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Research Article

Polyphenolic Profile and *in vitro* Antioxidant Activity of Three Algerian Date (*Phoenix dactylifera*) Varieties

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Abstract: As known, dates are packed with antioxidants and bioactive compounds and provide various health benefits, as evidenced by their varying nitrite levels. Algerian dates, on the other hand, have not been thoroughly investigated for their bioactive compounds and overall antioxidant capacity. This research aims to tap into this potential by meticulously measuring total polyphenols, flavonoids, and condensed tannins in three popular Algerian varieties (*Phoenix dactylifera* L.) Ksiba, Hamraya, and Deglet Nour, and the determination of their antioxidant activity using (scavenging assays of 2,2-diphenyl-1-picryhydrazyl (DPPH) and 2,2′-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radicals, reducing power, and total antioxidant capacity. The analysis showed that the three varieties of date fruits are rich in total phenolics with an amount ranging between 71±51 and 7975±389 mg of gallic acid equivalent (GAE).100 g^{-1} of dry weight (DW), the flavonoid amount ranged from 31±3 to 767±4 mg of quercetin equivalent (QE) 100 g^{-1} DW and condensed tannins between 6± 2 and 653 ±64 mg of catechin equivalent (CE) 100 g^{-1} dry DW. The antiradical activity was quite promising and ranged between 0.5 and 24 µg AAE mg^{-1} extract for DPPH and between 2 and 113 µg AAE mg^{-1} extract for ABTS, while the reducing power and total antioxidant capacity values ranged from 16 to 154 µg ascorbic acid (AAE) mg^{-1} and 39 to 68 µg AAE mg^{-1} extract respectively. The results of this study show that Algerian date fruit can be regarded as a potential natural source of antioxidants, with ethyl acetate serving as the best extractant solvent, resulting in higher polyphenol content and antioxidant activities.

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1. Introduction

Date palm fruits (*Phoenix dactylifera* L.), commonly known as dates, hold significant importance in the Saharan regions as a crucial food source. Beyond its economic value, the date palm stands out for its rich nutritional content. Renowned for being a powerhouse of carbohydrates and antioxidants (Sawaya et al., 1983; Biglari et al., 2008), dates are extensively consumed worldwide and constitute a staple in the diets of numerous Arab countries (Hasnaoui et al., 2012). Moreover, beyond its culinary uses, the date fruit has long been employed in traditional medicine (Benchelah and Maka,

2008). It serves as a tonic for the muscular and nervous systems, recommended for conditions like asthenia, demineralization, tuberculosis, and anemia (Delille, 2007).

In Algeria, for instance, there are more than 13 million date palm trees and 940 varieties that have been recorded to date, with a total production of approximately 1.13 million tons annually (Harkat et al., 2022; Khirani et al., 2020). While extensive studies have scrutinized the nutritional and chemical composition of dates, including their carbohydrates, proteins, lipids, fibers, vitamins, and minerals, a significant gap exists in the exploration of the biological impact of phenolic components found in date palm fruits. Existing research is limited to specific varieties (Sawaya et al., 1982; Mohamed et al., 2014).

In, particular, it has been repeatedly reported that a diet based on date fruits, which are naturally enriched in plant polyphenols is effective against many diseases. In fact, date fruits, contain many classes of bioactive components, including phytochemicals such as flavonoids, carotenoids, polyphenols, phytoestrogens, and sterols. Therefore, there has been a surge of interest among researchers in the secondary metabolites of dates due to their potential health benefits, notably in guarding against cardiovascular diseases (Mansouri et al., 2005; Biglari et al., 2008). Phenolic compounds have been associated with various biological activities such as antibacterial, antioxidant, antiviral, anti-carcinogenic, anti-inflammatory, anti-allergic, and vasodilator effects. They also demonstrate inhibition against lipid peroxidation and platelet aggregation (Packer, 2001; Hurst, 2008).

Colorimetric procedures, such as the Folin-Ciocalteu assay, are widely used to assess phenolic content. They are quick, straightforward, and inexpensive, making them excellent for high-throughput screening and routine analysis. However, they lack selectivity and may be impacted by interfering chemicals in the date fruit extract. On the other side, chromatographic methods like HPLC offer excellent resolution and specificity, it enables the identification and measurement of individual phenolic chemicals. In this context, Colorimetric and chromatographic approaches provide complimentary advantages for determining polyphenol content in date fruits. As a result, combining both strategies can be beneficial. Colorimetry provides a quick and initial assessment of overall phenolic content, whereas HPLC provides extensive information about the types and amounts of particular phenolics found in the date fruit sample (Benouamane et al., 2022; Dominguez-lópez et al., 2023).

