https://doi.org/10.31925/farmacia.2025.6.16 **ORIGINAL ARTICLE**

PHYTOCHEMICAL ANALYSIS, ANTIOXIDANT CAPACITY, AND ACUTE ORAL TOXICITY OF ALGERIAN DATE (*PHOENIX DACTYLIFERA* L.) SEED EXTRACTS IN WISTAR RATS

NADIA BOUABDALLAH, FATIMA ZOHRA BABA AHMED *, YOUCEF BENDIAF, LAMIA CHAOUI BOUDGHANE, SALIM HABI, ABDELWAHEB MEBARKI, SAMIRA BOUANANE

Laboratory of Physiology, Physiopathology and Biochemistry of Nutrition, Department of Biology, Faculty of Natural and Life Sciences, Earth and Universe, Abou-Bekr Belkaid University of Tlemcen 13000, Algeria

**corresponding author: fatimazohra_7@yahoo.fr*

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Abstract

Date seeds (*Phoenix dactylifera* L.) are known for their potential health benefits due to bioactive compounds. However, their toxicity is little known. This study evaluates the antioxidant activity and acute oral toxicity of aqueous and oil extracts obtained from Algerian date seeds. Antioxidant activity was performed by using DPPH radical scavenging and FRAP assays, alongside quantification of total phenolic, flavonoid, condensed tannin, and carotenoid contents. For toxicity assessment, Wistar rats received single oral doses of 300, 1000, or 2000 mg/kg bw of each extract. Animals were observed for 14 days before organ examination and biochemical analysis. Results showed a rich composition of secondary metabolites, with the oil extract exhibiting the highest antioxidant activity (IC_{50} = 0.48 ± 0.03 mg/mL), outperforming the aqueous extract (IC_{50} = 1.81 ± 0.06 mg/mL). No mortality, behavioural changes, or significant alterations in organ weights and biochemical parameters were observed at any dose. These findings confirm that Algerian date seed extracts exhibit noteworthy antioxidant properties and present no acute oral toxicity under the experimental conditions. Their phytochemical richness and demonstrated safety support their potential use as valuable raw materials for nutraceutical and pharmaceutical applications.

Rezumat

Semințele de curmal (*Phoenix dactylifera* L.) sunt cunoscute pentru potențialele beneficii pentru sănătate datorită compușilor bioactivi. Cu toate acestea, toxicitatea lor este puțin cunoscută. Acest studiu evaluează activitatea antioxidantă și toxicitatea orală acută a extractelor apoase și uleioase obținute din semințe de curmal algerian. Activitatea antioxidantă a fost evaluată folosind testele DPPH și FRAP, alături de cuantificarea conținutului total de fenoli, flavonoide, tanin condensat și carotenoizi. Pentru evaluarea toxicității, șobolanilor Wistar li s-au administrat doze orale unice de 300, 1000 sau 2000 mg/kgc din fiecare extract. Animalele au fost observate timp de 14 zile înainte de examinarea organelor și analiza biochimică. Rezultatele au arătat o compoziție bogată în metaboliți secundari, extractul uleios prezentând cea mai înaltă activitate antioxidantă (IC_{50} = 0,48 ± 0,03 mg/mL), mai mult decât extractul apos (IC_{50} = 1,81 ± 0,06 mg/mL). Nu a survenit decesul, nu s-au observat modificări comportamentale sau modificări semnificative ale greutății organelor și parametrilor biochimici la niciuna dintre doze. Aceste descoperiri confirmă faptul că extractele din semințe de curmale algeriene prezintă proprietăți antioxidante remarcabile și nu prezintă toxicitate orală acută în condițiile experimentale. Compoziția lor fitochimică și siguranța demonstrată susțin utilizarea lor potențială ca materii prime valoroase pentru aplicații nutraceutice și farmaceutice.

Keywords: Date (*Phoenix dactylifera* L.) seeds, antioxidant properties, acute toxicity study, Wistar rats

Introduction

Plants possess a wide range of phytochemical constituents and have great potential for the treatment of several human diseases [1]. These medicinal, edible or ornamental plants are characterized by the presence of thousands of secondary metabolites that offer a range of biological activities, including antioxidant, anti-inflammatory, antibacterial and antimutagenic properties. In addition, these plants are inexpensive and offer a clear economic advantage [2]. Phytochemicals with potential medicinal uses are generally found in various plant parts, including the bark, leaves, flowers, roots, fruits and seeds [3].

Recently, identifying new sources of natural antioxidants has become one of the most active fields of research worldwide [4]. Over the past few decades, interest in naturally occurring antioxidants has considerably increased, as these compounds may be utilized in food, cosmetic and pharmaceutical products [5]. Algeria ranks fourth in worldwide in date production with 1.188.803 tonnes, representing 12.61% of global production [6]. Date palms generate substantial agricultural residues, and date seeds, as by-products, constitute a significant source of waste, posing a challenge for manufacturing companies [7]. In many countries, date seeds (*Phoenix dactylifera* L.) are often discarded or partially incorporated into animal

feed, while human consumption remains low, limited mainly to infusions used to relieve various infections [8, 9]. They remain understudied despite their potential for added value [10]. Several studies suggest beneficial effects against oxidative stress- related diseases, including cardiovascular disorders, diabetes and certain neuro-degenerative conditions [11, 12], as well as protective effects on key organs [13] and potential applications for their oils. These health-promoting properties are largely attributed to bioactive compounds, including phenolics (flavonoids and phenolic acids), carotenoids, phytosterols and phytoestrogens [14].

To explore safer natural alternatives to synthetic antioxidants, this study evaluated the aqueous (AEDS) and oil (DSO) extracts of date seeds for their total polyphenol, flavonoid, condensed tannin, and carotenoid contents, as well as their antioxidant activity. Acute oral toxicity tests were also performed in Wistar rats to determine the safety of these extracts, providing essential data for their potential use in preclinical and human studies (Figure 1).

