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Composition and biological activities of seeds oils of two *Crataegus* species growing in Algeria

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- ✓ Antioxidant;
- ✓ Antibacterial

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Abstract

The oils extracted from seeds of two *Crataegus* species **(**Rosaceae), *Crataegus azarolus* L. and *Crataegus monogyna* Jacq. collected from Constantine (Eastern Algerian), were analyzed by GC and GC/MS. The main components of *C. azarolus* were tetradecamethylcycloheptasiloxane (39.43%), 3,4-dihydroxytetramethylsilyl mandelic acid (19.23%), dodecamethylcyclohexasiloxane (17.14%), decamethyl-cyclopentasiloxane (10.57%) and 3-isopropoxy-1,1,1,7,7,7-hexamethyl-3,5,5 tris(tri-methylsiloxy)tetrasiloxane (5.66%). It's the first report on *C. monogyna* seeds oil. The major constituents of *C. monogyna* seeds oil were found to be linoleic acid (44.2%), oleic acid (28.26%), oxalic acid, bis(trimethylsilyl) ester (9.74%), palmitic acid (6.56%) and tetracosamethylcyclododecasiloxane (5.04%). The Antioxidant activity was investigated by the use of β-carotene bleaching method. Antibacterial activity was performed according to disc diffusion and minimum inhibitory concentration (MIC) methods.

1. Introduction

Crataegus species (Rosaceae), known as "Hawthorn" are widely distributed throughout the northern temperate regions of the world with approximately 280 species [1]. Previous investigations indicated that Chinese *Crataegus* extracts have beneficial effects such as anti-inflammatory, hypolipidemic, antioxidant anticarcinogenic and antimicrobial [2-6]. Several ethnopharmacological surveys on the therapeutic uses of *Crataegus azarolus* L., the predominant species which populate the mountains of the Mediterranean basin, revealed its use in the Arab traditional medicine to treat cardiovascular diseases, as well as cancer and diabetes [7]. *Crataegus monogyna* Jacq. is commonly used for the treatment of circulatory and respiratory system disorders, insomnia and some nervous system disorders, such as memory loss, migraines, irritability and confusion [8-11]. In another hand, vegetable oils are known to be natural products that contained mixtures of esters derived from glycerol that have chains of fatty acid with 14 to 20 carbon atoms that have different degrees of unsaturation [12]. Vegetable oils have an important functional and sensory role in food products because of their fatty acids composition. They are also sources of energy and essential fatty acids like linoleic and linolenic that are responsible for growth and the health of organisms [13]. The main objectives of this study were to determine the composition of the vegetable oils from *Crataegus azarolus* and *Crataegus monogyna* grown at Constantine (Eastern Algerian) and to evaluate their antioxidant and antibacterial activities.

2. Experimental details

2.1. *Plant material*

Crataegus azarolus L. and *Crataegus monogyna* Jacq. were collected on September-October 2014 at Constantine (Eastern Algerian)

2.2. *Extraction*
Seeds oils of *Crataegus azarolus* L. and *crataegus monogyna* Jacq. were obtained by cold pressed extraction method yielding 2.1% and 1.95% of yellow seeds oils, respectively.

2.3. Gas chromatography
GC analyses of the vegetal oils were performed using an HP (Agilent technologies) 6800 plus chromatograph coupled with an HP (Agilent technologies) MSD 5973 selective detector, using an hp-INNOWAX column (30m×0.25 mm, film thickness 0.25 μm). The oven temperature was programmed at 120 °C for 2 min, then raised to 200 °C at 10 °C/min and held at this temperature, for 15 min, then raised to 240 °C for 2 min. The seeds oils were diluted in hexane (34/550) for the analyses.

2.4. *Gas chromatography-mass spectrometry*
GC-MS analyses were performed on an HP (Agilent technologies) 6800 plus chromatograph coupled with an HP (Agilent technologies) MSD 5973 selective detector, using an hp-INNOWAX column (30m×0.25 mm, film thickness 0.25 μm). The oven temperature was programmed at 120 °C for 2 min, then raised to 200 °C at 10 °C/min and held at this temperature, for 15 min, then raised to 240 °C for 2 min. Helium was used as the carrier gas at a rate 0.5 ml/min. 0.1 μL oil was introduced directly into the source of the MS, *via* a transfer line (280 °C) with a split ratio of 1:50 and a linear velocity of 30.0 cm/sec. Ionization was obtained by electron impact (70eV, source temperature 230 °C, resolution 1000).

