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Original article

# Chemical composition and antioxidant/antibacterial depictions of Zahidi date palm (*Phoenix dactylifera*) kernel Oil

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## ABSTRACT

Date palm "*Phoenix dactylifera*" variety *Zahidi* is a unique well-known cultivar. Mature fruits are semi-dry, medium size, cylindrically shaped and golden brown. However, there are three stages to harvesting the dates soft, hard or semi-soft. In this research, the oil processed from the seed/kernel of the date palm *Zahidi* is chemically analysed for its composition, antioxidant capacity and its antibacterial properties were investigated. Date seeds were ground and extracted for the oil. Wet chemical analyses were implemented on the extracted oil via various analytical techniques such as high-performance liquid chromatography (HPLC) and gas chromatography-mass spectrometry (GC–MS). Oil fractions of ethanol, methanol and acetone were tested for antibacterial activity, which was evaluated employing the Kirby–Bauer disc-diffusion method. Antibacterial action was examined for four bacterial strains; two of them representing Gram-positive such as *Bacillus subtilis*, and *Staphylococcus aureus*, and the other two for Gram-negative such as *Pseudomonas aeruginosa* and *Escherichia coli*. The antibiotics tetracycline, chloramphenicol, streptomycin, gentamycin and vancomycin were used as positive standards. The Minimum Bactericidal Concentration (MBC) and Minimum Inhibitory Concentration (MIC) of the bacterial strains were tested and evaluated. The antioxidant activity was evaluated as free-radical scavenging capacity (RSC) towards 2,2 - diphenyl -1-picrylhydrazyl (DPPH<sup>•</sup>) radicals for the methanol fraction (MF), remaining lipid fraction (LF) and non-fractioned oil, i.e., the total fraction (TF). The RSCs for the MF, LF and TF were 42.5, 9.1 and 68.3, respectively. *Zahidi* seed/kernel oil extract inhibited all of the test microorganisms, with MICs that ranged from 10 to 42 mg/mL and MBCs were found to be ranged between 21 and 167 mg/mL. These outcomes suggest that the extracts could be developed as a foundation of expected antimicrobial complexes to fight unwanted bacteria in foods. Overall, the results showed that the oil is suitable for food, cosmetics, personal care products and pharmaceuticals.

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## 1. Introduction

*Phoenix dactylifera*, which is normally known as the date palm and other members of the genus *Phoenix* are broadly cultivated for the dietetic delicious fruits. Because of the extended history of farming for edible fruit, the exact native spreading of *P. dactylifera* is unidentified, but the tree possibly originated someplace in Northern Africa and probably South-West Asia. The plant is about, a 15–25 m tall tree and habitually has a clump of one or more trunks from a one-root system, however, it as well grows as a single tree. The leaves are pinnate, 3–5 m long and have spines on the

petiole with approximately 150 leaflets, which are about 30 cm long and around 2 cm wide. The full span of the crown is ranging from 5 to 10 m (Neuhaus, 2013). The fruit type depends on the glucose, fructose and sucrose content. (*P. dactylifera*-; <https://www.thefullwiki.org/date>).

Date kernels were ground, soaked and either mixed with other feedstuff or used directly as feedstuff for animals. The extracted oil is edible and suitable for other uses such as cosmetics, and soap and may be medicinal. The seeds could as well be chemically treated and utilized for oxalic acid production (Joshi et al., 1991), burned to produce charcoal and can be looped in necklaces. Date kernel/seeds/pits were ground and used in means of coffee beans or mixed with coffee. Stripped fruit clusters are used as household products (Mohammed et al., 1983).

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The tannin content in date seeds is high tannin and is used medicinally as a deterrent and astringent to treat intestinal problems. As an infusion, decoction, syrup or paste, dates may be administered for sore throats, colds, bronchial catarrh and to relieve fever as well as some other complaints (Vyawahare et al., 2009). One traditional belief is that it can counteract alcohol intoxication. The kernel powder is also used in some traditional medicines (Al-Hooti et al., 1997).

Gum that exudes from the injured trunk of the palm tree is used for treating diarrhoea and Genito-urinary disorders. The roots are used to treat toothache. The pollen yields an oestrogenic component, restrone, and has a gonadotropic effect on young rats ([https://treepicturesonline.com/date\\_palm\\_tree\\_pictures.html](https://treepicturesonline.com/date_palm_tree_pictures.html)).

