25 Jan 23-Amended Final copy [Elwathig] (without references).docx

WORD COUNT	4966	TIME SUBMITTED	29-JAN-2023 03:09AM
		PAPER ID	96003802

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

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•) 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 that could fight the unwanted bacteria in foods. Overall, the results showed that the oil is suitable for food, cosmetics, personal care products and pharmaceuticals.

Keywords: Antioxidant; antibacterial; date palm (*Phoenix dactylifera);* DPPH•; GC-MS; lipid fraction; seed oil.

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 meters. The type of fruits are depending on the glucose, fructose and sucrose content. (*P. dactylifera*-; http://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 [1], 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 [2].

The tannin content in date seeds is high tannin and is used medicinally as a detersive 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. One traditional belief is that it can counteract alcohol intoxication. The kernel powder is also used in some traditional medicines [3].

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, estrone, and has a gonadotropic effect on young rats (http://treepicturesonline.com/date_palm_tree_pictures.html).

Systematic evaluation of the bioactivity was first reported by Maruzzella & Sicurella (1960) [4] and Kienholz (1959) [5] 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 [6]. 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 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•) 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 [7].

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*).

MATERIALS AND METHODS

Raw Materials

The fruits of dates that are known as (*tamer*) at the mature stage for the variety of Zahidi were produced in Saudia Arabia. Zahidi dates are medium-soft semi-dry at the stage of the *tamer*. [3]. The samples of date fruits were analysed for moisture content, ash, protein and sugar content agreeing to the standard methods such as (AOAC, 2000), which stands for Association of Official Agricultural Chemists [8].

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 x 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 [9].

Unsaponifiable Matter

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) [10].

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 sulphoxide (DMSO). Furthermore,

the effects of 5 antibiotics, tetracycline, chloramphenicol, streptomycin, gentamycin and vancomycin were also investigated as a positive control [4, 11].

Media Preparation

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 minutes. Then the sterilised flasks were removed, left to cool down and then kept at 4° C in a chiller while waiting for further use.

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 minutes. Then the sterilised flask was removed and left to cool down for about 10 to 15 minutes, 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 [12].

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, Kulliyyah of Engineering at the International Islamic University Malaysia (IIUM) that were ordered from the American Type Culture Collection.

Antibacterial Activity Test Kernel Extract

The antibacterial test/assay was performed as designated by Adebolu and Oladimeji (2005) [13], Raju Gautam *et al*. (2007) [14] and Karuppusamy *et al*. (2009) [15]. 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 µL 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 minutes. Ten microliters (10μL) of each extract -[(300 µg/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.

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 [16]. In this study, the discs were impregnated with ten (10 µL) 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 [16]. 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 µg/disc), gentamycin (10 µg/disc), chloramphenicol (30 µg/disc), streptomycin (10 µg/disc), and vancomycin (30 µg/disc). Ten percent (10%) of Dimethyl sulfoxide (DMSO) was used as the negative control.

Determining Minimum Inhibitory Concentration (MIC) of the Optimised Ethanolic Extract of Zahidi Kernel Oil

The essays that were described by Andrews *et al*. (2001) [17] and Rios *et al*. (1988) [18] 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 [19]. 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 µg/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.

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 μL of bacterial culture. The discs were infused with approximately 10 μL 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.

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 µL from all tubes in which no visible bacterial evolution was observed on a nutrient agar plate after 24 hours 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 [18]. 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 hours. 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.

Antioxidant Activity

The antioxidant properties of the date palm kernel oil were assessed as both the RSC and protective effects against LP [20].

Total Phenolic Content (TPC) or Folin–Ciocalteu Index (FCI)

FCI assay procedure was implemented following the method designated by Musa, et al., [21]. About 0.5 mL of a diluted Folin–Ciocalteu reagent was added to 100 µL 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).

DPPH RSC Assay

Following the methods labelled by Musa, et al., [21], 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 ± 0.01 unit at 517 nm by a spectrophotometer (Spectro Nanostar,

Germany). About 100 μL 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 equation (1):

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

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

Phenolic Acids Extraction and Quantification

High-performance liquid chromatographic (HPLC) following the method designed by Alasalvar et al., (2005) [22] 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 0.45μm PTFE filter (National Scientific Company, Rockwood, TN, USA) and then an HPLC analysis was performed. A 20-µL aliquot of a sample or standard extract was inserted by injecting it into a Prodigy ODS-2 column (250 mm x 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.