The current study aims to assess the quantity of phenolic compounds in three Algerian date varieties - Hamraya, Ksiba, and Deglet Nour - utilizing colorimetric and chromatographic analyses of diverse fruit extracts. Additionally, it seeks to estimate their antioxidant potential using various methods.

2. Material and Methods

2.1. Plant material and morphological characterization

The plant material used in this study consisted of three varieties of dates**,** Deglet Nour, Ksiba, and Hamraya growing in the Biskra region in Algeria. Deglet Nour variety was collected from the Tolga locality (GPS data: 34° 42′ 18″ N, 5° 23′ 01″ E), while the two other varieties Ksiba and Hamraya were collected from the Sidi Okba locality (GPS data: 34° 45′ 08″ N, 5° 53′ 25″ E) at full maturity and stored at 4 °C.

The morphological characterization of the whole date (IPIGRI, 2005) was carried out on a randomly sampled batch. The color was visually appreciated; the consistency was to the touch. The dimensions of the whole fruit and its kernel (length and width) were determined through a caliper, and the weight (pulp and core) was determined using a precision balance.

Elasticity is measured by inserting a date sample between the molar teeth, chewing, and calculating the force with initial chews. (sample pushback) (i.e. bite down evenly, evaluating the force required to compress at different degrees) (Singh et al., 2015).

2.2. Extracts preparation

The organic extracts were prepared following Diallo (2005) method. Initially, 200 grams of date paste were combined with a methanol-water blend (80/20: v/v) totaling 600 mL. Each mixture underwent an 18-hour agitation period and subsequent filtration through paper. The elimination of methanol was performed via a rotary evaporator operating at 45°C until complete evaporation.

The resultant crude extracts were reconstituted in 100 mL of water and sequentially subjected to extraction with four solvents of increasing polarities: Hexane, dichloromethane, ethyl acetate, and finally, butanol.

2.3. Extracts composition and characterization

The overall levels of polyphenols, flavonoids, and condensed tannins in three different Algerian date fruit extracts were measured. Total polyphenols were determined using the Folin-Ciocalteu method, as described by Waterman and Mole (1994) and Mansouri et al. (2005), and the results were expressed in gallic acid equivalents per 100 g of dry weight (DW). The flavonoid content was determined using methods described by Lamaison and Carnat (1991) and Bahorun (1997), with the results reported as quercetin equivalents per 100 g DW, and Condensed tannins were measured using Braca's (2002) method, and the results were presented as micrograms of ascorbic acid equivalents per milligram of extract (g AAE mg^{-1} extract).

High-Performance Liquid Chromatography (HPLC) was used to analyze different extracts to delve deeper into the polyphenolic composition of the three date fruit varieties. Organic extracts of date fruit were dissolved in methanol before HPLC analysis, which was carried out under the following conditions: C18 column (4.6 x 150 mm, 5 m), detector with a wavelength of 254 nm, mobile phase of acidified water/methanol, injection volume of 20 microliters, flow rate of 1 ml/min, and column temperature maintained at 25°C. Each sample's compound identification was based on detecting differences in retention times between the components determined and the standards.

2.4. Antioxidant activity

2.4.1. Antiradical activity

The antiradical activity of three different Algerian date extracts was measured against 2,2-diphenyl-1-picryhydrazyl (DPPH) as described by Braca (2002) and against 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) using the methods of Re et al. (1999). The activity was measured using an ascorbic acid standard curve in both tests, and the results were expressed as g ascorbic acid equivalents per mg of extract (g AAE mg^{-1} extract).

2.4.2. Reducing power

The study also examined the electron-donating mechanism as another aspect of antioxidant activity. The total antioxidant activity of the various extracts derived from the selected date fruits was determined using the method proposed by Prieto et al. (1999). Furthermore, their reducing power was assessed using the Bougandoura and Bendimerad (2012) methodology. These results were quantified and expressed as micrograms of ascorbic acid equivalents per milligram of extract (g AAE mg^{-1} extract).

