Date (*Phoenix dactylifera* var. Khalas) Seed Extracts Rich in Bioactive Compounds and Antioxidant Activities: Potential Preventive Effects Against Atherosclerosis and Lipid Oxidation in Model Systems

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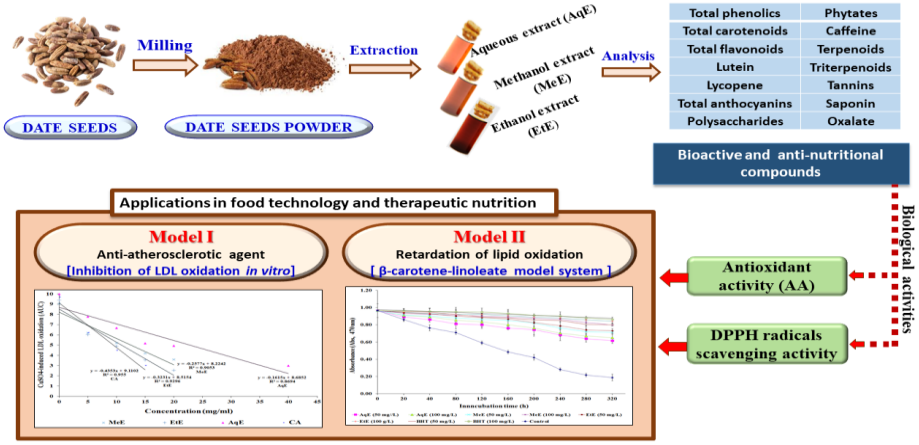
ABSTRACT

Date seed extracts (DSE) derived from ethanol, methanol, and water were analyzed for their content of bioactive compounds, antioxidant properties, and their potential roles in preventing atherosclerosis and lipid oxidation in model systems. The ethanolic extract (EtE) was found to contain the highest concentrations of bioactive compounds, including total phenolics, total flavonoids, lycopene, terpenoids, tannins, kaempferol, and phytates. In contrast, the methanolic extract (MeE) contained total carotenoids and polysaccharides, while the aquatic extract (AqE) was noted for compounds such as lutein, total anthocyanins, triterpenoids, saponin, oxalate, and caffeine. Additionally, all DSE exhibited significant antioxidant and radical-scavenging activities. In the DPPT assay, the IC50 values for AqE, EtE, and MeE were determined to be 17.63 ± 0.24, 12.10 ± 0.20, and 14.09 ± 0.13 µg/mL, respectively, in comparison to the BHT standard, which was 9.25 ± 0.19 µg/mL. Consequently, the free radical scavenging activity (FRSA) of the DSE and the standard was ranked as follows: standard (BHT) > EtE > MeE > AqE. Both 50 and 100 ppm concentrations of all DSE demonstrated the capability to inhibit lipid oxidation in both β-carotene linoleate and low-density lipoprotein (LDL) oxidation model systems (Anti-atherosclerotic effect). Therefore, it can be inferred that DSE may serve as a viable alternative source of natural antioxidants. Furthermore, DSE may serve as a potential drug in the prevention of atherosclerosis by blocking LDL oxidation.

Keywords: chemical composition, extractive value, antioxidant, DPPH, scavenging, Anti-atherosclerotic effect.

INTRODUCTION

The date palm (*Phoenix dactylifera* L.), a member of the *Arecaceae* family, is a flowering plant species widely cultivated for its edible sweet fruit, known as dates. This plant thrives primarily in tropical and subtropical regions, particularly in the Middle East, North Africa, Australia, South Asia, and California (Qadir *et al.*, 2020). Nearly 2,000 varieties of dates exist globally, with many harvested during an eight-month growing season each year. Since the 20th century, the global production and economic value of dates have seen a significant increase, reaching over US $14 billion in 2020, with a production volume of approximately 9.5 million tons (FAOSTAT, 2020). As such, date palm cultivation plays a crucial role in the economic and social landscapes of date-producing nations (Mrabet *et al.*, 2020 and Ghafoor *et al.*, 2022).



Graphical Abstract

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Beyond their consumption as whole fruits, dates are also processed into various value-added products, including date paste, syrup, vinegar, powder, confectionery, sweets, and bakery goods (Yousif & Alghamdi, 1999 and Achour *et al.,* 2022). The processing of dates generates substantial quantities of date seeds as by-products, which account for 10 to 15% of the fruit's total weight, depending on the variety, quality, and grade (Hussein *et al.,* 1998; Najjar *et al.,* 2020 and Kamal *et al.,* 2022). These seeds are often discarded or repurposed primarily as animal feed for cattle, sheep, camels, and poultry (Al-Farsi & Lee, 2008 and Qadir *et al.,* 2020). However, date seeds are rich in various bioactive compounds with significant biological and pharmacological properties, including antioxidant, anti-inflammatory, antidiabetic, antibacterial, and antiviral activities (Ataei *et al.,* 2020; Saryono *et al.,* 2020 and Moslemi *et al.,* 2022). Due to these health benefits, date seeds hold great potential as a nutritional and therapeutic agent for treating chronic diseases (Hilary *et al.,* 2020).

Lipid oxidation is a detrimental process that occurs when unsaturated fatty acids interact with oxygen in a free radical chain reaction. This process results in the formation of fatty acyl hydroperoxides, as well as non-volatile and various volatile degradation products (Benjakul *et al.,* 2013). This oxidative process can transpire at multiple phases of food production, encompassing harvesting, processing, and storage, which can result in the emergence of off-flavors, depletion of essential fatty acids, loss of fat-soluble vitamins, and reduction of other bioactive constituents, as well as the generation of potentially detrimental compounds. Such alterations have the potential to make lipids or lipid-rich foods unsuitable for human consumption (Shahidi and Zhong, 2010).

Synthetic antioxidants like butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG), and tert-butylhydroquinone (TBHQ) are commonly used to control lipid oxidation due to their high efficacy. However, growing consumer concerns about the safety and potential health risks associated with these chemical additives have led to a shift towards reducing their use (Chastain *et al.,* 1982 and Chen *et al.,* 1984). In response, there is increasing demand for natural and "label-friendly" food alternatives (McClements and Decker, 2000), prompting the food industry to explore natural antioxidant sources.

