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ORIGINAL RESEARCH PAPER

PHENOLIC COMPOUNDS RECOVERY FROM DATE SEED AND ASSESSMENT OF THEIR ANTIOXIDANT AND ENZYME INHIBITORY PROPERTIES

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Abstract

Date fruit is a highly important crop cultivated in arid and semi-arid regions, known for its nutritional value and health benefits. Dates' benefits extend beyond their edible portion and include seeds, which are a by-product of different industries, often considered waste, and have untapped potential. This work aimed to optimize the extraction conditions of antioxidant compounds from date seeds and assess their ability to inhibit disease-related enzymes. The optimization was carried out by examining the main parameters, including solvent type (acetone, ethanol, methanol, and water), solvent concentration (25–100%), sample-to-solvent ratio (10–60/20 mg/ml), and extraction time (15–90 min). Phenolic content (PC) was determined using the Folin-Ciocalteu method, while antioxidant activity was evaluated by ABTS and DPPH scavenging assays, as well as ferric reducing power (FRP). Based on the statistical analysis, 75% acetone, 10 mg/20 ml, and 45 min were found to be the best extraction conditions, manifesting a PC of 128.65 mg GAE/g dw, antiradical powers of 244.91 mg TE/g dw (ABTS) and 64.27 mg GAE/g dw (DPPH), and a FRP of 64.27 mg GAE/g dw. Moreover, the extract of date seed demonstrated a promising inhibitory property against some key enzymes such as tyrosinase, acetylcholinesterase, and α-glucosidase, which may be considered a good candidate for managing diabetes, Alzheimer's disease, and neurodegenerative disorders.

Keywords: date seed; polyphenols; extraction; antioxidant activity; enzyme inhibitory potential

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Introduction

Date palm (*Phoenix dactylifera* L.) is among the most important crops in arid and semi-arid zones of the world (Mrabet *et al*., 2015). Date palm plays a pivotal socioeconomic role in the oasis peoples of the Middle East due to its high nutritional value and pharmacological virtues (Baliga *et al*., 2011; Djaoudene *et al*., 2020). The interest in this plant is also due to its great adaptation to the severe conditions of these very hot regions as well as to its multiple uses in many fields.

Phoenix dactylifera L. fruit worldwide production is estimated at 9.66 million tons a year, according to the Food and Agriculture Organization of the United Nations' Statistics Division. It is concentrated mainly in the Middle East and North Africa. Algeria is the world's 4th largest producer of dates, with around 1.18 million tons in 2021 (FAOSTAT. Food and Agriculture Organization of the United Nations 2021). In Algeria, phoeniciculture is of great socio-economic and environmental importance because it is considered the linchpin on which life in the Saharan regions revolves. With over 18 million palm trees and more than 1,000 cultivars, Algeria occupies an important place among the world's date-producing and exporting countries (Benamara *et al*., 2017). This rise in consumption and production is directly proportional to the quantity of fruit waste generated.

The fruit of this plant is an essential raw material not only for its direct consumption, but also for the many industries of processing, giving rise to numerous derived products including paste, powder, syrup, juice, and confectionery (Khan *et al*., 2016; Al Juhaimi *et al*., 2018). Processing date fruit results in the generation of huge quantities of seeds, constituting about 10–15% of the initial weight, which were generated as waste that was thrown away or sometimes used as fertilizer or feedstock alimentation (Ben-Youssef *et al*., 2017; Khalid *et al*., 2017). Nevertheless, more recently, they have been widely used to make functional foods like cookies, bread, muffins, cheese, beef burgers, gluten-free cookies, and biscuits, as well as in food supplementation, cosmetics, and pharmacology (Sayas-Barberá *et al*., 2020; Alharbi *et al*., 2021; Habib *et al*., 2022; Alqahtani *et a*l., 2023).

Furthermore, date seeds include a variety of nutritive and functional substances; they are a good source of fiber and contain considerable amounts of lipids, carotenoids, protein, vitamins, and minerals. Likewise, date seeds are also thought to be a source of phytochemicals such as polyphenols, including flavonoids (Al Juhaimi *et al*., 2018; Salomón-Torres *et al*., 2019; Mrabet *et al*., 2020; Kamal *et al*., 2023).

In recent years, the issue of waste has attracted increasing public attention. By-products from agriculture and food processing can be recovered and used in practical ways to enhance scarce resources while reducing waste disposal issues. In this line, the seeds of *Phoenix dactylifera* have been considered long ago as a problem. Due to their abundance in high-added value bioactive compounds, they have the potential to be exploited as food additives and/or nutraceuticals, which would help to advance agriculture and increase profits in the food processing sector (Sirisenaa *et al*., 2016; Al-Meqbaali *et al*., 2017). Therefore, the use of this by-product is very attractive for the date industry which can increase the income of the date palm sector.

