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Date Industry by-Product: Date Seeds (*Phoenix dactylifera* L.) as Potential Natural Sources of Bioactive and Antioxidant Compounds

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Abstract: The chemical composition, carotenoids, anthocyanins, total phenolic content, total flavonoid content, and antioxidant activity of three date seed cultivars, mainly Barhi, Ruthana, and Qatarah, were investigated. Date seed bioactive compounds were extracted by using different extraction solvents. The chemical analysis revealed that the Barhi cultivar has the highest moisture and fat content, while Ruthana has the highest carbohydrate content. There were no significant differences in protein and ash contents among the three date seeds. Ethanol (100%) showed the highest bioactive compound contents and antioxidant activity, whereas water showed the lowest content among the six solvents. Generally, all the date seed cultivars contain high amounts of phenolic, flavonoid, and antioxidant activity. The carotenoid content was highest in the Barhi (1.98 mg/g DW) followed by Qatarah (1.25 mg/g DW) and Ruthana (0.89 mg/g DW) seed, while the anthocyanin content was highest in the Ruthana cultivar (5.51 ± 0.71 cyanidin 3-glucoside/g DM.) and lowest in Barhi date seed (3.335 ± 0.23 cyanidin 3-glucoside/g DM.). Among the three date seeds, the Ruthana seed showed the highest phenolic (93.36 ± 0.30 mg GAE/g) and flavonoid compounds (59.9 ± 0.44 mg CE/g), DPPH activity (78.6 ± 0.92%), and total reducing power (60.3 ± 0.09%). The optimum solvent for the extraction of the antioxidants and their activity in each date seed was validated by using partial least squares regression (PLS). The results revealed a variation in the valid and optimum extracts for each seed; 100% ethanol extract for Barhi seed and mixture of methanol:ethanol:water (M 40:E40:W20) extract for Ruthana and Qatarah cultivars were the best solvent systems. The high natural antioxidant content of date seeds indicates that they can be considered functional ingredients for food and medicinal uses.

Keywords: antioxidant activity; date seeds; extraction solvents; total phenol flavonoids; PLS

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1. Introduction

Date palm (*Phoenix dactylifera* L.) is a tropical plant that is grown in Middle Eastern and North African countries [1]. The date fruit is an essential component of the diet and has important social, environmental, and economic significance in Saudi Arabia. The Kingdom of Saudi Arabia is the leading date producer, with an annual production exceeding 1.5 million tons, which represents 17% of the total global production [2]. There are more than 5000 different varieties of dates worldwide, of which around 400 cultivars are grown in Saudi Arabia [3,4].

Several researchers have reported that dates are rich sources of carbohydrates, energy, vitamins, and minerals [5]. The top-quality date varieties, such as Sukkari, Ajwa, and Barhi, are consumed in the Rutab stage. In contrast, middle- and low-quality varieties are used to produce date paste, jam, syrup, vinegar, and other products [6]. Date seeds (also called stones, kernels, or pits) represent 10–15% of the ripe date weight, based on maturity,

variety, and the quality of the date [7]. It has been demonstrated that date seeds contain several functional components, such as dietary fiber, minerals, vitamins, fatty acids, and amino acids [8]. They are also a rich source of bioactive compounds such as carotenoids, polyphenols, flavonoids, and tocopherols [9–11]. Hilary et al. [12] reported that date seeds contain about 51.1 g of polyphenolic compounds per kg, higher than other poly phenol-rich foods like flaxseed, tea, and grapes. Similarly, Habib et al. [13] observed a high total polyphenol content of 4768.87 mg GAE/100 g in date seed. The cinnamic acid derivatives and benzoic acid hydroxylated derivatives were the predominant phenolic acids in date seeds [14,15], while rutin, catechin, quercetin, luteolin, and kaempferol were the main flavonoids [16,17]. These natural antioxidants' presence in date seeds is reported to provide protection effects against many oxidative-stress-induced diseases, such as inflammation, hyperlipidemia, and diabetes [18–20].

In vitro and in vivo studies on date seeds have shown their effective antioxidant properties. Abuelgassim et al. [21] reported that date seed extracts had high scavenging activity against ABTS, DDPH, and hydroxyl radicals. Date seeds have also shown higher antioxidant properties than date flesh, making them a viable natural source of antioxidants [22]. Oral administration of date seed extracts was found to lower oxidative stress damage and improve the defense systems of the organs [23,24]. Recently, Alahmadi and Banayah [25] reported the hypolipidemic and hypoglycemic effects of date seed extract in diabetic rats. They attributed the lipid- and glucose-lowering actions to the antioxidant properties of the date seeds. The antioxidant properties of date seeds may be due to poly phenolic compounds that act as reducing agents, free radical scavengers, and hydrogen donors, which may explain their efficiency in treating different conditions [26].

