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Chemical composition and antibacterial activities of *Capparis spinosa* essential oils from Algeria

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Abstract. *Benachour H, Ramdani M, Lograda T, Chalard P, Figueredo J. 2020. Chemical composition and antibacterial activities of* Capparis spinosa *essential oils from Algeria. Biodiversitas 21: 161-169.* The essential oils of *Capparis spinosa* L. collected from six locations in Algeria were obtained by hydro-distillation. The chemical composition of oils was performed by GC-MS. The disc diffusion method is applied for the antibacterial activity. The extraction produced low yield (0.03%). The result of chromatographic analysis (GC/MS) leads to the identification of 33 components; palmitic acid (38.19%), nonanal-n (12.61%), cymene-2,5-dimethoxy-para (8.94%) and octacosane (5.49%) were the major components of these oils. The result of cluster analysis based on essential oils constituents showed the presence of three chemotypes,i.e., the chemotype of Nonanal-n-Cymen 2,5 dimethoxy para-Dodecanal, the chemotype of Nonanal-n-Hexadecanoic acid-tetracosane and the chemotype Tetracosane-n-pentyl furane-2-octacosane. In-vitro antimicrobial activity of caper oils against nine bacterial species showed that the oils have no activity against *E. coli* and have modest activities against eight other bacterial species tested; however, the desirability test shows that the oils used were not effective on the bacteria tested.

Keywords: Algeria, antimicrobial activity, *Capparis spinosa*, chemotype, desirability test, essential oil

INTRODUCTION

Capparis spinosa L. (caper), of the *Capparaceae* family, is native to the Mediterranean region (Tesoriere et al. 2007). In Algeria, this species is easily found and wildly growing in all areas, especially in sunny places and on dry and rocky areas (Quezel and Santa 1962).

Caper has been used in traditional phytomedicine as a tonic, aperitif, diuretic, and anti-inflammatory (Al-Said et al. 1988; Lieutaghi 2004; Panico et al. 2005; Zhou et al. 2010). It is also widely used as a condiment by the Greeks and Romans (Boga et al. 2011). In Algeria, the different parts of the plant are used to treat itching, mosquito bites, urticarial and also to treat asthma and digestive problems (Benseghir-Boukhari and Seridi 2007).

Phytochemical analysis showed that *C. spinosa* is rich in polyphenols and flavonoids (Bonina et al. 2002; Satyanarayana et al. 2008; Allaith 2014). Chemical compounds of *C. spinosa* essential oils from Iran showed a different part of the plant has different major chemical compounds. Leaf oil is characterized by thymol (26.4%), isopropyl isothiocyanate (11%), hexenal-2 (10.2%) and butyl isothiocyanate (6.3%), fruit oil is mainly composed of isopropyl isothiocyanate (52.2%) and methyl isothiocyanate (41.6%), while root oil contains methyl isothiocyanate (53.5%) and isopropyl isothiocyanate (31.4%) (Afsharypuor et al. 1998).

Capparis spinosa from Turkey is characterized by the presence of methyl isothiocyanate, i.e., 26.5% in young shoots; 49.6% in floral buds (Ozcan and Chalchat 2007). The aromatic profile of *C. spinosa* from Italy shows 22.2% aldehydes, 21% esters, and 8.42% sulfur compounds (Romeo et al. 2007), while the essential oil of *C. spinosa* from Croatia contains 92.06%) methyl isothiocyanate (Kulisic-Bilusic et al. 2009).

Caper from Jordan contains isopropyl isothiocyanate, methyl isothiocyanate, and butyl isothiocyanate (Muhaidat et al., 2013). The essential oil from Syria is rich in thymol, octanoic acid, methyl isothiocyanate, and hexenal-2 (El-Naser 2016). The oxygenated monoterpenes of Moroccan caper populations are represented by carvacrol, borneol, p-cymene, α-pinene and linalool (Fadili et al. 2017). Phytochemical investigation of Algerian populations of *C. spinosa* showed that the major components were n-nonanal and palmitic acid (Benachour et al. 2017). The analysis of *C. spinosa* buds oil from Italy showed the presence of major compounds: docosane, nonacosane, palmitic acid and heptacosane (Mollica et al. 2018).

Capparis spinosa leaves from Egypt extracted with various solvents showed significant antibacterial activities against *Klebsiella pneumonia* and *Enterococcus faecalis* (Sherif et al. 2013). Aqueous extracts of the roots and fruits of *C. spinosa* showed potent antimicrobial activity against a wide range of microorganisms (Mahboubi and Mahboubi 2014; Gull et al. 2015). A study by Al-Azawi et al. (2018) showed that methanolic extract of *C. spinosa* leaves had potent antibacterial activity, particularly against *S. aureus*, *K. pneumonia*, and *E. coli*.

Al-Bayati and Al-Jarjry (2007) showed that the ethanolic and chloroform extracts of the aerial parts of the Iraqi caper have no antibacterial activity, while the root extracts have high antibacterial properties against Gram-positive bacteria. Compounds extracted from *C. spinosa* leaves showed activity on several bacterial species (Arean et al. 2018).

The objective of this work was to determine and compare the chemical composition of the essential oil of *Capparis spinosa* from several Algerian populations and to evaluate their antibacterial activities.