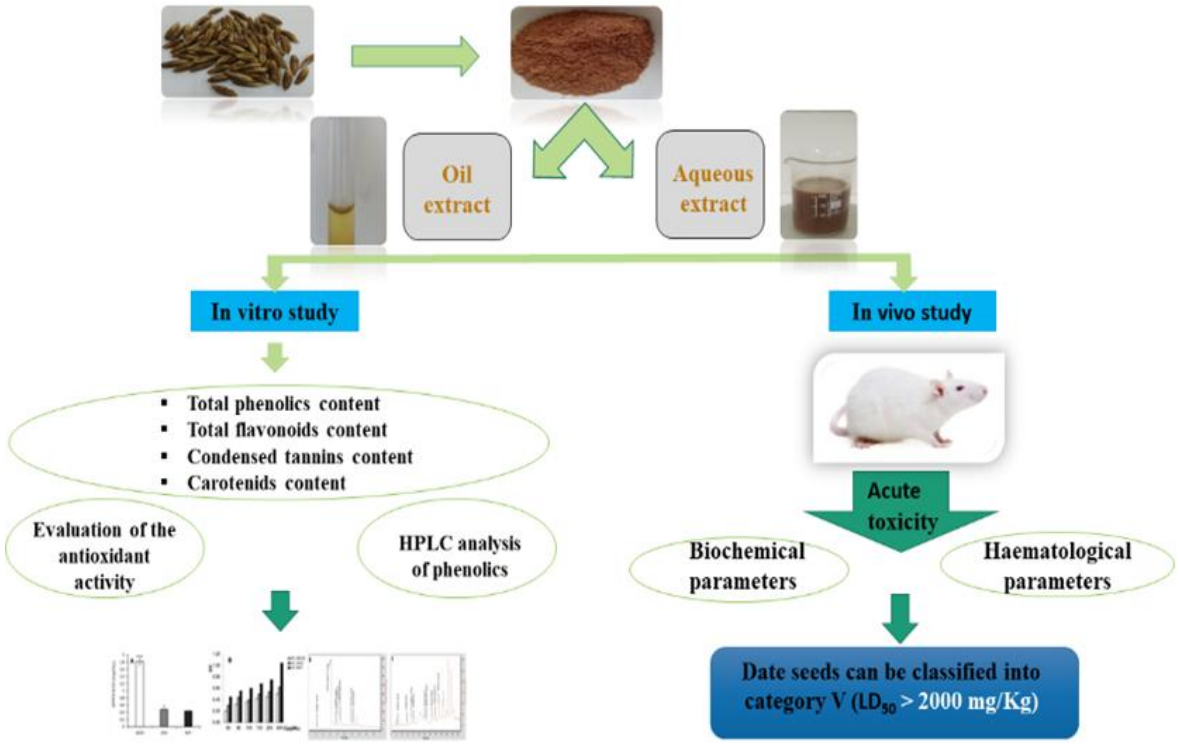

Figure 1.
Study design

Materials and Methods

Materials

Plant material

The date variety "*Deglet Nour"* was collected from the Wilaya (Province) of Biskra in Algeria, between the months of September and October 2020. The seeds were separated from the date flesh, then cleaned and air-dried at room temperature (20 - 25°C). Once desiccated, these seeds were ground. The resulting powder was sieved through a 250 µm mesh and then stored at room temperature for further analysis.

Preparation of the aqueous and oil extracts of date seeds

The AEDS was obtained by cold maceration. For this, 10 grams of date seed powder were macerated in 100 mL of distilled water, at room temperature, for 48 hours. During this time, the mixture was regularly and continuously stirred. Afterwards, the extract, which was obtained through filtration, was dried out through evaporation to obtain a completely dry extract. All final extracts were stored at the temperature of 4°C until further testing [15].

Similarly, the DSO was obtained using a Soxhlet apparatus with hexane as the solvent, according to the standard protocol of NF V03-924, with some minor modifications [16]. After five hours of extraction, the hexane was removed from the solution, using a rotary evaporator at 40°C under vacuum. The extracted oil was then stored at the temperature of 4°C, until additional investigation.

The oil extraction and aqueous extraction yield (R) was calculated using the formula:

$$R(\%)=\frac{P}{M}\text{x } 100$$

Where P is the weight of extract obtained and M is the total weight of vegetal material.

Phytochemical analysis

Total phenolics content

The total phenolics content (TPC) was determined using the Folin-Ciocalteu method, as described by

Chouikh [17], while introducing slight modifications. For this, 200 µL from each extract were mixed with 1 mL of Folin-Ciocalteu phenol reagent (1:10 in water) and stirred for 3 minutes. Next, the resulting solution was mixed with 0.8 mL of 7.5 % sodium carbonate (W/V) and then left to stand for 30 minutes. The absorbance was measured at 760 nm, using a UV-Vis spectrophotometer (Optizen UV-Vis 3220, Japan). A standard range was prepared with gallic acid under the same conditions. In addition, the phenolic compound content was expressed in mg of gallic acid equivalent per gram of dry weight (mg EGA/g DW).

Total flavonoids content

The total flavonoids content (TFC) was determined by colorimetric assay using aluminium chloride ($AlCl_3$), as reported by Ariffin *et al*. [18], with a few minor modifications. To do this, 0.5 mL from each extract was mixed with 1.5 mL of distilled water, and then with 0.15 mL of sodium nitrite ($NaNO_2$). After 5 minutes, 0.15mL aluminium chloride (10%) was added to the mixture. Then, after 6 minutes of incubation, at room temperature, 0.5 mL of sodium hydroxide (1M) was added. The absorbance was then measured at 510 nm with a UV-Vis spectrophotometer (Optizen UV-Vis 3220, Japan). A standard calibration interval of Quercetin, ranging from 0 to 20 µg/mL, was used as a standard to determine the concentration of total flavonoids. Afterwards, the total flavonoids content (TFC), which was expressed as mg of Quercetin equivalents per gram of dry weight (mg EQ/g DW), was calculated using an equation derived from the standard Quercetin concentration-response curve.

Total condensed tannins content

The condensed tannins content (CTC) was determined using the vanillin method that was described by Julkunen-Titto. [19]. When proanthocianidins react with vanillin in an acidic medium, a red coloured complex is formed; this reaction occurs mainly with the free terminal flavan-3-ol units of the condensed tannins. The intensity of the colour obtained is proportional to the concentration of these units. Therefore, a volume of 50 µL from each sample was added to 1500 µL of the vanillin/methanol solution at 4%. Next, 750 µL of hydrochloric acid (HCl) were poured in the mixture which was then incubated for 20 minutes, at room temperature. Thereafter, the absorbance was measured at 550 nm. Finally, the standard catechin calibration curve, extending from 0 to 1000 µg/mL, was utilized in order to evaluate the CTC.

Total carotenoids content

The extraction of carotenoids was carried out according to the method of Sass-Kiss *et al*. [20]. For this, 20 mL of the hexane/acetone/ethanol mixture (2:1:1) were added to 0.5 g of each sample. Next, the upper phase was recovered after 30 minutes of stirring. Then, 10 mL of hexane were added for a second extraction. Afterwards, the two phases were mixed in order to determine the total amount of carotenoids using spectrophotometry, at wavelength 450 nm. The β-carotene was used as a standard for constructing the standard curve which served to assess the amount of carotenoids. The results were expressed in mg/100g of sample weight.

In vitro antioxidant assay

2, 2-diphenyl-1-picryl-hydrazyl (DPPH) radical scavenging activity test

The scavenging activity of the stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) was assessed using the technique described by Sánchez-Moreno *et al*. [21] with some modifications. In this assay, 1950 µL of DPPH-methanol mixture (0.025 g/L) were mixed with 50 µL of various extracts, at different concentrations. After incubation for 30 minutes, at ambient temperature, the absorbance was measured at wavelength 515 nm against a blank. Then, the free radical scavenging activity of DPPH, which was expressed as percentage (%), was calculated using the following formula:

$$I\ (\%)=(A_c - A_s / A_c)\ x100$$

Where Ac and As refer to the absorbance values of the control and actual samples, respectively. The relative activity of the samples was compared to the standard antioxidant butylated hydroxytoluene (BHT). All samples were analysed in triplicate. The concentration of extract producing 50% inhibition (IC_{50}) was derived from the graph showing inhibition percentages at varying concentrations.

