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Comparative study of the phenolic content of Deglet Nour date seed extracts and evaluation of their antioxidant activities using different solvents

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Abstract
This study focused on the extraction and characterization of phenolic compounds of Deglet Nour date seeds, as well as the evaluation of their antioxidant activities. Three extraction solvents were compared: ethanol (80%), acetone (50%) and water. The extraction solvent showed an effect on the studied parameters. The highest extraction yield was obtained with ethanol (30.91%), followed by acetone (30.72%) and water (28.92%). The total phenolic compound content was significantly higher in the ethanolic extract (46.69 g GAE/100 g phenolic extract), followed by the acetone (38.36 g GAE/100 g) and water (33.52 g GAE/100 g) extracts. A similar variation was observed for flavonoid concentration, with significant differences depending on the type of solvent. Analysis by infrared spectroscopy identified the presence of characteristic phenolic and carbonyl compounds, with intensities varying according to the solvent used. Antioxidant activity assessed by DPPH and ABTS tests revealed that the ethanolic extract possessed the best activity, with respective IC_{50} of 0.20 and 0.17 mg/mL.
These results highlight the potential of date seed extracts as a natural source of bioactive phenolic compounds, able to confer strong antioxidant activity and contribute to protection against oxidative stress.

Keywords: Date seeds, phenolic compounds, antioxidant activity, infrared spectroscopy

Introduction

Faced with growing concerns about food safety, environmental protection and reduction in the use of chemical preservatives, scientific research is focusing progressively on exploring natural sources for the development of new antimicrobial and antioxidant agents (Oulahal et Degraeve 2022) [33]. In this context, plant extracts from different agricultural and agri-food matrices are attracting considerable interest because of their abundance of bioactive compounds, such as polyphenols, flavonoids, tannins and essential oils (Dar *et al*., 2023) [14]. Besides, many plants and residues from plant processing, long considered to be waste products, have demonstrated a high biological potential (Leichtweis *et al*., 2021) [27]. For instance, pomegranate peels (Mo *et al*. 2022) [30], olive leaves (Medina *et al*., 2019) [28], extracts of thyme, rosemary and cloves have been widely investigated for their strong antioxidant potential, attributed to their high content of phenolic compounds able of scavenging free radicals and slowing oxidative processes in foods (El-Refai *et al*., 2020) [17]. With consumers becoming increasingly wary of synthetic chemical additives, these natural compounds appear to be an attractive and eco-friendly alternative, addressing health, economic, and environmental concerns (Ciobanu *et al*., 2024) [13].

In this context of research into multifunctional natural agents, date seeds emerge as a particularly interesting plant resource that is still largely under-exploited. Derived from the fruit of the date palm (*Phoenix dactylifera L*.), these seeds represent around 10-15% of the total weight of the date (Omrani *et al*., 2024) [32]. They are produced in large quantity in the countries of North Africa and the Middle East, where dates play a strategic role in the agricultural economy (Tengberg, 2012) [41]. In Tunisia. for example. the "Deglet Nour" variety, which is widely exported, generates large volumes of lignocellulosic residues every year, of which the seeds make up a significant proportion (Souli *et al*., 2022) [38]. Although often relegated to low value uses such as animal feed or composting. These seeds are rich in fiber, lipids and phenolic compounds (Benabderrahim *et al*., 2018) [8]. Several studies have highlighted the richness of these extracts in flavonoids, phenolic acids, condensed tannins and powerful antioxidants (Bettaieb *et al*., 2023) [10]. The study of these compounds is becoming particularly valuable, as natural antioxidants derived from agricultural co-products are becoming widely used as alternatives to synthetic antioxidants, such as BHT or BHA, whose use is becoming restricted for health and legislative reasons (Felter *et al*., 2021) [18]. Given the limitations of traditional chemical treatments, which are being challenged (Dwivedi *et al*., 2017) [16], date seed phenolic extracts offer a powerful ability to scavenge free radicals and delay oxidation (Bentrad et Gaceb-Terrak., 2020) [9]. Incorporating them into edible films or natural formulations opens the way to innovative, eco-friendly and cost-effective preservation strategies (Muñoz-Tebar *et al*., 2023) [31]. This recovery, in line with the aims of circular economy, would help to reduce post-harvest losses and boost the competitivity of the date industry by creating high value-added outlets for a local co-product.

