CHEMICAL COMPOSITION AND BIOLOGICAL ACTIVITIES OF *DAUCUS AUREUS* ESSENTIAL OILS FROM EASTERN ALGERIA

WAFAA ZAIBET [1], HOCINE LAOUER [1], SMAIN AMIRA [2], GUIDO FLAMINI [3], MESSAOUD RAMDANI [1] AND SALAH AKKAL [4]*

[1]*Laboratory of Valorization of Natural and biological Resources, University Ferhat Abbas, Setif 1, Algeria*
[2]*Laboratory of Phytotherapy Applied to Chroniques Diseases,University Ferhat Abbas, Setif 1, Algeria*
[3]*Dipartimento di Farmacia, Università di Pisa,via Bonanno Pisano 6, 56126 Pisa (PI), Italy*
[4]*Laboratory of Chemistry, Chemical and Biological Analyze, University of Mentouri, Constantine, Algeria*

ABSTRACT

The aim of this study was to investigate the chemical composition of the essential oils of three populations of *Daucus aureus* from three sites in Eastern Algeria (Setif, Constantine and Oum Elbouaghi) and to test their antibacterial and antioxidant activities. The essential oils were obtained by hydrodistillation and analyzed by GC and GC/MS. The major components were sabinene (30.6% and 36.2%), *n*-nonane (8.0% and 7.9%), α-pinene (5.5% and 6.3%) and 4-terpineol (4.4% and 6.0%) in *D. aureus* from Setif and Constantine populations essential oils, respectively; whereas, α-pinene (19.4%), β-pinene (12.0%) and *p*-cymene (12.2%) were the major components in Oum Elbouaghi essential oil population. The chemical compositions of *D. aureus* from Eastern Algeria are markedly different from those from Western Algeria, and likely represent new chemotypes. The antimicrobial activity of the essential oils was evaluated against four bacteria and one fungus, using the disc-diffusion method and minimal inhibitory concentration (MIC), whereas, the antioxidant activity of the essential oils was evaluated using the DPPH test. The results showed that the oils have an antimicrobial activity against the microorganisms tested, with minimal inhibitory concentration (MIC) values between 0.97 and 3.23 mg/mL and weaker antioxidant and DPPH radical scavenging activities were found in comparison to butylated hydroxyl toluene (BHT).

Keywords: *Daucus aureus,* GC and GC/MS, essential oils, minimal inhibitory concentration, chemotype.

1. INTRODUCTION

Apiaceae represent one of the best-known plant families, and are often used as spices, vegetables or drugs due to the presence of useful secondary metabolites such as essential oils [1]. Throughout history, essential oils of Apiaceae family have been widely used as antibacterial, antifungal, antiviral, antiparasitic, insecticidal and antispasmodic [2,3,4]. It is reported that the genus *Daucus* was the richest genus of the Apiaceae concerning its essential oil content [5]. The leaves are used in their raw form or in an infusion as depurative and diuretic agents [6]. Some species are used in traditional medicine for the treatment of skin disorders, e.g. burns, furunculous [7] dropsy, inflammation and gastric ulcer [8,9,10]. Previous studies dealing with members of this genus revealed that the main constituents were: sabinene with monoterpene hydrocarbons for *D. carota* ssp. *gummifer* from Spain [11], whereas α-pinene with monoterpene hydrocarbons predominated in *D. carota* ssp. *carota* from Poland [12]. However, monoterpene hydrocarbons (α -pinene, limonene, and sabinene) were dominating in *D. muricatus* and *D. sahariensis* from Algeria [13,14]. In an ancient Italian variety, Pastinocello carrot (*D. carota* ssp. *major*), also oxygenated monoterpenes (geranyl acetate, geraniol, *epi*-α-bisabolol) plays an important role, together with lesser amount of monoterpene hydrocarbons (α-pinene and myrcene) [15].

The phytochemical analysis of *D. aureus* extract from Western Algeria was previously reported [16]. The analysis of the extract revealed the presence of tannins, flavonoids, steroids and saponins. The chemical composition of the essential oils of *D. aureus* from Western Algeria has also been previously reported [17]. The oils were characterized by the presence as main components of sesquiterpene hydrocarbons (54.3%) in the aerial parts **(Table 1)**.

The aim of the present work was to investigate the chemical composition of essential oils of three populations of *D. aureus* from three sites in Eastern Algeria (Setif, Constantine and Oum Elbouaghi), and to test their antibacterial and antioxidant activities. To the best of our knowledge, the antioxidant activity of the essential oils of *D. aureus* was not previously reported.

2. MATERIALS AND METHODS

2.1. Plant material

D. aureus is a biannual plant of the Mediterranean region [18]. The aerial parts of *D. aureus* were collected during their flowering stage from three regions; namely: Setif, Constantine and Oum Elbouaghi in June 2013 **(Figure 1)**. The plant was identified by Dr Boulaacheb Nacira, University Ferhat Abbas Setif 1. A voucher specimen was deposited at the Biological and Ecological Department herbarium. The plants were air dried in the shade then 200g of plant material was hydrodistilled for 3 hours using a Clevenger-type apparatus. The obtained oils were stored at +4°C in the dark until use.

