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Original Article

Chemical Analysis and Antimicrobial Activity of *Teucrium polium* L. Essential Oil from Eastern Algeria

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ABSTRACT

Objective: The investigation of the chemical composition and antibacterial activity of essential oils from *Teucrium polium* and the chromosome number identification.
Material and Methods: This study was carried in the Department of Biology and Ecology (University Ferhat Abbas) and ENSCCF (France) between September 2013 and October 2014. Aerial parts were collected from three localities from Eastern Algeria (Beni Aziz, Boutaleb and Amouchas). The air-dried aerial parts were subjected to hydro-distillation using a Clevenger-type apparatus. The analysis and identification of the essential oil components of *Teucrium polium,* was performed using the GC-MS. The disk diffusion method is utilised for determination of the antibacterial activity.
Results: The average yield of essential oil of the samples is 0.37%. These analyses led to the identification of 57 components. The chemical composition of the essential oil of *T. polium* is dominated by the presence of a major product, α-pinene (14.1 - 18%), β-pinene (15.3 - 18.1%), germacrene-D (3.8 - 19%), myrcene (8.2 - 10.4%), limonene (5.3 - 8.7%), sabinene (2.7 - 4.3%), caryophyllene-(E) (1.8 - 2.3%) and spathulenol (1 - 2.9%). The essential oil of the Beni Aziz population has a high antibacterial activity against *Escherichia coli*, *Staphylococcus aureus* and the yeast *Saccharomyces cerevisiae*. The population of Boutaleb has significant activity against *Bacillus cereus* and no action against *S. aureus* and the yeast *S. cerevisiae*.
Conclusion: The observed differences and variability of the essential oil of *T. polium* of Algeria are likely due to different environmental and genetic factors. The number of components is different in these

three populations of *T. polium* from eastern Algeria. Our investigation allows us to support the presence of several chemotypes in *T. polium*. The chemotype to germacrene-D is located in the regions of Amouchas and Boutaleb. Beni Aziz is the region that has favoured the development of Δ-elemene chemotype. The results of this work show that the essential oil of *T. polium* possesses antimicrobial properties, which can be used as natural antimicrobial agents.

Keywords: *Teucrium polium,* Essential oil, Antibacterial activity, Chemotype, Algeria.

INTRODUCTION

In the literature, several studies on the essential oils composition of *Teucrium polium* were performed[1-14] (Table 1). This species is rich in essential oil[13] and alkaloids[15]. Chemical analyzes of this species have revealed the presence of the esters and caffeic acid[16], cardiac glycosides[17], phenylethanoid[18] and the flavonoids[19,20]. Chemical analysis has allowed the identification of sterols, triterpene[17] and furanoid diterpenes[21].

The cases of hepatic toxicity caused by *Teucrium* have been described[22]. Consumption of this plant causes hepatitis[23,24]. The flower buds of *T. chamaedrys*, used in the preparation of tea, are considered by the Italian Ministry of Health as toxic[25]. The hepatic toxicity has been attributed to neoclerodane diterpenoids[26,27]. The therapeutic benefit of *Teucrium* is often attributed to their antioxidant properties[20,28,29].

The leaves and flowering tops of *T. polium* have an antiseptic and anti-inflammatory properties[30] and anti-oxidants[31]. The extract methanoic of *T. polium* protects RBCs against lipid peroxidation[32]. It has properties, sudorific, tonic and antipyretic[33], vulnerary, expectorant and ermifuge[34]. The essential oils of *T. polium* have a powerful effect against diabetes[35-39], while others have noticed the negative effects of the crude extract on liver and kidney function[23,40]. In traditional medicine the aerial parts of the plant are used in the treatment of abdominal cramps, headaches and diabetes[41]. The aqueous extract of the plant has an antispasmodic effect, anorexia and hypolipidemic animal[35,36].

It has been found that the extract of leaf of several plants have an important antimicrobial activity against human pathogens[42,43]. Various studies have shown significant antibacterial activity of *T. polium*[9]. This plant show an efficiency against *Staphylococcus epidermidis* compared to the gentamicin[44]. The test of MIC and MBC against *S. pneumoniae*, *S. aureus* and *Escherichia coli* have shown that these bacteria are sensitive to very low concentrations of the essential oil of *T. polium*[45]. The antimicrobial effects of this species were evaluated on bacteria and showed that extracts were quite effective on *Streptococcus pyogenes* and *S. epidermidis*[46].

