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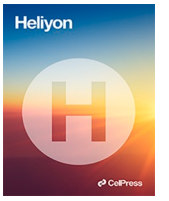

Research article

Optimizing the extraction of phenolic antioxidants from date palm fruit by simplex-centroid solvent mixture design

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ABSTRACT

Date palm (*Phoenix dactylifera* L.) fruits are rich in various bioactive compounds, such as phenolic acids, hydroxycinnamates, flavonoid glycosides, coumarins, alkaloids, and proanthocyanidin oligomers. The focus of this study was to develop a simplex-centroid mix design method to identify the most suitable mixture of solvents (water, acetone, and methanol) to extract bioactive compounds from date fruits. Three extraction solvents (water, methanol, and acetone) were investigated during this study to determine, total phenolic content (TPC), total flavonoid content (TFC), antioxidant activity (free radical DPPH) and ferric reduction ability (FRAP). The results showed that polar mixtures were effective in extracting antioxidant phenolics. The optimum solvent for extraction was Binary mixture water-acetone (50%) presenting TPC, TFC, DPPH and FRAP values of 502.88 mg GAE.100 g^{-1} DW, 206.23 mg QE.100 g^{-1} DW, 77 0.01% and 1688.66 μmol.100 g^{-1} respectively. The results also confirmed a strong correlation between the amount of polyphenols in a given extract and the antioxidant activities observed in the DPPH and FRAP assays. This study presents a pragmatic and efficient way to choose a solvent combination to extract polyphenols from date palm fruits.

1. Introduction

Date palm (*Phoenix dactylifera* L.) is an important fruit-bearing tree cultivated widely in arid and semi-arid regions globally. In Morocco, the date palm plays an important socio-economic and environmental role. It occupies 61,332 ha, or about 7% of the world's date palm area. Date production reached 143,160 (t) in 2020 dominated by the Khalts (36%), 'Mejhoul' (27%) and 'Boufeggous' (19%) cultivars. Production and consumption of dates in Morocco is concentrated in oasis areas where this fruit is considered an essential part of the diet. The export of dates from Morocco remains very modest compared to other countries such as Tunisia; it is ranked among the largest exporters of dates.

Dates fruits are known to contain a wide range of essential nutrients with health benefits. They provide carbohydrates in form of sugars, functional dietary fibre, proteins and lipids. Date fruits also contain many essential vitamins and minerals like riboflavin,

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thiamine, biotin, folic and ascorbic acid, calcium, iron, fluorine, and selenium [1–4].

Moreover, dates are a significant source of bioactive compounds [5,6]. It contains a variety of polyphenols, including phenolic compounds such as cinnamic and coumaric acids and their derivatives, for example ferulic, sinnapic, syringic, vanillic, gallic, caffeic, protocatechuic and dactilyferic acids. It also contains flavonoid glycosides (luteolin, methyl luteolin, quercetin, and methyl quercetin) flavones, flavanols (catechin, epicatechin), flavaxanthin and anthocyanins, making dates an interesting substrate from a pharmaceutical and therapeutic use [7,8]. The presence of polyphenols and flavonoids in fruit and vegetables is receiving substantial interest, due to their high antioxidant capacity, which acts as free radical scavengers and inhibits protein oxidation, cholesterol oxidation and DNA breakage [9].

In the food industry, phenolic compounds are considered as bio preservatives (antimicrobial and antioxidant effects) and as an alternative to synthetic additives [10]. Extraction of these compounds from food is a critical step for the isolation and purification of bioactive components. However, it is influenced by extraction solvents, sample/solvent ratio, time, temperature, physical and chemical properties of the sample matrix [11].

There is no consensus in the literature neither about a single, successful standard extraction method or the most efficient extraction solvent. Though, it has been reported in several studies that solid-liquid extraction with different types of solvents is the most effective [12] and a higher polarity of solvents usually means better solubility of polyphenols into extraction solvents [13]. In various extraction systems, isolated solvents are utilized, which are known to produce ineffective extraction. In such cases, it is useful to use solvent mixtures, which can vary from binary, ternary and multi-component mixtures [14]. Moreover, a mixture solvent offers a polarity variation, which helps in extracting phenolic compounds at different degrees of polarity [12].

