
Research Article

Evaluation of antioxidant activity in crude polysaccharide extracts from two date varieties (Tazerzait “Azerza” and Deglet-Nour)

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Abstract

The fruit of the date palm, *Phoenix dactylifera* L. is extensively consumed in Arabian countries and is esteemed for its nutritional attributes, boasting high carbohydrate content and specific vitamins. This study aims to assess the antioxidant potential of polysaccharide extracts from the date palm fruit (*Phoenix dactylifera* L.) from two selected cultivars, Tazerzait “Azerza” and Deglet-Nour, originating from the Ghardaia region. Crude polysaccharides were extracted from the dates using distilled water followed by ethanol precipitation. Yields from this process were 3.14% for the Tazerzait cultivar and 4.75% for the Deglet-Nour cultivar, indicative of significant polysaccharide content, known for their diverse health benefits. Antioxidant activity was evaluated in vitro through DPPH and ABTS assays. Polysaccharide extracts exhibited noteworthy antioxidant activity, notably the Tazerzait variety. In the DPPH assay, the Tazerzait extract displayed an IC₅₀ value of 4.66 mg/mL, indicating effective scavenging of free radicals. Moreover, in the ABTS assay, the extract demonstrated even more potent activity, with an IC₅₀ value of 0.49 mg/mL, underscoring its robust antioxidant properties. These results underscore the strong antioxidant potential of Tazerzait polysaccharides. Polysaccharide extracts from the date palm fruit, particularly from the Tazerzait cultivar, exhibit significant antioxidant activity, highlighting their potential as natural antioxidants. This suggests avenues for their utilization in pharmaceutical and food industries to enhance human health.

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1. Introduction

The Algerian Saharan zone presents a specific flora, characterized by an important floristic diversity, containing many endemic species highly adapted to the climate of the zone (Miara et al., 2019). The date palm "*Phoenix dactylifera* L.", is the most important fruit tree of the Arecaceae family which is located in the arid, tropical and subtropical areas of the world precisely those stretching from North Africa to the Middle East (Krueger, 2021). In addition, dates are well considered for their nutritional properties in many populations because of their high carbohydrate content and certain vitamins (Belmir et al., 2016). They are also an excellent source of dietary fiber and contain high amounts of minerals, fats and proteins (Al-Alawi et al., 2017). In this regard, researchers have reassessed the importance of date carbohydrates and considered their many applications, especially in the biomedical field, due to their wide range of therapeutic properties, abundant and renewable resources, non-toxic and biodegradable (Alvi et al., 2022).

Polysaccharides, also called polyholosides, are macromolecules composed of a large number of monosaccharide units. They play essential roles in storing energy and maintaining the structural integrity of organs (Muhamad et al., 2019). Polysaccharides are derived from different sources and have been widely studied and used for their techno-functional properties in pharmaceuticals, cosmetics, paper industry, food processing, and oil extraction (Olawuyi et al., 2021). Polysaccharides also have their own biological activities, including antioxidant activity, anticancer activity, immunomodulatory, and antibacterial activity (Mohan et al., 2020). Therefore, there is a need to discover and develop bioactive polysaccharides as functional foods or drugs for human health (Giavasis, 2014). Indeed, the current study is a part of broader research focusing on natural antioxidants. The specific aim is to assess the antioxidant activity of extracts derived from raw polysaccharides found in date fruit. This investigation concentrates on two carefully chosen date varieties: Tazerzait "Azerza" and Deglet-Nour from the Ghardaia region in Algeria.

This study is noteworthy for several aspects of novelty and significance, including its focus on dates sourced specifically from the Ghardaia region in Algeria, where unique environmental factors like soil composition, climate, and cultivation practices influence plant extracts' properties. It explores the antioxidant activity of polysaccharides from two different date varieties, Tazerzait "Azerza" and Deglet-Nour, each with distinct aspects and tastes, providing valuable insights into the health benefits associated with different types of dates. The study's emphasis on polysaccharides as target compounds for antioxidant evaluation is notable, given that polysaccharides are complex carbohydrates with diverse biological activities, including antioxidant properties, and research on their antioxidant potential, especially from these specific date varieties, is relatively scarce. By assessing the antioxidant activity of date polysaccharide extracts, the study enhances understanding of dates' potential health-promoting properties beyond their traditional use as a food source. The findings could have significant applications in the food and pharmaceutical industries, potentially leading to the development of functional foods, nutraceuticals, or natural antioxidants for food preservation. In fact, exploring the antioxidant activity of polysaccharide extracts from specific varieties of dates can offer valuable insights into their potential health benefits. Using established tests like DPPH and ABTS to evaluate antioxidant activity provides a solid foundation for understanding the effectiveness of these extracts as natural antioxidants.

