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CHEMICAL AND ANTIMICROBIAL PROPERTIES OF ESSENTIAL OILS OF ABIES NUMIDICA, ENDEMIC SPECIES OF ALGERIA

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ABSTRACT

The analysis and identification of the essential oils components of *Abies numidica*, endemic species of Algeria, was performed using the (GC-MS). The yield of essential oil samples is 0.4%. These analyses led to the identification of 36 components representing 95.5% of the total oil. The chemical composition of the essential oil is dominated by the products, α-pinene (22.6%), Limonene (19.7%), β-pinene (12.3%), Camphene (11.2%) and β-phellandrene (7.8%). Seven components are represented with a higher rate than 1%. The essential oil has a very effective antibacterial activity against bacterial strains *Bacillus cereus*, *Escherichia coli* and *Enterococcus faecalis*, a modest activity against *Staphylococcus aureus*, *S. epidermidis* and *Micrococcus luteu*s and low activity against *Klebsiella pneumoniae*. The action of the oil is very strong on the yeast *Saccharomyces cerevisiae* with a diameter inhibition of 27.7 mm.

Key words: *Abies numidica*, *Pinaceae*, Essential oil, Antibacterial activity, Babors, Algeria.

INTRODUCTION

Although the systematic of *Abies* is very complex, Quézel, (1985) distinguishes two groups; the fir with needles acute includes *Abies pinsapo, A. maroccana* and *A. cephalonica* and the fir with needles obtuse or emarginated, includes *Abies numidica* De Lannoy, *A. nebrodensis* and *A. cilicica*. Alizoti *et al*., (2011) recognizes that the *Abies* genus in the Mediterranean is represented by ten species (Figure 1).

Investigations on the compositions of the essential oils of firs trees have been performed, including several species, (Baran *et al*., 2007; Duquesnoy *et al.*, 2007; Hyun *et al.*, 2007). In general, the components of essential oils of *Abies* species have been determined using various chromatographic. The main major components of essential oils of *Abies* species are; α-pinene, β-pinene, limonene, camphene, β-phellandrene and bornyl acetate (Bagci and Digrak, 1996; Seung-II *et al*., 2007; Satou *et al.*, 2011a, b; Tumen *et al.*, 2010). The analysis of transplanted trees of *Abies numidica* from Seraidi (Annaba), shows that the bornyl acetate, camphene, α-pinene and Δ^3-carene are the majors components of the oil (Tlili Ait-Khaki *et al*., 2013) (table 1).

The volatile components obtained from *Abies* needles have been commonly used in aromatherapy, due to their relaxing and antimicrobial activities (Satou *et al.*, 2011b). The antimicrobial properties of essential oils have been known for a long time and it is also known that essential oils are more active against yeasts than bacteria (Eyiip and Digrak, 1996). However, little information is available on the antimicrobial and antifungal activities of the essential oils extracted from the *Abies* trees. The antimicrobial activities of the essential oils of nine *Abies* species against some Gram-positive and Gram-negative bacteria and two yeasts are determined (Bagci and Digrak, 1996) and the differences among the