The antioxidant properties and polyphenolic compounds of date seeds were reported to be affected by many factors, such as genetic diversity, fertilizer and soil type, water availability, maturity stages, storage conditions, the solvent system, and extraction methods [1,27]. The date industry generates thousands of discarded by-products, such as date pomace and seeds, that are rich with bioactive compounds. New aspects of using these by-products to produce high-nutritional-value food products have recently attracted interest. Therefore, the aim of this study is to chemical composition and validate the extraction conditions of bioactive compounds from seeds of Ruthana, Barhi, and Qatarah date varieties by using different solvent systems.

2. Materials and Methods

2.1. Materials

Three date varieties, Barhi, Ruthana, and Qatarah, were obtained from the local markets in Riyadh, Kingdom of Saudi Arabia, during the Rutab stage. The seeds were separated from the flesh, washed with water, and air-dried at room temperature. The seeds were grounded to fine powder by using a coffee grinder (Huge Grinder China) and kept at 4 °C for extraction and analysis.

2.2. Chemical Composition

The chemical composition of the seeds (moisture, protein, oil, ash, and carbohydrate) was determined by using the standard methods of the AOAC [28].

2.3. Total Carotenoids Content Determination

The total carotenoids of the date seeds were determined spectrophotometrically, according to Jacques et al. [29]. Carotenoids were extracted and fractioned with acetone and petroleum ether, respectively. The extracted carotenoids were detected at 450 nm and expressed as mg/g DW.

2.4. Total Anthocyanin Content Determination

The anthocyanin content of the date seeds was determined according to Lapornik et al. [30]. Anthocyanin was extracted with a methanolic solution (70%). Then, the extract was reacted

with an ethanol-HCL solution (0.01%), 2% HCL (pH 0.8), and citric buffer (pH 3.5). The methanol solution (70%) was used as a blank at a wavelength of 520 nm. The result was expressed in milligrams of cyanidin 3-glucoside/g DM.

2.5. Preparation of Extracts

The seeds' phytochemicals were extracted in a ratio of 1:5 (w/v) by using different systems: methanol (100%), methanol (50%), ethanol (100%), ethanol (50%), water, and methanol:ethanol:water (40:40:20). The extraction was repeated under the same conditions and then dried by using a rotary evaporator [31].

2.6. Phytochemical Compounds of the Seeds

The total phenolic content (TPC) was determined as described by Waterhouse [32] by using the Folin–Ciocalteu and gallic acid standard curve at 765 nm (R^2 = 0.9672). The result was expressed as mg of gallic acid equivalents (GAE)/g (DW). The total flavonoid content (TFC) was determined by using the method of Kim et al. [33]. The TFC was expressed as milligrams of catechin (CE) per gram equivalent to the catechin standard curve (R^2 = 0.974).

2.7. Antioxidant Activity of the Seeds

The percentage of inhibition of DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity of the seed extracts was estimated by using the spectrophotometer absorbance at 517 nm following the Chang et al. method and expressed as % inhibition [34].

The total reducing power (TRP) was carried out according to Gulçin et al. [35] by using an ultraviolet–visible (UV–VIS PD-303 UV) spectrophotometer. The absorbance was measured at 700 nm and expressed as mg of ascorbic acid equivalents (AAE)/g sample (DW).

2.8. Statistical Analysis

An analysis of variance (ANOVA) test, followed by Tukey's test as the post hoc test, was applied to the analysis and the degree of significance. Values are presented as the mean ± SD, and the degree of significance was established at $p \leq 0.05$. The link between variables was expressed by using Pearson's correlation coefficient (r). The relationships between the different extracts, their phytochemical contents, and their antioxidant activities were analyzed by using linear partial least squares regression analysis (PLS) [36].

3. Results

3.1. Date Seeds Chemical Composition

The chemical composition of three selected date seed varieties (moisture, fat, crude protein, ash, and carbohydrate content) is shown in Table 1. There were significant differences ($p \leq 0.05$) in moisture and fat content among the three date varieties of seeds. The highest values of moisture content (12.4%) and fat content (8.7) were observed in the Barhi seeds, while the Ruthana seeds showed the lowest values of moisture content and fat content, 6.6 and 6.3%, respectively. In contrast, the protein and ash contents showed no significant differences among the seed varieties, and ranged from 7.88 to 9.94% for protein and from 0.94 to 1.04% for ash. Similar to moisture and fat, carbohydrates showed significant ($p \leq 0.05$) differences among the date seed varieties, ranging from 70.0 to 76.44%. Ruthana seed showed the highest content whereas Barhi seed had the lowest carbohydrate content.

Table 1. Chemical composition of date cultivars seeds.

