Chemical Composition and Antibacterial Activity of Essential Oils Isolated From Leaves of Different Woody Trees Grown In Al-Jabel Al-Akhdar Region, Libya

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ABSTRACT

The present study reports the variation in the essential oils (EOs) composition from needles and scale leaves of *Pinus halepensis*, *Cupressus sempervirens* and *Juniperus phoenicea* which collected from three different altitudes at Al-Jabel Al-Akhdar area, Libya. The impact of the altitude on the quantity and quality of EOs was studied. In addition, the antibacterial activity was evaluated using microdilution broth assay technique. The results showed that the highest percentages of the EOs yield were found in *P. halepensis* with 0.41, 0.60, and 0.43% for the altitudes of 125m (I), 391m (II), and 851m (III), respectively, while the lowest percentages of the yield were found in *J. phoenicea*, with 0.15, 0.07, and 0.18% at altitudes of I, II, and III, respectively. Based on *P. halepensis* EOs analyses using GC/MS, 35 components have been detected, which represents 89.92, 91.56, and 86.44% for the altitudes of I, II and III, respectively. Furthermore, high percentages from the components of α-pinene, β-pinene, α-terpineol and caryophyllene were identified at the three tested altitudes. For *C. sempervirens* EOs, 33 components were identified representing 84.94, 93.37 and 99.48% for levels I, II and III, respectively. The highest percentages of the EOs components in this species were α-pinene, terpinen-4-ol and α-terpiny acetate at the three tested elevations. However, the detected main components in *J. phoenecea* EOs were α-pinene, α-myrcene, α-terpinyl acetate and γ-terpinene. The highest antibacterial activity was observed for the oils from *P. halepensis*at and *J. phoenicea* at altitude I against the *Agrobacterium tumefaciens, Erwinia carotovora, Corynebacterium fascians* and *Pseudomonas solanacearum*. While the EOs extracted from scale leaves of *C. sempervirens* obtained from trees growing at level II were the most active against the tested bacteria. The results of this work revealed the impacts of the environmental conditions on the EOs composition which affected significantly in its quantitative and qualitative performances. Consequently, their biological activities were varied considerably. Moreover, the obtained data offer the opportunity to choose EOs with preferential compound for pharmaceutical, pesticides, perfume and food industries in such important region in Libya.

Keywords: Plant essential oils; *Pinus halepensis*; *Cupressus sempervirens*; *Juniperus phoenicea*; GC/MS analyses; antibacterial activity.

INTRODUCTION

In Al-Jabal Al-Akhdar region, the largest phytogeographical, is the richest vegetation and highest species diversity in Libya, The vegetation in this area is classified into three main groups; the first is coastal and low altitude vegetation dominated by shrubs and trees, which constitute about 60% of the plant life forms, the second is mid altitude vegetation with the highest species richness and dominated by shrubs and trees with over 60% of the plant life forms, and the third is characterized by mountain top vegetation dominated by herbs and few low shrubs constituting up to 90% of the plant life forms. In addition, the flora in this area comprises the richest vegetation and the highest number of species known in Libya (Hegazy *et al.* 2011). Although the AL-Jabal AL-Akhdar region represented 1% of the total area of the country, it is characterized by the large bio-diversity, as the number of plant species reach up of 1100 species from the total of plant species (2000 species) with about 75 species of plants that grow only in this region (Al-Sodany *et al.* 2003 and El-Barasi and Saaed 2013). Recently, an increase interest in natural substances extracted from plants has been observed in literatures due to their less hazardous effect on environment, as well as to find effective alternatives to the synthesized chemicals used as drugs and pesticides. In this context, plant secondary metabolites, such as essential oils, have been widely investigated because of their non-toxic nature and a wide spectrum of biological activities (Daferera *et al*. 2000; Mohamed *et al.* 2009; Mohareb *et al*. 2013; Abdelgaleil *et al.* 2014 and Djouahri *et al* 2014). Essential oils are liquid

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products, which can be extracted by steam or water distillation of plant parts (leaves, stems, bark, seeds, fruits, roots and plant exudates). They are an important crop in many parts of the world and their profitability is closely linked to both the profile and concentration of the oil in each plant. Furthermore, several studies showed great influence of the physical factors such as temperature, atmospheric pressure, wind velocity, increasing precipitation and altitude on the amount and composition of the EOs (Friend and Woodward 1990 and Korner 2007). On the other hand, several authors have been demonstrated the antimicrobial activity of the EOs (Trabelsi,. *et al.* 2014; Zeraib *et al*. 2014 and Ghabraie *et al.* 2016).

