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Essential Oil from the Leaves of *Fraxinus syriaca:* Chemical Composition and Antimicrobial Activity

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ABSTRACT

Since ancient times, aromatic plants have been used in food and in folk medicine. The aim of the present work was to describe the chemical composition and antimicrobial activity of essential oil of *Fraxinus syriaca* l*eaves* growing in jedioua relizane region in the North West of Algeria. . The essential oil was analyzed by GC/GC-MS and resulted in the identification of Twenty one compounds representing 99.04 % of the oil were identified. The major constituents of the isolated oils were the hydrocarbon and oxygen compounds are: Pentcosane 19.04%, sabinene hydrate 11.10%, Eucalyptol 10,73% Bornyl acetate 9.18% and α-Pinene9.13%.The antimicrobial effect of *Fraxinus syriaca* essential oil "in vitro" condition was determined using the agar diffusion method and it was found that it was active which may find its application in future research for the food and pharmaceutical industry.

Key words: *Fraxinus syriaca*, leaves, Essential oil, GC/SM, antimicrobial activity.

INTRODUCTION

Essential oils are composite mixtures of volatile compounds most frequently present at low concentrations in plants. Numerous species of medicinal plants from Algeria are important aromatic and ornamental plants, as well as being medicinal.(Chouitahet al. 2017). Indiscriminate use of antibiotics for the control of pathogenic bacteria has increased their resistance to antibiotics. Multidrug-resistant bacteria are an increasing problem in the recent years and pose serious therapeutic difficulties because of their higher level of resistance. An alternative to this serious problem is to use essential oils of diverse plants for bacterial control (Chávez-González et al. 2016). In recent years, EOs and secondary metabolites of plants have received much attention with application in several areas such as pharmaceutical, food, cosmetic and agricultural industries. Essential oils are chemically characterized as complex mixtures of low molecular weight compounds and, some of them, are highly volatile and capable of generating flavors and/or aromas (trnmbetaIt et al. 2005) .in the traditional medicine, Fraxinus syriaca leaves and flowering aerial parts of species have been used extensively for their antiseptic, antitussive and carminative properties in the treatment of cystitis, insomnia , bronchitis, indigestion, hypertension and several types of inflammation (Miara et al. 2013).

The objective of the present work was to evaluate the chemical composition and antimicrobial of essential oil extracted from fruit of *Fraxinus syriaca*is . This study will contribute to the valorization of medicinal and aromatic plants of the Algerian floral.

MATERIALS AND METHODS

Plant material collection

The leaves of *Fraxinus syriaca* collected from jedioua relizane situated in the North West of Algeria in may2014. 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 2014-52499.

Essential oil distillation

The leaves of *Fraxinus syriaca* were shade, dried, and stored in a tightly closed container for further use. 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 Na2SO4 and stored in a scaled vial in the dark; at 4°C (Hammiche and Maiza 2006). The essential oil yield was calculated on a dry weight by gravimetric method.

Analysis of the essential oils

Following physicochemical analyses are determined: the refractive index, density, polarimeter deviation; point of freezing, solubility in ethanol at 9oC; and the acidity. The oil was analysed by GC on a Perkin-Elmer 8500 gas chromatograph equipped with a FID, fitted with a Supelcowax-10 fused silica capillary column (30 m x 0.32 mm; film thickness, 0.25 µm). The column temperature was programmed from 75 oC to 200 oC at a rate of 2.5 oC/min. The injector and detector temperatures were programmed at 230 oC and 300 oC, 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 x 0.25 mm x 0.25 m 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 oC 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) (Liolios C.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 x 0.32 mm x 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 oC 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 and Koenig 1998) and also by computer matching them with the NIST/EPA/NIH Mass Spectral Library data .

Microbial strains

Antimicrobial activity was carried out according to the disc diffusion assay, tested in vitro against Escherichia coli, Bacillus cereus, Salmonella typhimurium, and S.pneumonia suspensions were adjusted to1×107 CFUmL− (equivalent to 0.5 McFarland). Antimicrobial tests were carried out using the disc diffusion method. The Muller-Hinton mutriet agar and dimethyl sulfoxide (DMSO) solutions (in ratio 1:25 v.v-1) were vortexed for 2 min and immediately 20 ml were poured into sterile Petri dishes (90 mm diameter) and left to set for 30 min. 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 inhi- bition 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 per- formed 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 25 Fraxinus syriacais /ml. The initial test concentration was serially diluted two fold. Each well was inoculated with 5 µg/ml of suspension containing 107 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 DUISCUSSION

Complete knowledge of the chemical constituents of *Fraxinus syriaca* will facilitate synthesis of other chemicals and compounds for their potential applications in the cosmetics, food, and pharmaceutical sectors, (Teresa Fernández et al ,2013) The essential oil obtained by hydrodistillation of aerial parts *Fraxinus syriaca*was light yellow in color and possessed a distinct sharp odor. The yields were 0.28%.Determination of density was done by double weighing d = 0.978, the Specific rotation = +6.5 by polarimetry and the refractive index n = 1.4654 by an interferometric method (Table 1).

