[15] with minor medications. Two hundred microliters of Rabbit blood collected in heparin bulbs was mixed with 10 µL of date fruit extract, and then 600 µL of AAPH (10%) was added. The mixture was incubated at 37ºC. The absorbance of the mixture was measured at 450 nm every 5 min. The date fruit syrup extract was replaced by Trolox and saline (0.9% NaCl) in the positive and negative control respectively. The Protective effects of date fruit extract on free radical induced hemolysis of erythrocytes were estimated from the time required for half-hemolysis.

Statistical analysis

Statistical analysis was performed using StatView 5.0 software. The experimental results were reported as mean ± SE (standard error) (n=5) on a dry weight. Analysis of variance (ANOVA) and post-hoc Bonferroni. ($p<0.0018$) tests were used to compare the experimental groups. Pearson's correlation coefficient (r) was used to measure the association between two variables. Differences at $p< 0.05$ were considered significant.

RESULTS AND DISCUSSION

Phenolic content

Phenolic compounds are ubiquitous constituents of plants and their major sources in human diet are fruit, vegetables and various beverages. Acetone is one of the most widely used solvents and was found

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TABLE 1 : Total phenolic, ûavonoids and condensed tannins content of studied date fruit syrup

	TPC g GA/100g DW	TFC mg RE/100gDW	CTC g CE/100gDW
Bousthammi syrup	5.84 ± 0.31[a]	758.68 ± 11.27	3.82 ± 0.14
Bouslikhen syrup	5.39 ± 0.17	664.31 ± 10.42[a]	3.19 ± 0.23[bc]
Iklane syrup	4.52 ± 0.34	655.71 ± 8.62[a]	3.32 ± 0.11[c]
Jihl syrup	6.70 ± 0.25	932.82 ± 9.62	2.48 ± 0.09[a]
Lhafs syrup	5.96 ± 0.24[a]	863.57 ± 9.38	2.26 ±0.13[a]
Tamaajount syrup	3.72 ± 0.12	528.19 ± 6.81	2.87 ± 0.19[b]

Values in average (n =5) ± SE. Averages, in the same column, with same letters are not signiûcantly different using post hoc Bonferroni tests (p < 0.0018). TPC: Total Phenolic content; TFC: Total Flavonoid content; CTC: Condensed Tannins content

TABLE 2 : Antioxidant activities of analysed date fruit syrups

	FRAP mmol TE /100g DW	ABTS mmol TE /100gDW	DPPH µg/mL
Bousthammi syrup	7.08 ± 0.33	6.23 ± 0.21[b]	760.59 ± 22.20
Bouslikhen syrup	7.69 ± 0.20	6.57 ±0.19[b]	562.83 ± 34.21
Iklane syrup	5.98 ± 0.24	5.46 ± 0.17	649.34 ± 45.64
Jihl syrup	9.55 ± 0.13	8.27 ± 0.34[a]	381.99 ± 17.98
Lhafs syrup	9.09 ± 0.27	7.93 ± 0.27[a]	447.31 ± 6.28
Tamaajount syrup	5.30 ± 0.17	4.68 ± 0.22	1095.58 ± 56.37

Values in average (n =5) ± SE. Averages, in the same column, with same letters are not signiûcantly different using post hoc Bonferroni tests (p < 0.0018)

to be more effective for extracting phenolic from date fruit[16]. In order to evaluate the potential antioxidant capacity of the extracts from date fruit syrups, it was reasonable to determine the content of various polyphenols in aqueous acetone.

TABLE 1 illustrate the phenolic, flavonoid, and condensed tannins content of analysed date fruit syrups. Among these date fruit syrups, the highest phenolic content (6.70 g GAE/100 g of syrup dry weight) was observed from *Jihl*syrup,however, *Tamaajount* syrup exhibited the lowest phenolic contents (3.72 mg GAE/100 g syrup dry weight). On the other hand Total flavonoid contents (TFC) ranged over 528.19 – 932.82 mg RE/100 g of syrup DW. Highest TFC was recorded for *Jihl* syrups, while the lowest was for *Tamaajount* syrup. Our results are very higher compared with previous investigations who found that phenolic content and flavonoid content of date fruit syrup ranged between 368.35 - 529.28 GAE/ 100 g FW and 39.56 - 194.51 mg CE/100g of syrup FW respectively for[17] and between 434.3 -769.6 CE mg/100 g FW and between 310.5 - 554.0 mg QE/ 100 g for respectively[18]. This differences in phenolic content may be due to the method of syrup preparation include temperature, time of incubation, pH as well as variety. Regarding condensed tannins content the highest amount of these compound was showed in *Lhafs*syrup, whereas *Iklane* syrup had the lowest values. The important amount of phenolic content depicted in the syrups, compared to date fruit which contain an amount of phenolic and flavonoid ranged respectively between (331.86-537.07 mg GAE/100g DW) and (68.88 - 208.53 mg of RE/100 g DW)[8],show that the thermal processes during syrups preparation may lead to improve the extraction of phenolic compounds from date fruit. The same observation on the effect of temperature of phenolic content was observed by[19] and[20]. However the low amount of flavonoid observed in this this study compared to total phenolic content could be due to their degradation during the boiling at syrups preparation the same observation was reported by[21] who have been observed a loss of about 22% in total flavonoids in boiled celery at a temperature of 50°C during 90s.