Considerable research has focused on natural antioxidants derived from plant extracts, which have been utilized as alternatives to synthetic antioxidants (Shi *et al.,* 2002; Benjakul *et al.,* 2013; Flores *et al.,* 2014 and Zhang *et al.,* 2014). Shams Ardekani *et al.* (2010) found that Iranian date seeds possess strong radical-scavenging properties, making them a promising source of natural antioxidants for both medicinal and commercial applications. Similarly, Maqsood *et al.* (2015) demonstrated that date seed extracts can inhibit lipid peroxidation, suggesting their potential as a natural antioxidant source.

Despite these studies, there remains a lack of comprehensive information on the optimal extraction methods and conditions that maximize the concentration of bioactive compounds in date seeds, along with the associated biological activities. Therefore, the primary objective of the current study is to investigate the impact of different extraction media on the yield of bioactive compounds from date seeds and to assess the antioxidant activities of these extracts using *in vitro* assays. Additionally, this study aims to explore the potential preventive effects of date seed extracts against atherosclerosis and lipid oxidation in model systems.

MATERIAL AND METHODS

Materials

Date seeds

Khalas date seed (*Phoenix dactylifera* L.) was kindly obtained by special arrangement with some date factories in Siwa Oasis, Matrouh G., Egypt. The date seed samples were verified by the date's experts in the Faculty of Agriculture, Minoufiya Uni., Shebin El-Kom, Egypt.

Chemicals

Sigma Chemical Co., St. Louis, MO provided standards for bioactive compounds such as catechin (CA), gallic acid (GA), lycopene, linalool, α-tocopherol, ursolic acid, butylated hydroxytoluene (BHT), DPPH (2,2-diphenyl-1-picrylhydrazyl), $CuSO_4$, dimethyl sulfoxide (DMSO), and standard vitamins. All additional chemicals, reagents, and solvents, unless otherwise mentioned, were analytical grade and obtained from El-Ghomhorya Co., for Trading Drugs, Chemicals, and Medical Instruments in Cairo, Egypt.

Instruments

In the study, absorbance for various assays was measured using a UV-160A spectrophotometer from Shimadzu Corporation, Kyoto, Japan. Additionally, mineral content was determined using a Perkin-Elmer atomic absorption spectrophotometer, Model 2380, from Waltham, MA, USA. Micro-Kjeldahl semiautomatic apparatus, Velp company, Italy was used for total nitrogen determination. Soxhlet semiautomatic apparatus Velp company, Italy, was

used for crude fat determination. Homogenizer apparatus Velp Company, Italy, was used for β-Carotene-linoleate model system preparation.

Biological model for biological assay

Adult male albino rats (165±10 g) were obtained from the Laboratory Animal Colony at the Vaccine and Immunity Organization in Cairo, Egypt. The rats were housed in a controlled environment that adhered to the guidelines of the National Research Council's Institute of Laboratory Animal Resources, Commission on Life Sci (NRC, 1996). A standard diet (BD) formulated according to AIN specifications (1993) was provided to the rats.

Methods

Preparation of Khalas date seeds powder

Khalas date seed (*Phoenix dactylifera* L.) specimens underwent manual cleaning and sorting, followed by washing with water to eliminate all adhering components. The date seeds were initially washed and then dried in a hot air oven at 80°C for three hours (Horizontal Forced Air Drier, Proctor and Schwartz Inc., Philadelphia, PA). Following drying, the seeds were pulverized into a fine powder using (a high-speed mixer Moulinex, ElAraby Co., Benha, Egypt). The powdered material that passed through a 40-mesh sieve was collected, packaged in polyethylene bags, and refrigerated at 4°C for subsequent analysis.

Preparation of Khalas date seed extracts

Twenty grams of dried Khalas date seed powder were extracted using 80% aqueous solutions of methanol and ethanol and petroleum ether, diethyl ether, hexane, chloroform, and water (180 ml) on an orbital shaker (Unimax 1010, Heidolph Instruments GmbH & Co. KG, Germany). The extraction was performed for 120 minutes at 80°C for the organic solvents and 120 minutes at 100°C for water. Resultant mixes were then filtered with Whatman No. 5 filter paper and a Buchner funnel. The remaining solvents were evaporated under decreased pressure at 60°C for water extracts and 40°C for organic solvent extracts with a rotary evaporator (Laborata 4000; Heidolph Instruments GmbH & Co. KG, Germany). Finished extracts were kept at 4°C until further use.

Chemical analysis of Khalas date seed powder

Samples of Khalas date seed powder were subjected to an analysis of their chemical composition, encompassing moisture, protein (total nitrogen multiplied by 6.25, utilizing the micro-Kjeldahl method with semi-automatic apparatus from Velp Company, Italy), fat (extracted using a Soxhlet apparatus with petroleum ether as the solvent, Velp Company, Italy), fiber, ash, and dietary fiber. These analyses were conducted following the methods outlined by the AOAC (1995). The carbohydrate content was determined by difference, using the formula: Carbohydrates (%) = 100 - (% protein + % moisture + % fat + % ash + % fiber).

Bioactive compounds determination

The total phenolic content in Khalas date seed powder extracts was quantified using the Folin-Ciocalteu reagent, following the methods of Singleton & Rossi (1965) and Wolfe *et al*. (2003). The findings are represented as gallic acid equivalents (GAE). Total carotenoid concentration in the 80% acetone extract was assessed following the technique outlined by Lichtenthaler (1987), expressed in µg of carotenoids per gram of dry extract. Lycopene levels were quantified according to the protocol specified by Anthon and Barrett (2007), with results noted as µg per gram of dry extract. The colorimetric technique published by Zhishen *et al.* (1999) was used to quantify total flavonoid content. Results were represented as catechin equivalents (CAE) (standard curve equation: $y = 0.0003x - 0.0117$, $r^2 = 0.9827$), in milligrams of CA per gram of dried extract. Total polysaccharides were extracted and measured using Vazirian *et al.* (2014) technique, with the findings reported as milligrams of starch equivalents per gram of dry weight (dw). Total terpenoids were extracted and quantified using the method described by Ghorai *et al.* (2012), with linalool as the standard. The results were expressed as milligrams of linalool equivalents per gram of dry weight. Total triterpenoids were extracted and measured following the protocol of Schneider *et al.* (2009), using ursolic acid as the standard. The results were reported as milligrams of ursolic acid per 100 grams of dry extract. Saponin content was determined using the method outlined by Fenwick and Oakenfull (1981), with gallic acid serving as the standard for the calibration curve, from which the saponin content of the samples was calculated. Lutein extraction from molokhia leaves was performed according to the methods of Bangbang *et al.* (2022), and results were expressed in micrograms per 100 grams. Total anthocyanin content was assessed using the procedure described by Sharif *et al.* (2010), with results expressed as milligrams of cyanidin-3,5-diglucoside per 100 grams. Oxalate levels were measured according to the method of Oke (1966), while kaempferol content was quantified using the approach outlined by Fouda *et al.* (2019). Tannins were determined using the method of Van-Burden and Robinson (1981), with gallic acid as the standard for the calibration curve to estimate tannin content. Finally, phytate levels were determined using the colorimetric method of Latta and Eskin (1980).

