

Original article:

CHEMICAL COMPOSITION AND ANTIBACTERIAL ACTIVITY OF ESSENTIAL OILS FROM THE TUNISIAN *ALLIUM NIGRUM* L.

Lamia Sakka Rouis-Soussi¹, Asma El Ayeb-Zakhama¹, Aouni Mahjoub², Guido Flamini^{3*}, Hichem Ben Jannet⁴, Fethia Harzallah-Skhiri^{1*}

¹ Laboratory of Genetic, Biodiversity and Valorisation of Bioresources (LR11ES41), High Institute of Biotechnology of Monastir, Rue Tahar Haddad, BP.74, 5000 Monastir, Tunisia

² Laboratory of Transmissible Diseases and Biologically Active Substances, Faculty of Pharmacy of Monastir, Avenue Avicenne, 5000 Monastir, Tunisia

³ Dipartimento di Farmacia, Via Bonanno 33, 56126 Pisa, Italy

⁴ Laboratory of Heterocyclic Chemistry, Natural Products and Reactivity, Team: Medicinal Chemistry and Natural Products, Faculty of Sciences of Monastir, Avenue de l'Environnement, 5000 Monastir, Tunisia

* Corresponding author: Prof. Fethia Harzallah-Skhiri, E-mail: fethiaprosovis@yahoo.fr, Tel: +21673463711, Fax: +21673405404, Prof. Guido Flamini, E-mail: guido.flamini@farm.unipi.it

ABSTRACT

The chemical composition of the essential oils of different *Allium nigrum* L. organs and the antibacterial activity were evaluated. The study is particularly interesting because hitherto there are no reports on the antibacterial screening of this species with specific chemical composition. Therefore, essential oils from different organs (flowers, stems, leaves and bulbs) obtained separately by hydrodistillation were analyzed using gas chromatography–mass spectrometry (GC–MS). The antibacterial activity was evaluated using the disc and microdilution assays. In total, 39 compounds, representing 90.8–96.9 % of the total oil composition, were identified. The major component was hexadecanoic acid (synonym: palmitic acid) in all the *A. nigrum* organs oils (39.1–77.2 %). We also noted the presence of some sesquiterpenes, mainly germacrene D (12.8 % in leaves oil) and some aliphatic compounds such as *n*-octadecane (30.5 %) in bulbs oil. Isopentyl isovalerate, 14-oxy- α -muurolene and germacrene D were identified for the first time in the genus *Allium* L. All the essential oils exhibited antimicrobial activity, especially against *Enterococcus faecalis* and *Staphylococcus aureus*. The oil obtained from the leaves exhibited an interesting antibacterial activity, with a Minimum Inhibitory Concentration (MIC) of 62.50 μ g/mL against these two latter strains. The findings showed that the studied oils have antibacterial activity, and thus great potential for their application in food preservation and natural health products.

Keywords: *Allium nigrum* L., essential oils, organs, GC–MS analysis, antibacterial activity

INTRODUCTION

Plants and their essential oils, used since antiquity in folk medicine and for the preservation of food, are known sources of natural

secondary metabolites having many biological activities, such as antimicrobial action among others (Deans and Svoboda, 1990).

The increasing failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic microbial infectious agent has led to the screening for several medicinal plants for their potential antimicrobial activity (Bansal et al., 2010; Nostro et al., 2004; Scazzocchio et al., 2001). Their preparations have found applications as naturally occurring antimicrobial agents in pharmacology, pharmaceutical botany, phytopathology, medical and clinical microbiology (Magiatis et al., 2002). Thus, medicinal plants and herbs have assumed greater importance in recent days, due to the tremendous potential that they offer in formulating new drugs against many diseases and illnesses that affect the humankind (Pradeep et al., 2003).

Allium L. is the largest genus of Alliaceae (formerly Liliaceae) (Cuénod et al., 1954; Le Floch et al., 2010); it includes over 600 species, distributed all over Europe, North America, Northern Africa and Asia, each differing in taste, form and color, but close in biochemical, phytochemical and nutraceutical properties (Benkeblia and Lanzotti, 2007). For many centuries, several of these species have been used as vegetables and spices, and as folk medicines for curing various diseases (Hacisferogullari et al., 2005).

Chemical composition and antibacterial activity of the essential oils of many *Allium* species has been largely reported, i.e. *A. cepa* L., *A. sativum* L. (Benkeblia, 2004), *A. jesdianum* Boiss. (Amiri, 2007), *A. roseum* L. (Najjaa et al., 2007), *A. sphaerocephalon* L. (Lazarevic et al., 2011) and *A. rotundum* L. (Dehpour et al., 2012).