The use of DPPH and ABTS assays to evaluate the antioxidant activity of date polysaccharide extracts offers several advantages. These assays are widely recognized and accepted methods for assessing the antioxidant potential of natural compounds. By employing both DPPH and ABTS assays, researchers can obtain complementary information about the antioxidant capacity of the polysaccharide extracts, enhancing the robustness of the findings (Fernandes & Coimbra, 2023). Additionally, these assays provide quantitative data, allowing for comparison of antioxidant activity between different extracts and enabling researchers to identify the most promising candidates for further investigation.

In the DPPH assay, the antioxidant capacity of a substance is determined by its ability to neutralize the DPPH radical, a stable free radical compound. When an antioxidant is introduced to a solution containing DPPH radicals, it donates hydrogen atoms or electrons, thereby reducing the DPPH radicals to form a stable non-radical molecule (Gulcin & Alwasel, 2023). The degree of discoloration or reduction in absorbance of the DPPH solution is measured spectrophotometrically at a specific wavelength (at 515–520 nm) and the lower absorbance value indicates higher antioxidant activity (Sridhar & Charles, 2019). In contrast, the ABTS assay measures antioxidants' ability to scavenge ABTS radicals, formed by the reaction between ABTS and potassium persulfate. These radicals exhibit a blue-green color and have a characteristic absorbance at a specific wavelength. Antioxidants added to the ABTS radical solution reduce the radicals, leading to a decrease in absorbance (Ilyasov et al., 2020). Similar to the DPPH assay, spectrophotometry is employed to measure the reduction in absorbance, with lower values indicating higher antioxidant activity. Both assays yield quantitative measures of antioxidant activity, typically expressed as IC50 values representing the concentration of the antioxidant necessary to inhibit 50% of the free radicals in the assay system (Sridhar & Charles, 2019).

By subjecting the polysaccharide extracts to these rigorous tests, the study aims to provide valuable insights into the antioxidant capabilities of date fruit extracts, with a specific focus on the selected varieties. This research is instrumental in advancing our understanding of natural antioxidants and their potential applications in various industries, including food, cosmetics, and pharmaceuticals. Moreover, it contributes to the broader scientific endeavor of harnessing nature's bounty to combat oxidative stress and promote human health and well-being.

2. Materials and methods

2.1 Plant material

The plant material used is composed of four varieties of dates: Tazerzait "Taz" and Deglet-Nour "Dgl", at the final stage of maturation "Tmar". The harvest of the varieties was carried out in the palm groves of the wilaya of Ghardaia in the month of September-October 2022. The dates, freshly collected, are selected and stored at 4°C in closed polypropylene (PP) food boxes for later use. These varieties are chosen based on their wide consumption, taste quality and market availability.

The following table presents the characteristics of the varieties studied (Table 1).

Table 1. Characteristics of Tazerzait and Deglet-Nour varieties

Varieties	Characteristics	Pictures
Tazerzait (Taz)	- Tazerzait or Azerza date is a semi-dry variety; the shape of the fruit is slightly elongated ovoid. - The color is yellow-orange in the rotab stage and changes to honey brown in the tmar stage. - It is characterized by: an average weight of 10.5 g, an average length of 4 cm, and an average diameter of 2 cm. - The smooth blistered epicarp with a mesocarp has a fibrous texture (Mansouri et al., 2005).	

Continued Table 1

Varieties	Characteristics	Pictures
Deglet-Nour (Dgl)	- Deglet-Nour is a semi-soft date; - Tapered to ovoid shape - Light yellowish color ; - It is characterized by: an average weight of 12g, an average length of 6 cm, an average diameter of 1.8 cm. - The smooth, slightly wrinkled and shiny epicarp and a mesocarp has a fine, slightly fibrous texture (Boudries et al., 2007).	