As per the knowledge of the authors, there are no investigations on mathematical approaches for optimizing the solvent mixtures to create antioxidant-rich extracts from dates fruits. Hence, the goal of this research is to find the best solvent mixture combination (water, acetone, and methanol) for extracting antioxidant ingredients from four Moroccan dates cultivars using a simplex-centroid mixture design. To choose the optimal extraction solvent, total phenol, flavonoids, and antioxidant capabilities were determined by free radical scavenging activity (DPPH) and ferric reducing antioxidant (FRAP).

2. Materials and methods

2.1. Chemicals and reagents

Quercetin and gallic acid were obtained from Sigma-Aldrich (St-Louis, MO, USA), Acetone and Methanol (98%) from Riedel-de Haën - Honeywell (Seelze, Germany), Folin-Ciocalteu, sodium hydroxide (≥98%), trichloroacetic acid, and aluminium chloride (99%) from Sigma-Aldrich (St-Louis, MO, USA), Ferric chloride, sodium carbonate, sodium nitrite and ferric chloride from EMSURE (Darmstadt, Germany), 2,2-diphenyl-1-picryl-hydrazyl-hydrate from Sigma-Aldrich (Taufkirchen, Germany) and potassium ferricyanide from HiMedia (Mumbai, India).

2.2. Plant material

Dates cultivars (*Phoenix dactilyfera* L.); 'Mejhoul', 'Aziza Bouzid', 'Assiane', 'Boufeggous', 'Boufeggous Agharas' were collected at the Tamar stage from Figuig oasis, in the South East of Morocco. Details of selected cultivars, sampling site, tree age, etc are provided in Table 1. Approximately one kilogram of each sample was collected according to a factorial scheme 20 × 5 x 1 (dates x trees x plot) in which the trees were selected randomly. Samples were freeze-dried, crushed, and stored at −20 °C for further extraction and analysis.

2.3. Mixture design and statistical analysis

Simplex-centroid conception performed by Statistica 13.3.0, TIBCO Software, Palo Alto, CA, USA was used to explore the effect of different solvents on phenolic compounds extraction in dates fruits and to determine the optimal solvents mixtures to maximise the phenolic antioxidants. For the simplex-centroid design, the different solvents were evaluated from a triangle, with pure components at the top, representing 100% of each of the solvents. Each solvent in the system was studied at six levels according to the twelve tests presented in Table 2. The linear (Eq. (1)), quadratic (Eq. (2)), and special cubic (Eq. (3)) mathematical models were evaluated.

Table 1
Geographical location, age and height of studied dates trees.

Dates cultivars	Geographical location		Altitude	Age tree (year)	Height tree (m)
	Latitude	Longitude			
'Boufeggous Agharass'	N32°09'34.32	W1°18'27.32	890	20	6
'Boufeggous'	N 32°05'29.86	W 1°13'22.09	836	24	6
'Aziza Bouzid'	N32°06'37.18	W1°13'09.02	914	25	7
'Assiane'	N32°06'04.87	W1°13'19.93	904	20	7
'Mejhoul'	N32°09'44.12	W1°13'11.18	860	16	5

Table 2
Experimental variables and responses used in the simplex-centroid mixture design.

Run number	Independent variable			TPC (mg GAE.100 g)		TFC (mg QE/100 g)		RSA (%)		FRAP (μmol/100 g)	
	X_1 (Water)	X_2 (Methanol)	X_3 (Acetone)	Observed	Predicted	Observed	Predicted	Observed	Predicted	Observed	Predicted
1	1	0	0	316.72	311.50	119.47	118.15	28.70	24.39	1236.44	1216.60
2	0	1	0	222.37	231.52	95.95	113.42	47.90	50.98	1009.32	1105.20
3	0	0	1	80.29	70.24	91.72	98.64	32.05	33.49	379.63	432.15
4	1/2	1/2	0	428.20	440.59	124.15	115.79	65.51	70.14	1649.20	1661.90
5	1/2	0	1/2	502.88	496.06	206.23	212.25	77.01	79.94	1688.66	1656.69
6	0	1/2	1/2	219.45	227.01	111.09	106.03	37.12	42.23	810.58	768.67
7	2/3	1/6	1/6	463.90	477.21	137.67	162.53	59.90	68.73	1650.65	1683.93
8	1/6	2/3	1/6	390.67	360.86	126.50	124.85	72.81	64.68	1533.45	1345.24
9	1/6	1/6	2/3	297.78	325.59	147.69	152.77	65.38	62.24	1178.20	1121.37
10	1/3	1/3	1/3	464.26	449.04	173.70	154.21	76.69	72.28	1462.11	1489.59
11	1/3	1/3	1/3	456.61	449.04	155.32	154.21	75.00	72.28	1497.05	1489.59
12	1/3	1/3	1/3	444.59	449.04	178.30	154.21	75.66	72.28	1361.65	1489.59