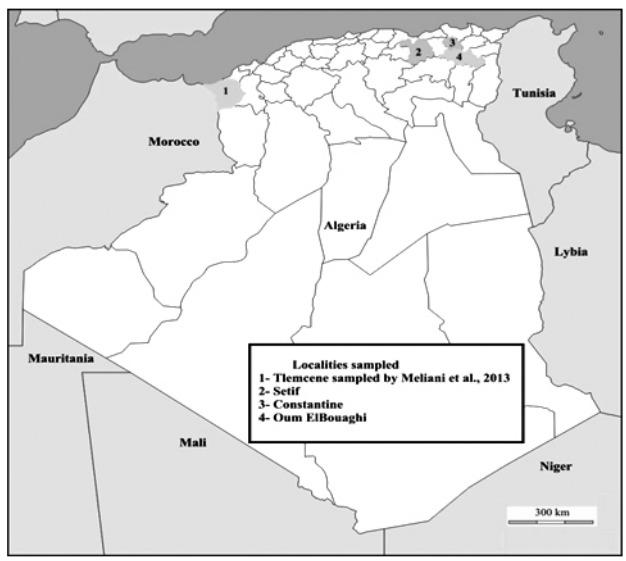

Figure 1: *D. aureus* populations studied.

2.2. Microbial strains and media of culture

Strains of bacteria and fungi were obtained from the Laboratory of Microbiology, Faculty of Nature and Life Sciences, University Setif 1. The obtained oils were assayed against *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC 6633 and fungal strain *Candida albicans* ATCC 1024. Culture of these strains were routinely incubated for a period of 24 hours at 37°C in Mueller-Hinton broth (MHB) for the bacteria and for 48 hours at 28°C in Sabouraud dextrose agar (SDA) for the yeasts.

2.3. Antimicrobial activity

The anti-bacterial activity of the essential oils was determined by the disc diffusion method [19, 20]. The inoculums containing 2.0×10^6 CFU/mL of bacteria and 10^7 CFU/ mL yeast were spread on a Muller-Hinton agar and Sabouraud dextrose agar (SDA), respectively. Sterile absorbing paper discs (6 mm in diameter) were impregnated with 10 µl of different oil dilutions (1/2, 1/5 and 1/10 v/v) in Dimethyl sulfoxide (DMSO) (Sigma- Aldrich), and then placed on the surface of inoculated Petri dishes (90 mm). The diameter of inhibition was

measured after 24 h of incubation at 37°C for bacteria and after 7 days of incubation at 28°C for the fungi. Gentamicin [10µg/mL (Sigma Aldrich)], Nystatine [100 µg/mL] and DMSO were used as positive and negative controls, respectively. The diameters of the inhibition zones were measured in mm. All the growth inhibition tests were performed in triplicate.

Table 1: Percentage major components, in essential oil, of *D. aureus* growing in Western Algeria [17]

Populations*	Group 1							Group 2						
	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14
β-pinene	1.5	1.1	0.9	0.6	0.5	1.2	2.7	2.9	2.8	2.7	2.1	2.2	1.5	1.6
Myrcene	2.6	2.8	1.2	2	2.2	2.6	2.2	2.4	2.3	2.1	2.6	2.7	1.2	1.7
(E)-Decenal	0.9	0.2	0.6	0.5	1.2	1.1	1.5	1.6	1.2	1.1	2.2	1.7	1.5	1.4
Germacrene D	56.7	67.2	66.1	65.4	58.9	44.5	39.5	18.2	17.9	18.9	11.3	17.5	15.4	17.9
7-epi-α-selinene	0.9	0.6	1.2	0.9	1.7	1.4	1.9	2.6	2.7	2.6	2.9	2.5	1.9	2.5
Germacrene B	1.1	0.5	0.9	0.7	1.1	2.1	2.2	2.5	2.7	2.9	2.8	2.7	2.8	2.5
Spathulenol	1.3	0.6	0.8	1	1.2	2	2.1	5.2	5.7	5.6	7.7	6.2	8.4	8.4
Caryophylene-oxyde	1.2	1.9	0.9	1.1	1.8	3.2	4.4	7.3	8.3	7.3	8.3	8.9	8.6	9.5
Globulol	0.8	0.3	0.2	0.5	0.8	1.3	0.8	2.3	3.2	3.8	3.4	2.6	3.6	2.4
Viridiflorol	1.2	1.1	0.9	0.9	1.7	1.6	2.3	4.5	5.5	5.8	4.2	3.5	4.7	3.2
Guaiol	0.7	0.3	0.2	0.5	0.4	1.1	1.2	1.3	2.1	1.9	1.4	1.2	2.3	2.5
Copaborneol	1.2	0.3	0.6	0.3	0.6	1.6	0.8	1.1	2.6	2.7	2.7	1.1	2.3	1.2
Cadin-4-en-7-ol	1.2	1.7	1.6	1.5	1.2	1.6	2.9	4.1	4.5	4.7	4.3	5.5	5.6	4.4
β-Cadinol	2.5	1.2	1.5	2.2	2.2	1.5	1.9	3.9	2.3	4.2	4.6	5.2	4.3	4
Bulnesol	0.7	0.2	0.1	0.3	0.5	0.9	1.1	1.5	1.6	1.9	1.6	1.6	1.1	1.7
(Z)-α-Santanol	1.4	2.1	2.2	1.5	1.4	2.8	2.7	6.5	5.1	5.6	4.3	5.1	4.9	4.7

* S1= Saf Saf; S2= Chetouane; S3= Ain Fezza; S4= Laourit; S5= Mansourah; S6= Zarifet; S7= Khemis; S8= El Emir Abdelkader; S9= Ghazaouet; S10= Sidi youchaa; S11= Beni Saf; S12= Oulhaça; S13= Rachgoun; S14= Oulad Benayed (B'hira).