2.2 Extraction of hydrosoluble polysaccharides

The extraction is a very important step for the isolation of active ingredients from plant material but it is influenced by the chemical nature of the components, the method of extraction employed and the presence of interfering substances (Altemimi et al., 2017). Hot water or boiling water extraction is the classic and most practical method of extracting laboratory, and it is widely used in food and medical industry (Teo et al., 2010), due to their environmentally friendly process, higher extraction efficiency, cost effectiveness and structure preservation (Plaza & Turner, 2015). Most bioactive polysaccharides are polar in nature, so polar solvents, such as hot water and alkaline aqueous solution are used to extract polysaccharides and it requires long time and high temperature (Shi, 2016).

The polysaccharide extracts are obtained by the following process: first fifty grams (50 g) of pitted dates cut into small pieces and introduced into 1000 ml of boiling distilled water for 3 hours. Then the extract is filtered (using filter paper No. 4) and then centrifuged at 5000 rpm for 15 minutes. The supernatant obtained is concentrated using a rotary evaporator at 60°C (until less than 100 ml). This supernatant is treated with 4 times of ethanol volume (96%, Honeywell Fluka™) for 48 hours at 4°C. After centrifugation at 5000 rpm for 15 minutes, the precipitate is collected and dried at 45°C (in a clean oven) to obtain the crude extract of the polysaccharides (Li & Huang, 2021).

2.3 Yield calculation

The extraction yield (Y) was calculated by the following equation(1):

$$Y (\%) = \frac{W}{W_0} \times 100 \dots\dots\dots (1)$$

where W (g) is the dried powder of *P. dactylifera* fruit weight and W_0 (g) is total weight of plant material (Nurcahyani et al., 2020).

2.4 Antioxidant activity of polysaccharides

DPPH radical scavenging activity

The DPPH radical scavenging protocol was carried out following the method of Carvalho et al., (2022). One volume of 50 µL of the extract, at different concentrations, is mixed with 1.950 mL of the DPPH solution (Sigma Aldrich®), at 6×10^{-5} M, in ethanol at 96% (v/v). After reacting for 30

minutes at room temperature, the absorbance is immediately measured at 515 nm. For comparative purposes, two standard antioxidants were used, ascorbic acid (Vit C at 99% from Sigma Aldrich®) and Butylated hydroxytoluene (BHT) (Sigma Aldrich®).

The IC₅₀ value (mg/mL) is the concentration at which this scavenging activity is 50%. The values obtained represent the average of three tests.

The radical-scavenging activity was calculated as the percentage inhibition of DPPH from the following equation (2):

$$\text{Inhibition (\%)} = \frac{A_c - A_s}{A_c} \times 100 \dots\dots\dots (2)$$

where; A_c is the absorbance of DPPH solution without sample, A_s is the absorbance of the test sample mixed with DPPH solution (Carvalho et al., 2022).

ABTS radical scavenging activity

The ABTS radical scavenging protocol was performed following the method of Olszowy and Dawidowicz, (2018). The cation radical (ABTS•+) is generated by mixing a solution of ABTS (7 mM) with an aqueous solution of potassium persulfate (2.45 mM) and allowing the mixture to stand in the dark at room temperature for 12-16 hours before use.

At the time of use, the ABTS•+ solution is diluted to an absorbance of 0.70 ($\pm$ 0.02) at 734 nm. Then, 0.2 mL of the extract, at different concentrations is added to 2 mL of the ABTS•+ solution. After reacting for 20 minutes at room temperature, the absorbance is immediately measured at 734 nm. Ascorbic acid and BHT have been used as positive control with the same concentrations as the extracts. The values obtained represent the average of three tests.

