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Chemical composition and antibacterial activity of essential oils of *Thymelaea hirsuta* from Algeria

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Abstract. *Souhila B, Takia L, Messaoud R, Pierre C, Gilles F. 2019. Chemical composition and antibacterial activity of essential oils of Thymelaea hirsuta from Algeria. Biodiversitas 20: 2868-2876.* The objectives of this study were to determine the chemical composition and to evaluate the antibacterial activity of *Thymelaea hirsuta* (L.) Endl., essential oils from seven sampling locations in M'sila region (Algeria). Extraction of essential oils was carried out by the hydro-distillation; the analysis of chemical composition of essential oil was carried out by GC-MS. Antimicrobial activity was performed by disc diffusion method at the essential oil concentration of non-diluted and diluted (1:2, 1:4 and 1:8 v:v of DMSO) against eight species of bacteria. The results showed that the average yields of essential oils were 0.3 ± 0.12%. A total of 45 components were identified, averaging 98.2 ± 1.85% of the total oils. The main components were nonanal-n (10.39 ± 3.21%), hexadecanoic acid (9.77 ± 2.81%), nonanoic acid (9.13 ± 6.49%), triacontane (7.2 ± 3.34%), isopropyl tetradecanoate (6.16 ± 1.99%) and tridecane (4.87 ± 3.1%). Based on the UPGMA cluster analysis, there were two clades of *T. hirsuta*. *T. hirusta* has a chemical polymorphism with different chemotypes marked in nature. There were four chemotypes identified in the essential oil of *T. hirsuta* in the region of M'sila. The essential oil of *T. hirsuta* has antibacterial activity against eight tested bacteria on the concentration-dependentt manner.

Keywords: Algeria, antibacterial activity, chemotypes, essential oils, *Thymelaea hirsuta*

INTRODUCTION

The genus *Thymelaea* consists of 31 species with circum-Mediterranean distribution (Galicia-Herbada 2006). In Algeria, there are eight species of *Thymelaea* including *T. hirsuta* (L) Endl. (Quezel et Santa 1963). Previous studies showed that *T. hirsuta* has no toxic effects on humans (Bnouham et al. 2007; Azza et al. 2012). Various parts of *T. hirsuta* have been widely used in the paper industry (Schmidt et al. 1983).

In traditional medicine, *T .hirsuta* is used as antiseptic, anti-inflammatory and in the treatment of hypertension (Le Floc'h 1983; Azza et al. 2012; Bnouham et al. 2012; Azza and Oudghiri 2015). It is also used in hypoglycemic, antidiabetic drugs and as an antioxidant (Ziyyat et al. 1997; Djeridane et al. 2006; El Amrani et al. 2009; Akrout et al. 2011; Trigui et al. 2013; Yahyaoui et al. 2017, 2018a and b). *T. hirsuta* extracts are used as anti-melanogenesis (Kawano et al. 2007), anti-tumor (Akrout et al. 2011), anti-cholinesterase and anti-cytotoxic (Yahyaoui et al. 2018a). In Algeria, *T. hirsuta* is used as an antioxidant (Amari et al. 2014), and for the treatment of Leishmanicide and eczema (Boudjelal et al. 2013).

The aqueous extracts of *T. hirsuta* from eastern Algeria have an inhibitory activity to the growth of *S. aureus* and *P. aeruginosa* (Deramchia et al. 2017). Kadi et al. (2017) reported that *T. hirsuta* extracts from the Batna are effective against several bacterial species (*P. aeruginosa*, *E. coli*, and *S. aureus*). The essential oil of *T. hirsuta* has a potent antioxidant activity (Kadri et al. 2011), while essential oils of *T. hirsuta* from Tunisia have significant antibacterial activity against *S. aureus*, *Enterobacter cloacae*, *Bacillus cereus*, *Enterococcus faecalis*, *Bacillus subtilis*, *Micrococcus luteus* and *Escherichia coli* (Felhi et al. 2017).

Thymelaea hirsuta is rich in polyphenols, flavonoids, tannins, and alkaloids (Djeridane et al. 2006; Akrout et al. 2011; Trigui et al. 2013; Amari et al. 2014; Bouzouina et al. 2016; Yahyaoui et al. 2018 a and b). The essential oil of *T. hirsuta* from Tunisia is very rich in heptane, germacrene-D, γ-eudesmol (Kadri et al. 2011; Benchobba et al. 2014). On the other hand, Yahyaoui et al. (2014) reported that the oils from Tunisian are composed of hexadecanoic acid, stylopsal, 4-8-dimethylhecosan, and 5-7-dodecadienal (Z, Z) (Table 1).

The aims of this study were to determine the chemical composition and to evaluate the antibacterial activity of essential oils of *T. hirusta* as a source of the natural antibiotic agent.