RESULTS AND DISCUSSION

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) [23].

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% ± 1.00%	
2	Fats & oils	12.35% ± 00.5%	Dry weight
3	Carbohydrate	08.13% ± 0.42%	g/100 g
4	Crude fibre	62.70% ± 0.94%	g/100 g
5	Copper	0.9227 ± 0.03 mg/g	
6	Calcium	2.0352 ± 0.14 mg/g	
7	Iron	0.9108 ± 0.02 mg/g	
8	Manganese	0.4285 ± 0.01 mg/g	
9	Magnesium	4.9900 ± 0.32 mg/g	
10	Potassium	6.4700 ± 0.45 mg/g	
11	Protein	1.9809 ± 0.02 mg/g	
12	Glucose	0.7351 ± 0.05 g/L	

13	Fructose	0.6009 ± 0.03 g/L	
No	**Chemical**	**Fatty Acid Methyl Ester**	**Area (%) relative to total area**
1	C8	Caprylic	00.30 ± 0.01
2	C10	Capric	00.34 ± 0.01
3	C12	Lauric	20.83 ± 0.45
4	C14	Myristic	12.05 ± 0.06
5	C16	Palmitic	12.19 ± 0.07
6	C16:1	Palmitoleic	00.50 ± 0.02
7	C17	Heptadecanoic	00.28 ± 0.01
8	C18	Stearic	04.71 ± 0.01
9	C18:1	Oleic	37.69 ± 0.40
10	C18:2	Linoleic	07.52 ± 0.22
11	C18:3	Linolenic	00.23 ± 0.01
12	C20	Arachidic	00.53 ± 0.00
13	C20:1	Arachidonic	Traces
14	C20:2	Eicosadienoic	00.10 ± 0.01
15	C22	Behenic	00.28 ± 0.01
16	C22:1	Erucic	00.23 ± 0.00
17	-	Others	Traces
18	-	Sat: Unsat Ratio	(52.68:47.32)
19	-	Unsaponifiable matter	1.60 ± 0.03

*Results obtained are the mean of 3 replicates ± standard deviation

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 [23-25]. 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 [26]. The result is also in covenant with that published by Saafi *et al*. (2008) [27]. Besbes *et al*. (2004) [28] 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 ($C_{12:0}$) (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 Basuny and Al-Marzooq (2011) [25], 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 ($C_{12:0}$) and lower chain fatty acids mean that this oil work would be useful as an antimicrobial agent, as previously reported [29] [30]. 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) [31] also reported high lauric acid content in date seed (kernel) as did Saafi *et al*., (2008) [27].

Evaluation of Antibacterial Activity

Antibacterial activity test of date palm seed oil

Figure 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. Figure 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.

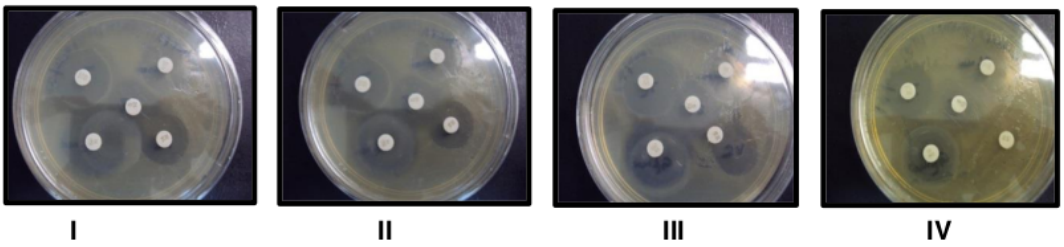

Figure 1: Positive controls: (tetracycline) Upper left; (chloramphenicol) Upper right; (streptomycin) Middle; (gentamycin) Lower left; and (vancomycin) Lower right. Plates I: *B. subtilis*, II: *S. aureus*, III: *P. aeruginosa*, IV: *E. coli*.

The observation and measurement of the diameter of inhibition zones formed (Table 2 and Figure 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 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 that 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.