In Tunisia 11 species belonging to the genus *Allium* are distributed in the North East, the North West, and in the Center of the country on mountainous regions and in the South of the country (Cuénod et al., 1954). Among them we are interested in *A. nigrum* L. (black garlic, black onion, broad leaved garlic), a perennial herbaceous ornamental plant that grows in wheat fields and widespread in the North West of Tunisia. The name derives from the greenish-black lobed ovaries in the centre of each flower.

Some are blacker than others. This species has a large bulb (3–4 cm) with an ovoid-spherical shape and an entire smooth white tunic. It differs from other *Allium* species for its relatively broad leaves (Burnie, 1995). This species was reported to be used as a food spice (Lentini and Venza, 2007).

This study was carried out to investigate the chemical composition of the essential oils obtained from *A. nigrum* organs in the aim to characterize the oil profile of this Tunisian species and to evaluate the antimicrobial activity of those oils employing the disc and microdilution assays. To the best of our knowledge the present study is the first one performed on this *Allium* species.

MATERIALS AND METHODS

Plant material

Allium nigrum was collected at the flowering stage, in April 2012, in the region of Beja (North West of Tunisia, Latitude 36°43'32''N, Longitude 9°10'54''E), characterized by a humid climate. The specimens were identified according to the Flora of Tunisia (Cuénod et al., 1954). A voucher specimen (An9) has been deposited in the Herbarium of the Laboratory of Genetic Biodiversity and Valorisation of Bioresources, High Institute of Biotechnology of Monastir, Tunisia. The fresh plants were separated into flowers, stems, leaves and bulbs.

Essential oil extraction

For each extraction, 3 x 100 g of fresh plant material cut into small pieces was subjected to hydrodistillation for 4 h using a Clevenger type apparatus. The obtained oils were dried under anhydrous sodium sulphate and weighed. Essential oil yield was expressed as percentage (v/w) of the fresh plant material. All the oils were stored at 4° C until chemical and biological analyses.

Chemical analysis

Gas chromatography (GC) analysis was accomplished with a HP-5890 series II instrument equipped with HP-wax and HP-5 capillary columns (both 30 m x 0.25 mm,

0.25 µm film thickness) with the following temperature program: 50° C for 1 min, ramp of 5° C/min to 280° C. Both injector and detector temperatures were maintained at 250° C and 280° C; carrier gas nitrogen (1.2 mL/min); detector dual FID; split ratio, 1:30. The volume injected was 0.1 µL (1 % *n*-hexane solution).

Gas chromatography-electron ionization mass spectrometry (GC-EIMS) analysis was performed with a Varian CP-3800 gas chromatograph equipped with a HP-5 capillary column (30 m x 0.25 mm; film thickness 0.25 µm) and a Varian Saturn 2000 ion trap mass detector. Analytical conditions: injector and transfer line temperature were 220° C and 240° C, respectively. Oven temperature programmed from 60° C to 240° C at 3° C/min; carrier gas was helium at a flow rate of 1 mL/min; injection of 0.2 µL (10 % *n*-hexane solution); split ratio 1:30. MS were recorded at 70 eV. The acquisition mass range was 30-300 m/z at a scan rate of 1 scan/s.

Compound identification

Identification of the constituents was based on comparison of the retention times with those of authentic samples, comparing their linear retention indices relative to the series of *n*-hydrocarbons, and on computer matching against commercial (NIST 98 and ADAMS) and home-made library mass spectra built up from pure substances and components of known oils and MS literature data (Adams, 2007; Wiley registry of mass spectral data, 1998). Moreover, the molecular weights of all the identified substances were confirmed by GC-CIMS, using MeOH as CI ionizing.

Antibacterial activity

Bacterial strains

The *in vitro* antibacterial activity of the essential oils of the different *A. nigrum* organs were tested against two Gram-positive; *Enterococcus faecalis* (ATCC 29212), *Staphylococcus aureus* (ATCC 25923), and four Gram-negative bacteria; *Pseudomonas*

aeruginosa (ATCC 27853), *Escherichia coli* (ATCC 25922), *Citrobacter freundii* (clinical strain) and *Proteus mirabilis* (clinical strain). The microbial strains were obtained from the culture collection of the Laboratory of Infectious Diseases and Biological Active Agents, Faculty of Pharmacy, Monastir, Tunisia.

