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ORIGINAL ARTICLE

Fatty Acids Composition, Total Phenolics Content, Antioxidant and Antibacterial Activities of Algerian *Ziziphus lotus* L. (Desf.) Fruit Oil

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Abstract

Oil seeds are valuable natural sources of lipophilic compounds for pharmaceutical, cosmetic, and biodiesel applications. *Ziziphus lotus* L. (Desf.), which is also called jujube, is a common plant in Algeria, and its fruit is well-known for its nutritional and medicinal benefits. Algerian traditional medicine uses the plant for its anti-diabetic, sedative, analgesic, anti-inflammatory, and hypoglycemic activities.This study was conceived to investigate the fatty acid composition, total phenolic content, antioxidant, and antibacterial activities of fixed oil obtained from Algerian *Z. lotus* fruits. Gas chromatography-mass spectrometry (GC/MS) analysis revealed that the major components of fatty acids were oleic (61.8%) and linoleic acid (16.2%), with a total of 78% unsaturated fatty acids. According to the Folin–Ciocalteau assay, the total phenols of the oil sample were determined at 25.08 mg GAE/100 g oil. The fixed oil showed significant antioxidant activity (IC50 values of 79.61 and 618 g mL^{-1} for DPPH and reducing power assays, respectively). Furthermore, the antibacterial activity of fixed oil was determined using the disc-diffusion and microdilution assays against two Gram-positive and two Gram-negative bacteria. The results revealed that the oil exhibited interesting antibacterial activity against *Streptococcus pneumonia* (IZ = 19 ± 0.4 mm and MIC = 0.2 ± 00 mg mL^{-1}), *Staphylococcus aureus* (IZ = 15 ± 0.1 mm and MIC = 0.4 ± 00 mg mL^{-1}), and *Escherichia coli* (IZ = 14 ± 0.2 mm and MIC = 0.5 ± 00 mg mL^{-1}). Results suggest that *Z. lotus* fruit oil could be explored as a novel and potential natural antioxidant and antibacterial for use in functional foods and medicine.

Keywords *Ziziphus lotus* L. · Fruit oil · Fatty acids · Phenolic content · Antioxidant activity · Antibacterial activity

1 Introduction

In the pharmaceutical, cosmetic, and food sectors, the need for innovative sources of oil is expanding because they are important natural sources of nutritious bioactive chemicals [1]. Vegetable oils with a high proportion of small lipid components are vital to human health, and their composition is crucial from a nutritional standpoint [2]. Because of recent advancements in the sector of oil seeds, the seeds' oil quality is mostly controlled by the fatty acid, triacylglycerol, and antioxidant properties [3]. Therefore, n-3 fatty acids are crucial to physiology, particularly during the early stages of development [4], because of their antithrombotic, anti-inflammatory, antiarrhythmic, and plaque-stabilizing properties, they play an essential role in preventing cardiovascular disease [5]. For this reason, many fruits and seeds, including the papaya, prickly pear, mangosteen, honeydew, rambutan, date palm, durian, etc., have been the subject of investigation

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into their potential applications in food or industrial uses, such as new bioactive compound sources [6].

Z. lotus known as "Sedra" belonging to the family of Rhamnaceae, is a medicinal plant largely found in the Mediterranean regions, including Algeria [7]. Several traditional uses were described for this plant, including for digestive problems, fatigue, liver problems, excess body fat, urinary issues, diabetes, skin infections, high body temperature, diarrhea, and sleeplessness [8, 9]. In Algeria, *Z. lotus* is used in traditional medicine to treat diabetes, pain, anxiety, and inflammation [10]. The fruits are widely used in folk medicine as a digestible agent, tonic, aphrodisiac, laxative, and for burning sensations, thirst, and vomiting; they are also reportedly used to treat tuberculosis and blood sickness [11–13]. In fact, Benammar et al. [14] found that the fruit pulp has the richest source of linoleic acid (18:2n − 6) with a value of 36.63 ± 1.26%, a precursor of n-6 fatty acids, and a more prominent antioxidant capacity than other parts of the plant. Relatively recently, it was shown that the protein hydrolysates that were extracted from *Z. jujuba* have antioxidant properties [15]. In addition, *Z. jujuba* seed oil was observed to reduce glucose and cholesterol levels in the serum of fed hyperlipidemic rats. [16]. The potential supply of lipids from fruits and fruit by-products is worth investigating.