$$\text{Linear}: \ y_n(x) = \sum_{i=1}^{q} \beta_i x_i \tag{1}$$

$$\text{Quadratic}: \ y_n(x) = \sum_{i=1}^{q} \beta_i x_i + \sum_{i<j}^{q-1} \sum_{j}^{q} \beta_{ij} x_i x_j \tag{2}$$

$$\text{Special cubic}: \ y_n(x) = \sum_{i=1}^{q} \beta_i x_i + \sum_{i<j}^{q-1} \sum_{j}^{q} \beta_{ij} x_i x_j + \sum_{i<j}^{q-2} \sum_{j<k}^{q-1} \sum_{k}^{q} \beta_{ijk} x_i x_j x_k \tag{3}$$

y_n represent the predicted response function. Four responses (n = 4) have been studied: total phenols, flavonoids and antioxidant activity by FRAP and DPPH. Independent variable x_i, x_j *and* x_k corresponds to components used in the mixing plan (water, methanol, and acetone), with $1 > x_{ijk} > 0$. β_i, β_{ij} and β_{ijk} represent respectively; the linear coefficient related to the pure component *i,* the quadratic binary interaction coefficient for components i and j in addition to the cubic ternary interaction coefficient for components *i, j* and *k*.

Variance analysis (ANOVA) and regression analysis have been used to evaluate mathematical prediction models (p-value <0.05). The response surfaces were generated from fitted models. The desirability function [15] was used to optimize simultaneously the four response variables.

The mathematical models were validated by conducting three additional tests under the most suitable conditions determined by the desirability function. The *t*-student test was employed to assess the experimental values with those estimated responses at a 95% confidence interval.

2.4. Antioxidant compounds

The extraction of antioxidant components was realized according to the experiment indicated in Table 2. The antioxidant extract was obtained by mixing 0.6 g of dates pulp powder with 6 ml of solvent. Next, the mixture was vortexed for 2 min and centrifuged (5000 g for 10 min). Twelve extracts were obtained, including tree replicates of the centre point (1/3, 1/3, 1/3) and analyzed for antioxidant potential and total phenols and flavonoids of dates. All determinants were randomly assessed, and were conducted in triplicates. The data was recorded as mean ± SD.

2.5. Total phenolic content (TPC)

Total phenolic compounds of dates fruits were quantified according to the Folin–Ciocalteu's method [16], where 2.0 ml of diluted Folin-Ciocalteu reagent was added to 50 μL of each extract. Mixtures were kept at room temperature for 5 min, and then, 2.0 ml of sodium carbonate solution 10% (Na_2CO_3) was added. Incubation was made for 60 min at room temperature and absorption was measured at 760 nm (nm). Total polyphenolic content was calculated using a calibration curve and presented as milligrams gallic acid equivalents per 100 g on a dry weight basis (mg GAE.100 g^{-1} DW).

2.6. Total flavonoid content (TFC)

The total flavonoid of the extract was determined according to Ref. [17] method. 500 μl of the extract was mixed with 120 μl of sodium nitrite 5% and 50 μl aluminium chloride 10%. The mixture was incubated for 6 min, at room temperature, and then 2 mL of sodium hydroxide 1 M and 500 μl of distilled water were added. After mixing, absorbance was immediately measured at 510 nm, which was further calibrated to a standard curve to obtain quercetin solution. The flavonoid content was expressed as milligrams quercetin equivalents per 100 g dry weight basis (mg QE 0.100 g^{-1} DW).