In addition to their nutritional and biological properties, the interest in these natural compounds is also driven by advances in extraction and processing methods. The selection of an appropriate solvent is crucial to maximize the recovery of bioactive molecules while preserving their functional properties. Solvents such as aqueous ethanol and aqueous acetone are commo

nly used due to their high efficiency in extracting polyphenols and other antioxidant compounds, allowing researchers to obtain extracts suitable for further functional and nutritional evaluation (Galanakis, 2021).

The objective of this study is to compare the effect of three extraction solvents for recovering phenolic compounds from date seeds, by assessing their total polyphenol and flavonoid content and their antioxidant activity, in order to identify the most suitable method for the production of a natural and sustainable source of antioxidants that meets the expectations of consumers and the agri-food industry.

Materials

All the reagents and standards were of analytical grade were purchased from VWR (France).

Materials and methods

Extraction of phenolic compounds from date seeds

The seeds used in this study were provided by Boudjbel SA VACPA from the date variety "Deglet Nour" harvested in 2022 at the Tamr stage. They were washed, dried and crushed in a Yangui hammer mill (power: 4 KW. voltage: 380 V 50-HZ. capacity: 250 kg/hour) to be filtered through a 1 mm filter.

The obtained powder was subjected to an extraction by maceration using three solvents according to the protocols described by Radfar *et al*.(2019) [35] and Al-Farsi et Lee (2008) [5], with some modifications.: distilled water, 80% ethanol and 50% acetone. The solid/solvent ratio was fixed at 1:60 (w/v), and the extraction was carried out under constant stirring for 2 h at 45°C. After the extraction, the mixtures were centrifuged at 3940 × g for 20 min at room temperature, then filtered on No. 1 filter paper. The extracts obtained were concentrated to dryness using a rotary evaporator at 40°C. The dry residues were then stored at -18°C, in the dark. until use.

Yield of extraction

The extraction yield of phenolic compounds was determined using the formula (1):

$$Yield\ of\ extraction\ (\%) = [\frac{Weight\ of\ dry\ extract(g)}{Weight\ of\ dry\ plant\ material\ (g)} * 100] \quad \text{(Equation 1)}$$

Total phenols content

The total extractable phenolic compounds content was estimated using the Folin-Ciocalteu colorimetric method described by (Hayder *et al*., 2021) [21]. This consists of mixing 0.3 ml of the extract solution with 1.5 ml of Folin-Ciocalteu reagent (diluted 1/10) and 1.2 ml of sodium carbonate solution (Na_2CO_3, 7.5%). After one hour's incubation in darkness, absorbances were read at 760 nm. The concentration of total extractable phenolic compounds was determined by reference to the gallic acid calibration curve performed in parallel under the same conditions.

Total flavonoids content

The flavonoid content of date seed extracts was estimated using the method described by Hayder *et al*. (2021) [21]. 1 ml of phenolic extract was mixed with 1 ml of a 2% Aluminum trichloride ($AlCl_3$) solution. After incubation for 30 minutes, the absorbance of the mixtures was measured at 430 nm using a spectrophotometer. Rutin was used as the standard compound for the calibration curve.

Mid Infrared spectroscopy measurements

Measurements were carried out on the various phenolic extracts in the 800 to 4000 cm-1 range. The analysis was conducted using a Fourier transform spectrometer IRTracer-100 (Shimadzu. France) fitted with an attenuated total reflection (ATR) accessory equipped with a grip (Pike Technologies. Inc. Madison. USA). The ATR cell featured a ZnSe crystal with a 45° angle of incidence and a total of 10 internal reflections (Omrani *et al*., 2024) [32]. For each spectrum, 32 scans were recorded with a resolution of 4 cm-1 and an empty background was recorded before each spectrum was acquired.