The chemical composition of the extracted *Fraxinus syriaca* was analyzed by GC-MS (Fig. 1), which resulted in identification of twenty one volatile compounds. The analysis o f the volatile constituents was carried out using GC-MS systems, equipped with columns of different polarities (HP-5 and Aquawax, respectively). The chemical compositions are summarized in Tables 2. The identified components represented 99.04% of all the components found in the oil samples. These percentages were based on normalization of peak areas without application of the response correction factor. The major components included: Pentcosane 19.04%, sabinene hydrate 11.10%, Eucalyptol 10.73% Bornyl acetate 9.18% and α-Pinene9.13%. Other components were present with smaller percent. This is the first report of the chemical compos ion of *Fraxinus syriaca.* Thus, further investigations are necessary to study the potential of the essential oil leaves. The antibacterial activity of *Fraxinus syriaca*against four food borne pathogenic bacteria is presented in Table 3. *Fraxinus syriaca*exerted antibacterial activity against *Salmonella typhimurium TCC14028, S. aureus* ATCC 49444 and *Bacillus cereus ATCC*-49444, however, it exerted no antibacterial effect on *Escherichia coli* ATCC 10876.; whereas the negative control [5% dimethylsulphoxide (DMSO)] exerted no inhibitory activity. Among chemical components in several essential oils, has been shown to exert a distinct antimicrobial action (Veldhuizen et al. 2006). In general, the higher antimicrobial activity of essential oils is observed on gram-positive bacteria than gram-negative bacteria (Kokoska and al 2002; Okoh and al 2010). Lipophilic ends of lipoteichoic acids in cell membrane of gram positive bacteria may facilitate the penetration of hydrophobic compounds of essential oils (Cox and others 2000)bay other feeding essential oils is associated with the protecting role of extrinsic membrane proteins or cell wall lipopolysaccharides, which limits the diffusion rate of hydrophobic compounds through the lipopolysaccharide layer (Burt 2004).

Thus, further investigations are necessary to study the potential of the essential oil leaves. Including the preservation of raw and processed food, pharmaceuticals, alternative medicines, and natural therapies (Zuzarte et al. 2011).

Table 1. Physicochemical composition of *Fraxinus syriaca.*

Specification	Density D20	Refractive index	Optical activity N20	Solubility in ethanol 90(%)	Freezing Point (°C)
***Fraxinus syriaca*was**	0.937	1,4564	+5,5	1:2	-20

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

Volatile compounds	Ri	Area %
. α-thujene	930	0.7
α-Pinene	939	9.13
camphene	954	5.5
Verbenene	968	3.5
Tetrachloroethylene	887	3.57
Brassicol	667	2.22
Terpinolene	865	2.88
Limonene	948	0.85
1-Butanamine, N-butyl-	1013	1.80
Dolcymene	1042	4.16
Eucalyptol	1059	10.73

sabinene hydrate	1177	11.10
Terpinen-4-ol	1181	0.12
Bornyl acetate	1289	1.20
β-caryophyllene	1427	0.12
γ-cadinene	1529	0.77
Eugenol	1392	8.19
Pentcosane	2249	19.4
Palmitic acid	1968	02.30
phytol	1738	03.50
Myristic acid	1769	07.3
Total		99.04

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 microliter plate.

Microorganism	**Diameter of inhibitionzones (mm)**	**MIC (mg/mL)**
Escherichia coli ATCC 25922	16.5±10.20	00.18
Salmonella typhimurium TCC14028	15.0±0.33	14.60
Escherichia coli (G-) ATCC 35218	15.3±0.10	12.50
Staphylococcus aureus (G+) ATCC 25923	11 ±0.03	14.50
Bacillus subtilis ATCC-6633	12.5±0.8	15.00

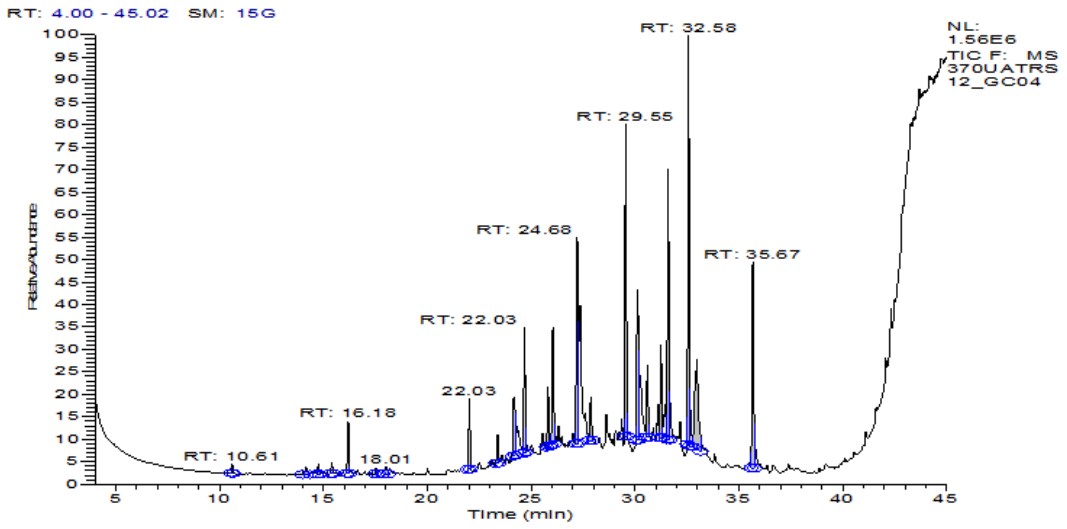

Fig. 1. Gas Chromatogram (GC-FID) of essential oil of *Fraxinus syriaca*

Our results from *Fraxinus syriaca* essential oil found in Algeria may be regarded as Pentcosane chemo type and its broad spectrum of activity against bacteria supports the traditional uses of this plant as disinfectant (Table 3). The oil could be exploited to treat topical infections or preventative measures may halt progression to more serious infection requiring systematic antibiotic therapy, and reduce the risk of development of resistance to valuable antibiotics.

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