The extract of *T. polium* the Mediterranean region has a high inhibitory effect against several bacteria[47]. The same effect is observed on the extracts from the region of Iraq[48], and in the region of Iran[46]. The *T. polium* extracts are effective on *Bacillus anthracis*, *Bordetella* *bronchiseptica* and *Salmonella typhi*[47]. The same observations were made on *Klebsiella pneumoniae*[13]. The extracts of *T. polium* of Turkey have high activity against *Staphylococcus aureus*, but do not have activity against *E. coli*[49]. The methanol extracts of *T. polium* of London show high activity on *Bacillus subtilis*, *Micrococcus luteus* and *Paracoccus pantotrophus*[50].

In the present study, the aim was to identify the chemical composition of the oils of *Teucrium polium* obtained from plants growing in the eastern Algeria as well as to evaluate their antimicrobial activity and identification of the chromosome number.

MATERIALS AND METHODS

Plant material

Teucrium polium samples were collected from natural populations of Setif region which is located in the North East part of Algeria (Figure 1). Aerial parts were collected in September 2013, from three localities (Beni Aziz, Boutaleb and Amouchas) (Figure 2). Voucher specimens were deposited in the herbarium of the Department of Ecology and Biology, Setif University, Algeria.

Extraction of the essential oil

The air-dried aerial parts of the three populations were subjected to hydro-distillation for 3 h with the distilled water using a Clevenger-type apparatus. The oil obtained was collected and dried over anhydrous sodium sulphate and stored in screw capped glass vials in a refrigerator at 4°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 Model 5890, coupled to a Hewlett-Packard model 5971, equipped with a DB5 MS column (30 m X 0.25 mm; 0.25 µm), programming from 50°C (5 min) to 300°C at 5°C/min, with a 5 min hold. Helium was used as the carrier gas (1.0 ml/min); injection in split mode (1:30); injector and detector temperatures, 250 and 280°C, respectively. 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 33-450.

The identification of the components was based on comparison of their mass spectra with those of NIST mass spectral library[51,52] and those described by Adams, as well as on comparison of their retention indices either with those of authentic compounds or with literature values[53].

Antibacterial and antifungal activities

The antimicrobial activity of *Teucrium polium* essential oils has been investigated on different bacteria and yeast. The Extract Essential oil was tested against the following bacteria; two gram negative bacteria: *Escherichia coli* ATCC 25922 and *Bacillus cereus* ATCC 10876 and two gram positive bacteria; *Staphylococcus aureus* ATCC 6538 and *Micrococcus luteus* ATCC 533 and the yeast *Saccharomyces cerevisiae* ATCC 763. The *in vitro* antibacterial and antifungal activity of the examined extract was assessed the determination of the activity by the disk diffusion method, according to recommendations of the Clinical and Laboratory Standards Institute.

The bacterial inocula were prepared from overnight broth culture in physiological saline (0.9% of NaCl) in order to obtain an optical density ranging from 0.08-01 at 625 nm. Muller-Hinton agar (MH agar), and the Sabouraud broth for yeast, were poured in Petri dishes, solidified and surface dried before inoculation. Sterile discs (6 mm Φ) were placed on inoculated agars, by test bacteria, filled with 10 µl of mother solution. 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

Data were treated with the Unweighted Pair Group Method with Arithmetic mean (UPGMA), using the set of terpenoids that was found in sufficient quantity to be considered for statistical analysis. The analysis was carried out on the original variables and on the Manhattan distance matrix to seek hierarchical associations among the species. The cluster analysis was carried out using software Statistica 10.

RESULTS

The hydrodistillation of the aerial parts of *Teucrium polium* of Beni Aziz, Amouchas and Boutaleb populations gave essential oils of yellow color. The average essential oil yield is 0.37%. The essential oil tested in this study was analyzed using GC-MS to identify its major components. Total ion chromatography of the oils revealed 56 significant peaks representing an average of 93.9% of the total oil (Figure 3). The mass spectrum of each peak was analyzed, followed by a search of the library of mass spectra of known chemicals. The compounds identified in these oils and their relative abundances are presented in order of their appearance (Table 2).

