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Chemical Composition and Antimicrobial Activity of *Elaeoselinum thapsioides* (Desf) Maire from Algeria

Ounoughi Abdelkader[1,2], Ramdani Messaoud[2*], Lograda Takia[2], Chalard Pierre[3], Figueredo Gilles[4]
[1]Université Larbi Ben M'Hidi, Faculté des Sciences, Oum el Bouaghi 04000, Algeria
[2]Laboratory of Natural Resource Valorisation, SNV Faculty, Setif 1 University, 19000 Setif, Algeria
[3]SIGMA Clermont, Campus Des Cezeaux, CS 20265, 63178 Aubière Cedex, France
[4]LEXVA Analytique, 460 Rue Du Montant, 63110 Beaumont, France

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***Corresponding author**
Ramdani Messaoud

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Abstract: The essential oil, isolated by hydrodistillation, from the aerial parts of *Elaeoselinum thapsioides* collected during the flowering phase from Setif region, is analezed by GS/GC-MS. Oil gas chromatography revealed the presence of 32 chemical components representing an average of 99.55% of the total oil. The populations of *E. thapsioides* studied are very heterogeneous in the chemical composition of essential oils, it shows significant differences. The population of Tachouda is characterized by a high rate of myrcene (26.56%), cymene-ortho (12.43%), α-pinene (11.68%), sabinene (10.44%), β-pinene (6.40%), limonene (5.04%), γ-terpinene (4.45%) and germacrene-D (3.72%), while the population of Megres shows the presence of limonene (36.30%), α-pinene (31.50%), trimethyl benzalhyde-(2, 3, 4) (5.83%), Δ3-carene (3.50%) and myrcene (3.50%). The Antibacterial activity of essential oils of *E. thapsioides* is evaluated by the disc method. The oil has no effect against *P. aeruginosa* ATCC 2785 and *K. pneumonia* ATCC 700603, while the essential oils have a high activity against *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922.
Keywords: Elaeoselinum thapsioides, essential oil, Antibacterial activity, Setif, Algeria.

INTRODUCTION

The species of the genus *Elaeoselinum* have been studied either in terms of the composition of their essential oils [1] or their terpenic metabolites [2, 3]. Tetracyclic diterpenes have been reported in several species of *Elaeoselinum* [4, 5]. The chemical composition of essential oils of species of *Elaeoselinum* presents some variability (Table 1), but most of its species contain the major components, α-pinene, β-pinene, sabinene, myrcene and limonene.

Other species are separated by high-rate component, the *E. thapsioides* from the Setif region (Algeria) is isolated by the trimethyl benzaldehyd-(2,3,4) with a level of (36%) [6] and *E. gummiferum* of Malaga (Spain) by β-phellandrene (7%) [1].

The analysis of essential oils of *E. asclepium* showed that this species is rich in α-pinene and other compounds detected in appreciable quantities, β-pinene and sabinene [7, 8]. The main constituents of *E. asclepium* essential oil, from Greece are sabinene, α-pinene, myrcene, β-pinene, germacrene-D, trans-β-farnesene, terpinen-4-ol, β-phellandrene and γ-terpinene [9], populations of Spain, of the same species; contain the same majority components [10]. According to Carretero *et al.* [11], the essential oil of *E. asclepium* ssp *millefolium* has the α-pinene and β-pinene as the major components.

The studies concerning the species *E. thapsioides* are very rare, the analysis of the essential oils of a population, harvested from the Setif region (Algeria), allowed Djarri [6] and Djarri *et al.* [12], identify the major constituents, 2,3,4-trimethylbenzaldehyde (36%), limonene (19.4%), α-pinene (13.6%), Δ3-carene (5%) and (Z)-β-ocimene. Chemical analysis of the essential oil of *E. fontanisii* (synonym of *E. thapsioides*) collected from different parts of Morocco, has shown that its main constituents are terpene hydrocarbons and oxygenated monoterpenes, while sesquiterpene derivatives represent minor constituents. The major components in this species are α-pinene, sabinene, myrcene, limonene, terpinen-4-ol, α-terpinene and γ-terpinene [13]. The oils of *E. gummiferum* collected in Spain, contain the major compounds, α-pinene, myrcene, β-pinene, α-fenchene and phellandrene [1]. The analysis of the essential oil of *E. fetidum*, harvested in Spain has shown that it is rich in α-pinene and β-pinene [1].