2.7. Free radical scavenging activity

DPPH free radical-scavenging activity of dates was determined using the method defined by Ref. [18]. DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) technique is an antioxidant assay based on electron transfer that generates a violet solution in methanol, where the free radicals are reduced in the presence of antioxidant molecules, giving out a yellow colour. DPPH stock solution (8 mg DPPH/200 mL methanol) was diluted with methanol to achieve an absorbance of 1.1 at 515 nm. An aliquot of 0.1 mL sample extracts, and blank (methanol), was allowed to react with 1.6 mL of DPPH solution for 20 min in dark conditions. The ability to scavenge the DPPH radical was calculated using the following equation:

%RSA = [(A0 - A1) / A0]*100 $A_{0:}$ control absorbance, A1: absorbance of samples

The graph representing the percentage reduction of DPPH as a function of the concentrations of the extracts allows the determination of IC_{50} which represents the concentration of the extract necessary to reduce the absorbance by 50%. The IC_{50} was expressed as a gram of date per litter (g date. L-1).

2.8. Ferric reducing antioxidant

The ferric reducing power (FRAP) of extracts was measured using the potassium ferricyanide ferric chloride method described by Ref. [19]. Substances, with reduction potential, react with potassium ferricyanide ($C_6N_6FeK_3$) to form potassium ferrocyanide ($C_6FeK_4N_6$), which subsequently reacts with ferric chloride to form a ferric ferrous complex which shows maximum absorbance at 700 nm. An amount of 1.25 mL phosphate buffer (0.2 M, pH 6.6) and 1.25 mL of 1% (w/v) potassium ferricyanide was added to 0.5 mL of extract. Mixtures were incubated at 50 °C for 20 min and then 1.25 mL of trichloroacetic acid (10%) was added to stop the reaction. 1.25 mL of mixture was mixed with 2.5 mL of distilled water and 0.5 mL of 0.1% $FeCl_3$(w/v). The intensity of the blue-green colour was measured at 700 nm. The ferric reducing power was expressed as micromole per 100 g of sample dry weight (μmol.100 g^{-1} DW).

3. Results and discussion

Responses on the experimental variable for the simplex-centroid mixture design are presented in Table 2, which shows the proportion mixture as an independent variable and the responses as observed and predicted amount of TPC, TFC, RSA, and FRAP.

ANOVA of quadratic models shown in Table 3, allowed tracing plot contour (Fig. 1) of total phenolic (TPC) and total flavonoid contents (TFC), DPPH antioxidant activity, and FRAP antioxidant activity, as a function of solvent proportions. Contour plot vertices present response value and triangle edges indicate concentration, which respectively represents individual components and their binary and ternary mixtures.

According to ANOVA results, quadratic model is satisfactorily reproduced for TFC ($p = 0.00002$) TPC ($p = 0.002$), DPPH ($p = 0.0007$), and FRAP ($p = 0.001$). Inversely, linear, and special models were outside the confidence level ($p > 0.05$). A global adjustment was applied to investigate the quadratic model (Table 4). The degree of model fit was verified by the coefficient R^2. This coefficient expresses the proportion of the total response variation predicted by the model. The closer the value of R^2 is to 1, the model fits perfectly with the real data. R^2 results of the quadratic regression models were 0.986, 0.918, 0.962, and 0.979, respectively for TPC, TFC, DPPH, and FRAP. Thus, the results predicted by the models were close to the experimental results. This was verified by the lack of fit test which showed non-significant results ($p > 0.05$) indicating the validity of the models, except for DPPH model which presented a lack of fit ($p < 0.05$), but with a very satisfactory R2 value (0.962). Positive coefficients in equations revealed that the highest phenolic component extraction of dates fruits is coinciding with binary mixtures use. Those differences in the extraction efficiency of solvents have been attributed to their polarities, material matrix, and extractable compounds. Binary mixtures present a higher efficient interaction, and the mixture coefficient was greater than when a solvent was isolated, which indicates that both components act in synergy.

3.1. Extraction of the total phenolic compounds (TPC)

Results obtained of TPC extracted from dates fruits using different solvents varied from 80.29 to 502.88 mg GAE.100 g^{-1} DW (Table 2). The contour plot (Fig. 1A) shows that the solvents mixture displays a higher capacity to extract phenolic compounds than the isolated one. The mathematical equation for TPC illustrates the efficiency of compound extract following decreasing order: water-acetone; water-methanol; water; acetone-methanol, methanol, and acetone.