The inhibition percent of ABTS•+ was calculated according to the following equation (3):

$$\text{Inhibition (\%)} = \frac{A_c - A_s}{A_c} \times 100 \dots\dots\dots (3)$$

where; A_c is the absorbance of ABTS•+ solution without sample, A_s is the absorbance of the test sample mixed with ABTS•+ solution (Olszowy & Dawidowicz, 2018). However, the antioxidant activity can be classified based on IC₅₀ values as follows: IC₅₀ values below 1 mg/mL indicate high antioxidant potency, suggesting that substances with IC₅₀ values in this range are highly effective at scavenging free radicals. IC₅₀ values between 1 mg/mL and 10 mg/mL suggest moderate antioxidant potency, indicating significant antioxidant activity, though not as potent as those with lower IC₅₀ values. IC₅₀ values above 10 mg/mL indicate low antioxidant potency, meaning that while substances in this range may still exhibit some antioxidant activity, they are generally less effective compared to those with lower IC₅₀ values.

This classification provides a quantitative measure of the efficacy of antioxidants in neutralizing free radicals and protecting against oxidative damage (Fan et al., 2018; Wang et al., 2016).

3. Result and discussion

3.1 Yield of polysaccharide extraction

The crude extracts recovered after evaporation to dryness under reduced pressure are weighed to determine the dry weight. The yield is determined in relation to the initial weight of the plant material having undergone the extraction. The yield percentage of each extract varieties is calculated and the results are shown in (Figure 1).

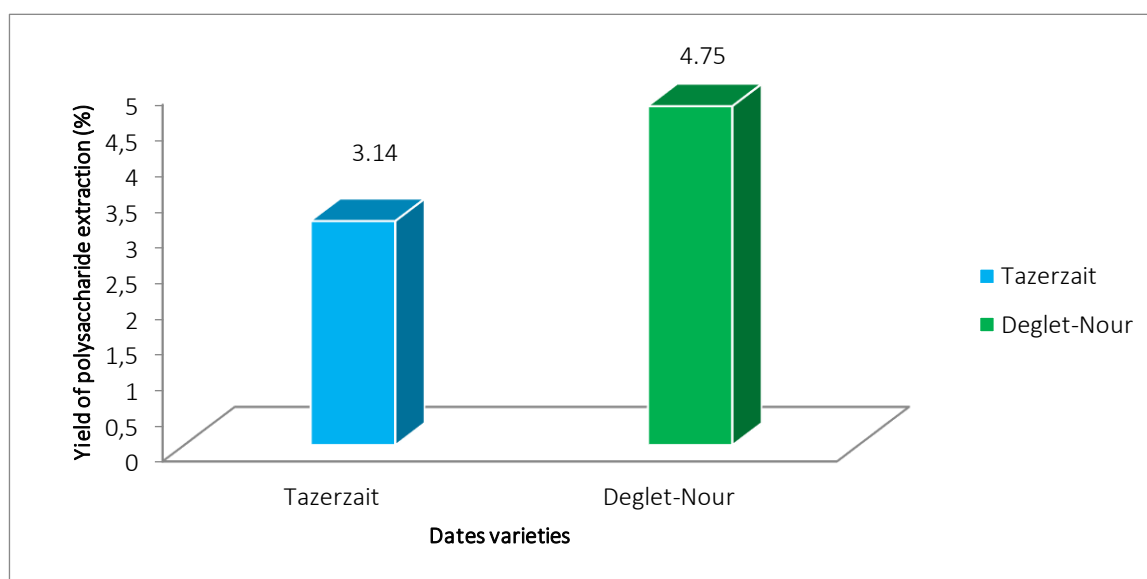


Figure 1. Yield of crude polysaccharide extracts of date varieties

The results obtained show that the Deglet-Nour cultivar has the highest yield, estimated at 4.75%, compared with the Tazerzait cultivar, which has the lowest yield at 3.14%. The yield of the extraction of hydrosoluble polysaccharides depends on different parameters including the plant material studied (particle size), the physico-chemical characteristics of the solvents used and particularly their polarity, it also depends on the conditions and duration of storage, harvesting period as well as the method and conditions of realization of the extraction (Kurd & Samavati, 2015). According to Pérez et al. (2018) classical hot water extraction combined with some additional methods such as: microwaves, is widely used for the extraction of polysaccharides from olives (Pérez et al., 2018). These methods can reduce solvent consumption and improve polysaccharide extraction yield (Ji et al., 2017).