Disc-diffusion assay

The above-mentioned bacteria were incubated at 37 ± 0.1° C for 24 h after injection into nutrient broth (Pronadisa; Hispanlab S.A., Madrid Spain). *In vitro* antibacterial activity of the essential oils was evaluated with the disc diffusion method using Mueller Hinton Agar (MHA) with determination of the diameter inhibition zones (DIz) (Rios et al., 1988). The Mueller-Hinton agar medium (MHA; Bio-Rad, Marnes-la-Coquette, France) was sterilized in a flask, cooled to 45–50° C, and distributed into sterilized Petri dishes (90 mm diameter). After cooling the medium, the nutrient-agar plates were swabbed with 0.1 mL (diluted to 10⁶ CFU/mL) of the respective broth culture of the microorganisms. Sterilized Whatman paper discs (n°3, 6 mm diameter) were impregnated with 10 µL of each essential oil and then placed on the surface of the inoculated plates. The plates were kept at 4° C for 2 h before incubation at 37° C for 24 h (Bradshaw, 1992). Then the diameters of the inhibition zones were measured. Positive control discs of gentamicin (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.

Determination of the minimum inhibitory concentration

A microdilution broth susceptible assay was used for the determination of the minimum inhibitory concentration (MIC) (Bassole et al., 2003). All tests were performed in Muller-Hinton broth (MHB) medium. The inoculum suspensions of the bacterial strains were prepared from 12 h broth cultures and

adjusted to obtain a final density of 10^6 CFU/mL. The essential oils, dissolved in 10 % dimethylsulfoxide (DMSO), were first diluted to the highest concentration (1 mg/mL) to be tested and the 96-well plates were prepared by dispensing 100 μ L of MHB, into each well. Then, 95 μ L of MHB and 5 μ L of the inoculum were added. A 100 μ L aliquot from the stock solutions of the essential oils were added into the first wells. Serial two-fold dilutions were made in nine consecutive wells to obtain an oil-concentration range from 3.9 μ g/mL to 1000.0 μ g/mL. The last well, containing 195 μ L of MHB without oil and 5 μ L of the inoculum, was used as a negative control. The final volume in each well was 200 μ L. The plates were covered with a sterile plate sealer and incubated at 37° C for 24 h. The bacterial growth was indicated by the presence of a white pellet on the well bottom. Microbial growth was determined by reading the absorbance at 600 nm and confirmed by plating 5 μ L samples from clear wells on nutrient agar medium. The essential oils tested in this study were screened three times against each microorganism. The MIC was defined as the lowest concentration able to inhibit any visible bacterial growth.

Statistical analysis

All the results are expressed as mean \pm standard deviation of three replications. The data were processed using Microsoft Excel 2007, then subjected to one way analysis of variance (ANOVA) and the significance of differences between means were calculated by *Duncan* multiple range test using SPSS for Windows (Standard Version 14.0 SPSS Inc., Chicago. IL.), *p* values < 0.05 were regarded as significant.

RESULTS

Oil yield

The oil yield for the four organs of *A. nigrum* varied significantly from 0.025 (flowers) to 0.005 (w/w) (bulbs). Stems and leaves yielded 0.011 and 0.010 % of oil, respectively.

Essential oil composition

The essential oils of *A. nigrum* organs obtained by hydrodistillation were analyzed by GC–FID and GC–MS. The composition of the volatile oils is reported in Table 1. Thirty-nine compounds were identified, representing 90.8–96.9 % of the total oils composition.

Flowers oil composition

Globally, 7 compounds were identified in the flowers oil. The main constituent was hexadecanoic acid (synonym: palmitic acid) (61.0 %), followed by its methyl (23.1 %), and ethyl (5.5 %) esters. *n*-Eicosane, *n*-octadecane and *n*-nonadecane, were found in smaller proportions 3.2, 1.3 and 0.6 %, respectively. Nonanal was detected only in the flowers oil (2.2 %).

Bulbs oil composition

In the bulbs, 7 compounds were identified. Among them, 5 out of the 7 compounds were found in both oils from bulbs and flowers. Bulbs oil constituents were also dominated by hexadecanoic acid (39.1 %). On the other side, *n*-octadecane was present at higher percentages in bulbs oil, reaching 30.5 %. On the contrary, methyl hexadecanoate was detected at a lower percentage (7.5 %). Three out of five constituents were present at similar percentages: *n*-nonadecane (3.5 %), ethyl hexadecanoate (3.4 %) and *n*-eicosane (3.2 %). Furthermore, appreciable amounts of *n*-hexadecane were also identified in the bulbs oil (5.9 %).