Antioxidant activities

Antioxidant activity (AA)

The antioxidant activity (AA) of Khalas date seed powder extracts, along with standards such as α-tocopherol and BHT, was tested using the β-carotene bleaching (BCB) assay, using a modified version of Marco's technique established in 1968. In a typical test, 1 mL of β-carotene solution (0.2 mg/mL in chloroform) was put into 50 mL round-bottom flasks with 0.02 mL of linoleic acid and 0.2 mL of Tween 20. The mixtures were then treated with 0.2 mL of either 80% methanol (control), plant extract, or standard. After evaporating the solvent under vacuum at room temperature, 50 mL of oxygenated distilled water was added and stirred to generate a liposome solution. The samples were treated to thermal auto-oxidation at 50°C for 2 hours. A spectrophotometer (Beckman DU-50) was used to measure absorbance at 470 nm at 10-minute intervals. The rate of β-carotene bleaching was calculated using a linear regression model over time. Each sample was tested in triplicate, with different doses of BHT and α-tocopherol in 80% methanol as controls.

Antioxidant activity was calculated using four different methods as follows: 1) The absorbance was plotted against time, and the absolute value of the slope was expressed as the antioxidant value (AOX) - (Al-Saikhan *et al.,* 1995). 2) Antioxidant activity (AA) was also calculated as a percentage inhibition relative to the control using the equation (Al-Saikhan *et al.,* 1995): AA = ($R_{control}$ - R_{sample}) / $R_{control}$ x 100, where $R_{control}$ and R_{sample} represent the bleaching rates of β-carotene in the absence and presence of the plant extract, respectively. 3) The oxidation rate ratio (ORR) was determined according to Marinova *et al.* (1994) using the equation ORR = R_{sample} / $R_{control}$. 4) The antioxidant activity coefficient (AAC) was calculated as described by Mallett *et al.* (1994) using the formula AAC = (Abs_{S120}-Abs_{C120})/(Abs_{C0}-Abs_{C120}) x 100, where Abs_{S120} is the absorbance of the antioxidant mixture at 120 minutes, Abs_{C120} is the absorbance of the control at 120 minutes, and Abs_{C0} is the absorbance of the control at zero time.

DPPH radical scavenging assay

The antioxidant activity of Khalas date seed powder extracts was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay, as described by Desmarchelier *et al.* (1997). A solution was prepared by mixing 2.4 mL of a 0.1 mM DPPH solution in methanol with 1.6 mL of the date seed extract at varying concentrations (12.5-150 µg/mL). After vigorous mixing and incubation in the dark at room temperature for 30 minutes, the absorbance of the solution was measured at 517 nm using a spectrophotometer (UV-160A; Shimadzu Corporation, Kyoto, Japan). Butylated hydroxytoluene (BHT) served as a positive control. The percentage of DPPH radical scavenging activity was calculated using the following formula:

DPPH radical scavenging activity (%)= A0(A0 −A1)×100

Where "A0" represents the absorbance of the control, and "A1" represents the absorbance of the sample or BHT. The inhibition percentage was plotted against concentration, and the IC_{50} value was derived from the resulting graph.

Anti-atherosclerotic effect of DSE *in vitro* [Inhibition of low-density lipoprotein (LDL) oxidation]

The inhibition of LDL oxidation (anti-atherosclerotic effect) for Khalas date seed powder extracts was assessed in vitro using the method described by Princen *et al.* (1992). Serum was collected from adult male Sprague Dawley rats and diluted to 0.6% concentration using phosphate buffer (50 mM, pH 7.4). To each 5.0 mL aliquot of diluted serum, 10 µL of DMSO or 10 µL of DMSO containing various concentrations of Khalas date seed powder extracts was added. The reaction was initiated by adding 20 µL of a 2.5 mM $CuSO_4$ solution. Absorbance at 234 nm was measured every 20 minutes for a total duration of 140 minutes at room temperature. The final results were determined by calculating the net area under the curve.

***β*-Carotene-linoleate model system**

The antioxidant capacity within a *β*-carotene-linoleate model system was assessed following the procedure established by Chandrasekara and Shahidi (2010). In summary, an oil-in-water emulsion of *β*-carotene was created by dissolving 1 mg of β-carotene in 10 mL of chloroform. A 0.2 mL aliquot of this β-carotene solution was combined with 20 mg of linoleic acid and 200 mg of Tween 40, and the chloroform was subsequently eliminated under a nitrogen gas flow. Following this, 50 mL of oxygenated distilled water was incorporated into the mixture, which was then homogenized using a Velp Company homogenizer (Italy) at a speed of 13,000 g for one minute. The resultant emulsion was stored at ambient temperature (24–26 °C) in a dark environment. Date seed extracts, dissolving in 50% (v/v) ethanol at concentrations of 500 and 1000 ppm (500 µL), were mixed with 4.5 mL of the prepared emulsion to achieve final concentrations of 50 and 100 ppm. These mixtures were incubated at 50°C in the dark. At various time points (0, 20, 40, 80, 120, 160, 200, 240, 280, 320 minutes), samples were taken randomly to measure absorbance (Abs) at 470 nm. The control and reference/standard were prepared similarly, with distilled water and BHT (50 and 100 ppm) substituted for the samples. A lesser reduction

in Abs indicated a greater capacity to inhibit the oxidation process within the system.