2.5. Statistical analysis

Data were presented as the mean ± standard deviation (SD) (n=3). Normality distribution of the data was validated using the Shapiro test and the determination of significant differences among groups was made via two-way analysis of variance (ANOVA) and the Tukey test was selected as a post hoc ($p<0.05$) using GraphPad Prism 7.00 software (GraphPad Software Inc., San Diego, CA, USA). The Correlation tests were also performed using the same software to determine the relationship between phenolic compounds and antioxidant activity. The correlation was defined by Pearson’s correlation coefficient after validation of the normality distribution of the data using the Shapiro test.

3. Results and Discussion

3.1. Physical characteristics of fruits

Table 1 shows the physical characteristics of the studied varieties. Generally, Deglet Nour, Hamraya and Ksiba dates have an aromatic taste. When ripe, dates are brown, red, and somewhat dark brown, respectively, with a brown color of seeds and a semi-soft consistency, the peel is smooth and shiny (Figure 1).

Figure 1. Three Algerian date varieties; a) Deglet Nour; b) Ksiba and c) Hamraya.

Sensory properties are important in evaluations, which are typically conducted by expert panels, trained panels, and consumer panels. Ben Ismail et al. (2013) carried out a sensory profiling study on seven date fruit cultivars in Tunisia, revealing significant morphological and physicochemical diversity among the tested varieties. According to Ismail et al. (2001), understanding the physicochemical, mechanical, structural, textural, and sensory properties of dates is critical for their processing, storage stability, and consumer acceptance. Dates' preferred sensory quality attributes at the "tamar" stage of maturity were ranked as high for color and appearance, medium for fruit size, chewiness, solubility, and flesh thickness, and low for elasticity, mouthfeel, and shear force.

Table 1. Physical properties of Ksiba, Hamraya, and Deglet Nour varieties

Character of the fruit	Ksiba	Hamraya	DegletNour
Fruit shape	Egg-shaped	Egg-shaped	Egg-shaped
Color	Dark brown	Red	Brown
Consistency	Semi-soft	Semi-soft	Semi-soft
Plasticity	Tender	Tender	Tender
Texture	fibrous	fibrous	Smooth
Core shape	egg-shaped	egg-shaped	egg-shaped
Core color	Brown	Brown	Brown
Fruit quality	Common	Common	Common

3.2. Total polyphenols content (TPC)

Colorimetric analysis revealed significant differences in the total polyphenol content of the three date fruit varieties (Table 2). Notably, ethyl acetate proved the most effective extraction solvent for all three, showcasing the variety-specific influence on polyphenol extraction. Hamraya emerged as the richest in terms of polyphenol abundance, boasting a content ranging from 371 ± 20 mg GAE 100 g^{-1} DW (n-butanol extract) to a whopping 7975 ± 389 mg GAE 100 g^{-1} DW (ethyl acetate extract). This translates to a remarkable 95% confidence interval of -2220 to 8438, underscoring Hamraya's potential as a potent source of polyphenols. In contrast, Ksiba displayed the lowest polyphenol levels, with values ranging from 71 ± 51 mg GAE 100 g^{-1} DW (hexane extract) to 5200 ± 404 mg GAE 100 g^{-1} DW (ethyl acetate extract) and a 95% CI of -2129 to 5447. Deglet Nour GAE? Our findings align with Benmeddour et al. (2013) observations, who reported total polyphenol contents between 226 and 955 mg GAE 100 g^{-1} DW for ten Algerian date cultivars. This suggests similarities in polyphenol profiles within geographically close regions.

However, Bensaçi et al. (2021) reported lower values (154-278 mg GAE 100 g^{-1} DW) for four Algerian date varieties from Ouargla, possibly due to the influence of extraction solvent (hydromethanolic mixture) and/or regional variations. Interestingly, Kchaou et al. (2013) documented values between 199 and 576 mg GAE 100 g^{-1} on a fresh weight basis for some Tunisian cultivars, highlighting the impact of moisture content on reported values.