Phytochemicals such as polyphenols, including flavonoids, are major plant-based biocompounds recognized for their beneficial effects, particularly their antioxidant potential. These beneficial biomolecules, which have the ability to inhibit some key enzymes like α-amylase and acetylcholinesterase associated with postprandial hyperglycemia and neurodegenerative diseases, have generated a great deal of interest as a potential treatment for type 2 diabetes mellitus and Alzheimer disease (Yao *et al*., 2013). Date seed has been known to contain valuable polyphenolic compounds (Messaoudi *et al*., 2013). Moreover, it has been demonstrated that date seed bioactive compounds have antioxidant and anti-inflammatory effects; thus, they confer protective effects against various chronic diseases, like cancer, diabetes, cardiovascular, and neurodegenerative diseases (Khan *et al*., 2018; Alkhoori *et al*., 2022). The phytochemical profile of date seed varies greatly because of several aspects such as variety and origin, stage of maturation, processing, and analytical methods (Al-Farsi and Lee 2011; Maqsood *et al*., 2020).

Due to their beneficial properties, the antioxidants extraction is an important process, since there isn't a unique procedure or satisfactory method for their recovery from different resources due to the chemical complexity of compound structures and their polarity, the variation in sensitivity to extraction conditions, and differences in the structural organization and composition of botanical matrices. However, various factors, including the extraction method, nature and concentration of the solvent, temperature, duration of extraction, and sample-to-solvent ratio, are known to influence the extraction process of phenolics (Ng *et al*., 2012). In this regard, previous studies have reported that the extraction process can affect the quantity and quality of bioactive compounds of the date seed extracts as well as their biological activities (Thouri *et al*., 2017; Li *et al*., 2020; Pourshoaib *et al*., 2022). Therefore, it is important to investigate the effectiveness of various extraction systems, such as to allow selecting the most efficient procedure. To the best of our knowledge, there are only a few studies investigating the optimization of phenolic compounds extraction from Algerian *P. dactylifera* seed extracts, and a few reports evaluating the enzyme inhibitory capacity of these extracts.

Therefore, the aim of this work was the determination of the optimal experimental parameters (nature and concentration of the solvent, sample-to-solvent ratio, extraction time) for the recovery of phenolic compounds from date palm seeds and then to obtain the best *in vitro* antioxidant capacity. This investigation also attempts to assess the potential of the studied date by-product extract for preventing type 2 diabetes by inhibiting α-glucosidase and managing some neurodegenerative disorders by reducing acetylcholinesterase activity. Finally, the extract is also tested for its in vitro skin hyperpigmentation potential by measuring its ability to inhibit tyrosinase activity.

Materials and methods

Chemicals

Sodium carbonate (Na_2CO_3), sodium phosphate dibasic (Na2HPO4), and sodium phosphate monobasic (NaH_2PO_4) were from Biochem, Chemopharma (Georgia,

USA); potassium persulfate ($K_2S_2O_8$) and Folin–Ciocalteu reagent were purchased from Biochem, Chemo-pharma (Montreal, Quebec); ferric chloride was from Panreac (Barcelona, Spain); ABTS (2,2–azino-bis (3-ethylbenzthiazoline- 6–sulfonic acid)) and DPPH (2,2–Diphenyl-1-picrylhydrazyl) were from Sigma Chemical (Sigma–Aldrich GmbH, Germany); gallic acid ($(HO)_3C_6H_2CO_2H$) was from Prolabo (Montreuil, France); acetone, ethanol, and methanol were obtained from VWR-Prolabo (CE–EMB). Mushroom tyrosinase (EC 1.14.18.1), acarbose, β-D-glucopyranoside (pNPG), kojic acid, acetylthiocholine iodide (ATCI), galantamine, 5,5'-dithiobis (2–nitrobenzoic acid) (DTNB), 3,4–Dihydroxy-L–phenylalanine (L-DOPA), acetylcholinesterase (Electriceel, EC 3.1.1.7, Type VIS), α-glucosidase (*Saccharomyces cerevisiae*, EC 3.2.1.20, Type I), and 2-nitrophenyl were purchased from Sigma-Aldrich (Madrid, Spain).

Biological material and preparation of extracts

Date fruits in the ripe stage of the cultivar locally known as Ourous were harvested in southern Algeria's Ghardaia region's M'zab oasis. Date seeds were separated from the pulp and soaked in distilled water to remove all traces of fruit flesh, then wiped with absorbent paper. The seeds were dried in a ventilation-equipped oven for 24h at 50 °C. With an electric grinder, the dried seeds were reduced to a very fine particle size powder (Ø<250µm).

For bioactive compounds extraction, 20 mg of date seed powder was mixed in 20 ml of solvent and subjected to magnetic steering. The extract was obtained by centrifugation (2822×g for 15 min). In order to establish the best extraction conditions, the solvent type, its concentration, the sample-to-solvent ratio, and the extraction time were tested at different levels and examined in a sequential manner.

After having determined the optimal extraction conditions, the organic solvent was eliminated under vacuum, and the remaining water in the extract was frozen at 20°C and then lyophilized (ChristAlpha 1-4 LD). The resulting powder residue was kept at -20°C in an airtight container to test the enzyme inhibitory activity. Figure 1 summarizes the experimental approach followed in this study.