2.4. Determination of the minimum inhibitory concentration (MIC)

The MICs of the essential oils for the test microorganisms were determined using the broth microdilution method (96-well microtiter plates) as previously described [21]. All tests were performed in MHB or SDA medium. The inocula of the bacterial strains were adjusted to 0.5 McFarland standard turbidity. The essential oils were first dissolved in a solution of 10% DMSO, and serial two-fold dilutions of the oil were prepared in a 96-well plate corresponding to concentrations ranging from 0.97 mg/mL to 6.46 mg/mL. The MIC was defined as the lowest concentration of the essential oil at which the microorganism does not demonstrate visible growth. Indication about microorganism growth was shown by the turbidity of the mixture. In these tests the gentamicin and nystatine were used as experimental positive controls for bacteria and fungi, respectively. While the solution of dimethyl sulphoxide (DMSO)-sterile distilled water served as the negative control.

2.5. GC and GC/MS analysis

The essential oil chemical composition assessments and the identification of the main constituents were conducted by GC and GC-MS analyses. GC/MS analyses were performed with a Varian CP-3800 gas-chromatograph equipped with a DB-5 capillary column (30 m x 0.25 mm; coating thickness 0.25 µm) and a Varian Saturn 2000 ion trap mass detector. Analytical conditions were: injector and transfer line temperatures 220 and 240 °C. respectively; oven temperature programmed from 60 to 240°C at 3°C/min; carrier gas helium at 1 mL/min; injection of 0.2 µL (10% hexane solution); split ratio 1:30. The constituents' identification was based on comparison of the retention times with those of authentic samples. comparing their linear retention indices relative to the series of n-hydrocarbons and on computer matching against commercial (NIST, 98 and Adams) and home-made library mass spectra built up from pure substances and components of known oils and MS literature data [22, 23]. Moreover, the molecular weights of all the identified substances were confirmed by GC/CIMS, using MeOH as CI ionizing gas.

2.6. Free radical scavenging assay

The antioxidant activity of volatile compounds was measured in terms of hydrogen donating or their ability to scavenge 2.2-diphenyl-1-picrylhydrazyl (DPPH), a stable radical. The radical scavenging abilities of *D. aureus* oils were evaluated according to the slightly modified method of Que et al [24]. 600 µl of various dilutions of the samples were mixed with 600 µl of 0.004% methanol solution of DPPH. The measurement of the residual absorbance was carried out at 517 nm after 30 min at room temperature for all samples. Methanol was used as a blank. While methanol with DPPH solution was used as a control. Inhibition ratio (percent) was calculated from the following equation:

% inhibition= [(absorbance of control – absorbance of sample)/ absorbance of control] × 100%.

All determinations were taken in triplicate and special care was taken to minimize the loss of free radical activity of the DPPH. Butylated hydroxytoluene (BHT) was used as a positive control.

2.7. Statistical analysis

Data were first subjected to Principal Components Analysis (PCA) to examine the relationships among the terpenes compounds and to identify the possible structure of the populations. The cluster analysis using the unweighted pair-group method with arithmetic mean (UPGMA) was carried out on the original variables and on the Manhattan distance matrix to investigate the hierarchical associations among the populations. Only the compounds whose rate is higher than 1%, were used to determine the chemical relationship between the different *D. aureus* essential oil samples. These ananlyses were performed using Statistica software 10.

3. RESULTS AND DISCUSSION

The oils obtained by hydrodistillation from the aerial parts of *D. aureus*

from the three sites in Eastern Algeria (Setif, Constantine and Oum Elbouaghi), have a green-yellow colors. The yields were 0.15%, 0.16% and 0.13%, respectively. The essential oils were obtained by hydrodistillation and analyzed by Gas Chromatography and Gas Chromatography –Mass Spectrometry (GC/MS). The volatile components of the essential oils of *D. aureus* and their percentages are shown in **Table 2**. The monoterpene hydrocarbons were the major components and ranged between 49.1% and 59.9%.

Table 2. Percentage composition of essential oils of *D. aureus.*

Populations		Constantine	Setif	Oum Elbouaghi
Yield (%)	RI	0.16	0.15	0.13
Number of compounds		58	58	45
Total (%)		**90.1**	**82.9**	**94.1**
2-heptanone	890	0.2	0.2	0.4
n-nonane	899	7.9	8.0	18.3
α-thujene	931	1.5	1.2	1.1
α-pinene	939	6.3	5.5	19.4
camphene	954	0.1	0.2	0.6
thuja-2.4(10)-diene	957	0.2	0.2	0.5
benzaldehyde	962	tr	tr	tr
1-heptanol	970	tr	tr	tr
sabinene	977	36.2	30.6	3.1
β-pinene	980	0.2	1.8	12.0
3-octanone	988	-	tr	tr
myrcene	992	1.0	0.8	1.0
δ-2-carene	1001	-	0.4	-
octanal	1003	tr	tr	tr
α-phellandrene	1005	0.9	tr	3.4
α-terpinene	1018	2.5	1.5	0.2
p-cymene	1027	3.1	1.2	12.2
limonene	1031	1.3	1.5	3.5
(Z)-β-ocimene	1041	tr	-	-
(E)-β-ocimene	1051	tr	tr	-
γ-terpinene	1062	4.2	2.8	0.5
cis-sabinene hydrate	1069	0.2	0.4	tr
1-octanol	1072	tr	tr	0.3
terpinolene	1089	1.5	0.8	0.4
2-nonanone	1092	tr	tr	tr
3-nonanol	1094	tr	-	tr
n-undecane	1099	-	-	tr
linalool	1000	0.4	0.8	0.4
nonanal	1103	0.2	0.1	0.6
β-thujone	1115	tr	tr	tr
cis–p-menth-2-en-1-ol	1122	0.4	0.6	tr
α-campholenal	1126	0.4	0.3	1.6
cis-p-mentha-2.8-dien-1-ol	1138	tr	-	-
Trans-pinocarveol	1140	0.6	0.7	0.8
Cis-verbenol	1142	-	0.4	0.4
trans-verbenol	1144	0.2	tr	0.8
β-pinene oxide	1158	tr	0.1	-
(E)-2-nonenal	1160	0.2	0.3	0.5
pinocarvone	1163	0.3	0.2	1.5
δ-terpineol	1166	0.2	0.2	0.2