Ferric Reducing Antioxidant Power (FRAP)

The ferric reducing antioxidant power (FRAP) was measured by the formation of Perl's Prussian blue at 700 nm with the antioxidant extract, following the steps outlined by Chouikh [17]. This was done as follows. First, 1 mL from each sample was mixed with 2.5 mL of a phosphate buffer solution (0.2M, pH = 6.6) and 2.5 mL of a potassium ferricyanide solution [$K_3Fe(CN)_6$] (1 %). The tubes, which contain 1 mL of the samples, which were at different concentrations, mixed with 2.5 mL of a phosphate buffer solution (0.2M, pH = 6.6) and 2.5 mL of a 1% potassium ferricyanide solution [$K_3Fe(CN)_6$], were incubated for 20 minutes, at temperature 50°C. Next, aliquots of trichloroacetic acid (2.5 mL, 10% aqueous solution) were added to the resulting mixture which was then centrifuged at 3000 rpm, for 10 minutes. In the end, 2.5 mL of the supernatant from each extract were mixed with 2.5 mL of distilled water and 0.5 mL of a freshly prepared ferric chloride solution ($FeCl_3$, 6 H_20) (0.1%). The absorbance was measured at 700 nm. Antioxidant activity was expressed as the half-maximal effective concentration (EC_{50}), which was calculated by linear regression analysis. All samples were tested in triplicate, and their activity was compared to the standard antioxidant butylated hydroxytoluene (BHT).

High-performance liquid chromatography (HPLC) analysis of phenolic compounds of AEDS and DSO

Considering the fact that date seeds are quite rich in bioactive molecules, it seemed appropriate to fraction the aqueous extract using four solvents, namely chloroform, diethyl ether, ethyl acetate, and n-butanol, as suggested by El Haci *et al*. [22], with a slight modifications. This was done to get separate and clear chromatograms showing the different phenolic compounds present in these seeds (Figure 2).

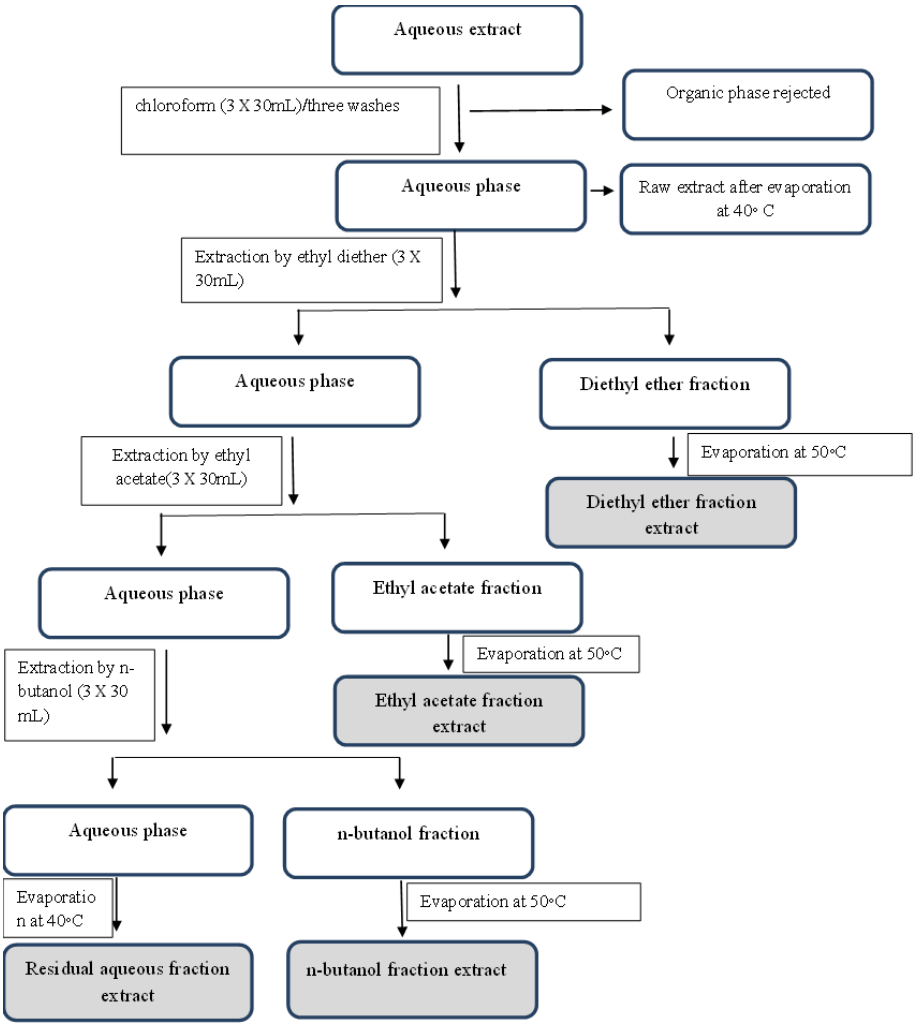

Figure 2.
Experimental protocol of aqueous date seeds extract fractionation

Qualitative analysis of the phenolic compounds present in AEDS and DSO was carried out using the hexane as the extraction solvent and by applying the robust and sensitive reverse phase HPLC method. The PerkinElmer Flexar HPLC system used here is equipped with a binary pump, manual injector, degasser, and a UV/Vis variable wavelength detector, a Hypersil ODS C18 Column (Column Length 150mm, Column Inside Diameter (ID) 4.6mm, Column Particle Size 5µm). The mobile phase consisted of acidified ultrapure water (3%) acetic acid (A), and MeOH (B). The gradient elution system was as follows: 0 min: 95% A - 5% B, 20 min: 5% A - 95% B.

The flow rate for this column was adjusted at 1 mL/min. Then, an aliquot of 20 µL from each sample was injected into the HPLC column which was maintained at 25°C. The detection wavelength was selected at 254 nm. The phenolics, which were identified on the basis of their retention times, were then compared with those encountered in authentic standard samples. The mean of the results obtained from three successive injections was used for all samples to identify the compounds under investigation [23].

In vivo acute toxicity assessment

Animals

Toxicity testing was performed according to the Organization for Economic Cooperation and Development (OECD) test guideline 423 for the Testing of Chemicals, as described by Quattara *et al*. [24], while introducing some new minor modifications. All experimental procedures were carried out in strict accordance with the guidelines provided by the Ethical Committee for Experimental Animal Care and in compliance with the Recommendations for the

Appropriate Care and Use of Laboratory Animals (INSERM, CEEA, 2017). The study protocol was approved by the Animal Ethics Committee of the University of Tlemcen (Approval No. 165-2019).

Thirty-six male Wistar rats weighing 200 ± 20 g were maintained at a temperature of 25 - 30°C, with a relative humidity of 60%, under a 12-hour light/ dark cycle. The animals had free access to water and standard diet [(338 kcal/100 g), 21% protein, 4% lipid, 54.5% carbohydrate, 4.5% fiber, 5% minerals, and 2% vitamins (ONAB, Algeria)], and were allowed a one-week acclimatization period before the beginning of the experiment [25].

Determination of the LD_{50} and observation of the signs of acute intoxication

The acute toxicity study following a single dose of aqueous date seeds and date seeds oil extracts (*Phoenix dactylifera* L.) aims to determine the median lethal dose (LD_{50}), i.e. the dose causing death in 50% of treated animals, over a period of 14 days. In accordance with OECD recommendations, these substances should be administered to groups of animals at increasing doses, giving a mortality rate ranging from 0 to 100%. The doses used should vary between 5 mg/kg and 5 g/kg body weight, in order to position the substances on the comparative chemical toxicity scale of Hodge and Sterner [26].

