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Research Article

Chemical Composition and Antioxidant Activity of the Fruit Essential Oil of *Zizyphus lotus* (L.) Desf. (Rhamnaceae)

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ABSTRACT

The essential oil of the fruit of *Zizyphus lotus* (L.) Desf. belonging to the Rhamnaceae family, was obtained by steam distillation and analyzed by GC-FID and GC-MS. 38 components were identified corresponding to 92% of the total oil. Fatty acids represented the major fraction (78.9%), followed by hydrocarbons (10.8%) while terpenic fraction constituted only 1.1% of the oil (α- and β-eudesmol). The fatty acids fraction contained 23 saturated and unsaturated compounds (67.8 and 11%, respectively) from C_8 to C_{18}. The major constituents are in decreasing order: ethyl hexadecanoate (12%), decanoic acid (11%), ethyl dodecanoate (9.4%), ethyl hexadec-9-enoate (7.9%), dodecanoic acid (6.5%), ethyl tetradecanoate (6.1%) and tetradecanoic acid (5%). Several studies described the fatty acid composition of different parts of Z*izyphus* species in the fixed oil. Our study is the first report devoted to the chemical composition of the essential oil of the fruit of this species. The antioxidant property of this oil was evaluated using β-carotene bleaching method.

Keywords: *Zizyphus lotus* L. (Desf.), Rhamnaceae, Essential oil composition, Fatty acids, Antioxidant capacity.

INTRODUCTION

Zizyphus species have wide distribution and uses worldwide in traditional medicines for many purposes[1-3]. The genus belonging to the Rhamnaceae family, contains about 100 species deciduous or evergreen trees and shrubs distributed in the tropical and subtropical regions of the world[4]. In Algeria, this genus is represented by four species[5]. *Zizyphus lotus* (L.) Desf. also, known as Jujube « Sedra » is a Mediterranean species widely distributed in North Africa, Septentrional Sahara and Southern Europe countries[6,7]. Leaves of *Zizyphus* species, have hypoglycemic effect and antiseptic activity[8,9]. The flower infusion is used as febrifuge and disinfectant for eyes[10]. The fruit of *Z. lotus* is a valuable source of nutrients as well as antioxidant[11,12], antimicrobial and antifungal[13], immunosuppressive[14], anti-inflammatory[15], and antiulcerogenic[12,16] compounds. The fruits have also antitumoral effects[17], while the roots extract has antifungal and antibacterial activities[18]. Fruit of *Zizyphus lotus* of North Africa is delicious and consumed directly due to its high nutritional value. This organ is rich in minerals (Ca, Mg, Na, K and phosphorus), carbohydrates, fatty acids and proteins[10]. The fruit pulp of this plant contained higher contents of vitamin A and C compared to the other parts[19]. In continuation of our ongoing program on medicinal and aromatic species from Algeria[20-24], we investigated the chemical composition of the essential oil of *Zizyphus lotus* (L.). Several studies on fixed oil of *Zizyphus lotus* showed that oleic and linoleic acids were the major components of the seeds and fruit[10,25-27]. Our results on the essential oil of the fruit were quite different and showed diverse proportions of fatty acids. To the best of our knowledge, this is the first study devoted to the chemical composition of the essential oil of the fruit of this species.

MATERIALS AND METHODS

Plant material

The fruits of *Zizyphus lotus* (L.) Desf. were collected on December 2014 in the region of Ouled Fadhel Batna (Aures), and authenticated by professor Mohamed Kaabeche, Setif 1 university, Algeria.

Extraction of the essential oil

The aerial parts (450 g) of the fruit of *Zizyphus lotus* were subjected to steam distillation in a Kaiser Lang apparatus for three hours. The obtained essential oil was collected and dried over anhydrous sodium sulphate and kept at 4°C until analysis. The yield of the oil was calculated in relation of the dry weight of the plant.

GC-FID Analysis

The essential oil was analyzed on an Agilent gas chromatograph (GC-FID) Model 6890, equipped with a HP-5MS fused silica capillary column (5%-diphenyl-95%-

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Table 1: Composition of *Zizyphus lotus* fruit essential oil.