2.5. Identification of components
Retention indices of all the components were determined by Kovats method. The compounds assayed by GC were identified by comparing their retention indices with those of reference compounds in the literature and confirmed by comparison of their mass spectra with those of reference substances for major components of the oils [14-15].

2.6. Antioxidant activity
The antioxidant activity was evaluated using the β-carotene-linoleic acid test system [16, 17]. β-Carotene (0.5 mg) in 1 mL chloroform was added to 25 mL of linoleic acid and 200 mg of Tween 40 emulsifier mixture. After evaporation of chloroform under *vacuum*, 100 mL distilled water saturated with oxygen was added, followed by vigorous shaking. Then 4.0 mL of this mixture was transferred into different tubes containing different concentrations of the sample. As soon as the emulsion was added to each tube, the zero time absorbance was measured at 470 nm using a spectrophotometer. The emulsion system was incubated for 2 h at 50 °C. A blank devoid of β-carotene was prepared for background subtraction. Vitamin E was used as a standard. The bleaching rate (R) of β-carotene was calculated according to the following equation:

$$R = \ln(a/b)/t,$$

where ln is natural log, a is absorbance at the time zero, and b is absorbance at the time (120 min).
The antioxidant activity (AA) was calculated in terms of percent inhibition relative to the control using the following equation:

AA (inhibition %) = [(Absorbance of the control – Absorbance of the sample) /Absorbance of the control]*100.

2.6. *Antibacterial activity*
Susceptibility of the bacterial strains to the seeds oil was investigated using the disk diffusion method and by comparing their antibiogram inhibition zones to those reported by the National Committee for Clinical Laboratory Standards (NCCLS). Disks containing freshly prepared seeds oil were used for antibacterial activity assays. The diameters of inhibition zones were measured and compared with those suggested by NCCLS (sensitive P≥15 mm). The susceptibility of the strains to the seeds oil was further evaluated by agar dilution method, different concentrations of the seeds oil were included in Mueller-Hinton agar plates (sensitive MIC ≤ 32 μg/ml). The minimum inhibitory concentration (MIC) was defined as the concentration at which no colony was observed after incubation [18]. The agar plates were prepared and inoculated with bacterial suspension. After incubation at 37°C for 18–24h, the inhibition zones were measured and averaged. The essays were performed in triplicate. MICs of the seeds oils were also determined by an agar dilution method.

3. Results and Discussion

3.1. Chemical composition

From GC and GC/MS nine components were identified in the seeds oil of *C. azarolus* L., representing 97.81 % of the total oil content (figure 1). The seeds oil is characterized by the main presence of tetradecamethylcycloheptasiloxane (39.43%), 3,4-dihydroxy tetramethylsilyl mandelic acid (19.23%), dodecamethylcyclohexasiloxane (17.14%), decamethylcyclopentasiloxane (10.57%) and 3-isopropoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris(trimethylsiloxy)tetrasiloxane (5.66%) (table1).