In this context, the aim of this study was to evaluate the fatty acid compositions, the total phenolic contents (TPC), the antioxidant and antibacterial activities of *Z. lotus* fruit oil from the south-western region of Algeria.

2 Results and Discussion

2.1 Fatty Acids Composition

The main classes of fatty acids detected in the *Z. lotus* fruit oil are presented in Table 1. *Z. lotus* fruit oil is characterized by a high amount of monounsaturated fatty acid (MUFA) (66.28%) compared to polyunsaturated fatty acids (PUFA) and saturated ones (SAFA), which were at almost similar levels (16.7 and 16.99%, respectively). The total unsaturated fatty acid represents 82.98% of the total *Z. lotus* fruit oil. It was important to highlight that this oil was characterized by an unsaturated/saturated (U/S) ratio of 4.88, superior to that of soybean oil (3.69) [17]. Having a high U/S ratio is beneficial for lowering serum cholesterol, preventing atherosclerosis, and protecting against cardiovascular disease [18].

Twelve different fatty acids, among them, seven were unsaturated, were identified during the analysis of Z. lotus fruit oil. The majority of them were oleic (61.8%), linoleic (16.2%), palmitic (10.4%), stearic (4.69%), and gadoleic (3.13%) acids. *Z. lotus* fruit oil also has small amounts (3.75%) of arachidic, behenic, *trans*-vaccenic and *α*-linolenic

Table 1 Fatty acid composition of *Z. lotus* fruit oil

Position of the unsaturation and indice	Fatty acids	Percentage of fatty acids (%)
Saturated		
C16:0	Palmitic acid	10.40
C17:0	Margaric acid	0.06
C18:0	Stearic acid	4.69
C20:0	Arachidic acid	1.32
C22:0	Behenic acid	0.52
Monounsaturated		
C16:1	Palmiloleic acid	0.21
C17:1	*cis*-9-Heptadecenoic acid	0.09
C 18:1	Oleic acid	61.80
C18:1 n-7	*trans*-vaccenic acid	1.05
C20:1	Gadoleic acid	3.13
Polyunsaturated		
C18:2	Linoleic acid	16.20
C18:3	*α*-Linolenic acid	0.50
SAFA		16.99
MUFA		66.28
PUFA		16.70
U/S		4.88

SAFA saturated fatty acid, *MUFA* monounsaturated fatty acid, *PUFA* polyunsaturated fatty acid, *U/S* unsaturated/saturated fatty acids

acids. Previous studies have shown that palmitic, stearic, linoleic, and oleic acids are found in high amounts in all parts of *Z. lotus*, especially the seeds, pulp, fruits, leaves, root, and stem [14, 19–21]. As reported in a study by Ghazghazi et al. [21], Most of the fats in Tunisian *Z. lotus* fruits were made up of oleic acid (88.12%) and elaidic acid (7.88%). With relative quantities of 88.12%, 61.93%, and 49.88%, oleic acid was the most significant fatty acid of *Z. lotus* fruits, seeds, and almonds. [18]. Oleic acid plays an essential part in the prevention of cardiovascular disease and is important to the normal growth and development of human skin [22]. In terms of nutrition and medicine, this oil is very desired because to its high concentration of MUFA, which has been shown to reduce blood cholesterol levels, alter immunological function, lessen LDL's oxidation susceptibility, and increase HDL's fluidity [23]. However, before this oil may be used in human nutrition, its safety must be assessed.

2.2 Physicochemical Properties

The comparison of the physicochemical properties of Z. Lotus fruit oil with those of olive oil and soybean oil is presented in Table 2.