MATERIALS AND METHODS

Plant materials

Thymelaea hirsuta, synonym (*Passerina hirsuta* L and *Passerina metnan* Forsk), is belonging to the family

Thymelaeaceae (Schmidt et al. 1983), a perennial shrubby plant can reach 2-3 meters in height. The small leaves are densely imbricated, coriaceous ovoid acute, glabrous below. The flowers are deciduous calyx, yellowish, polygamous, are at the tops of the branches (Figure 1). The fruit is a hairless berry (Quézel et Santa 1963).

Aerial parts of *T. hirsuta* were collected during the flowering stage in May 2017 from M'sila region (Figure 2). The geographical coordinates of sampling locations were noted using a GPS (Table 2).

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 before analysis. The yield based on the dry weight of the samples was calculated.

Essential oil analysis

The essential oils were analyzed on 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), programming from 50°C for 5min – 5°C/min until 300°C. 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).

Table 1. Chemical composition of *Thymelaea hirsuta* essential oil of Tunisia

Localities	Gafsa and Sidi Aich	Tunisia 1	Tunisia 2
References	**A**	**B**	**C**
Hexadecanoic acid	0	0	15.4
Heptane	28.34	34.2	0
Citronellyl formate	9.98	12.04	0
Trans-*β*-caryophyllene	3.25	3.92	0
Germacrene-D	12.98	15.66	0
γ-cadinene	2.55	3.08	0
γ-Eudesmol	11.81	14.25	0
Tetradecamethyl-heptasiloxane	11.83	0	0
4, 8-dimethylhecosane	0	0	12.9
13-methylhexacosane	0	0	5
Stylopsal	0	0	15.5
5,7-dodecadienal (Z, Z)-	0	0	12.2

Note: A. Kadri et al. (2011); B. Benchobba et al. (2014); C. Yahyaoui et al. (2014)

Table 2. Geographical coordinates of sampling locations

Localities		Lat (N)	Lon (E)	Alt. (m)
1	Bouti sayeh	35° 63′ 18″	3° 72′ 55″	647
2	Ain Lehdjel	35° 66′ 60″	3° 85′ 98″	623
3	Mergueb	35° 60′ 14″	3° 93′ 95″	630
4	Sidi Hadjress	35° 66′ 80″	3° 98′ 24″	502
5	Ouanougha	35° 58′ 51″	4° 11′ 10″	922
6	Ouled Mansour	35° 43′ 46″	4° 23′ 47″	478
7	Ben Zouh	35° 51′ 75″	4° 08′ 65″	713

Figure 1. *Thymelaea hirsuta* from M'sila region, Algeria (Photograph: Bounab, 2017)

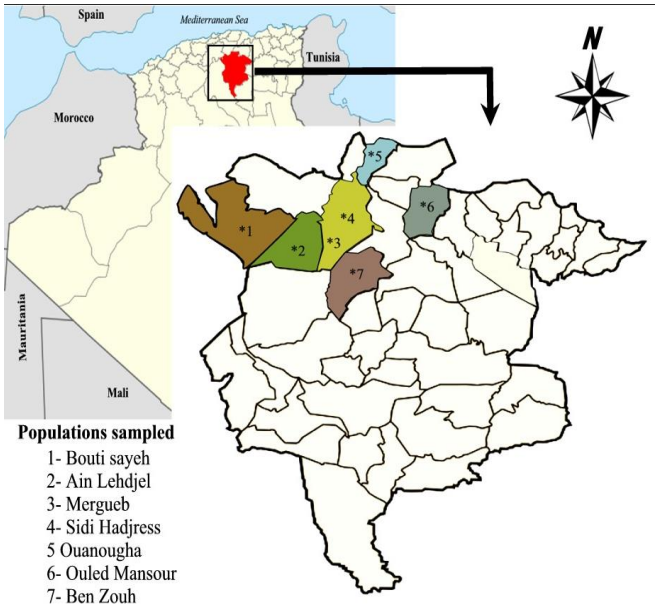

Figure 2. Locations of collected samples of *Thymelaea hirsuta*

Antibacterial activity assessment

The in vitro evaluation of the antimicrobial activity was performed by the disc diffusion method against four Gram-positive bacteria (*Bacillus subtilis* ATCC 6633, *Bacillus cereus* ATCC 11778, *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 51299) and four Gram-negative bacteria (*Escherichia coli* ATCC 25922, *Salmonella enterica* ATCC43972, *Klebsiella pneumoniae* ATCC 700603, and *Pseudomonas aeruginosa* ATCC 27853). The bacterial inoculums were prepared from overnight broth culture in physiological saline (0.8% NaCl) to obtain an optical density range from 0.08-01 at 625 nm. Muller Hinton agar (MH agar) and MH agar supplemented with 5% sheep blood for fastidious bacteria were poured in Petri dishes, solidified and surface dried before inoculation. Sterile discs (6 mm) were placed on inoculated agars with test bacteria, added with 10 µl of stock solution of essential oil and diluted essential oil (1:2, 1:4 and 1:8 v:v of DMSO). DMSO was used as 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

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 analyses were carried out using STATISTICA 10 software. Statistical significance of antibacterial activity results were analyzed by the ANOVA three-way Completely Randomized (Populations, doses, and bacteria) by using the statistical software package (CoStat). All analyses are performed at the 5% significance level ($P < 0.05$).