The essential oils of *E. fontanisii* and *E. thapsioides* showed moderate antibacterial activity [12, 13].The oil of *E. asclepium* has antimicrobial and antioxidant activities and a have a higher effets on *Candida albicans* [8].

E. asclepium essential oil shows strong effect on *Candida albicans*. Moreover, this oil shows fungicidal activity against *C. albicans* [15]. The study by Denecke et al. [16] showed that essential oils of *E. asclepium* have some toxicity, whereas the works of Rosselli et al. [5] and Bouchekrit [17] have demonstrated the opposite. The essential oil of *E. asclepium* from Greece has shown low larvicidal activity [18].

Aim of the study

The aims of this study were to examine the chemical composition of the essential oil isolated from *E. thapsioides*, and to test the efficacy of the essential oil as an antibacterial potential.

MATERIALS AND METHODS

Plant material

Samples of *Elaeoselinum thapsioides* (Desf.) Maire. were collected in the flowering stage, in two eastern Algeria localities, Megres and Tachouda (Figure 1). *E. thapsioides* It is a perennial plant up to 3 m tall, found on dry and rocky terrain. The leaves are petiolate, very glabrous, and smooth with short and acute linear divisions, all more or less spread out in a plane. The umbels with pedicels are longer than the fruit. This species occurs in lawns and scrub throughout Algeria, his vulgar name is "Becibsa".

This species has a synonym (*E. fontanesii* Boiss) [21] and *Laserpitium thapsioides* Desf. [22]. *E. fontanesii* Boiss is indicate in Algeria [22-24]. Voucher specimens of populations sampled were deposited in the herbarium of the Department of Ecology and Biology, Setif-1 University, Algeria.

Extraction of the essential oil

The air dried materials were subjected to hydro-distillation for 3h using a Clevenger apparatus type. The oil obtained was collected and dried over anhydrous sodium sulphate and stored in screw capped glass vials in a refrigerator at 4-5°C prior to analysis. Yield based on dried weight of the samples was calculated.

Essential oil analysis

The essential oils were analysed on a Hewlett-Packard gas chromatograph CPG/FID 7890, coupled to a gas chromatograph: CPG/MS 7890/5975C, equipped with a Colonn Apolar: DB5 MS: 40 m 0,18 mm 0,18 µm, 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 [25, 26] and those described by Adams as well as on comparison of their retention indices either with those of authentic compounds or with literature values [27].