MATERIALS AND METHODS

Plant materials

Capparis spinosa is a shrub growing to 1 m or more climb and fall. It is a woody, spiny with opposite leaves alternate and sub-orbicular or oval 1 to 4 cm in length. Buds are 8 to 13 mm long, and the flowers are axillary solitary, white or pinkish-white, slightly unequal, and very numerous long stamens that extend beyond the calyx. The berries are ovoid oblong or pyriform, seeds convolute black kidney-shaped 3 mm languor (Quezel and Santa 1962; Mayor 1965; Ozenda 1977; Satyanarayana et al. 2008).

The aerial parts of *Capparis spinosa* were collected from six locations in Setif and Mila of Algeria (Figure 2) during July 2017. Their geographical coordinates are listed in Table 1.

Essential oil extraction

The air-dried materials were subjected to hydro-distillation for 3h using a Clevenger apparatus type. Voucher specimens were deposited in the herbarium of the Department of Biology and Ecology, Setif University, Algeria. The oil obtained was collected and dried over anhydrous sodium sulfate and stored in screw-capped glass vials in a refrigerator at 4-5°C for further analysis. The yield based on the dry weight of the samples was calculated using the equation as follows:

$$\text{Yield of essential oil (\%)} = \frac{\text{weight of essential oil (g)}}{\text{weight of plant sample (g)}} x100$$

Figure 1. *Capparis spinosa* collected in Dehamcha region (Setif)

Table 1. Geographical coordinates of sampling locations

Provinces	Localities	Latitude (N)	Long (E)
Setif	Dehamcha	36° 22′ 56″	5° 35′ 43″
	Aïn Sebt	36° 28′ 54″	5° 42′ 40″
	Djemila	36° 23′ 17″	5° 24′ 39″
	Ain El Kebira	36° 21′ 53″	5° 30′ 07″
Mila	Zeghaia	36° 28′ 05″	6° 10′ 21″
	Ferdjioua	36° 24′ 32″	5° 56′ 45″

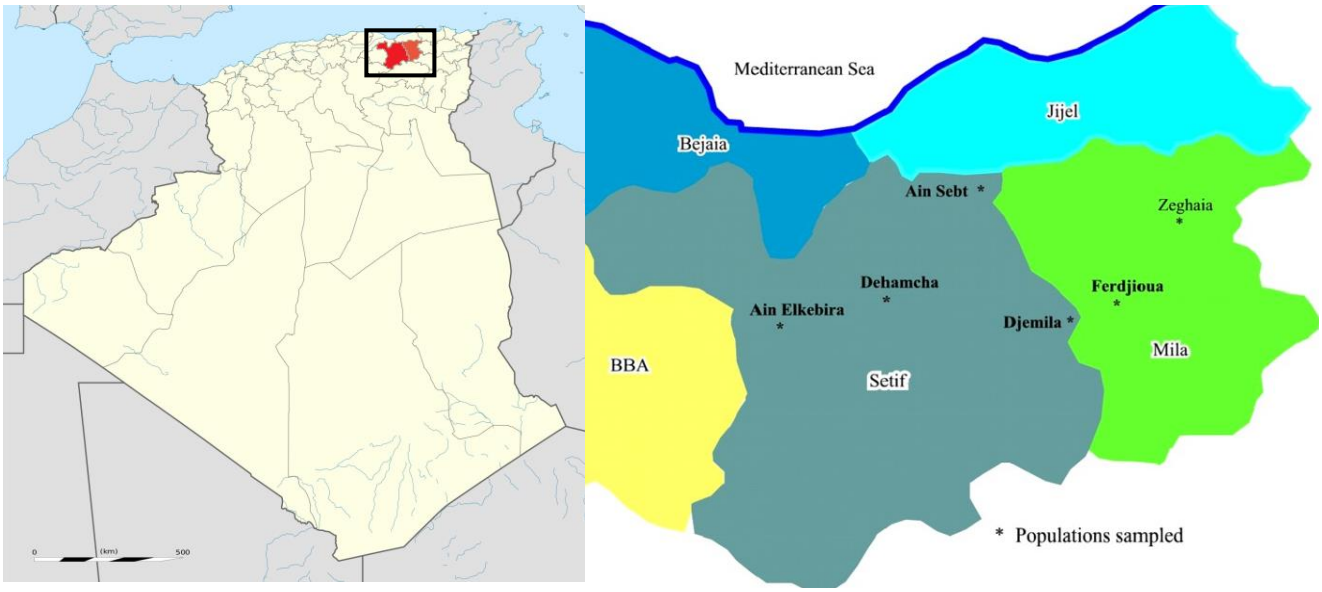

Figure 2. Sampling locations of *Capparis spinosa* in Setif (Djemila, Dehamcha, Ain Lekbira, Ain Sebt) and Mila (Ferdjioua, Zeghaia) provinces, Algeria

Essential oil analysis

The essential oils were analyzed in a Hewlett-Packard gas chromatograph CPG/FID 7890, coupled to a gas chromatograph: CPG/MS 7890/5975C, equipped with a Column Apolar: DB5 MS (40m x 0.18mm; 0.18µm), programmed as follows: the initial temperature was set at 50°C for 5min, then increased to 300°C at a rate of 5°C/min. Helium was used as the carrier gas (1.0 ml/min); injection in split mode (1:30), injector, and detector temperature is 280°C with split 1/100. The mass spectrometer worked in EI mode at 70 eV; electron multiplier, 2500 V; ion source temperature, 180°C; MS data were acquired in the scan mode in the *m/z* range 33450. The identification of the components was based on comparison of their mass spectra with those of NIST mass spectral library (Masada 1979; NIST 2002) and those described by Adams as well as on comparison of their retention indices either with those of authentic compounds or with literature values (Adams 2007).