RESULTS AND DISCUSSION

Chemical analysis

The hydro-distillation of *Thymelaea hirsuta* essential oil gave a pale yellow viscous liquid. The average yield of essential oil of the samples was 0.3 ± 0.12%. The highest value (0.4%) was obtained from the samples collected in Benzoh, Bouti Sayeh, and Ain Lehjel. The results of the analysis and identification of the essential oil components of *T. hirsuta* using GC-GC/MS was shown in Figure 3.

The chemical analysis of the essential oil of *T. hirsuta* by (GC / MS) showed a total of 45 identified compounds with the average of (92.7 ± 4.8%) of the total oil. The identified compounds and their abundance were presented in Table 3.

The chemical composition of *T. hirsuta* was dominated by nonanal-n (10.39 ± 3.21%), with the highest level was in the sample collected from Ouanougha (16.7%). The second highest compound was hexadecanoic acid (9.77 ± 2.81%), followed by nonanoic acid (9.13 ± 6.49%), triacontane (7.2 ± 3.34%), isopropyl tetradecanoate (6.16 ± 1.99%) and tridecane (4.87 ± 3.1%).

The chemical classes of *T. hirsuta* essential oils showed significant variations between samples collected from different locations. Acids and aldehydes are dominant with an average of 23.25 ± 9.67% and 20.25 ± 5.12% respectively, followed by alkanes (16.28 ± 4.27%) and monoterpenes (15.08 ± 1.5%) (Figure 4).

Result of UPGMA Cluster Analysis (Figure 5) revealed two clades of *T. hirsuta* used in this study. The first clade includes samples from two locations of sampling (Bouti-Sayeh and Benzoh), that are characterized by the highest level of nonanoic acid (21.3 and 12.2%).

The second clade groups consisted of samples collected from five sampling locations, which are characterized by hexadecanoic acid, in which samples from Sidi Hadjress deviates from the group by the presence of a high level of triacontane (7.2%). Based on this statistical analysis, four chemotypes were identified that characterize *T. hirsuta* in Algeria (Table 4).

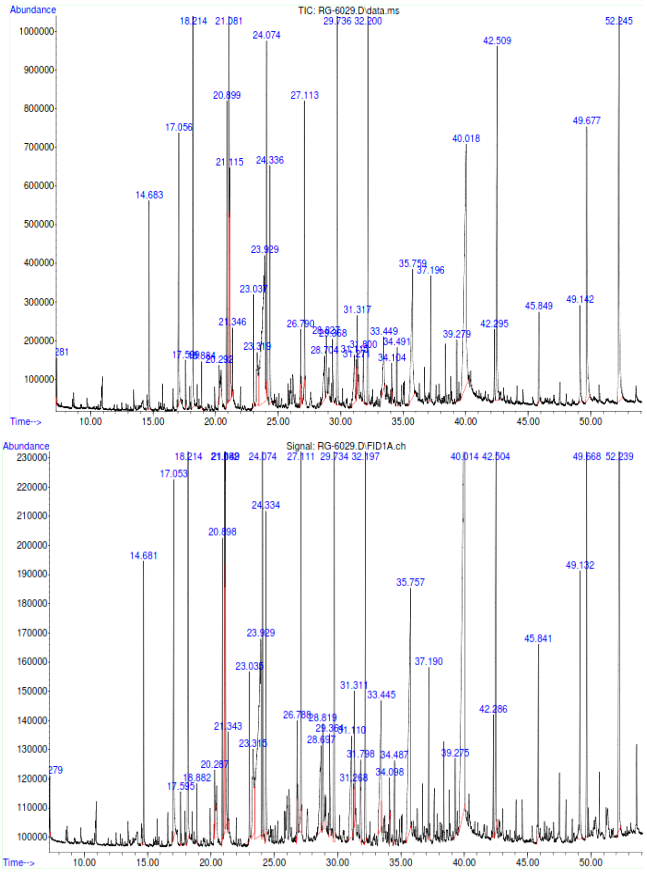

Figure 3. GC/Masse and GC/FID profiles of *Thymelaea hirsuta*

Table 3. Chemical composition of *Thymelaea hirsuta* essential oils from 7 sampling locations in M'sila region, Algeria