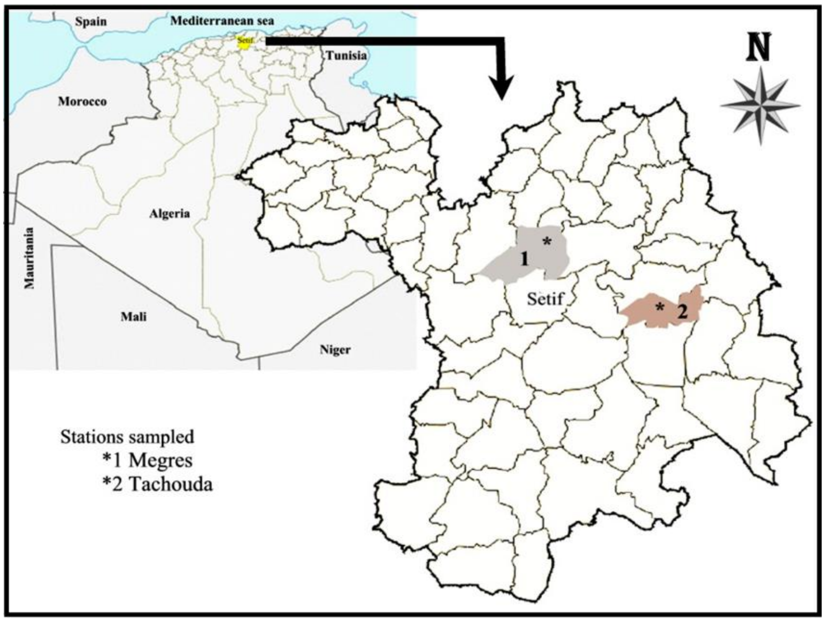

Fig-1: Populations of *Elaeoselinum thapsioides* sampled

Table-1: Main components of *Elaeoselinum* species from literature

Elaeoselinum species and localities		References	α-pinene	β-pinene	Sabinene	α-fenchene	Myrcene	Limonene	α-terpinene	γ-terpinene	β-phellandrene	Terpinen 4-ol	β-farnesene-T	Germacrene-D	α-copaene	Δ3-carene	Trimethyl benzaldehyde 2,3,4
fontanesii	Morocco 1	[13]	25	4	11	0	25	33	0	0	0	0	0	0	0	0	0
	Morocco 2		51	9	20	0	3	9	1	0	0	2	0	0	0	0	0
gummiferum	Spain 1		46	8	16	0	1	0	2	0	0	3	0	0	0	0	0
	Spain 2		65	11	15	0	1	0	0	0	0	1	0	0	0	0	0
foetidum	Spain 1		81	14	1	0	1	1	0	0	0	0	0	0	0	0	0
	Spain 2	[14]	77	17	1	0	1	1	0	0	0	0	0	0	0	0	0
asclepium	Spain 1	[1]	37	14	0	10	23	0	0	0	7	0	0	0	0	0	0
	Spain 2	[10]	67	21	5	0	1	0	0	0	0	0	0	0	0	0	0
	Spain 3	[11]	81	9	0	0	0	0	0	0	0	0	0	0	0	0	0
	Greece	[9]	27	6	35	0	3	0	0	0	2	4	5	5	0	0	0
	Italy 1	[7]	77	4	12	0	1	0	0	0	1	0	0	0	0	0	0
	Italy 2		92	4	0	0	0	0	0	0	0	0	0	0	0	0	0
	Italy 3		61	8	13	0	0	0	0	0	0	0	0	0	0	0	0
asclepium	Algeria Skikda	[8]	44	16	28	0	1	2	0	0	0	1	0	0	0	0	0
tenuifolium	Spain 1	[13]	3	1	24	0	48	7	4	2	0	4	0	0	0	0	0
	Spain 2	[19]	1	1	17	0	66	9	0	0	2	1	0	0	0	0	0
	Portugale 1	[20]	0	1	2	0	77	6	0	1	2	1	0	1	3	0	0
	Portugale 2		0	1	11	0	48	20	0	3	1	4	0	1	4	0	0
	Portugale 3		0	1	0	0	85	2	0	0	0	0	0	1	0	0	0
	Portugale 4		1	1	6	0	59	14	0	0	2	2	0	0	0	0	0
thapsoides	Algeria Setif	[6]	14	0	0	0	3	19	0	0	0	0	0	0	0	5	36

Antibacterial activity

The antimicrobial activities of the essential oil of *E. thapsioides* were evaluated against three Gram negative bacteria (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 2785 and *Klebsiella pneumonia* ATCC 700603), and one Gram positive bacteria *Staphylococcus aureus* ATCC 2592. The bacterial inoculums were prepared from overnight broth culture in physiological saline (0.8 % of NaCl) in order to obtain an optical density ranging from 0.08-01 at 625 nm. MullerHinton 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 F) were placed on inoculated agars, by test bacteria, filled with 10 µl of mother solution and diluted essential oil (1:2, 1:5 and 1:10 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. All tests were performed in triplicate. Then, Petri dishes were incubated at 37°C during 18 to 24h aerobically (bacteria). After incubation, inhibition zone diameters were measured and documented.

STATISTICAL ANALYSIS

Cluster analysis (UPGMA) was carried out on the original variables of *Elaeoselinum* species essential oils and on the Manhattan distance matrix to seek for hierarchical associations among the species. The cluster analyses were carried out using Statistica v10 software.

RESULTS

The hydrodistillation of the dried aerial parts of the two populations of *Elaeoselinum thapsioides* gave yellow oil with a no aromatic odor. The essential oil yield average is 0.19% (v/w). The analysis and identification of the components of the essential oil of this species was performed using the (GC-GC/MS) (Figure 2).