Systematic evaluation of the bioactivity was first reported by Maruzzella and Sicurella (1960) and Kienholz (1959) who used the inverted Petri dish technique to avoid contamination and ensure proper microbial growth. This technique, in which a volatile compound placed in a cup or a paper disc was exposed to the inverted agar medium plate inoculated with test strains at about a 5-mm distance, was convenient and had been used by subsequent researchers (Danial and Alkhalaf, 2018). However, the use of oil from the seeds of date palms as an antimicrobial agent has not yet been evaluated for its antioxidant activities.

The fruit Zahdi (Zahidi) is one of the oldest cultivars that is consumed in prodigious quantities in the Middle East. The date is of medium size, cylindrical, light golden-brown and semi-dry and is harvested and sold in 3 stages: soft, semi-dry and dry. The fruit is very sweet, keeps well mouth-feel and is often used for culinary purposes, in addition, it could be stored in a good condition for many months.

This research was conducted to test date kernel oil for its *in-vitro* bioactivities such as antioxidant and antimicrobial properties as well. GC-MS analysis will be applied to evaluate the chemical profile of the extracted oil. Antioxidant activity will be evaluated as free-radical scavenging capacity (RSC) to 2,2-diphenyl-1-picrylhydrazyl (DPPH<sup>•</sup>) radicals composed with effects on lipid peroxidation (LP). The antibacterial activity was inspected in four (4) bacterial strains. This aspect was particularly important because of the augmented fight of some bacterial strains against the most known antibiotics and antiseptics/antibacterial agents for the protection of food and/or food products (Adam, 2002).

The primary objectives of this research were to study the bioactivity and the composition of the antioxidants and the antibacterial properties of the oil processed from the kernels of the Zahidi variety date palm (*P. dactylifera*). If the product has high antimicrobial activity and antioxidant as well; it could be used as a natural, halal food additive in fats, oils and related material industry, locally and globally.

## 2. Materials and methods

### 2.1. Raw materials

The fruits of dates that are known as (*tamer*) at the mature stage for the variety of Zahidi were produced in Saudi Arabia. Zahidi dates are medium-soft semi-dry at the stage of the *tamer* (Al-Hooti et al., 1997). The samples of date fruits were analysed for moisture content, ash, protein and sugar content agreeing to the standard methods such as ASSOCIATION OF OFFICIAL ANALYTICAL COLLABORATION (AOAC) INTERNATIONAL (AOAC, 2000), which stands for Association of Official Agricultural Chemists (Carrapiso and García, 2000).

### 2.2. Analysis of the oil

Analysis of the oil samples was performed by using GC-MS (Hewlett Packard 5973-6890) operating in electrospray ionisation mode at 70 eV equipped with a split-split-less injector at 200 °C. The detector was flame ionisation at 250 °C. The carrier gas was helium at the rate of 1 mL per minute. A capillary column was hp 5MS (30 m × 0.25 mm; film thickness 0.25 µm). Temperatures of 60 °C to 280 °C and 60 °C–260 °C programs were designed and both were increased at the rate of 3 °C/min. The fraction of the split was 1/10 or 1:10 ratio. The identification of the separated compounds was based on co-elution and MS analysis (Gupta and Girish, 1988). The unsaponifiable matter was determined by extraction from date seed oil after saponification at room temperature according to the methods outlined by Mohamed and Awatif (1998).

### 2.3. Antibacterial activity testing

Bacterial strains, representing Gram-positive and Gram-negative were used in this testing. The bacterial strains used were *Escherichia coli*, *Pseudomonas aeruginosa*, for G-negative and *Staphylococcus aureus*, *Bacillus subtilis* for representing G-positive strains. To evaluate the oil extract for antibacterial activities; the disc-diffusion method was performed in triplicate. The solvents used were evaporated to eliminate the effects on microbial growth and dissolve the residue in dimethyl sulfoxide (DMSO). Furthermore, the effects of 5 antibiotics, tetracycline, chloramphenicol, streptomycin, gentamycin and vancomycin were also investigated as a positive control (Maruzzella and Sicurella, 1960; Abimbola, 2011).

### 2.4. Media preparation

#### 2.4.1. Broth

Luria-Bertani broth was used following the instructions of the producer. A twenty-five (25 g) of broth powder was well mixed with 1 L of distilled water in a Schott bottle, and then the was covered and sterilised using an autoclave at 121 °C for 15 min. Then the sterilised flasks were removed, left to cool down and then kept at 4 °C in a chiller while waiting for further use.

#### 2.4.2. The agar

A 37 g of agar powder (Luria-Bertani Agar) was mixed with 1 L of distilled water in a Schott bottle, then covered with aluminium foil and sterilised at 121 °C in an autoclave for 15 min. Then the sterilised flask was removed and left to cool down for about 10 to 15 min, then the solution was transferred into clean sterilised Petri dishes up to about one-third (1/3) full and allowed cool down by handling the lids loosely on top of the dishes avoiding condensation (Danial and Alkhalaf, 2016).