Location of collected samples		**Ouled Mensour**	**Ounougha**	**Mergueb**	**Bouti Sayah**	**Ain Lehdjel**	**Sidi Hadjress**	**Ben Zouh**	**Average**	**SD**
Yield (%)		0.18	0.18	0.16	0.4	0.4	0.38	0.4	0.3	0.12
Number of compounds	KIc	30	33	37	28	33	28	27	31	4
Total %		99.7	98	94.9	100	98.8	96.6	99.4	98.2	1.85
Octane-n	800	0.98	0.73	0.26	0.8	0.61	1.42	0	0.69	0.46
Octanal-n	1003	3.14	3.27	1.51	2.34	2.23	3.17	1.43	2.44	0.78
Octen-1-al (2E)	1057	0	0	0	0	0	0	0.52	0.07	0.2
Octanol-n	1072	3.87	3.95	2.66	3.2	3.89	3.63	2.54	3.39	0.6
Linalool oxide cis-	1086	0.61	0.45	0.26	0	0.41	0	0	0.25	0.25
Undecane-n	1096	0.58	0.56	0	0	0	0	0	0.16	0.28
Nonanal-n	1106	11.6	16.7	8.03	6.82	8.99	10.4	10.2	10.4	3.21
Octyl formate	1127	0.98	1.09	0.27	0.8	0.63	1.2	0	0.71	0.44
Nonanol-n	1173	0	1.4	0.4	0	0.58	0	0	0.34	0.52
Naphtalene	1193	4.56	2.35	1.66	1	0.99	2.01	5.6	2.6	1.79
Methyl salicylate	1199	1.85	1.9	3.11	1.06	1.85	0	1.22	1.57	0.96
Dodecane	1200	3.46	2.18	3.55	2.51	2.01	5.76	2.12	3.09	1.34
Butanoate-3Mpentyl-3M	1202	0	0	0	0	1.93	0	1.39	0.47	0.83
Decanal-n	1208	0.78	0.95	0.44	0	0	0.9	0.52	0.51	0.4
Geraniol	1230	0	0.81	0	0	0	0	0	0.12	0.31
Decenal (2E)	1266	0.84	1.2	0.87	1	0.94	0.84	1.81	1.07	0.35
Decanol-n	1275	0	0.5	1.81	0	0.2	0	0	0.36	0.67
Nonanoic acid	1296	6.66	7.16	8.32	21.3	8.28	0	12.2	9.13	6.49
Tridecane	1302	8.51	6.49	3	0	3.7	3.95	8.41	4.87	3.11
Decanoic acid	1306	2.25	0	2.05	0	0	0	0	0.61	1.05
Undecanol n	1311	2.39	3.02	0	0	0	2.07	0	1.07	1.36
Tetradecane	1400	0.87	0.5	0.64	0.63	0.52	0.7	0	0.55	0.27
Dodecanal	1413	2.8	2.85	2.51	1.66	2.18	2.09	1.91	2.29	0.45
Dodecanol-n	1480	1.13	0	0.98	4.03	4.14	2.63	2.85	2.25	1.59
Pentadecane	1500	0	0	0.8	0	0	0	0.59	0.2	0.34
Dodecanoic acid	1511	0	0.98	2.05	2.88	2.84	0	16.7	3.64	5.89
Tridecenol (2E)	1554	4.56	4.59	3.89	2.71	2.95	3.45	3.69	3.69	0.73
Hexenyl benzoate (2Z)	1556	0	0	1.13	0.69	0.99	2.97	0	0.82	1.06
Hexadecane	1571	0	0	0.76	0	0	0	0.97	0.25	0.43
Tetradecanal	1580	3.64	4.73	3.66	2.17	2.57	2.95	4.62	3.47	0.98
Tetradecanol-n	1650	2.63	0.56	2.49	4.11	2.92	0	3.69	2.34	1.53
Heptadecane	1700	0	0	0.23	0	0	0	0	0.03	0.09
Octadecane-1	1717	0	0	0.57	0	0	0	0	0.08	0.22
Isopropyl tetradecanoate	1745	6.55	3.72	5.81	8.65	7.64	7.34	3.39	6.16	1.99
Hexadecanol	1843	1.64	1.04	0.9	1.2	1.27	1.38	1.46	1.27	0.25
Cyclohexadecanolide	1877	0	1	0.51	0.66	1.02	0.88	1.5	0.79	0.47
Hexadecyle acetate	1946	0.92	1.43	0	1.06	0.63	1.5	0	0.79	0.62
Hexadecanoic acid	1976	10.7	6.77	13.6	11.6	10.7	9.5	5.46	9.77	2.81
Eicosene-1	2077	0	0	0	0	0	0.8	0.87	0.24	0.41
Eicosane	2104	3.35	4.53	0.78	4.65	3.92	6.88	3.03	3.88	1.86
Docosene-1	2115	0	0	3.74	0	0	0	0	0.53	1.41
Hexacosane	2305	1.04	1.76	1.29	1.2	2.21	2.19	0	1.38	0.77
Octacosane	2507	1.38	1.68	2.03	1.4	3.06	2.51	0	1.72	0.97
Nonacosane	2541	0	0	0	1.91	2.04	2.71	0	0.95	1.21
Triacontane	2711	5.4	7.09	8.38	7.94	9.99	10.8	0.8	7.2	3.34
Chemical classes										
Monoterpenes		16.83	16.32	13.39	15.25	16.36	13.16	14.23	15.08	1.50
Esters		10.3	8.14	10.32	12.26	13.67	13.01	6	10.53	2.75
Hydrocarbons		10.94	10.17	14.8	11.65	13.63	16.94	7.37	12.21	3.18
Aldehydes		22.8	29.7	17.02	13.96	16.91	20.35	21.01	20.25	5.12
Acids		19.61	14.91	25.9	35.78	21.82	9.5	34.36	23.13	9.67
Alkanes		19.19	17.7	12.89	10.39	15.42	23.38	15.02	16.28	4.27
Others		0	1	0.51	0.66	1.02	0.88	1.5	0.8	0.47