44 components are identified in the oil of Amouchas, corresponding to a percentage of 92% of the total oil. In the oil of the Beni Aziz population, 39 terpenoid components are identified, representing 97.5%, while the population of Boutaleb contains 40 products, representing 92.3% of total oil of the population.

The essential oil of *Teucrium polium* is dominated by the presence of major products, α-pinene (14.1-18-17.4%), β-pinene (15.3-18.1-15%), germacrene D (14.7-3.8-19%), myrcene (8.6-10.4-8.2%), limonene (5.3-8.7-6%), sabinene (2.7-4.3-3.6%), Caryophyllene-(E) (2.9-2.2-1.8%), Spathulenol (2.7-1-2.9%), β-bourbonene (1.4-1.6-1.7%) and Caryophyllene oxide (1.5-1-1.5%) in the populations of Amouchas, Beni Aziz and Boutaleb, respectively.

The Amouchas population is isolated by the presence of α-cadinol (3.6%), the population of Beni Aziz by α-thujopsein-2-ol (3.5%), while the population of Boutaleb contains the dauca 5-8-diene with a rate of (1.6%), which is absent from the other two populations. The α-humulene and α-cadinol-epi are present in populations of Amouchas and BeniAziz. The Δ-cadinene and α-muurolene-epi are found in Amouchas and Boutaleb.

The Antibacterial activity of essential oils of *T. polium* 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).

The results show that the essential oil of the population of Beni Aziz has high activity against *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 6538 and the yeast *Saccharomyces cerevisiae* ATCC 763. This action is low against Bacillus cereus ATCC 10876. The population of Boutaleb has significant activity against *Bacillus cereus* ATCC 10876, an average activity against *Escherichia coli* ATCC 25922 and no action against *Staphylococcus aureus* ATCC 6538 and the yeast *Saccharomyces cerevisiae* ATCC 763 (Figure 4).

The actions of essential oils of the populations studied have a moderate effect on the bacterium *Micrococcus luteus* ATCC 533. The essential oil of Amoucha population has high activity against *Bacillus cereus* ATCC 10876 and a weak action against the other bacteria. The yeast *Saccharomyces cerevisiae* ATCC 763 is resistant to the essential oil of this population.

DISCUSSION

The main goal of this study was to evaluate the chemical composition of Algerian germander. The yield average of the oil is 0.37%. These results fit into the overall context of yield of *T. polium*. In western Algeria a low yield of 0.21% was found[9]. However, Aburjai[54] and Kabouche[1] have obtained a yield 0.8% and 1.7%, respectively. The yield of this species is found very high 6% in Iran[37], 3.3% in Turkey[2]. While in a population of Iran a yield of 0.5% was found[10]. This difference in yield of essential oils of *T. polium* may be related to several factors, such as geographical area, climate, stage of development of the plant and the harvest season.

The oil predominantly contained α-pinene, limonene, β-pinene and camphene. The β-pinene is the major component in populations Beni Aziz (18.4%), Amouchas (15.71%) and Boutaleb (15.01%); the same rate of β-pinene was observed in populations of Turkey[2] of Tlemcen[8,9] and Iran[4,5,10,13]. This rate is very low (0.3%) in the Sultanate Oman[11].

The population of Boutaleb contains a rate of germacrene-D (19.02%), Beni Aziz (3.76%) and Amouchas (14.70%); the same rate is observed in the population of Tlemcen[8], while, the lowest rate is observed in populations of Iran[10,13] and Greece[3]. The limonene is the major component of the Greece population (37.7%), his rate is lower in the populations of Amouhas, Beni Aziz Boutaleb and in populations of Tlemcen[8], Turkey[2] and Iran[13]. The population of the Sultanate Oman presents the ledeneoxide (II) as the major component with a rate of (20.47%)[11]. The comparison of chemical components of essential oils in our samples, with those of *T. polium* shows that the α-pinene is the major product of the oil[2,4]. The rate of α-pinene is lower in populations of Tlemcen[8,9] and in Iran populations[5,13] while it is very low in the population of the Sultanate Oman[11] and in Greece[3].