Stems oil composition

Also in this oil, hexadecanoic acid was the main component (77.2 %). All the other compounds (15) were detected at lower percentages (0.4–2.1 %). Among them, 8 characterize this oil, such as 1-nonadecene (1.8 %), hexadecanal (0.6 %), 1-pentadecanol (0.6 %) and 1-hexadecyl acetate (0.5 %).

Leaves oil composition

The leaves oil was the richest in constituents (26), with 20 of them exclusively of this

oil. It was dominated by hexadecanoic acid (47.8 %) and germacrene D (12.8 %). Isopentyl isovalerate, β -caryophyllene, *T*-cadinol, and 2,3,6-trimethylbenzaldehyde were detected in appreciable amounts (5.4 %, 3.2 %, 3.2 %, 2.5 %, respectively). Methyl hexadecanoate, and ethyl hexadecanoate, identified in all the organs, reached here the lowest percentages (1.5 and 1.2 %, respectively). Three constituents, ethyl tetradecanoate, hexahydrofarnesyl acetone and *T*-cadinol were found both in the leaves and stems oils. Other important constituents were caryophyllene oxide (1.7 %), α -cadinol (1.5 %), linalool (1.3 %), α -humulene (0.9 %), *T*-muurolol (0.9 %), bicyclogermacrene (0.8 %), δ -cadinene (0.8 %) and 14-oxy- α -muurolene (0.8 %).

Variation of the chemical classes within plant organs

The chemical classes of the studied oils are reported in Table 1. Their contents varied significantly according to the plant organs. The main class was represented by carboxylic acids (39.1-77.7 %). The main representative was hexadecanoic acid, particularly in the stems oil. Aliphatic derivatives predominated in the essential oil of bulbs, with a mean percentage of 43.1 %. The alkanes *n*-octadecane and *n*-hexadecane reached the highest percentages in this oil, respectively 30.5 and 5.9 %. Compounds belonging to this class were in low percentages in the other organs (5.1, 5.1 and 1 %, respectively for flowers, stems and leaves oils). The third major class was that of fatty acid methyl esters (3.7-28.6 %), represented by methyl hexadecanoate (1.5-23.1 %) and ethyl hexadecanoate (1.2-5.5 %). Their highest percentages were reported in flowers oil; 23.1 and 5.5 %, respectively. The class of sesquit-

erpenes occupied the fourth position (0.9 and 30.0 % in stems and leaves, respectively). It was represented in leaves oil, by two sesquiterpene hydrocarbons; germacrene D (12.8 %) and β -caryophyllene (3.2 %) and an oxygenated sesquiterpene, the *T*-cadinol (3.2 %), as main compounds. Esters class predominated in leaves oil by isopentyl isovalerate (5.4 %). The other classes are in low contents and are not further discussed here.

Antibacterial potential of the essential oils

The results of the antibacterial assays are shown in Table 2. The highest antibacterial activities were observed against *Enterococcus faecalis* (9.0 ± 1.0 – 17.33 ± 0.57 mm), and *Staphylococcus aureus* (10.66 ± 1.15 – 19.33 ± 0.57 mm). The essential oil obtained from the leaves was the most effective one, with a minimum inhibition concentration (MIC) of 62.5 μ g/ml and a diameter of the inhibition zone of 17.33 ± 0.57 mm against *Enterococcus faecalis* and 19.33 ± 0.57 mm against *Staphylococcus aureus*. No essential oil showed inhibitory activity against *Citrobacter freundii* and *Pseudomonas aeruginosa*. The leaves oil was also effective against *Escherichia coli* and *Proteus mirabilis*, with a diameter of the inhibition zone varying from 12.66 ± 0.57 mm (MIC >500 μ g/ml) to 15.33 ± 0.57 mm (MIC = 125 μ g/ml), respectively; however they were less active than gentamicin. Bulbs oil was also effective against *Proteus mirabilis* (12.66 ± 1.52 mm, MIC = 250 μ g/ml). The Gram negative bacteria *Escherichia coli* and *Proteus mirabilis*, the major opportunistic pathogens, were resistant to flowers and stems essential oils.

Table 1: Composition (%) of the essential oils from Tunisian *Allium nigrum* L.