Therefore, the objective of the current research was to study the chemical composition using GC/MC and antibacterial activity of the essential oils from leaves of *Pinus halepensis*, *Cupressus sempervirens* and *Juniperus phoenicea* collected from three different altitudes in Al-Jabel Al-Akhdar area, Libya. The percentages of EOs of leaves from the selected trees, the influence of the altitude on the quantity and quality of EOs, identification of the most effective compounds were studied in details.

MATERIALS AND METHODS

1. Plant materials

Needles and scale leaves of three tree species namely, *Pinus halepensis* Mill, *Cupressus sempervirens* L. and *Juniperus phoenicea* L. were collected from three locations in AL-Jabal AL-Akhder, Libya. The trees were chosen and represented by three classes of different levels of altitude. The plant samples were collected from three places in AL-Jabal AL-Akhder region Sidi Alhemre (851m high), Wadi Alkuf (391m high), and Alwsita near the Sea level. The leaves were taken from the first branch of each tree during August, 2014.

2. Isolation of essential oils

The air-dried leaves (100g) of the three species were cut into small pieces and subjected to hydrodistillation for 3 h with 500 ml of distilled water using a Clevenger-type apparatus. The essential oil was collected and stored in a refrigerator at 4°C prior to analysis. The oil yield was calculated based on dry weight (v/w, %) (Lograda *et al.* 2013).

3. Analysis of essential oils

The composition of the EOs was analyzed by gas chromatography mass spectrometry (GC/MS) with the following specifications: A Trace GC Ultra/Mass Spectrophotometer ISQ (Thermo Scientific) instrument equipped with a FID and a DB-5 narrow bore column. Helium was used as the carrier gas (flow rate of 1 ml min^{-1}), and the oven temperature program was: 45-165 º C (4 ºC min^{-1}) and 165-280 ºC (15 ºC min^{-1}) with post run (off) at 280 ºC. Samples (1 µl) were injected at 250 ºC, with split/split-less injector (50:1 split ratio) in the split less mode flow with 10 ml min^{-1}. The GC/MS was equipped with a ZB-5MS Zebron capillary column (length 30m× 0.25 mm ID, 0.25-µm film thickness; Agilent).

All mass spectra were recorded in the electron impact ionization (EL) at 70 electron volts. The mass spectrometer was scanned from *m/z* 50-500 at five scans per second. Peak area percent was used for obtaining quantitative data with the HP-Chem Station software (Agilent Technologies) without using correction factors. Identification of the essential oil constituents was performed on the basis of MS library of NIST and Wiley, (Davies 1990 and Adams 1995).

4. *In-vitro* antibacterial assay

4.1. Tested bacteria

Four plant pathogenic bacteria namely, *Agrobacterium tumefacie*ns (Family: Rhizobiaceae), *Erwinia carotovora* (Family: Enterobacteriaceae), *Corynebacterium fascians* (Family: Nocardiaceae) and *Pseudomonas solanacearum* (Family: Pseudomonadaceae) were provided by Department of Pesticide Chemistry and Technology, Faculty of Agriculture, Alexandria University, Egypt. The bacterial strains were maintained on nutrient agar (NA: Peptone 5g, Beef extract 3g, NaCl 8g), medium at 37ºC.

4.2. Broth microdilution technique

The *in vitro* antibacterial activities as minimum inhibitory concentrations (MICs) of the EOs were determined by microdilution broth assay method using 2,3,5-triphenyltetrazolium chloride (TTC, Sigma) as a chromogenic marker. Nutrient broth (NB) medium was used to grow the bacterial strains to a final inoculum size of 5×10^5 CFU/mL. The EOs were dissolved in dimethyl sulfoxide (DMSO) and diluted with distilled water to obtain a final stock solution of 1000 mg/L. For the broth microdilution test, 20µL of each bacterial suspension in NB medium was added to the wells of a sterile 96- well microtitre plate already containing 40 µL of serially diluted compounds and 140 µL NB medium. The final volume in each well was 200 µL. Control wells were prepared with culture medium, bacterial suspension, and solvent. The contents of each well were mixed on a micro plate shaker at 200 rpm/L min prior to incubation for 24 h at 37± 2ºC .To indicate respiratory activity, the presence of color was determined after adding 10 µL / well of TTC dissolved in water (0.01%, w/v) and incubated under appropriate cultivation condition for 30 min in dark. The absorbance was measured at 492 nm in an Ultra Micro

plate Reader (Robonik, PVT.LTD). Positive controls were wells with a bacterial suspension. Negative controls were wells with growth medium and the tested compounds. All measurements of MIC values were repeated in triplicate. The MIC was the lowest concentration, where no viability was observed after 24 h on the basis of metabolic activity.