Samples	Moisture %	Fat %	Protein %	Ash %	Carbohydrates %
Barhi	12.4 ± 0.05 ^{a}	8.7 ± 0.146 ^{a}	7.9 ± 0.785 ^{a}	1.04 ± 0.115 ^{a}	70.0 ± 0.624 ^{c}
Ruthana	6.6 ± 0.064 ^{c}	6.3 ± 0.155 ^{c}	9.7 ± 0.673 ^{a}	0.97 ± 0.040 ^{a}	76.4 ± 0.422 ^{a}
Qatarah	7.6 ± 0.075 ^{b}	7.9 ± 0.155 ^{b}	9.9 ± 1.258 ^{a}	0.94 ± 0.006 ^{a}	73.6 ± 1.181 ^{b}

Values are means (±SD) of triplicate samples. Values with the same letter are not significantly different ($p \leq 0.05$) as assessed by Tuckey. Lower caps letters indicate significant differences among cultivars.

3.2. Total Carotenoids (TCC) and Anthocyanin Content (TAC)

The date seed contents of the TCC and TAC are presented in Figure 1. The TCC of the seeds was found to be 1.98, 0.89, and 1.25 mg/g for the seeds of the Barhi, Ruthana, and Qatarah varieties, respectively. The TCC of the Barhi seed was significantly ($p \leq 0.05$) higher than that of the Ruthana and Qatarah seeds, which had a similar TCC. Total anthocyanin results revealed that the Ruthana seeds showed significantly ($p \leq 0.05$) higher TAC (5.51 ± 0.71 mg/g) content than Barhi (3.335 ± 0.23 mg/g) and Qatarah (3.835 ± 0.47 mg/g), but there was no significant difference between Qatarah and Barhi.

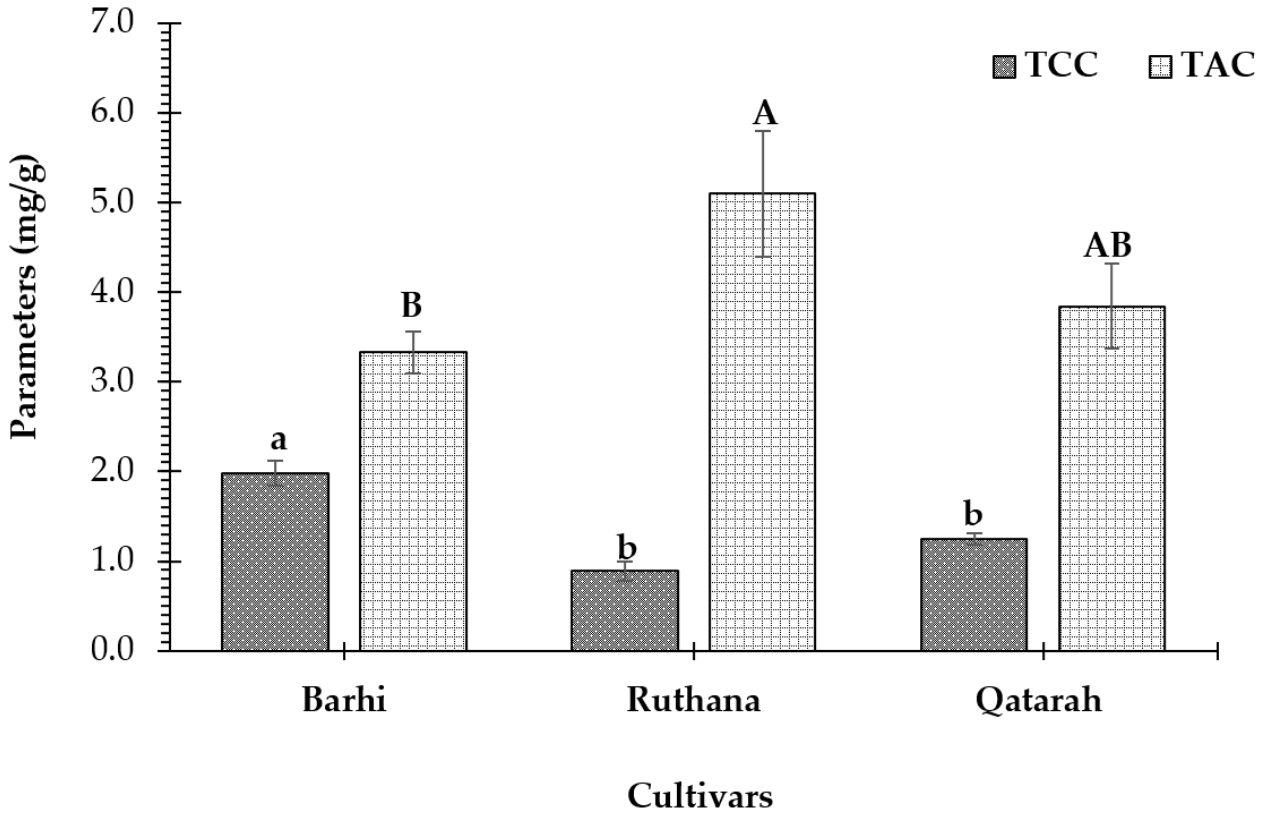

Figure 1. Total carotenoids content (TCC) and total anthocyanin content (TAC) of date cultivar seeds. Values are means (±SD) of triplicate samples. Values with the same letter are not significantly different ($p < 0.05$) as assessed by Tuckey. Capital letters indicate significant differences in TAC among date cultivars, whereas lower caps letters indicate significant differences in TCC among date cultivars.