Antioxidant activity of phenolic extracts

DPPH test

The free radical scavenging activity was evaluated using the DPPH assay (Abdessemed *et al*., 2024) [4] with slight modifications. Briefly, 50 µL of extract solution prepared at different concentrations were mixed with 950 µL of a DPPH ethanolic solution (2×10^{-4} M) and incubated in the dark at room temperature for 30 min. The absorbance was measured at 517 nm against a blank, and the scavenging activity was expressed as percentage inhibition (PI) using the formula (2):

$$PI(\%) = \frac{DO\ controle - DO\ sample}{DO\ controle} * 100 \quad \text{(Equation 2)}$$

Three replicates were carried out for each concentration and the results are expressed as IC_{50} (50% effective concentration), corresponding to the concentration required to inhibit 50% of the DPPH radicals present in the reaction medium. determined from the PI = f(C) curve. BHT was used as a reference antioxidant for comparison.

ABTS test

The ABTS test is used to assess the antioxidant activity of an extract by measuring its ability to neutralize the $ABTS^{-+}$ cation radical. This is generated by reaction between a solution of ABTS (7 mM) and a solution of potassium persulphate (2.45 mM). The mixture was incubated in the dark for 12 to 16 hours at room temperature to allow complete formation of the radical. Before use. the solution obtained was diluted with ethanol to adjust its absorbance to 0.7 nm. For the test. 1.5 mL of the radical solution was mixed with 50 µL of the extract under investigation. After incubation for 10 minutes in darkness. the absorbance of the mixture is measured at 734 nm (Dieng *et al*., 2017) [15]. Three replicates were performed for each concentration. Results are expressed as percentage inhibition (PI) and IC_{50}, using the same principles as those applied for the DPPH assay.

Statistical analysis

All the measurements were made in triplicate and results are presented as mean ± standard deviation. They were processed statistically by a single-factor variance analysis followed by a two-by-two comparison of the averages according to Tukey's test and a Pearson's correlation test was used to examine the relationship between two variables with the Minitab statistical software (v.19. Minitab Inc. Pennsylvania. USA). P-value < 0.05 was considered as statistically significant.

Results and discussion

Yield of extraction

Statistical analysis revealed no significant difference between the extraction yields of ethanol and acetone solvents, while both showed a significantly higher yield compared to aqueous extraction. Ethanol (80%) showed the highest yield (30.91%), followed by acetone (30.72%), while aqueous maceration showed the lowest yield (Figure 1).

These results are in agreement with those reported by Al-Farsi and Lee (2008) [5] and Radfar *et al*. (2019) [35], who observed that extracting phenolic compounds from date seeds with ethanol (60-80%) resulted in higher yields than those obtained by aqueous extraction. Similarly, Shi *et al*. (2023) [37] observed that ethanol 70% was the most effective solvent for extraction of phenols from date seeds, while water proved to be more effective for the pulp. Moreover, several studies have highlighted the suitability of acetone as an extraction solvent. Jdaini *et al*. (2022) [23] reported that 50% acetone was more effective than methanol and water for extracting polyphenols from date seeds. Meanwhile, Suresh *et al*. (2013) [39] showed that acetone (50%) offered a better yield than ethanol (50%) and water.

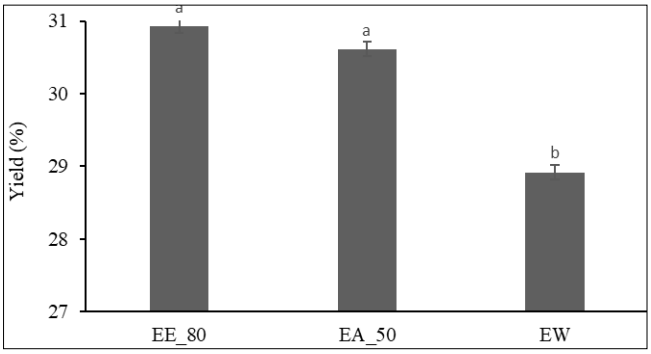

Fig 1: Extraction yield of Deglet Nour date seed using different solvents

EE_80; EA_50 and EW: Date seeds extract obtained with Ethanol (80), Acetone (50) and Water, respectively. Each value is mean ± standard deviation (n = 3). Values bearing the same letter within the same column are not significantly different ($p<0.05$)