3.2 Antioxidant activity of polysaccharides

DPPH radical scavenging activity

The DPPH test is a method frequently used to assess the antioxidant potential of different natural compounds due to its speed, reliability and low cost (Munteanu & Apetrei, 2021). The DPPH radical scavenging mechanism is based on the reduction of DPPH to DPPH-H in the presence of a hydrogen-donating antioxidant, resulting in the discoloration of purple color and a decrease in absorbance at a wavelength $\lambda = 515$ nm, this makes it possible to evaluate the activity of the various extracts with respect to this radical. Lower absorption indicates higher DPPH radical scavenging activity.

Figure 2 represents the variations in the percentage of inhibition of the DPPH radical as a function of the various concentrations of the crude extracts of the polysaccharides of the dates studied. According to (Figure 2), it can be seen that the shapes of the curves are different from one extract to another and that the rate of reduction of DPPH increases with the increase in the concentration of the extract.

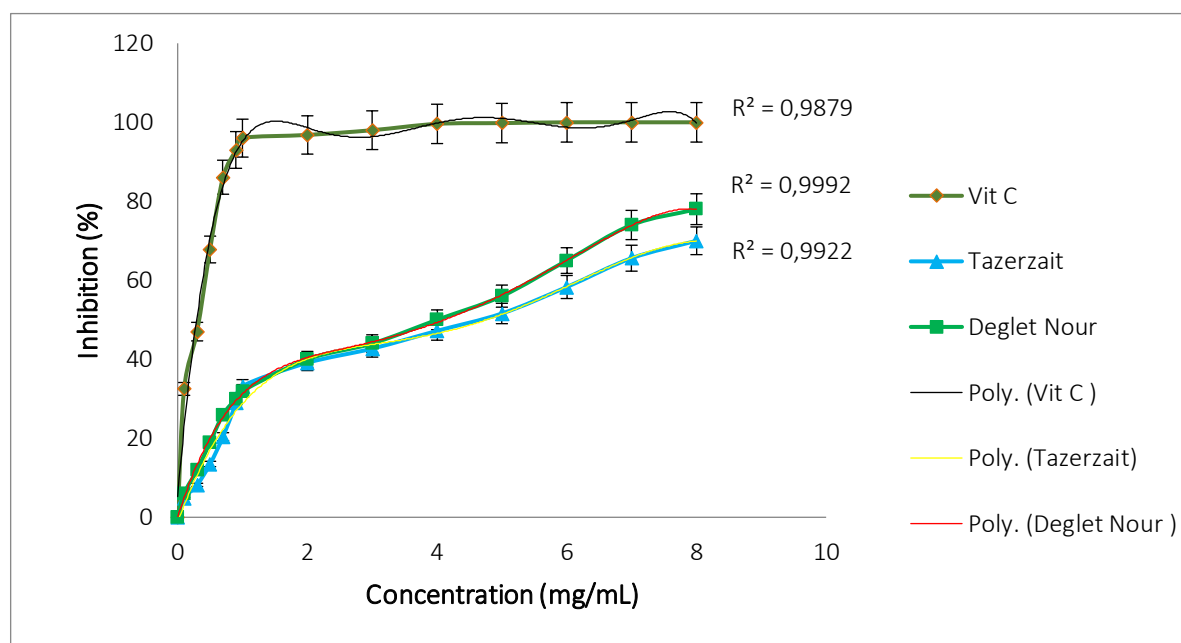


Figure 2. Variations in the percentage of inhibition of the DPPH radical according to the different concentrations of the crude extracts of date polysaccharides

From this profile, the IC₅₀ value of each extract is determined. This value is inversely related to its antioxidant capacity, a low IC₅₀ indicates the highest antioxidant activity (Do et al., 2014). Vitamin C is used as standard antioxidants in this work, it present anti-radical activity with IC₅₀ equal to 0.33 ± 0.004 mg/mL. This activity is higher than the values of all the extracts tested. Among the crude polysaccharide extracts tested, the extract of the Deglet-Nour variety represents the most active extract with an IC₅₀ of 4.01 ± 0.011 mg/mL, followed by the extract of the Tazerzait variety with an IC₅₀ of 4.66 ± 0.013 mg/mL.