3.3. Total Phenolic and Flavonoid Contents of Date Seeds

Table 2 illustrates the date seeds' total phenolic content (TPC) as influenced by different solvent extractions. As shown in the table, the TPC of the seeds was significantly different ($p \leq 0.05$) among the cultivars. The highest TPC was observed in the Ruthana seed (53.64 to 93.36 mg GAE/g) followed by Barhi seed (34.21 to 77.71 mg GAE/g), and the Qatarah seeds had the lowest content of TPC (13 to 48.21 mg GAE/g). Moreover, for each cultivar, the TPC of the seeds was found to be significantly ($p \leq 0.05$) affected by the solvent system. The ethanol (100%) solvent shows the highest TPC yield for the seeds of the Barhi, Ruthana, and Qatarah cultivars, which were found to be 77.71, 93.36, and 48.21 mg GAE/g, respectively. While the aqueous system showed the lowest values of the TPC for the three date seeds, it was found to be 34.21 mg GAE/g in the Barhi seeds, 53.64 mg GAE/g in the Ruthana seeds, and 13.0 in the Qatarah seeds (Table 2).

Table 2. Effect of solvents on the total phenolic content (mg/g) of three date cultivars seeds.

Extracts	Date Cultivars		
	Barhi	Ruthana	Qatarah
Methanol (100%)	57.07 ± 0.10^{dB}	65.28 ± 0.20^{cA}	33.14 ± 0.40^{dC}
Methanol (50%)	63.64 ± 1.11^{cA}	58.86 ± 0.20^{dB}	37.5 ± 0.10^{cC}
Ethanol (100%)	77.71 ± 0.20^{aB}	93.36 ± 0.30^{aA}	48.21 ± 1.11^{aC}
Ethanol (50%)	65.93 ± 0.30^{bB}	75.14 ± 0.40^{bA}	46.64 ± 0.30^{aC}
Water	34.21 ± 0.10^{eB}	53.64 ± 0.30^{eA}	13.0 ± 0.20^{eC}
M40:E40:W20	63.64 ± 0.30^{cB}	66.43 ± 0.61^{cA}	39.71 ± 0.20^{bC}

Values are means (±SD) of triplicate samples. Values with the same letter are not significantly different ($p \leq 0.05$) as assessed by Tuckey. Capital letters indicate significant differences among date cultivars, whereas lower caps letters indicate significant differences among extracts.

The date seed total flavonoid content (TFC) is shown in Table 3. Similar to the total phenolic content, the TFC of the date seeds was significantly ($p \leq 0.05$) influenced by the date cultivars and solvent system. The Ruthana cultivar seeds showed the highest TFC values, while the lowest was in the Qatarah cultivar seeds. Regarding the impact of the extraction solvent, methanol (100%) and mixture solvent extracts showed a significantly ($p \leq 0.05$) higher TFC compared to other solvents. It was found to be 35.3 and 36.8 mg CE/g in the Barhi seeds, 59.9 and 59.3 mg CE/g in the Ruthana seeds, and 35.9 and 35.8 mg CE/g in the Qatarah seeds, respectively.

Table 3. Effect of solvents on the total flavonoid content (mg/g) of three date cultivars seeds.

Extracts	Date Cultivars		
	Barhi	Ruthana	Qatarah
Methanol (100%)	35.3 ± 0.35^{aB}	59.9 ± 0.44^{aA}	35.9 ± 0.18^{aB}
Methanol (50%)	32.0 ± 0.00^{bB}	56.8 ± 0.18^{bA}	31.8 ± 0.18^{bB}
Ethanol (100%)	22.8 ± 0.18^{cB}	25.8 ± 0.27^{dA}	19.9 ± 0.09^{cC}
Ethanol (50%)	32.2 ± 0.53^{bB}	40.5 ± 0.09^{cA}	16.8 ± 0.35^{dC}
Water	17.5 ± 0.71^{dB}	21.9 ± 0.09^{eA}	15.5 ± 0.05^{eC}
M40:E40:W20	36.8 ± 0.35^{aB}	59.3 ± 0.27^{aA}	35.8 ± 0.18^{aC}

Values are means (±SD) of triplicate samples. Values with the same letter are not significantly different ($p \leq 0.05$) as assessed by Tuckey. Capital letters indicate significant differences among date cultivars, whereas lower caps letters indicate significant differences among extracts.