Extraction conditions

Solvent type

Date seed powder was extracted with 50% (v/v) ethanol, methanol, acetone, and water; the solid-to-solvent ratio and extraction time were set at 20 mg/20 ml and 15 min, respectively.

Solvent concentration

According to the previous step, the best extraction solvent nature was selected and used to study the adequate solvent concentration (25, 50, 75, and 100% (v/v)) while keeping the sample-to-solvent ratio and extraction time at the same levels.

Sample-to-solvent ratio

In this step, the extraction was performed by applying different sample-to-solvent ratios (10/20, 20/20, 40/20, and 60 mg/20 ml), where the solvent type at its best concentration was fixed as determined from the last steps, while 15 min was kept as the extraction time.

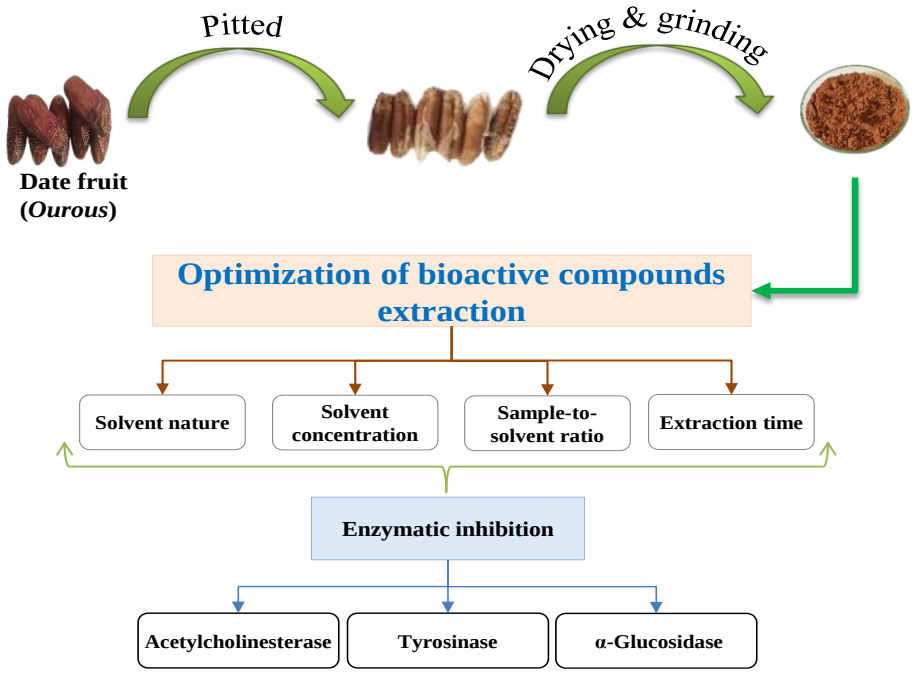

Figure 1. Scheme of the applied experimental approach followed in this study.

Extraction time

The extracts were prepared by changing the extraction duration (15, 30, 45, 60, and 90 min) using the optimal solvent concentration and solid/solvent ratio.

Evaluation of bioactive compounds content and antioxidant capacity

Polyphenols content

Polyphenols content was determined as described by Al-Farsi *et al*. (2005). A volume of 750 µl of Folin–Ciocalteu (diluted ten times with distilled water) was mixed with 100 µl of extract. Next, the mixture was supplemented with 750 µl of 6% sodium carbonate. Absorbances were measured at 760 nm after one hour of ambient incubation. The standard calibration curve made with established gallic acid concentrations was used to calculate phenolic concentrations. Results were given as milligrams of gallic acid equivalents per gram of dry weight (mg GAE/g dw).

DPPH radical scavenging assay

The DPPH radical scavenging test is employed as previously reported (Brand-Williams *et al*., 1995). Concisely, 1000 µl of 60 µM methanolic DPPH solution was mixed with 100 µl of extract. The mixture was left for 30 minutes of incubation, and the absorbance was measured at 517 nm. The radical scavenging activity was given as mg GAE/g dw.

ABTS cation radical scavenging assay

The test is based on the ABTS cation radical discoloration procedure (Re *et al*., 1999). The testing solution of ABTS°+ was obtained by dilution of the activated

radical form with potassium persulfate and adjustment of the absorbance to 0.70 at 734 nm. The reaction mixture consisted of adding 1000 µl of ABTS reagent to 100 µl of extract. The absorbances were recorded after six minutes, and the results were reported as mg Trolox equivalent (TE)/g dw.

Ferric Reducing Power (FRP)

The ferric reducing power (FRP) was assessed using the Oyaizu procedure (Oyaizu 1986). A mixture of 100 µl of extract, 250 µl of 0.2 M phosphate buffer (pH 6.6), and 250 µl potassium ferricyanide (1%) was formed. 250 µl TCA (trichloroacetic acid, 10%) was added after incubation at 50 °C for 20 minutes. Then, 850 µl distilled water and 170 µl FeCl3 (ferric chloride, 0.1%) were added. The absorbances were read at 700 nm and the results were reported as mg GAE/g dw, referring to gallic acid as a standard.