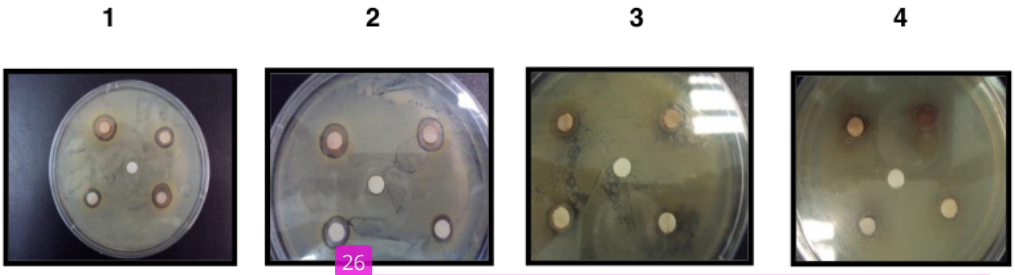

Figure 2: Inhibition zones; 1 (*B. subtilis*), 2 (*S. aureus*), 3 (*P. aeruginosa*), 4 (*E. coli*); Upper left = Zahidi+ethanol extract, upper right = Zahidi+methanol extract, lower left = Zahidi+acetone extract, lower right = Zahidi+distilled water extract "middle for all" (Negative controls = DMSO)

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*			
	B. subtilis	***S. aureus***	***P. aeruginosa***	***E. coli***
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

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 ($C_{12:0}$) and other short-chain fatty acids, as previously reported [29, 30].

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) [32]. 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.

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 Gautman *et al*. (2007) [32], Othman *et al*. (2011) [33] and Mahmud *et al*. (2019) [34]. 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.

Antioxidant Activity

TPC

The TPCs in the oil extracted from Zahidi seeds was 32.96 ± 1.66 mg GAE/100 g oil, which is similar to the outcome obtained by Besbes *et al*. (2004) [28] 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. Table 3 shows the major phenolic compounds in Zahidi seed oil determined by GC-MS.

Table 3: Major phenolic compounds in Zahidi date kernel oil analysed by GC-MS*

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

* Results are average of 3 replicates ± standard deviation

DPPH Assay

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 equation (2)

$$DPPH^{\cdot} + (AH)_n = DPPH - H + (A^{\cdot})_n \quad \text{..... (2)}$$

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*., (2004b) [35] on oils extracted from the kernels of Deglet Nour and Allig cultivars of *P. dactylifera* L. obtained from Tunisia.

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/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.

25 Jan 23-Amended Final copy [Elwathig] (without references).docx

ORIGINALITY REPORT

19%
SIMILARITY INDEX

PRIMARY SOURCES

1	www.mdpi.com Internet	82 words — 2%
2	docksci.com Internet	79 words — 2%
3	aligarhpmd.blogspot.com Internet	66 words — 1%
4	academic.oup.com Internet	47 words — 1%
5	Ahmed A M Elnour, Mohamed E S Mirghani, Nassereldeen A Kabbashi, Khalid Hamid Musa et al. " Comparative Study of the Characterisation and Extraction Techniques of Polyphenolic Compounds from gum ", Food Quality and Safety, 2022 Crossref	39 words — 1%
6	Al-Farsi, M.A.. "Optimization of phenolics and dietary fibre extraction from date seeds", Food Chemistry, 20080601 Crossref	34 words — 1%
7	www.eisrjc.com Internet	34 words — 1%

8	ci.thejakartapost.com Internet	33 words — 1%
9	Mohamed Ali Al-Farsi, Chang Yong Lee. "Optimization of phenolics and dietary fibre extraction from date seeds", Food Chemistry, 2008 Crossref	32 words — 1%
10	"Fruit Oils: Chemistry and Functionality", Springer Science and Business Media LLC, 2019 Crossref	29 words — 1%
11	living-in-theworld77.blogspot.com Internet	29 words — 1%
12	Larry Oscar Chañi-Paucar, Ádina L. Santana, Juliana Q. Albarelli, Maria Angela A. Meireles. "Extraction of polyphenols by sub/supercritical based technologies", Elsevier BV, 2022 Crossref	26 words — < 1%
13	www.science.gov Internet	26 words — < 1%
14	"Dates", Wiley, 2013 Crossref	23 words — < 1%
15	Daljit S Arora, Jasleen Kaur. "Antimicrobial activity of spices", International Journal of Antimicrobial Agents, 1999 Crossref	23 words — < 1%
16	link.springer.com Internet	22 words — < 1%
17	Aleksandra Popović, Jovana Šućur, Dejan Orčić, Pero Štrbac. "EFFECTS OF ESSENTIAL OIL	21 words — < 1%

FORMULATIONS ON THE ADULT INSECT TRIBOLIUM CASTANEUM (HERBST) (COL., TENEBRIONIDAE)", Journal of Central European Agriculture, 2013
Crossref

