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Essential Oil from the Leaves of *Crataegus oxyacantha:* Chemical Composition and Antimicrobial Activity

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ABSTRACT: In this study, we performed the chemical characterization of *Crataegus oxyacantha* essential oil from Algeria and the assessment of its bioactivity in terms antibacterial, activity as starting point for possible applicative uses.

Leaves were analyzed its essential oil by gas chromatography-mass spectrometry (GC-MS) combined with the retention indices (RI). The antimicrobial activities of the EO on same food borne pathogens was tested. Twenty-five compounds, representing 97.04% of the total essential oil, were identified. eugenol (24.27%) Longifolenaldehyde (17.46%), β-Selinene (15.6 %) were the main components. The antimicrobial activity of the essential oil was assayed against Gram-positive strains (*Bacillus cereus, Staphylococcus aureus*) and, Gram-negative strains (*Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi)* all strains were sensitive to the essential oil.

Keywords: *Crataegus oxyacantha*, leaves, Essential oil, antimicrobial activity, GC/SM.

INTRODUCTION

Pathogenic bacteria have developed several defense mechanisms on antimicrobial agents and resistance to old and novel manufactured drugs (Asbahani *et al.*, 2014, Asghari *et al.,* 2012). Antibiotic resistance is a serious and important phenomenon in contemporary medicine and has evolved as one of the concerns of preeminent public health in the 21st century. Essential oils are complex mixture of labile and volatile compounds susceptible to oxidation and degradation (Bringas-Lantigua, Valdés, & Pino, 2012). They are aromatic oily liquids obtained from plant material (flowers, buds, seeds, leaves, bark, herbs, wood, fruits, and roots). They are usually complex mixtures of natural compounds, both polar and non polar, composed of dozens to hundreds of compounds, including monoterpenes, sesquiterpenes and their oxygenated derivatives as the main components (Asbahani *et al.,* 2014). In the Algerian traditional medicine the plant is widely used for treating gastrointestinal disorders and as carminative (Chouitah O, 2017). Scientific evidences supported several therapeutic effects such as antihypertensive, antitumor, antimicrobial and vagolytic (Asgary *et al.,* 2000; Tosun *et al.,* 2004; Niazmand *et al.,* 2010; Ali *et al.,* 2011), individually since it is linked to pathogenic microorganisms. The aim of this study was to identify the chemical analysis of essential oil. The other goal of this research was to investigate the antimicrobial effects of *Crataegus oxyacantha* essential oil against the growth of some food pathogenic bacteria. This study will contribute to the valorization of medicinal and aromatic plants of the Algerian floral.

MATERIALS AND METHODS

A. Plant material collection

Plants were collected in April 2016 their natural Habitats is in North West of Algeria (Jdioua Relizane). This plant was identified by botanists of Faculty science. A voucher specimen is deposited in the Herbarium of the Department of Botany and Ecology at the Agronomic Institute under code number 2016-52.

The essential oils were obtained by hydro-distillation from the plant material using a Clevenger –type apparatus for 3h. The essential oil was dried over anhydrous Na_2SO_4 and stored in a scaled vial in the dark; at 4°C in a refrigerator (Hammiche V., Maiza K., 2006). The essential oil yield was calculated on a dry weight by gravimetric method.

B. Analysis of the essential oils

Physicochemical analyses are determined the refractive index, density, polarimeter deviation; point of freezing, solubility in ethanol at 9°C; and the acidity. The chemical composition was determined by GC-MS (Christine and Suzanne, 2000) using an Rxi-5ms fused-silica column (30 m × 0.25 mm × 0.25 μ m).

The column temperature was programmed from 75°C to 200°C at a rate of 2.5°C/min. The injector and detector temperatures were programmed at 230°C and 300°C, respectively. Helium was used as carrier gas at flow rate of 0.6 mL/min. The GC-MS analysis was carried out using two different GC-MS systems. The first was a Hewlett Packard 5973-6890 GC-MS operating on EI mode (equipped with a HP 5MS 30 m ×0.25 mm × 0.25 µm film thickness capillary column). Helium (1 mL/min) was used as carrier gas. Temperature program: initial temperature of the column was 60 °C (for 5 min), then raised to 280°C at 3 °C/min, and held there for 30 min (total time: 93.33 min). The compounds were identified by comparison of their retention indexes (RI) (Phlomis Bovei *et al*, 2007) retention times (RT) and mass spectra with those of authentic samples and/or the NIST/NBS, NIST02, Wiley 575 libraries spectra and the literature (Mohamed Sabry *et al*, 2016). The percentage composition of the essential oil is based on peak areas obtained without FID factor corrections. The second GC-MS system analysis was a Finnegan Trace GC Ultra system, operating on EI mode and equipped with AT™ Aquawax 30 m × 0.32 mm × 0.25 µm film thickness capillary column. Helium was used as the carrier gas, at a flow rate of 1.5 mL/min (constant flow) and a 1:10 split ratio. Temperature program: initial temperature of the column 60°C (for 5 min), then raised to 235°C at 3°C/min, retention indices (RI) determination, a hydrocarbon series was analyzed on GC together with the essential oil on a polar columns, and their linear retention indices were determined and compared with those reported in the literature (Joulain, D. and Koenig, W.A., 1998) and also by computer matching them with the NIST/EPA/NIH Mass Spectral Library data.