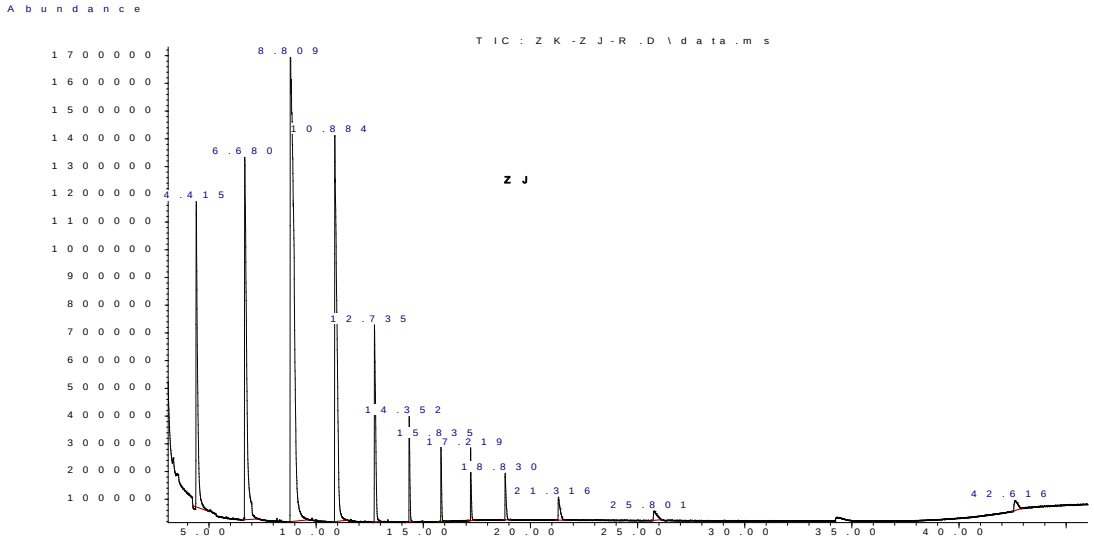

Figure 1. The GC-MS Chromatogram of seeds oil of . *azarolus* L.

Table 1: Composition of oil seeds from fruits of *Crataegus azarolus* L.

N°	Compounds	RI[a]	(%)
1	Decamethylcyclopentasiloxane	1034	**10.57**
2	Oxalic acid, bis(trimethylsilyl) ester	1100	0.77
3	Dodecamethylcyclohexasiloxane	1240	**17.14**
4	Tetradecamethylcycloheptasiloxane	1447	**39.43**
5	3-Isopropoxy-1,1,1,7,7,7-hexamethyl-3,5,5 tris(trimethylsiloxy)tetrasiloxane	1648	**5.66**
6	Mandelic acid, 3,4-Dihydroxy tetramethylsilyl	1853	**19.23**
7	Phenyllactic acid bis(trimethylsilyl)	1886	0.72
8	Eicosamethylcyclodecasiloxane	2067	1.92
9	Tetracosamethylcyclododecasiloxane	2480	2.37
	Total (%)		**97.81**

[a] RI (retention index) as determined on a DB-5MS column,

Thus, it appears that *Crataegus azarolus* L. seeds are a good source of siloxanes which are widely used in various industrial processes and consumer products such as detergents, shampoos, cosmetics, paper-coatings and textile because of several beneficial properties and are moreover feedstock chemicals for the production of silicones [19].

The present composition of the seeds oil of *C. azarolus*, collected from Constantine, is quite different from that of the seeds oil of *C. azarolus* growing in Batna (Algeria) which was mainly characterized by palmitic, oleic and linoleic acids [20], neither that from Kurdistan region (Iraq); mainly represented by linoleic acid and palmatic acid [21].

Ten components were identified in the seeds oil of *C. monogyna* Jacq., representing 98.17 % of the total oil content (figure 2, table 2). The results showed that the major constituents of *C. monogyna* seeds oil were found to be linoleic acid (44.2%), oleic acid (28.26%), oxalic acid bis(trimethylsilyl) ester (9.74%), palmitic acid (6.56%) and tetracosamethylcyclododecasiloxane (5.04%). Thus, *C. monogyna* seeds oil was dominated by fatty acids. It can be concluded that *C. monogyna* seeds oil is an excellent source of essential fatty acids omega-6 (linoleic acid 44.22 %) and omega-9 (oleic acid 28.26 %).