Fruit of *Z. lotus* furnished 5.49 ± 0.01% of oil; this value was significant and comparable with that of Ait Bouzid et al. [26] ranged from 1.59 ± 0.34% to 2.91 ± 0.91%. The acid

Table 2 Comparison of physicochemical properties of *Z. lotus fruit* oils with those of olive oil

Parameter	Z. lotus	Olive oil [24]	Soybean [25]
Density	0.89 ± 0.008	–	–
Refractive index	1.4627 ± 0.002	–	1.477 ± 0.002
Acidity indexmg KOH/g oil	5.61 ± 0.01	0.84 ± 0.00	1.72 ± 0.08
Saponification index (mg KOH/g oil	222.156 ± 0.002	189.30 1.16	179.45 ± 0.68

value of *Z. lotus* oil was 5.61 ± 0.01, it was higher than that of olive oil and soybean oil, which might be attributed to the presence of a large amount of free acids [27]. The acidity index showed that pre-refining and packaging precautions must be taken in order to limit the deterioration of the physicochemical and functional quality of this oil [28]. The saponification value was higher than that of olive oil (189.30 1.16), and soybean oil (179.45 ± 0.68). Triacylglycerols containing short-chain fatty acids have higher saponification values than those with longer chain fatty acids, and a high saponification value indicates that the fatty acids present in the oils have a high number of carbon atoms [25]. Therefore, the value obtained for *Z. lotus* fruit oil in this study shows that it has the potential for use in the production of soap and shampoos.

2.3 Total Phenolics Content (TPC)

More than 8,000 phenolic compounds have been found, and this is usually credited as the cause of vegetable oils' beneficial properties [29]. Recently, polyphenols have been regarded to be of significant scientific and clinical importance, making them an important category of naturally occurring chemicals. Oil samples were analyzed for their total phenolics content (TPC) with the use of the Folin-Ciocalteu reagent. Using a calibration curve with GA concentrations, the TPC was calculated as mg gallic acid equivalent (GAE) per gram of dry weight (mg GAE/g DW) and indicated significantly significant TPC (Table 2) (25.08 1.02 mg GAE/100 g oil) compared to the Tunisian seed oil of *Z. lotus* (18.45 0.81 mg/100 g oil) [19].

2.4 Antioxidant Activity

The present study aimed to investigate the antioxidant ability of the fruit oil of *Z. lotus*. The obtained results of these biological tests by DPPH radical scavenging and ferric-reducing antioxidant power (FRAP) assays are shown in Table 3. *Z. lotus* fruit oil exhibited a moderate antioxidant activity according to DPPH and ferric ion assays with IC_{50} values of 79.61 ± 0.83 and 618 ± 0.5 µg/mL, respectively, compared to the ascorbic acid reference value (6.31 ± 0.57 and 66.73 ± 0.33 µg/mL, respectively) ($P < 0.0001$). This could be justified by the synergistic effects between the various antioxidant components of the oil (unsaturated fatty acids, phospholipids, carotenoids, tocopherols, phenolic compounds, etc.) that lead to the reduction of free radicals through a hydrogen atom transfer and/or electron transfer [30], which could extend the shelf life of oils and particularly those rich in polyunsaturated fatty acids [31]. Interestingly, several in vitro studies have demonstrated the capacity of the different parts of *Z. lotus* for scavenging free radicals, for instance, in lipid peroxidation, resulting in cell damage prevention [32].

Means of three replicates ± SD (standard deviation) with different letters in the same column present significant differences between the results obtained for fruit oil and those for standard ($p < 0.05$) according to the Tukey's test. IC_{50} (µg mL^{-1}) the concentration at which 50% of DPPH is inhibited. EC_{50} value is the effective concentration at which the absorbance was 0.5.