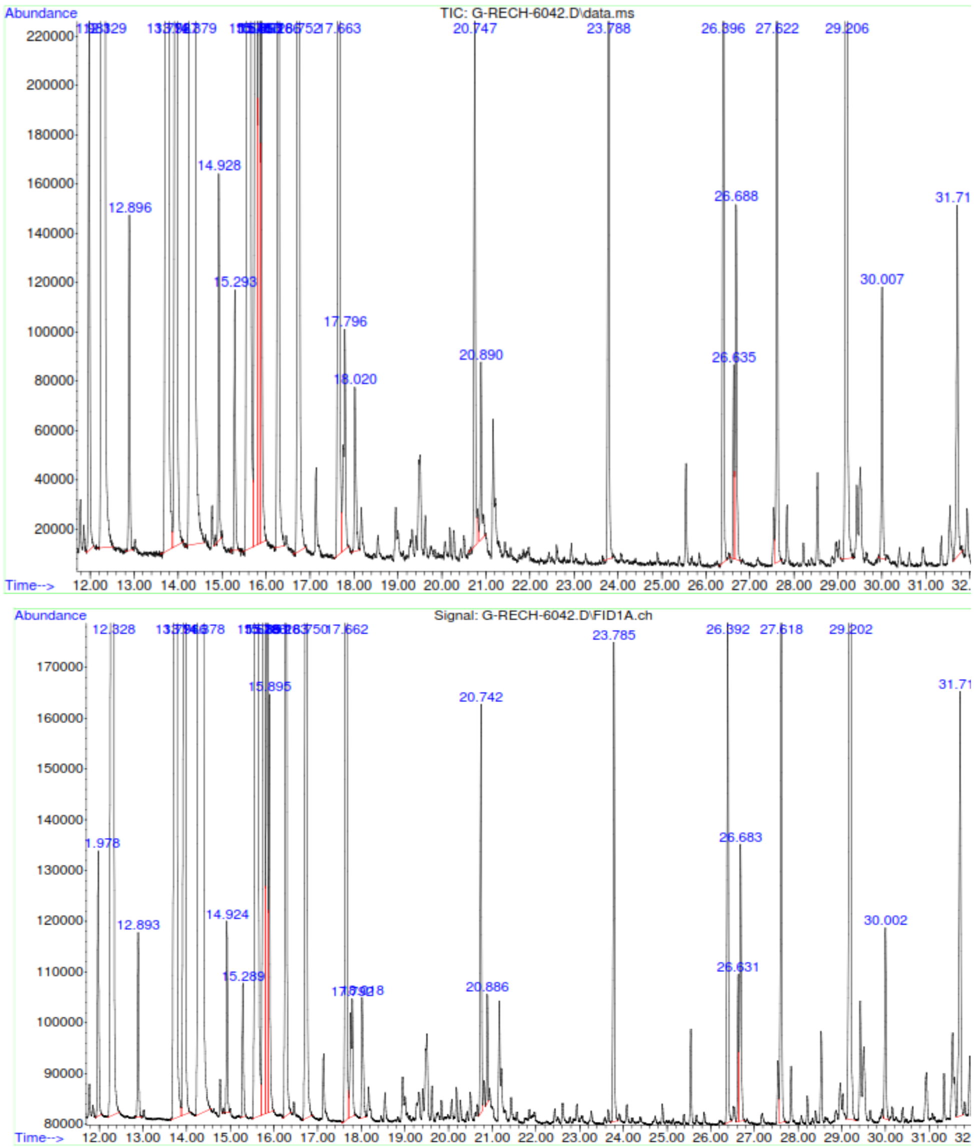

Fig-2: GC/MAS and FID profiles of *Elaeoselinum thapsioides*

The compound identified in these oils and their relative abundances are presented in order of their appearance (Table 1). This analysis allowed the identification of 32 chemical components representing an average of 99.55% of the total oil.