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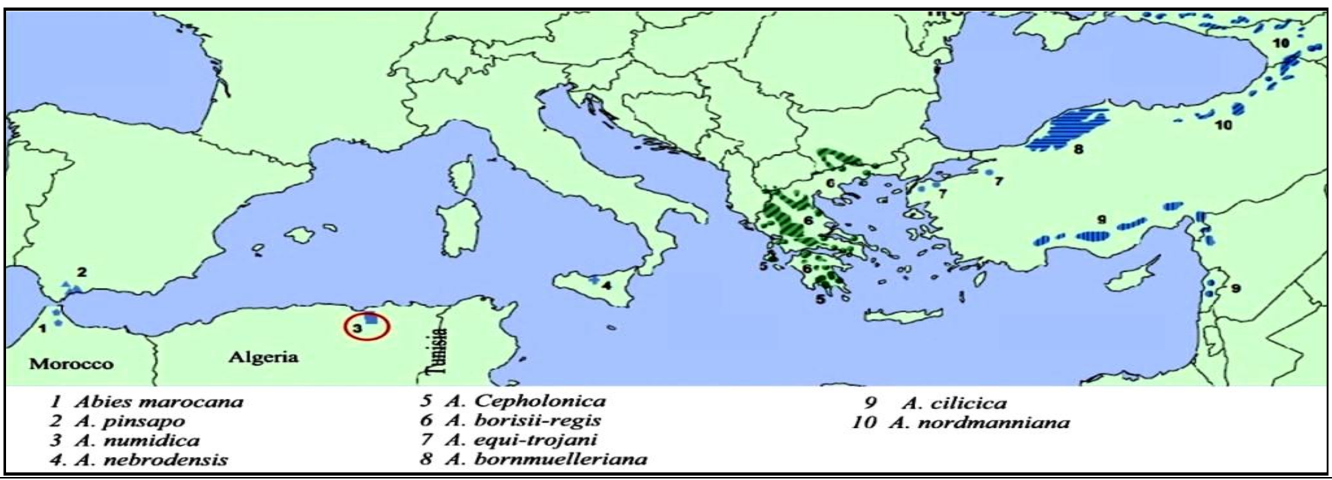
antimicrobial activity levels of naturally grown and exotic form of *Abies* species were determined. Bagci and Digrak, (1996) found that *A. koreana* essential oil was more effective against bacteria and yeast than other plantation *Abies* forms. On the other hand, *A. concolor* and *A. pinsapo* were inactive against most of the bacteria. The studies on essential oils of *Abies* cones, root, stems and leaves indicated the antibacterial and antifungal activities of nine *Abies* species (Bagci and Digrak, 1994; Bagci and Digrak, 1996; Kizil *et al.,* 2002).

Furthermore, the antibacterial activities of the seed lipids of *A. nordmanniana* subsp. *nordmanniana* were generally greater than those of essential oils of *A. nordmanniana* (Bagci and Digrak, 1994; 1996; 1999). Meanwhile, in comparison to the essential oils of *Abies nordmanniana* species, its seed lipids were found to have less antifungal activities (Digrak *et al*., 2002), the studies have shown that the essential oils of *Abies* species inhibit the development of bacteria and yeasts to a variable extent.

The essential oil of *A. numidica* needles from Seraidi, has an antibacterial effect on some pathogenic bacterial strains (Tlili Ait-Khaki *et al.,* 2013). Embryos of *A. numidica* express defense reactions manifested by inhibition of fungal growth (Hrib *et al.,* 2011).

To the best of our knowledge, the chemical composition of essential oils of *A. numidica* growing naturally in Algeria has not been studied yet. The aim of this work is to investigate the chemical composition and antibacterial activity of essential oil from the species *A. numidica* endemic to Algeria.

Figure 1. Distribution range of Mediterranean firs (Alizoti *et al*., 2011)



MATERIALS & METHODS

Plant material

Abies numidica samples were collected from natural populations of Babors region which is located in the North East part of Algeria at 2000 m elevation (Figure 2). Aerial parts were collected in September 2013. 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 Ecology and Biology, Setif University, Algeria. 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.

A. numidica is endemic to Algeria, it occupies a small area (less than 1 km^2) in the mountains of Babors (Setif region), at an altitude of 1300-2000 m. The climate is particularly humid and cold, with annual precipitation of 2500 mm, much of which falls as snow during the winter. The summers are dry and typical of a Mediterranean climate with an average of 18°C and a winter minimum of -1°C, with extreme frosts of between -8 to -10°C (Yahi *et al*., 2013). This species is often mixed with *Quercus mirbecki*, *Cedrus atlantica*, *Taxus baccata*, *Ilex aquifolium* and other species.