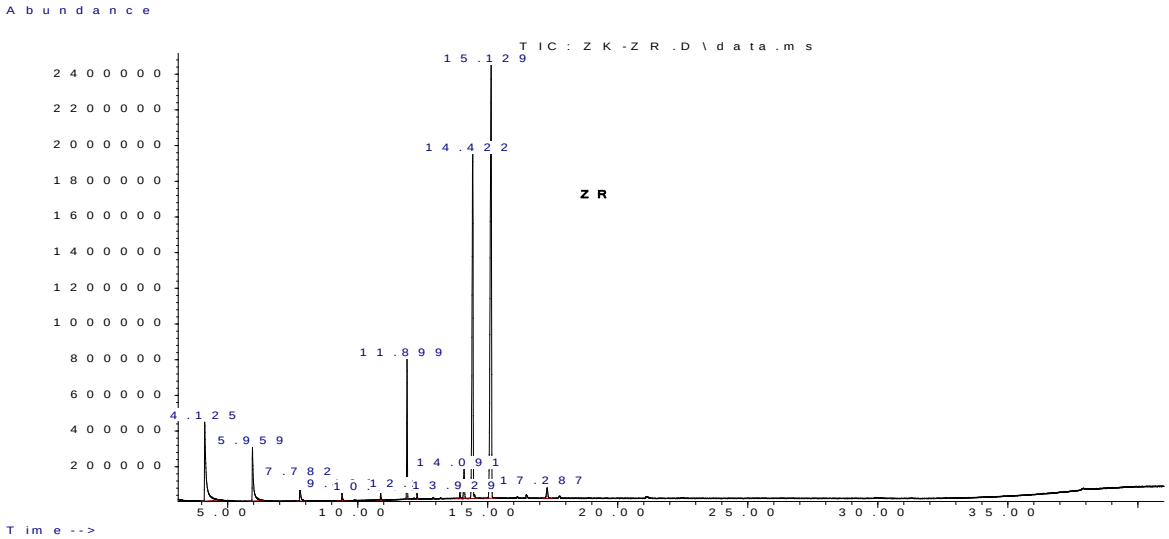

Figure 2. The GC-MS Chromatogram of seeds oil of *C. monogyna*

Table 2 : Composition of oil seeds from fruits of *C. monogyna* Jacq.

N°	Compounds	RI[a]	(%)
1	Acetic acid, [(trimethylsilyl)oxy] -, trimethylsilyl ester	880	0.36
2	Oxalic acid, bis(trimethylsilyl) ester	1100	**9.74**
3	Malonic acid, bis(2-trimethylsilyl ethyl ester	1468	0.49
4	*trans*-2-Hexenedioic acid, trimethylsilyl	1501	0.34
5	Palmitic acid	1968	**6.56**
6	Oleic acid	2105	**28.26**
7	Linoleic acid	2197	**44.22**
8	Stearic acid	2167	2.11
9	Eicosanoic acid	2366	1.05
10	Tetracosamethylcyclododecasiloxane	2480	**5.04**
	Total (%)		**98.17**

[a] RI (retention index) as determined on a DB-5MS column,

3.2. *Antioxidant activity*

As shown in table 3, the seeds oil of *C. monogyna* exhibited a higher antioxidant activity than the seeds of *C. azarolus* with 77.75 % inhibition at 4 mg/mL which is close to the standard vitamin E (80.5%), at the same concentration. This can be explained by the richness of *C. monogyna* with linoleic, oleic and palmitic acids which are known for their antioxidant activity [22-30].

Table 3: Antioxidant activity of the seeds oils of *C. azarolus and C. monogyna* (Inhibition %)

Oils	Inihibition (%)		
	4mg/mL	2mg/mL	1mg/mL
C.azarolus L.	58.40	56.19	47.34
C.monogyna Jacq.	75.77	61.44	49.47
Standard	(vitamin E, 80.5% at 4 mg/mL and 73.9% at 2 mg/mL)		

3.3. *Antibacterial Activity*

The antibacterial activity of seeds oils was tested against a range of bacteria, namely, *Escherichia coli* ATCC 25922, *Escherichia coli* (HS), *Staphylococcus aureus* ATCC 43300, *Staphylococcus aureus* (HS), *Pseudomonas aeruginosa* ATCC 27853, *Pseudomonas aeruginosa* (HS), *Enterobacter aerogenes* (HS), *Klebsiella pneumoniae* (HS), *Salmonella enterica* and *Morganella morganii*. The reference strains were obtained from the Pasteur Institute (Algiers). The hospital strains (HS) were obtained from the laboratory of bacteriology, Benbadis Hospital, Constantine, using conventional methods (clinical isolation). Antibacterial activity was evaluated by measuring the inhibition zone in mm against the tested strains and by the use of ampicillin and gentamicin as control.