Phenolic compounds extracted from dates fruits using distilled water was 316.72 mg GAE.100 g^{-1} DW. These findings are similar to those obtained by Ref. [20]; reporting 376 mg GAE 100 g^{-1} DW for the 'Mabroom' cultivar. For isolated methanol extract, the TPC of dates fruits was 222.37 mg GAE.100 g^{-1} DW, higher than the values obtained by Ref. [21]. Water-acetone mixture extracted more than 500 mg GAE.100 g^{-1} DW from dates fruits. According to Ref. [22]; acetone mixtures were more effective than methanol ones for polyphenol extraction. Those results are consistent with those of [23]; which found that Acetone 60% extract almost 493.15 mg

Table 3
ANOVA results of regression models from simplex-centroid design for solvent optimization.

ANOVA Source	Sum of squares	DF	F-value	*p*-value	R^2
TPC					
Linear	61526.3	2	2.21	0.165	0.329
Quadratic	122607.4	3	93.68	0.00002	0.986
Special cubic	154.3	1	0.31	0.599	0.987
TFC					
Linear	1995.80	2	0.80	0.479	0.15
Quadratic	10143.74	3	18.48	0.002	0.917
Special cubic	36.96	1	0.17	0.693	0.919
RSA					
Linear	64.255	2	0.08	0.924	0.017
Quadratic	3374.611	3	26.99	0.0007	0.932
Special cubic	69.836	1	1.94	0.222	0.951
FRAP					
Linear	762828	2	3.63	0.069	0.446
Quadratic	862827	3	21.05	0.001	0.951
Special cubic	10752	1	0.75	0.424	0.958

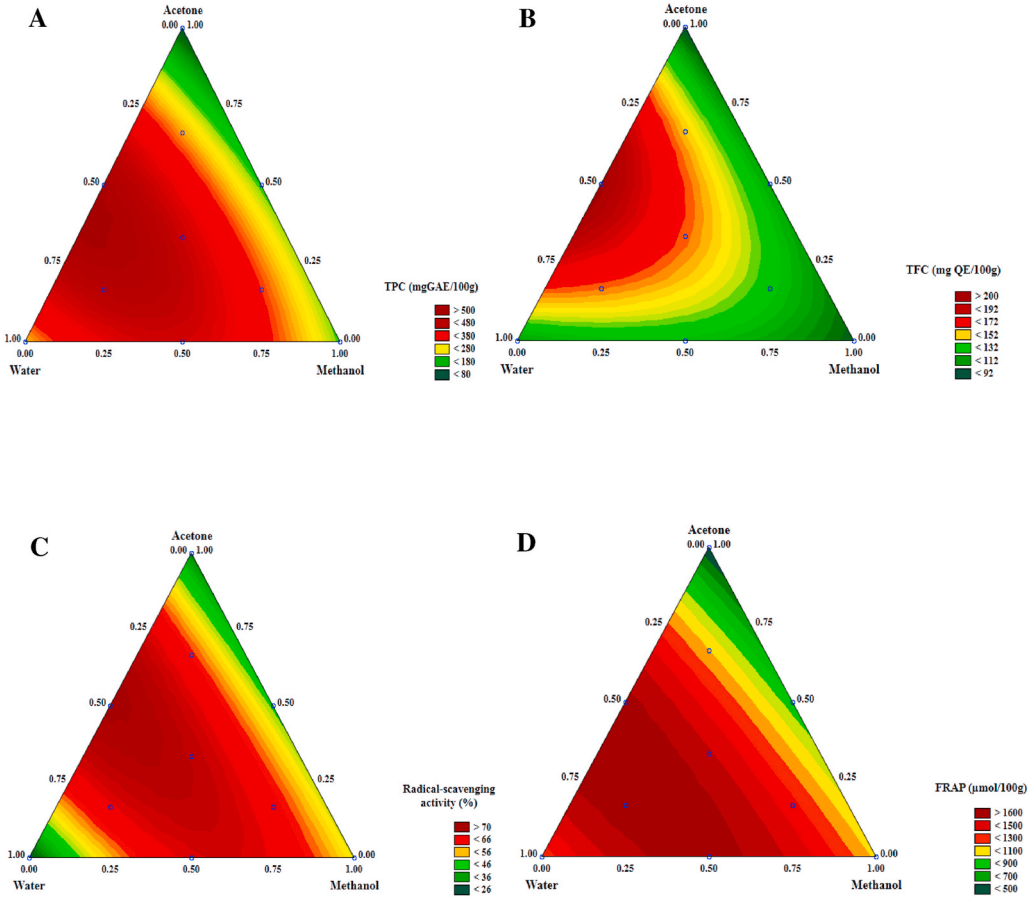

Fig. 1. Contour plot as a function of solvent proportion; (**A**) total phenolic content (TPC) (**B**) total flavonoid content (TFC) antioxidant activity by (**C**) DPPH and (**D**) FRAP.