Data from correlations and linear regression analysis (Table 4) displayed a positive statistical correlation between the antioxidant activity and total phenolics content with coefficients of determination $R^2 = 0.968$ ($p = 0.115$) and $R^2 = 0.549$ ($p = 0.469$), respectively, for DPPH and reducing power assays. Many investigations found strong

Table 3 Total phenolics content (TPC) and antioxidant activity of the fruit oil from *Z. lotus*

Sample	Total phenolics content (TPC) mg GAE/100 g oil	DPPH radical IC_{50} (µg mL^{-1})	Reducing power EC_{50} (µg mL^{-1})
Fruit oil	25.08 ± 1.23	79.61 ± 0.81[b]	618.00 ± 0.50[b]
Ascorbic acid	/	6.31 ± 0.57[a]	66.73 ± 0.33[a]

Table 4 Correlation analysis between the different antioxidant assays and phenolic contents of *Z. lotus* fruit oil

Antioxidant activity	Regression equation	R^2	r	P
DPPH assay	$IC_{50} = 63.30 + 0.65 \times TPC$	0.968	0.984	0.115
Reducing power assay	$EC_{50} = 610.44 + 0.30 \times TPC$	0.549	0.741	0.469

linear relationships between phenolic content and antioxidant capacity, suggesting that polyphenol-rich extracts had more antioxidant activity [22, 33, 34].. Similarly, the unsaponifiable substances and unsaturated fatty acids found in vegetable oils give them their antioxidant properties [35, 36]. Fatty acids' antioxidant properties are directly related to their chemical composition. Unsaturated fatty acids are hydrocarbon chains that include one or more carbon–carbon double bonds in addition to a methyl group at one end and a carboxyl function at the other. This means that, like phenols, these unsaturated compounds may eliminate hydrogen from the carbon atoms that are located between a double bond and the carboxylic acid group [37].

R^2: Coefficient of determination, r: Pearson correlation coefficient, P: Probability level.

2.5 Antibacterial Activity

The fruit oil of *Z. lotus* was evaluated for its antibacterial activity using disc diffusion and microdilution methods against two Gram-positive bacteria (*S. pneumoniae* and *S. aureus* ATCC 27,923) and two Gram-negative bacteria (*E. coli* ATCC 25,922 and *S. typhimurium*). Their potency was qualitatively and quantitatively assessed by the presence or absence of inhibition zones (IZ) and minimum inhibitory concentration (MIC) values (Table 4). Results showed that the vegetable oil was found to be active against three strains of pathogenic bacteria. However, the tested fruit oil has a higher significant activity against the Gram-positive strains (*S. aureus* and *S. pneumoniae*) than to the Gram-negative bacteria (*E. coli*), with IZ values of (15 ± 0.1 and 19 ± 0.4 mm) versus (14 ± 0.2 mm), respectively. MIC values for all bacterial strains were in the range of 0.2–0.5 mg mL^{-1}. These differences may be related to hydrophobic lipopolysaccharide in the outer membrane, which provides protection against different agents [38, 39] Table 5.

Means of three replicates ± SD (standard deviation) with different letters in the same column present significant differences between the bacteria strains ($p < 0.05$) according to the Tukey's test.

Results of correlations and linear regression analysis (Table 6) revolted a positive relationship between the antibacterial activity and total phenolics content with the coefficients of determination ranged between $0.057 \leq R^2 \leq 0.893$ ($P > 0.05$).

Previous studies on the species harvested from diverse regions of the world showed the antimicrobial activity of *Z. lotus* fruits, leaves, and seeds extract against *S. aureus*, *E. coli*, *S. typhi*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia* and *Bacillus subtilis* [40–42]. Aziz et al. [43] reported that the antimicrobial activity of *Z. lotus* seems to be mediated by the presence of phenolic compounds. Elaloui et al. confirmed that the ethanol extracts were found to be the most effective against *E. coli, S. aureus,* and *K. pneumoniea*, while aqueous extracts had moderate activity against the same strains [30]. The growth inhibition of bacteria by *Z. lotus* oil may be linked to its content of phenolic compounds and fatty acids. The antimicrobial effect of phenols has long been known. In fact, Ghazghazi et al. [21] demonstrated that methanolic *Z. lotus* leaf extract with a high level of phenolic content (6.64 ± 0.01 mg GAE/g DW) exhibited the best antibacterial activity when compared with fruit extract (2.97 ± 0.01 mg GAE/g DW). The MIC were in the range of 12.5–25 and 12.5–50 mg mL^{-1}, respectively. In addition, Zheng et al. [44] reported that the long-chain unsaturated fatty acids, such as linoleic acid, palmitoleic acid, oleic acid, linolenic acid, and arachidonic acid can inhibit the process of biosynthesis in bacteria in vivo, which will affect the composition of the bacterial cell membrane. This can cause