#### 2.4.3. Preparation of inoculum

As usual, a loopful of inoculum was taken from the pure culture of each bacteria grown on slants and inoculated into 10 mL of the prepared broth (Luria-Bertani). Then produced broth was incubated overnight at 37 °C. Then the obtained growth was used as an inoculum for the antimicrobial activity tests/assays. The cultures were obtained from the Microbiology Laboratory at the Department of Chemical Engineering and Sustainability, Kulliyah of Engineering at the International Islamic University Malaysia (IIUM) that were ordered from the American Type Culture Collection.

#### 2.4.4. Antibacterial activity test kernel extract

The antibacterial test/assay was performed as designated by (Adebolu and Oladimeji (2005), Gautam et al. (2007) and Karuppusamy and Rajasekaran (2009)). The broth suspension was diluted to give an optical density at 625 nm ( $OD_{625}$ ) equal to (=) 0.1 to achieve a microbial suspension of about  $10^6$  CFU/mL. Separate agar plates (Luria–Bertani) were coated with different bacterial strains. Using a pipette, 100  $\mu$ L of the prepared bacterial solution was added to the agar plates and evenly spread using a sterilised bent glass rod. The solution was almost absorbed into the agar for 5 min. Ten microliters (10  $\mu$ L) of each extract – [(300  $\mu$ g/disc) at a concentration of 30 mg/mL]– was applied to blank discs and observed/tested on the bacterial plates by means of Kirby–Bauer antimicrobial disc analysis.

#### 2.4.5. Disc-Diffusion method (Kirby–Bauer)

Although many antibiotics were discovered, most of them become medically useless because of unacceptable poisonousness or serious evil side effects. One of the most universally used methods for antimicrobial susceptibility testing is the Kirby–Bauer disc-diffusion method (Bauer et al., 1966). In this study, the discs were impregnated with ten (10  $\mu$ L) of the extracts plated onto the prepared agar plates which were prepared by swabbing the bacterial strain and incubating. Then the plates were placed into the incubator for 24 h 37 °C waiting for growing and inhibition of the bacteria to happen. A clear area around the disc that contains the extract indicates no bacterial evolution. This clear area is termed a zone of inhibition, which was used to quantify the susceptibility. The diameter of the zone of inhibition determined the result of the extracts. A great inhibition zone indicates that the organism was susceptible, whereas a lesser diameter of clear zones of inhibition indicates that the bacterial strain resisted the extract and continue growing (Bauer et al., 1966). The discs of antibiotics used in this research were pre-ordered from Merck Company (Merck Malaysia, Bandar Sunway, Petaling Jaya, Selangor DE, Malaysia). However, the discs used for standards were having low doses of tetracycline (30  $\mu$ g/disc), gentamycin (10  $\mu$ g/disc), chloramphenicol (30  $\mu$ g/disc), streptomycin (10  $\mu$ g/disc), and vancomycin (30  $\mu$ g/disc). Ten percent (10%) of Dimethyl sulfoxide (DMSO) was used as the negative control.

#### 2.5. Determining Minimum Inhibitory Concentration (MIC) of the optimised ethanolic extract of zahidi kernel oil

The essays that were described by Andrews (2001) and Ríos and Marzo (1988) with few modifications were followed for determining the MIC of the optimised ethanolic extract of Zahidi date kernel oil. MIC is the lowest concentration of extract that inhibits the growth of the microorganism (Maurya et al., 2013). The MIC of the optimised extract was evaluated and measured via the micro broth dilution assay and the agar disc diffusion method as well. The extract was serially diluted in the DMSO to get concentrations ranging from 333 mg/mL to 156  $\mu$ g/mL (12-fold serial dilution). The lowest concentrations of the extract that resulted in the absence of visible bacterial evolution within 24 h were verified as the MIC.

#### 2.6. Determination of MIC via the agar disc diffusion method

Dilutions of the bacterial cultures were performed at an  $OD_{625} = 0.1$  to attain a bacterial suspension of  $10^6$  CFU/mL. Petri plates containing nutrient agar were inoculated with 100  $\mu$ L of bacterial culture. The discs were infused with approximately 10  $\mu$ L of the different concentrations of the optimised extract. The antibacterial activity against each bacterial strain was quantified by measuring the diameter of the zone of inhibition around the discs in millimetres. This assessment was achieved to relate it to

the measurement of the MIC using the Broth Dilution Method for validation.