C. Microbial strains

For this purpose, the antimicrobial activity of the essential oil was assayed against Gram-positive strains (*Bacillus cereus, Staphylococcus aureus*) and, Gram-negative strains (*Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi)*. The minimum inhibitory concentration (MIC) values were determined for all oils by using the microplate dilution method. The essential oil was prepared in a proper solvent (DMSO). Suspensions were adjusted to1×10^{7} CFUmL− (equivalent to 0.5 McFarland). Antimicrobial tests were carried out using the disc diffusion method in The Muller-Hinton mutriet agar. Paper discs (6 mm diameter) were impregnated aseptically with 3 µl of essential oil at final concentrations of 1-20 µg/ml and placed on the inoculated agar surfaces. After aerobic incubation for 24 hours at 37°C, the antimicrobial activity was estimated by measuring the diameters of inhibition zone (Bakkali, F. *et al.,* 2008). The control test by aqueous DMSO alone showed no toxicity in the concentrations used for these bacteria. The antibacterial mini- mum inhibitory concentrations (MICs) were performed according to the Mueller-Hinton broth microdilution method in 96 multiwell microtiter plate. The essential oils were dissolved in the aqueous DMSO and the initial concentration was 25g/ml. The initial test concentration was serially diluted two fold. Each well was inoculated with 5 µg/ml of suspension containing 10^{7} CFU/ ml of bacteria and incubated for 24 hours at 37°C. The MIC of the tested material was determined as the lowest concentration at which no visible growth of the microorganism had occurred. Each test was carried out in triplicate.

RESULTS AND DISCUSSION

The yield of the essential oil obtained by hydro distillation of leaves of *Crataegus oxyacantha is* 0.45%. The Eos color was pale yellows and possessed a distinct sharp odor. The physicochemical analyses shown the density was done by double weighing d = 0,830, the Specific rotation = +3.5 by polarimetry and the refractive index n = 1.4644 by an interferometric method.

Table 1: Physicochemical composition of *Crataegus oxyacantha* L.

Specification	Density D20	Refractive index	Optical activity N20	Solubility in ethanol 90(%)	Freezing Point (°C)
Crataegus oxyacantha	0.830	1,4644	+3,5	1:3	-18

The total ion chromatogram of EOs obtained by GC-MS is shown in Fig. 1. The identified components of EOs analyzed by GC-MS are listed in Table 2, which resulted in identification of twenty volatile compounds representing 90.04% of the total essential oil, were identified. eugenol (24.27%) Longifolenaldehyde (17.46%), β-Selinene (15.6 %) 2-Methoxyl-6-antranilat (9.5%), were the main components. Other components were present with smaller percent.

Generally, the tested Gram-positive bacteria were more sensitive to the volatile oil than Gram-negative bacteria (Filomena Nazzaro ; 2013). The antibacterial activity of *Crataegus oxyacantha* against five food borne pathogenic bacteria is presented in Table 3. *Crataegus oxyacantha* exerted antibacterial activity against *Bacillus cereus, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and *Salmonella typhi.*

Table 2: The major identified components in essential oil from *Crataegus oxyacantha* L.: analyzed by GC-MS technique with retention indices on HP-5MS capillary Column.

S. No	Compound	TR	RI	Area%
1.	Camphene	3.096	**945**	**1.6**
2.	3-Carene	8.946	**1013**	**0.5**
3.	-Cymene	9.161	**1027**	**0.2**
4.	trans-Bicyclo[4.4.0]Decane	10.925	**1057**	**1.5**
5.	2-Methoxyl-6-antranilat	11.724	**1127**	**9.03**
6.	1-(5,5-Dimethyl-1-cyclopenten-1-yl)-2-methoxybenzene	11.969	**1128**	**2.04**
7.	β-Selinene	12.103	**1195**	**15.6**
8	Longifolenaldehyde	12.208	**1226**	**17.46**
9	Eugenol	12.998	**1290**	**24.27**
10.	8-Isopropenyl-1,5 dimethyl 1,5-cyclodecadiene	13.869	**1339**	**2.65**
11.	Carvacrol methylether	16.388	**1344**	**2.78**
12.	Metyl 2-amino-3-methoxyl benzoate	16.736	**1356**	**0.86**
13.	limonene	17.837	**1365**	**3.25**
14.	citronellyl butyrate-	19.130	**1382**	**0.04**
15.	1,2-Benzenedicarboxylic Acid	19.266	**1390**	**7.8**
16.	-Cadinene	19.360	**1450**	**3.52**
17.	macrene D	19.499	**1477**	**4.56**
18.	caryophyllene oxide	41.874	**1484**	**0.2**
19.	β -guaiene	41.888	**1490**	**0.1**
20.	caryophyllene oxide	41.900	**1530**	**04**
	Total			**90.86**