The comparison of the populations studied to those in the world is based on the construction of clades. The UPGMA has divided the populations into several clades (Figure 5). This fragmentation of populations of the species into several groups demonstrates that populations of *T. polium* are different chemically, which lead us to suspect the presence of chemotypes. It is noted that the Algerian populations of *T. polium* are confined in the same subgroup. The populations studied (Amouchas, Boutaleb and Beni Aziz) are grouped together with one populations of Iran and a population of Turkey. The populations from Tlemcen are characterized by germacrene-D, β-pinene and spathulenol[8,9] thus forming the chemotype of the western region of Algeria.

In eastern Algeria, on samples of Ain Melilla, the chemotype to α-cadinol-α-pinene and β-pinene was identified[1], this chemical composition is observed in Saudi Arabia[14]. The Algerian Sahara (Tamarast) contains a chemotype of limonene, β-caryophyllene and δ-Cadinen[12]. Our investigation on the populations of the Setif region allowed us to identify a chemotype (α-pinene; β-pinene; germacrene-D and myrcene), this chemical composition is close of chemotype of western Algeria.

The essential oil of *T. polium* exhibits antibacterial and antifungal activity moderate, except Boutaleb population which has no effect on *Staphylococcus aureus*

ATCC 6538 and *Saccharomyces cerevisiae* ATCC 763. Vahdani[45] have shown that the essential oils of *T. polium* from Iran have antimicrobial activity against *Staphylococcus aureus* and *Escherichia coli*. *Bacillus cereus* is very sensitive to the *T. polium* oil of Tlemcen populations[1]. The essential oil of the aerial parts of *T. polium* shows significant activity against *Bacillus subtilis*, *Micrococcus luteus* and *Paracoccus pantotrophus*, while it has no effect on *E. coli* strain[46].

CONCLUSION

The observed differences and variability of the essential oil of *T. polium* of Algeria are likely due to different environmental and genetic factors. Our results showed that the number of components were different in these three populations of *T. polium* from eastern Algeria. The GC/MS analysis results of the samples led to identification of 58 compounds. Our investigation of the chemical compositions of the essential oil of *T. polium* led us to the identification of a chemotype (α-pinene; β-pinene; germacrene-D and myrcene) in the region of Setif. The results of this work show that the essential oil of *T. polium* possesses antimicrobial properties, which can be used as natural antimicrobial agents.

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Table 1. Chemical composition of essential oil of *Teucrium polium*