Antibacterial activity assessment

The in-vitro evaluation of antimicrobial activity was performed by the disc diffusion method against five Gram-negative bacteria (*Escherichia coli* ATCC 25922, *Salmonella enterica* ATCC 43972, *Klebsiella pneumoniae* ATCC 700603, *Proteus mirabilis* ATCC 35659 and *Pseudomonas aeruginosa* ATCC 27853), and four Gram-positive bacteria (*Bacillus cereus* ATCC 11778, *Staphylococcus aureus* ATCC 25923, *Staphylococcus aureus* MRSA ATCC 43300 and *Enterococcus faecalis* ATCC 51299). These bacteria were obtained from the Pasteur Institute of Algiers and M'Sila and the Microbiology laboratory of Setif University Hospital. The bacterial inoculums were prepared from overnight broth culture in physiological saline (0.8% NaCl) to obtain an optical density ranged from 0.08-01 at 625 nm. Muller Hinton agar (MHA) was poured in Petri dishes, solidified, and surface dried before bacteria inoculation. Sterile discs (6 mm) were placed on inoculated MHA, added with 10 µl of stock solution of essential oil and diluted essential oil (1:2, 1:5 and 1:10 v:v of DMSO). DMSO was used as a negative control. Bacterial growth inhibition was determined as the diameter of the inhibition zones around the discs. The Petri dishes were incubated at 37°C for 18 to 24h aerobically. All the tests were performed in triplicate, and the means were calculated as final results. The sensitivity to essential oil was classified by diameter of inhibition halos as follows: not sensitive (-) for diameter less than 8 mm; sensitive (+) for diameter 9-14 mm; very sensitive (++) for diameter 15-19 mm and extremely sensitive (+++) for diameter larger than 20 mm (Ponce et al. 2003).

Statistical analysis

Data were first subjected to Principal Components Analysis (PCA) to examine the relationships among the terpenes compounds and identify the possible structure of the population and study of variations in the composition of the essential oil of *Capparis spinosa*. Cluster analysis (UPGMA) was carried out on the original variables and on the Manhattan distance matrix to seek for hierarchical associations among the populations. The cluster analysis was carried out using Statistica v10 software. All data of antibacterial activity were expressed as mean ± SD. Statistical significance was analyzed by the three-way ANOVA and posthoc tests by using a co-stat v 6.4 software package. Differences with $P < 0.05$ were considered significant.

Desirability test

A desirability test is a tool for evaluating the effect of factors or variables, which can predict the effect of each factor of the design. The scales range from 0 (most unfavorable/undesirable values) to 1 (most desirable values).

RESULTS AND DISCUSSION

Oil extraction by hydro-distillation of the aerial part of *C. spinosa* from six locations produced pale yellow oil. The yields of essential oils from these populations were very low, with an average of 0.03 ± 0.01%. The chemical analysis of the essential oils by GC/MS led to the identification of 33 components representing an average of 99.42 ± 0.63% of the total oil (Table 2).

The chemical composition of *C. spinosa* essential oils revealed palmitic acid as a major compound with an average of 38.19 ± 9.23%, with the highest concentration found in the oil originated from the Ain Sebt population (51.20%). The other major compound was nonanal-n with an average of 12.61 ± 7.34%, with the highest content observed in the population of Dehamcha (22.33%). Other constituents with high concentrations were cymene-2,5-dimethoxy-para (8.94 ± 5.23%), octacosane (5.49 ± 1.91%) and tetracosane-n (4.37 ± 3.90%). The main components of the oils were fatty acids with an average of 38.95 ± 9.16%), aldehydes (19.74 ± 7.74%), and alkanes (15.16 ± 5.46) (Figure 3).

Capparis spinosa populations from different locations showed significant differences in the quality and quantity of the chemical composition of essential oils. The concentration of the components showed significant chemical variability (Figure 4), namely hexadecanoic acid (38.19 ± 9.23%) was the component with the most considerable variation in populations, followed by nonanal (12.61 ± 7.34) and cymene-2,5-dimethoxy-para (8.94). ± 5.23%).

The three-dimensional spatial projection of the six populations of *C. spinosa* showed that the chemical composition of the oils was very heterogeneous, which made it impossible to distinguish homogeneous groups (Figure 5).

To analyze the relationships between the populations, the UPGMA cluster analysis based on the Un-weighted pair-group average and the distance between City-block (Manhattan) was carried out. The results of analysis confirm differences in the chemical composition of

essential oils divides *C. spinosa* populations into two distinct clades (Figure 6).

UPGMA analysis results showed that there were two clusters of *C.spinosa.* The first cluster was the population of Ferdjioua, which separates from other populations characterized by high levels of tetracosane-n (11.72%), Pentyl furan-2 (10.15%), and octacosane (8.52%). These compounds were under-represented in other populations. The second cluster is characterized by the presence of hexadecanoic acid, which was divided into two subclades; i.e., the first subclade united the two populations of Ain El Kbira and Ain Sebt), which was characterized by octacosane. The second subclade consists of the populations of Djemila, Zeghaia, and Dehamcha was characterized by significant levels of nonanal-n. The population of Djemila separated from the other two populations because of the presence of octacosane-n. Based on the cluster analysis of essential oil constituents, so three chemotypes were identified (Table 3).