18	bjp.sagepub.unboundmedicine.com Internet	18 words — < 1%
19	www.scholarsresearchlibrary.com Internet	18 words — < 1%
20	P. A. Kumar. "Cecembia lonarensis gen. nov., sp. nov., a novel haloalkalitolerant bacterium of the family Cyclobacteriaceae, isolated from a haloalkaline lake and emended descriptions of the genera Indibacter, Nitritalea, Belliella and Aquiflexum", INTERNATIONAL JOURNAL OF SYSTEMATIC AND EVOLUTIONARY MICROBIOLOGY, 09/17/2010 Crossref	17 words — < 1%
21	www.actabp.pl Internet	16 words — < 1%
22	www.chiriottieditori.it Internet	16 words — < 1%
23	pdfs.semanticscholar.org Internet	14 words — < 1%
24	spaj.ukm.my Internet	14 words — < 1%
25	orgmedchemlett.springeropen.com Internet	13 words — < 1%
26	cell.ijbio.ir Internet	12 words — < 1%

27	coek.info Internet	10 words — < 1%
28	www.docdroid.net Internet	10 words — < 1%
29	www.researchsquare.com Internet	10 words — < 1%
30	www.rroij.com Internet	10 words — < 1%
31	www.scientific.net Internet	10 words — < 1%
32	bmjopen.bmj.com Internet	9 words — < 1%
33	storage.googleapis.com Internet	9 words — < 1%
34	www.hindawi.com Internet	9 words — < 1%
35	Ahmed A M Elnour, Mohamed E S Mirghani, Nassereldeen A Kabbashi, Khalid Hamid Musa et al. " Comparative study of the characterisation and extraction techniques of polyphenolic compounds from gum ", Food Quality and Safety, 2022 Crossref	8 words — < 1%
36	Jinhee Lee, Hyunjoo Yoon, Sunah Lee, Heeyoung Lee, Yohan Yoon. "Effect of Fat Contents on Thermal Resistance, Antibiotic Sensitivity, and Caco-2 Cell Invasion of Listeria monocytogenes", Korean Journal for Food Science of Animal Resources, 2013 Crossref	8 words — < 1%

37	M.F.P. Domingos-Lopes, C. Stanton, P.R. Ross, M.L.E. Dapkevicius, C.C.G. Silva. "Genetic diversity, safety and technological characterization of lactic acid bacteria isolated from artisanal Pico cheese", Food Microbiology, 2017 Crossref	8 words — < 1%
38	Mattila, Pirjo, and Jorma Kumpulainen. "Determination of Free and Total Phenolic Acids in Plant-Derived Foods by HPLC with Diode-Array Detection", Journal of Agricultural and Food Chemistry, 2002. Crossref	8 words — < 1%
39	Xiaojie Lu, Yue Jing, Yanyi Li, Naisheng Zhang, Yongguo Cao. "Eurotium cristatum produced β-hydroxy acid metabolite of monacolin K and improved bioactive compound contents as well as functional properties in fermented wheat bran", LWT, 2022 Crossref	8 words — < 1%
40	bulletin.mfd.org.mk Internet	8 words — < 1%
41	Ioanna Fitsiou, Olga Tzakou, Monica Hancianu, Antonia Poiata. " Volatile Constituents and Antimicrobial Activity of Moench and Miller Oils ", Journal of Essential Oil Research, 2007 Crossref	8 words — < 1%
42	mod0.cs.uaf.edu Internet	8 words — < 1%
43	tnsroindia.org.in Internet	8 words — < 1%
44	worldwidescience.org Internet	8 words — < 1%

45	www.euroasiacongress.org Internet	8 words — < 1%
46	www.orientjchem.org Internet	8 words — < 1%
47	A. Basta. "Essential oil composition ofPrasium majus L. from Greece", Flavour and Fragrance Journal, 09/2007 Crossref	6 words — < 1%
48	Edible Medicinal and Non-Medicinal Plants, 2012. Crossref	6 words — < 1%
49	Maran Marimuthu Mani, Arul Aruldass Claira, Mageswary Sandrasagaran Uma, Mohamad Suriyati et al. "Antimicrobial activity and phytochemical screening of various parts of Ixora coccinea", Journal of Medicinal Plants Research, 2014 Crossref	6 words — < 1%

EXCLUDE QUOTES	OFF	EXCLUDE SOURCES	OFF
EXCLUDE BIBLIOGRAPHY	OFF	EXCLUDE MATCHES	OFF