A. numidica is a tree up to 25 m high. The bark is grey and smooth on young trees, becoming brown-gray, scaly and fissured with age. Crown conical, densely branched and branches horizontally spread. The shoots are yellow-green to brown, glossy and glabrous. Buds ovate, large, dark-brown, not resinous or slightly resinous. The needles are densely packed, on the upper side of the shoot, along the central part of the shoot are absent thus forming a V-shape, 15-20 mm long. Female flowers green-yellow; the cones are cylindrical, 15-20 cm long, 3.5-5.5 cm wide. The seed somewhat tomentose dorsally, reflexed, with an entire margin, and with 3 cm wide (Vidakovic, 1991).

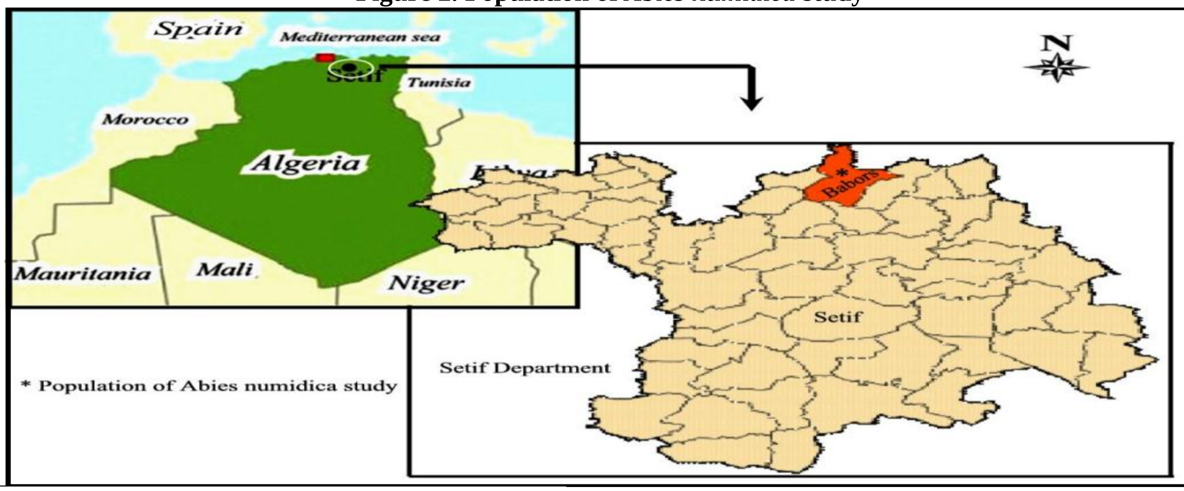
Messaoud Ramdani *et al.* / *International Journal of Phytopharmacology*. 5(6), 2014, 432-440.