Table 6 Correlation analysis between the antibacterial activity and phenolics content of the fruit oil from *Z. lotus*

Antibacterial activity	Regression equation	R^2	r	P
E. coli (ATCC 25,922)	IZ = 13.77 + 9.23 × TPC	0.057	0.003	0.964
S. aureus (ATCC 27,923)	IZ S = 13.18 + 7.25 × TPC	0.893	0.798	0.297
S. pneumoniae	IZ = 11.73 + 0.29 × TPC	0.893	0.798	0.297

R^2 Coefficient of determination, r Pearson correlation coefficient, P Probability level

Table 5 Antibacterial activity of the fruit oil from *Z. lotus*

	Bacteria	(IZ) (mm ± SD) *Z. lotus*	MIC (mg mL^{-1}) *Z. lotus*	(IZ) (mm ± SD) Chloramphenicol	(IZ) (mm ± SD) Cefoxitin	(IZ) (mm ± SD) Gentamycin
Gram(−)	*E. coli* (ATCC 25,922)	14.00 ± 0.2^a	0.4 ± 00	24.00 ± 0.52^a	–	–
	S. typhimurium	–	–	25.13 ± 0.22^b	–	–
Gram(+)	*S. aureus* (ATCC 27,923)	15.00 ± 0.1^b	0.5 ± 00	–	17.33 ± 0.76	–
	S. pneumoniae	19.00 ± 0.4^c	0.2 ± 00	–	–	44.63 ± 0.52

the fluidity and permeability of bacterial cell membranes to become unstable and undergo lysis. Linoleic acid, is the most inhibiting against Gram-positive organisms. Dilika et al. [45] described the antibacterial activity of linoleic and oleic acids isolated from the leaves of *Helichrysum pedunculatum*. The MIC of each of the fatty acids alone against *S.aureus* and *M. kristinae* was 1 mg mL^{-1}, but when administered in combination, the MIC was 0.05 mg mL^{-1}, indicating a strongly synergistic effect.

3 Materials and Methods

3.1 Chemical Reagents

All the solvents, i.e., methanol, hexane, and dimethylsulfoxide (DMSO), were purchased from Biochem Chemopharma CO (Canada), as were Potassium ferricyanide, ferrous chloride, ferric chloride, Folin–Ciocalteu's reagent (FCR), trichloroacetic acid (TCA). 1,1-Diphenyl-2-picrylhydrazyl (DPPH) and butylated hydroxytoluene (BHT) were purchased from Sigma-Aldrich (Germany). All solvents and reagents used were of the highest analytical grade and purity.

3.2 Plant Material

The fruits of *Z. lotus* were harvested from the south-western part of Algeria (El Aghouat city) at coordinates (N 33°48'00" and E 2°51'54") between August and September 2018. Botanical identification of plant materials was authenticated by Pr. Omar Idoude, affiliated with the Department of Biology Science, Kasdi Merbeh-Ouargla University, Algeria, where the voucher specimens have been preserved (DP2018-1).

3.3 Oil Extraction

100 g of ground *Z. lotus* fruits were placed into a cellulose paper cone and extracted with hexane using a Soxhlet extractor for 3 h at 60 °C. The oil was then recovered by evaporating the solvent using a rotary evaporator. The residue was weighed and stored until it was analyzed. The weight of oil extracted from 100 g of fruit powder was determined to calculate the lipid content. The result was expressed as the lipid percentage in the dry fruit powder.

3.4 Physicochemical Properties of Z. lotus Fruit Oil

Density was determined by filling a pycnometer with oil, measuring its mass, and devising the result by the volumetric mass of water.

Refractive index was determined using a manual refractometer.

Acid index was estimated according to the standard [46].

Saponification index was defined according to the standard [47].