#### 2.7. Evaluation of the Minimal Bactericidal Concentration (MBC) of optimised ethanolic extract of zahidi seed oil

The MBC was used to determine the capability of the extract to inhibit the growth or kill the test organisms. The MBCs for the optimised extract were measured via sub-culturing 10  $\mu$ L from all tubes in which no visible bacterial evolution was observed on a nutrient agar plate after 24 h at 37 °C. The maximum dilution that produced no bacterial growth, was defined as the lowermost concentration that killed/inhibit 99.9% of the test bacterial strain that was stated as the MBC (Ríos and Marzo, 1988). Each tube's contents were homogenised by gently shaking and then 0.01 mL of the contents of each tube were sub-cultured by streaking on nutrient agar plates [Luria–Bertani]. Then the plates were upturned and incubated at 37 °C for 18–24 h. After that, the plates were then detected for the growth of colonies. The extract concentrations of the tube producing zero colonies or in other words no growth observed at all on the nutrient agar plates [Luria–Bertani] were stated as the MBC.

#### 2.8. Antioxidant activity

The antioxidant properties of the date palm kernel oil were assessed as both the RSC and protective effects against LP (Yousif et al., 1976).

#### 2.9. Total phenolic content (TPC) or Folin–Ciocalteu index (FCI)

FCI assay procedure was implemented following the method designated by Musa et al. (2011). About 0.5 mL of a diluted Folin–Ciocalteu reagent was added to 100  $\mu$ L of the extracted sample and let to stay for 5 min prior to the addition of 1 mL of  $Na_2CO_3$  (7.5%) (w/v). Then and after two (2) hours, the absorbance was measured at 765 nm by a spectrophotometer and the obtained result was documented as mg of gallic acid equivalent (GAE).

##### 2.9.1. DPPH RSC assay

Following the methods labelled by Musa et al. (2011), the antioxidant capacity was evaluated by employing the DPPH RSC. DPPH reagent was newly prepared by dissolving 40 mg DPPH in one (1.00) litre of methanol, which is sufficient to get an absorbance of  $1.00 \pm 0.01$  unit at 517 nm by a spectrophotometer (Spectro Nanostar, Germany). About 100  $\mu$ L of the extract –(sample)– was then mixed with 1 mL of the newly made DPPH solution and reserved in the dark for about 30 min. The DPPH scavenging activity was then calculated and measured according to Eq. (1):

$$DPPH\% \text{ of inhibition} = \frac{(A_{control} - A_{sample})}{A_{control}} \times 100 \quad (1)$$

where:  $A_{control}$  is the absorbance of the control and  $A_{sample}$  is the absorbance of the sample.

##### 2.9.2. Phenolic acids extraction and quantification

High-performance liquid chromatographic (HPLC) following the method designed by Alasalvar et al. (2005) with slight adjustment was used to determine the phenolic acids in the Zahidi date palm kernel extract. The sample of the extract was filtered through a 0.45  $\mu$ m PTFE filter (National Scientific Company, Rockwood, TN, USA) and then an HPLC analysis was performed. A 20- $\mu$ L aliquot of a sample or standard extract was inserted by injecting it into a Prodigy ODS-2 column (250 mm  $\times$  3.2 mm, Phenomenex, Cheshire, UK). The equipment contained a Quat pump, a diode array detector

(G1315A) and a degasser from (HP “hp” Agilent 1100 Series, Waldbronn, Germany). The filtered gradient elution was used with a mobile phase that contained 50 mM solution (A), which is a phosphoric acid, pH 2.5 and solution (B), which is acetonitrile. 0–5 min, isocratic elution 95% A and 5% B; 5–55 min, linear gradient to 80% A and 20% B; 55–60 min, linear gradient to 95% A and 5% B. The flow rate of the mobile phase was 0.9 mL/min. The wavelengths of the diode array detector were set at 254, 270, 280 and 329 nm to monitor the phenolic acids. Phenolic acid standards (2-hydroxyl cinnamic acid, vanillic acid, 4-hydroxyl benzoic, coumaric acid, 4-hydroxyl cinnamic acid, gallic acid and caffeic acid) were used for the identification of phenolic acids. However, the retention time, peak spectra and internal standards were used cautiously to identify & quantify phenolic acids. The phenolic acids were measured by calibration curves for standard concentrations that was ranging between 1 & 100 mg/mL of alcohol (methanol) and conveyed in milligrams of phenolic acid per 100 g of the sample of the extract.