Table 2. Total polyphenol content of the extracts of the three date varieties (in milligram gallic acid equivalent (GAE) 100 g^{-1} dry weight date fruit)

Extract	DegletNour	Ksiba	Hamraya
Hexane	$1390\pm207^{1,2,a}$	$71\pm51^{1,b}$	$1725\pm339^{1,a}$
Dichloromethane	$1668\pm137^{2,b}$	$554\pm76^{1,2,c}$	$2365\pm212^{2,a}$
Ethyl acetate	$5972\pm434^{3,b}$	$5200\pm404^{3,c}$	$7975\pm389^{3,a}$
***n*-butanol**	$947\pm76^{1,4,a}$	$811\pm66^{2,b}$	$371\pm20^{4,bc}$

Results are expressed as mean of triplicates with standard deviation. Columns with different superscript numbers are significantly different ($p<0.05$). Rows with different superscript letters are significantly different ($p<0.05$).

Various factors might be responsible for the observed differences such as variety, growing condition, maturity, season, geographic origin, fertilizer, soil type, storage conditions, agricultural methods, process and stabilization conditions, climatic conditions, use of different analytical methods, and use of different phenolic acid standards (Besbes et al., 2004; Al-Farsi et al., 2007; Besbes et al., 2009; Ahmed et al., 2021; Benmehaia et al., 2022). Compared to popular fruits like cranberries (607 mg CE 100 g^{-1} DW), plums (551 mg CE 100 g^{-1} DW), and apricots (333 mg CE 100 g^{-1} DW) (Vinson et al., 2005), Algerian date varieties pack a remarkable punch of total polyphenols content.

3.3. Flavonoid content (TFC)

The flavonoid contents of the different extracts from the three varieties of dates are shown in Table 3.

Table 3. Average flavonoid content of the extracts of the three date varieties (in micrograms equivalent of quercetin (QE) 100 g^{-1} dry weight date fruit)

Extract	Deglet Nour	Ksiba	Hamraya
Hexane	$98\pm2^{1,a}$	$31\pm3^{1,b}$	$71\pm1^{1,c}$
Dichloromethane	$176\pm3^{2,a}$	$113\pm1^{2,b}$	$139\pm 2^{2,c}$
Ethyl acetate	$208\pm4^{3,a}$	$767\pm4^{3,b}$	$710\pm 21^{3,c}$
n-butanol	$117\pm6^{4,a}$	$162\pm2^{4,b}$	$49\pm4^{4,c}$

Results are expressed as mean of triplicates with standard deviation. Columns with different superscript numbers are significantly different ($p<0.05$). Rows with different superscript letters are significantly different ($p<0.05$).

Continuing the trend observed for total polyphenols, ethyl acetate exhibited superior efficacy in extracting flavonoids from all three date fruit varieties (Table 3). Ksiba variety displayed the highest total flavonoid content, attaining a remarkable 767 ± 4 mg quercetin equivalents (QE) 100 g^{-1} dry weight (DW) within a 95% confidence interval (CI) of -268 to 804. Conversely, Deglet Nour exhibited the lowest content, averaging at 208 ± 4 mg QE 100 g^{-1} DW. Comparative analysis with existing literature reveals significant variations in flavonoid content among date cultivars. Bensaçi et al. (2021) reported considerably lower levels, ranging from 3 to 12 mg QE 100 g^{-1} DW (95% CI: 68.4 to 231) in four different Algerian cultivars. This observation aligns with the findings of Biglari et al. (2008) and Hasnaoui et al. (2012) who documented low total flavonoids in Moroccan and Iranian date cultivars. Our findings, however, resonate more closely with the work of Benmeddour et al. (2013), Kchaou et al. (2014), and Bouhlali et al. (2015). These studies reported total flavonoid content ranging from 15 to 300 mg QE 100 g^{-1}, 59 to 214 catechin equivalents (CE) 100 g^{-1} extract, and 69 to 209 mg rutin equivalents (RE) 100 g^{-1} DW in Algerian, Tunisian, and Moroccan date varieties, respectively.

3.4. Condensed tannins content (CTC)

The condensed tannin contents of the three varieties are presented in Table 4.

Table 4. Average condensed tannins content of the extract of the three date varieties (in micrograms equivalent of catechin (CE) 100 g^{-1} dry weight date fruit)

Extract	Deglet Nour	Ksiba	Hamraya
Hexane	$416\pm19^{1,a}$	$184\pm16^{1,b}$	$6\pm2^{1,c}$
Dichloromethane	$59\pm2^{2,a}$	$168\pm0^{1,b416}$	$150\pm11^{2,b}$
Ethyl acetate	$378\pm99^{1,a}$	$215\pm67^{1,b}$	$653\pm64^{3,c}$
n-butanol	$89\pm2^{2,a}$	$66\pm23^{2,a}$	$46\pm5^{1,a}$

Results are expressed as mean of triplicates with standard deviation. Columns with different superscript numbers are significantly different ($p<0.05$). Rows with different superscript letters are significantly different ($p<0.05$).