In vitro enzyme inhibitory properties

Acetylcholinesterase (AChE) inhibition

The AChE inhibition was carried out using Ellman's method using 96–well microplates (Ellman et al., 1961). A volume of extract (25 µl) prepared in 50 mM Tris–HCl (pH 8) was put in microplates and added with an equal volume of 15 mM ATCI, 3 mM DTNB (prepared in Tris–HCl (50 mM, pH 8), containing 20 mM $MgCl_2$ and 100 mM NaCl), and 50 µl Tris–HCl (50 mM, pH 8 and 0.1% bovine serum). To complete the reaction, 25 µl of AChE (0.22 U/ml) was added. The absorbance was measured for 11 min at 405 nm. Galantamine was used as a reference inhibitor.

Tyrosinase (TYR) inhibition

The TYR inhibition was determined using 96-well microplates as reported by Masuda et al. (2005). A volume of extract (10 µl) at different concentrations prepared in DMSO was mixed with 40 µl of L-DOPA, 80 µl of 75 mM phosphate buffer (pH 6.8), and 40 µl of tyrosinase. Tyrosinase inhibitory potential was expressed as a percentage inhibition based on the absorbance at 475 nm. Kojic acid was used as a positive inhibitor.

α-Glucosidase (α-GLU) inhibition

The ability of the extract to inhibit α-GLU was determined according to Kazeem et al. (2013). The reactional medium contained 100 µl of α-GLU (1 U/ml) and 50 µl of extract or acarbose (positive inhibitor). After 10 minutes of preincubation, 50 µl of pNPG (3 mM) dissolved in 20 mM phosphate buffer (pH 6.9) were added, and the mixture was incubated for 20 min at 37°C. The absorbance was recorded at 405 nm and the results were expressed as percentages.

Statistical analysis

The data were reported as the mean ± standard error of at least triplicate experiments. To assess the differences between the results obtained from the tested levels of each parameter, the data were subjected to a one-way analysis of variance following the Fisher LSD-test (Least Significant Difference) with Statistica v.8 software, and the level of significance was considered at $P \leq 0.05$.

Results and discussion

Influence of extraction process parameters

The extraction of phenolic compounds from different plant resources is considered a complex process. Thereby, an optimization study is recommended in order to guarantee maximum extraction in quantitative and qualitative terms with a minimum of economic loss. Following several preliminary tests, four parameters showed a significant influence on the recovery of polyphenols from the date seed.

Solvent type

Determining the best extraction solvent is a crucial step, as it influences both the quantitative and qualitative features of the extract and, consequently, its biological properties. The variation in solvent polarity results in a large difference in phenolic extraction efficiency. The impact of the solvent type on PC recovery and antioxidant potential was studied first, and the results are shown in Figure 2. The extracts obtained from the used solvents had significantly different PC and antioxidant activities. Indeed, the best extraction was obtained with acetone (97 mg/g dw), followed by ethanol (92 mg/g dw). Moreover, as represented in Figure 2B, 2C and 2D, the extract performed with acetone demonstrated the highest antioxidant activity (40 mg GAE/g dw and 178 mg TE/g dw for the DPPH and ABTS scavenging assays, respectively). However, ethanol extract displayed the best FRP, slightly higher than that of acetone. In contrast, the extract prepared using water revealed the weakest potency of PC extraction and the lowest antioxidant activities. Therefore, the more polar solvent, which was water with a polarity index of 10.2, was not adequate for the extraction of antioxidant compounds from date seeds. On the other hand, in spite of the similar polarities of the three organic solvents used, with a polarity index of 5.1 for methanol and acetone and 5.2 for ethanol, the highest efficiency for PC and antioxidant activities was globally expressed by acetone. Thus, extraction effectiveness was attributed to the polarity of the solvent and its nature, including its chemical structure and physicochemical properties.

The investigations conducted on the extraction of bioactive compounds from date seeds, particularly phenolics and their classes such as flavonoids, also found that acetone was the most efficient solvent compared to other organic solvents and water (Al-Farsi & Lee 2008). In contrast, a previous study on the effect of the solvent system on the extraction of phytochemical compounds from date seeds (*var*. Kabkab) showed that extraction with water and aqueous-ethanol offered the highest phenolics content and antioxidant activities (Pourshoaib *et al*., 2022). In this regard, Thouri *et al.* (2017) also reported that water and methanol were better solvents as compared to aqueous acetone and absolute acetone for phenolic compounds extraction from Tunisian date seeds (var. Korkobbi and Arechti). Moreover, acetone permitted the best extraction of phenolic compounds from other seeds, such as prickly pear seeds (Chaalal et al., 2012). The same fact was also mentioned by Benchikh and Louaileche (2014), who showed that acetone was more efficient than ethanol, methanol, and water for the extraction of phenolic compounds from carob (*Ceratonia siliqua* L.) pulp. However, a considerable influence of the solvent on

phenolic recovery from Jabuticaba fruit seeds was observed, and the highest polyphenols content was obtained by ethanol (Paludo *et al.*, 2019).