Country	Greece	Iran						Oman	S. Arabia	Turkey	Croatia	Algeria			
												Tlemecen		Tamanrast	Ain mila
Authors	3	13	7	4	5	10	11	14	2	6	9	8	12	1
α-pinene	0.2	5.5	30.8	12.5	4.2	0	0.5	3.7	12	0	4	7.2	0.2	9.5
β-pinene	0	11	12	7.1	3.7	11	0.3	4.6	18	0.3	11.7	16.6	0.3	8.3
Myrcene	0	0.5	8.9	1.5	0	10.1	0.1	0.8	6.8	0.1	0.9	3	0.1	0.5
Linalyl acetate	0	0	0	0	0	0	11.2	0	0	0	0	0	0.1	0
Caryophyllene	9.8	0	5.6	0	0	0	0	0	17.8	0	0	0	0.1	0
Limonene	0	4.2	7.9	1.9	37.7	0	1.1	4.3	3.5	5.9	1.8	5.6	11.2	0.6
Linalool	0.8	0	0	10.6	0	4	1.6	1.2	0	1.9	0.1	0.6	0	0.3
2,4-di-tetra-butylphenol	0	0	0	0	10.8	0	0	0	0	0	0	0	0	0
Terpinen-4-ol	0.6	0	0	0.2	1.7	0	0	3.7	0	0	0.1	0.5	0.1	0
m-cymene	0	0	0	0	2.5	0	0	2.9	0	0	0	0	0	0
α-terpineol	0.7	0	0	0.3	0	0	0.1	0.6	0	0	1.9	0	0	0.6
Carvacrol	10	0	0	5.2	0	0	0	0	0	0	8.9	1.1	0	0
Hedycaryol	0	0	0	0	3.6	0	0	0	0	0	0	0	0	0
β-caryophyllene	0	29	0	7	0	0	0	0.7	0	0	0.4	0	9.2	0.4
α-humulene	3.8	2.9	0	2.8	0	0	0.4	0	0	4.6	0.2	0.6	0	0
Bicyclogermacrene	0.7	5	4.5	0	0	8.3	0	0	0	0	13	5.5	0	0
δ-cadinene	0	1.1	0	0	0	0	2.4	0	0	0	4.3	0.7	0	0
Spathulenol	2.7	1.5	1.1	0.2	0	15.1	0	3.4	3.3	0	6.5	6.4	0	3.4
α-trans-bergamatene	0	0	0	0	0	0	6.8	0	0	4.1	0	0	0	0
Torreyol	7.6	0	0	0	0	0	0	0	0	0	0	0	0	0
p-cymene	0.3	0.1	0	0.5	8.2	0	0	0	0	0	0	0.6	0	0
Germacrene-D	3.1	6.5	6.9	5.0	0	8.2	0.3	1.6	5.3	8.7	25.8	14.8	0	1.6
α-camphene	0	0	0	5.7	0	0	0	0	0	0	0	0	0	0
Camphor	0	0	0	5.2	0	0	0	0	0	1.4	0	0.7	0	0
Bornyl acetate	0	0	0	5.4	0	0	0	0.4	0	1.1	0	0	0	0.4
γ-cadinene	3	0	0	3.7	1.5	0	0	0	0	0	0	1.1	10.2	0
α-cadinol	4.5	0	0	1.7	0	0	1.7	46.8	0	0	0	2.2	4.3	46.8
Germacrene-B	0.7	0	0	0	0	10.1	0.4	0	0	0	0	0	3.1	0
Ledeneoxide (II)	0	0	0	0	0	0	20.5	0	0	0	0	0	0	0
β-eudesmol	0	0	2	0	0	0	11.6	0	0	0	0	0	0	0.6

Table 2. Composition chimique de l'huile essentielle de *Teucrium polium*

Populations		Amouchas	Beni Aziz	Boutaleb	Populations		Amouchas	Beni Aziz	Boutaleb
Yield (v/v)		0.5	0.4	0.2	Yield (v/v)		0.5	0.4	0.2
Number of compounds	KI	44	40	43	Number of compounds	KI	44	40	43
Total (%)		92	97.5	92.3	Total (%)		92	97.5	92.3
α-thujene	926	0.4	1.0	0.7	α-himachalene	1450	0.4	0	0
α-pinene	934	14.1	18	17.4	α-clovene-neo	1454	0	0.3	0
Camphene	950	0	0.3	0	α-humulene	1460	1.8	4.2	0.8
Sabinene	973	2.7	4.3	3.6	Aromadendrene-allo	1464	0	0.1	0.2
β-pinene	977	15.3	18.1	15.0	Caryophyllene,9-epi-E	1465	0.5	0	0
Myrcene	989	8.6	10.4	8.2	Cadina-1(6),diene-trans	1476	0.3	0.2	0.1
Δ^2-carene	1005	0.4	0	0.3	γ-curcumene	1479	0	0	0.2
Limonene	1030	5.3	8.7	6.0	Germacrene-D	1486	14.7	3.8	19.0
β-ocimene-E	1047	0.7	0.8	0.7	γ-Cadinene	1512	0.3	0.7	0.1
γ-terpinene	1059	0.6	0.4	0.2	β-curcumene	1514	0.5	0	0.3
Terpinolene	1089	0.3	0	0.2	Δ-cadinene	1522	2.5	1.0	2.3
Linalool	1097	0.4	0.3	0.2	Bourbonanone-1-nor	1564	0.5	0.6	0.7
Cymene-para	1091	0	0.4	0	Spathulenol	1582	2.7	1.0	2.9
α-campholenal	1128	0	0.2	0	Caryophyllene oxide	1584	1.5	1.0	1.5
Pinocarveol-trans	1143	0.7	0.4	0.4	α-thujopsene 2-ol	1586	0	3.5	0
Pinocarvone	1164	0.3	0.3	0.4	Viridiflorol	1591	0.3	0	0.1
Terpinen-4-ol	1182	1.2	0.6	0.2	Longiborneol	1601	0.4	0	0
Myrtenal	1197	0.9	0.7	0.7	Rosifoliol	1613	0.2	0	0
Decanone 2	1293	0	0.4	0	Humulene epo-II	1616	0.5	0.3	0.4
Δ-elemene	1334	0	4.5	0	Junenol	1630	0.3	0	0.3
α-copaene	1379	0.4	0.4	0.3	Cubenol-epi-	1633	0.2	0	0.1
β-bourbonene	1387	1.4	1.6	1.7	β-atlantol	1635	0	0.3	0
β-elemene	1391	0.5	0.3	0.5	Dauca-5,8-diene	1647	0	0	1.6
Caryophyllene-E	1424	2.9	2.3	1.8	α-cadinol-epi	1647	1.4	2.2	0
β-copaene	1434	0.2	0.7	0.4	α-murrolol-epi	1650	0	2.0	2.2
α-guaiene	1438	1.4	1.4	0.7	α-cadinol	1661	3.2	0	0
Aromadendrene	1443	0.3	0	0.1	α-selin 11-ol	1665	0.2	0	0
Muurola-3,5 diene	1449	0.2	0	0.2	β-bisabolol-ep	1672	0.6	0	0.1