Table-2: Chemical composition of *Elaeoselium thapsoides* Essential oil

Localities		Tachouda	Megres
Yield %		0.8	1
Number of compounds		26	23
Total %	KI	99.3	99.8
α-thujene	921	0.61	0.00
α-pinene	931	11.68	31.50
Camphene	946	0.39	0.30
Sabinene	969	10.44	0.56
β-pinene	974	6.40	0.97
Myrcene	985	26.56	3.50
Mesitylene	990	0.00	0.81
α-phellandrene	1002	0.39	0.54
Δ3-Carene	1006	0.30	5.83
Cymene-ortho	1023	12.43	0.80
β-phellandrene	1029	2.36	0.00
Limonene	1030	5.04	36.30
β-ocimene-(Z)	1030	0.87	2.74
β-ocimene-(E)	1042	2.82	1.37
γ-terpinene	1054	4.45	0.15
Terpinolene	1081	3.39	0.49
Cymenene-para	1086	0.51	0.00
Furan 3-(4-methyl-3-pentenyl)	1092	0.35	0.00
Ocimene-allo	1123	0.00	0.22
α-campholenal	1125	0.00	0.22
Terpinol-L	1184	0.91	0.00
Benzene acetonitale	1189	0.22	0.00
Bornyl acetate	1288	1.08	1.76
Trimethyl benzaldehyde 2,3,4	1322	0.00	9.30
α-copaene	1383	1.16	0.00
β-bourbonnene	1392	0.30	0.00
Caryophyllene (E)	1430	1.34	0.24
Germacrene-D	1491	3.72	0.86
β-selinene	1499	0.00	0.71
α-selinene	1505	0.00	0.29
α-cadinene	1524	0.47	0.00
Caryophyllene 14-hydroxy	1595	1.14	0.00

23 components are identified in the oil of Megres population, corresponding to 99.78% of the total oil. In the oil of the Tachouda population, 26 components are identified, representing 99.31% of total oils of the population. According to our results the chemical composition the essential oil of *E. thapsioides* is dominated by the presence of major products, limonene (36.3-5.04%), α-pinene (31.5-11.68%), myrcene (3.5-26.56%), cymene-ortho (0.8-12.43%), β-pinene (0.97-6.4%), trimethyl benzaldehyde-(2,3,4) (9.3-0.00%) in the populations of Megres and Tachouda, respectively.

The Megres population is isolated by the presence of high level of limonene (36.3%), α-pinene (31.5%) and trimethyl benzaldehyde-(2,3,4) with a rate of (9.3%) which is absent from the Tachouda populations, while the population of Tachouda is characterized bay a high level of myrcene (26.56%), cymene-ortho (12.43%) and γ-terpinene (4.45%).

The Antibacterial activity of essential oils of *E. thapsioides* is evaluated by the disc method. The diameters of inhibition of the bacterial strains are expressed by measuring the diameter of the inhibition halos in mm after 24 hours of incubation in an incubator at 37°C (Table 3).

Table-3: Inhibition diameter of essential oil of *E. thapsioides*

Bacteria	Ant.	Megres			Tachouda		
		Dilution					
		1/2	1/5	1/10	1/2	1/5	1/10
Staphylococcus aureus ATCC 25923	18	32	18	14	28	11	7
Escherichia coli ATCC 25922	22	28	18	18	20	14	11
Pseudomonas aeruginosa ATCC 2785	24	0	0	0	0	0	0
Klebsiella pneumonia ATCC 700603	19	0	0	0	0	0	0

Ant.: Gentamicine

The results show that *P. aeruginosa* ATCC 2785 and *K. pneumonia* ATCC 700603 are resistant to the oil of *E. thapsioides,* while the essential oil of the two populations (Megres and Tachouda) have a high activity against *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922 (Figure 3).