Evaluation antioxidant activities

Phenolics, such as flavonoids, phenolic acids, and tannins, are considered to be major contributors to the antioxidant capacity[22]. The evaluation of the

antioxidant activity of date fruit syrups required different methods because of their chemical complexity. In the present study, therefore three complementary methods were followed to evaluate the reducing ability and the capacity to scavenge free radicals.

The DPPH and ABTS radical scavenging assays are usually employed to evaluate the ability of antioxidants to scavenge free radicals. As the reaction between antioxidant molecules and radicals progresses, the absorbance of the reaction system decreases. Hence, the change of absorbance is used as a measure for the scavenging of DPPH and ABTS+ radicals, and the more rapidly the absorbance changes, the more potential antioxidant activity the samples possess[23]. The free radical-scavenging activity of analysed date fruit syrups is shown in TABLE 2 the highest scavenging activity based on ABTS assay (8.27 mmol TE/100g of syrups DW) and DPPH assay (381.99 µg of syrup DW/mL) was found in *Jihl* syrup. The lowest scavenging ability based on ABTS assay (4.68 mmol TE/100g of syrups DW) and DPPH assay (1095.58 µg of syrup DW/mL) was depicted in *Tamaajount* syrup.

The FRAP assay is a simple, inexpensive and widely employed method used to evaluate of antioxidant capacity of medicinal plants[24] which, is based on the ability of antioxidants to reduce ferric(Fe3+) ions to ferrous (Fe2+) ions in the presence of TPTZ, forming an intense blue ferrous (Fe2+)-TPTZ complex at an acid pH (3.6). The change is monitored spectrophotometrically at 593nm[25]. As seen from the data in the table, the FRAP values varied from 5.30 to 9.55 mmol TE/100g of syrups DW, In general, the studied date fruit syrups had very high antioxidant capacities. The highest FRAP value was observed in *Jihl* syrup and *Tamaajount* syrup had the lowest value.

Correlation between antioxidant capacities, phenolic content, flavonoids and condensed tannins content.

Correlation analysis was used to explore the relationships amongst antioxidant capacities, total phenolic, flavonoids and condensed tannins measured for all the syrup samples (TABLE 3). The result showed a positive linear correlation between the antioxidant capacities and total phenolic content ranged between $R^2 = 0.703$ for DPPH assay and $R^2 = 0.869$ for ABTS assay. Which is better than the correlation between flavonoids and antioxidant activity ranged between $R^2 = 0.722$ for DPPH assay and $R^2 = 0.906$ for ABTS assay. However very low correlation was found between condensed tannins content and antioxidant activity varied between $R^2 = 0.171$ for DPPH assay and $R^2 = 0.328$ for FRAP assay Therefore, flavonoids and phenolic acid compounds are the dominant contributor to the antioxidant activity. the strong correlation in this study confirm several studies which have revealed that the phenolic content in the plants are associated with the antioxidant activities, probably due to their redox properties, which allow them to act as reducing agents, hydrogen donors, and singlet oxygen quenchers[26].