A total of thirty-six rats were randomly assigned into six groups, with six rats in each group (n = 6 per group), as follows: Group 1 (Control): received 1 mL of physiological saline *per* 100 g of body weight by gavage; Groups 2 - 6 (Experimental): received 1 mL *per* 100 g of body weight by gavage of either the aqueous extract (AEDS) or the oil extract (DSO) of date seeds at doses of 300 mg/kg, 1000 mg/kg, or 2000 mg/kg.

Mortality was assessed within 24 hours. The animals were observed regularly over a 14-day period and weighed every seven days. The initial dose of 300 mg/kg was selected according to OECD guidelines, as this dose is recommended for animal welfare in cases where no prior in information on the test substance is available.

As no death was recorded after the first day at the dose of 300 mg/kg, the same protocol was repeated 24 hours later with the dose of 1000 mg/kg dose, and then another after 24 hours with the dose of 2000 mg/kg of body weight.

The five parameters used to observe the animals were the changes in the animals' weight (due to variations in their food and water intake, urine volume, and faeces weight), the changes in their external physical appearance, the measurable clinical signs (variations in their heart and respiratory rates and their nature), the behavioural changes (balance disorders, posture, scratching, appearance of faeces, occurrence of diarrhoea, aggressiveness, bleeding, hypersalivation), and the behavioural responses to external stimuli (noise and light intensity variations).

The rats in each group were fasted for 12 hours, and were then weighed at the end of the experiment. Afterwards, they were anesthetized with an intraperitoneal injection of a 10% chloral solution (0.3 mL *per* 100 g of body weight) and then sacrificed. An abdominal aortic puncture was used for collecting blood. Part of this blood was collected in tubes containing anticoagulant (EDTA) for the haematological testing, while the remaining blood was kept in Heparinized tubes for the biochemical testing. Once all the organs (liver, heart, spleen, kidneys, and lungs) were thoroughly cleaned with 9‰ NaCl, they were weighed.

In toxicity studies, the LD50 is determined using the following method of Behrens and Karber [27].

$$LD_{5O}=LD_{100}-\frac{(axb)}{n}$$

Where LD_{100} is the dose that gave 100% mortality; a is the difference between two successive doses; b is the average number of deaths between two successive doses; n: average number of animals *per* batch.

Haematological parameters

Red blood cells and leukocytes were counted using the Thomas automatic cell-counting system, along with an optical microscope at a magnification of 10x40. The haemoglobin was determined by the DRABKIN colorimetric method (cyanomethemoglobin).

Biochemical parameters

Enzymatic assays (Kit Spinreact) were utilized for the purpose of measuring the plasma levels of glucose, total cholesterol, and triglycerides. As for the renal function, it was assessed through the determination of plasma levels of creatinine and urea in blood (Kit Spinreact). Regarding the liver function, it was evaluated *via* the enzymatic activity of transaminases, i.e. aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP), and by measuring the total plasma protein level in blood (Kit Spinreact).

Statistical analysis

The results were presented as mean ± standard deviation (SD). All variables were tested for normal distribution using the Shapiro- Wilk test. Data not normally distributed were logarithmically transformed. Mean comparisons among the four rat groups were conducted using a one-way ANOVA. The Tukey and post hoc tests to identify specific significant differences between each pair complete this analysis. Differences were considered significant at $p < 0.05$.

Results and Discussion

Yield, contents of phenolic compounds, flavonoids, condensed tannins and carotenoids

The extraction yields for AEDS and DSO are presented in Table I. They were found to be significantly high.

In a similar study that was conducted on another variety of date seeds, i.e. Kabkab date seeds, using water as the solvent, the yield was reported to be 10.27% [28]. As for Herch *et al*., they found out that the oil extracted from the Kentichi date variety showed yields ranging from 5.12% to 7.11% [29]. These yield differences are generally influenced by various factors, including the specific plant material characteristics, extraction conditions, and solvent properties (such as pH and polarity) [28].

Furthermore, Table I shows that the TPC and TFC were significantly higher in date seed oil than in date seed aqueous extract. These findings exceed those previously found for Ajwa date seeds, which showed a TPC ranging from 31.55 to 39.32 mg GAE/g, and that for flavonoids between 18.4 and 29.56 mg CE/g (Khalid *et al*., 2017), as well as those for Sukkari date seeds with TPC and TFC values of 98.1 mg GAE/g and 70 mg CE/g, respectively [30]. These phenolic and flavonoid content variations can be attributed to various factors, including the date variety, geographical origin, soil conditions, sunlight exposure, as well as the analytical methods and extraction techniques used [31]. Therefore, it can be asserted that date seed oil, particularly the one obtained from the Deglet Nour cultivar, could be a promising source of natural phenolic compounds [32].

Table I

Extraction yields, phenolic compounds content, and carotenoids concentration in the aqueous extract of date seeds (*Phoenix dactylifera* L.) (AEDS) and date seeds oil (DSO)

Extracts	AEDS (%)	DSO (%)
Yields (%)	6.35 ± 1.45	9.25 ± 0.40*
TPC (mg GAE/g DW)	92.25 ± 1.68	304.11 ± 1.99**
TFC (mg QE/g DW)	121.11 ± 1.14	261.2 ± 1.82**
CTC (mg CE/g DW)	57.92 ± 1.42	70.3 ± 0.62*
Carotenoids (mg/100g FM)	0.185 ± 0.02	2.61 ± 0.51**

All values are expressed as means of three replicates ± standard deviation (SD). *: significant difference at $p < 0.05$; **: highly significant difference at $p < 0.01$. TPC: Total Phenolics Content. TFC: Total Flavonoids Content. CTC: Condensed tannins content. GAE: Gallic Acid Equivalent. QE: quercetin Equivalent. CE: Catechin Equivalent. DW: Dried Weight.FM: Fresh Matter.

Moreover, Table I indicates that the CTC were moderately significant. These values are comparable to those reported by Boudghane et al for the variety Deglet Nour; they were found equal to 87.62 mg GAE/g and 50.90 mg CE/g DW [33].

The data in Table I indicate that the carotenoid content in the DSO is significantly higher than that in AEDS. The oil extract results were found quite consistent with those previously reported in several studies which indicated that the carotenoid content was between 1.46 and 3.53 mg/100 g [34]. The values obtained were lower than those found in Tunisian date seeds (5.51 mg/100 g) [35]. Hence, the "Deglet Nour" date seeds may be considered as a potential source of carotenoids [36].

Antioxidant evaluation

2,2-diphenyl-1-picryl-hydrazyl (DPPH) radical scavenging activity test

It was revealed that the DPPH radical scavenging activities of AEDS and DSO are dose-dependent, which may certainly be assigned to their chemical composition. The IC_{50} value was determined for each sample for the purpose of comparing the antiradical activity of these extracts. Low IC_{50} values imply high antioxidant activity.

The data presented in Figure 3A show that the oil extract possessed the strongest and the most significant free radical scavenging activity (IC_{50} = 0.48 ± 0.03 mg/mL), followed by the aqueous extract (IC_{50} = 1.81 ± 0.06 mg/mL). However, these IC_{50} values were significantly lower than that of BHT (IC_{50} = 0.43 ± 0.08 mg/mL) which is used as a positive control. A similar study was conducted by Arechti and Korkobbi on the aqueous extracts of two date seed varieties which exhibited an IC_{50} of 0.35 mg/mL [37]. Another study was carried out on the aqueous extract from Kabkab date seeds which showed a relatively high antioxidant activity. The findings suggested that Kabkab date seeds are strong radical scavengers (IC_{50} = 0.91 mg/mL) [28].