3.4. Date Seeds Antioxidant Activity

Different solvent systems were found to influence the antioxidant activity of the date seed, as shown by the DPPH scavenging activity and the total reducing power (TRP) results in Table 4. There were significant ($p \leq 0.05$) differences in the DPPH scavenging activity among the three date cultivars and the extracts. The DPPH of the Ruthana seed ranged from 45.63 to 81.88%, followed by Barhi (37.88 to 67.99%) and Qatarah (21.39 to 42.07%). Irrespective of the seeds, the solvent mix (M40:E40:W20) had the highest DPPH scavenging activity for all the date cultivar seeds. It was found to be 67.9, 81.9, and 42.1% for the Barhi, Ruthana, and Qatarah cultivars, respectively. The aqueous extract, on the other hand, had the lowest DPPH values in the Barhi (37.3%), Ruthana (45.0%), and Qatarah (21.4%) seeds.

The total reducing power (TRP) of the date seeds is demonstrated in Table 5. Similar to the DPPH results, both the cultivars and extract solvents exhibited a significant ($p \leq 0.05$) impact on the date seed TRP. The Ruthana date cultivar seed had the highest value of the TRP among the cultivars, followed by Bahi and Qatarah. The highest TRP values were obtained by using the mixed solvent system for extraction, while the water system showed the lowest. The Ruthana cultivar showed the highest TRP value (60.3 mg AAE/g) when seeds were extracted with the mixed solvent, while the lowest (26.4 mg AAE/g) was observed in the water-extracted seeds. Similarly, both the Ruthana and Qatarah seeds exhibited the highest values of 60.3 and 33.3 mg AAE/g f when the seeds were extracted

with the mix solvent. The lowest values were obtained after the seeds were extracted with water.

Table 4. Effect of solvents on the DPPH scavenging activity (%) of three date cultivars seeds.

Extracts	Date Cultivars		
	Barhi	Ruthana	Qatarah
Methanol (100%)	42.1 ± 0.46 ^{cB}	78.6 ± 0.92 ^{aA}	31.1 ± 0.69 ^{bC}
Methanol (50%)	43.4 ± 0.46 ^{cB}	54.4 ± 1.37 ^{cA}	42.0 ± 0.71 ^{bC}
Ethanol (100%)	51.6 ± 1.37 ^{bB}	60.2 ± 0.46 ^{bA}	28.9 ± 0.57 ^{bcC}
Ethanol (50%)	49.2 ± 0.87 ^{bB}	61.5 ± 0.46 ^{bA}	27.8 ± 0.38 ^{cC}
Water	37.9 ± 1.24 ^{dB}	45.6 ± 0.92 ^{dA}	21.4 ± 0.5 ^{dC}
M40:E40:W20	67.9 ± 0.90 ^{aB}	81.9 ± 0.92 ^{aA}	42.1 ± 1.37 ^{aC}

Values are means (±SD) of triplicate samples. Values with the same letter are not significantly different ($p \leq 0.05$) as assessed by Tuckey. Capital letters indicate significant differences among date cultivars, whereas lower caps letters indicate significant differences among extracts.

Table 5. Effect of solvents on the total reducing power activity (%) of three date cultivars seeds.

Extracts	Date Cultivars		
	Barhi	Ruthana	Qatarah
Methanol (100%)	33.4 ± 0.05 ^{eB}	59.8 ± 0.15 ^{bA}	26.5 ± 0.03 ^{cC}
Methanol (50%)	33.9 ± 0.04 ^{dB}	38.6 ± 0.05 ^{eA}	31.1 ± 0.05 ^{bC}
Ethanol (100%)	43.5 ± 0.05 ^{bB}	45.5 ± 0.09 ^{dA}	26.2 ± 0.07 ^{dC}
Ethanol (50%)	37.4 ± 0.07 ^{cB}	47.4 ± 0.03 ^{cA}	24.2 ± 0.00 ^{eC}
Water	26.4 ±0.02 ^{fB}	35.2 ± 0.03 ^{fA}	21.7 ± 1.04 ^{fC}
M40:E40:W20	47.3 ± 0.00 ^{aB}	60.3 ± 0.09 ^{aA}	33.3 ± 1.04 ^{aC}

Values are means (±SD) of triplicate samples. Values with the same letter are not significantly different ($p \leq 0.05$) as assessed by Tuckey. Capital letters indicate significant differences among date cultivars, whereas lower caps letters indicate significant differences among extracts.

3.5. Multivariable Analysis

The multivariable analysis was described in terms of the correlation between phenolic components and antioxidant activities (Table 6) and the partial least squares regression analysis (Figure 2). There was a significant ($p \leq 0.05$) positive correlation between the phenolic and flavonoid contents (r = 0.337). Also, the phenolic content has a significantly ($p \leq 0.01$) higher positive correlation with the DPPH (r = 0.718) and TRP (r = 0.722). Similarly, a strongly positive correlation was observed between the flavonoid, DPPH (r = 0.748), and TRP (r = 0.743). At the same time, the DPPH and TRP were also revealed to have a strong positively increasing relationship (r = 0.980) at the $p \leq 0.01$ level, as evident in Table 6.

Table 6. Correlation between total phenolic and flavonoid content with antioxidant activities.