Table 1. Major components, in essential oil of *Abies* species

Authors	**Rudolff and Granat, 1982**	**Shaw, 1952**	**Bagci *et al.*, 1999**		**Tumen *et al.*, 2010**				**Koedam *et al.*, 1980**	**Szymon *et al.*, 2007**	**Hyun *et al.*, 2007**	**Seung II *et al.*, 2007**	**Seun-Ah *et al.*, 2009**	**Chalchat *et al.*, 2001**	**Fady *et al.*, 1992**				**Satou *et al.*, 2011a**	**Satou *et al.*, 2011b**	**Satou *et al.*, 2011a**				**Tlili-Ait Kaki *et al.*, 2013**
Country	**Canada**		**Turky**						**Netherland**	**Poland**	**Korea**			**Montenegro**	**Greece**				**Japon**						**Algeria**
Abies species	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**	**K**	**L**	**M**	**N**	**O**	**P**	**Q**	**R**	**S**	**T**	**U**	**V**	**W**	**X**	**Y**
Tricyclene	0.7	0	0	0	0	0	0	0	0	0	0	0	0	2.1	27.5	19.3	23.2	19.1	0	0.9	0	0	0	0	0
α-pinene	7	8.4	6.8	10.1	53	64.2	70.6	65.7	16.3	19.6	11.1	23.2	12.9	17.3	0	0	0	0	0	25.5	0	0	0	0	13.2
camphene	6.1	0	0	0	0	0	0	0	24.8	19.7	10.2	0	0	16.7	23.6	21.7	17.7	8.6	12.1	7.6	11.5	20.1	11.5	7.1	24
β-pinene	54.2	36.2	6.7	29	10.9	8.2	8.6	9.6	0	0	0	5.8	19.8	32.8	0	0	0	0	16.1	4.5	0	14.1	18.3	0	2
Myrcene	1.1	0	0	0	21.3	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1.4	0	0	0	0	0.5
Δ3-Carene	0	11.1	14.2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.7	0	0	0	0	12.3
p-cymene	0	0	0	0	0	0	0	0	0	0	0	0	13.9	0.1	0	0	0	41.2	0	0.1	27.8	0	0	4.9	0
β-phellandrene	6.5	0	0	6.7	0	0	0	0	0	0	0	0	0	4.9	19.4	31.9	39.4	0	0	0.1	0	0	0	0	0
Limonene	3.1	11.1	0	0	0	0	0	7.2	5.5	9.1	23.5	0	0	6.1	0	0	0	0	5.1	28.8	0	0	0	0	5.6
Terpinolene	0.2	0	0	0	0	0	0	0	0	0	0	0	7.5	0.3	0	0	0	0	5	0.2	0	0	0	22.3	0
Borneol	0.2	10.1	0	0	0	0	0	0	0	0	0	27.9	0	2.1	0	0	0	0	0	7	0	0	0	0	2
Bornyl acetate	12.8	14.6	0	0	0	0	0	0	38.3	19.7	17.9	0	0	9	0	0	0	0	0	10.2	0	0	0	0	29.6
β-caryophyllene	0.3	0	7.8	8.8	0	0	0	0	0	0	0	0	30.3	1.3	0	0	0	0	20.6	0.8	0	30.1	26	0	0
α-elemene	0	0	0	0	0	0	0	0	0	0	0	0	0	0	18.8	15.1	8	18.5	0	0	0	0	0	0	0
Cineol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2.1

(A, B)= balsamea ;; (**C, D, E**)= cilicia; **F**= equi torjanie; **G**= bornmülleriana; **H**= nordmanniana; **I**= arnoldiana; (**J, K, L**)= koreana; (**M, N**)= alba; (**O, P, Q**)= cephalonica; **R**= boresii regi; (**S, T**)= sachalinensis; **U**= firma ; **V**= homolepis ; **W**= veitchii ; **X**= mareisii ; **Y**= numidica

Figure 2. Population of *Abies numidica* study



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 (Masada, 1996; 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, 2001).

Antibacterial and antifungal Activities

The antimicrobial activity of *Abies numidica* essential oils has been investigated on different bacteria and yeast. Tree samples were collected from naturally grown *A. numidica*, which is located in Babors in Setif. The Extract Essential oil was tested against the following bacteria; four gram negative bacteria: *Escherichia coli* ATCC 25922; *Klebsiella pneumoniae* ATCC 532; *Enterococcus faecalis* ATCC 29212; *Bacillus cereus* ATCC 10876 and three gram positive bacteria; *Staphylococcus aureus* ATCC 6538; *Staphylococcus epidermidis* ATCC 12228; *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 and diluted essential oil (1:1, 1:2, 1:4, and 1:8 vs: 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.

Determination of Minimum Inhibitory Concentration (MIC)

The minimal inhibition concentrations (MIC) values were determined for essential oil of *A. numidica* and tested against bacterial strains. 100 µl of the inoculum was spread onto 20 ml Mueller-Hinton agar supplemented with the oil at concentrations ranging from 2-6 µl/ml in Petri dishes, with each one its equivalent in DMSO. These serially cultures were then incubated at 37°C for 24 h. The MIC is defined as the lowest concentration at which the microorganism does not demonstrate visible growth. As control, DMSO was used. Tests were carried out in triplicate.

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

Messaoud Ramdani *et al.* / *International Journal of Phytopharmacology*. 5(6), 2014, 432-440.