3.5 Identification of Fatty Acids by Gas Chromatography (GC)

Fatty acid composition was determined as fatty acid methyl esters (FAMEs). The sample (0.05 g) was weighed and dissolved in 1 mL of hexane. To the mixture, a potassium methoxide solution was added (0.2 mL $KOCH_3$ 2 mol L^{-1} in anhydrous methanol) and then mixed for 1 min using a vortex mixer. After sedimentation of potassium glycerolate, 1 µL of the clear supernatant was injected into a fused silica capillary column DB23 capillary column (60 m length, 0.32 mm i.d., and 0.25 µm film thickness; HP Agilent Technologies, Wilmington) and analyzed using a model 5890 Series II instrument (Hewlett-Packard, 105 Palo-Alto, Ca, USA) gas chromatograph equipped with a flame ionization detector. Injection and detection temperature was 250 °C. The oven temperature was set at 130 °C, increased to 170 °C at 6.5 °C/min, then augmented again to 215 °C at 2.8 °C/min, and held there for 12 min. Finally, it was increased to 230 °C at 40 °C/min and maintained for 20 min.

Injector and detector temperatures were set at 270 °C and 280 °C, respectively. Nitrogen was used as the carrier gas at 1 ml/min, and the split ratio was set at 1:5.

The fatty acid methyl esters (FAMEs) were identified by comparing their retention times with respect to pure standard FAMEs purchased from Sigma and analyzed under the same conditions. Each *Z. lotus* fruit fatty acid was quantified by calculating its peak area relative to the total peak area and expressed as fatty acid content relative to total lipid content (%) [48].

3.6 Extraction of Phenolics Content

To extract the phenolic compounds from Z. lotus oil, we adopted the protocol of Pirisi et al. [49]. Briefly, 10 g of olive oil was dissolved in 50 ml of hexane, followed by addition of 20 ml of aqueous methanol (60%) and vigorous mixing for 2 min. The methanolic phase was removed and placed in a beaker each time the two phases were separated. The combined extracts were laid out to dry in a vacuum rotary evaporator at 40 ºC. The residue was dissolved in 1 ml of methanol.

3.7 Determination of Total Phenolics Content (TPC)

Total phenolics content analysis of the *Z. lotus* fruit oil were performed using Folin-Ciocalteu's phenol reagent and the spectrophotometer method of Obiang-Obounou [50]. Briefly, 0.1 mL of phenolic fraction of oil was added to

450 µL of distilled water and 500 µL of the Folin- Ciocalteu's phenol reagent. The mixture was shaken and allowed to stand for 5 min, before the addition of 1.5 mL of 6% Na_2CO_3. After 90 min of incubation in the darkness at room temperature (25 °C), absorbance was measured at 725 nm using a UV–Vis spectrophotometer (Spectro Sean 800 V). Gallic acid was used for the calibration curve.

The results were expressed as gallic acid equivalents (GAE) per 100 g of oil (mg GAE/100 g *Z. lotus* oil). Measurements were performed in triplicate.

3.8 Antioxidant Activity

3.8.1 DPPH Radical Scavenging Activity

The antioxidant activity of *Z. lotus* fruit oil was evaluated by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging capacity assay [51]. Briefly, 1 mL of phenolic fraction was diluted in ethanol at different concentrations and mixed with 1 ml of DPPH solution prepared at 0.4 mM in ethanol. After 30 of min incubation in the darkness at 25 °C, the absorbance of the sample at 520 nm was read. A mixture of 1 mL of DPPH. solution and 1 mL of ethanol was used as a negative control. The decrease in absorption induced by the samples was compared to that of ascorbic acid, used as a positive control. The antioxidant potential of the samples was given as a percentage of inhibition (I%) of free radical DPPH at different concentrations. I% was expressed on the basis of the following formula:

$$I\% = \left[\left(A_{\text{negative control}} - A_{\text{sample}}\right)/A_{\text{negative control}}\right] \times 100 \quad (1)$$

$A_{\text{negative control}}$ represents the absorbance of the negative control. A_{sample} represents the absorbance of the studied sample.

As for the inhibitory concentrations (IC_{50}), they are calculated from the curves of linear regression. All the measurements were performed in triplicate.