For the Hamraya and Ksiba varieties, ethyl acetate was the best solvent in the extraction of condensed tannins, with the Hamraya variety presenting the highest CTC content among the three varieties (653±64 mg CE 100 g^{-1} DW; 95% CI: -262.1 to 689.6). However, for Deglet Nour the amounts of CTC extracted using hexane and ethyl acetate were statistically similar and higher than the remaining solvents. The tannin content in this study is significantly higher than that reported by Bouhlali et al. (2015) and Alahyane et al. (2018). Their research found a range of 57-92 mg CE 100 g^{-1} dry weight and 5-152 mg CE 100 g^{-1} dry weight, in some Moroccan cultivars. On the other hand, Benmeddour et al. (2013) reported similar results for the condensed tannin content in several Algerian date varieties (82 to 525 mg CE 100 g^{-1} DW). however, our CTC values, are lower than those reported by Sawaya et al. (1982). In their study of 25 Saudi Arabian cultivars, they observed significant variations in total tannin content, ranging from 600 to 2700 mg per 100 g of dry weight at the final stage of fruit maturity. Note that, tannic acid and procyanidin B2 were detected as hydrolyzable and condensed tannins, respectively by HPLC analysis (Dassamiour et al., 2022) and during storage of dates at room temperature, the relative quality of simple polyphenols and soluble tannins decreased. Flavones disappeared by giving brown oxidized compounds (Mohamed et al., 1985). Also, the level of these phenolic components was observed to be stable during storage at 4°C, as reported by Benmehaia et al. (2022).

3.5. HPLC analysis

Dichloromethane and ethyl acetate extracts of the three varieties were analyzed for their phenolic content using HPLC, and the results are shown in Table 5.

Table 5. Identified compounds by HPLC in Dichloromethane and Ethyl acetate extracts of the three date varieties

Phenolic compounds	Reten. Time [min]	Deglet Nour		Ksiba		Hamraya	
		Dichloromethane	Ethyl acetate	Dichloromethane	Ethyl acetate	Dichloromethane	Ethyl acetate
P.coumaric acid	25.21	-	+	-	-	+	-
3-hydroxy-4-cinnamic acid	28.28	+	-	-	-	+	+
cafeic acid	20.52	+	+	+	+	-	-
Ferulic acid	26.6	-	-	-	-	-	-
Gallic acid	6.5-7	-	-	+	-	-	-
M Anisic acid	33.03	+	+	-	-	+	+
Salicylic acid	30.74	-	-	-	-	-	-
Syringic acid	21.96	+	-	-	-	-	-
Trans-2.4-diméthoxycinnamic acid	39.28	+	-	-	-	-	-
Trans-cinnamic acid	25.17	-	-	-	+	-	-
Vanillic acid	22.7	-	-	-	-	-	-
Catechin	21.55	-	-	-	-	-	-
Epicatechin	22.50	-	-	-	-	+	-
Euleropein	32.36	-	-	-	-	-	-
Kaempferol	41.1	-	-	-	-	-	-
Myricetin	34-41	+	+	+	+	+	+
Quercetin	36.85	-	-	-	-	-	-
Berberine	24.52	-	-	-	-	-	-
Resorcinol	10.40	-	-	-	-	-	-
Rutin	30.68	-	-	-	-	-	-