Table 2. Chemical composition of *Capparis spinosa* essential oils collected from six locations in Algeria

		Mila		**Setif**				**Average**	**SD**
		Ferdjioua	**Zeghaia**	**Djemila**	**Dehamcha**	**Ain Lekbira**	**Ain Sebt**		
Yields (%)	KI	0.03	0.02	0.03	0.02	0.02	0.04	0.03	0.01
Compounds Nb		24	25	29	24	27	26	26	2
Total		99.63	99.29	99.97	99.68	98.21	99.75	99.42	0.63
Hexanal	803	0.31	0.93	0.41	0.73	0.49	0.54	0.57	0.23
Hexanol-n	873	0.10	1.89	0.99	0.57	2.34	1.03	1.15	0.83
Heptanal	899	0.22	0.54	0.00	0.45	2.15	0.27	0.61	0.78
Heptanol-n	970	0.55	0.27	0.82	0.24	0.51	0.27	0.44	0.23
Hepten-2-one-6-methyl-5	988	0.00	0.00	0.90	0.00	0.00	0.00	0.15	0.37
Pentyl furan-2	991	**10.15**	1.77	0.65	0.00	0.89	0.65	2.35	3.86
Octanal-n	1005	0.53	1.12	0.88	1.83	0.84	2.06	1.21	0.60
Octen-1-ol-2E	1032	0.00	0.00	1.47	0.00	0.00	0.00	0.25	0.60
Octanol- (10,11)-n	1078	0.00	0.00	2.89	0.00	0.00	0.00	0.48	1.18
Nonanal-n	1116	1.49	**17.72**	**10.84**	**22.33**	**8.47**	**14.79**	12.61	7.34
Decanol-n	1212	0.51	0.96	1.25	0.86	0.74	0.99	0.89	0.25
Decanal-n	1214	**6.79**	3.28	1.23	4.93	1.39	2.60	3.37	2.16
β-cyclocitral	1238	1.24	0.71	1.60	1.10	1.35	0.84	1.14	0.33
Carvacrol, methyl ether	1246	0.00	0.00	0.58	0.00	0.00	0.00	0.10	0.24
Nonanal, dimethyl acetal	1279	1.77	1.53	0.62	0.53	0.36	1.34	1.03	0.59
Cymene-2,5-dimethoxy-para	1308	**8.63**	**8.73**	**16.40**	**11.29**	**0.27**	**8.33**	8.94	5.23
Arbozol-Endo-	1400	1.61	1.55	2.93	1.34	4.98	0.84	2.21	1.52
Arbozol-Exo-	1425	2.28	0.92	0.97	1.18	0.78	0.38	1.09	0.64
Geranyl acetone	1455	0.47	0.00	1.68	1.30	1.18	0.34	0.83	0.65
Dodecanol-n	1480	0.96	**6.80**	**5.45**	**3.99**	**3.85**	0.54	3.60	2.46
Tridecanone-2	1487	0.00	1.10	2.20	0.57	0.44	0.73	0.84	0.76
Tridecanal	1722	1.33	1.94	2.11	1.10	1.01	0.80	1.38	0.53
Pentadecanone-2	1930	0.00	0.62	0.90	0.00	0.51	0.00	0.34	0.39
Methyl hexadecanoate	1948	1.47	0.36	0.91	0.49	0.76	0.57	0.76	0.40
Hexadecanoic acid	1993	**33.60**	**33.99**	**30.40**	**31.30**	**48.65**	**51.20**	38.19	9.23
Eicosane-1	2101	3.00	0.00	0.00	1.87	1.90	0.73	1.25	1.21
Eicosane	2109	1.30	0.96	1.18	1.83	0.00	1.34	1.10	0.61
Docosane	2208	1.10	0.00	2.03	0.57	1.29	0.00	0.83	0.80
Tetracosane-n	2419	**11.72**	**0.00**	**3.62**	**3.67**	**4.23**	**2.98**	4.37	3.90
Heptacosane	2607	0.00	0.82	0.95	0.00	1.63	0.42	0.64	0.63
Octacosane	2624	**8.52**	**4.29**	**3.10**	**5.58**	**6.65**	**4.78**	5.49	1.91
Nonacosane	2753	0.00	1.81	0.00	0.00	0.57	0.38	0.46	0.70
Dotriacontane	2805	0.00	4.69	0.00	0.00	0.00	0.00	0.78	1.91
Chemical classes									
Fatty acid		35.07	34.35	31.31	31.79	49.41	51.77	38.95	9.16
Aldehydes		10.67	25.53	15.47	31.37	14.35	21.06	19.74	7.74
Alkanes		25.64	12.57	12.35	13.52	16.27	10.63	15.16	5.46
Alcohols		2.12	9.92	8.51	5.66	7.44	2.83	6.08	3.13
Sesquiterpenes		8.63	8.73	16.40	11.29	0.27	8.33	8.94	5.23
Monoterpenes		1.71	0.71	3.86	2.4	2.53	1.18	2.07	1.12
Ketones		0	1.72	4	0.57	0.95	0.73	1.32	1.42
Furan		10.15	1.77	0.65	0.00	0.89	0.65	2.35	3.86
Acetals		1.77	1.53	0.62	0.53	0.36	1.34	1.03	0.59
Others		3.89	2.47	6.79	2.52	5.76	1.22	3.78	2.14

The antibacterial activity of *C. spinosa* essential oils was estimated by measuring the diameter of inhibition halos (Table 4). All bacteria tested were sensitive to gentamicin and cefotaxime, except *E. coli* was resistant to all oils of *C. spinosa* populations at all test concentrations. Essential oils at ½ dilution were the most effective for inhibiting the growth of all tested bacterial species.