Note: KIc = Kovats retention index calculated

Table 4. Chemotypes of *Thymelaea hirsuta* in M'sila region, Algeria

Chemotypes				Locations of sampling
1	Nonanoic acide	Decanoic acid	Nonanal-n	Benzouh
2		Hexadecanoic acid	Isopropyl tetra decanoiate	Bouti sayeh
3	Hexadecanoic acid	Nonanoic acid	Nonanol- n	Ouled Mansour, Ouanougha
			Triacantane	Mergueb, Ain Lehdjel
4		Triacantane		Sidi Hadjress

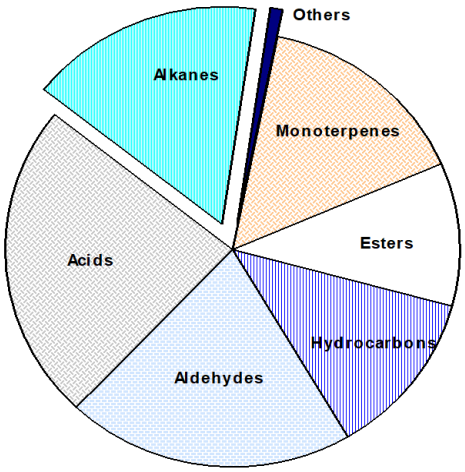

Figure 4. Chemical classes of *Thymelaea hirsuta* oils

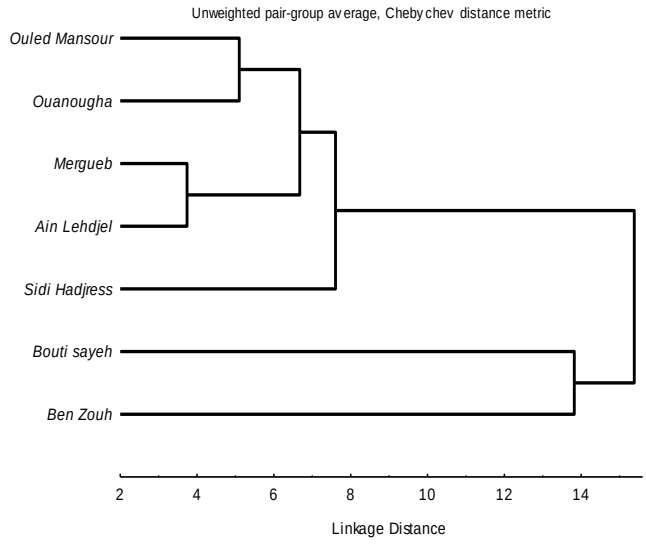

Figure 5. UPGMA cluster analysis of *Thymelaea hirsuta* in M'sila, Algeria

Antibacterial activity

The in vitro evaluation of antimicrobial activity was performed by the diffusion method against eight species of bacteria. All bacteria species tested showed high sensitivity to the essential oil of *T. hirsuta* (Table 5).

The ANOVA statistical analysis (three Way Completely Randomized) showed that sampling locations, doses, and bacterial species, and their interactions are very highly significant ($P<0.001$) (Table 6).

The antibiotic susceptibility test showed that the activity of antibiotic standard (gentamicin) varied according to the species of bacteria. The antibiotic (gentamicin) tested showed a higher growth inhibition than the oils against the bacteria used (group a) (Table 7). Of the four tested concentrations, pure oil exhibited the most potent effect compared to diluted oils (group b).