Ferric reducing antioxidant power

The ferric reducing antioxidant power was estimated by quantifying the reducing substances present in the sample. These substances could provide electrons to reduce the ions Fe^{3+} to Fe^{2+} (FRAP) and to eventually lead to the formation of Perls' Prussian blue at 700 nm. Low $A_{0.5}$ values, as an indirect measure, implied high reducing activity. Figure 3B clearly indicates that the oil extract exhibited the highest reducing activity (EC_{50} = 195.76 ± 0.7 µg/mL), followed by the aqueous extract (EC_{50} = 340.14 ± 0.04 µg/mL). It should be emphasized that these EC_{50} values are significantly lower than those of BHT (EC_{50}= 48.25 ± 0.05 µg/mL) that is used as a positive control. These findings are in good agreement with those reported in a previous study that found a positive correlation between the high FRAP values and the substantial electron transfer capacity towards the FRAP reagent [38]. A similar study revealed that the aqueous date seed extract showed potential reducing activity values ranging from 270 to 860 µg/mL [39].

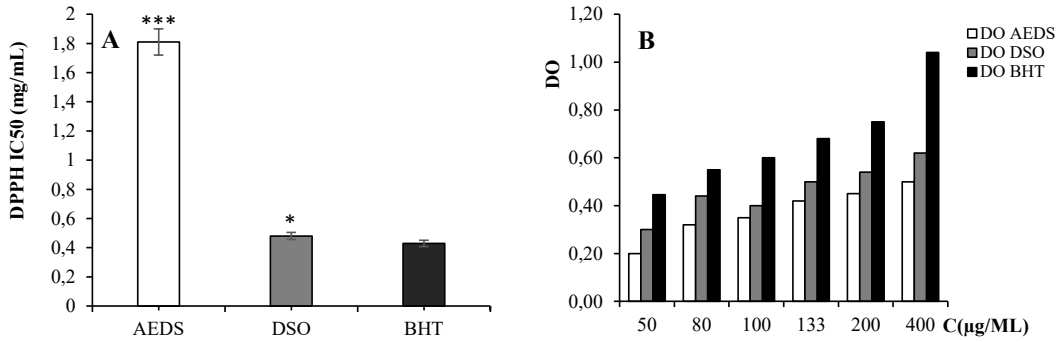

Figure 3.

In vitro antioxidant activity of aqueous date seeds extract (AEDS) and oil date seeds extract (DSO)
A: DPPH IC_{50} concentrations of date seeds extracts. **B** : reducing power by the ferric ion extracts of date seeds and standard BHT. Data are presented as means of three replicates ± SD; *: significant difference at $p < 0.05$; ***: highly significant difference at $p < 0.001$

High-performance liquid chromatography

The high-performance liquid chromatography with diode-array detection (HPLC-DAD) technique was employed to perform preliminary metabolic profiling with the goal of identifying putative essential classes of chemicals and/or particular components in AEDS and DSO. The majority of the detected metabolites are presented in Table II. Their identification was further confirmed by comparing the obtained results with the standard (reference) values. Gallic acid, caffeic acid, cinnamic acid, catechin, chlorogenic acid, caffeine, (+)- epicatechin, coumarin and quercetin, were the most remarkably representative family in the aqueous extract and oil extract. Likewise, Bouhlali *et al*. conducted a similar study and found out that caffeic acid, gallic acid, and caffeine were the most predominant bioactive constituents in the aqueous and oil extracts of date seeds [40]. Furthermore, several other studies confirmed that the most bioactive date seed components, such as gallic acid, caffeic acid [41], ferulic acid [42], quercetin, catechin [43], epicatechin [44, 45], cinnamic acid [45], caffeic acid, gallic acid [46, 47, 48] and Kaempferol [47] could be identified by means of the HPLC-DAD technique.

Table II

Retention time of different phenolic compounds of AEDS and DSO

	Retention time (min)					
	Chloroform	**Diethyl ether**	**Ethyl acetate**	**n-butanol**	**Aqueous extract**	**Oil**
Gallic acid	-	2.4	2.4	2.3	2.5	2.5
Catechin	-	-	5.2	5.2	-	5.6
Chlorogenic acid	-	6.5	-	6.8	6.8	-
Caffeic acid	7.6	7.6	7.5	-	7.6	7.4
Caffein	-	8.3	8.3	8.3	8.1	8.3
(+)-Epicatechin	9.1	8.9	9.1	-	-	-
Riboflavin	-	-	9.7	-	-	-
Ferulic acid	-	11.8	11.7	-	-	-
Coumarin	12.3	12.4	-	12.1	12.1	12.1
Salicylic acid	-	-	13	12.8	-	12.8
Quercetin	-	-	14.4	-	-	14.5
Cinnamic acid	-	-	15.2	-	-	-
Kaempferol	15.9	-	-	-	-	15.9
Tannic acid	16.9	-	16.7	-	-	16.9

The phenolic compounds identified exhibit antioxidant activity through various molecular mechanisms. Their ability to neutralise reactive oxygen species (ROS) is mainly based on: the donation of hydrogen atoms or electrons from their phenolic groups, the chelation of transition metal ions, and the interruption of radical chain reactions. Antioxidant efficacy is directly correlated with their chemical structure, particularly the number and position of hydroxyl groups, the conjugation of double bonds, and the extent of the aromatic system. These properties give these molecules a protective role against oxidative damage at the cellular and molecular levels, thus helping to limit oxidative stress, that is, the imbalance between the production of ROS and the body's antioxidant defences, linked to the development of various diseases, including

cardiovascular diseases, neurodegenerative diseases, cancer and age-related conditions [49].

In vivo evaluation of acute toxicity of date seed extracts

Determination of LD_{50}

Plant extracts represent a promising source of active molecules for the development of new drugs. However, in-depth toxicological assessments are required to guarantee their safe use, which is motivating intensified research in this field. Although the literature provides a wealth of information on various extracts of date seeds (*Phoenix dactylifera* L.), there are no data concerning their toxicological profile, which constitutes a "new and innovative element" justifying the present study. Our work is therefore devoted to the in vivo evaluation of the acute toxicity of date seeds (*Phoenix dactylifera* L.) in Wistar rats, in order to determine their potential for clinical trials in the treatment of metabolic diseases.

In our study, a single-dose acute oral toxicity evaluation was carried out and the results indicated that AEDS and DSO, at doses of 300, 1000 mg/kg and 2000 mg/kg body weight (bw), did not lead to mortality among rats, as clearly indicated in Table III. However, Table IV showed that the AEDS and DSO, at 300 and 1000 mg/kg bw, did not engender any clinical signs, as the rats appeared normal, indicating that these doses are well tolerated and do not disrupt the animals' normal physiological functions. On the other hand, the dose of 2000 mg/kg produced a few clinical signs, including slight behavioural changes with mild agitation, minor alterations in physical appearance such as raised hair, moderately red eyes, and transient difficulty in breathing (dyspnoea), all of which resolved within three days. These signs were mild and reversible, and therefore do not warrant classification of the extracts as toxic according to standard risk assessment principles, which differentiate between observed effects and formal toxicity classification.