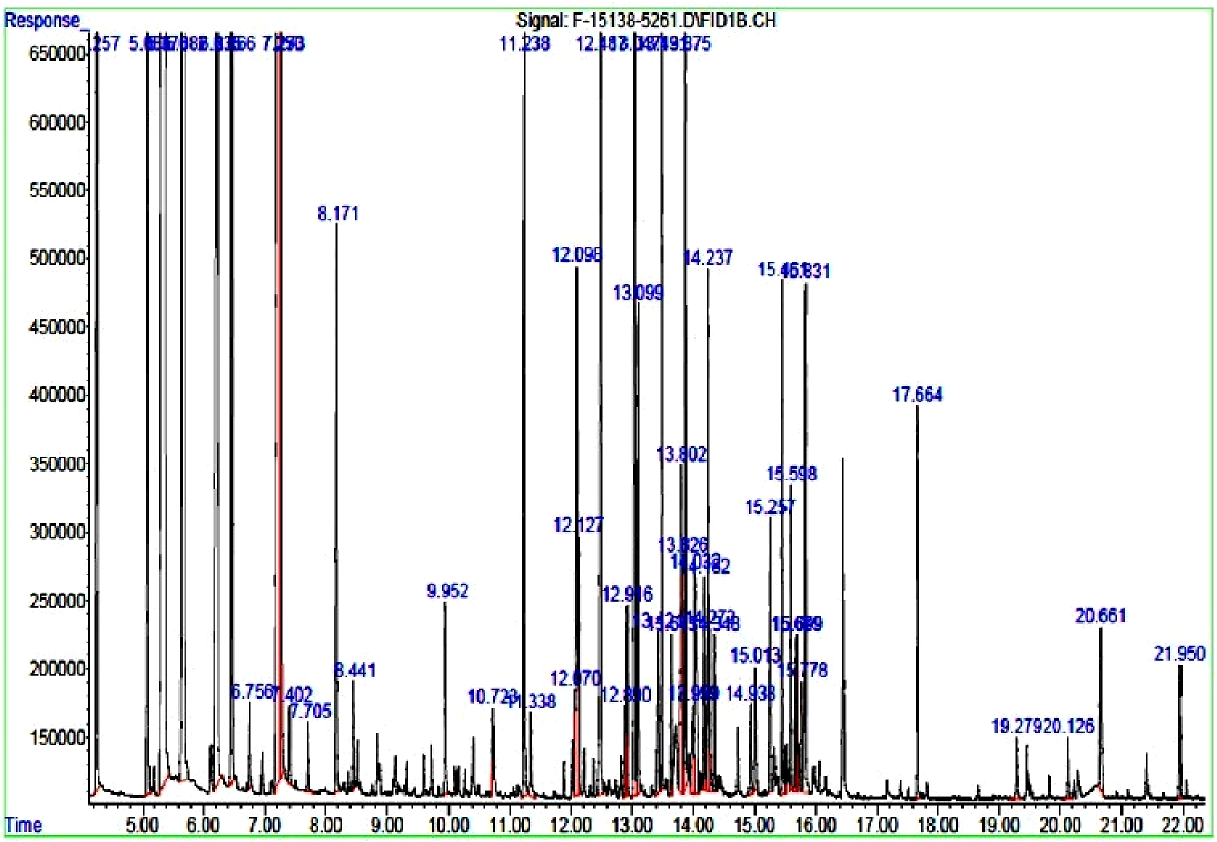
distance matrix to seek hierarchical associations among the species. The cluster analysis was carried out using software Statistica 10.

RESULTS

The hydrodistillation of the essential oil of *Abies numidica* gave a viscous liquid with a greenish color. The yield of the sample essential oil is 0.4%. *A. numidica* essential oil tested in this study was analyzed using GC-MS to identify its major components. Total ion chromatography of the oils revealed 36 significant peaks representing 95.5% 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). The oil predominantly contained α-pinene (22.6%), limonene (19.7%), β-pinene (12.3%), camphene (11.2%) and β-phellandrene (7.8%). The chemical composition of this species contains other components of a lower rate, santene, tricyclene, myrcene, bornyl acetate, geranyl acetate, caryophyllene-(E) and valencene.