The most effective oils against the tested bacteria were those of *T. hirsuta* collected from Sidi Hajress, Ouanougha and Bouti Sayeh, forming the group (a). On the other hand, the least antibacterial activity is the oil from *T. hirsuta* collected from Ouled Mansour (Table 8).

The antibacterial activity of *T. hirsuta* essential oil was qualitatively assessed by the diameter of inhibitory zone. The results indicated that the tested oils exhibited a significant antibacterial activity against all the tested bacteria, but on a concentration dependent-manner (Table 9). The highest average of inhibition zone diameters was recorded against *Salmonella enterica* (18.64 mm), while the lowest average of inhibition zone diameter was recorded against *Enterococcus foecalis* (12.07 mm).

Discussion

Hydro-distillation of the aerial parts of *T. hirsuta* gives an average yield of essential oils of 0.3 ± 0.12%, and this result was similar to the study by Kadri et al. (2011). Variations in essential oil yields from different sampling locations may be due to several factors, especially interaction with the environment (the type of climate, soil), harvest time and extraction methods (Viljoen et al. 2006; Sefidkon et al. 2007).

The chemical composition of *T. hirsuta* essential oils was dominated by nonanal-n, hexadecanoic acid, nonanoic acid, triacontane, and isopropyl tetradecanoate. The composition of the essential oils in this study was different from that of collected in Tunisia, whose major compounds were heptane, germacrene-D, eudesmol and citronellyl formate (Kadri et al. 2011; Benchobba et al. 2014). Other studies by Yahyaoui et al. (2014) showed that the essential oil of *T. hirsuta* grown in Tunisia contained stylopsal, hexadecanoic acid, and 5,7-dodecadienal- (Z, Z) (2014).

UPGMA cluster analysis allowed us to compare chemical compounds of *T. hirsuta* from this study with those of the literature (Figure 6). Two clades are different and well separated. The first clade includes samples collected from Gafsa, Sidi Aich and Tunisia (1), which are characterized by the presence of heptane in high proportions, germacrene-D and γ-eudesmol (Kadri et al. 2011; Benchobba et al. 2014). These populations may represent a chemotype with heptane and germacrene-D compounds, as in sample collected from Tunisia.

Table 5. Inhibition diameter (mm) of *Thymelaea hirsuta* essential oils against eight bacteria species