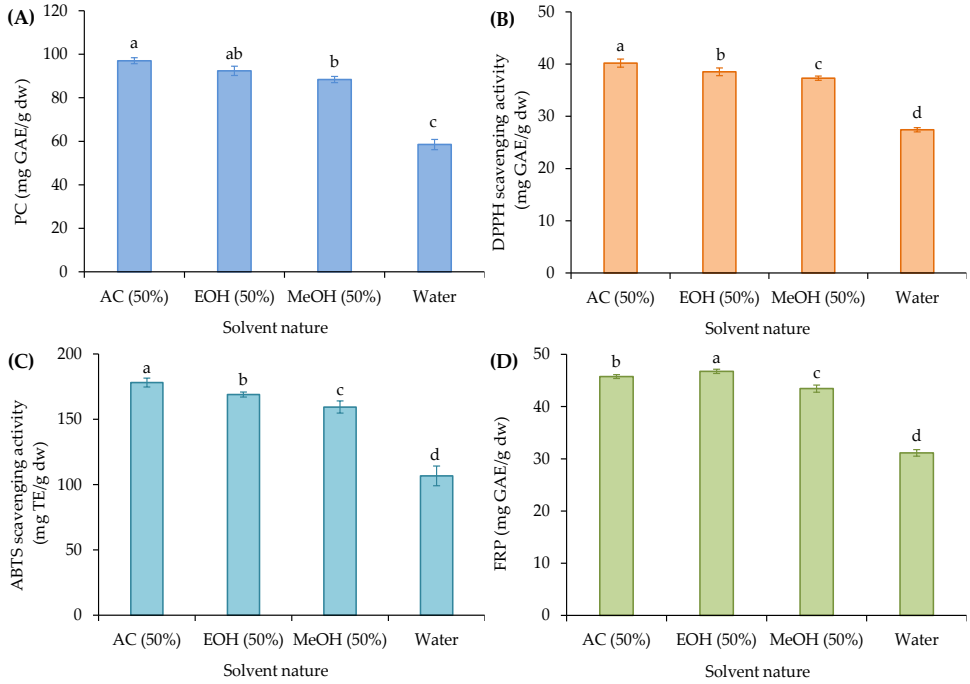

Figure 2. Effect of solvent nature on polyphenols recovery (**A**), DPPH (**B**) and ABTS (**C**) radical scavenging activities, and ferric reducing power (**D**) of date seed. AC - acetone; EOH - ethanol; MeOH - methanol; for each parameter, the results with different letters are statistically different (ANOVA, LSD-test, $P \leq 0.05$) with a>b>c>d.

Solvent concentration

Acetone was selected as the best solvent; therefore, the influence of its concentration was studied, and Figure 3 shows the obtained results. The statistical analysis attested that acetone concentration significantly ($P \leq 0.05$) influenced polyphenols concentration (Figure 3A) and antioxidant activities of date seeds (Figure 3B, 3C, and 3D). The best values were recorded for 75% acetone with 100.49 mg GAE/g, 41.59 and 47.61 mg GAE/g, and 187.36 mg TE/g for PC, DPPH, FRP, and ABTS, respectively. Pure acetone was the least efficient solvent for recovering phenolics, and under this condition, pure acetone was inefficient for extracting date seed polyphenols, indicating that the combination of acetone with water was better than 100% acetone.

Numerous previous investigations have tested the influence of different solvent concentrations on phenolic recovery and antioxidant properties. In fact, Afifi *et al.* (2017) revealed that 50% ethanol was the better solvent for phenolic extraction from date seeds. Furthermore, Santana *et al.* (2017) indicated that 70% ethanol recovered the highest level of phenolics from yellow passion fruit seeds (*Passiflora edulis*

Sims). In addition, Vural *et al*. (2018) reported that the highest phenolic recovery from grape seeds was recorded for 61.76% ethanol. The results of Al-Farsi and Lee (2008) and Kchaou *et al*. (2013) were in concordance with those of the present findings, suggesting that acetone-water combinations are efficient solvents for phenolic extraction. On the other hand, the results of the study conducted by Thouri *et al*. (2017) showed that 100% methanol exhibits a higher ABTS cation radical scavenging activity from two date seed cultivars.

The solvent's concentration is a crucial factor; therefore, the polarity can improve the extraction efficiency of phytochemicals by modifying the permeability, in particular the osmotic process of the plant material, allowing the penetration of the solvent and the interaction with the plant matrix (Sheng *et al*., 2018).