Compound name	RI ^a	Content (%) ^b			
		Flowers	Stems	Leaves	Bulbs
Linalool	1101	- ^c	-	1.3	-
Nonanal	1104	2.2	-	-	-
Isopentyl isovalerate	1105	-	-	5.4	-
2,3,6-Trimethylbenzaldehyde	1355	-	-	2.5	-
n-Tetradecane	1400	-	0.5	-	-
β-Caryophyllene	1419	-	-	3.2	-
α-Humulene	1455	-	-	0.9	-
Germacrene D	1482	-	-	12.8	-
Valencene	1493	-	-	0.7	-
Bicyclogermacrene	1496	-	-	0.8	-
n-Pentadecane	1500	-	-	0.5	-
trans-γ-Cadinene	1514	-	-	0.6	-
δ-Cadinene	1524	-	-	0.8	-
Caryophyllene oxide	1582	-	-	1.7	-
n-Hexadecane	1600	-	0.9	-	5.9
T-Cadinol	1641	-	0.5	3.2	-
T-Muurolol	1646	-	-	0.9	-
β-Eudesmol	1650	-	0.4	-	-
α-Cadinol	1654	-	-	1.5	-
Acorenone	1689	-	-	2.1	-
2-Pentadecanone	1698	-	-	0.5	-
n-Heptadecane	1700	-	-	0.5	-
Pentadecanal	1716	-	-	0.5	-
14-oxy-α-Muurolene	1766	-	-	0.8	-
Tetradecanoic acid	1769	-	0.5	-	-
1-Pentadecanol	1776	-	0.6	-	-
1-Octadecene	1793	-	0.5	-	-
Ethyl tetradecanoate	1795	-	0.5	1.0	-
n-Octadecane	1800	1.3	1.4	-	30.5
Hexadecanal	1820	-	0.6	-	-
Hexahydrofarnesyl acetone	1844	-	1.3	1.4	-
1-Nonadecene	1894	-	1.8	-	-
2-Heptadecanone	1897	-	-	0.6	-
n-Nonadecane	1900	0.6	-	-	3.5
Methyl hexadecanoate	1928	23.1	2.1	1.5	7.5
Hexadecanoic acid	1963	61.0	77.2	47.8	39.1
Ethyl hexadecanoate	1993	5.5	1.5	1.2	3.4
n-Eicosane	2000	3.2	-	-	3.2
1-Hexadecyl acetate	2018	-	0.5	-	-
Class					
Carboxylic acids		61.0	77.7	47.8	39.1
Aliphatics		5.1	5.1	1.0	43.1
Fatty acid methyl esters		28.6	4.1	3.7	10.9
Sesquiterpenes hydrocarbons		-	-	19.8	-
Oxygenated sesquiterpenes		-	0.9	10.2	-
Other esters		-	0.5	5.4	-
Ketones		-	1.3	2.5	-
Others		2.2	1.2	4.3	-
Total		96.9	90.8	94.7	93.1

^a Retention index (RI) determined relatively to the retention time of a series of n-alkanes

^b Content (%): Relative percentage calculated by GC/FID on an apolar capillary column HP-5.

^c -: not detected

Table 2: Antibacterial activity of essential oils from Tunisian *Allium nigrum* L.

Micro-organisms	Essential oils								Gentamicin Diz
	Flowers		Stems		Leaves		Bulbs		
	Diz ^a	MIC ^b	Diz	MIC	Diz	MIC	Diz	MIC	
<i>Escherichia coli</i> ATCC 25922	– ^c	–	–	–	c)12.66 ± 0.57a	> 500	–	–	18.0 ± 0.1b
<i>Pseudomonas aeruginosa</i> ATCC 227853	–	–	–	–	–	–	–	–	15.0 ± 0.1a
<i>Enterococcus faecalis</i> ATCC 29212	9.00 ± 1.00a	> 500	12.33 ± 1.15b	> 500	17.33 ± 0.57c	62.50	13.00 ± 1.00b	250	20.0 ± 0.1d
<i>Staphylococcus aureus</i> ATCC 25923	10.66 ± 1.15a	250	12.00 ± 2.00b	250	19.33 ± 0.57c	62.50	13.33 ± 1.52b	125	24.0 ± 0.0d
<i>Proteus mirabilis</i>	–	–	–	–	15.33 ± 0.57b	125	12.66 ± 1.52a	250	17.0 ± 0.0c
<i>Citrobacter freundii</i>	–	–	–	–	–	–	–	–	12.0 ± 0.1 a

^a Diameter of inhibition zone (Diz) in mm including the diameter of disc 6 mm

^b Minimum Inhibitory Concentration (MIC) expressed in µg/mL

^c -: no inhibition zone

Values were expressed as mean ± SD (n=3), values in the same line with different superscripts (a-d) are significantly different at p < 0.05; ATCC: American Type Culture Collection; Gentamicin as antibiotic (10 µg/disc)