Statistical Analysis

All experiments were conducted in triplicate. The data underwent analysis of variance (ANOVA), and mean comparisons were executed utilizing Duncan's multiple range test (Steel and Torrie, 1980). Statistical evaluations were performed using the Statistical Package for the Social Sciences (SPSS for Windows: SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

Proximate composition of Khalas date seeds powder (DSP)

The data presented in Table (1) illustrates the proximate composition of Khalas date seed powder (DSP). It is evident from this data that carbohydrates (57.75 ± 1.80) constitute the predominant component, followed by crude fiber (17.21 ± 0.99), crude fat (9.11 ± 0.97), total protein (6.09 ± 0.21), and ash (1.52 ± 0.14). These findings are partially consistent with observations from various studies (Habib & Ibrahim, 2009; Ali *et al.*, 2015 and Oladipupo *et al.*, 2021). Furthermore, Al-Farsi *et al.* (2007) reported that the proximate composition of Omani date seed powder varied, showing protein levels ranging from 2.3–6.4%, fat from 5.0–13.2%, ash from 0.9–1.8%, and dietary fiber from 22.5–80.2%. Additionally, moisture content is a critical parameter influencing the shelf life of food products. The moisture content results indicate that DSP can be stored for an extended duration, rendering it less prone to microbial spoilage and enzymatic reactions. Concerning carbohydrates, Al Juhaimi *et al.* (2012) identified soluble sugars in DSP carbohydrates, including glucose, fructose, stachyose, sucrose, and galactose.

This specific sugar composition in DSP has been reported to assist in regulating blood sugar levels in diabetic individuals, alongside its beneficial insulin-producing characteristics (Anbarshahi, 2022). In this regard, El Fouhil *et al.* (2013) observed the potential hypoglycemic effects of DSP by noting an increased serum c-peptide level in diabetic rats treated with date seed extract and insulin, as opposed to those treated solely with insulin. This observation supports the notion of enhanced endogenous insulin secretion through compensatory β-cell hypertrophy, as determined by immunohistochemistry analysis. Moreover, DSP is distinguished by its high fiber content, which plays a vital nutritional role by adding bulk to stool and facilitating movement through the digestive system.

The findings of this study, in conjunction with previous research, suggest that variations in the proximate composition of DSP compared to other studies may arise from several factors, including the date type/species, developmental stage, sample origin, and agricultural management practices (Al-Farsi *et al.*, 2007; Ali *et al.*, 2015; Khalid *et al.*, 2016 and Oladipupo *et al.*, 2021). In conclusion, the results of the current study indicate that DSP serves as a valuable source of diverse nutrients for human consumption, encompassing carbohydrates, fiber, protein, and ash.

Extractive value of Khalas date seeds powder ((DSP)

The extractive value of DSP was determined by successive extraction in different organic solvents and water using a Soxhlet's apparatus which is illustrated in Table (2). Such data indicated that the extractive value of DSP in some organic solvents (methanol and ethanol) and water was higher than in the rest of the tested organic solvents (petroleum ether, hexane and chloroform). Such data confirmed that DSP components were found in both lipophilic and hydrophilic phases. Such findings align with the established principle of "like dissolves like," which signifies that substances sharing analogous chemical characteristics regarding polarity will dissolve in one another. In particular, non-polar solvents typically dissolve non-polar solutes, whereas polar solvents are more effective at dissolving polar solutes; conversely, non-polar and polar substances are immiscible, meaning they do not combine. Consequently, the selection of an appropriate solvent system is a critical step in enhancing the extraction of bioactive compounds from DSP extracts. As noted by Sulaiman *et al.* (2011), variations in the polarities of extraction media can significantly affect the solubility of chemical constituents within the sample and its overall extraction yield. Various studies have indicated that extractive values serve as a reliable indicator for assessing the quality of drugs or food products, and any fluctuations in the content of chemical constituents may lead to alterations in these extractive values (El-Wazeer, 2011; Hegazy, 2014; Abd El-Khader, 2018; Elhassaneen *et al.*, 2018; El-Soukoty, 2021 and Elhassaneen *et al.*, 2022). Therefore, extractive values are instrumental in identifying adulteration and serve as a measure of the purity of drugs or food products.

Table 1. Proximate composition of Khalas date seeds powder (DSP)

Component (g/100g FW)	Range	Mean ±SD
Moisture	7.92 - 9.02	8.32 ± 0.43
Dry matter	90.31 - 91.88	91.68 ± 0.72
Total protein	5.91 - 6.43	6.09± 0.21
Crude fat	8.16 - 10.14	9.11 ± 0.97
Crude Fiber	16.10 - 18.30	17.21 ± 0.99
Ash	1.30 - 1.68	1.52 ± 0.14
Carbohydrate	55.92 - 59.47	57.75 ± 1.80

Each value represents the mean of three replicates ±SD., (FW, fresh weight).

Table 2. Extractive value of Khalas date seed samples using different organic solvents and water

Extraction medium	Range	Mean extract (%) ±SD
Methanol (80%)	5.788 - 6.001	5.879 ± 0.080 [ab]
Ethanol (80%)	5.948 - 6.087	6.017 ± 0.075[a]
Petroleum ether	3.750 - 3.827	3.791 ± 0.037 [c]
Hexane	3.617 - 3.605	3.641 ± 0.019 [d]
Chloroform	3.022 - 3.070	3.048 ± 0.020[d]
Water (Tap water)	5.046 – 4.230	5.129 ± 0.091 [b]

Each value is the average of four replicates ± SD. Values with different superscript letters in the same column are significantly different at $p \leq 0.05$.

Bioactive and antinutritional compounds in Khalas date seed extracts

Bioactive and antinutritional compounds in date seed extracts are shown in Table (3). Total phenolics were reported as the most abundant compound in all extracts while phytates recorded the small quantity. Also, bioactive and anti-nutritional compounds were recorded in different quantities that differed depending on the type of extract. The most abundant compounds including total phenolics, total flavonoids, lycopene, terpenoids, tannins, kaempferol and phytates were recorded in ethanolic extract. Other compounds i.e. total carotenoids and polysaccharides were found in the methanolic extract while the rest of the compounds (lutein, total anthocyanin, triterpenoids, saponin, oxalate and caffeine) were recorded in the aquatic extract. In similar study, Shams Ardekani *et al.* (2010) determined the total phenolic components of 14 distinct kinds of date palm seed extracts using 5 solvents [water, methanol, methanol (50%), DMSO, and water: methanol: acetone: formic acid (20:40:40:0.1)]. The "Zahedi" variety's DMSO extract had the greatest total phenolic content (3541 mg/100 g dry plant) among the 14 cultivars and 5 solvents tested. The variation in the bioactive and antinutritional compounds content determined were noticed in the date seeds extracts depending on many factors including the determination methods parameters i.e. extraction methods and extraction medium etc., (Al-Farsi *et al.*, 2005; Khanavi *et al.,* 2009 and Shams Ardekani *et al.,* 2010). The findings of the current study affirm that the presence of bioactive compounds within various extracts is influenced by the polarity of those compounds, aligning with the established principle of "like dissolves like." This principle indicates that substances with comparable polar chemical properties are capable of dissolving in one another. Specifically, non-polar solvents are effective in dissolving non-polar solutes, while polar solvents dissolve polar solutes; conversely, non-polar and polar substances are immiscible, meaning they do not combine. In this regard, Sulaiman *et al.* (2011) highlighted that variations in the polarities of extraction media can impact the solubility of the chemical constituents present in a sample. Overall, numerous prior investigations, including this one, have established that bioactive compounds such as phenolics, flavonoids, and lycopene identified in palm seed extracts are crucial in preventing and/or managing various diseases, including diabetes, atherosclerosis, obesity, cancer, bone disorders, and aging (Elhassaneen *et al.,* 2016a, 2019). The enhancement of immune function through polysaccharides is believed to be a primary mechanism underlying their antitumor effects (Wasser, 2005). Triterpenoids can also serve as precursors for the development of more potent bioactive derivatives, including experimental antitumor agents. Oxalates are produced naturally within the human body as metabolic waste (Masao, 2008). It has been reported that tannins contribute to reductions in feed intake, feed efficiency, growth rate, net metabolizable energy, and protein digestibility in experimental subjects (Chung *et al.,* 1998). Kaempferol, a flavonoid phytoestrogen, is known to