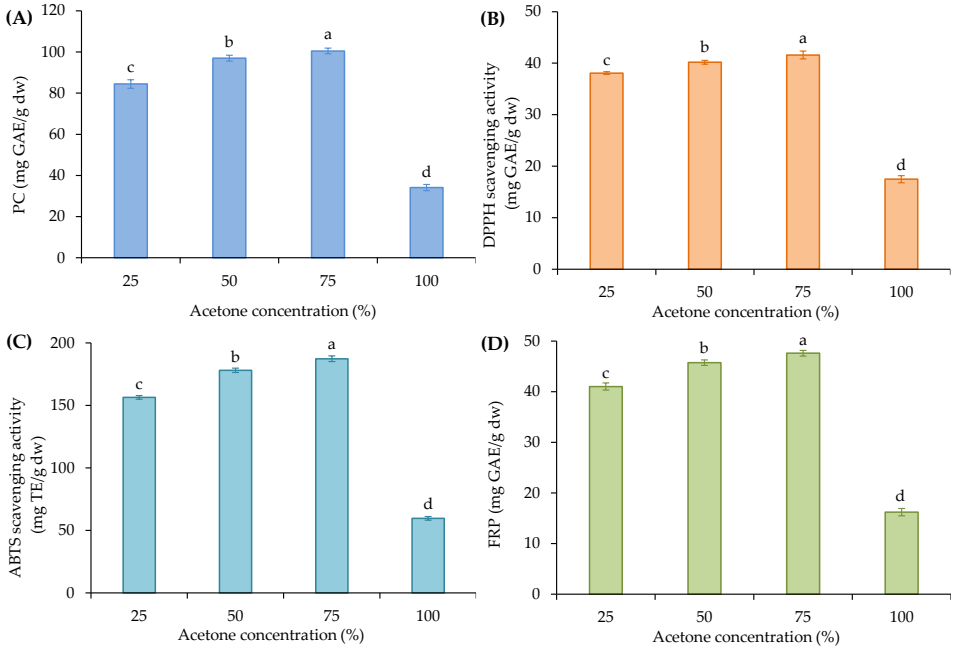

Figure 3. Effect of solvent concentration on polyphenols recovery (**A**), DPPH (**B**) and ABTS (**C**) radical scavenging activities, and ferric reducing power (**D**) of date seed. The results with different letters are statistically different (ANOVA, LSD-test, $P \leq 0.05$) with a>b>c>d.

Sample-to-solvent ratio

The recovery of phenolics and antioxidant activity of the plant matrix is influenced by the equilibrium and mass transfer principles between the solid and the solvent during the extraction exchanges. Hence, the determination of an appropriate solid-to-liquid ratio (SLR) is a critical step, as low SLRs result in rapid saturation of solvent, and the use of high SLRs is economically unprofitable.

According to the obtained results, the sample-to-solvent ratio significantly influenced ($p \leq 0.05$) the phenolic recovery (Figure 4A) and antioxidant activity of

date seeds (Figure 4B, 4C, and 4D). The highest levels of antioxidant parameters were achieved with the lowest sample-to-solvent ratio. PC, FRP, DPPH, and ABTS radical scavenging capacities overall decreased with the increase of the sample-to-solvent ratio from 10/20 to 60/20. According to Cacace and Mazza (2003), this process is consistent with the driving force that operates during mass transfer from the solid to the solvent and is closely linked to the concentration gradient. However, the solvent-to-solid ratio affects diffusivity to the point of establishing equilibrium. Also, the solute solubility is influenced by the variation in the activity coefficient, which changes with the variation in the interactions of the compounds with the solvent and the composition of the solution (Tan *et al*., 2011; Belwal *et al*., 2016).

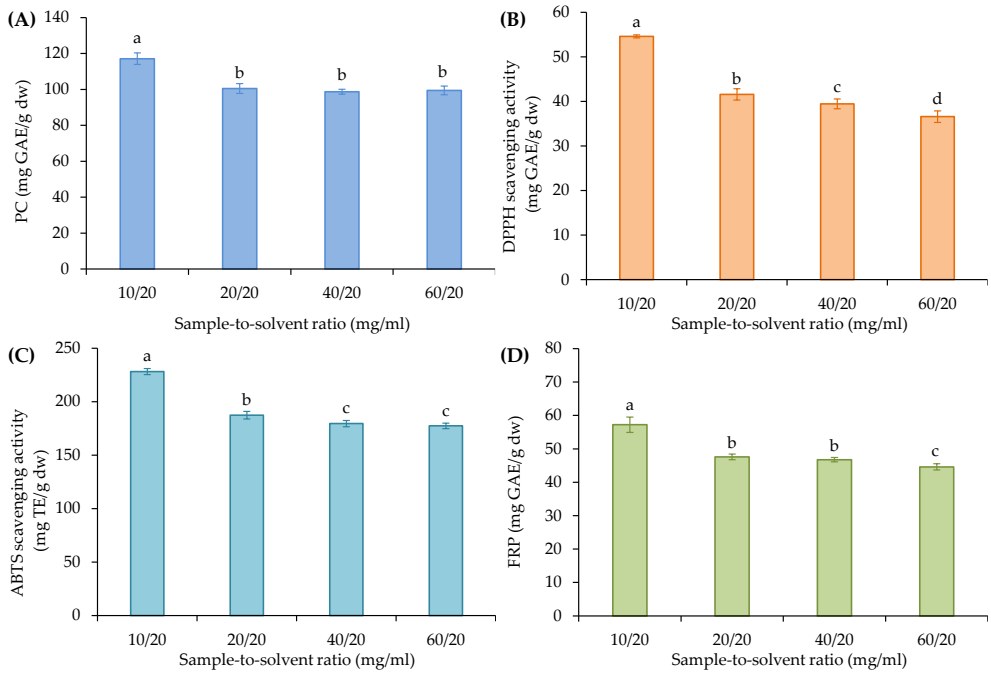

Figure 4. Effect of sample-to-solvent ratio on polyphenols recovery (**A**), DPPH (**B**) and ABTS (**C**) radical scavenging activities, and ferric reducing power (**D**) of date seed. The results with different letters are statistically different (ANOVA, LSD-test, $P \leq 0.05$) with a>b>c>d.