Growth inhibition of *C. spinosa* essential oil against *P. mirabilis, E. faecalis*, was very low at all dilutions. Growth inhibition against *K. pneumoniae, S. aureus* MRSA was low at all dilutions except essential oils from Ferdjioua and Ain Elkbira populations with inhibition diameters of 29.1 and 20.85 mm against *K. pneumonia* at ½ dilution respectively. The essential oil derived from Djemila inhibits the growth of the *Staphylococcus aureus* MRSA strain with an inhibition diameter of 35.33 ± 3.05mm. The bacterial species of *S. aureus, B. cereus, P. aeruginosa, S. enterica* were very sensitive to the oil of *C. spinosa.*

Table 3. *Capparis spinosa* chemotypes

Chemotypes	Populations
Tetracosane-n-Pentyl furane-2-octacosane	Ferdjioua
Nonanal-n- Cymen 2,5 dimethoxy para-Dodecanal	Djemila, Zeghar and Dehamcha
Nonanal-n- Hexadecanoic acid-tetracosane	Ain Elkbira and Ain Sebt

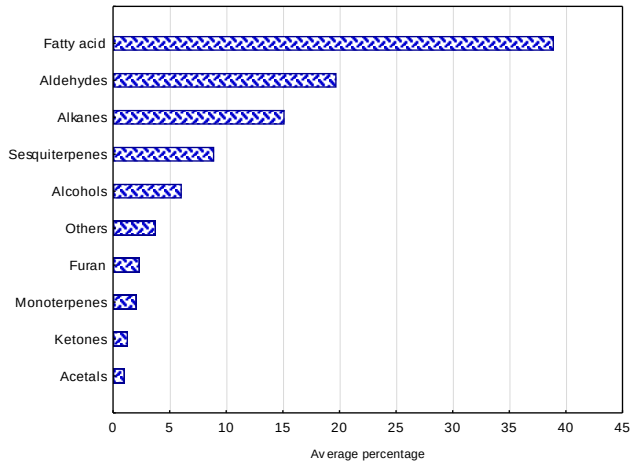

Figure 3. Chemical classes of *Capparis spinosa* oils

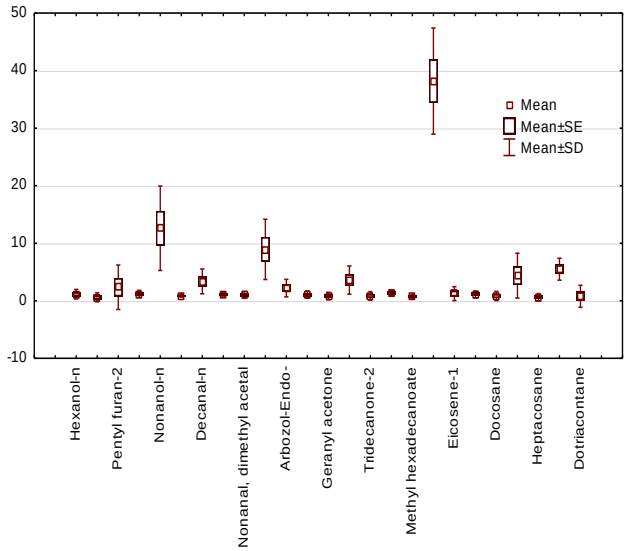

Figure 4. Variation in concentration of essential oil compounds of *Capparis spinosa*

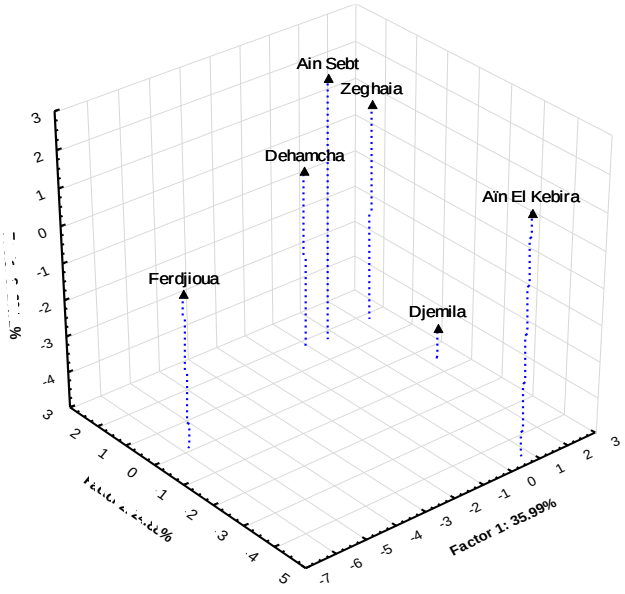

Figure 5. Spatial projection of *Capparis spinosa* populations

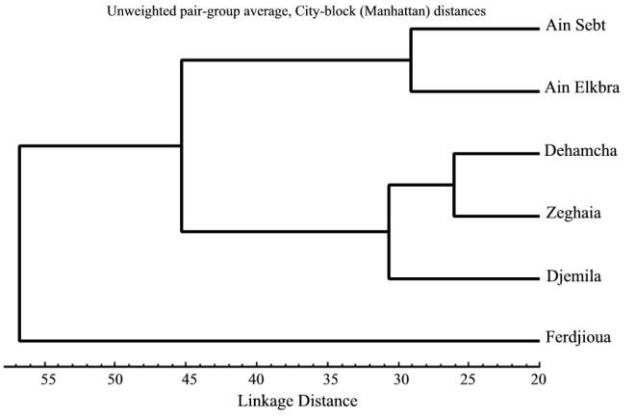

Figure 6. UPGMA of *Capparis spinosa* essential oils

Table 4. Inhibition diameter (mm) of *C. spinosa* essential oils against nine bacterial species