Table 3. Bioactive and antinutritional compounds content of Khalas date seed extracts (DSE)

Compound	Methanol extract	Ethanol extract	Aquatic extract
Total phenolics (mg gallic acid. g^{-1})	127.21 ± 3.39 ^{b}	144.42 ± 10.11^{a}	83.68 ± 2.76 ^{c}
Total carotenoids (mg catechin. g^{-1})	0.675 ± 0.16 ^{a}	0.664 ± 0.13 ^{a}	0.565 ± 0.1^{b}
Total flavonoids (mg RE. g^{-1})	29.50 ± 1.66 ^{b}	51.83 ± 2.09 ^{a}	4.84 ± 0.04 ^{c}
Lutein (µg. g^{-1})	4.98 ± 0.31 ^{b}	3.63 ± 0.99 ^{b}	13.52 ± 0.51 ^{a}
Lycopene (µg. g^{-1})	2.19 ± 0.09 ^{a}	2.89 ± 0.06 ^{a}	0.53 ± 0.04 ^{b}
Total anthocyanins (mg cyanidin-3,5-diglucoside. g^{-1})	1.83 ± 0.11 ^{b}	1.78 ± 0.10 ^{b}	5.77 ± 0.19 ^{a}
Polysaccharides (mg starch. g^{-1})	117.45 ± 2.10 ^{a}	108.43 ± 1.54 ^{b}	49.76 ± 2.17 ^{c}
Terpenoids (mg linalool. g^{-1})	16.83 ± 2.15 ^{b}	23.53 ± 0.99 ^{a}	5.06 ± 0.59 ^{c}
Triterpenoids (mg ursolic acid.g^{-1})	12.16 ± 1.04 ^{b}	11.91 ± 0.99 ^{b}	15.34 ± 2.05 ^{a}
Tannins (mg catechine equivalent.g^{-1})	19.67 ± 0.99 ^{b}	25.33 ± 3.06 ^{a}	6.17 ± 0.45 ^{c}
Saponin (mg . g^{-1})	21.87 ± 1.21 ^{b}	21.58 ± 1.90 ^{b}	36.53 ± 0.86 ^{a}
Oxalate (mg . g^{-1})	17.99 ± 0.45 ^{a}	18.33 ± 0.87 ^{a}	19.65 ± 1.02 ^{a}
Kaempferol (mg. g^{-1})	0.651 ± 0.11 ^{a}	0.836 ± 0.10 ^{a}	0.163 ± 0.099 ^{b}
Caffeine (mg. g^{-1})	3.55 ± 0.09 ^{b}	3.36 ± 0.11 ^{b}	9.91 ± 1.01 ^{a}
Phytates (mg. g^{-1})	0.29 ± 0.05 ^{a}	0.38 ± 0.09 ^{a}	0.08 ± 0.01 ^{b}

Each value is the average of four replicates ± SD. Values with different superscript letters in the same row are significantly different at p≤ 0.05.

lower the risk of chronic diseases, particularly cancer, enhances the body's antioxidant defenses against free radicals, and modulates apoptosis, angiogenesis, inflammation, and metastasis (Allen and Yi, 2013). Saponins, a type of triterpene, have been associated with chemopreventive properties, such as lowering blood cholesterol, inhibiting the proliferation of cancer cells, and bolstering the immune system (Lewu *et al.,* 2010 and Jukanti *et al.,* 2012). Lastly, phytic acid/phytate exhibits a strong affinity for dietary trace elements, including calcium, iron, and zinc, thereby inhibiting their absorption in the small intestine; however, phytochemicals such as polyphenols and tannins can influence the binding of these minerals (Hurrell, 2003; Prom-u-thai *et al.,* 2006 and Schlemmer *et al.,* 2009).

Biological activities of Khalas date seed extracts

Antioxidant activity

The antioxidant properties of Khalas date seed extracts are detailed in Table (4). Analysis of this data indicates that Khalas date seed extracts exhibited significant variability in antioxidant activity (AA= 86.98 to 94.76%). The EtE demonstrated robust activity, likely attributable to its elevated levels of bioactive compounds, while the AqE also revealed substantial content in both AA (86.98%) and the total bioactive compounds analyzed. These findings are partially consistent with observations reported in multiple studies (Barros *et al.,* 2007 and Shams Ardekani *et al.,* 2010). Furthermore, the results of the current study align partially with those documented for various plant parts rich in bioactive compounds, as noted in DSE (Elhassaneen *et al.,* 2016b; Mashal, 2016; Sayed Ahmed, 2016; Aly *et al.,* 2017; Hallabo *et al.,* 2018; El-Nassag *et al.,* 2019; Abd Elalal *et al.,* 2021; Elhassaneen *et al.,* 2019, 2021; Gharib *et al.,* 2022 and Elhassaneen *et al.,* 2023a). All of these researchers indicated that the presence of various bioactive compounds (phenolics, carotenoids, flavonoids, lutein, lycopene, anthocyanins, polysaccharides, terpenoids, triterpenoids, and Kaempferol) found in high concentrations in DSP extracts correlates strongly with antioxidant activity. Therefore, the observed variations in antioxidant activity levels of the DSE can likely be attributed to their differing bioactive compound compositions. Also, antinutritional compounds (Saponins, triterpene and phytate) determined in DSE have been reported to pose chemopreventive roles through their antioxidant activity (Schlemmer *et al.,* 2009; Lewu *et al.,* 2010 and Jukanti *et al.,* 2012).