Torres-León *et al*. (2017) obtained higher antioxidant capacity from mango seed by using a sample-to-solvent ratio of 1:60 (g/ml). Vural *et al*. (2018) found that phenolic recovery was very dependent on the sample-to-solvent ratio. The authors established that a ratio of 1:30 (g/ml) was the most adequate for extracting the highest phenolic amount from grape seeds.

Extraction time

The contact duration of the sample with the solvent is another major parameter influencing the extraction process. The determination of this parameter not only

minimizes the time required for extraction but also reduces the energy loss. Time is a crucial factor in bioactive compounds recovery due to its implication in concentration equilibrium during extraction (Spigno et al., 2007). As illustrated in Figure 5, the obtained results showed that extraction time significantly influenced the phenolic content of date seed powder. PC, FRP, and DPPH, and ABTS radical scavenging capacities increased significantly with the increase of agitation time from 15 min until 45 min, when a maximum of 128.65 mg GAE/g, 61.90 mg GAE/g, 64.27 mg GAE/g, and 244.91 mg TE/g were recorded, respectively, and then stabilized until 60 min.

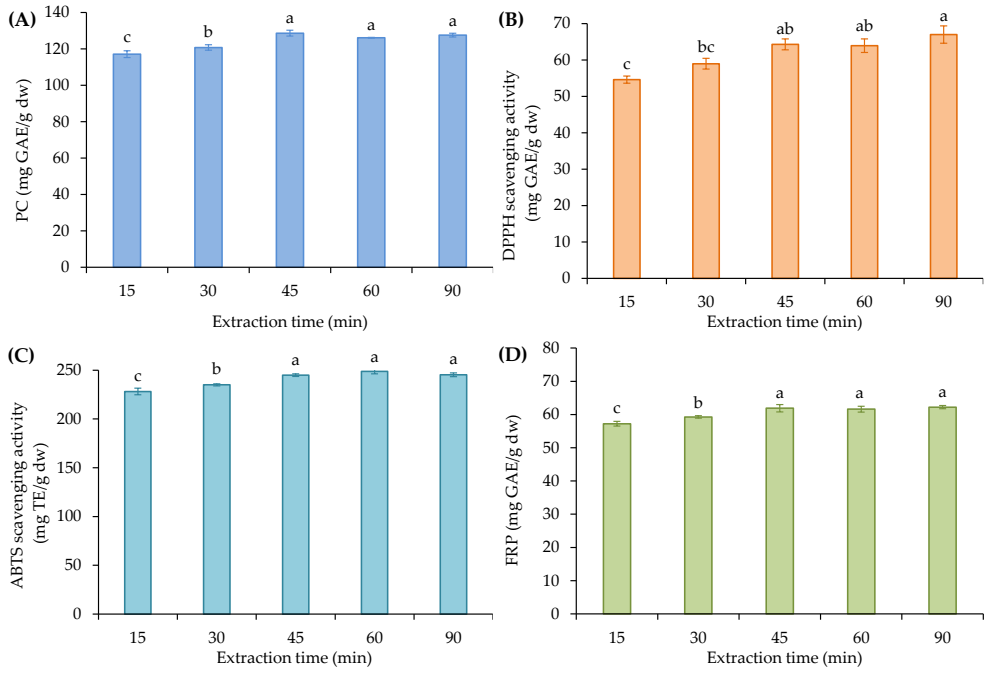

Figure 5. Effect of extraction time on polyphenols recovery (**A**), DPPH (**B**) and ABTS (**C**) radical scavenging activities, and ferric reducing power (**D**) of date seed. The results with different letters are statistically different (ANOVA, LSD-test, $P \leq 0.05$) with a>b>c>d.

A similar trend was shown by Santana *et al*. (2017) in the extraction of phenolic compounds from yellow passion fruit seeds. Such a phenomenon could be explained by Fick's 2nd law of diffusion, which dictates that an ultimate equilibrium of concentrations between the sample and the solvent is achieved after a given time in the extraction process (Pinelo *et al*., 2006; Tan *et al*., 2013).

Consequently, longer extraction times did not always result in more effectiveness. Furthermore, exposure to light and air (oxygen), particularly at high temperatures, during extended extraction may result in phenolic oxidation or degradation. On the other hand, the increased extraction time is uneconomical and time-consuming from an industrial point of view, potentially increasing the loss of solvent by evaporation,

which directly negatively influences the sample-to-solvent ratio of extraction. Thus, an extraction time of 45 min was selected as the optimum due to practical and economic considerations, despite the higher yield of PC (Tan *et al*., 2013; Lai *et al*., 2014).