Total phenols content

Significant differences between the three extracts (figure 2) were observed. The results showed that the ethanolic extract had the highest content of total phenolic compounds (46.69 g GAE/100 g phenolic extract), followed by the acetone extract (38.36 g GAE/100 g), while the aqueous extract had the lowest concentration (33.52 g GAE/100 g). According to Bouhlali *et al* (2020) [11], the most abundant phenolic compounds in date seeds are caffeic acid, chlorogenic acid, p-coumaric acid, ferulic acid, gallic acid, syringic acid and vanillic acid, along with flavonoids such as quercetin, rutin and luteolin. The highest content of total phenols obtained for ethanol (80%) is due to the intermediary polarity of this solvent, which enhances the solubilization of this wide range of phenolic compounds, thus ensuring more efficient extraction (Hikmawanti *et al*., 2020). As shown by Galanakis *et al* (2013) [19], ethanol has low activity coefficients for these compounds, reflecting high solubility and better recovery than acetone, which is less suitable for polar compounds but more effective for extracting high molecular weight polyphenols and condensed tannins (Teng *et al*., 2019) [40], while water alone can only extract very hydrophilic fractions, explaining the lower content observed in the aqueous extract.

The total phenolic contents obtained in this study are close to those found by John and Shahidi (2019) [24] who noted that the total content of soluble phenolic compounds in date seeds reached 82.62 mg GAE/g of seeds, significantly higher than that observed for dates (between 2.49 and 8.36 mg GAE/g). This study also showed that phenolic compounds are mainly present in soluble rather than bound form, and that date seeds have a phenolic compound content comparable to that of date palm leaves (106 mg GAE/g of sample). In addition, Gouda *et al*. (2024) [20] also observed a high content of phenolic compounds in date seeds of the Khalas variety, but with slightly lower values than those obtained for Deglet Nour (127.21, 144.42 and 83.68 mg GAE/g for methanolic, ethanolic and aqueous extracts, respectively). These results illustrate the valuable phenolic content of date by-products, especially date seeds, representing a promising source of bioactive phenolic compounds for potential industrial applications.

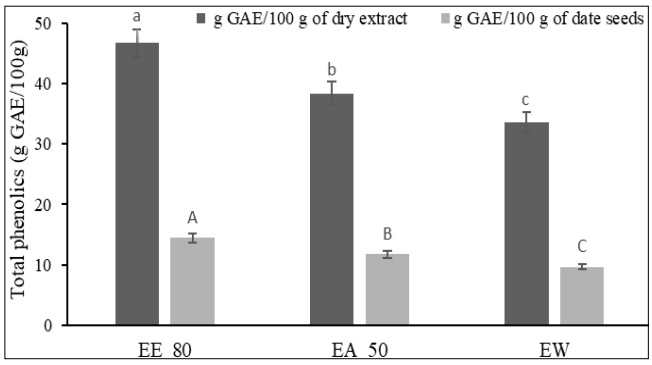

Fig 2: Total phenols of date seeds extracts using different solvents

EE_80; EA_50 and EW: Date seeds extract obtained with Ethanol (80), Acetone (50) and Water, respectively. Each value is mean ± standard deviation (n = 3). Values bearing the same letter within the same column are not significantly different ($p<0.05$)