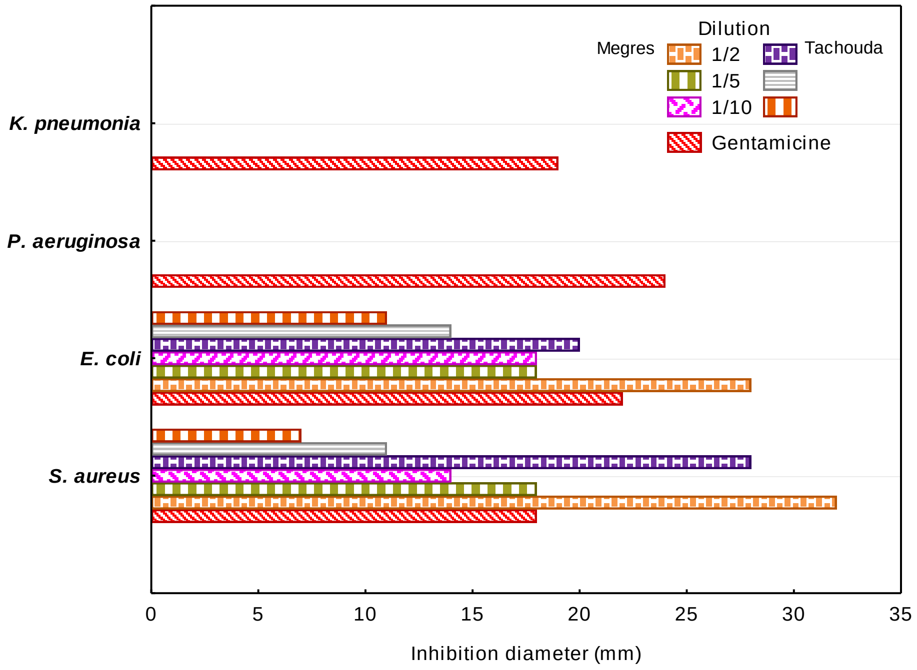

Fig-3: Inhibition diameter of *E. thapsioides* essential oils

DISCUSSION

The essential oil yield in this study (0.19%) was relatively low compared to the species yields in the same genera: The works of Djarri [6] on the samples of *E. Thapsoides*, from Setif (Algeria), show a high yield (0.5%). The aerial parts of *E. gummiferum* give a yield of 2.66% [1]. The essential oil extracted from *E. asclepium* give a high yield 1.98% [15]. The fruits of *E. asclepium* give a yield of (4.66%) [10, 11] and (3.8%) [7]; on the other hand on the aerial parts of the same species Evergetis *et al*. [9] find a yield of 0.02%.

The yield of *E. fetidum* is 5% for fruits and 0.55% for leaves and stems [14]. *E. tenuifolium* has a high yield for fruits and flowers (4.93 - 4.63%) respectively, and a low rate for leaves (0.66%) [19]. The variations in yields could be attributed to several factors such as the extraction technique and the collection period of the plant material [28. Our results show that the population of Megres contains the following major components: limonene (36.3%), α-pinene (31.5%), trimethyl benzaldehyde- (2,3,4) (9.3%), Δ3-carene (5.83%) and myrcene (3.5%).

The study of *E. thapsioides*, specimen collected from the region of Setif (Algeria), allowed Djarri [6] to identifying the same components with some variability in attendance rate (trimethyl benzaldehyde (2,3,4) (36%), limonene (19.4%), α-pinene (13.6%), Δ3-carene (5.0%), (Z) -β-ocimene (3.3%) and myrcene (3.1%). While the chemical composition of the essential oils of the Tachouda population is characterized by a high rate of myrcene (26.56%), cymene-ortho (12.43%), α-pinene (11.68%), sabinene (10.44%), β-pinene (6.4%), limonene (5.04%) and γ-terpinene (4.45%). This composition is very different from the composition of Megres population and that of Djarii [6], but it is close to the chemical composition of the essential oil of *E. fontanisii* collected from different parts of Morocco, which presents major components (α-pinene, sabinene, myrcene, limonene, terpinen-4-ol, α-terpinene and γ-terpinene) [13].

UPGMA statistical analysis, using data from the literature, allowed us to recognize the interspecies relations of the genus *Elaeoselinum*. The population of *E. thapsioides* of Megres is very close to the population of Sétif studied by Djarii (2011), whereas the population of Tachouda is close to that of *E. fontanesii* of Morocco (Figure 4).