Analysis of the phenolic compounds of dichloromethane and ethyl acetate in Ksiba, Hamraya, and Deglet Nour extracts by HPLC allowed the identification of the following phenolic acids: gallic acid, coumaric acid, manisic acid, caffeic acid, syringic acid, 3-hydroxy-4-cinnamic acid, Trans-cinnamic acid and trans-2,4-dimetoxic acid. The presence of two types of flavonoids was highlighted in the ECh and EAc extracts as myricetin and epicatechin compounds(Table 5). The table illustrates the diversity in polyphenolic compounds present in the different date varieties and their extracts. Some compounds are unique to specific varieties or extraction solvents, while others are common. Dichloromethane and ethyl acetate extracts vary in terms of the polyphenols they contain as the presence or absence of certain compounds can be attributed to the choice of solvent, highlighting the importance of solvent selection in polyphenol extraction. Each date variety exhibits a unique polyphenolic profile. For example, Hamraya contains quercetin and myricetin, which are not found in the other two varieties, Deglet Nour and Ksiba. While, some compounds, like 3-hydroxy-4-cinnamic acid and M Anisic acid, are common in both dichloromethane and ethyl acetate extracts of all three varieties. The presence of gallic acid and catechin, varies between varieties, indicating that the specific date variety influences the composition of polyphenols. Several compounds, including ferulic acid, salicylic acid, vanillic acid, kaempferol, berberine, resorcinol, and rutin, are absent in all the extracts, suggesting that they may not be prevalent in these date varieties. In conclusion, the HPLC results reveal that the three date varieties (Deglet Nour, Ksiba, and Hamraya) exhibit variations in their polyphenolic composition. The choice of extraction solvent (dichloromethane or ethyl acetate) also influences the presence of specific compounds. This information is valuable for understanding the diversity of bioactive compounds in different date varieties and can have implications for their potential health benefits and various applications in the food and pharmaceutical industries.

3.6. Antioxidant activity

The antioxidant effect of the date fruit extracts was measured using different methods including FRAP, total antioxidant TAC, DPPH, and ABTS free radical scavenging activity.

3.6.1. DPPH and ABTS radical scavenging capacities

The results of the radical scavenging ability (Table 6) revealed that the Ksiba and Hamraya had strong free radical scavenging ability, especially when ethyl acetate was used as solvent. The two varieties presented a similar DPPH scavenging capacity of 24±0.1 µg AAE.mg^{-1} extract (95% CI: -4.506 to 30.88). For Deglet Nour both dichloromethane and ethyl acetate gave the best DPPH scavenging capacity (17±0.04 µg AAE mg^{-1} extract; 95% CI: -0.32to 22.33), yet, it remains lower compared to the two other varieties. Hexane and *n*-butanol extracts of the three varieties exhibited low DPPH anti-radical activity.

Table 6. DPPH and ABTS Antioxidant capacities of three Algerian date cultivars

	DPPH (µg AAE mg^{-1} extract)				**ABTS (µg AAE mg^{-1} extract)**			
Variety/ Solvent	Hxn	DCL	EA	n-Btn	Hxn	DCL	EA	n-Btn
Deglet Nour	3±0.04^{1,c}	17±0.04^{2,a}	17±0.1^{2,a}	7±0.04^{2,b}	7±0.3^{1,d}	82±2^{1,b}	113±0.2^{1,a}	18±0.2^{1,c}
Ksiba	0.5±0.01^{2,d}	10±0.2^{3,b}	24±0.1^{1,a}	8±1^{1,c}	2±0.4^{2,d}	57±3^{3,b}	91±0.4^{2,a}	15±0.6^{2,c}
Hamraya	0.75±0.2^{2,d}	21±0.04^{1,b}	24±0.1^{1,a}	7±1^{2,c}	5±0.1^{1,c}	74±0.2^{2,b}	79±0.5^{3,a}	6±0.3^{3,c}

Results are expressed as mean of triplicates with standard deviation. Columns with different superscript numbers are significantly different ($p<0.05$). Rows with different superscript letters are significantly different ($p<0.05$).

Our findings regarding DPPH radical scavenging activity (32-86%) for the three date varieties align with Benmeddour et al. (2013) who reported similar values in ten Algerian cultivars. This finding further corroborates the observations of Mansouri et al. (2005) and Hasan et al. (2010) highlighting the effective DPPH scavenging capabilities of date fruits. The ABTS method assesses an antioxidant's ability to neutralize the green-blue cationic radical ABTS+ (Antolovich et al., 2002). Interestingly, all three varieties exhibited stronger ABTS scavenging compared to DPPH, with ethyl acetate extracts demonstrating the highest activity. Deglet Nour emerged as the most efficient scavenger (113 ± 0.2 µg