Total flavonoids content

Figure 3 showed that date seeds are rich in flavonoids, with significantly different levels depending on the type of solvent. The highest concentrations were observed in the ethanolic extract (10.83 g RE/100 g phenolic extract), followed by the acetone 50% (9.48 g RE/100 g) and aqueous (8.46 g RE/100 g) extracts. These results are in line with those reported by Al-Farsi and Lee (2008) [5], who observed flavonoid contents ranging from 6.39 to 15.93 g/100 g of phenolic concentrate for the Mabseeli variety. Similarly, Metoui *et al*. (2018) [29] compared the flavonoid content of 11 date seed varieties, reported in catechin equivalents (CAE), and found values ranging from 1.43 to 3.83 g CAE/100 g dry matter. For the Deglet Nour variety, a content of 2.31 g CEA/100 g was reported, which is comparable to the concentrations obtained in the present study for Deglet Nour seeds, which varied between 2.45 and 3.35 g CEA/100 g of seeds depending on the extraction solvent used.

The flavonoid content of date seed extracts is within the range of plants considered to be particularly rich in these compounds. They are higher than those observed in pomegranate peel, which reaches up to 70.5 mg EQ/g extract (Abdu *et al*., 2020) [2], and comparable to the values reported for grape seeds, which range from 40 to 52 mg EQ/g dry matter depending on the used variety (Krasteva *et al*., 2023) [26].

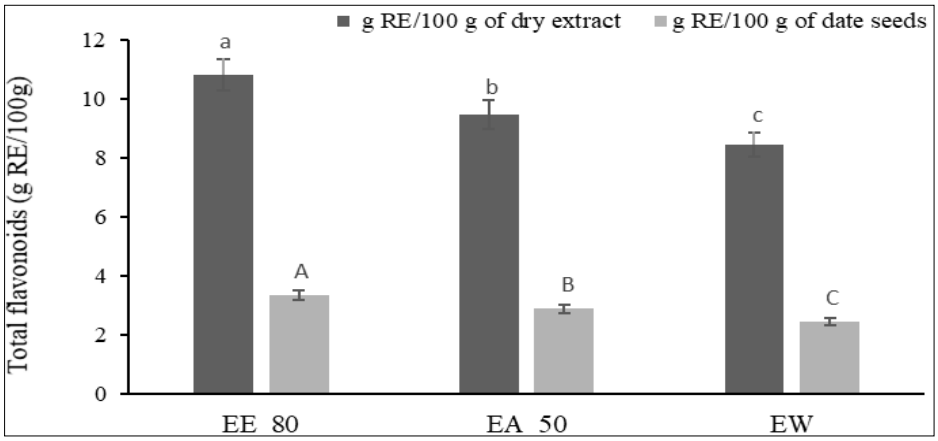

Fig 3: Total flavonoids of Deglet Nour date seeds extracts using different solvents

EE_80; EA_50 and EW: Date seeds extract obtained with Ethanol (80), Acetone (50) and Water, respectively. Each value is mean ± standard deviation (n = 3). Values bearing the same letter within the same column are not significantly different ($p<0.05$)

Mid-Infrared spectroscopy

MIR analysis of date seed extracts obtained with ethanol (80%), acetone (50%) and water, as well as that of date seed powder, revealed several characteristic absorption bands, indicating the presence of various bioactive compounds, particularly phenolic compounds. Although the spectra showed a comparable overall profile, the relative intensity of the bands varied depending on the solvent used, and some differences in peaks were observed. Date seed powder showed the lowest absorbance, which can be explained by the concentration of bioactive components during extraction (Figure 4).