	TPC	TFC	DPPH	TRP
TPC	1	0.337 *	0.718 **	0.722 **
TFC		1	0.748 **	0.743 **
DPPH			1	0.980 **
TRP				1

* Correlation is significant at the 0.05 level (2-tailed). ** Correlation is significant at the 0.01 level (2-tailed).

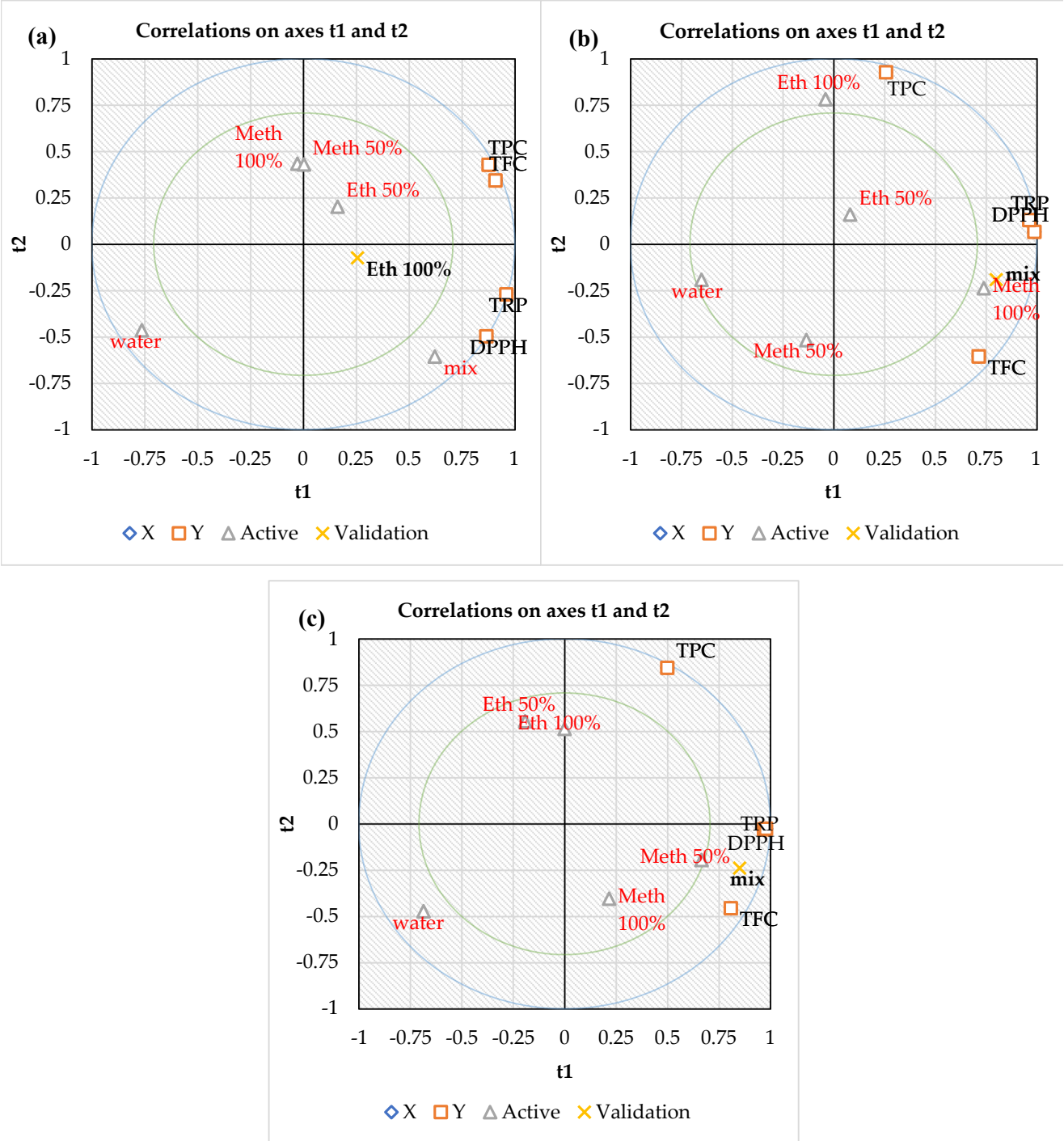

Figure 2. Partial least squares regression analysis (PLS) of validation of different solvents system used in the extraction of the phytochemical compounds and antioxidant activities of the date seeds of Barhi (**a**), Ruthana (**b**), and Qatarah (**c**) cultivars.