### 2.9.3. Statistical analyses

Each value is the mean of three replications. Values of different parameters were expressed as the mean  $\pm$  standard deviation ( $\pm$ SD). Student's *t*-test was performed on the statistical analysis using MS Excel at the level of  $P < 0.05$ , to evaluate the significance of differences between mean values.

**Table 1**  
Proximate determination of date (*Phoenix dactylifera*) kernels (variety Zahidi) composition and fatty acid profile of Zahidi date palm kernel oil.\*

No.	Compound	Total-Content	Comments
1	Moisture	09.85% $\pm$ 1.00%	
2	Fats & oils	12.35% $\pm$ 00.5%	Dry weight
3	Carbohydrate	08.13% $\pm$ 0.42%	g/100 g
4	Crude fibre	62.70% $\pm$ 0.94%	g/100 g
5	Copper	0.9227 $\pm$ 0.03 mg/g	
6	Calcium	2.0352 $\pm$ 0.14 mg/g	
7	Iron	0.9108 $\pm$ 0.02 mg/g	
8	Manganese	0.4285 $\pm$ 0.01 mg/g	
9	Magnesium	4.9900 $\pm$ 0.32 mg/g	
10	Potassium	6.4700 $\pm$ 0.45 mg/g	
11	Protein	1.9809 $\pm$ 0.02 mg/g	
12	Glucose	0.7351 $\pm$ 0.05 g/L	
13	Fructose	0.6009 $\pm$ 0.03 g/L	
No	Chemical	Fatty Acid Methyl Ester	Area (%) relative to total area
1	C8	Caprylic	00.30 $\pm$ 0.01
2	C10	Capric	00.34 $\pm$ 0.01
3	C12	Lauric	20.83 $\pm$ 0.45
4	C14	Myristic	12.05 $\pm$ 0.06
5	C16	Palmitic	12.19 $\pm$ 0.07
6	C16:1	Palmitoleic	00.50 $\pm$ 0.02
7	C17	Heptadecanoic	00.28 $\pm$ 0.01
8	C18	Stearic	04.71 $\pm$ 0.01
9	C18:1	Oleic	37.69 $\pm$ 0.40
10	C18:2	Linoleic	07.52 $\pm$ 0.22
11	C18:3	Linolenic	00.23 $\pm$ 0.01
12	C20	Arachidic	00.53 $\pm$ 0.00
13	C20:1	Arachidonic	Traces
14	C20:2	Eicosadienoic	00.10 $\pm$ 0.01
15	C22	Behenic	00.28 $\pm$ 0.01
16	C22:1	Erucic	00.23 $\pm$ 0.00
17	–	Others	Traces
18	–	Sat: Unsat Ratio	(52.68:47.32)
19	–	Unsaponifiable matter	1.60 $\pm$ 0.03

\* Results obtained are the mean of 3 replicates  $\pm$  standard deviation.

## 3. Results and discussion

### 3.1. Date composition

The results of the proximate analysis of Zahidi date seeds are shown in Table 1. The stated composition was 62.7% crude fibre, 12.4% fats, 1.9 mg/g protein and some minerals. This composition is comparable to the nutritional properties of another other variety (Barhi) of date seeds reported by Mahmud et al. (2017).

### 3.2. Oil analysis

The fatty acid composition of Zahidi date kernel oil was determined and is presented in Table 1. The highest fatty acid content (37.7%) was for oleic acid (C18:1), which was reliable with the results of earlier studies for other varieties of date seeds (Mahmud, et al., 2017; Al-Hooti et al., 1998; Amany Mohamed Mohamed and Maliha Ali, 2011). High oleic acid content oils are considered to be good for human health since the amount of saturated fatty acids is relatively reduced, they have better oxidative stability than that of highly unsaturated fatty acids and are possible to have reduced low-density lipoprotein cholesterol in the blood (Habib et al., 2013). The result is also in covenant with that published by Saafi et al. (2008). Besbes et al. (2004b) described that oleic acid was the main unsaturated fatty acid but slightly higher (41.3%–47.7%) than that in Zahidi date kernel oil in the present study, whereas the main saturated fatty acid was lauric acid (C12:0) (17.8%) in date kernels of the variety Deglet Nour, from Tunisia, but was 20.8% in Zahidi oil. These differences in the fatty acid profiles are subject to the date varieties and of course, the climatic conditions according to the origins where those varieties are grown.