Table 4
ANOVA results for overall fit of reduced quadratic model.

ANOVA Source	Sum of squares	DF	F-value	p-value	Equation
TPC					
Model	184133.7	6	84.42	0.00001	$y = 311.50w + 231.52\,m + 70.24a + 676.30wm + 1220.76wa + 304.52am + 0$
Total error	2617.5	6			
Lack of fit	2420.8	4	6.15	0.144	$r^2 = 0.986$
Pure error	196.7	2			
TFC					
Model	242936.95	4	231.96	<0.00001	$y = 118.15w + 113.42\,m + 98.64a + 415.41wa + 0$
Total error	2094.61	8			
Lack of fit	1798.93	6	2.03	0.366	$r^2 = 0.918$
Pure error	295.69	2			
RSA					
Model	3438.87	5	230.54	<0.00001	$y = 24.39w + 50.98\,m + 33.49a + 129.81wm + 204.01wa + 0$
Total error	278.50	7			
Lack of fit	277.05	5	76.38	0.013	$r^2 = 0.962$
Pure error	1.45	2			
FRAP					
Model	21545162.29	5	417.63	<0.00001	$y = 1216.60w + 1105.20\,m + 432.15a + 2003.99wm + 3329.25wa + 0$
Total error	72225.19	7			
Lack of fit	62343.13	5	2.52	0.3078	$r^2 = 0.979$
Pure error	9882.05	2			

a: acetone, *m*: methanol, *w*: water.

GAE.100 g^{-1} DW of TPC [24]. report that the highest level of phenolic compounds for all dates varieties was found in 70% acetone extract. The use of water and organic solvent may facilitate the extraction of molecules soluble in water and/or organic solvent [25]. indicated that a mixture solvent use is rather than a mono-solvent for extracting phenolic compounds in vegetable samples. According to Refs. [26,27] water increases cell tissue permeability and mass transfer by molecular diffusion in addition to recovery of water-soluble bioactive compounds. Nevertheless, the use of water in a mixture with organic solvents provides a moderate polar medium which is most favourable for extraction [28], once the chemical nature of phenolic compounds changes from simple to highly polarized [29].

Optimized proportions were 50% of water and 50% of acetone. Results obtained are in agreement with [30] finding, who found that 50% acetone mixed with water is suitable for the extraction of phenolic compounds from '*Eucalyptus. Camaldulensis*'. Also, for strawberry extracts [31], report that the highest TPC was obtained for acetone/water (50/50, v/v) and (70/30, v/v) extraction solution.

3.2. Extraction of total flavonoid compounds (TFC)

The total flavonoid content (TFC) of dates extracted by different solvents fluctuates between 91.72 and 206.23 mg QE.100 g^{-1} DW. The quadratic model showed that only the acetone and water mixture have a high affinity to the flavonoid, compared to the isolated solvent (water, methanol, and acetone). While water-methanol and acetone-methanol mixtures are the least efficient for flavonoid extraction, with coefficients equal to 85.94 and 69.81 mg QE.100 g^{-1} DW respectively. The flavonoid extracted rate in this study by water-acetone mixture is higher than the rate obtained by Bouhlali et al., (2017), who found that the flavonoid content of 'Mejhoul' cultivar was equal to 77.73 mg QE.100 g^{-1} DW when water and methanol mixture was used.

The current study found that acetone-water (50%) had a better flavonoid extraction in comparison with methanol-water (50%) and acetone-methanol (50%) (Fig. 1B), which is correlated with results reported by Ref. [32]; finding that 50% acetone had higher extraction yields of flavonoids than those of isolated solvents [33]. report that a combination of acetone and water (50%) showed the highest extraction yields, followed by absolute methanol and 50% ethanol [32]. confirm that 80% acetone, 80% ethanol, and 80% methanol acetone extract more flavonoids than water or absolute methanol, ethanol, or acetone. Those differences can be assigned to flavonoids solubility and different polarities of solvents, which selectively extract targetable flavonoid compounds from materials.