Species of bacteria	Dilutions	Bouti Sayeh	Mergueb	Ain Lehjel	Ouanougha	Ouled Mansour	Benzoh	Sidi Hajress
Pseudomonas aeruginosa ATCC 27853	1	14 ±1.15	15 ± 2	12 ± 2	15 ± 2	14 ± 2	12 ± 1	15 ± 2
	1/2	12 ± 1	12 ± 1.52	9 ± 0.6	12 ± 1	11 ± 1.15	9 ± 1.52	12 ± 1
	1/4	11 ± 1.15	10 ± 0.57	9 ± 5.3	9 ± 1.5	9 ± 0.57	9 ± 5.19	11 ± 1.5
	1/8	9.33 ± 0.6	9 ± 0	0	8 ± 0.6	9 ± 0	0	9.33 ± 0.6
	Gentamicin = 28 ± 1							
Klebsiella pneumoniea ATCC 700603	1	17 ± 1.52	18 ± 1.15	14 ± 2.9	16 ± 1.5	14 ± 3.6	19 ± 1.52	15 ± 1.2
	1/2	14 ± 0.57	12 ± 1	10 ± 0.6	12 ± 0	10 ± 0	13 ± 1.15	11 ± 0.6
	1/4	12 ± 0.54	9 ± 1.15	9 ± 0.6	10 ± 0	9 ± 0	12 ± 1	8 ± 0.6
	1/8	10 ± 1.52	9 ± 1.73	0	9 ± 0	0	10 ± 0.57	0
	Gentamicin = 31.33 ± 1.52							
Escherichia coli ATCC 25922	1	20 ± 2.08	10 ± 1.15	13 ± 1.2	14 ± 1	17 ± 2	16 ± 2.64	31 ±3.6
	1/2	13 ± 1.73	9 ± 0.57	9 ± 0.6	11 ± 0	11 ± 1.15	10 ± 0.57	22 ± 3.8
	1/4	10 ± 0.57	8 ± 0.57	9 ± 5.2	10 ± 1	10 ± 1	9 ± 0.57	17 ± 3.1
	1/8	9 ± 1.15	0	0	0	0	0	13 ± 1.5
	Gentamicin = 30.66 ± 1.15							
Salmonella enterica ATCC43972	1	24 ± 3.05	24 ± 3.78	21 ± 2.5	24 ± 4	11 ± 1.52	20 ± 1.15	27 ± 3.5
	1/2	20 ± 2.08	11 ± 1.15	15 ± 3.2	22 ± 3	10 ± 0	15 ± 3	10 ± 0.6
	1/4	14 ± 3.21	10 ± 0.57	12 ±1.5	15 ± 5.7	9 ± 1.15	12 ± 2.08	10 ± 0
	1/8	11 ± 1.52	8.33 ± 0.6	10 ± 1.2	11 ± 1.7	8 ± 0	10 ± 3.88	0
	Gentamicin = 35.33 ± 0.57							
Bacillus cereus ATCC 11778	1	21 ± 3.51	11 ± 1.52	22 ± 3.1	17 ± 1.5	21 ± 2.51	15 ± 1.52	24 ± 4.01
	1/2	9 ± 0.57	8 ± 0.57	15 ± 1.5	13 ± 0.6	14 ± 3.60	11 ± 1	14 ± 4.7
	1/4	0	8.33 ± 0.6	11 ± 0.6	11 ± 1	11 ± 2	9 ± 1	12 ± 3.2
	1/8	0	0	9 ± 0.6	9 ± 0.6	9.33 ± 0.6	9.33 ± 0.6	10 ± 1.5
	Gentamicin = 33 ± 1							
Staphylococcus aureus ATCC 25923	1	20 ± 1.52	16 ± 1.52	14 ± 1.7	17 ± 1.2	12 ± 1.52	12 ± 1	21 ± 2.5
	1/2	14 ± 1	13 ± 0.57	12 ± 1	13 ± 0.6	11 ± 1	10 ± 0.57	15 ± 1.5
	1/4	11 ± 1.52	11 ± 1.52	10 ± 1	10 ± 0.6	9 ± 1.15	9 ± 0.57	12 ± 1.2
	1/8	9 ± 1	9 ± 0	9 ± 0	9 ± 0.6	0	0	10 ± 1
	Gentamicin = 30.33 ± 0.57							
Bacillus subtilis ATCC 6633	1	16 ± 1.52	24 ± 2	25 ± 3.1	21 ± 2.1	13 ± 1.52	17 ± 2	14 ± 2.1
	1/2	13 ± 1.73	19 ± 3.05	16 ± 1.5	17 ± 0.6	11 ± 1.15	13 ± 0.6	11 ± 2.5
	1/4	11 ± 1.52	14 ± 1.52	14 ± 1.7	13 ± 0.6	9 ± 1.52	11 ± 0.6	10± 2.1
	1/8	10 ± 1.73	11 ± 1.52	12 ± 1	10 ± 1.2	8.33 ± 0.6	9 ± 1	10 ± 1.7
	Gentamicin = 26 ± 1							
Enterococcus faecalis ATCC 51299	1	16 ± 1.15	17 ± 2	14 ± 0.6	18 ± 1.7	13 ± 1.15	16 ± 1.15	14 ± 1
	1/2	13 ± 0.57	14 ± 1	12 ± 1.2	14 ± 1.2	11 ± 1	14 ± 1	11 ± 1.52
	1/4	12 ± 1	11 ± 1.15	10 ± 0.6	11 ± 1.2	10 ± 1	11 ± 1.52	10 ± 0.6
	1/8	9 ± 1	9.33 ± 0.6	9 ± 0	9 ± 0.6	8.33 ± 0.6	9 ± 1.15	9 ± 0
	Gentamicin = 12.9 ± 0.1							

Table 6. Main effects and interactions of essential oils of *Thymelaea hirsuta*

Sources	df	F	P
Main effects			
Sampling locations	6	35.21	.0000 ***
Doses	4	4496.93	.0000 ***
Species of bacteria	7	148.79	.0000 ***
Interactions			
Sampling locations * Doses	24	5.66	.0000 ***
Sampling locations * Species of bacteria	42	30.88	.0000 ***
Doses * Species of bacteria	28	84.45	.0000 ***
Sampling locations * Doses * Species of bacteria	168	7.06	.0000 ***

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

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

Rank	Doses	Mean inhibition zones	n	Significant groups
1	Gentamicin	28.45	168	a
2	1	17.54	168	b
3	0.5	12.96	168	c
4	0.25	10.75	168	d
5	0.125	6.92	168	e

Note: LSD 0.05 = 0.3429650445

Table 8. The effectiveness of *T. hirsuta* essential oils from several sampling locations against eight bacteria species

Rank	Sampling location	Mean inhibition zones	n	Significant groups				
1	Sidi Hajress	16.31	120	a				
2	Ouanougha	16.13	120	a				
3	Bouti Sayeh	15.99	120	a				
4	Mergueb	15.12	120		b			
5	Ain Lehjel	15.07	120			bc		
6	Benzoh	14.71	120				c	
7	Ouled Mansour	13.92	120					d