4-terpineol	1178	6.0	4.4	0.5
p-cymen-8-ol	1184	0.2	0.1	3.0
α-terpineol	1190	0.2	0.2	tr
Myrtenal	1193	0.6	0.5	1.8
decanal	1204	-	-	tr
trans-piperitol	1206	0.3	0.3	0.2
cuminaldehyde	1240	tr	tr	0.2
carvone	1243	tr	tr	0.5
carvotanacetone	1248	tr	-	tr
geraniol	1256	tr	0.5	-
(E)-2-decenal	1262	tr	-	tr
isobornyl acetate	1285	0.1	tr	0.4
Trans-sabinyl acetate	1291	-	0.2	-
thymol	1293	tr	-	0.2
trans-pinocarvyl acetate	1298	-	-	tr
(E.E)-decadinal	1315	tr	-	tr
α-cubebene	1351	tr	-	0.2
α-longipinene	1353	-	-	tr
piperitenone oxide	1363	tr	-	-
α-copaene	1376	0.3	tr	0.4
β-bourbonene	1384	0.1	-	tr
Geranyl acetate	1386	-	3.9	-
β-cubebene	1390	0.3	tr	0.2
β-elemene	1394	tr	-	-
α-cedrene	1409	tr	-	-
β-cedrene	1418	0.2	0.2	0.2
β-copaene	1429	tr	-	-
γ-elemene	1433	tr	-	-
aromadendrene	1440	tr	-	-
(E)-geranylacetone	1452	tr	-	tr
Trans-muurola-3.5-diene	1454	tr	-	-
α-humulene	1456	tr	tr	tr
alloaromadendrene	1461	tr	-	tr
β-acoradiene	1471	tr	-	-
γ-muurolene	1480	0.1	-	tr
germacreneD	1482	0.2	0.2	0.1
Ar-curcumene	1484	0.3	-	tr
Cis-β-guaiene	1490	0.1	0.2	-
viridiflorene	1493	-	-	tr
bicyclogermacrene	1494	0.2	0.4	-
α-muulorene	1499	0.1	0.3	tr
germacrene A	1503	tr	0.2	-
δ-amorphene	1505	tr	-	-
β-bisabolene	1509	0.3	0.2	0.3
δ-cadinene	1524	1.4	0.4	0.4
α-cadinene	1538	tr	0.1	-
α-calacorene	1542	0.2	0.2	tr
α-agarofuran	1546	0.1	-	-

elemol	1550	-	0.3	-
longipinanol	1569	0.8	0.4	-
spathulenol	1576	1.0	0.8	0.5
trans-sesquisabinene hydrate	1579	0.1	0.2	-
caryophyllene oxide	1581	0.4	0.4	0.6
β-oplopenone	1606	0.3	0.6	-
humulene epoxide	1608	0.2	tr	0.2
1.10-di-epi-cubenol	1614	2.8	3.4	0.2
helifolen-12-al	1620	0.2	0.3	-
epi-α-cadinol	1641	0.7	tr	tr
cubenol	1643	tr	0.5	-
α-cadinol	1654	0.9	0.7	0.3
cis-calamenen-10-ol	1661	tr	0.1	-
occidentalol acetate	1678	0.8	0.3	-
eudesma-4 (15).7-dien-1-β-ol	1688	0.2	0.5	-
juniper camphor	1692	-	0.5	-
hexahydrofarnesylacetone	1845	0.2	tr	-
Monoterpene hydrocarbons		59.0	49.1	57.9
Oxygenated monoterpenes		10.1	13.8	12.5
Sesquiterpene hydrocarbons		3.8	2.4	1.8
Oxygenated sesquiterpenes		8.5	9.0	1.8
Apocarotenoids		0.2	–	–
Non-terpene derivatives		8.5	8.6	20.1

RI: Retention index relative to n-alkanes on DB-5 capillary column; tr: trace, less than 0.05.

The main constituents of essential oils from Constantine and Setif were sabinene (36.2 and 30.6%. respectively), followed by α-pinene (6.3 and 5.5%) and 4-terpineol (6.0 and 4.4%). In contrast α-pinene (19.4%) was the main component of the essential oil from Oum Elbouaghi. followed by β-pinene (12.0%).

The oxygenated monoterpenes percentages were less variable ranging from 10.1% to 13.8%. In the case of oxygenated sesquiterpenes, the percentages were the same for the essential oils from Setif and Constantine (9.0 and 8.5%), whereas the essential oil from Oum Elbouaghi contained a considerable less amount of these chemicals (1.8%).

Sesquiterpene hydrocarbons were present in small amounts in the essential oils of the three populations. The content of the non-terpene compound from Oum Elbouaghi essential oil was higher than those of the other populations studied (20.1% against 8.6% and 8.5 %.). These constituents were dominated by *n*-nonane: 18.3%, 8.0% and 7.9% of the oils composition from Oum Elbouaghi, Setif and Constantine, respectively.

In a previous study, the essential oils obtained from fresh aerial parts of *D. aureus* from different sites in Tlemcene (Western Algeria) yielded between 0.01 and 0.2% [17], whereas the yield of the aerial parts of *D. aureus* from Setif, Oum Elbouaghi and Constantine were 0.15%, 0.16% and 0.13% respectively.