Figure 3. The chromatogram of *Abies numidica* volatile oil



The antimicrobial activities of essential oils of *A. numidica* used showed significant activities against yeasts than bacteria (Table 3). Gram negative bacteria (*Escherichia coli* ATCC 25922, *Enterococcus faecalis* ATCC 29212 and *Bacillus cereus* ATCC 10876) are strongly inhibited by the essential oil of *A. numidica*, with an inhibition diameter greater than that of gentamicin (Figure 4). While Gram-positive bacteria *Staphylococcus aureus* ATCC 6538, *Staphylococcus epidermidis* ATCC 12228 and *Micrococcus luteus* ATCC 533, are less sensitive to the oil with an inhibition diameter slightly smaller than that of gentamicin. Although *Klebsiella pneumoniae* ATCC 532 is a gram-positive bacterium, it is more resistant to the action of the oil. The essential oil of *A. numidica* is very effective against the yeast *Saccharomyces cerevisiae* ATCC 763, which is resistant to gentamicin.

The lowest value of MIC of *A. numidica* essential oil is (0.063 mg / ml), it was observed against *Escherichia coli* ATCC 25922 and *Bacillus cereus* ATCC 1087. The highest concentration (0.5 mg/ml) is observed against *Staphylococcus aureus* ATCC 6538 and *Micrococcus luteus* ATCC 5336, The MIC which inhibits the yeast *Saccharomyces cerevisiae* ATCC 763 is 0.13 µg/ml.

DISCUSSION

The yield of the essential oil of *Abies numidica* is 0.4%, this result is consistent with literature results. The same yield of 0.37% was found in samples of *A. numidica* planted in Seraidi (Tlili Ait-Khaki *et al.*, 2013). *A. cilicica* shows a similar yield (0.42%), the highest essential oil yield was found in *A. equitrojani* with 0.59% (Tumen *et al.*, 2010).

Table 2. Chemical composition of essential oil of *Abies numidica*

Yield (v/v)	KI	0,4		Yield (v/v)	KI	0,4
Number of compounds		**36**		**Number of compounds**		**36**
Total		**95.54**		**Total**		**95.54**
Santene	878	**3.32**		Longifolene	1415	0.26
Tricyclene	922	**1.54**		Caryophyllene (E)	1425	**2.35**
α-pinene	936	**22.62**		Guaiadiene 6-9	1429	0.67
Camphene	951	**11.23**		β-farnesene-(E)	1453	0.01
β-pinene	979	**12.29**		α-himachalene	1455	0.25
Myrcene	989	**2.23**		α-humulene	1461	0.99
α-phellandrene	1006	0.12		9-epi-caryophyllene-(E)	1473	0.22
Limonene	1033	**19.74**		Germacrene D	1485	0.61
β-phellandrene	1034	**7.8**		γ-himachalene	1487	0.3
Terpinolene	1085	0.77		Valencene	1491	**1.06**
Linalool	1099	0.17		α-muurolene	1502	0.2
α-terpineol	1196	0.29		β-himachalene	1505	0.47
Bornyl acetate	1286	**1.1**		γ-cadinene	1517	0.28
Undecanone <2>	1292	0.1		Caryophyllene oxide	1588	0.16
Citronellyl acetate	1349	0.67		Longiborneol	1611	0.39
α-longipinene	1353	0.36		1-epi-cubenol	1640	0.43
Geranyl acetate	1378	**2.15**		Manool oxide	2002	0.09
Dodecanal	1411	0.11		Tricosane	2299	0.19

Table 3. Inhibition diameter and CMI of essential oil of *Abies numidica*

Germs	Inhibition diameter*		CMI (µl/ml)	Gen.
	Average	**SD**		
Bacillus cereus ATCC 10876	**27.3**	2.5	0.063	24
Escherichia coli ATCC 25922	**24.7**	1.5	0.063	18
Klebsiella pneumoniae ATCC 532	11.7	1.5	0.13	30
Enterococcus faecalis ATCC 29212	**21**	1.0	0.13	19
Staphylococcus epidermidis ATCC 12228	14	2.7	0.25	19
Staphylococcus aureus ATCC 6538	12.7	2.5	0.5	14
Micrococcus luteus ATCC 533	13	2.7	0.5	18
Saccharomyces cerevisiae ATCC 763	25.7	2.1	0.13	0
(*)Average inhibition diameter (mm) of three trials with SD; Gen. = Gentamicine				