C. Azarolus seeds oil exhibited the best antibacterial activity against *Escherichia coli* ATCC 25922, *Escherichia coli* (HS), *Salmonella enterica* (HS) and *Enterobacter aerogenes* with 14 mm, 13 mm, 14 mm and 13 mm inhibition zone diameters, respectively. However, *C. monogyna* oil was more selective against *Escherichia coli* ATCC 25922, *Salmonella enterica* (HS) and *Staphylococcus aureus* ATCC 43300 with 13mm, 12 mm, 14mm, respectively. Thus, from table 4, it can be concluded that *C. azarolus* and *C. monogyna* seeds oils possess a moderate antibacterial activity.

Table 4: Antibacterial Activity of the seeds oils of *C. azarolus and C. monogyna* (Inhibition zone diameters)

Microorganisms	Inhibition zone (mm)			
	C. azarolus oil[a]	***C. monogyna oil***[a]	**Amp**[b]	**Genta**[b]
Escherichia coli ATCC 25922[c]	14	13	18	22
Escherichia coli (HS)[d]	13	-	-	-
Pseudomonas aeruginosa ATCC 27853[c]	-	-	-	12
Pseudomonas aeruginosa (HS)[d]	-	-	-	-
Enterobacter aerogenes (HS)[d]	13	-	14	22
Klebsiella pneumonia (HS)[d]	-	-	-	-
Salmonella enterica (HS)[d]	14	12	-	-
Staphylococcus aureus ATCC 43300[c]	12	14	-	14
Staphylococcus aureus (HS)[d]	-	-	-	-
Morganella morganii (HS)[d]	13	-	-	-

[a]: seeds oils (128 µg/mL)
[b]: Control: Amp: Ampicillin (10 µg/mL); Genta: Gentamicin (10 µg/mL)
[c]: Obtained from the Pasteur Institute (Algiers)
[d]: Clinical isolates from the laboratory of bacteriology (CHU Constantine, Algeria)

MICs of the seeds oils were also determined by an agar dilution method. From table 5, the values were ranged from 20-40 µg/mL. The results of the MICs were in agreement with those of the disc diffusion method. Besides, its good antioxidant activity and because of its main fatty acids content (oleic, linoleic and palmitic acids) content which have been reported to possess an anticorrosive activity [31,32], the present seed oil of *Crataegus monogyna* could be tested as a corrosion inhibitor [33].

Table 5: Antibacterial activity of the seeds oils of *C. azarolus and C.monogyna* (MICs: Minimum inhibitory Concentration)

Microorganisms	MIC (µg /mL)			
	C. azarolus oil	***C. monogyna oil***	**Amp**[a]	**Genta**[a]
Escherichia coli ATCC 25922	40	40	10	10
Escherichia coli (HS)	40	-	-	-
Pseudomonas aeruginosa ATCC 27853	-	-	-	-
Pseudomonas aeruginosa (HS)	-	-	-	5
Enterobacter aerogenes (HS)	20	-	-	-
Klebsiella pneumonia (HS)	-	-	10	5
Salmonella enterica (HS)	40	40	-	-
Staphylococcus aureus ATCC 43300	20	40	5	-
Staphylococcus aureus (HS)	-	-	-	15
Morganella morganii (HS)	20	-	-	-

[a]: Control: Amp: Ampicillin (10 µg/mL) ; Genta: Gentamicin (10 µg/mL)

Conclusions

The seeds oils of *Crataegus azarolus* L. *and C. monogyna* Jacq., collected from Constantine (Eastern Algerian), were analyzed by GC and GC/MS. The main constituents of the oil of *C. azarolus* L. were found to be tetradecamethylcycloheptasiloxane (39.43%), mandelic acid, 3,4-dihydroxytetramethylsilyl (19.23%), dodecamethylcyclohexasiloxane (17.14%), decamethylcyclopentasiloxane (10.57%) and 3-isopropoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris(trimethylsiloxy)tetrasiloxane (5.66%). This oil seems to be a source of siloxanes. In addition, it is the first report on the composition of *C. monogyna* oil. Its major components were

linoleic acid (44,2%), %), oleic acid (28.26%), oxalic acid bis(trimethylsilyl) ester (9.74%), palmitic acid (6.56%) and tetracosamethylcyclododecasiloxane (5.06%). The seeds oils of *C. azarolus* L. and *C. monogyna* Jacq. showed a moderate antibacterial activity whereas *C. monogyna* oil exhibited a high antioxidant activity.

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