A partial least squares regression analysis (PLS) was achieved to confirm the most optimum solvent for extracting phytochemicals and their antioxidant activity in the date seeds for each cultivar. The shared effects of diverse extracts (methanol 100 and 50%, ethanol 100 and 50%, water, and M40:E40:W20) on the TPC, TFC, DPPH, and TRP of each date seed cultivar are exhibited in Figure 2. There was a deviation in the valid and optimum extracts for each seed. The PLS chart for the Barhi seed (Figure 2a) shows that the ethanol and methanol extracts have more antioxidant activities and TPC; however, the

100% ethanol extract was the most valid extract for these compounds. The PLS in Figure 2b shows that the extract of M40:E40:W20 showed the highest validation in the extraction of the compounds in the Ruthana seed. Similarly, M40:E40:W20 was the most valid and optimum extract for the Qatarah seed (Figure 2c). PLS has generally shown a variation in the validation of the different extracts for each seed. The most valid and optimum extract for the Barhi seed was 100% ethanol, whereas it was M40:E40:W20 for the Ruthana and Qatarah seeds.

4. Discussion

This study was conducted to assess the proximate composition, phytochemical content, and antioxidant properties of Barhi, Ruthana, and Qatarah date seeds. Furthermore, the optimization of phenolic compounds and antioxidants' extraction by using different solvent systems was also performed. During the ripening stages of the date (Kimri, Khalal, Rutab, and Tamr), the moisture content decreased from 20% at the Kimri stage to about 6–12% at the Tamr stage [37]. The moisture contents analysis showed 12.4, 6.6, and 7.6% for Barhi, Ruthana, and Qatarah, respectively. Our findings agree with those reported for Emirati date seeds [38,39], Moroccan date seeds [40], and Omani date seeds [10]. The variation in the moisture content among the cultivars might be due to the climate conditions and the relative humidity of the atmosphere. Nevertheless, the low moisture content of the seeds limits the growth of bacteria and allows for better preservation [41]. Carbohydrates are the main component of date seeds, and their quantity varies with different varieties [42]. The carbohydrate contents of the Barhi, Ruthana, and Qatarah date seeds were 70.0, 74.6, and 73.6%, respectively. These results agree with those found by [38], who analyzed six date cultivar pits (seeds), namely Mabroom, Lulu, Khalas, Fard, Khodari, and Abu Maan, and reported that the total carbohydrate content ranged from 70.14 to 78.70% but was lower than that of the Omani date seeds (83.14–86.89%), namely Shahal, Mabseeli, Um-Sellah [10], and Tunisian date seeds (Allig and Deglet Nour) (ranged between 81.0% and 83.1%) [43]. Interestingly, date seeds contain a significantly higher amount of protein and fat compared with date flesh [44,45]. Date seeds were found to contain a significant amount of fat; it ranged from 6.3% for the Ruthana to 8.7% for the Barhi variety. This agrees with that of [44,45], who reported the fat content of various Saudi date seeds, including Barhi, ranging from 7.83 to 8.69%. The date seed fat analysis revealed relatively high percentages of fatty acids, tocopherols, phenolic compounds, phytosterols, and carotenoids with excellent thermal and oxidative stability and a lower acidity content (0.5%) compared to soybean oil (0.86%), sunflower oil (1.4%), and palm Olean7 (4–5.5%), making it convenient for use in cosmetic and food applications [46]. The protein content of the date seeds did not significantly vary among the cultivars. The protein content of the seeds reported in this study were higher than those of the Tunisian date variety [47]. Date seed protein is reported to contain the most essential amino acids and has a higher concentration of sulfur amino acids than soybean, peanut, and cottonseed [48]. The differences in chemical composition could be due to the variability in the cultivars being studied, environmental conditions, agronomic practices, use of fertilizer, postharvest treatments, and storage conditions.

Researchers from different regions have investigated date seeds' phytochemical compounds and their biological activities. Date seeds were reported to be a rich source of biologically active compounds such as phenolic compounds, tocopherols, carotenoids, and phytosterols that are responsible for several pharmacological activities, such as hypolipidemic, antidiabetic, anti-inflammatory, anticancer, antioxidant, and antimicrobial activities [18,49]. In this study, the Ruthana seed showed a significantly high TPC, followed by Barhi and Qatarah. The total phenolic content (TPC) ranges from 13.0 to 93.357 mg GAE/g. The 100% ethanol showed the highest phenolic content, while water had the lowest. This finding agrees with the results reported by [20,50], who assessed the efficiency of different solvent systems and found that ethanol is the most effective solvent for extracting phenolic compounds from date seeds, whereas, other researchers [16,18] observed that 50% and 70% acetone were more efficient in extracting phenolic compounds from date seeds. The TPC of

the 100% methanol extract of the date seeds found in this study is higher than that reported for 14 varieties of Iranian date seeds [11] but was lower than that of the methanolic extract of some Tunisian date seeds [49]. These results revealed that it is hard to find a certain solvent system that extracts all phenolic compounds; therefore, employing an organic solvent alone or water can be more efficient in extracting most of the phenolic components of date seed samples. Similar to the TPC, Ruthana showed a significantly high total flavonoid content (TFC) while Qatarah showed the lowest. The results showed that 100% methanol was the most effective solvent, followed by the mixture of methanol:ethanol:water (40:40:20) and methanol 50% for the extraction of the TFC from date seed varieties, whereas water was the least efficient for TFC extraction. The TFC values of the date seeds were in agreement with those reported by [16] for the Saudi date seeds but higher than those of the Tunisian date seed varieties [40]. The high flavonoid yield of the mixture could be attributed to the high polarity of the solvent, which favors the extraction of flavonoids [49].