	Dilutions	Djemila	Dehamcha	Ferdjioua	Ain Sebt	Zeghaia	Ain El Kbira
Escherichia coli	1/2	0	0	0	0	0	0
	1/5	0	0	0	0	0	0
	1/10	0	0	0	0	0	0
	Gentamicin	29±0.5	29±0.5	29±0.5	29±0.5	29±0.5	29±0.5
	Cefotaxime Céfot	21±1	21±1	21±1	21±1	21±1	21±1
	Cefotaxime axime						
Staphylococcus aureus	1/2	40.33±0.57	13.66±1.44	42.66±1.52	42.5±1.32	42±2.64	42.33±2.51
	1/5	28.5±0.5	9.33±0.28	15.5±0.5	36.75±.25	27.5±1.32	9.16±0.28
	1/10	18.66±1.15	8.33±1.04	0	13.5±1.32	16±1	7.83±0.76
	Gentamicin	30.33±0.57	30.33±0.57	30.33±0.57	30.33±0.57	30.33±0.57	30.33±0.57
	Cefotaxime Céfot	27.5±0.5	27.5±0.5	27.5±0.5	27.5±0.5	27.5±0.5	27.5±0.5
	Cefotaxime axime						
Klebsiella pneumoniae	1/2	15±0.5	10.5±0.5	29±1	13.66±1.52	11.66±0.57	20.83±0.76
	1/5	9,83±0,28	8.75±0.75	8.5±0.5	9.5±0.5	9.83±0.28	10±0
	1/10	9.5±0.5	7.5±0.5	7.33±0.57	8.16±0.28	0	9.66±0.76
	Gentamicin	30.83±0.76	30.83±0.76	30.83±0.76	30.83±0.76	30.83±0.76	30.83±0.76
	Cefotaxime Céfot	30.33±0.57	30.33±0.57	30.33±0.57	30.33±0.57	30.33±0.57	30.33±0.57
	Cefotaxime axime						
Bacillus cereus	1/2	42±2	25.33±1.52	43±2	42.66±2.08	41.66±1.52	39±1.73
	1/5	36.66±1.52	12.33±2.08	13.66±1.15	15±2	39±1.73	16±1
	1/10	9.83±0.28	10.33±0.57	9±1	10±1	9.33±0.57	10.33±0.57
	Gentamicin	33±1	33±1	33±1	33±1	33±1	33±1
	Cefotaxime Céfot	33±1	33±1	33±1	33±1	33±1	33±1
	Cefotaxime axime						
Staphylococcus Aureus MRSA	1/2	35.33±3.05	10.16±0.28	12.66±0.57	14.83±0.76	15.33±0.57	11±1.32
	1/5	13.16±0.28	8.83±0.28	9.83±0.28	10.5±0.5	10.5±0.5	8.83±0.28
	1/10	9.16±0.28	7.33±0.28	8.16±0.28	8±0	7.66±0.28	7.66±0.28
	Gentamicin	30.5±0.5	30.5±0.5	30.5±0.5	30.5±0.5	30.5±0.5	30.5±0.5
	Cefotaxime Céfot	27.83±0.76	27.83±0.76	27.83±0.76	27.83±0.76	27.83±0.76	27.83±0.76
	Cefotaxime axime						
Pseudomonas aeruginosa	1/2	42.66±2.51	28.33±2.92	23.16±2.08	42.5±6.50	15.43±3.78	41.16±4.01
	1/5	17±1	15±0.5	9±0.5	16.5±0.86	0	39±6.08
	1/10	18.83±4.07	0	0	10.5±0.5	9.16±0.76	20.66±1.04
	Gentamicin	29.83±0.76	29.83±0.76	29.83±0.76	29.83±0.76	29.83±0.76	29.83±0.76
	Cefotaxime Céfot	31±1	31±1	31±1	31±1	31±1	31±1
	Cefotaxime axime						
Salmonella enterica	1/2	43±2	12.66±0.6	24.5±1.3	28.33±2.1	17.33±0.6	31.66±1.5
	1/5	14±1	11±0.5	12.16±0.6	17.16±0.8	15.83±0.3	16±1
	1/10	10.33±0.57	8.5±0.5	9.66±0.57	11.33±0.76	11.83±0.76	14.5±0.5
	Gentamicin	35.33±0.6	35.33±0.6	35.33±0.6	35.33±0.6	35.33±0.6	35.33±0.6
	Cefotaxime Céfot	30.66±0.6	30.66±0.6	30.66±0.6	30.66±0.6	30.66±0.6	30.66±0.6
	Cefotaxime axime						
Proteus mirabilis	1/2	9.83±0.28	8.33±1.15	9.33±0.57	14.33±1.15	12.16±1.04	10.5±0.5
	1/5	8.83±0.76	7.66±0.57	8.33±0.57	9.16±0.76	9.16±1.89	8.66±0.76
	1/10	7±0	0	7.33±0.57	7.5±0.86	7.41±0.38	7.66±1.15
	Gentamicin	30.33±0.57	30.33±0.57	30.33±0.57	30.33±0.57	30.33±0.57	30.33±0.57
	Cefotaxime Céfot	33±1	33±1	33±1	33±1	33±1	33±1
	Cefotaxime axime						
Enterococcus faecalis	1/2	14±1	14±1	15.33±1.52	11.66±0.57	14.33±0.57	16±1
	1/5	12.33±1.52	11±1	12.33±0.57	11±1	13.33±0.57	12±1
	1/10	9.33±0.57	8.66±0.57	11.33±1.15	9.83±0.76	11.66±0.57	10.66±0.57
	Gentamicin	25.33±0.57	25.33±0.57	25.33±0.57	25.33±0.57	25.33±0.57	25.33±0.57
	Cefotaxime Céfot	39.33±1.15	39.33±1.15	39.33±1.15	39.33±1.15	39.33±1.15	39.33±1.15
	Cefotaxime axime						

Statistical analysis showed that the Main Effects (Populations, doses, bacterial species, and their interactions are highly significant ($P < 0.001$) (Table 5).