AAE mg^{-1} extract), followed by Ksiba and Hamraya. Similar to DPPH, hexane, and n-butanol extracts exhibited weaker ABTS activity, ranging from 5 ± 0.1 µg AAE mg^{-1} for Hamraya's hexane extract to 18 ± 0.2 µg AAE mg^{-1} for Deglet Nour's n-butanol extract. Our ABTS findings resonate with Hasan et al. (2010) and Benmeddour (2013) who also reported potent ABTS scavenging activities in date fruits. The DPPH values were higher compared to Siddeeg et al. (2019) who documented lower activity (43-76%) in the ethanolic and methanolic flesh extracts of the Sukkari variety (Saudi Arabia/Iraq). Notably, Siddeeg et al. (2019) also confirmed strong ABTS activity in their date samples. The observed disparities in scavenging activity potentially stem from varietal differences, extraction solvents employed, and specific date fruit regions (Deghima et al., 2020). The abundant phenolic and flavonoid compounds in date fruits are recognized as potent hydrogen/electron donors, likely contributing to their effective radical scavenging capabilities.

3.6.2. Reducing power

The reducing power of the different solvents' extracts from the three varieties is presented in Table 7.

Table 7. The reducing power of the three varieties expressed as µg ascorbic acid equivalent (AAE) mg^{-1} of extract

Extract	Reducing power (µg AAE mg^{-1} extract)		
	Ksiba	Hamraya	Deglet-Nour
Hexan	21 ± 0.2^{4b}	18 ± 0.3^{3c}	30 ± 1^{3a}
Dichloromethan	29 ± 0.0^{3b}	60 ± 0.6^{1a}	19 ± 0.1^{4c}
Ethyl acetat	50 ± 0.2^{1c}	55 ± 0.4^{2b}	154 ± 1^{1a}
n-butanol	31 ± 0.4^{2c}	16 ± 0.5^{4b}	38 ± 0.3^{2a}

Results are expressed as mean of triplicates with standard deviation. Columns with different superscript numbers are significantly different ($p<0.05$). Rows with different superscript letters are significantly different ($p<0.05$).

This method was developed to assess extracts' ability to reduce ferric iron ions (Fe^{3+}) found in ferrous iron complexes (Fe^{2+}). In fact, Fe^{3+} is involved in the formation of the hydroxyl radical (Benzie and Strain., 1996). The ethyl acetate extracts of studied varieties presented an important reducing power, with the highest value registered for the Deglet Nour variety (154±1 µg AAE mg^{-1} extract; 95% CI:-39.97 to 160.5) and the lowest registered for the Ksiba variety (50±0.2 µg AAE mg^{-1} extract; 95% CI:13.20 to 52.30). While for Hamraya variety the dichloromethane was the solvent with the best reducing power (60±0.6 µg AAE mg^{-1} extract; 95% CI:-0.1212 to 74.62). Benmeddour et al., (2013) reported higher values (272 to 1175 mg GAE 100 g^{-1} DW) for ten Algerian cultivars studied. Comparable results were reported by Zeroual et al. (2020), who observed that the best reducer was obtained by ethyl acetate extract with 118 µg EAA mg^{-1} extract from the Deglet Nour variety. The reducing power of a sample is determined by the electron transfer capacities of the reducers (antioxidants) in that sample. Because our extracts are high in electron-donating polyphenols, we may speculate that these compounds are responsible for our extracts' reducing power, which allows them to scavenge free radicals and function as chain-breaking antioxidants. They may also limit peroxide production and help in the regeneration of other damaged antioxidants (Deghima et al., 2020).

3.6.3. Total antioxidant capacity

The total antioxidant capacity of the different organic extracts from the three varieties is shown in Table 8.

The phosphomolybdate method quantifies the total antioxidant capacity (TAC) of the extracts, exploiting the reduction of Mo(VI) to Mo(V) in the presence of antioxidants and the subsequent formation of a green-colored phosphate-Mo(V) complex (Sahua and Laloo, 2011). Notably, the solvent employed significantly impacted the TAC values across varieties. Ksiba exhibited the highest TAC values (62 ± 0.1 and 61 ± 0.1 µg AAE mg^{-1} extract) for hexane and dichloromethane extracts, respectively. This may be attributed to the potential presence of lipophilic antioxidants in this variety (Deghima et al., 2020). Conversely, dichloromethane and ethyl acetate emerged as the superior solvents for Deglet Nour and Hamraya varieties. Overall, Deglet Nour achieved the highest TAC value (68 ± 3

µg AAE mg^{-1} extract, 95% CI: 37.99 to 69.01) associated with its dichloromethane extract. It is noteworthy that beyond polyphenols, other bioactive compounds such as carotenoids and α-tocopherol present in non-polar extracts (hexane and dichloromethane) might contribute to their overall antioxidant activity (Deghima et al., 2020). Our findings contrast with Ali Haimoud et al. (2016) who documented lower TAC values for dates, ranging from 43 to 90 µmol ascorbic acid g^{-1} extract, with Ali Ourached cultivar showcasing the highest recorded level.