The Ruthana seed exhibited the highest TPC and TFC, ranging from 53.64 to 93.36 mg GAE/g and 21.89 to 59.89 mg CE/g, respectively, depending on the solvent system compared to the others. These values are higher than those of the Ajwa seed TPC and TFC, which range from 31.55 to 39.32 mg GAE/g and 18.4 to 29.56 mg CE/g, respectively [50], but lower than those of the Sukkari date seed, which contained 98.1 mg GAE/g phenolic and 70 mg CE/g flavonoids [16]. Several factors, including the date variety, geographic location, soil, sunlight, analytical and extraction methods, as well as the solvent system, might be responsible for the variation in the phenolic and flavonoid contents of the date seeds. The phenolic compounds in seeds are affected by the ripening stages of dates; they increase in the Rutab stage and decline progressively as the dates mature to the Tamr stage, and this was attributed to their embryo-protective effect, while at the Tamr stage, these compounds are broken down [11,31].

Carotenoids are major phytochemicals that act as colorants, vitamin precursors, and antioxidants. In the seed, carotenoid presence is necessary for the synthesis of abscisic acid, a phytohormone that regulates plant growth and development and significantly affects plant defense against bacteria and fungus. Carotenoids were found to protect against the lipid peroxidation of the membranes during seed aging and loss of viability [51]. Both the total carotenoids and anthocyanin levels contribute to the antioxidant activity of date seeds. The total carotenoid content (TCC) of the three date seeds reported in this study is higher than that of the Emirati date seed varieties [52]. The total anthocyanin content (TAC) of the three date seed varieties is in agreement with a previous study that revealed the TAC in Deglet Nour seed [53] but is higher than the TAC values of some Tunisian date seed varieties [47]. Similar to the other bioactive compounds, carotenoid and anthocyanin content were reported to vary according to cultivars, ripening stages, storage conditions, and postharvest processing [54].

Date seed was reported to contain higher antioxidant activity than date fruit [44]. The antioxidant activity of the seeds was reported to increase until the Rutab stage and then decrease in the Tamr stage due to a decrease in phenolic compounds, mainly because of polyphenol oxidase activity [55]. In plant extracts, the antioxidant activity is determined by using DPPH free radical scavenging activity and TRP, which are associated with the presence of antioxidant molecules that neutralize free radical chains by donating a hydrogen atom. This study found significant antioxidant activity differences among the tested cultivars, similar to the phenolic and flavonoid content. A strong correlation between the phenolic components and the antioxidant activity of the date seeds was observed in this study. The Ruthana seeds had the highest antioxidant activity values (81.88% for the DPPH and 60.29 mg AAE/g for the TRP), which contain more polyphenols and flavonoids whereas the Qatarah seeds, had the lowest phenolic compounds and antioxidant activity (42.07% for the DPPH and 33.27 mg AAE/g for the TRP). Several studies revealed an association between the antioxidant activity of the samples and their phenolic content: the higher the levels of the total phenolic content and flavonoids, the greater the antioxidant activity [47,50,56]. Our results revealed that the antioxidant capacity of the date seeds was

strongly and significantly correlated with the phenolic content (r = 0.718 for the DPPH and r = 0.788 for the TRP) and flavonoid contents (r = 0.748 for the DPPH and r = 0.743 for the TRP).

Moreover, similar to phenolic compounds, the antioxidant activity of extracts is found to be affected by the solvent system used. The mixture extracts showed the highest antioxidant activity as assayed by the DPPH or TRP, while water extracts showed the lowest values. Our data agree with those reported by [50], who found that antioxidants are more dissolved in polar solvents and their aqueous mixtures due to the wide range of polyphenolic compounds that can dissolve in them compared to water. These results indicate that solvent polarity increases the extraction efficiency of the antioxidant compounds and antioxidant activity compared to water or a pure solvent.

5. Conclusions

In the current study, seeds from three date cultivars, mainly Barhi, Ruthana, and Qatarah, were investigated for their proximate composition, bioactive content, and antioxidant activity, as well as the most efficient solvent for extracting bioactive and antioxidant compounds. The results revealed that date seed cultivars were rich in carbohydrates, energy, phytochemicals, and antioxidant compounds. The Ruthana cultivar seed exhibited the highest content of phenolic compounds and antioxidant activity compared to the other cultivars. A partial least squares regression analysis (PLS) showed that the valid and optimum extraction solvent for the Barhi seeds was the 100% ethanol extract, while the valid and optimum extraction solvent was the M40: E40: W20 extract for the Ruthana and Qatarah seed cultivars. Therefore, this study showed that the date seed produced by the date industry as a by-product is a rich source of natural antioxidants that might be used as a functional food component for therapeutic purposes.

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