The chemical groups in the essential oil of *Abies* species were investigated. The monoterpene hydrocarbons group, was found to be the highest level in *A. cilicica* (93.1%), *A. nordmanniana* (85.7%), *A. equi-trojani* (77.2%) and *A. bornmümmerian* (84.0%) (Tumen *et al.*, 2010). The same result is obtained in essential oil of *A. numidica* with a percentage (81.7%).

The composition of the oil studied has given a differences qualitative and quantitative distinct in the two oils of *A. numidica* natural and planted. The planted trees in Séraidi are characterized by the presence of camphene, Δ^3-Carene, bornyl acetate, borneol and ceniol (Tlili Ait-Khaki et al., 2013). The natural population of *A. numidica* of Babors is rich in β-pinene, myrcene, β-phellandrene, limonene and β-caryophyllene. Despite the presence of differences in the chemical composition of the essential oils of two populations, the UPGMA grouped them in the same subset (figure 5). The chemical composition of essential oil of the native population is very close to *A. cilicia* of Turky (Bagci *et al.*, 1999) and to *A. koreana* from Korea (Hyun *et al.*, 2007). This small group is opposed by his chemical composition to *A. numidica* of

Seraidi. The chemical composition of the essential oil of *A. numidica* is very different from the Mediterranean species. In this group it is closest to the species *A. borisii regis*. The antimicrobial activity of essential oils of *Abies* species varid at different concentrations against bacteria and yeasts. The antimicrobial activities of essential oils were found to be more active against yeasts than bacteria; the same result was reported by Bagci and Digrak, (1996). The least active essential oils against the bacteria were from *A. pinsapo* and *A. alba* and against yeasts were from *A. alba, A. firma* and *A. pinsapo (*Bagci and Digrak, 1996). The essential oil of *A. numidica* is very effective against bacterial strains *Bacillus cereus, Escherichia coli* and *Enterococcus faecalis*, while the action of this oil is very low against *Klebsiella pneumoniae* and *Micrococcus luteus*. *Staphylococcus epidermidis* and *S. aureus* are resistant to the oil of this species.