The *C. spinosa* essential oil from the Djemila region exhibits high antimicrobial activity against bacterial species tested with an average inhibition diameter of 22.74 mm, while the oil of the Dehamcha population is the least active against bacterial species with an average inhibition diameter of 17.91 mm (Table 6).

Two antibiotics used in this study (Gentamicin and Cefotaxime) were able to inhibit bacterial growth better than *C.spinosa* oils. The diameter of inhibition of the

essential oil of *C. spinosa* at the dilution of ½ was lower than that of antibiotics with an average diameter of inhibition of 21.32mm (Table 7) while essential oils at 1/10 dilution were not significant in inhibiting the growth of bacteria with an average inhibition diameter of 8 mm.

The highest resistance to *C. spinosa* essential oil was observed in *E. coli* with an average inhibition zone of 10 mm; moreover, the higher sensitivity is observed in *B. cereus* with a zone of inhibition average of 27.37 mm (Table 8).

The desirability profile of *C. spinosa* essential oils against the bacteria tested had a prediction value of 0.38287 (Figure 7).

The predictive value of essential oils was low (less than the prediction value), except for the oil from the Djemila population that has a value higher than 0.38287. The doses of the oil used in this study have low activity against the bacteria tested, with the values lower than the predicted value. The desirability test showed that the bacterial species tested have lower values than the predicted value.

Discussion

The essential oils of *Capparis spinosa* obtained by hydro-distillation have low yields with an average of 0.03 ± 0.1%. A study by Afsharypuor et al. (1998) showed that the yield of *C.spinosa* essential oil ranged from 0.02 to 0.9%, depending on the part of the plant. The same observation was made by Kulisic-Bilusic et al. (2009) and Muhaidat et al. (2013).

Chromatographic analysis of *C. spinosa* oils revealed that hexadecanoic acid is the major component with an average of 38.19 ± 9.23%; however, hexadecanoic acid had low concentration in *C. spinose* originating from Iraq (4.7%) (Afsharypuor et al. 1998) and Syria (4.7%) (El-Naser 2016).

The chemical composition of the essential oils of the *C. spinosa* in this study is different from that which has been analyzed previously from Turkey (Ozcan and Chalchat 2007) and Croatia (Kulisic-Bilusic et al. 2009). The essential oil in this study is characterized by Methyl isothiocyanate as a major compound, the oil of *C. spinosa* leaves from Iran, and Syria was characterized by thymol is a major compound (Afsharypuor et al. 1998, El-Naser 2016). Oils from the Moroccan population showed carvacrol as the major component (Fadili et al. 2017). A previous study of 15 *C. spinosa* populations showed that essential oils were rich in nonanal-n, palmitic acid, and isopulegyl acetate (Benachour et al. 2017). Differences in the chemical composition of the same species were due to various parameters, including environment, geographical origin, harvest period, location, and temperature and drying time (Burt 2004; Aminzadzh et al. 2010; Aboukhaid et al. 2017; Yeddes et al. 2018).

Table 5. Main effects and interactions of essential oils of *C. spinosa*

Source	df	F	P
Main Effects			
Populations	5	290.28	0000 ***
Doses	8	12951.85	0000 ***
Bacteria	8	1851.01	0000 ***
Interaction			
Populations * Doses	40	88.31	0000 ***
Populations * Bacteria	40	84.45	0000 ***
Doses * Bacteria	64	434.74	0000 ***
Populations * Doses * Bacteria	320	43.46	0000 ***

Note: *** Very highly significant (P < 0.001)

Table 6. The effectiveness of *Capparis spinosa* essential oils from several sampling locations against nine bacteria species

Rank	Populations	Mean	n	Significant groups
1	Djemila	22.74	135	a
2	Ain El Kbira	21.54	135	b
3	Ain Sebt	21.26	135	b
4	Zeghaia	20.36	135	c
5	Ferdjioua	19.78	135	d
6	Dehamcha	17.91	135	e

Note: LSD 0.05 = 0.24813651563

Table 7. Effect of oil dilutions on the inhibitory zone of tested bacteria

Rank	Doses	Mean	n	Significant groups
1	Gentamicin	30.5	162	a
2	Cefotaxime	341	162	a
3	1/2	21.32	162	b
4	1/5	12.67	162	c
5	1/10	8.09	162	d

LSD 0.05 = 0.33291006999

Table 8. Sensitivity groups of tested bacteria with essential oils of *Capparis spinosa*

Rank	Bacteria species	Mean ranges	n	Significant groups	S*
1	*Bacillus cereus*	27.37	90	**a**	+++
2	*Staphylococcus aureus*	25.39	90	**b**	+++
3	*Pseudomonas aeruginosa*	23.59	90	**c**	+++
4	*Salmonella enterica*	23.53	90	**c**	+++
5	*Enterococcus faecalis*	20.23	90	**d**	+++
6	*Klebsiella pneumoniae*	18.88	90	**e**	++
7	*S. aureus MRS*	18.63	90	**e**	++
8	*Proteus mirabilis*	17.78	90	**f**	++
9	*Escherichia coli*	10.00	90	**g**	+