Enzyme inhibitory activity

Polyphenols from date seeds are well known for their potential to prevent chronic diseases and have been proposed as possible therapeutic agents (Hilary *et al*., 2021). The potential of the date seed extract of the Ourous cultivar to inhibit three enzymes (AChE, TYR, and α-GLU) involved in metabolic and neurodegenerative disorders was assessed, and the results are depicted in Table 1.

Acetylcholinesterase is a cholinesterase that involves in the regulation of cerebral acetylcholine levels. However, the accumulation of malignant amyloid plaques in the brain tissue of patients with Alzheimer's disease has been shown to be associated with an increase in cholinesterase levels. Therefore, AChE inhibitors should be useful agents to increase cholinergic activity and thus prevent the development of neurodegenerative diseases (Dundar *et al*., 2019). Moreover, it is noteworthy that Ourous seed extract showed moderate inhibitory activity against acetylcholinesterase. However, different results were found in the study of Sekeroglu *et al*. (2012) who indicated that date seed extracts at 300 µg/ml exhibited a good inhibition against AChE (52.96%).

Tyrosinase, a multifunctional copper-containing oxidase, has been linked to skin disorders like hyper-pigmentation. Because of the critical role of tyrosinase in browning processes and melanogenesis, research is being carried out to identify tyrosinase inhibitors that are both natural and non-toxic, particularly for food (as antibrowning compounds), cosmetic, and dermatological (as depigmentation agents) applications (Zolghadri *et al*., 2019). The findings revealed that the date seed extract exhibited high inhibition of tyrosinase (77.34%). In the search for efficient tyrosinase inhibitors from natural products, many phenolic compounds are being isolated from various natural sources and tested for their ability to inhibit tyrosinase. However, eight flavonoids isolated from *Juniperus communis* fruits have been described as potential tyrosinase inhibitors (Jegal *et al*., 2016).

Diabetes is one of the world's major public health epidemics. It is a metabolic disorder of multiple aetiology, defined by chronic hyperglycemia with changes in the metabolism of proteins, lipids, and carbohydrates as a result of defective insulin secretion, insulin action, or both. One therapeutic approach to treating diabetes is to reduce postprandial hyperglycemia. This is done by delaying and reducing glucose digestion and absorption by preventing the activity of carbohydrate enzymes, including α-glucosidase and α-amylase. Inhibition of these enzymes delays carbohydrate digestion, leading to a reduction in the rate of glucose absorption (Kumar *et al*., 2012; Ganesan and Xu, 2019). As shown in the results illustrated in Table 1, the acetonic date extract of the Ourous cultivar presented a strong α-glucosidase inhibition activity at 12.5 µg/ml dry extract with a value of 70.74%. Previous studies on date seeds conducted by Thouri *et al*. (2017) and Khan *et al*. (2016) showed a lower inhibitory effect against α-glucosidase. Furthermore, the

study of Shakoor *et al*. (2020) found that date seed extract (DSE) of Khalas cultivar exhibited good inhibition activity against α-amylase and α-glucosidase enzymes, and the maximum inhibition activity was observed at 400 mg/mL and 900 mg/mL of DSE, respectively.

Table 1. Inhibitory activity of the acetonic extract from the date palm by-product of Ourous cultivar against acetyl-cholinesterase (AChE), tyrosinase (TYR), and α-glucosidase (α-GLU).

	AChE	**TYR**	**α-GLU**
Inhibition (%)	23.72 ± 1.71	77.34 ± 2.23	70.74 ± 0.45
Extract concentration in the well (µg/ml)	125	125	12.5
	Galantamine	***Kojic acid***	***Acarbose***
Reference inhibitors (%)	94.20 ± 0.13	88.91 ± 1.60	71.59 ± 3.02
Reference concentration in the well (µg/ml)	10	20	936

Conclusions

Overall, this work shows that the date palm by-product is a good natural source of antioxidants. The recovery of these bioactive compounds represents a crucial step in the valorization of date seeds. Optimization of extraction conditions represents a promising tool to recover high added-value compounds from such seeds. The input parameters (solvent type and its concentration, sample-to-solvent ratio, and extraction time) displayed a high influence ($P \leq 0.05$) on the recovery effectiveness of the desired bioactive compounds.

In this study, the optimized date seed extract showed promising effects on metabolic and neurological disorders. Strong inhibition efficiency of *Phoenix dactylifera* seed extract against α-glucosidase activity provides significant potential use as an anti-hyperglycemic agent. Furthermore, more research based on toxicology limits as well as in vivo and clinical investigations are required to confirm these extracts' potential as food matrices with various biological properties and potential health benefits.

It is important to highlight that the antioxidant performance and healthy properties open the possibility of using these extracts in multiple fields, from the medicine and/or pharmaceutical industry to the agri-food industry. This strategy enables the sustainable valorization of date palm thus contributing to the circular economy.

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