It was shown that the toxicity of a substance depends on its median lethal dose (LD_{50}) value which represents the dose that kills 50% of the animals tested. In accordance with the method of Behrens and Karber [27], the absence of mortality observed in our study precludes the determination of a precise LD_{50} value. These results suggest that the LD_{50} of AEDS and DSO is greater than 2000 mg/kg. In this regard, Ugochukwu *et al*. carried out a study and found out that the LD_{50} of date seed ethanol extract is equal to 2000 mg/kg, which means that the results reported here are consistent with those revealed in other prior studies [50]. In light of this research, it may then be asserted that date seed aqueous and oil extracts can be classified in the fifth category, according to the Globally Harmonized System for the classification of chemicals [25]. These extracts may therefore be considered as a non-toxic substance by the oral route (Table III).

Table III

Mortality of rats treated with different doses of AEDS and DSO

	Doses (mg/kg)	**Mortality rate**	**Percentage of mortality (%)**
AEDS			
Lot 1 (n = 6)	300	0	0
Lot 2 (n = 6)	1000	0	0
Lot 3 (n = 6)	2000	0	0
DSO			
Lot 4 (n = 6)	300	0	0
Lot 5 (n = 6)	1000	0	0
Lot 6 (n = 6)	2000	0	0

After gavage administration of three different doses (300 mg/kg bw, 1000 mg/kg bw and 2000 mg/kg bw) of aqueous extract date seeds (*Phoenix dactylifera* L.) (AEDS) and date seed oil (DSO), the rats were observed for 14 days. Mortality rates were recorded for each dose administered.

Table IV

Clinical signs observed in rats following administration of AEDS and DSO at different doses

Doses / **Clinical signs**	**300 mg/kg bw** **1000 mg/kg bw**	**2000 mg/kg bw**
Physical appearance	Normal	Slight change
Coat	Smooth, normal grooming	Slight hair straightening
Posture	Normal	Minor change
Eyes	Normal	Slightly red
Salivation	Normal	Minor change
Breathing	Normal	Slight dyspnoea
Behaviour	Normal	Minor change with slight agitation
Urine and faeces	Normal	Slight change
Rectal temperature:	38°C	38°C

After gavage administration of three different doses (300 mg/kg bw, 1000 mg/kg bw and 2000 mg/kg bw) of aqueous extract date seeds (*Phoenix dactylifera* L.) (AEDS) and date seeds oil (DSO), the rats were observed for 14 days, several times a day. The animals were observed for physical appearance, behavioural changes, coat, posture, eye signs, rectal temperature and changes in heart and respiratory rates.

Changes in body weight, food ingestion and excretion

Variations in body weight are frequently used as endpoints in acute toxicity studies in rats, as they can reflect the pathophysiological impact of exposure to a toxic substance. Indeed, notable changes in body weight, such as unusual weight loss or gain, are often early signs of deterioration in the state of health of the animals, and can be linked to acute toxicity. In our study, variations in body weight were determined in control and experimental rats and recorded daily at the same time in the morning during the experimental period. Only the values at D0 (at the time of administration), D7 (finding of the first week) and D14 (finding of the observation period) are reported in Figure 4. Our results show that at the beginning of the experiment, the different batches of rats have homogeneous weights, varying from 186 to 210 g. Oral administration of AEDS and DSO at different doses (300, 1000 and 2000 mg/kg) did not cause significative variations in the body weight of experimental rats compared with controls at different times (D7 and D14). Figure 4 also indicates that the relative growth (%) did not change during the two weeks following the administration of aqueous and oil extracts. These findings agree well with those previously reported by Oluele *et al*. who also did not observe any significant body weight changes among rats that were administered oil extracts from date seeds [51]. As for Figure 5, it suggests that no substantial differences were noticed in food consumption, water intake, and amount of faeces or urine excreted between the experimental rats and the control group rats throughout the 14-day study period, after gavage with AEDS and DSO extracts. According to Stevens and Gallo, monitoring food and water intakes is a highly effective approach for assessing the growth rates and identify possible toxic effects [52]. In our study, experimental rats maintained food and water intake levels comparable to those of control rats, without significant variations. Consequently, we can conclude that the administration of date seed extracts did not induce acute toxicity in rats, in the absence of disturbances observed in their growth and feeding behaviour.

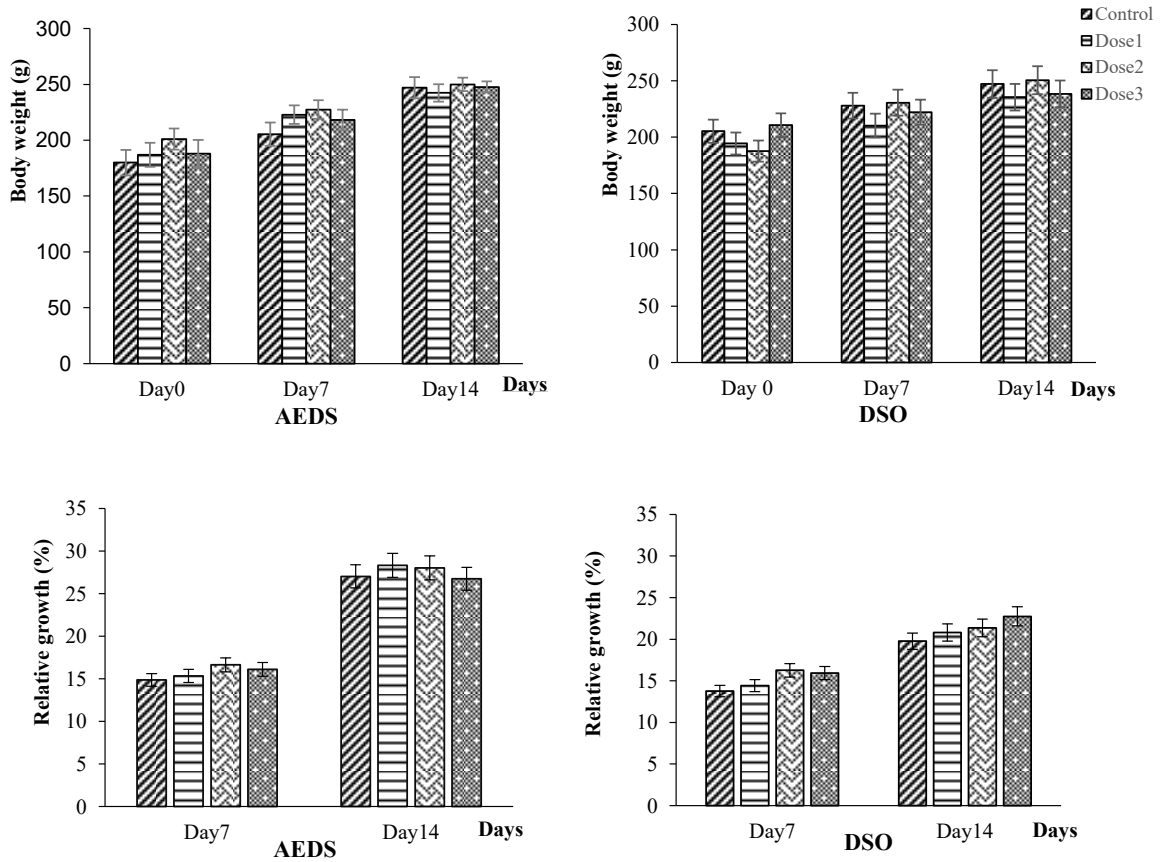

Figure 4.