ABTS radical scavenging activity

The antioxidant activity towards the ABTS radical was evaluated, by UV-Visible spectrophotometer, following the reduction of this radical, which is accompanied by its transition from green color to light green color until total disappearance of the color, at a wavelength $\lambda = 734$ nm. This method provides a measure of the antioxidant activity of the compounds, determined by the decolorization of ABTS^{•+}, by measuring the loss of color when an antioxidant is added to the blue-green chromophore ABTS^{•+}, which is a stable radical not found in the human body. The antioxidant reduces ABTS^{•+} to ABTS and discolors it (Ilyasov et al., 2020). The graphical representations of the antioxidant activity, towards the ABTS radical of the extracts of polysaccharides of varieties of dates are presented in (Figure 3).

Figure 3 shows that the polysaccharide extracts of the different varieties of dates present a variable potential in the scavenging of the radical cation ABTS^{•+}. These antioxidant activity profiles reveal that the extracts have a dose-dependent antiradical activity. Tazerzait extract inhibits the ABTS^{•+} radical, more significantly than Deglet-Nour extract, this is demonstrated by the exponential pattern where the IC₅₀ value equal to 0.49 ± 0.007 mg/mL

According to these results, ascorbic acid with an IC₅₀ equal to 0.42 ± 0.002 mg/mL and Tazerzait extract are excellent electron donors compared with Deglet-Nour extract, which has an IC₅₀ equal to 1.47 ± 0.005 mg/mL. The antioxidant capacity of a compound is all the higher as its IC₅₀ is small (Rebaya et al., 2015). From the results of the antiradical activity (DPPH and ABTS), date

polysaccharide extracts have a relatively high activity but lower than that of Vit C for all concentrations tested. These activity profiles obtained (ABTS and DPPH) reveal that the extracts have a dose-dependent antiradical activity.

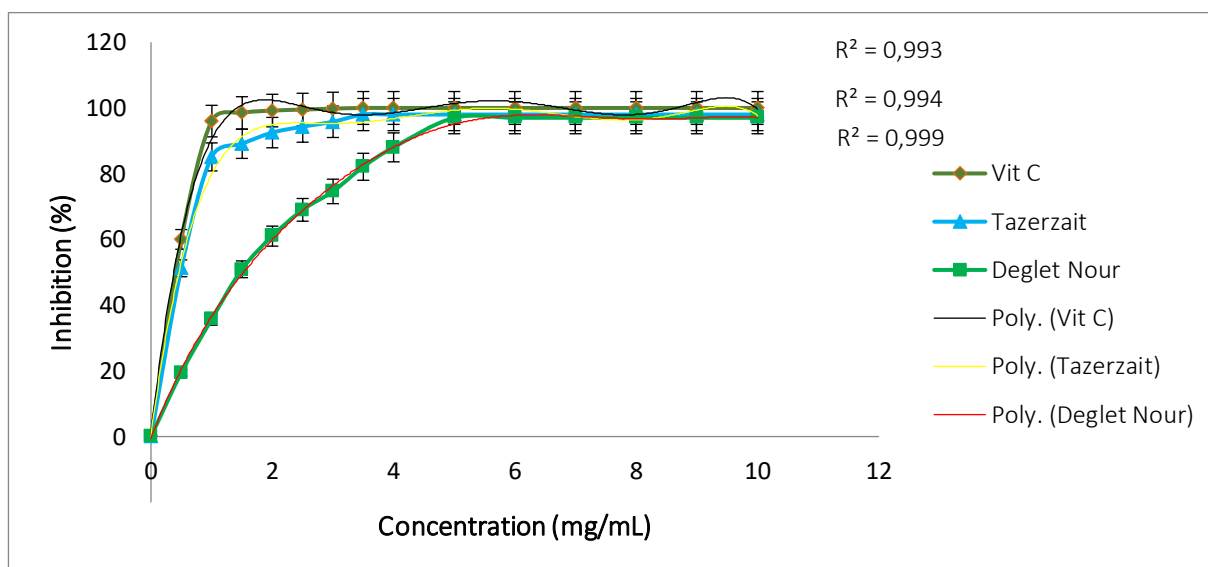


Figure 3. Variations in the percentage of inhibition of the ABTS radical according to the different concentrations of the crude extracts of date polysaccharides