The comparison of the chemical compositions of *D. aureus* essential oils obtained from three sites shows significant differences. Monoterpene hydrocarbons were the most abundant chemicals identified in the aerial parts. The amount of sabinene significantly increased in Oum Elbouaghi and Constantine essential oils (3.1%-36.2%), respectively. Compared to Oum Elbouaghi essential oil, the fraction of oxygenated sesquiterpenes was increased in Constantine and Setif essential oils; this is mainly due to an increase of 1,10-di-epi-cubenol, β-oplopenone and longipinanol percentages. Some components such as trans-sabinyl acetate, geranyl acetate, elemol and juniper camphor were present only in Setif population, probably because of the influence of factors such as the geographical and other ecological parameters [25]. Many volatiles identified in the present study were reported as the main components in other *Daucus* species. 4-terpineol was detected in *D. carota* ssp. *carota* from Lithaunia (4.6-7.5%) [26], α-pinene was identified in *D. gingidium* ssp. *gingidium* from Italy (40.0-46.0%) [27], sabinene was detected in *D. carota* ssp. *gummifer* from Spain [11]. Limonene was detected in *D. muricatus* and *D. sahariensis* from Algeria (14.2-24.0%) [13,14].

In order to investigate the differences between the essential oils of the different samples from different regions in Algeria, we have chosen the Principal Component Analysis (PCA) to examine the relationships between populations. This analysis presented three axes comprising 92.05% of the total variation present in the original data. This analysis clustered populations in two groups, Eastern and Western groups. The ordination of population's used obtained for the three vectors is shown in **Figure 2**.

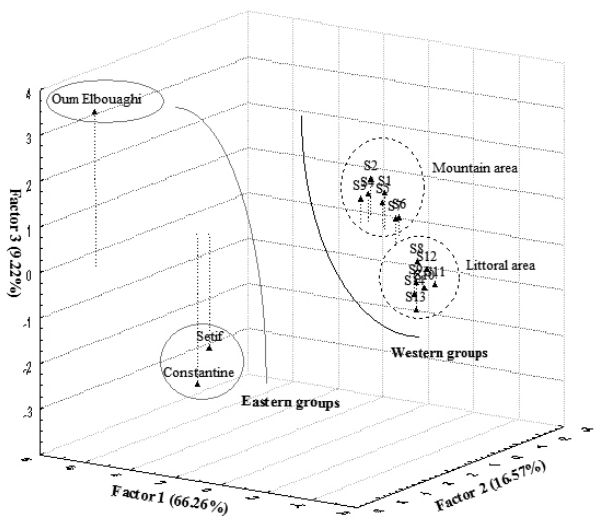

Figure 2: Score plot of the first three components for the PCA of composition of *D. aureus* populations.

The principal component analysis (PCA) allowed recognizing four distinct essential oil types. The first group was represented by Western populations. This group is divided into two subgroups based on the chorology. The populations (S1-S7) originated from the mountains of Tlemcene are characterized by the presence of high levels of germacren-D (59.8% ± 8.6). Whereas populations (S8-S14) consisted of oil samples that were oxygenated sesquiterpene which is rich and originated from the littoral zone near the Mediterranean Sea. This later group contains lower rates of germacrene-D (19% ± 8.7) and with a significant presence of caryophyllene oxide components (7.8% ± 1.6), spathunelol (6.2% ± 2.1), α-santalol (4.9% ± 1.1) viridiflorol (4.2% ± 1.2) and cadin-4-by-7-ol (4.5% ± 0.8).

The second group; formed by three populations located in Eastern Algeria (Setif, Oum Elbouaghi and Constantine). The statistical analysis clustered the essential oil samples of this groups are characterised by a very low rate of germacren-D, and is also divided into two subgroups; The subgroup of Oum Elbouaghi which is rich in α-pinene (19.4%) and β-pinene (12.0%), opposing the subgroup formed by the populations of Constantine and Setif, with the presence of a high rate of sabinene (36.2 and 30.6%) and 4-terpineol (6.0-4.4%).

We noted that all components present a quantitative and qualitative variability. The terpenoids variability reflects the heterogeneity of the genetic structure of *D. aureus*. Genetic analyses were carried out using terpenoids including some compounds that have been shown in other species to be under the control of single locus with two alleles. The dendrogram based on UPGMA clustering (Manhattan distance), shows the presence of two groups **(Figure 3)** that confirms results obtained from PCA analyses.

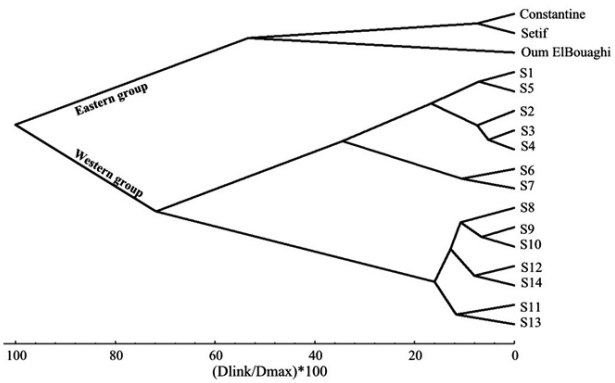

Figure 3: UPGMA cluster of *D. aureus* populations

These results suggest that this variability observed in the composition of essential oils can be attributed to the different geographical localities, the climate, the harvesting seasons, the drying procedure, the distilled part of the plant and also to the technique for processing [28, 29]. There is another important factor, that influences the chemical composition of essential oils; namely: the genetic composition of the plant. Therefore, all these biotope factors (genetic and epigenetic) influence the biochemical synthesis of essential oils in a given plant.