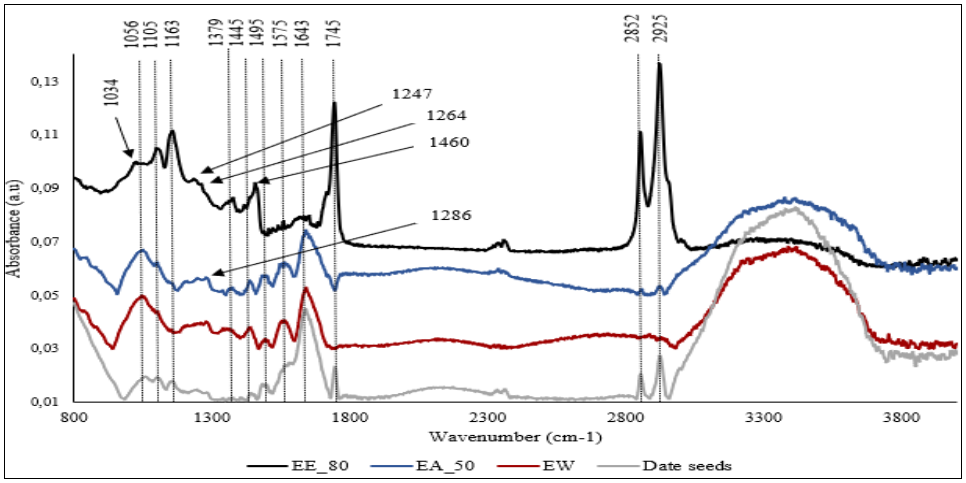

Fig 4: Mid-infrared spectra acquired between 800 and 4000 cm^{-1} of date seeds, EE_80, EA_50 and EW

EE_80; EA_50 and EW: Date seeds extract obtained with Ethanol (80), Acetone (50) and Water, respectively.

The ethanol extract displayed the most intense and well-defined bands overall, reflecting its higher solubilization capacity for a broad range of compounds. The acetone extract showed intermediate intensities, while the aqueous extract presented the lowest absorbance, confirming its lower efficiency for less polar molecules.

The peak recorded at 1034 cm^{-1} in the ethanol extract and at 1056 cm^{-1} in the acetone, aqueous extracts and date seed powder spectra is attributed to the -COH groups of sugars present in glycosylated polyphenols. The bands at 1105 and 1163 cm^{-1} also correspond to the -COH groups of glycosylated phenols (Scano, 2021) [36]. In the region 1247–1286 cm^{-1}, the ethanol extract displayed clear signals at 1247 and 1264 cm^{-1}, but the 1286 cm^{-1} band was almost absent, while this latter band was more evident in the acetone and especially in the aqueous extract. This suggests that some phenolic hydroxyl groups or carbohydrate-linked structures are better solubilized by acetone or water than by ethanol.

According to Tondi and Petutschnigg (2015) [42], the peaks at 1445, 1460 and 1495 cm^{-1} can be associated with gallic acid, ellagic acid, flavanone and epicatechin, while the peak at 1379 cm^{-1} is linked to phenols such as resorcinol, pyrocatechol, hydroquinone, pyrogallol and catechol. The band at 1460 cm^{-1}, observed only in the ethanol extract and seed powder, as well as the intense band at 1745 cm^{-1}, corresponding to the carbonyl groups (C=O) of carboxylic acids, confirm that ethanol has a better extraction capacity

for phenolic acids, flavanones and hydrolysable tannins than acetone or water. This observation reflects the greater affinity of ethanol for compounds that are slightly less polar than free phenols. The peak at 1643 cm^{-1}, which is more intense in the acetone, aqueous extracts and powder spectra, corresponds to the C=O stretching vibrations of carbonyl compounds, generally associated with a high flavonoid content (Kannan, 2011) [25]. Although the ethanol extract has the highest flavonoid content according to spectrophotometric analysis, the carbonyl band at 1643 cm^{-1} is not very pronounced. This variation can be explained by the chemical nature of flavonoids (glycosylated or aglycone forms), by the overlap of signals with other major compounds, or by the sensitivity limits of MIR compared to quantitative methods.

The signal at 1575 cm^{-1} is attributed to the C=C stretching of the aromatic rings of phenols (Abbas *et al*., 2017) [1], while the peaks at 2925 and 2852 cm^{-1}, present only in the spectra of the ethanol extract and seed powder, correspond to the symmetric and asymmetric vibrations of the lipid CH groups. Their absence in the acetone and aqueous extracts confirms that these solvents are less efficient at extracting lipid-associated compounds.