Peak N°	RT	[b]RI	[a]Components	%
1.	9.734	1167	Octanoic acid	3.1
2.	10.825	1260	(E)-Dec-2-enal	0.6
3.	11.075	1287	Nonanoic acid	2.4
4.	12.52	1390	Decanoic acid	11.0
5.	12.619	1397	Ethyl decanoate	4.8
6.	13.2	1443	Ethyl-(2E)-dec-2-enoate	0.7
7.	13.604	1476	Undecanoic acid	2.1
8.	13.858	1496	Ethyl undecanoate	2.8
9.	14.808	1565	Dodecanoic acid	6.5
10.	15.049	1594	Ethyl dodecanoate	9.4
11.	15.782	1662	β-Eudesmol	0.6
12.	15.804	1664	α-Eudesmol	0.5
13.	16.143	1695	Ethyl tridecanoate	1.1
14.	16.198	1697	Pentadecan-2-one	0.7
15.	16.947	1770	Tetradecanoic acid	5.0
16.	17.066	1781	(Z)-Tetradec-9-enoic acid	0.3
17.	17.132	1788	(E)-Tetradec-9-enoic acid	0.3
18.	17.22	1796	Ethyl tetradecanoate	6.1
19.	17.698	1843	6,10,14-Trimethyl-pentadecan-2-one	1.3
20.	17.898	1863	Phtalate	0.8
21.	18.218	1894	Ethyl pentadecanoate	0.8
22.	18.53	1926	Methyl hexadecanoate	0.2
23.	18.975	1972	Ethyl (Z)-hexadec-9-enoate	7.9
24.	19.08	1983	Ethyl (E)-hexadec-9-enoate	0.6
25.	19.22	1997	Ethyl hexadecanoate	12.0
26.	20.11	2085	Ethyl heptadecanoate	0.3
27.	20.77	2163	Ethyl (Z)-octadec-9-enoate	0.7
28.	20.827	2170	Ethyl (E)-octadec-9-enoate	0.5
29.	21.011	2193	Ethyl octadecanoate	0.3
30.	21.922	2300	Tricosane	0.3
31.	21.988	2308	Tricosan-2-one	0.6
32.	22.744	2399	Tetracosane	0.3
33.	23.536	2500	Pentacosane	0.5
34.	24.262	2595	Hexacos-9-ene	0.2
35.	24.297	2600	Hexacosane	0.3
36.	25.046	2702	Heptacosane	3.7
37.	25.746	2800	Octacosane	0.5
38.	26.446	2903	Nonacosane	3.3
Total identified				92.0
Grouped compounds				
hydrocarbons				10.8
Fatty acids				78.9
sesquiterpenes				1.1

[a]Compounds are listed in order of their RI

[b]RI (retention index) measured relative to *n*-alkanes (C_8-C_{20}) using HP-5MS column.

dimethylpolysiloxane, 25 m x 0.25 mm, film thickness 0.25 µm), programmed from 50°C (5 min) to 250 °C at 3°/min and held for 10 min. Injector and flame ionization detector temperatures were 280 and 300 °C, respectively. The essential oil was diluted in acetone (3.5%, *v/v*) and injected in split mode (1/60), helium was used as a carrier gas (1.0 mL/min). Solutions of standard alkanes (C_8-C_{20}) were analyzed under the same conditions to calculate retention indices (RI) with Van del Dool and Kratz equation.

GC-MS Analysis

Mass spectrometry was performed on an Agilent gas chromatograph-mass spectrometer (GC-MS) Model 7890/5975, equipped with HP-5MS capillary column (25 m x 0.25 mm, film thickness 0.25 µm) programmed with the same conditions as for GC-FID. The mass spectrometer (MS) ionization was set in positive electron impact mode at 70 eV and electron multiplier was set at 2200 V. Ion source and MS quadrupole temperatures were 230 °C and 180 °C, respectively. Mass spectral data were acquired in the scan mode in the *m/z* range 33-450. The essential oil constituents were identified by matching their mass spectra and retention indices (RI) with those of reference

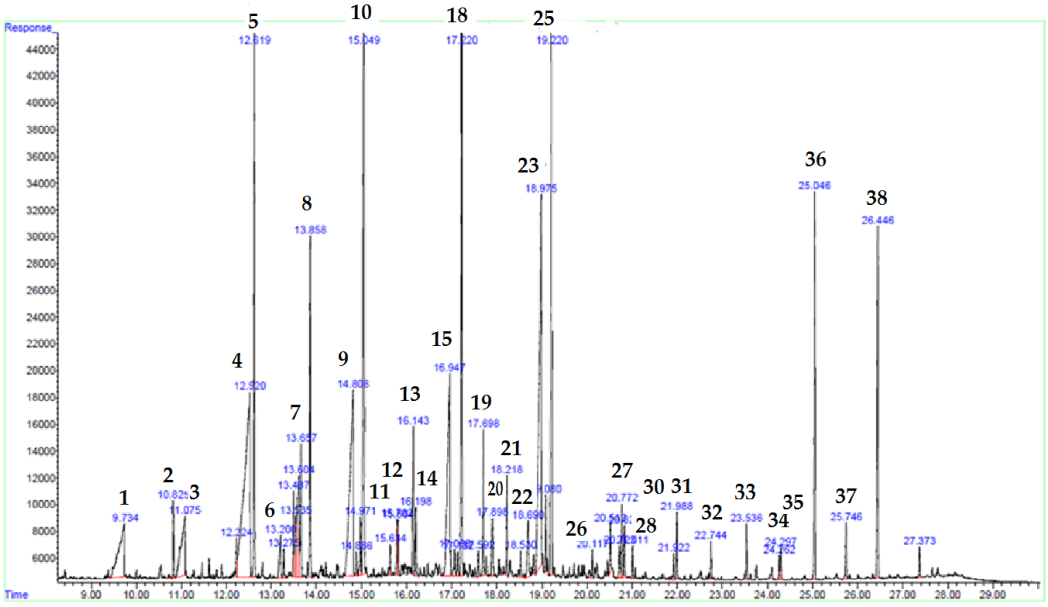

Figure 1: GC-FID Chromatogram of *Zizyphus lotus* fruit essential oil.