Hannover [30] provides evidence that terpene chemotypes are strongly controlled by genetic factors; he also reported instances of environmental variation in terpene expression under extreme habitat conditions. The chemovariation observed appears to be environmentally determined [31]. The hierarchical cluster analysis of essential oils compositions revealed the *D. aureus* essential oils from Eastern Algeria (Setif, Constantine and Oum Elbouaghi) are chemically distinct from other *D. aureus* essential oils (Western Algeria) and represents a two new chemotype rich in sabinene, 4-terpineol, α-pinene and β-pinene.

The essential oils were evaluated for their antimicrobial activity against two Gram-positive and two Gram-negative bacterial strains and one pathogenic yeast **(Table 3).** Using the agar diffusion method, the highest oil dilution (1/2) from Oum Elbouaghi population and Setif population of *D. aureus* showed a high antimicrobial activity against *Staphylococcus aureus,* a common food-borne pathogen, with wide inhibition zones of 14 and 26 mm. respectively, followed by *E. coli* ATCC 2592 with inhibition halos of 13 and 10 mm. respectively. The antifungal activity of the essential oils on the pathogenic yeast *C. albicans* showed a moderate antifungal activity for the Oum Elbouaghi *D. aureus* essential oil, with an inhibition zone of 12 mm. However, no inhibition was shown against *P. aeruginosa.*

The MIC values confirmed the activity against the tested microorganisms, as shown in **Table 4**. The MIC values of Setif and Oum Elbouaghi essential oils ranged from 0.97 to 2.54 mg/mL. The dilutions of the essential oils from Constantine population were less effective against all micro-organisms tested, except *B. subtilis* which showed a moderate antibacterial sensitivity (14 mm) at 1/2 (v/v) dilute oil, with MIC value of 3.23 mg/mL.

The monoterpene hydrocarbons sabinene and α-pinene were the main components identified in this essential oils and may be responsible, in part, for the antimicrobial activity, since sabinene [32] and α-pinene [33] have been reported to present notable antimicrobial activity against bacterial infections. The results show that the essential oil of *D. aureus* from Oum Elbouaghi inhibits both Gram- positive (*S. aureus*) and Gram- negative (*E. coli*) bacteria and inhibits the yeast *C. albicans*. The antibacterial activity could have resulted from the presence of α-pinene and β-pinene compounds that are known to possess antibacterial activity [34,35,36].

Table 3: Antimicrobial activity of the essential oils of *D. aureus*.

Populations	Inhibition zone in diameter (mm)									Controls*
	Constantine			Oum ELbouaghi			Setif			
Microorganisms	Dilution									
	1/2	1/5	1/10	1/2	1/5	1/10	1/2	1/5	1/10	
E .coli ATCC 2592	-	-	-	13±0.4	11±0.9	9±0.4	10±0.4	9±0.0	7±0.4	23±0.5
P. aeruginosa ATCC27853	-	-	-	-	-	-	-	-	-	26±0.1
S. aureus ATCC 25923	8±0.4	7±0.0	-	14±0.9	11±0.8	10±0.0	26±0.0	13±1	10±0.5	26±0.0
B. subtillus ATCC 663313	14±0.8	7±0.8	7±0.0	7±0.0	-	-	7±0.4		-	35±0.1
C. albicans ATCC1024	8±0.4	7±0.4	-	12±0.4	9±0.0	-	8±0.4	7±0.	-	21±0.9

*Controls: Gentamicin for all bacteria and Nystatin for *Candida albicans*

The essential oil of *D. aureus* from Setif showed a minimum inhibitory concentration (MIC) value lower than the essential oil *D. aureus* from Oum Elbouaghi with respect to the same bacteria, while the essential oil of *D. aureus* from Constantine exhibited antimicrobial activity only against *B. subtilis* and no antimicrobial activity against *C. albicans,* suggesting its selectivity. The high concentration of sabinene in the essential oils of *D. aureus* from Setif and Constantine could be, at least in part, responsible for the antimicrobial activity against the tested microorganisms [37].

Our results on the antimicrobial activity of the *Daucus* essential oils are not in completely agreement with those of Meliani et al [17], who found a MIC of 0.125 ± 0.02 mg/mL against *C. albicans*. The same group of authors found lower MIC values against *S. aureus* and *E.coli* (4.8 ± 0.6 mg/mL and > 5mg/mL), respectively. Differences in MIC values are probably due to the different percentages of essential oils constituents. Antimicrobial activity investigated in this study was also detected for the essential oils of other plants belonging to the Apiaceae family [12,13,38, 39, 40]. Probably, similar components detected in our experiments could be responsible for these properties, such as sabinene and α-pinene [12, 41].

Table 4: Minimal inhibitory concentrations (MIC) of the essential oils of *D. aureus*.

Microorganisms	MIC (mg/mL)			
	Constantine	Oum Elbouaghi	Setif	Controls*
E. coli ATCC 2592	> 6.46	1.81	2.29	0.25
P. aeruginosa ATCC 27853	> 6.46	> 5.73	> 6.36	0.56
S. aureus ATCC 25923	> 6.46	0.97	1.14	0.12
B. subtillus ATCC 663313	3.23	> 5.73	> 6.36	0.18
C. albicans ATCC1024	> 6.46	2.54	> 6.36	0.12

* Controls:.Gentamicin for all bacteria and Nystatine for *C. albicans*

Table 5: DPPH scavenging activity of essential oils of *D. aureus* collected from the three regions.