Note: LSD 0.05 = 0.40580171325

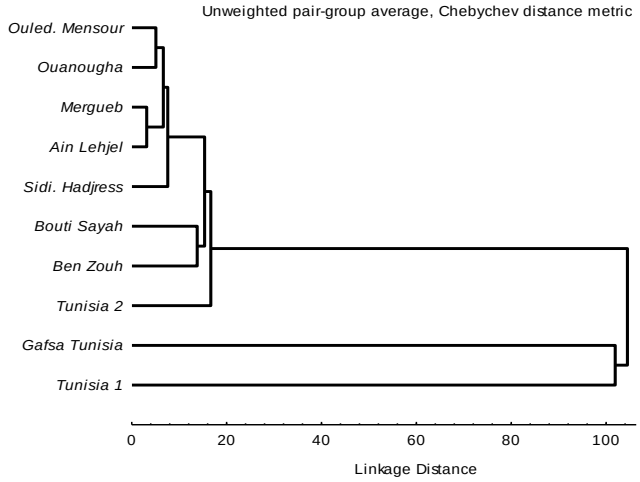

Figure 6. UPGMA of essential oils of *Thymelaea hirsuta* populations

The second clade was clustered between *T. hirsuta* in this study with that of collected in Tunisia (2). The Tunisian (2) was characterized by the presence of hexadecanoic acid and stylopsal (Yahyaoui et al. 2014). This composition may represent a second Tunisian chemotype.

The essential oil of *T. hirsuta* from the M'sila region has antibacterial activity against the tested bacterial species. These bacterial species are highly sensitive, except *Enterococcus foecalis* which shows low sensitivity to the essential oil of *T. hirsuta* with inhibition diameters of 10 to 18 mm for all tested dilutions. This sensitivity gives it the status of sensitive bacteria (+), but statistically, it is the least sensitive, among the bacteria tested, to the oils of the seven populations studied. It is classified in the last group (e) with an average zone of inhibition of 12.07mm.

Compared to the reference antibiotic, similar degrees of inhibitory effects of pure essential oils were observed on Gram-negative bacteria; with inhibitory zones of 10-31 mm. All the dilutions have shown more potent effect against Gram-positive bacteria than Gram-negative ones. All dilutions showed, overall, a more potent effect against Gram-positive bacteria than Gram-negative bacteria.

Table 9. Sensitivity groups of tested bacteria with essential oils of *Thymelaea hirsuta*

Rank	Species of bacteria	Mean inhibition zones	n	Significant groups					S*
1	*Salmonella enterica*	18.64	105	a					++
2	*Bacillus subtilis*	16.33	105		b				++
3	*Bacillus cereus*	16.03	105		b				++
4	*Staphylococcus aureus*	15.36	105			c			++
5	*Escherichia coli*	15.20	105			c			++
6	*Klebsiella pneumoniae*	15.13	105			c			++
7	*Pseudomonas aeruginosa*	13.83	105				d		++
8	*Enterococcus foecalis*	12.07	105					e	+

Note: LSD 0.05 = 0.43382027941; * S.= Sensitivity (++ Highly significant (P <0.01); + Significant (P <0.05)

It is noted that the concentration of essential oils directly influences the inhibitory activity, when the concentration of the oil increases, the diameters of inhibitions are important, similar remarks were made by Emiroglu et al. (2010).In addition, the presence of oxygenated terpenes and especially oxygenated monoterpenes in essential oils may be responsible for the pronounced activity of oils (Akrout et al. 2010; Taran et al. 2010; Ben Marzoug et al. 2011; Bencheqroun et al. 2012). Antibacterial activity appears to result from a combination of the diversity of molecules present in Essential oils and several modes of action (Calsamiglia et al. 2007, Goetz and Ghedira 2012). On the other hand, the method used to study the antibacterial effect of essential oil can be considered as a factor influencing the zones of inhibition (Fazeli et al. 2007).

Kadi et al. (2017) and Deramchia et al. (2017) reported that essential oils of *T. hirsuta* had antibacterial activity. Trigui et al. 2013; and Felhi et al. (2017) also reported that *T. hirsuta* extracts from Tunisia were also able to inhibit the growth of bacteria.

In conclusion, this study showed that the yield of *T. hirsuta* essential oils by hydrodistillation was very low (0.3 ± 0.12 %). There were two clades of *T. hirsuta* in Algeria. The 1st clades have a high content of nonanoic acid, and the 2nd clade has a high content of hexadecanoic acid. Four chemotypes have been identified from the essential oil of *T. hirsuta* from Algeria. The essential oil of *T. hirsuta* was able to inhibit the growth of eight species tested bacteria. The preliminary results obtained are promising in expanding the therapeutic arsenal of plants with antibacterial properties. However, the in vitro methods used to confirm the antibacterial activity of essential oils are insufficient and require further, more advanced, additional testing. More detailed future studies should be performed to identify the antimicrobial activity of essential oils of *T. hirsute.*

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