5. Statistical analysis

Statistical analysis was performed using the SPSS 21.0 software program (Statistical Package for Social Sciences, USA). The experimental design was a split plot with three replications in each elevation. The main plots were occupied by three species (*P. halepensis, J. phoenicea* and *C. sempervirenus*). The sub-plots were allocated to three levels at altitude (I, II and III), respectively. In the antibacterial assessment, three plates were used at each concentration. The data were analyzed by one-way analysis of variance (ANOVA). Mean separations were performed by Student-Newman-Keuls (SNK) test and differences at $P < 0.05$ were considered as significant. The ANOVA and L.S.D procedures were done according to Steel and Torrie (1980).

RESULTS AND DISCUSSIONS

1. Yield of the essential oils

The results in Table (1) showed that the highest percentages of the yield were found in *P. halepensis* Mill., 0.41, 0.60, and 0.43% for the altitudes of 125m (I), 391m (II), and 851m (III), respectively, while the lowest percentages of the yield were found in *J. phoenicea* L., with 0.15, 0.07, and 0.18% at altitude of I, II, and III, respectively. Significant differences were found among the means of EO yields at the studied altitudes for *P. halepensis* MIill. and *C. sempervirens* L., while no significant differences among EO yields in *J. phoenicea* L. at the different altitudes. From the obtained data, it can be noted that the EO content varied significantly based on tree species and locations. The yield of EOs isolated from needles of *P. halepensis* Mill. obtained in our study is lower than that reported by Amri *et al.* (2013) and higher than that reported by Abi-Ayad *et al.* (2011) and Raho (2014) but it was relatively similar to that reported by Dob *et al*. (2005); Fekih *et al.* (2014), and Hamrouni *et al*. (2015). Also, the yield of EOs from leaves of *C. sempervirens* L. obtained in this study was lower than that reported by Mazari *et al.* (2010); Elansary *et al.* (2012) and Amri *et al.* (2013), and lower than the yield of EOs from cone of Tunisian *C. sempervirens* L. which reported by Ben Nouri *et al.* (2015). The yield of EOs isolated from leaves of *J. phoenicea* L. obtained in this study was lower than that reported by Stasis *et al.* (1996); Mazari *et al.* (2010); Derwich *et al.* (2010) and Amalich *et al.* (2015), but it was relatively similar when compared to that reported by Angioni *et al.* (2003) and Achak *et al.* (2009).

2. Chemical composition of essential oils

2.1. Chemical composition of essential oils of *P. halepensis*

Figure (1) showed that the EOs were mixture of numerous compounds. The data in Table (2) presented the constituents of EOs of *P. halepensis* with their retention times and peak area and some of them presented in trace amounts were discussed, in total 35 components were identified representing 89.92, 91.56, and 86.44% for needles of the trees growing at altitude I, II and III, respectively of the total EOs composition. The major components for level I were β-pinene (19.93%), α-pinene (13.56%), caryophyllene (8.45%), germacrene D (8.40).While the major components for level II were caryophyllene (24.85%), α-terpineol (11.44%), β-pinene (7.90%),β-pinene (7.90%), also the major components for level III caryophyllene (27.23%), phenethylisovalerate (7.97%), α-caryophyllene (6.34%).

Some compounds such as fenchol, borneol, pinocarveol, myrtenol and geraniol were found in the needles of the trees growing at altitude I and II, while compounds of copaene, phenyl ethyl tiglate, guaiol, muurolol, cembrene and thunbergol were found in those growing at altitude III only.

Generally, the samples showed variable composition, which are related to different sites and their characteristics. These data are in agreement with those obtained by Dob *et al.* (2005) Fekih *et al.* (2014) and Raho (2014) and were relatively similar with that reported by Govindarajan *et al.* (2016).