Samples	**IC50 (µg/ml)***
Constantine	783 ± 0.16
Oum Elbouaghi	970 ± 0.53
Setif	713 ± 0.04
BHT (butyl hydroxyl toluene)	41.3 ± 0.40

* Each value in the table is represented as mean ± S.D. (n = 3)

The components at lower concentrations, such as γ-terpinene, α-phellandrene, and limonene may also contribute to the antimicrobial activity of the oils [42,43,44]. Therefore, the synergistic effects of the diverse major and minor components of the essential oils should be taken into consideration to account for the oil biological activity [45]. Essential oil antimicrobial activity is highly correlated with the composition in monoterpenes compared with other chemical families and in oxygenated molecules against hydrocarbons [46,47]. The antimicrobial activities of the essential oils of *D. aureus* confirm that the Apiaceae species are a source of biologically active compounds.

The DPPH radical is a free radical, which has been widely used as a tool to estimate free radical scavenging activity of antioxidants. The DPPH free radical scavenging activity of the essential oils of *D. aureus* is shown in **Table 5**, in which lower IC_{50} values indicate higher antioxidant activity. The essential oils showed a weak antioxidant activity compared to BHT activity (41.3±0.397µg/ml). The essential oil from Oum Elbouaghi revealed the lowest antioxidant activity, IC_{50}= 970±0.53µg/ml; the values for the Setif and Constantine sites were 713 ± 0.047 and 783 ± 0.16 µg/ml, respectively.

The essential oils of the present study did not show significant antioxidant activity. This can be attributed to the chemical composition of the essential oils. It has been reported that components with phenolic structures such as thymol or carvacrol, were responsible for the antioxidant activity of many essential oils and a scant antioxidant activity is given to monoterpene and sesquiterpene hydrocarbons [48,49]. These results, may explain the low antioxidant activity of the essential oils of *D. aureus* from Setif which is composed by 51.5% of monoterpene and sesquiterpene hydrocarbons, as well as those of *D. aureus* from Oum Elbouaghi and Constantine, which are composed by 59.7% and 62.8%, respectively of these chemical classes of substances. Djabou et al [16] concluded that, the plant extract of *D. aureus* possess modest medicinal and antioxidant properties.

CONCLUSIONS

The present study demonstrate that the most abundant constiutants of *D. aureus* essential oils from Setif and Constantine are sabinene (30.6% and 36.2%, respectively) and 4-terpineol (4.4% and 6.0%, respectively); whereas α-pinene (19.4%) and β-pinene (12%) are the major compounds in essential oil of *D. aureus* from Oum Elbouaghi. Our study of *D. aureus* from Eastern Algeria and the previous study on *D. aureus* allowed us to detect the presence of (α-pinene-β-pinene) chemotype and (sabinene-4-terpineol) chemotype, in the population of Eastern Algerian and germacrene-D-caryophyllene oxid-spathulenol chemotype in Western Algeria. The oils of *D. aureus* from Setif and Oum Elbouaghi were active against *E. coli* and *S. aureus* while the oil of *D. aureus* from Constantine was only active on *B. subtilis*. The results of DPPH test of the present study show that the essential oils have a weak antioxidant activity compared to BHT.

ACKNOWLEDGMENTS

We are grateful to Dr Boulaacheb Nacira from Setif 1 University for his help in plant specimen identification.

REFERENCES

1.- F**.** Bakkali, S. Averbeck, D. Averbeck, M. Idaomar, *Food Chem Toxicol* **46**, 446, (2008)
2.- M. Olle, I. Bender, *Agronomy Res* **8**, 687, (2010).
3.- H. Laour, A. Bouheda, S. Haroutounian, E. Evergetis, M. Bouchecrit, F. Sahli, *Pharmacognosy commun* **3**, (2013).
4.- G. Benelli, G. Flamini, G.Fiore, P. L. Cioni, B. Conti, *Parasitol Res* **112**, 1155, (2013).
5.- S. M. El-Sayed, T. M. Galal, T. K. Ashraf, M. M. El-Sayed, A. S. Amal, *Naturforsch C J Biosci* **59**, 373, (2004).
6.- M. A. Dib, M. Bendahou, A. Bendiabdellah, N. Djabou, H. Allali, B. Tabti, J. Paolini, J. Costa, *Gras YAceites* **61**, 271, (2010).
7.- S. B. Glisic, D. R. Misic, M. D. Stamenic, I. T. Zizovic, R. M. Asanin, D. U. Skala, *Food. Chem* **105**, 3462, (2007).
8.- H. W. Fu, L. Zhang, T. Yi, R. N. Chen, X. Wang, J. K. Tian, *Chem Pharm Bull* **58**, 125, (2010).
9.- N. Khalil, A. Ashour Singab, O. Salama, *J pharmacy Biological Sci* 10, **13**, (2015).
10.- M. Tawil, A. Bekdash, M. Mroueh, C. F. Daher, R. J. Abi-Habib, *Asian Pac J Cancer Prev* **16**, 761, (2015).
11.- M. G. Pinilla, M. J. Pérez-Alonso, A. Velasco-Negueruela, *J Ess Oil Res* **7**, 433, (1995).
12.- M. Staniszewska, J. Kula, M. Wieczorkiewicz, D. Kusewicz, *J Ess Oil Res* **17**, 579, (2005).
13.- A. Bendiabdellah, M. A. Dib, N. Djabou, H. Allali, B. Tabti, A. Muselli, J. Costa, *Chem Cent J* **6**, 48, (2012).
14.- T. Smaili, A. Zellagui, P. L. Cioni, G. Flamini, *Nat Prod Commun* **6**, 883, (2011).
15.- G. Flamini, E. Cosimi, P. L. Cioni, I. Molfetta, A. Braca, *Chem Biodivers* **11**,1022, (2014).
16.- N. Djabou, N. Meliani, M. E. A. Dib, A. Bendiabdellah, H. Allali, B. Tabti, *Int J Trad Her Med* **1**, 41, (2013).
17.- N. Meliani, M. E. A. Dib, N. Djaboua, J. Costab, H. Allalia, B. Tabtia, A. Muselli, *Nat Prod Commun* **6**, 835, (2013).
18.- P. Quezel, S. Santa, Nouvelle flore d'Algérie et des régions désertiques méridionales. Tome II. Edition Paris: CNRS. 1963.
19.- F. R. Cockerill, M. A. Wikle, J. Alder, M. N. Dudley, G. M. Eliopoulos, M. J. Ferrar, D. J. Hardy, D. W. Hecht, J. A. Hindl, J. B. Patel, Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, Approved Standard-9th ed.; Clinical and Laboratory Standards Institute: 950 West Valley Road, Suite 2500 Wayne, PA 19087, USA; p. 277, 2012.
20.- H. Laouer, Y. Adjal- Hireche, S. Prado, N. Boulaacheb, S. Akkal, G .Singh, P. Singh, V. A. Isidorov, L. Szczepaniak, *Nat Prod Commun* **4**, 1605, (2009).
21.- L. H. N. Bassole, A. S. Quattara, R. Nebie, C. A. T. Quattara, Z. I. Kabore, S. A. Traore, *Phytochemistry* **62**, 209, (2003).
22.- R. P. Adams, In identification of essential oil components by gaz chromatography/mass spectrometry, 4th ed.; Carol Stream IL: Allured Publishing, USA, 2007.
23.- Wiley registry of mass spectral data (7th ed). New York: Wileyand Sons,