Concerning the relationships between antioxidant assaysthe positive linear correlation between them which varied between $R^2 = 0.786$ for DPPH/FRAP and $R^2 = 0.994$ for FRAP/ABTS, suggested that antioxidant components in these date fruit syr-

TABLE 3 : Correlation phenolic and flavonoid content with antioxidant activities

	TPC	TFC	CT	FRAP	ABTS	DPPH	AhE
TPC	1						
TFC	0.899	1					
CT	0.067	0.200	1				
FRAP	0.864	0869	0.328	1			
ABTS	0.869	0.906	0.328	0.994	1		
DPPH	0.703	0.722	0.171	0.786	0.811	1	
AhE	0.931	0.926	0.220	0.936	0.939	0.720	1

TPC: total phenolic content; TFC:Total flavonoids content CT: condensed tannins AhE: anti-hemolytic effect

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TABLE 4 : Protective effect of date fruit syrup against AAPH induced erythrocyte hemolysis

	Hemolysis half-time (min)
Control	105.66 ± 3.46[a]
AAPH + blood	52.17 ± 2.61
AAPH +blood+ *Bouslikhen syrup*	267.13 ± 7.83[b]
AAPH +blood+ *Jihl syrup*	317.79 ± 10.63
AAPH +blood+ *Bousthammi* syrup	269.49 ± 9.02[b]
AAPH +blood+ *Tamaajount syrup*	226.44 ± 7.59
AAPH +blood+ *Lhafs syrup*	291.86 ± 9.76
AAPH +blood+ *Iklane syrup*	244.37 ± 8.21
AAPH+ Trolox 1%	109.73 ± 5.29[a]

Values in average (n =5) ± SE 'verages, the column with the same letters are not signiûcantly different using post hoc Bonferroni tests (p < 0.0018)

TABLE 5 : Evaluation of hemolysis induced by date fruit syrup extracts

	Hemolysis half-time (min)
Control	105.66 ± 3.46
Sang + Trolox	118.25 ± 6.29
Blood + *Bouslikhen syrup*	271.64 ± 5.72
Blood+ *Jihl syrup*	294.17 ± 5.21[a]
Blood+ *Bousthammi* syrup	257.03 ± 7.24[b]
Blood+ *Tamaajount syrup*	238.03 ± 6.82[c]
Blood+ *Lhafs syrup*	293.20 ± 5.92[a]
Blood+ *Iklane syrup*	247.52 ± 7.62[bc]

Values in average (n =5) ± SE. Averages, the column with the same letters are not signiûcantly different using post hoc Bonferroni tests (p < 0.0018)

ups could reduce oxidants (such as ferric ions) and scavenge free radicals. The strongest correlation between FRAP and ABTS assays may be due to the same mechanism that they have and their similar redox potential 0.70 V for Fe(II)/(III) and 0.68 V for ABTS/ABTS+•[27]. The difference of redox potential between ABTS and FRAP assay justified the high antioxidant activities depicted using FRAP assay than ABTS assay that means that any compound with lower Fe (II)/(III) redox potential can theoretically reduce Fe (III) to Fe (II) and contributes to the FRAP values resulting in falsely high FRAP values.

The anti-hemolytic effect of date fruit syrups

Erythrocytes membrane lipids are rich in polyunsaturated fatty acids; and therefore the exposure to free radical generated from the decomposition of AAPH at physiological temperature causes hemolysis. For that reason, the AAPH induced hemolysis provides a good approach to research the free radical induced membrane damage[28].

TABLE 4 show the protective effects of date fruit syrups and Trolox on the hemolysis induced by AAPH. This effect was found to be dose dependent in all date fruit syrups. The highest protective effect was found in *Jihl*syrups, which possessed the highest half hemolysis value (317.79 min) whereas, the lowest half hemolysis value (226.44 min) was found using *Tamaajount*syrups. The important half hemolysis value (52.17min) illustrated in the negative control may be due to the endogenous antioxidants in the erythrocytes which can trap radicals to protect them against free radical induced hemolysis as described previously[28].

The high half hemolysis value observed for all date fruit syrups compared to negativecontrolshow that these extracts did not provide just the protective effect but also the stabilising effect of erythrocytes membrane. The non-significant hemolysis observed when erythrocytes were treated only with date

fruit syrups as illustrated in TABLE 5 can be justified as nontoxic and harmless for the cells. The high positive correlation between phenolic/erythrocyte protective effect (R^2= 0.931), flavonoids/erythrocyte protective effect(R^2=0.926) show that phenolic and flavonoid content are the main contributor to the erythrocytes protective effect through their AAPH scavenging activity as show the high correlation between both DPPHand ABTSin one hand and protective effect. Our results are in agreement with other studies showing that polyphenols are able to protect erythrocytes from oxidative stress or increase their resistance to damage caused by oxidants[29, 30].

CONCLUSION

The results presented showed the six date fruit syrups are very rich on phenolic, flavonoid and condensed tannins content and exhibited the high antioxidant activity and very important protective effect of membrane erythrocytes against AAPH induced hemolysis. The important content of antioxidant may considered the date fruit syrups a very good functional food ingredient as well as an appropriate source this compound in pharmaceutical Feld.

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