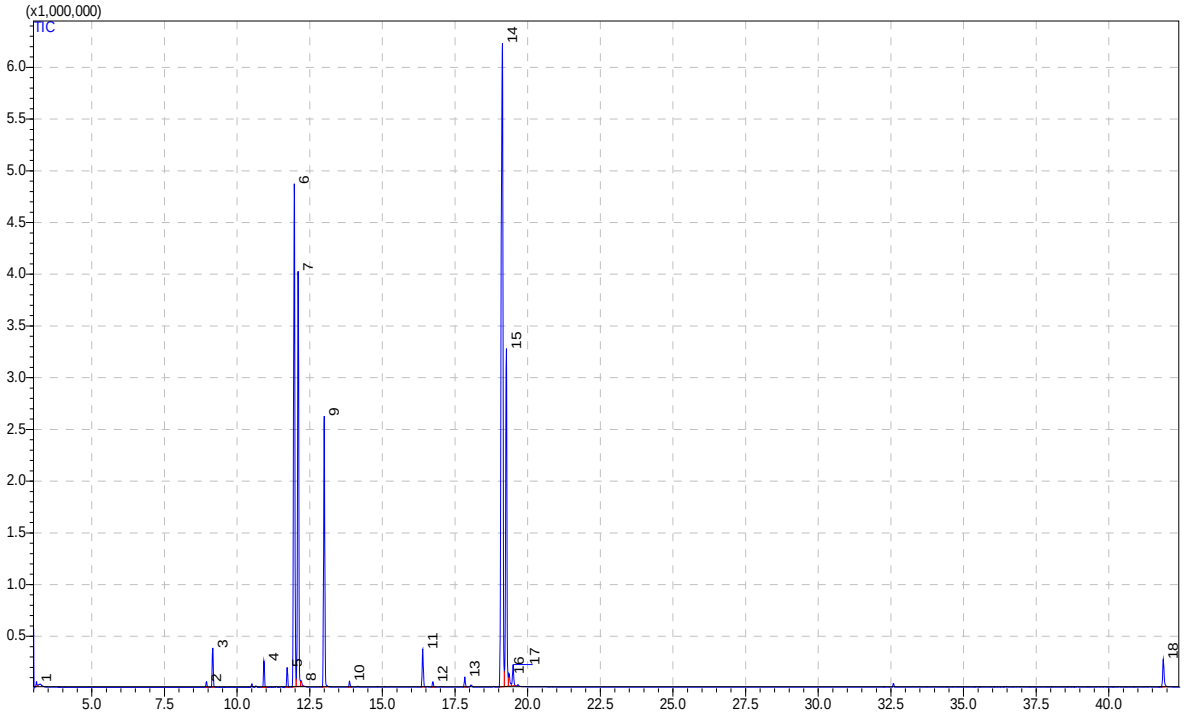

Fig. 1. Gas Chromatogram (GC-FID) of essential oil of *Crataegus oxyacantha.*

Table 3: Inhibition zone (mm) using direct contact technique in agar medium and MIC (mg/mL) for the essential oil using microdilution method in 96 multiwall micro liter plate.

Microorganism	Inhibition zone	Diameter MIC
Bacillus cereus	14.5±10.20	00.18
Staphylococcus aureus	15.0±0.83	15.02
Escherichia coli	16.0±0.70	15.50
Pseudomonas aeruginosa	13.0±0.88	11.60
Salmonella typhi	12.0±0.77	11.30

Whereas the negative control [5% dimethylsulphoxide (DMSO)] exerted no inhibitory activity. The toxic activity of the essential oil against the tested bacteria could be attributed to the presence of significant amounts of eugenol (24.27%).

Electron microscope observations further confirmed that essential oil destroyed cell membrane. Moreover, we found that essential oil could induce cells death of many bacteria through apoptosis pathway based on apoptosis analysis. These findings suggested that essential oil mainly exerted antibacterial activity by damaging cell membrane and membrane-mediated apoptosis pathway (Imran Khan *et al;* 2017).

The results of the present study provide a scientific validation for the traditional use of *Crataegus oxyacantha* as an antibacterial agent. Future work is needed to investigate and explore its application in the environmental and medical fields. In addition, to evaluating the efficacy of the individual ingredients separately to better understand the underlying mechanism.

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