3.3. Antioxidant activity by scavenging of DPPH free radical

Free radical scavenging activity DPPH assay is useful to investigate the antioxidant properties of dates obtained using various solvents and which can be applied to both hydrophilic and lipophilic antioxidants [34]. DPPH assay depends on the antioxidant compounds ability to lose hydrogen and the structural conformation of these components [35,36].

DPPH scavenging activities of different solvent extracts from 'Mejhoul' dates fruits ranged from 28.70% to 77.01% indicating that extraction solvent had a significant influence on DPPH ($p < 0.05$) (Table 2). According to the mathematical equation of the quadratic model, binary mixtures of water-acetone and water-methanol exhibited the highest DPPH scavenging activity. The antioxidant potential obtained followed the same tendency observed for total phenolic compounds. The current finding revealed that antioxidant activity was significantly correlated with phenolic content. Subsequently, the influence of solvent used on phenolic compounds extraction was a determinant for DPPH % antioxidant activity results. Results were in agreement with those reported in the literature, which revealed a positive correlation between phenolic compound quantity and DPPH free radical scavenging effect [34,37].

The contour plot (Fig. 1C) shows that extracts prepared from 'Mejhoul' dates using a combination between 50% acetone and 50% water present more potent antioxidant properties than absolute organic solvent extracts. Obtained results were following [38] data, reporting that water-acetone mixture extract from barley represents the highest DPPH scavenging activity, followed by ethanol and methanol extracts. The variation observed within DPPH scavenging activities from the fruit can be explained by phenolic compounds, which dissolve differently due to different polarities of used solvents.

3.4. Antioxidant activity by FRAP

The FRAP assay is used for the direct assessment of the total antioxidant activity of a sample. It is based not on free radical scavenging capacity but on reducing ability [39]. Reducing power is based on electron transfer-ability to reduce ferric Fe^{3+} to ferrous Fe^{2+} [40].

Antioxidant activity achieved by the FRAP method showed that values obtained from various solvents were significantly different ($p < 0.05$). FRAP values ranged between 379.63 and 1688.66 μmol.100 g^{-1} DW. Thereby, FRAP data was following the same behaviour results of TPC, TFC, and DPPH, showing that binary extracts water-acetone, and water-methanol reveal the highest antioxidant activity (Fig. 1D). For [41]; aqueous acetone 70% and 50% showed the highest FRAP value and suggested it as the most suitable solvents among all of the three solvents. According to Ref. [42] binary mixture of water-acetone (1/3–2/3) was the most efficient solvent to improve FRAP reducing capacity from Chia seeds.

In the present study, the high level of phenolic compounds, extracted by acetone 50% from 'Mejhoul' dates are responsible for high reducing ability. Redox properties of phenolic compounds act as reducing agents, hydrogen donators, and singlet oxygen quenchers. The redox potential of phenolic compounds is crucial within antioxidant potential [43].

Table 5
Antioxidant activity, TPC and TFC of different Moroccan dates cultivars.

	TPC mg GAE.100g^{-1} DW	TFC mg QE.100g^{-1} DW	IC_{50} DPPH g date.L^{-1}	FRAP μmol. 100g^{-1} DW
'Boufeggous Agharass'	342.75 ± 17.46^{d}	100.63 ± 1.31^{b}	3.87 ± 0.04^{b}	855.67 ± 16.77^{bc}
'Boufeggous'	290.91 ± 6.98^{c}	117.35 ± 1.31^{c}	4.35 ± 0.1^{c}	769.29 ± 15.33^{b}
'Aziza Bouzid'	209.25 ± 3.76^{a}	87.43 ± 4.84^{a}	5.18 ± 0.2^{e}	661.35 ± 40.41^{a}
'Assiane'	261.36 ± 5.37^{b}	104.81 ± 3.66^{b}	4.57 ± 0.11^{d}	932.15 ± 92.51^{c}
'Mejhoul'	496.09 ± 6.69e	190.80 ± 3.46^{d}	3.46 ± 0.06^{a}	1662.30 ± 27.48^{d}

Values are average (n = 5) ± standard deviation. Significant differences in the same row are shown by different litters (a-e)
TPC: Total phenolic Compounds, TFP: Total flavonoid compounds, DW: Dry weight, GAE: equivalent acid Gallic; QE: Equivalent quercetin.