Table 8. Total antioxidant capacity of the three varieties expressed as µg ascorbic acid equivalent (AAE) mg^{-1} of extract

Extract	Total antioxidant capacity (µg AAE mg^{-1} extract)		
	Ksiba	Hamraya	Deglet-Nour
Hexane	$62\pm0.1^{1,a}$	$41\pm0.5^{3,c}$	$49\pm0.6^{2,b}$
Dichloromethane	$61\pm0.1^{1,b}$	$63\pm3.8^{1,b}$	$68\pm3^{1,a}$
Ethyl acetate	$45\pm1.1^{2,c}$	$66\pm2.1^{1,a}$	$50\pm1.2^{2,b}$
n-butanol	$39\pm2.4^{3,b}$	$50\pm1.5^{2,a}$	$47\pm0.2^{2,a}$

Results are expressed as mean of triplicates with standard deviation. Columns with different superscript numbers are significantly different ($p<0.05$). Rows with different superscript letters are significantly different ($p<0.05$).

3.7. Correlation study

A correlation study was performed to link the bioactive compounds to the observed activities and the results are presented in Table 9.

Table 9. Linear correlation coefficients between phenol content and antioxidant activity of extracts of *Phoenix dactylifera* L.

	TPC	TFC	CTC
DPPH	-0.3505	-0.38591	-0.12956
FRAP	0.74907	0.67693	0.67297
ABTS	0.93210	0.82561	0.64201
TAC	0.35289	-0.06963	0.25612

TPC: Phenolic compounds; TFC: Flavonoids; CTC: condensed tannins; FRAP: Reducing power; DPPH: Scavenger activity of the radical DPPH; ABTS: Scavenger activity of radical ABTS; TAC: Total antioxidant capacity.

Statistically significant positive correlations were observed between the FRAP and ABTS antioxidant capacities and the total phenolic content ($r = 0.75$ and $r = 0.93$, respectively). These findings corroborate those of Mansouri et al. (2005) and Biglari et al. (2008) who demonstrated a substantial contribution of phenolic compounds to the overall antioxidant potential of date fruits.

A similar trend emerged for the relationships between flavonoid content and condensed tannins with both FRAP and ABTS capacities ($r = 0.68$ and $r = 0.67$ for FRAP; $r = 0.83$ and $r = 0.64$ for ABTS). Interestingly, a weak negative correlation was observed between total antioxidant capacity and flavonoid content ($r = -0.06963$). Intriguingly, negative correlations were observed between total condensed tannins, phenolic compounds, and flavonoids concerning the DPPH antioxidant activity ($r = -0.13$, $r = -0.36$, and $r = -0.39$, respectively). These findings resonate with those of Ramchoun et al. (2017) and Alahyane et al. (2019) who reported similar negative correlations between these bioactive compounds and the DPPH scavenging capacity in their studies.

4. Conclusion

The study of the physicochemical and biochemical characteristics of three date varieties, namely: Hamraya, and Ksiba, harvested in the region of Sidi Okba, and Deglet Nour harvested in Tolga, revealed different characteristics from one cultivar to another. The morphological and organoleptic characteristics showed that the half-marrow dates have a dark color, a soft texture, and a fibrous appearance. The presence of considerable amounts of total phenolic compounds was revealed, especially in the more polar extracts, like ethyl acetate where the amount of polyphenols reached 7975 ±389 mg GAE/ mg extract for the Hamraya variety whereas the condensed tannin and flavonoid levels were

relatively lower with ethyl acetate being the best extractant solvent, except for Deglet Nour where hexane was the best extractant with an amount of 416±19 mg CE/ mg extract. High antioxidant activity was found in ethyl acetate extracts and dichloromethane compared to the hexane and n-butanol extracts which was positively correlated with phenolic content. Finally, the 3-hydroxy-4-cinnamic acid and M. Anisic acid, are common in both dichloromethane and ethyl acetate extracts of all three varieties.

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