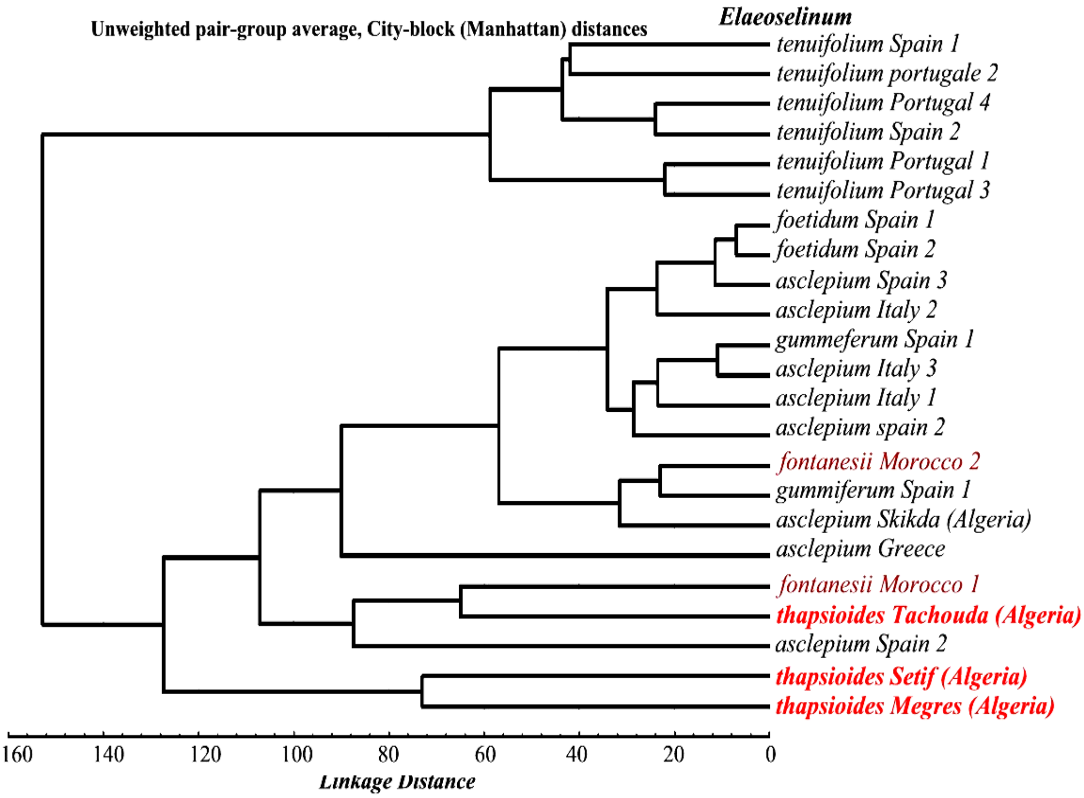

Fig-4: UPGMA cluster of *Elaeoselinum* species

So we can suggest that the population of Megres belongs to the species *E. thapsioides* and Tachouda population belongs to the species *E. fontanesii*, knowing that the latter species is not reported in Algeria and its distribution is limited to Morocco.

The antibacterial activities of the genus *Elaeoselinum* are little studied. The *E. asclepium* has antimicrobial effect on *Candida albicans* ATCC 1024 strain [8] and *E. tenuifolium* has antifungal activity [20]. To our knowledge, no investigation of antibacterial activity of *E. thapsioides* essential oils has been performed.

Our investigation shows a high activity of *E. thapsioides* essential oil on *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922, and no effect against *P. aeruginosa* ATCC 2785 and *K. pneumonia* ATCC 700603. The absence of antibacterial activity can be explained by the developed resistance of some strains that react differently to several essential oils; this is the case of *P. aeruginosa* [29, 30].

CONCLUSION

This study aims to identify the chemical composition of *E. thapsioides* essential oils and the evaluation of their antibacterial activities. The analysis of the chemical composition of the essential oil by GC/MS has allowed the identification of 32 compounds. The major components α-pinene, limonene, myrcene, cymene-ortho, sabinene, β-pinene are identified in *E. thapsioides* essential oils. Trimethyl benzaldehyde-(2,3,4) is identified in this species and it was found to be absent in *Elaoselinum* species. The population of Tachouda is close to that of *E. fontanesii* of Morocco. So we suggest that Tachouda population belongs to the species *E. fontanesii.* An additional characteristic of *E. thapsioides* essential oil was its prominent antibacterial activity against *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922. These results are very helpful to rehabilitation of Algerian flora and further studies are needed to prove the presence of the species *E. fontanesii* in Algeria and its relationship with *E. thapsioides.*

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