Table 1. Mean of essential oils yields(%) in the tree leaves at different levels of altitude in *P. halepensis* ,*C. sempervirens* and *J. phoenicea*

Altitude	Tree Species		
	P. halepensis	***C. sempervirens***	***J. phoenicea***
Level I (% ± SE)	0.41^{b}±0.21	0.29^{a}±0.04	0.15^{a}±0.03
Level II (% ± SE)	0.60^{a}±0.11	0.15^{ab}±0.001	0.07^{a}±0.01
Level III (% ±SE)	0.43^{b}±0.24	0.05^{b}±0.02	0.18^{a}±0.04

(1) Means followed by the same letter(s) are not significant, but different letter are significant for each column.
(2) LSD for altitude = 0.149

Table 2. Components of the essential oils from needles of *P. halepensis* at different altitudes in AL-Jabal AL-Akhdar region

Compound Name	Altitude (m)					
	Level I (125m)		Level II (391m)		Level III (851m)	
	RT(min)	(%)	RT(min)	(%)	RT(min)	(%)
α-pinene	6.95	13.56	6.93	7.32	6.92	3.66
β-pinene	8.39	19.93	8.39	7.90	8.26	1.05
α-myrcene	8.94	3.76	8.93	2.00	8.86	0.76
3-carene	9.50	1.90	9.48	0.40	9.45	2.77
Sylvestrene	10.16	4.63	10.16	5.42	10.12	0.77
Ocimene	10.91	0.46	10.91	3.10	10.91	2.60
Terpinolene	12.30	1.39	12.30	1.84	12.30	1.93
Linalool	12.78	1.18	12.77	1.70	12.78	1.95
fenchol, exo-	13.19	0.46	13.18	0.76	-	-
Pinocarveol	14.11	3.52	14.12	2.63	-	-
Borneol	15.09	0.66	15.09	1.34	-	-
terpinen-4-ol	15.53	0.37	15.55	2.33	15.54	1.76
cherry propanol	15.91	8.59	-	-	15.86	0.43
α-terpineol	16.17	1.02	16.20	11.44	16.08	1.96
(-)-myrtenol	16.29	0.58	16.30	0.50	-	-
Geraniol	18.43	0.79	18.37	0.46	-	-
bornyl acetate	19.44	1.02	19.45	1.57	19.44	0.53
Copaene	-	-	-	-	23.53	1.08
β-elemene	23.08	0.49	23.06	0.26	23.09	0.33
methyl eugenol	23.52	0.32	23.71	0.46	23.68	0.62
Caryophyllene	24.03	8.45	24.19	24.85	24.21	27.23
β –cubebene	24.27	1.06	24.26	0.97	24.38	1.55
γ-muurolene	24.74	0.38	24.80	0.77	24.79	0.67
α-caryophyllene	25.03	2.74	25.04	2.45	25.15	6.34
germacrene D	25.96	8.40	25.40	0.55	25.94	1.48
Phenethylisovalerate	26.22	1.03	26.20	7.63	26.55	7.97
α-muurolene	26.50	0.17	26.50	0.42	26.67	1.54
phenyl ethyl tiglate	-	-	-	-	28.88	0.78
caryophyllene oxide	28.94	1.26	28.94	1.43	29.00	1.94
Guaiol	-	-	-	-	29.45	1.86
Muurolol	-	-	-	-	30.66	1.22
aromadendrene oxide-(1)	30.65	0.35	-	-	-	-
α-cadinol	30.99	1.45	30.98	1.06	31.01	1.56
cembrene)	-	-	-	-	35.72	3.87
Thunbergol	-	-	-	-	36.84	6.23
TOTAL	89.92%		91.56%		86.44%	

In agreement with our results, several studies have demonstrated the variability of the production of secondary metabolites as a consequence of climatic and soil conditions (Fekih *et al.* 2014 and Hamrouni *et al.* 2015).

2.2. Chemical composition of essential oils of *C. sempervirens*

The chemical composition of essential oils isolated from *C. sempervirens* at different levels is shown in Table (3) and Figure (2). In total, 33 components were identified representing 84.94, 93.37 and 99.48% for scale leaves of the trees growing at altitude I, II and III, respectively, The major components for level I were terpinen-4-ol (20,82%), α-pinene (15.65%), α-terpinyl acetate (8.78%), and α-terpineol (7.49%), While the major components for level II and III were α-pinene (17.69 and 21.03%), α-terpinyl acetate (7.91 and 7.09%), germacrene D (5.87 and 4.06%) and terpinolene (5.49 and 5,47%). However compounds such as 3-Carene (11.93 and 12.41%), manoyl oxide (0.98 and 2.56%), totarol (2.13 and 1.75%) were found only in levels II and III, respectively. While cedrene, tau.cadino, 2-isopropyl-4-methylanisole and dehydroabietane were found in level III only. The compound of sylvestrene was detected only in level II.