DPPH radical scavenging activity

Fig (1) and Table (5) display the free radical scavenging activity (FRSA) of Khalas date seed extracts compared to butylated hydroxytoluene (BHT). The results show that the ethanol extract exhibited the highest scavenging activity, followed by the methanol and aqueous extracts. Specifically, the scavenging activities of the aqueous, ethanol, and methanol extracts at a concentration of 100 µg/mL were 74.74%, 87.91%, and 82.90%, respectively, whereas BHT exhibited an activity of 94.31%. Regarding IC50 values, the aqueous, ethanol, and methanol extracts had IC50 values of 17.63 ± 0.24,

12.10 ± 0.20, and 14.09 ± 0.13 µg/mL, respectively, while BHT had an IC50 of 9.25 ± 0.19 µg/mL. The FRSA of the date seed extracts and BHT standard was ranked in the following order: BHT > ethanol extract > methanol extract > aqueous extract.

The DPPH assay is a colorimetric method used to assess the antioxidant activity of compounds. It involves the decolorization of the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical, a stable free radical with a deep purple color, upon reaction with antioxidants. This method has been widely employed to assess the antioxidant potential of various plant extracts, including date seeds (Aaby *et al.*, 2004; Laura *et al.*, 2010; Aly *et al.*, 2017; Aminzare *et al.*, 2019; El-Gamal, 2020; Abd Elalal *et al.*, 2021; Alharbi *et al.*, 2021; Elhassaneen *et al.*, 2021; Fayez, 2022 and Elhassaneen *et al.*, 2023b). The observed FRSA in date seed extracts can be attributed to the presence of a range of bioactive compounds with antioxidant properties, including phenolics, carotenoids, anthocyanins, flavonoids, lutein, lycopene, polysaccharides, terpenoids, kaempferol, saponins, and tannins. These antioxidant properties are crucial for mitigating the harmful effects of free radicals in various diseases such as obesity, diabetes, cancer, anemia, neurological disorders, pulmonary conditions, nephropathy, and cardiovascular diseases (Gharib *et al.*, 2022; Elhassaneen *et al.*, 2023a and Mahran & Elhassaneen, 2023).

Table 4. Antioxidant activity of DSE

DSE	Antioxidant value[a] AOX (A/h)	Antioxidant activity[b] AA (%)	Oxidation rate ratio[c] (ORR)	Antioxidant activity coefficient[d] (AAC)
AqE	0.080± 0.007	86.98± 2.02 [e]	0.141± 0.004	1021± 5.17
MeE	0.030± 0.004	91.05± 1.89 [c]	0.050± 0003	1131± 16.23
EtE	0.009± 0.002	94.76± 1,09 [b]	0.020± 0.001	1190± 11.89
BHT, 50 mg/L	0.074± 0.002	87.27± 1.11 [d]	0.130± 0.003	997± 14.27
BHT, 200 mg/L	0.011± 0.001	98.05± 0.99 [a]	0.017± 0.001	1193± 13.71
a-tocopherol, 50 mg/L	0.007± 0.001	98.27± 0.78 [a]	0.011± 0.001	1199± 10.56

[e] Each value reflects mean ±SD. Values with different superscript letters in the same column are significantly different at p≤ 0.05.

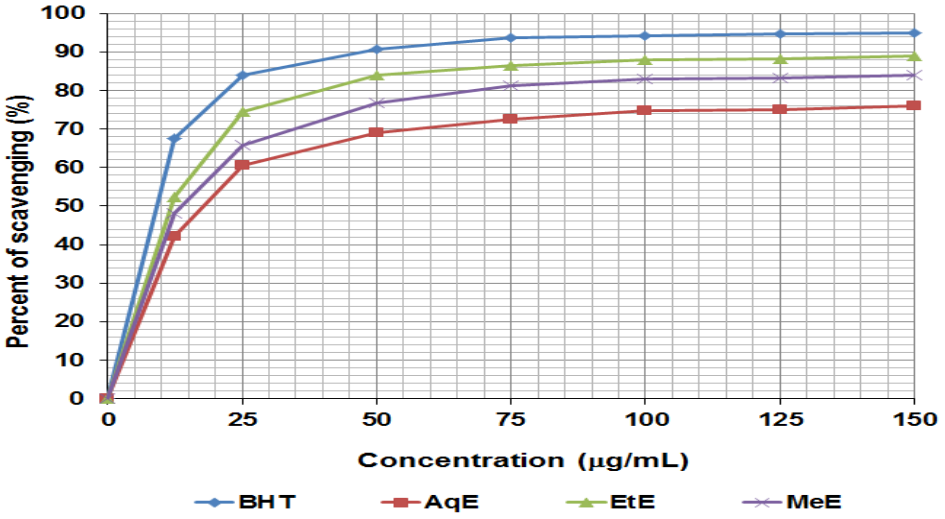

Fig. 1. DPPH radical scavenging activity (%) of Khalas date seeds powder extracts and standard (BHT)[*]

[*] Each value represents the mean value of three replicates.

Table 5. IC_{50} (DPPH) of Khalas date seeds powder extracts and BHT (Standard)

Name of sample	"BHT"	"AqE"	"EtE"	"MeE"
IC_{50} (µg/mL)	9.25 ± 0.19 [c]	17.63 ± 0.24[a]	12.10±0.20[b]	14.09 ± 0.13 [b]

Each value is the average of four replicates ± SD. Values with different superscript letters in the same row are significantly different at p≤0.05.

Applications in food technology and therapeutic nutrition

Anti-atherosclerotic effect of DSE *in vitro* [Inhibition of low-density lipoprotein (LDL) oxidation]

Fig (2) illustrates the dose-dependent inhibition, of ($CuSO_4$- induced) LDL oxidation by date seed extracts. The data reveal that date seed extracts significantly inhibit LDL oxidation induced by ($CuSO_4$), as evidenced by a reduction in conjugated diene ( ) production in a dose-dependent manner. Among the different extracts, the ethanol extract showed the highest protective effect against LDL oxidation, followed by the methanol and aqueous extracts, with the protective efficacy ranked as follows: ethanol > methanol > aqueous.