Evolution of the body weight (g) and relative growth (%) of control and experimental rats during the 14 days of observation. Each value represents the mean ± SD. Day0: initial weight; Day7: weight after one week; Day14: weight after 2 weeks. C: control rats; AEDS: rats treated with an aqueous extract of date seeds by gavage; DSO: rats treated with date seed oil by gavage Dose1: rats treated with 300 mg/kg bw; Dose2: rats treated with 1000 mg/kg bw; Dose 3: rats treated with 2000 mg/kg bw. The data were analysed using one-way ANOVA followed by Tukey and post hoc tests. The variations between control and experimental rats are not significant

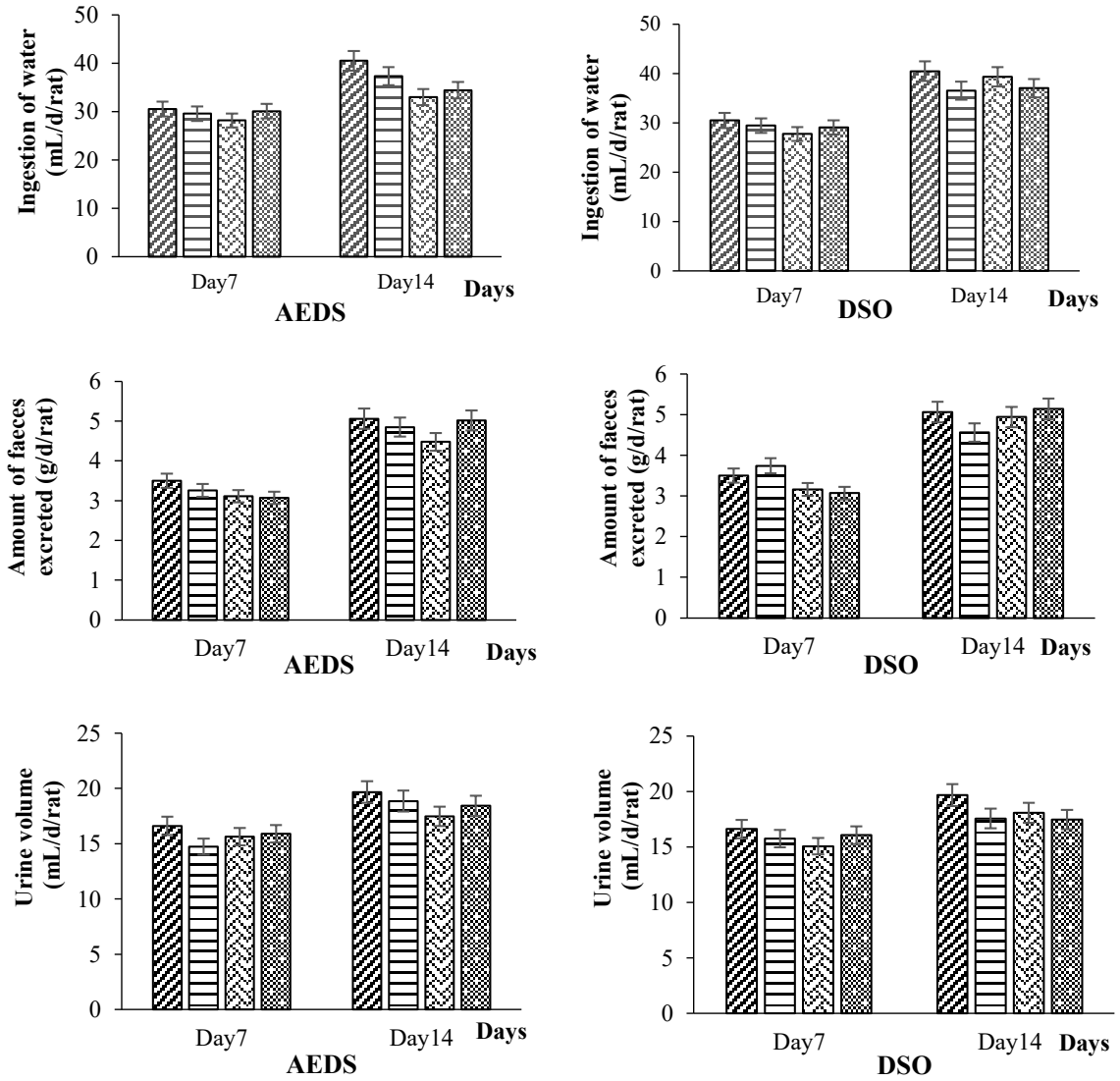

Figure 5.

Food consumption (g/d/rat), water ingestion (mL/d/rat), amount of faeces excreted (g/d/rat) and urine volume (mL/d/rat), of control and experimental rats during the 14 days of observation. Each value represents the mean ± SD. Day0: initial weight; Day7: weight after one week; Day14: weight after 2 weeks. C: control rats; AEDS: rats treated with an aqueous extract of date seed by gavage; DSO: rats treated with date seed oil by gavage Dose1: rats treated with 300 mg/kg bw; Dose2: rats treated with 1000 mg/kg bw; Dose 3: rats treated with 2000 mg/kg bw. The data were analysed using one-way ANOVA followed by Tukey and post hoc tests. The variations between control and experimental rats are not significant

Variations in haematological and biochemical parameters

The degree of toxic effects of medicines can be determined by conducting analyses on the haematological parameters which play a highly important role in the body's response to multiple changes such as stress and deprivation of nutrients. In addition, some haematological and biochemical parameters were analysed at the end of the experiment (D14) for the purpose of making sure that date seeds are not toxic. The haematological parameters were measured and the results showed that the administration of different doses of date seed extracts did not cause substantial changes in the numbers of white blood cells and red blood cells, and in the haemoglobin level in experimental rats as compared to control rats (Table V). Therefore, the different physiological mechanisms associated with these haematological parameters remained normal in these experimental rats [53]. A similar study was performed and the results suggested that date seed extracts have beneficial effects on mice that were exposed to toxic silver nanoparticles. It turned out in fact that date seeds have the potential to reduce and regulate the level of white blood cells which play a crucial role in the body's immune system that serves as the first line of defence in the body [54].

Table V

Haematological parameters in control and experimental rats

Parameters	White blood cells (10^3/mm^3)	Red blood cells (10^6/mm^3)	Haemoglobin (mg/dL)	Lymphocytes (10^3/mm^3)	Polynuclear (10^3/mm^3)	Monocytes (10^3/mm^3)
Control	3.54 ± 0.07	6.26 ± 0.06	11.81 ± 2.16	3.00 ± 0.94	0.41 ± 0.24	0.57 ± 0.09
AEDS						
Dose1	3.33 ± 0.09	6.22 ± 0.05	12.03 ± 2.14	4.02 ± 1.07	0.48 ± 0.04	0.60 ± 0.17
Dose2	3.99 ± 1.05	6.13 ± 0.09	11.85 ± 2.47	3.85 ± 1.04	0.46 ± 0.02	0.62 ± 0.04
Dose3	4.05 ± 1.57	6.11 ± 0.07	11.9 5 ± 1.57	3.65 ± 1.09	0.53 ± 0.14	0.55 ± 0.04
DSO						
Dose 1	3.88 ± 0.01	6 ± 0.08	12.22 ± 2.14	4.07 ± 0.14	0.48 ± 0.2	0.67 ± 0.05
Dose2	4.04 ± 0.21	6.14 ± 0.54	11.93 ± 2.43	3.87 ± 0.24	0.41 ± 0.13	0.61 ± 0.24
Dose3	4.25 ± 0.21	6.20 ± 0.45	12.24 ± 2.15	3.82 ± 0.52	0.44 ± 0.04	0.58 ± 0.34

All values are presented as means ± SD. All measurements were conducted at the end of the experiment (Day 14) C: control rats; AEDS: rats treated with an aqueous extract of date seeds by gavage; DSO: rats treated with date seeds oil by gavage Dose1: rats treated with 300 mg/kg bw; Dose2: rats treated with 1000 mg/kg bw; Dose 3: rats treated with 2000 mg/kg bw. The data were analysed using one-way ANOVA followed by Tukey and post hoc tests. The variations between control and experimental rats are not significant.