Table 3. Inhibition diameter of essential oil of *Teucrium polium*

Germs	Gentamicine	Beni Aziz	Boutaleb	Amouchas
Micrococcus luteus ATCC 533	18	14.6 ± 1*	10 ± 1	10 ± 1.5
Bacillus cereus ATCC 10876	24	16.5 ± 0.6	48.7 ± 1.2	30 ± 1
Escherichia coli ATCC 25922	18	33.5 ± 1.7	14 ± 1	15 ± 1.6
Staphylococcus aureus ATCC 6538	14	17 ± 0.2	0	7 ± 1
Saccharomyces cerevisiae ATCC 763	0	18.1 ± 0.9	0	0

(*)Average of inhibition diameter (mm) of three trials with standard deviation

Figure 1. *Teucrium polium* from boutaleb area

Figure 2. Populations of *Teucrium polium* studied

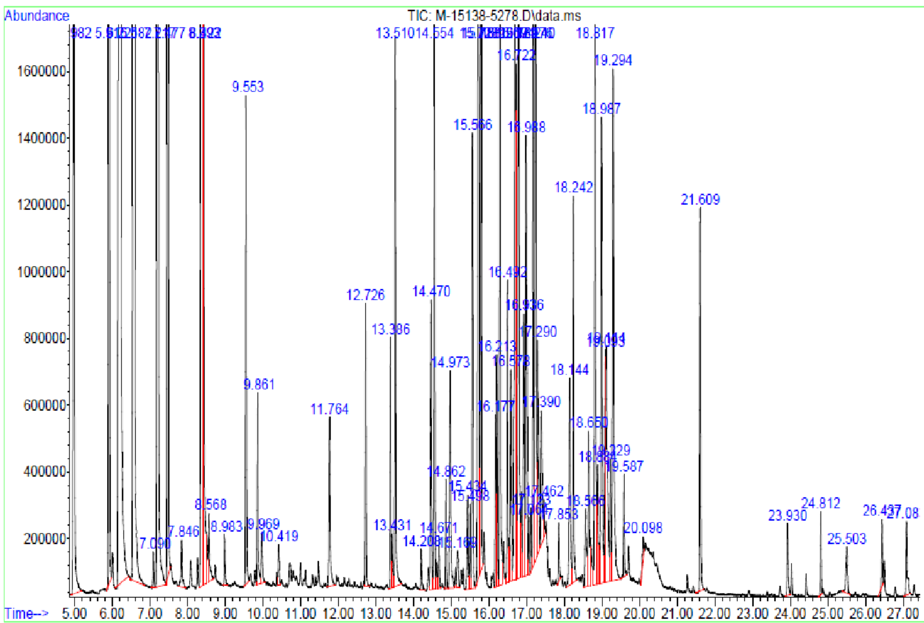

Figure 3. Profile GC / FID of essential oils of *Teucrium polium* (population of Amouchas)