Reactive oxygen species (ROS) can damage cellular membranes, including those of the cell wall, mitochondria, and lysosomes, through lipid peroxidation of polyunsaturated fatty acids (PUFAs), initiating a chain reaction (Lien *et al.*, 2008). The present study demonstrates that date seed extracts effectively inhibit lipid peroxidation, suggesting their potential to mitigate free radicals that trigger cellular damage by disrupting the lipid peroxidation chain reactions. In vivo studies have shown that phenolic compounds, which are abundant in date seed extracts, can protect LDL from oxidation by increasing levels of reduced glutathione (GSH) and glutathione reductase (GSH-Rd) in the liver and lungs, as observed in studies by Majid *et al.* (1991), Elbasouny *et al.* (2019), El-Gamal (2020) and Elhassaneen *et al.* (2020). Additionally, this extract was found to inhibit NADPH. dependent lipid peroxidation, further suggesting its potential to protect against oxidative stress. Similarly, other studies have reported LDL oxidation inhibition by plant extracts containing bioactive compounds similar to those in date seeds (Aly *et al.*, 2017; El-Gamal, 2020; Abd Elalal *et al.*, 2021; Elhassaneen *et al.*, 2021; Fayez, 2022 and Elhassaneen *et al.*, 2023a, 2023b). LDL oxidation in the vascular endothelium is a precursor to atherosclerotic plaque formation (Poznyak *et al.*, 2021). The ability of date seed extracts to inhibit LDL oxidation may be attributed to their high content of antioxidants and free radical scavengers, such as phenolics, anthocyanins, lycopene, polysaccharides, carotenoids, and terpenoids. Consequently, the findings suggest that date seed extracts could be a promising agent for preventing atherosclerosis by inhibiting LDL oxidation.

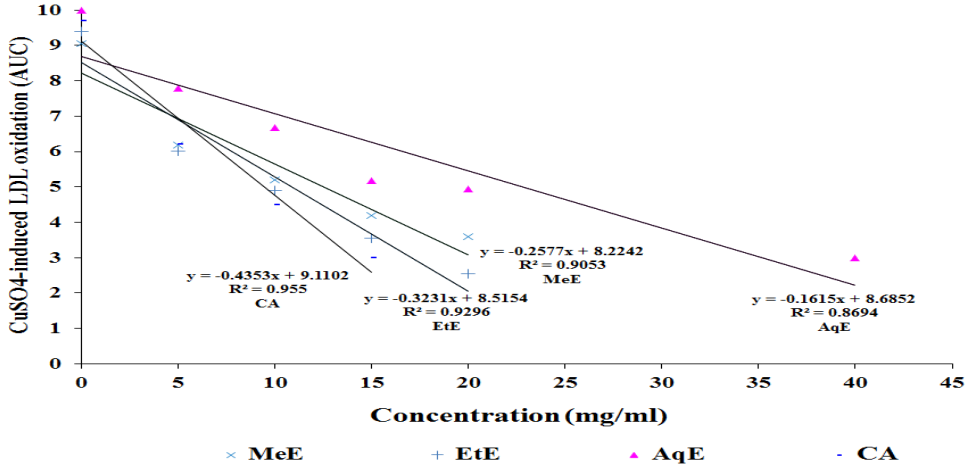

Fig. 2. Khalas date seed extracts prevent $CuSO_4$-induced LDL oxidation *in vitro* through dose-dependent inhibition

Effect of DSE on lipid oxidation of β-carotene-linoleate model system

Fig (3) depicts the impact of date seed extracts (DSE) on lipid oxidation in the β-carotene-linoleate model system, compared to a reference system with standard antioxidants (BHT) at 50 and 100 ppm. The absorbance (Abs) at 470 nm for all samples decreased over time (P≤0.05), indicating β-carotene oxidation due to free radicals generated from linoleic acid oxidation (Chandrasekara and Shahidi, 2010). This reduction in Abs reflects the effectiveness of the extracts and antioxidants in mitigating β-carotene degradation. As documented by Kittiphattanabawon *et al.* (2012), the presence of antioxidants notably mitigated β-carotene bleaching, primarily due to the free radical scavenging properties inherent to these antioxidants. The Abs values for the systems incorporating DSE and the standard (BHT) were significantly (p≤0.05) elevated compared to systems lacking extracts. No significant differences were noted in Abs among the systems containing 50 ppm of MeE and EtE, as well as 50 ppm BHT, nor between those with 100 ppm of MeE and EtE and 100 ppm BHT. Conversely, the AqE systems displayed a reduced level of activity when compared to those comprising the other extracts. The findings of the current investigation demonstrated that DSE, particularly EtE and MeE at a concentration of 100 ppm, effectively inhibited lipid oxidation within the β-carotene-linoleate model system. The DSE's capacity to impede lipid oxidation may be attributed to its bioactive compound composition and its associated ability to donate electrons and/or hydrogen atoms. Numerous prior studies have indicated that phenolics, carotenoids, flavonoids, lutein, lycopene, anthocyanins, polysaccharides, terpenoids, triterpenoids, and Kaempferol, abundantly present in DSP extracts, exhibit a strong correlation with antioxidant/scavenging activities (Barros *et al.*, 2007; Elhassaneen & Sanad, 2009; Shams Ardekani *et al.*, 2010; El-Nassag *et al.*, 2019; Elhassaneen *et al.*, 2019, 2021 and Abd Elalal *et al.*, 2021). Hence, the variations observed in antioxidant activity among the date seed extracts are likely attributable to the differing concentrations of these bioactive compounds. The disparities in lipid oxidation among the systems containing ethanol extract, methanol extract, and aqueous extract can be primarily ascribed to differences in polarity, which may have impacted the extraction efficacy of the compounds responsible for the antioxidant activities of the extracts.