Note: * S (+: sensitive, ++: very sensitive and +++: extremely sensitive); LSD 0.05 = 0.33291006999

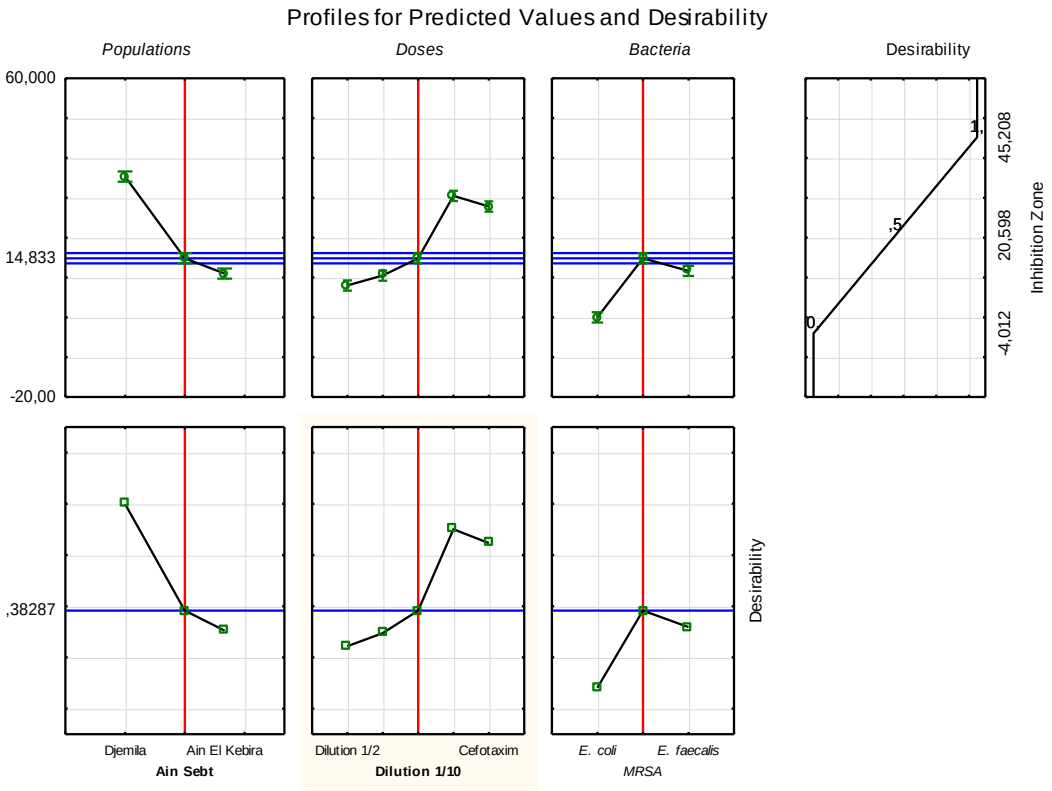

Figure 7. Profile of Predicted Values and Desirability for populations of *Capparis spinosa* essential oils

The antibacterial test showed that *B. cereus* was the most sensitive to the essential oil of *C. spinosa*, while *E. coli* was the most resistant to oils from the six localities of this study and to the three dilutions tested. These results are consistent with the conclusions of Proestos et al. (2006), and Meddour et al. (2011), who have shown that *E. coli* is resistant to methanolic extracts from the leaves of *C. spinosa*. Orooba (2012) showed that the ethanolic extract of flowers was effective against Gram-positive and Gram-negative bacteria. The presence of fatty acids in essential oils, such as palmitic acid, may be responsible for the antibacterial activities of the oils (Kabara et al. 1972; Bravo-Santano et al. 2019).In addition, the presence of monoterpene compounds, which have proven their effects, can be also responsible of this antibacterial action (Marchese et al. 2017). Gull et al. (2015) showed that methanolic extracts were more effective than ethanolic extracts and acetone against *S. aureus, E. coli, B. subtilis* and *P. multocida*. Arean et al. (2019) showed that *S. aureus, S. epidermidis, K. pneumoniae, E. coli* and *S. typhi* are very sensitive to extracts of *C. spinosa*

In conclusion, the essential oils of *C. spinosa* collected from 6 locations in Algeria have a chemical composition different from those described in the literature. GC-GC/MS analysis leads to the identification of 33 components in the essential oil of *C. spinosa*. The chemical compositions of these populations are dominated by hexadecanoic acid, nonanal-n, cymene-2,5-dimethoxy-para, and octacosane. The oil is mainly composed of fatty acids, aldehydes, and alkanes. The results showed great variability in the chemical composition of essential oils. Results of the cluster analysis showed the presence of three chemotypes, i.e.; chemotype which was characterized by (Nonanal-n – Cymen 2,5 dimethoxy para – Dodecanal) consisted of Djemila, Zeghar, and Dehamcha populations; the chemotype which was characterized by (Nonanal-n – Hexadecanoic acid – tetracosane) consisted of the populations of Ain Elkbira and Ain Sebt; while the chemotype which was characterized by (Tetracosane-n – pentyl furane-2 – octacosane) consisted of Ferdjioua population. The concentrated essential oil of *C.spinosa* was the most effective against all bacteria (Gram+ and Gram-), except for *E. coli* which is resistant to *C. spinosa* oils. While the desirability test shows that the bacteria have lower values than the predicted value.

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