The approximate ratio of saturated to unsaturated fatty acids in Zahidi kernel oil was 53: 47 (Table 1), so this kind of natural fat is suitable for use as shortening and production of ghee, butter and mayonnaise, as stated by Amany Mohamed Mohamed and Maliha Ali (2011), who successfully utilised the cultivar Khalas to substitute conventional oil in manufacturing mayonnaise. Our study disclosed that Zahidi date kernel oil might be utilised as a non-conventional oil in food processing to produce ghee, butter and mayonnaise products. Furthermore, the high relative percentages of lauric acid (C12:0) and lower chain fatty acids mean that this oil work would be useful as an antimicrobial agent, as previously reported (Batovska et al., 2009; Abbas et al., 2017). These characteristics of Zahidi seed oil, which is a waste by-product from the date palm industry, indicate that the oil could also be incorporated into cosmetic formulations, personal care, shampoo and pharmaceutical products. Akbari et al. (2012) also reported high lauric acid content in date seed (kernel) as did Saafi et al. (200).

### 3.3. Evaluation of antibacterial activity

#### 3.3.1. Antibacterial activity test of date palm seed oil

Fig. 1 shows the ability of some common antibiotics, such as tetracycline, chloramphenicol, streptomycin, gentamycin and vancomycin, to inhibit the growth of *B. subtilis* and *S. aureus*, representing the G-positive strains and *P. aeruginosa* and *E. coli*, representing the Gram-negative strains. Fig. 1 shows that all selected antibiotics affected the growth of all selected bacteria; however, gentamycin and tetracycline did a better job than the others as indicated by the inhibition zone diameters.

The observation and measurement of the diameter of inhibition zones formed (Table 2 and Fig. 2) showed that the extracts from the *P. dactylifera* seed oil (Zahidi) had significant potential as sources of antimicrobial agents. The results showed that Zahidi



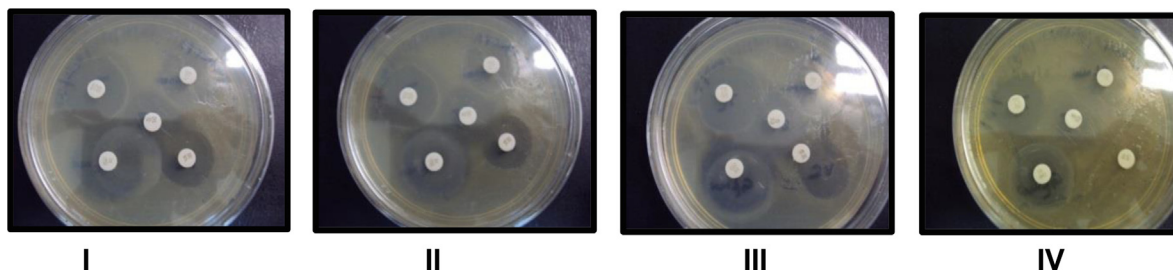


Fig. 1. Positive controls: (tetracycline) Upper left; (chloramphenicol) Upper right; (streptomycin) Middle; (gentamycin) Lower left; and (vancomycin) Lower right. Plates I: *B. subtilis*, II: *S. aureus*, III: *P. aeruginosa*, IV: *E. coli*.

Table 2  
Antibacterial activity of various solvents extracts of Zahidi seed oil against *B. subtilis*, *S. aureus*, *P. aeruginosa* and *E. coli*.

Extracts (Treatment)	Inhibition zone diameters (mm) for each type of bacterial strain*			
	<i>B. subtilis</i>	<i>S. aureus</i>	<i>P. aeruginosa</i>	<i>E. coli</i>
Zahidi + Ethanol	19 ± 0.5	20 ± 1.5	15 ± 0.5	12 ± 0.7
Zahidi + Methanol	17 ± 0.3	16 ± 0.5	12 ± 0.5	11 ± 0.4
Zahidi + Acetone	13 ± 0.5	18 ± 0.6	15 ± 0.2	11 ± 0.3
Zahidi + Distilled Water	15 ± 2.0	10 ± 1.5	08 ± 1.2	07 ± 1.5
DMSO	NI	NI	NI	NI

Abbreviation: NI = No inhibition, DMSO = Dimethyl sulphoxide. Distilled water extract is from the remaining seed cake.

\* Results are the mean of 3 replicates ± standard deviation.