DISCUSSION

The chemical composition of *A. nigrum* essential oil was clearly dependent on the organ of the plant. Germacrene D, 14-oxy- α -muurolene and isopentyl isovalerate were identified for the first time in the genus *Allium*. Considering the identified compounds, only hexadecanoic acid, methyl hexadecanoate and ethyl hexadecanoate were present in all of the plant organs, *n*-octadecane was found in flowers, stems and bulbs oils. *n*-Nonadecane and *n*-eicosane were present in flowers and bulbs oils. Two compounds were detected exclusively in stems and leaves (*T*-cadinol and hexahydrofarnesyl acetone), while *n*-hexadecane was detected only in stems and bulbs.

Hexadecanoic acid, identified as a major component in all of *A. nigrum* oils organs (39.1 % to 77.2 %), was also reported in some other *Allium* species. It represents 19.03 % and 16.75 % of the totality of the

essential oil in *A. jesdianum* Boiss aerial parts (Amiri, 2007) and *A. ursinum* L. leaves (Blazewicz-Wozniak and Michowska, 2011), respectively. It was found to be the most abundant component in some essential oils such as those from *Prunella vulgaris* L. flowers (70.0 %) (Yuhang et al., 2012), *Lycium chinense* fruits (62.89 %) (Min Chung et al., 2011) and from *Scutellaria diffusa* aerial part (30 %) (Cicek et al., 2011). This carboxylic acid has been mentioned for the first time in the essential oil of the genus *Allium* with a relatively high level and would characterize the essential oil of *A. nigrum*.

Many volatiles identified in the present study are reported as main components in other *Allium* species. Methyl hexadecanoate was detected in *A. roseum* stems (2.56 %) (Ben Jannet et al., 2007), nonanol has been identified in similar proportions in the flowers of *A. rotundum* (2.17 %) (Dehpour et al., 2012), and in lower amounts (0.2 %) in the

inflorescences of *A. sphaerocephalon* (Lazarevic et al., 2011).

On the other hand, flowers oils from *A. roseum* (Zouari et al., 2013) and *A. rotundum* (Dehpour et al., 2012) also contain aliphatic compounds, including *n*-eicosane (0.55 % and 2.94 %, respectively) and *n*-octadecane (0.44 % and 0.53 %, respectively). Also, *n*-hexadecane (0.9 %) and *n*-tetradecane (0.5 %) were detected in small amounts in *A. rotundum*, *A. tuberosum* and *A. roseum* (Dehpour et al., 2012; Guohua et al., 2013; Zouari et al., 2013).

Lazarevic et al. (2011) reported for the first time the presence of many terpenoid compounds in *Allium* oils including bicyclogermacrene and α -cadinol that we also found in the oil of the Tunisian *A. nigrum*. Furthermore, these authors identified α -muurolene in *A. sphaerocephalon* (1.1 %). In our work we reported the presence of a related volatile, 14-oxy- α -muurolene in leaves oil. Noteworthy, the essential oil of *A. nigrum* is completely devoid of sulfur derivatives.

A significant difference was observed between Gram positive and Gram negative bacteria in terms of susceptibility to the *A. nigrum* oils. Indeed, Gram positive bacteria were more sensitive to tested oils. Most studies reported that essential oils are relatively more active against Gram positive bacteria than against Gram negative ones (Lambert et al., 2001), which partially support our findings.

In the literature, Benkeblia (2004) reported that the essential oils of *Allium sativum* and *Allium cepa* showed an antimicrobial activity against *Staphylococcus aureus*, with diameters of the inhibition zones varying from 5.6 to 9.3 mm. Besides, the essential oil of *A. roseum* presented a growth inhibition zone of 10.0 mm against the same bacteria *S. aureus* (Najjaa et al., 2007). The microbiostatic effect against the Gram-negative *P. aeruginosa* was observed at a lower concentration (MIC = 0.08 mg mL⁻¹) in comparison with *S. aureus* and *E. coli* (MIC = 0.63 mg mL⁻¹) (Lazarevic et al.,

2011). However, in our study, no oil was able to inhibit the growth of *P. aeruginosa*. A relationship can be deduced between the antimicrobial activity and the chemical composition of the essential oils. Even though major components are usually responsible for the antimicrobial activity of many essential oils, the effect of minor ones cannot be neglected (Koroch et al., 2007). In this study, hexadecanoic acid, the dominant constituent of all *A. nigrum* oils, can be involved in this activity. Elsewhere, Ogunlesi et al., (2009) signaled that this component has an antibacterial potential.