Overall, the ethanol extract displayed the highest intensities in the characteristic region of phenolic and carbonyl groups, followed by the acetone extract, while the aqueous extract, which is richer in water-soluble compounds, showed the lowest intensities. These variations reflect the relative efficiency of the solvents used for phenolic compounds extraction from date seeds, highlighting the strong influence of solvent polarity on the extraction profile of bioactive compounds (Palaiogiannis *et al*., 2023) [34].

Antioxidant activity of phenolic extracts

Table 1 illustrated the antioxidant activity of the different date seed extracts, assessed by the DPPH and ABTS methods. The ethanolic extract showed the highest activity, with IC_{50} values of 0.20 mg/mL for the DPPH assay and 0.17 mg/mL for the ABTS assay. It was followed by the acetone extract, with IC_{50} values of 0.27 mg/mL (DPPH) and 0.26 mg/mL (ABTS). The aqueous extract showed the lowest antioxidant activity, with IC_{50} of 1.10 mg/mL for DPPH and 0.82 mg/mL for ABTS. In contrast, BHT has a more powerful activity with an IC_{50} of 20µg/mL.

Table 1. DPPH and ABTS radical scavenging activities (IC50 mg/ml) of date seed extracts

	IC50 (mg/mL)	
	DPPH	**ABTS**
EE_80	0,20 ± 0,00 [a]	0,1 ± 0,01[a]
EA_50	0,27 ± 0,01 [b]	0,26 ± 0,01[b]
EW	1,10 ± 0,02 [c]	0,82 ± 0,03[c]
BHT	0,02 ± 0,00 [d]	0,02 ± 0,00[d]

EE_80; EA_50 and EW: Date seeds extract obtained with Ethanol (80), Acetone (50) and Water, respectively. Each value is mean ± standard deviation (n = 3). Values bearing the same letter within the same column are not significantly different ($p<0.05$)

In addition, a significant negative correlation was found between total phenolic content and IC_{50} ($r = –0.819$), indicating that a higher phenolic compound content is associated with better antioxidant capacity.

These results are close to those reported by Bouhlali *et al*. (2017) [12], who obtained IC_{50} values of 0.17 g/L, 0.11 g/L and 0.13 g/L, respectively, for date seeds of the Moroccan varieties Boufgous, Bousthammi and Majhoul, respectively. Similarly, Abuelgassim *et al*. (2020) [3] reported that date seed extracts exhibited significant antioxidant activity, assessed using three separate methods targeting different types of free radicals. For the Sukkari variety, IC_{50} values were 431.17 µg/mL ($ABTS^{-+}$), 400 µg/mL (DPPH-) and 680 µg/mL (hydroxyl radical). For the Khalas variety, the IC_{50} obtained were 476 µg/mL, 302.24 µg/mL and 284.18 µg/mL respectively for the same tests. These results highlight the potential of date seeds to neutralize different types of radicals, reflecting a polyvalent antioxidant potential.

These results can be explained by the composition and structure of the polyphenols present in date seeds. Flavonoids, such as quercetin and catechins, and phenolic acids such as caffeic acid and ferulic acid, display hydroxyl groups and conjugated systems that confer a strong capacity to scavenge free radicals. These bioactive compounds are able to interrupt oxidation reactions by releasing hydrogen atoms, forming stable forms (Al Ghezi *et al*., 2020) [6].

These observations highlight the versatile potential of date seed polyphenolic compounds to neutralize different types of free radicals and contribute to their overall antioxidant effect.

Conclusion

The extraction of phenolic compounds from Deglet Nour date seeds showed that ethanol (80%) provided the highest extraction yield as well as the highest total phenolic and flavonoid contents. The resulting extract exhibited significant antioxidant activity, confirmed by DPPH and ABTS assays. MIR spectroscopic analysis was used to characterize the functional groups of the bioactive compounds, highlighting the rich chemical diversity of this extract. These results suggest that phenolic extracts from date seeds are a promising source of natural antioxidants, with potential interest for the valorization of by-products from the date sector and for various applications, including food processing and integration into bioactive packaging.

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