Table 2: Linoleic acid peroxidation activity (%) of the fruit of *Zizyphus lotus* (L .).

Inhibition of linoleic acid peroxidation (%)	
Zyziphus lotus(L.)	81.61±11.37
Negatif control	18.66±3.21
BHA	92.28±2.46

Values are mean ± SD of three samples analyzed individually in triplicate at p <0.05

compounds from libraries such as Adams[28] and McLafferty & Stauffer[29]. The proportions of the identified compounds were calculated by internal normalization.

β-carotene bleaching method

The test was carried out following the spectrophotometric method of Miller[30] based on the ability to decrease the oxidative bleaching on β-carotene in a β-carotene/linoleic acid emulsion.

The essential oil of *Zizyphus lotus* was prepared at a concentration of 2 mg/mL in methanol. Then, a solution of ß-Carotene was prepared by dissolving 0.5 mg of ß-carotene in 2 mL of chloroform. 25 μL of linoleic acid and 200 mg of Tween 40 were added. The chloroform was removed at 40 °C under reduced pressure, 100 mL of oxygenated water were added under vigorous shaking until the formation of an emulsion.3.5 mL of this emulsion was transferred into a tube containing 500 μL of essential oil. The zero time absorbance was read at 490 nm. Absorbance readings were recorded at 48 hours until the visual color of ß-carotene in the control sample disappeared. The antioxidant activity (Inhibition %) was calculated using the following equation:

Inhibition % = ($A_{\text{ß-carotene after 48 hours assay}}$ * 100) / $A_{\text{initial ß-carotene}}$)

Where; $A_{\text{ß-carotene after 48 hours assay}}$ is the absorbance of ß-carotene after 48 hours assay remaining in the samples, and $A_{\text{initial ß-carotene}}$ is the absorbance of initial ß-carotene at the beginning of the experiment. All tests were carried out in couple.

RESULTS AND DISCUSSION

The steam distillation of the essential oil of *Zizyphus lotus* (L.), gave a viscous liquid. The yield of essential oil was 0.005% (*w/w*) in relation to the dry weight of the plant. The analysis and identification of the compounds of the essential oil was performed using the (GC-MS). The general chemical profile of the essential oil, the percentage content and retention indices of the constituents are summarized in Table 1 and Figure 1. This investigation allowed the identification of 38 constituents corresponding to 92% of the total oil. Among the identified constituents, fatty acids compounds represented 78.9% , 10.8% were hydrocarbons and 1.1% were sesquiterpenes. The fatty acids fraction contained 23 saturated and unsaturated compounds (67.8 and 11%, respectively) from C_8 to C_{18} (Table 1). The major constituents are in decreasing order: ethyl hexadecanoate (12%), decanoic acid (11%), ethyl dodecanoate (9.4%), ethyl hexadec-9-enoate (7.9%), dodecanoic acid (6.5%), ethyl tetradecanoate (6.1%), tetradecanoic acid (5%), ethyl decanoate (4.8%), octanoic acid (3.1%), ethyl undecanoate (2.8%), nonanoic acid (2.4%) and undecanoic acid (2.1%). The major hydrocarbons were heptacosane (3.7%) and nonacosane (3.7%) while the only sesquiterpene found were α-and β-eudesmol (0.6% and 0.5% respectively). These results were quite different from those obtained previously on the fixed oil of the fruit of this species from two samples from Al Mader region (Aures) and Djelfa (Southwest of Algeria) which indicated that the major fatty acid was oleic acid (49.88% and 62.79%, respectively). To the best of our knowledge, this is the first report on essential oil composition of *Zyziphus lotus.*

β-carotene bleaching method : Table 2 shows the decrease in absorbance during the coupled oxidation of β -carotene and linoleic acid. The β-carotene bleaching test was selected for antioxidant activity determination because it is carried out in an emulsion, a situation frequent in foods.

On the other hand, it is generally agreed that the oxidation is initiated by free radical attack; therefore, assays to evaluate the radical scavenging activity are representative of the potential of a compound to retard oxidation. The antioxidant capacities were estimated to be 92.28% and 81.61% for BHA and *Z. lotus*, respectively. This result indicated the potent antioxidant activity of essential oils from *Z. lotus* which can be related to fatty acid compounds.

CONCLUSION

We report for the first time the essential oil composition of *Zyziphus lotus* collected from the area of Batna in the Northeast of Algeria. Analysis by GC-FID and GC-MS allowed the identification of 23 fatty acids representing 78.9% of the total oil. The major components were: ethyl hexadecanoate (12%), decanoic acid (11%), ethyl dodecanoate (9.4%), ethyl hexadec-9-enoate 7.9%, dodecanoic acid (6.5%), ethyl tetradecanoate (6.1%) and tetradecanoic acid (5%). Hydrocarbons represented 10.8% while sesquiterpene fraction reached only 1.1%. These results differ from those obtained previously on the fixed oil of the fruit of this species. The result of antioxidant capacity of the oil evaluated by the β-carotene bleaching method, showed the potent activity of the essential oil of this species.

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