A. nigrum leaves oil which was the richest in constituents, exhibited the greatest activity. It may be attributed to the presence of some components in moderate percentages, known for their antimicrobial activity, such as terpenes (Dorman and Deans, 2000), germacrene D (Ngassapa et al., 2003), caryophyllene oxide and linalool (Ulubelen et al., 1994; Pattnaik et al., 1997).

The tested bacterial strains showed different pattern of inhibition in presence of the oils obtained from flowers, stems and bulbs of *A. nigrum*. This can be explained by the presence of some *n*-alkanes such *n*-tetradecane, *n*-hexadecane, *n*-nonadecane, *n*-eicosane and *n*-octadecane. In fact, He (2009) indicated that some alkanes have a good antimicrobial effect especially on *Staphylococcus aureus* and *Escherichia coli*. In conclusion, the different active volatile components in each organ may be responsible for the variability of the antibacterial activity.

CONCLUSION

This is the first study reporting the volatile compounds profile of the Tunisian *A. nigrum* and its antibacterial activity. The tested oils were characterized by hexadecanoic acid, methyl hexadecanoate, *n*-octadecane, germacrene D and isopentyl isovalerate. *A. nigrum* oils contained compounds with wide-spectrum antibacterial activity, so this plant may be used as a natural

antimicrobial agent for human infectious diseases.

DECLARATION OF INTEREST

The authors declare that they have no conflict of interest. The authors are alone responsible for the content and writing of the paper.

REFERENCES

- Adams RP. Identification of essential oil components by gas chromatography/mass spectrometry. Carol Stream IL.: Allured Publishing, 2007.
- Amiri H. Chemical composition and antibacterial activity of the essential oil of *Allium Jesdianum* Boiss. and Buhse from Iran. J Med Plants 2007;6:39-44.
- Bansal S, Malwal M, Sarin R. Anti-bacterial efficacy of some plants used in folkloric medicines in arid zone. J Pharm Res 2010;3:2640-2.
- Bassole LHN, Quattara AS, Nebie R, Quattara CAT, Kabore ZI, Traore SA. Chemical composition and antibacterial activities of the essential oils of *Lippia chevalieri* and *Lippia multiflora* from Burkina Faso. Phytochemistry 2003;62:209-12.
- Ben Jannet H, Sakka Rouis L, Neffati M, Mighri Z. Chemical composition of flowers and stems essential oils from the Tunisian *Allium roseum* L. J Essent Oil Bear Pl 2007;10:151-6.
- Benkeblia N. Antimicrobial activity of essential oil extracts of various onions (*Allium cepa*) and garlic (*Allium sativum*). Lebensm Wiss Technol 2004;37:263-8.
- Benkeblia N, Lanzotti V. *Allium* thiosulfinates: chemistry, biological properties and their potential utilization in food preservation. Food 2007;1:193-201.
- Blazewicz-Wozniak M, Michowska A. The growth, flowering and chemical composition of leaves of three ecotypes of *Allium ursinum* L. Acta Agrobot 2011;64:171-80.
- Bradshaw LJ. Laboratory microbiology (4th ed.) San Diego, CA: Harcourt Brace, 1992.
- Burnie D. Wild flowers of the Mediterranean. London: Dorling Kindersley Book, 1995.
- Cicek M, Demirci B, Yilmaz G, Can Baser KH. Essential oil composition of three species of *Scutellaria* from Turkey. Nat Prod Res 2011;25:1720-6.
- Cuénod A, Pottier-Alapetite G, Labbe A. Flore analytique et synoptique de la Tunisie. Cryptogames vasculaires. Gymnospermes et monocotylédones. Tunis : Imprimerie SEFAN, 1954.
- Deans SG, Svoboda KP. Biotechnology and bioactivity of culinary and medicinal plants. Ag Biotech News Inform 1990;2:211-6.
- Dehpour AA, Yousefian M, Jafary Kelarijani SA, Koshmoo M, Mirzanegad S, Mahdavi V. Antibacterial activity and composition of essential oils of flowers *Allium rotundum*. Adv Environ Biol 2012;6:1020-5.
- Dorman HJD, Deans SG. Antimicrobial agents from plants: Antibacterial activity of plant volatile oils. J Appl Microbiol 2000;88:308-16.
- Guohua H, Chun S, Rengang M, Zhengzhi M, Yanhua L, Dongzhi W. Essential oil composition of *Allium tuberosum* seed from China. Chem Nat Compd 2013;48:1091-3.
- Haciseferogullari H, Özcan M, Demir F, Çalisir S. Some nutritional and technological properties of garlic (*Allium sativum*L.). J Food Eng 2005;68:463-9.
- He L. The studies on the bacteriostatic effects of loquat flower's extracts and its relative active compositions. Ms.D. Thesis. Sichuan Normal University, 2009 (in Chinese).
- Koroch AR, Juliani HR, Zygadlo JA. Bioactivity of essential oils and their components. In: Berger RG (ed): Flavors and fragrances: chemistry, bioprocessing, and sustainability (pp 87-116). Berlin: Springer, 2007.
- Lambert RJW, Skandamis PN, Coote P, Deans GJ. Mode of action of oregano essential oil, thymol and carvacrol. J Appl Microbiol 2001;91:453-62.
- Lazarevic JS, Dordevic AS, Zlatkovic BK, Radulovic NS, Palic RM. Chemical composition and antioxidant and antimicrobial activities of essential oil of *Allium sphaerocephalon* L. subsp. *Sphaerocephalon* (Liliaceae) inflorescences. J Sci Food Agr 2011;91:322-9.
- Le Floc'h E, Boulos L, Vela E. Catalogue synonymique commenté de la flore de Tunisie. République Tunisienne, Ministère de l'environnement durable, banque nationale de gènes, 2010.
- Lentini F, Venza F. Wild food plant of popular use in Sicily. J Ethnobiol Ethnomed 2007;3:1-12.
- Magiatis P, Skaltsounis AL, Chinou I, Haroutounian SA. Chemical composition and *in-vitro* antimicrobial activity of the essential oils of three Greek *Achillea* Species. Z Naturforsch 2002;57:287-90.