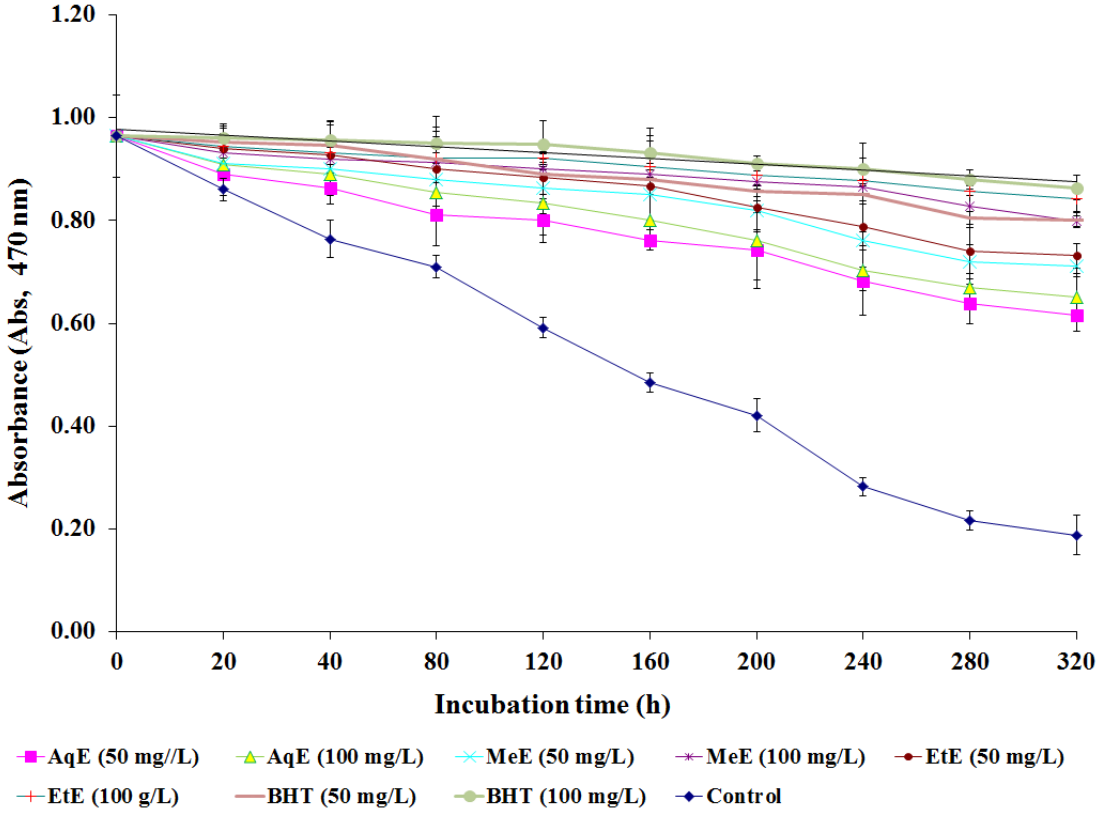

Fig. 3 Effect of adding DSEs at different doses on lipid oxidation in the β-carotene-linoleate model system. Bars represent standard deviation (n=3)

CONCLUSION

The yield, concentrations of bioactive compounds, and antioxidant activities of DSE were influenced by the choice of solvent extraction method. The application of organic solvents such as ethanol and methanol for extraction produced extracts exhibiting the highest yield, bioactive compound concentrations, and antioxidant capacities. All DSE at concentrations of 50 and 100 ppm effectively inhibited lipid oxidation in both β-carotene linoleate and low-density lipoprotein (LDL) oxidation (anti-atherosclerotic effect) model systems. Consequently, it can be inferred that DSE holds promise as an alternative source of natural antioxidants. Furthermore, DSE could be effectively utilized as a potential agent in the prevention of atherosclerosis by obstructing the LDL oxidation process. As such, they may represent a viable source of natural antioxidants to mitigate lipid oxidation in various food products, particularly in oil-in-water emulsions. Additionally, DSE could be effectively employed as a promising agent in the prevention of atherosclerosis through the inhibition of LDL oxidation.

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الملخص العربي

مستخلصات نوى التمر الغنية بالمركبات النشطة بيولوجيًا والأنشطة المضادة للأكسدة: التأثيرات الوقائية المحتملة ضد تصلب الشرايين وأكسدة الدهون في الأنظمة النموذجية المعملية خارج الجسم

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تم فى هذه الدراسة تجهيز مستخلصات مختلفة لنوى التمر (DSE) والتي تشمل المستخلصات الإيثانولية والميثانولية والمائية بهدف تقدير محتوياتها من المركبات النشطة بيولوجيا، والأنشطة المضادة للأكسدة وكذلك استكشاف تأثيراتها الوقائية المحتملة ضد تصلب الشرايين وأكسدة الدهون في الأنظمة النموذجية المعملية خارج الجسم. أوضحت النتائج أن المستخلص الإيثانولى قد سجل تركيزات عالية من المركبات النشطة بيولوجيًا (الفينولات الكلية والفلافونويدات الكلية والليكوبين والتربينويدات والتانينات والكامفيرول والفيتات)، فى حين سجل المستخلص الميثانولى تركيزات عالية من الكاروتينات الكلية والسكريات العديدة، اما المستخلص المائى فقد سجل تركيزات عالية من الليوتين، الأنثوسيانين الكلي، التربينويدات الثلاثية، السابونين، الأوكسالات والكافيين. أيضًا، تمتلك جميع أنواع المستخلصات السابقة أنشطة عالية مضادة للأكسدة لكسح الشقوق الحرة، حيث سجلت التركيزات بالنسبة لـ IC50 (تركيز المستخلص اللازم لكبح ٥٠% من التفاعلات) لاختبار الـ DPPH، ١٧,٦٣ ± ٠,٢٤ و ١٢,١٠ ± ٠,٢٠ و ١٤,٠٩ ± ٠,١٣ ميكروجرام/مل بالنسبة للمستخلصات المائية والإيثانولية والميثانولية على التوالي مقارنة بالمادة القياسية (بيوتايل هيدروكسى تولوين) التى سجلت قيمة مقدارها ٩,٢٥ ± ٠,١٩ ميكروجرام/مل. وبالتالي، كان نشاط مسح الجذور الحرة الخاص بالمستخلصات وفق لهذا المعيار عند الترتيب التالى: بيوتايل هيدروكسى تولوين > المستخلص الإيثانولى > المستخلص الميثانولى > المستخلص المائى. وعند التركيزات ٥٠ و ١٠٠ جزء في المليون من هذه المستخلصات يمكن أن تمنع أكسدة الدهون في كل من البيتا–كاروتين لينوليات، وأنظمة أكسدة البروتين الدهني منخفض الكثافة (تأثير مضاد لتصلب الشرايين). ولذلك، يمكن أن نستنتج أنه من الممكن استخدام مستخلصات نوى التمر كمصدر لمضادات الأكسدة الطبيعية. كما يمكن استخدامها بنجاح كعامل واعد في الوقاية من تصلب الشرايين من خلال تثبيط عملية أكسدة البروتين الدهني منخفض الكثافة.

الكلمات المفتاحية: التركيب الكيميائي، القيمة الإستخلاصية، مضادات الأكسدة، DPPH، الكسح، التأثير المضاد لتصلب الشرايين.