Figure 4. Antibacterial activity of *Abies numidica*

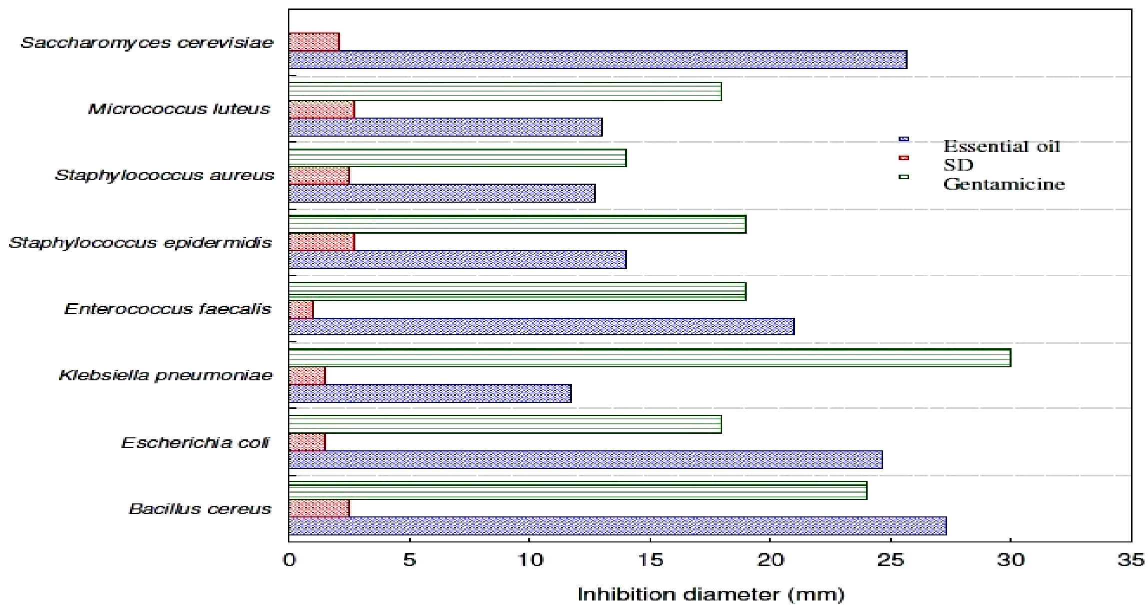
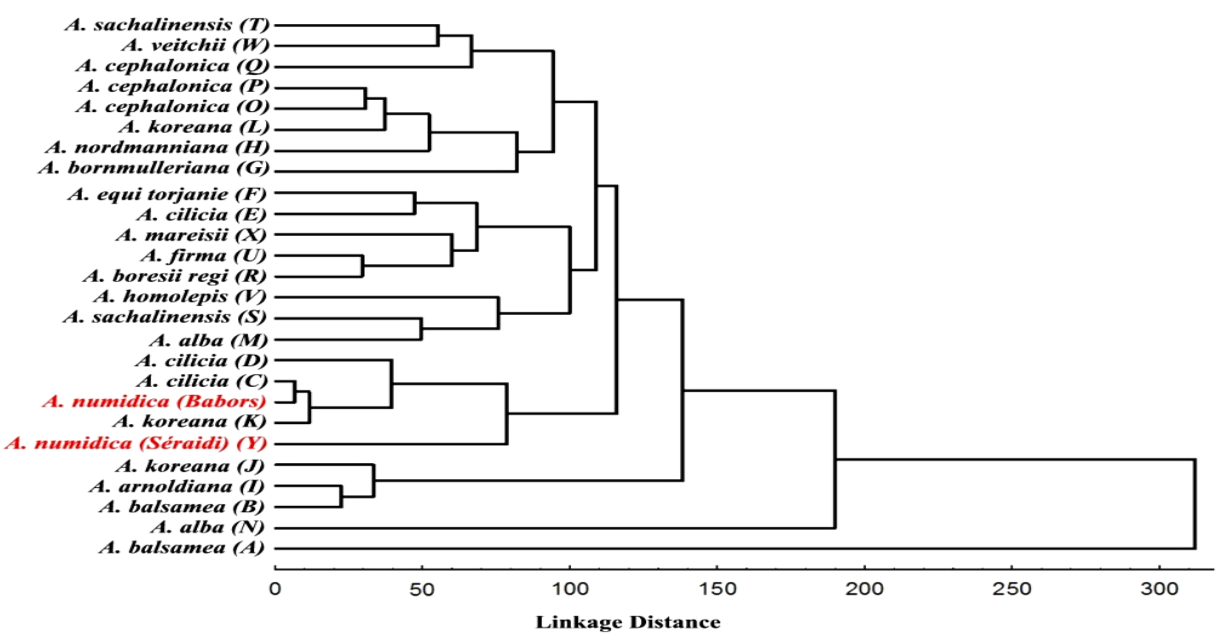


Figure 5. Dendrogram of *Abies* species, based on Manhattan similarity distance
(The letters in parentheses, see Table 1)



CONCLUSION

The chemical composition of the essential oil of *A. numidica* is dominate by α-pinene, camphene, β-pinene, limonene and β-phellandrene, this composition is differs slightly from the oil composition of planted trees in Seraidi. The results indicate that the essential oil of *A. numidica* used in this study inhibited development of bacteria and yeast to a variable extent. The essential oil is more active against bacteria *Bacillus cereus*, *Escherichia coli* and *Enterococcus faecalis.* On the other hand, it was found that the essential oils used were more active against yeast than bacteria. In addition, it was found that the antimicrobial activity of natural *A. numidica* were higher than the population planted in Seraidi (Algeria).

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