- Min Chung I, Nagella P, Ahn YS, Kim SJ, Ahmad A. Composition of the essential oil and petroleum ether extract of *Lycium chinense* Miller fruits and antioxidant activity of its several extracts. *J Med Plants Res* 2011;5:5973-81.
- Najjaa H, Neffati M, Zouari S, Ammar E. Essential oil composition and antibacterial activity of different extracts of *Allium roseum* L. a North African endemic species. *C R Chim* 2007;10:820-6.
- Ngassapa O, Runyoro DKB, Harvala E, Chinou IB. Composition and antimicrobial activity of essential oils of two populations of Tanzanian *Lippia javanica* (Burm. F.) Spreng (Verbenaceae). *Flavour Fragrance J* 2003;18:221-4.
- Nostro A, Blanco AR, Cannatelli MA, Enea V, Flamini G, Morelli I. Susceptibility of methicillin-resistant staphylococci to oregano essential oil, carvacrol and thymol. *FEMS Microbiol Lett* 2004;230:191-5.
- Ogunlesi M, Okiei W, Ofor E, Osibote AE. Analysis of the essential oil from the dried leaves of *Euphorbia hirta* Linn (*Euphorbiaceae*) a potential medication for asthma. *Afr J Biotechnol* 2009;8:7042-50.
- Pattnaik S, Subramanyam VR, Bapaji M, Kole CR. Antibacterial and antifungal activity of aromatic constituents of essential oils. *Microbios* 1997;89:39-46.
- Pradeep P, Leena P, Bohra A. Antibacterial potential of *Cedrus deodara*. *Adv Plant Sci* 2003;16:479-82.
- Rios JL, Recio MC, Villar A. Screening methods for natural products with antimicrobial activity: A review of the literature. *J Ethnopharmacol* 1988;23:127-49.
- Scazzocchio F, Cometa MF, Tomassini L, Palmery M. Antibacterial activity of *Hydrastis canadensis* extract and its major isolated alkaloids. *Planta Med* 2001;67:561-4.
- Ulubelen A, Topcu G, Eris C, Sonmez U, Kartal M, Kurucu S et al. Terpenoids from *Salvia sclarea*. *Phytochemistry* 1994;36:971-4.
- Wiley registry of mass spectral data (7th ed.). New York: Wiley and Sons, with NISTspectral data, CD-ROM, 1998.
- Yuhang C, Qiaosheng G, Zaibiao Z, Lixia Z, Xiaolei D. Comparative analysis of the essential oil of flowers, leaves and stems of *Prunella vulgaris* L. *J Essent Oil Bear Pl* 2012;15:662-6.
- Zouari S, Ketata M, Boudhrioua N, Ammar E. *Allium roseum* L. volatile compounds profile and antioxidant activity for chemotype discrimination-Case study of the wild plant of Sfax (Tunisia). *Ind Crop Prod* 2013; 41:172-8.