Likewise, the kidneys play an essential role in purifying and eliminating xenobiotics such as pharmaceutical compounds and metabolic waste. It is widely established that creatinine and urea are commonly used as indicators of renal function. If the levels of creatinine and urea in blood are not within the normal range, it means that the kidneys are not working properly [55]. The data in Table VI did not show any significant changes in the creatinine and urea levels in rats that were given various concentrations of aqueous and oil extracts. In this context, a previous study revealed no substantial changes in creatinine levels, with a slight decrease in urea levels in rats force-fed with 1500 mg/kg of body weight of aqueous date seed extract [56]. Other studies, which were conducted in this same context, did not find any significant alterations in serum biomarkers after rats were administered date seed essential oil at doses below 500 mg/kg of body weight [51].

Table VI

Composition and codification of metronidazole gel formulations

Parameters	Glucose (g/L)	Creatinine (mmol/L)	Urea (mmol/L)	Total protein (g/L)	TC (g/L)	TG (g/L)	AST (UI)	ALT (UI)	ALP (UI)
Controls	0.90 ± 0.31	7.78 ± 0.54	5.96 ± 0.40	50 ± 0.24	0.81 ± 0.24	0.67 ± 0.09	51.51 ± 0.67	69.5 ± 8.54	99.76 ± 1.53
AEDS (mg/kg)									
Dose 1	0.84 ± 0.44	7.68 ± 0.42	6.15 ± 0.04	50.08 ± 0.24	0.82 ± 0.04	0.66 ± 0.17	46.90 ± 2.40	70.54 ± 7.68	99.09 ± 1.64
Dose2	0.81 ± 0.32	7.71 ± 0.44	5.8 ± 0.06	50.47 ± 0.25	0.86 ± 0.02	0.68 ± 0.04	50.25 ± 6.50	82 ± 5.70	99.10 ± 1.44
Dose3	0.91 ± 0.01	7.70 ± 0.514	5.94 ± 0.04	50.86 ± 0.67	0.83 ± 0.14	0.65 ± 0.04	51.14 ± 0.81	62 ± 4.14	99.76 ± 1.53
DSO (mg/kg)									
Dose1	0.88 ± 0.01	7.67 ± 0.50	6.08 ± 0.10	50.14 ± 0.18	0.80 ± 0.2	0.67 ± 0.05	50.9 ± 1.40	67.84 ± 4.04	100.18 ± 1.83
Dose2	0.84 ± 0.21	7.64 ± 0.54	5.92 ± 0.4	50.04 ± 0.8	0.83 ± 0.13	0.61 ± 0.24	52.9 ± 2.37	66.33 ± 5.4	99.07 ± 1.53
Dose3	0.85 ± 0.01	7.70 ± 0.45	6.02 ± 0.11	50.44 ± 0.18	0.82 ± 0.04	0.66 ± 0.34	49.9 ± 1.40	70.12 ± 4.33	101.14 ± 1.12

All values are presented as means ± SD. All measurements were conducted at the end of the experiment (Day 14). C: control rats; AEDS: rats treated with an aqueous extract of date seed by gavage; DSO: rats treated with date seed oil by gavage Dose1: rats treated with 300 mg/kg bw; Dose2: rats treated with 1000 mg/kg bw; Dose 3: rats treated with 2000 mg/kg bw; TC: Total cholesterol; TG: Triglyceride; AST: Aspartate-Amino-Transferase; ALT: Alanine-Amino-Transferase; ALP: Alkaline phosphatase. The data were analysed using one-way ANOVA followed by Tukey and post hoc tests. The variations between control and experimental rats are not significant.

Furthermore, the hepatic function was evaluated by measuring the levels of AST, ALT, and ALP. These three enzymes are key biomarkers of liver diseases. Elevated levels of these enzymes can indicate cellular damage as they are involved in essential metabolic processes within cells [57]. The levels of these enzymes, which are given in Table VI, remained within normal ranges in the experimental rats, indicating that the liver function was not affected by the treatment. The findings of this study are in good agreement with others reported in the literature, which highlights the protective effects of date seeds on liver function [54].

Based on the above, one may assert that the administration of various doses of date seeds does not result in significant changes in serum levels of glucose, total cholesterol, triglycerides, or total protein (Table VI). These findings also indicate that the carbohydrate, protein, and lipid metabolism remained normal. These results are supported by previous research which showed no alterations in the biochemical parameters in rats treated with 1500 mg of aqueous date seed extract [56]. Furthermore, El-Kelawy and El-Shafey conducted another investigation and found out that incorporating a date seed meal

into rabbit diets did not adversely impact the blood parameters such as glucose, total lipids, triglycerides, HDL, LDL, and total protein [58]. The stability of these parameters may certainly be attributed to the nutritional composition of dates. It was indeed found that the date components counterbalanced any acute effects and helped to maintain these levels stable in rats. Other results also indicated that the weights of organs, i.e. lungs, liver, heart, spleen, and kidneys, remained unaltered, and no clinical or metabolic changes were noticed in the Wistar rats (Figure 7), hence corroborating the non-toxicity of date seeds.

The toxicological assessment of a substance is based on a progressive approach, ranging from immediate effects to the consequences of prolonged exposure. Acute toxicity studies are essential for identifying lethal doses and short-term risks. However, subchronic and chronic tests are necessary to detect cumulative alterations in order to predict the actual health impact and ensure the safety of exposed populations. Therefore, our work must be supplemented by other long-term studies in order to decide on the possibility of using date seeds and taking advantage of their therapeutic properties without causing adverse effects.

This study represents a key step towards promoting date seeds in functional or therapeutic food applications. Thanks to this assessment, we were able to identify the presence or absence of any immediate adverse effects, thus laying the initial foundations for ensuring their safe use. The results obtained, combined with existing data on their high polyphenol content, are crucial in supporting their potential use as health ingredients. Their acute non-toxicity could pave the way for further research (subchronic, functional) and the development of innovative nutraceuticals or dietary supplements, while ensuring consumer safety.

Conclusions

In vitro investigations have demonstrated the significant antioxidant potential. The primary bioactive compounds contributing to this antioxidant activity include polyphenols, flavonoids, condensed tannins, and carotenoids.

The acute toxicity results clearly demonstrate that date seeds are safe in the short term and open the door to exciting opportunities for their use. However, further studies are essential to fully assess their potential. This includes subchronic and chronic toxicity tests to understand the effects of prolonged exposure, research on the bioavailability and metabolism of their main active compounds, and *in vivo* validation of their efficacy in relevant models of metabolic or neurodegenerative diseases. This future research will help us better define the benefit-risk ratio of date seeds and optimize their use in functional foods or therapeutic applications.

Conflict of interest

The authors declare no conflict of interest.

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