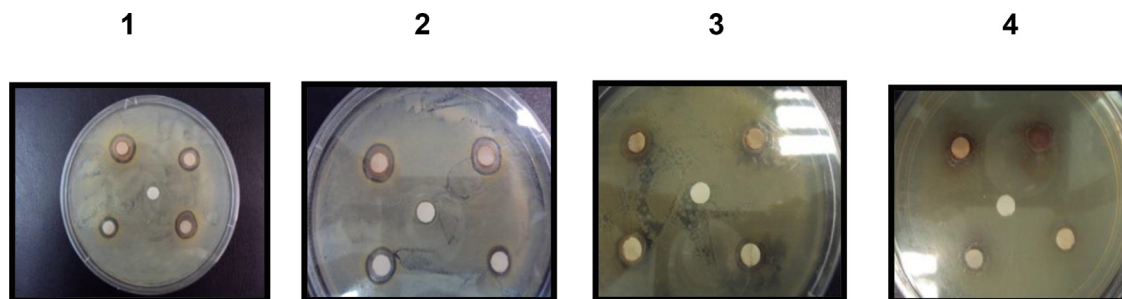


Fig. 2. Inhibition zones; 1 (*B. subtilis*), 2 (*S. aureus*), 3 (*P. aeruginosa*), 4 (*E. coli*); Upper left = Zahidi + ethanol extract, upper right = Zahidi + methanol extract, lower left = Zahidi + acetone extract, lower right = Zahidi + distilled water extract “middle for all” (Negative controls = DMSO).

seed oil extracts had greater antimicrobial activity hindering G-positive bacterial strains, such as *B. subtilis* and *S. aureus*, than did other G-negative strains, which were represented by *P. aeruginosa* and *E. coli* in this study. However, the solvents used to extract the active ingredients to be used were ethanol, methanol, acetone and distilled water, which were evaporated and replaced by DMSO which was utilised as a negative standard. The results showed that ethanol, methanol and acetone were good at extracting the active ingredients against Gram-positive bacteria from oil. The water extract showed some activity, but the activity was not consistent with that of G-positive bacteria and showed weak activity against G-negative bacteria.

We concluded that the oil extracted from Zahidi seed has a strong antibacterial act of inhibiting G-positive and G-negative bacterial strains. Such activity may be because of the presence of lauric (C<sub>12:0</sub>) and other short-chain fatty acids, as previously reported (Batovska et al., 2009; Abbas et al., 2017).

### 3.3.2. MIC of ethanolic extract of Zahidi seed oil

The MIC was determined using the agar disc diffusion assay showed that *S. aureus* has the greatest sensitivity to the Zahidi palm kernel extract. *S. aureus* need only 10 mg/mL of extract to inhibit its growth at the MIC, the next was *B. subtilis* and *P. aeruginosa*,

which had a MIC of 20 mg/mL, the most less sensitive was *E. coli*, which had a MIC of 44 mg/mL. These results are following the findings by Mirghani et al. (2011). The determination of the MIC of an ethanolic extract of Zahidi seed oil may be useful information for such industrial application of the date kernel oil in the food, pharmaceutical and cosmetics industries.

### 3.3.3. Determination of MBC

The MBCs of the selected ethanolic extract of Zahidi seed oil against *S. aureus*, *B. subtilis*, *P. aeruginosa* and *E. coli* were 20, 42, 81 and 165 mg/mL, respectively. These results are within the same range of results from the previous studies conducted by Mirghani et al. (2011), Othman et al. (2011) and Mahmud et al. (2019). The MBC of the selected ethanolic extract of Zahidi seed oil is important for applications of date seed oil to kill and/or inhibit the growth of unwanted microbial strains, such as in the food industry, or the treatment of infective diseases caused by resistant bacterial strains.

### 3.4. Antioxidant activity

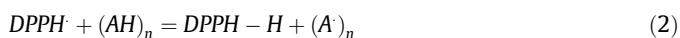
#### 3.4.1. TPC

The TPCs in the oil extracted from Zahidi seeds was  $32.96 \pm 1.66$  mg GAE/100 g oil, which is similar to the outcome obtained by Besbes et al. (2004b) who determined the TPC values in other varieties of date seeds oil from Tunisia, which were 520.8 and 220.3 mg/kg oil for the varieties Deglet Nour and Allig seed oils, respectively. Although phenolic compounds are part of the minor components of oils, they are factors for important oil characteristics, such as resistance against oxidation that may increase the shelf life, and they may have some effect on the oil's flavour as well. Table 3 shows the major phenolic compounds in Zahidi seed oil determined by GC–MS.

### 3.5. DPPH assay

#### 3.5.1. Phenolic acids extraction and quantification

For the DPPH assay, the disappearance of DPPH is a well-known index used for the estimation of the RSC, which is usually spectroscopically measured as a decrease in the absorbance due to the change in the purple colour to yellow because of the radical scavenging by the antioxidants in the sample or the standard used via the hydrogen donation that stabilises the DPPH-H, as described in the following Eq. (2)



In this study, the occurrence of an antioxidant, lessening the absorbance at 517 nm as was measured until the antioxidant was exhausted under the assay conditions for oils.