The different activities of radical scavenging polysaccharides in different evaluation systems could suggest that the chemical composition had some effect on the antioxidant activity (Fan et al., 2018). Thus, these results indicate that date polysaccharides can effectively inhibit DPPH- and ABTS-+ radicals, and the explanation could be concluded that date polysaccharides, can act as electron and/or hydrogen donor to scavenge free radicals. Compared with standard antioxidant (Vit C), the extracts of polysaccharides tested proved to be active, especially the extract of Tazerzait variety. This is in agreement with several studies, which stated that plant polysaccharides have shown strong antioxidant activity and can be explored as potential new antioxidants (Huang & Huang, 2020; Liu et al., 2018).

Indeed, in vitro studies have demonstrated the antioxidant properties of polysaccharides by free radical scavenging methods. Thus, the results obtained are in agreement with J. Wang, Hu, Nie, Yu, & Xie, (2016), Hafsa et al., (2017) and Venkatesan et al., (2019), who isolated hydrosoluble polysaccharides from different plants, fungi and algae, which they showed very significant antioxidant activity, and IC₅₀ ranged from 0.4 - 5 mg/ml and 1 - 3 mg/ml for DPPH and ABTS assay respectively (Hafsa et al., 2017; Venkatesan et al., 2019; Wang et al., 2016).

According to S. Dontha, (2016), the result of in vitro antioxidant activity depends largely on the chosen scavenging assay, because the different in vitro tests have a different mechanism and the antioxidant activity has been attributed to various mechanisms (Dontha, 2016). According to Martínez et al., (2021), factors affecting antioxidant properties include genetics, growing and environmental conditions, diseases and treatment of the plant (Martínez et al., 2021).

In summary, this study marks an important initial exploration into the potential antioxidant properties of date polysaccharides in combating oxidative stress-related diseases. These limitations include in vitro limitations, where the tests may not entirely reflect the actual biological effects of polysaccharide extracts once they are metabolized and distributed in the body. There is also the issue of sample representativeness, as the polysaccharide extracts used may not accurately represent the composition of dates consumed by humans, limiting the generalizability of the results. Additionally, while the extracts exhibit promising anti-radical activity in vitro, their

effectiveness and safety in preventing oxidative stress-related diseases in humans require evaluation through animal models and clinical trials. The study mentions the necessity of characterizing the constituent glycosides of the polysaccharides but lacks specific details on the characterization methods used, potentially compromising result reproducibility. Furthermore, additional testing is necessary to confirm the anti-radical properties of date polysaccharide extracts and explore their potential applications in the pharmaceutical and agri-food industries. Essentially, while this study establishes a ground work, addressing these limitations is crucial for furthering our comprehension and utilization of date polysaccharides as potent antioxidants.

4. Conclusion

In the framework of the development of natural antioxidants and their role in the prevention of diseases caused by oxidative stress, the crude extracts of the polysaccharides of two varieties of dates (Tazerzait and Deglet-Nour) were tested for their potential antiradical activity using the DPPH radical method and the ABTS test. Antioxidant activity tests show that the Tazerzait variety has the highest antiradical activity. This can be explained by the nature of the carbohydrates present in the extract which directly influenced its ability to scavenge free radicals. All these results obtained in-vitro are only a first step in the search for biologically active substances and natural sources. Additional tests will be necessary and should be able to confirm the highlighted performances. Indeed, in order to confirm the obtained results, a complementary and more promising study concerning the identification and the characterization of the glycosidic residues which constitute these polysaccharides, as well as their biological activities and their potentialities in the pharmaceutical and agroalimentary sectors.

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Authors' declaration and contributions

There is no actual or potential conflict of interest in relation to this article. Mohammed Habib Belhachemi (PhD in Applied Biochemistry) was designed and conducted all of the experiments and wrote the manuscript. Sara Belmir (PhD in Applied Biochemistry) was performed the results analysis and data visualization and wrote the manuscript. Mohammed Oulad mebarek, Mohammed Reffis and Fatima Zahra Achour (master degree in Applied Biochemistry) were assisted in conducting the experiments.

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