3.5. Antioxidant activity, phenolic and flavonoid content

The results of antioxidant activity, and total phenolic and flavonoid content from five Moroccan dates cultivars using an optimized mixture (water – acetone 50%) are presented in Table 5. A significant difference ($p < 0.05$) was observed between the different studied cultivars.

The highest TPC value was contributed to 'Mejhoul' cultivar (496.09 mg GAE.100 g^{-1} DW), followed by 'Boufeggous Agharass' (mg GAE 100 g^{-1} DW), 'Boufeggous' (290.91 mg GAE.100 g^{-1} DW), 'Assiane' (mg GAE.100 g^{-1} DW), and 'Aziza bouzid' (209.25 mg GAE.100 g^{-1} DW). TFC of Moroccan dates fluctuated between 87.43 mg QE.100 g^{-1} DW for 'Mejhoul' and 190.80 mg QE.100 g^{-1} DW for 'Aziza Bouzid'. The present study showed that TPC and TFC extracted from Moroccan dates, using a water-acetone mixture were higher compared to other studies using solvent methanol 80% [44]. report that the total phenol and flavonoid contents of 'Mejhoul' dates were 398.228 mg GAE.100 g^{-1} DW and 77.73 mg QE.100 g^{-1} DW respectively. TPC and TFC values determined by Ref. [45] for 'Boufeggous', 'Aziza Bouzid' and 'Assiane 'cultivars were also lower than those obtained in this study. The variation between cultivars may be attributed to variety within cultivars, growing conditions, maturity, seasons, geographic origin, fertilizer, and soil type and storage conditions.

IC_{50} of scavenging activity based on DPPH was higher for 'Mejhoul' dates (3.87 g date. L^{-1}), while and lower for 'Aziza Bouzid' (5.8 g date. L$^{-1)}$. These results are higher than those of [44]; reporting an IC_{50} equal to 5.25 g date. L^{-1} for the 'Mejhoul' cultivar. However [46], presented the value of DPPH as inferior to those obtained in this study. Correlation analysis of scavenging activity DPPH and TPC content showed high correlation (r = 0.913; $p < 0.05$). Several previous studies show a positive correlation between the concentration of phenolic compounds and the DPPH [34,37]. FRAP results of cultivars showed good reducing power, ranging respectively between 661.35 and 1662.30 μmol.100 g^{-1} DW for 'Aziza Bouzid' and 'Mejhoul' ($p < 0.05$). As demonstrated in this study, antioxidant activity obtained using FRAP assay presents a high positive correlation with total polyphenol contents (r = 0.950) as well as flavonoid content (r = 0.912). Thus, acetone-water mixtures were more effective than methanol for phenolic extraction from Moroccan Dates and for potential antioxidant activity.

4. Conclusion

This study attempted to optimize the extraction protocol for phenolic compounds using three solvents (water, methanol and acetone). The simplex-centroid solvent mixture design was effective in estimating the suitable mixture to extract total phenolic compounds in dates, as well as total flavonoids and antioxidant capacity measured by DPPH and FRAP. The moderately polar solvent mixture of water-acetone (50% v/v) was the most effective in extracting phenolic compounds from studied cultivars. The water-acetone binary mixture used for extraction was also critical for DPPH scavenging activity and free radical reducing ability (FRAP). Phenolic extracts obtained from date fruits can be recommended as an excellent substitute for food chemical additives, they can also be used in cosmetic and pharmaceutical industries.

Declarations

Author contribution statement

Kawtar Jdaini: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Fouzia Alla: Performed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Farid Mansouri: Conceived and designed the experiments, Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Aditya Parmar: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Mohamed Aziz Elhoumaizi: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Declaration of interest's statement

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Additional information

No additional information is available for this paper.

List of abbreviations

µL	Microliter
µmol	Micromole
ANOVA	Analysis of variance
CA, USA	California, United States of America
DNA	Deoxyribonucleic acid
DPPH	2, 2-Diphenyl-1-picrylhydrazyl
DW	Dry weight
FRAP	Ferric Reducing Antioxidant Power Assay
g	Gram
GAE	Gallic acid equivalent
IC_{50}	Half-maximal inhibitory concentration
L	Litter
mg	Milligram
min	Minute
ml	Milliliter
nm	Nanometer
pH	Potential of hydrogen
QE	Quercetin equivalent
RSA	Radical scavenging activity
SD	Standard deviation
TFC	Total flavonoid compounds
TPC	Total phenolic compounds
v	Volume

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