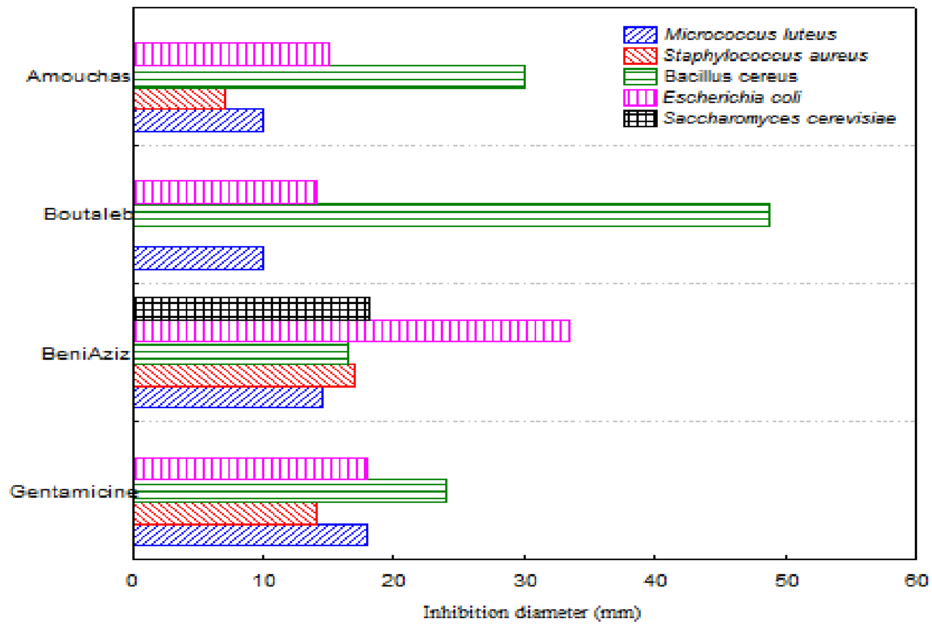

Figure 4. Inhibition diameter of the essential oil of *Teucrium polium*

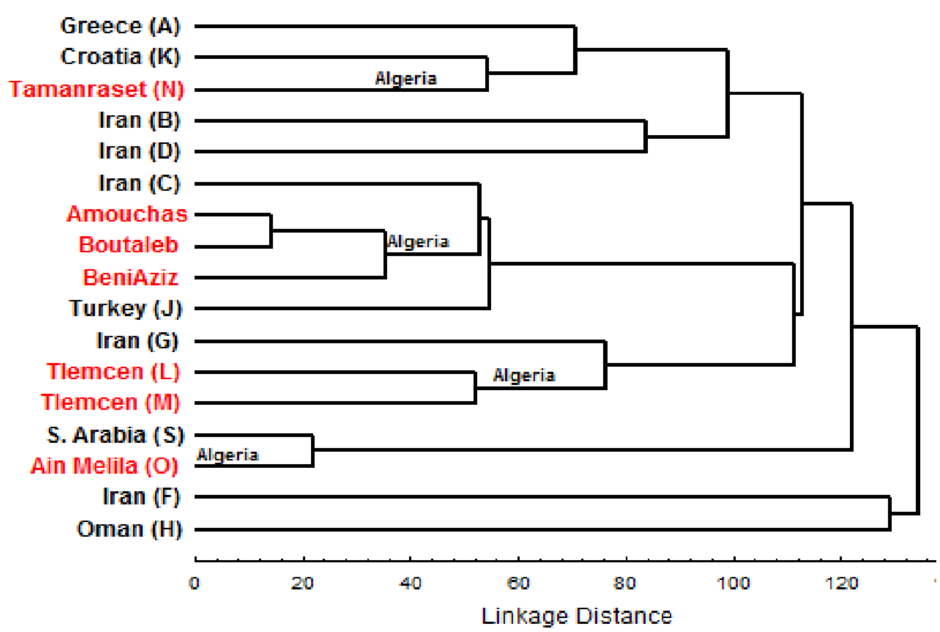

Figure 5. UPGMA of *Teucrium polium*, based on Manhattan similarity distance

(The letters in parentheses, See Table 1)