with NIST spectral data, CD-ROMP, 1998.
24.- S. Que, M. Linchun, P. Xin, *Food Res Int* **39**, 581, (2006).
25.- S. Burt, *Int J Food Microbiol* **3**, 223, (2004).
26.- D. Mockute, O. Nivinskiene, *J Ess Oil Res* **16**, 277, (2004).
27.- G. Flamini, P. L. Cioni, S. Maccioni, R. Baldini, *Food Chem* **103**, 1237, (2003).
28.- M. Moghaddam, R. Omidbiagi, F. Sefidkon, *J Ess Oil Res* **19**, 18, (2007).
29.- T. Ma, J. Luo, C. Tian, X. Sun, M. Quan, C. Zheng, L. Kang, J. Zhan, *Food Chem* **170**, 394, (2015).
30.- J. W. Hannover, *New Forests* **6**, 159, (1992).
31.- M. M. Lesjak, I. N. Beara, D. Z. Orcic, J. D. Ristic, G. T. Anackov, B. N. Bozin, N. M. Mimica-Dukić, *Food Sci Tech* **53**, 530, (2013).
32.- B. M. Damnjanovic, M. Sc. Thesis, Faculty of Technology and Metallurgy. Belgrade; 2000.
33.- S. B. Glisic, S. Z. Milojevic, S. L. Dimimitrijevic, A. M. Oriovic, D. Skala, *J Serb Chem. Soc* **4**, 311, (2007).
34.- B. Mercier, J. Prost, M. Prost, *Int J Occup Med Environ Health* **22**, 331, (2009).
35.- Y. Jiang, N. Wu, Y. J. Fu, W. Wang, M. Luo, C. J. Zhao, Y. G. Zu, X. L. Liu, *Environ Toxicol Pharmacol **32,*** 63*,* (2011).
36.- M. Karapandzova, G. Stefkov, E. Trajkovska-Dokic, A. Kaftandzieva, S. Kulevanova, *Maced Pharm Bull* **57**, 25, (2011).
37.- R. Arunkumar, S. A. Nair, K. B. Rameshkumar, A. Subramoniam, *Rec Nat Prod* **8**, 385, (2014).
38.- F. M. Abd Alla, K. A. Abdelshafeek, A. M. El-soll, W. M. ELsayed, *J Arab Soc Med Res* **8**, 96, (2013).
39.- D. A. Lanfranchi, H. Laouer, M. El Kolli, S. Prado, C. Maulay-Bailly, N. Baldovini, *J Agric Food Chem* **4**, 2174, (2010).
40.- I. Labed-Zouad, A. Labed, S. Laggoune, S. Zahia, A. Kabouche, Z. Kabouche, *Rec Nat Prod* **9**, 518, (2015).
41.- A. Zeraib, M. Ramdani, L. Boudjedjou, P. Chalard, G. Figuredo, *J Bio Sci Biotech* **3***,* 147, (2014).
42.- A. Giweli, A. M. Džamić, M. Soković, M. S. Ristić, P. D. Marin, *Molucules* **17**, 4836, (2012).
43.- J. Dai, L. Zhu, L. Yang, J. Qiu, *EXCLI J* **12***,* 479*, (*2013).
44.- D. V Biljana, D. Tatjana, Š. Danijela, D. Jovanka, *Czech J Food Sci* **3**, 277, (2011).
45.- A. Morey, N. Canillac, *Food Control* **13**, 289, (2002).
46.- A. Koroch, H. R. Juliani, J. A. Zygadlo, Bioactivity of essential oils and their components. In *flavours and fragrences* chemistry, Bioprocessing and Sustainability, Berger R.G., Ed; Springer Verlag: Berlin, Germany, 2007; pp. 87-115
47.- A. Srivastava, Y. N. Shukla, S. Kumar, *J Med and Ar Plant Sci* **22**, 349, (2000).
48.- B. Teixeira, A. Marques, C. Ramos, N. R. Neng, J. M. F. Noguira, J. A. Saraiva, M. L. Nunes, *Ind Crops Prod* **43**, 587, (2013).
49.- G. Ruberto, M. T. Baratta, *Food Chem*. **69**,167–174, (2000).