3.8.2 Reducing Power Assay

The ferric-reducing power of *Z. lotus* fruit oil was determined as described by Jlizi et al. [52]. 1 mL of different concentrations of the phenolic fraction, as well as a chlorogenic acid as reference for comparative purposes, were added to 2.5 mL of phosphate buffer (pH 6.6) and 2.5 mL of potassium ferricyanide [$K_3Fe(CN)_6$] solution (1%). Later, the mixture was incubated at 50 °C for 20 min and then 10% trichloroacetic acid was added. The mixture was shaken vigorously, and this solution was mixed with distilled water and $FeCl_3$ (0.1%, w/v). After 30 min incubation, absorbance was read at 700 nm. Increased absorbance of the reaction meant increased reducing power. Ascorbic acid was used as a positive control. The EC_{50} value is the effective concentration at which the absorbance was 0.5 for reducing power and was obtained by interpolation from linear regression analysis [53]. All the measurements were performed in triplicate.

3.8.3 Antibacterial Activity

3.8.3.1 Bacterial Strains In vitro antibacterial activity of the oil extract was tested against two Gram-positive strains, (*Streptococcus pneumonia* and *Staphylococcus aureus*) and two Gram-negative strains (*Salmonela typhi* and *Escherichia coli*). The microbial strains were obtained from the culture collection of the Laboratory of Microbiology, Faculty of Natural and Life Sciences, Kasdi- Merbah University of Ouargla.

3.8.3.2 Disc Diffusion Method The antibacterial activity of the *Z. lotus* fruit oil was evaluated with the disc diffusion method using Mueller Hinton Agar (MHA) with determination of the diameter inhibition zones (IZ). Inocula were prepared by diluting overnight (24 h at 37 °C) cultures in Muller Hinton broth medium to approximately 10^6 colony-forming units per milliliter (CFU mL^{-1}). Absorbent discs (diameter 6 mm, Whatman Paper No. 3) were impregnated with 10 µL of sample and then placed on the surface of the inoculated plates (90 mm diameter). The plates were kept at 4 °C for 1 h before incubation at 37 °C for 24 h [52]. Then the diameters of the inhibition zones were measured. Positive control discs of chloramphenicol, cefoxitin, and gentamycin (10 µg/disc, Bio-Rad) were included in each assay, and the developing inhibition zones were compared with those of the reference disc. The assays were performed in triplicate.

3.8.3.3 Minimum Inhibition Concentration (MIC) The estimation of the minimal inhibition concentration was carried out by a microliter plate dilution method [54]. Dilutions of the *Z. lotus* fruit oil were prepared to obtain concentrations ranging from 0.1–0.9 mg mL^{-1}. Dimethylsulfoxide (DMSO) solution was employed for sample dilution at a concentration of 10%. The MIC values were considered the lowest fruit oil fraction concentrations that prevent visible bacterial growth after 24 h of incubation at 37 °C. At the end of incubation period, the plates were evaluated for the presence or absence of growth. Chloramphenicol, cefoxitin, and gentamycin were employed as a positive control against the tested bacteria.

3.9 Statistical Analysis

The recorded data were expressed means ± SD (standard deviation) and were statistically analyzed using XLSTAT statistical software version 2009.6.01. The means were

compared using a Tukey's HSD test at a threshold of 5% to highlight homogeneous groups. In addition, we performed correlations and linear regression equations in pairs among the antioxidant activity, antibacterial activity, and total phenolic content according to Bravais-Person correlation test.

4 Conclusion

The present study revealed that *Ziziphus lotus* fruit oil, seems to be a promising source of fatty acids and polyphenolic compounds, this oil contained 20 fatty acids. Our findings indicate that the oil is characterized by oleic acid (61.8%) as the main fatty acid and that it possesses in vitro antibacterial and antioxidant properties.

Although more research is needed, so far as the nutritional composition is concerned, the *Z. lotus* fruit are a potential source of vegetable oil with economic benefit to populations, which may be used in various fields such as food, cosmetics, and pharmaceutics with pre-refining and packaging precautions.

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Data availability All data cited in the article are available from the corresponding author.

Declarations

Conflict of interest The authors have no competing interests to declare that are relevant to the content of this article.

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