The DPPH percent inhibition values were 42.5%, 9.1% and 68.3% used for MF, LF and TF, respectively. The total oil fraction showed the highest anti-scavenging activity for DPPH free radicals, which may be because of a synergistic effect of the whole oil constituents. Therefore, fractionation of Zahidi seed oil is not advisable if the maximum antioxidant activity of the oil is to be retained. The capacity for high scavenging activity may be predictable to the high amounts of TPC in Zahidi seed oil extract, which agrees with the conclusions of Besbes et al. (2004a) on oils extracted from the kernels of Deglet Nour and Allig cultivars of *P. dactylifera* L. obtained from Tunisia.

## 4. Conclusion

The composition of the oil processed from the seed of *P. dactylifera* (date palm) and its antioxidant and antibacterial properties were analysed in this study. The results indicated that Zahidi date palm kernel extracts inhibited definite foodborne decay and pathogenic bacteria (*B. subtilis*, *S. aureus*, *P. aeruginosa* and *E. coli*). The date palm seed extracts suppressed or inhibited the evolution of all tested bacteria, with MICs reaching values from 10 to 42 mg/

**Table 3**  
Major phenolic compounds in Zahidi date kernel oil analysed by GC–MS.\*

No.	Compound	Amount (mg/100 g)
1	2-hydroxyl cinnamic acid,	$8.33 \pm 0.20$
2	Vanillic acid,	$7.24 \pm 0.16$
3	4-hydroxyl benzoic	$6.19 \pm 0.14$
4	4-hydroxyl cinnamic acid,	$4.42 \pm 0.05$
5	<i>p</i> -coumaric acid	$2.11 \pm 0.04$
6	Gallic acid	$0.52 \pm 0.03$
7	3,4-dihydroxy phenylacetic acid	$0.50 \pm 0.02$
8	Caffeic acid	$0.43 \pm 0.01$
9	Hydroxytyrosol	$0.21 \pm 0.01$
10	Others	$2.17 \pm 0.35$

\* Results are average of 3 replicates  $\pm$  standard deviation.

mL and MBCs ranging between 21 and 167 mg/mL. The outcomes indicated that the extracts could be used as a foundation source of natural antibacterial amalgams, which could be useful for food preservation that prevents the evolution of unwanted bacteria. Moreover, date palm kernel extracts have excessive potential as antibacterial combinations. Thus, they also could be developed in the treatment of catching infections triggered by resistant bacteria or other germs.

The optimal settings to enhance the action of inhibiting the growth/ evolution of the bacterial species in this study were the temperature of extraction that was 35 °C for 23 h extraction time and agitation at 200 rpm. The temperature and time of extraction and the speed of agitation meaningfully affected the total antibacterial contents extracted from date kernels.

The major phenolic acids of interest in this study were detected in the optimised ethanolic extract of Zahidi date seed oil and were 2-hydroxyl cinnamic acid (8.33 mg/100 g oil), vanillic acid (7.24 mg/100 g oil), 4-hydroxyl benzoic (6.19 mg/100 g oil), 4-hydroxyl cinnamic acid (4.42 mg/100 g oil), *p*-coumaric acid (2.11 mg/100 g oil) and gallic acid (0.52 mg/100 g oil) in addition to other phenolic compounds in small amounts, such as 3,4-dihydroxy phenylacetic acid, caffeic acid, hydroxytyrosol and others. However, the number of TPCs was approximately 33 mg GAE/100 g oil.

### 4.1. Recommendation

Since Zahidi date seeds contain antimicrobial compounds that can inhibit the growth of microorganisms. Ordinary people can use date palm seeds in many ways to benefit from these properties such as to crush the kernels into a fine powder and sprinkle it on food to prevent the growth of harmful bacteria and fungi. Zahidi date seeds are also rich in antioxidants, so they could be used by ordinary people to protect the body against oxidative stress. Crush the kernels into a fine powder and add it to smoothies, juices, or other drinks for an antioxidant boost. However, it is important to note that while date palm seeds may have potential antimicrobial and antioxidant properties, it is always best to consult with a healthcare professional before using them for any medicinal purposes. It is also, advisable to start with small amounts and monitor for any adverse reactions because some people may have allergies or sensitivities to certain components of the kernel/seed.

### Compliance with ethical standards

Not applicable.

### Human and animal rights

This manuscript does not contain any experiments involving humans or animals.

### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jksus.2023.102817>.

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