The chemical compositions revealed that these leaves have compositions relatively similar to those of other *C. sempervirens* EOs isolated from Tunisian plants Boukhris *et al.* (2012) and Ben-Nouri *et al.* (2015). The oils were predominantly composed of α-pinene, (−) terpinen-4-ol and (±)-α-terpinyl acetate as major constituent, while α-myrcene, limonene, germacrene D and cedrol were found to be the second most important constituents of the *C. sempervirens*oil. Similar result was found by Mazari *et al.* (2010), who reported that α-pinene was the major component of leaves EOs, but it is presented in upper content (60.5%) compared with our study (15.05 , 15.63 and 17.89%). Also the percentage of α-pinene was the same as obtained by Elansary *et al.* (2012), while, α-pinene was reported as the second and the third major component, respectively, in the previous researches (Sacchetti *et al.* 2005 and Tapondjou *et al.* 2005).

2.3. Chemical composition of essential oils of *J. phoenecea*

The constituents of EOs of *J. phoenecea* with their retention times and percentages are shown in Table (4) and Figure (3). In total, 26 components were identified representing 85.92, 91.08 and 89.13% for branch lets of trees growing at altitude of I, II, and III, respectively, of the total EOs composition. The principal components of *J. phoenecea* EOs were α-pinene (26.25, 20.44 and 24.12%) and α-terpinyl acetate (12.06, 12.34 and 12.14%) for scale leaves of trees growing at altitudes of I, II and III, respectively, also components γ-terpinene(16.86 and 19.39%) and α-thujene (6.67 and 7.8%) for elevations II and III, respectively.

However, compounds of α-cubebene and T-muurolol were found in scale leaves of trees growing at altitudes of II and III. It is also noted that a carotol was detected in level I and III, respectively. The chemical analysis in this part revealed that these leaves have oil component relatively similar to those of other *J. phoenicea* EOs analyzed in Libya by Alfitori (2009) and also in Morocco by Derwich *et al.* (2010) and Amalich *et al.* (2015) who reported that the major component was α-pinene and the other main constituents were α-myrcene, γ-terpinene, linalool and germacrene D. In addition, Robert *et al*. (1996) studied the composition of *J. phoenicea* oil collected from the Portugal, Spain and Greece and they reported that the yields and the total oil obtained were (0.41 and 98.3%), (0.66 and 99%) and (0.58 and 88%), respectively, and the composition was characterized by a high content of α-pinene (34.1, 53.5 and 41.8%), β-phellandrene (19.2, 5.9 and 3.5%) and β- caryophyllene (0.22, 1.0 and 0.5%).

Such a discrepancy in chemical composition could be understood since several authors have documented significant species with specific variations in the concentrations of detected compounds and/or presence of others in high concentration. Moreover, the essential oil composition of *J. phoenicea*, as occurs with other medicinal and aromatic plants, is highly influenced by genetic and environmental factors (climatic, seasonal, geographical and geological).

3.3. Antibacterial activity of essential oils isolated from needles of *P. halepensis,* branchlets of *C .sempervirens* and *J. phoenicea*

The antibacterial activity of EOs isolated from leaves or branch lets of *P. halepensis*, *C. sempervirens* and *J. phoenicea* against *A. tumefaciens, E. carotovora, C. fascians* and *P. solanacearum*are are presented in Table (5).

The EOs obtained from *P. halepensis* showed that the plant sample collected from the trees growing at altitude Icontained the most active product against the growth of *A. tumefaciens, E. carotovora, C. fascians*and *P. solanacearum* among the three altitudes, with MIC values of 680, 485, 500, and 390 mg/L, respectively. However, the activity was decreased with the needles collected from the trees growing at altitude II (MIC 710, 465, 875 and 580 mg/L, respectively) and then level III which showed the lowest activity (MIC 755, 875, 945 and 585 mg/L, respectively).

The EOs obtained from *C. sempervirens* showed that the scale leaves collected from the trees growing at altitude II contained the most active product against *A. tumefaciens*, *E. carotovora, C. fascians* and *P. solanacearum* with MIC values of 530, 585, 405, and 390 mg/L, respectively. However, the activity was decreased with the altitudes I and III, respectively.

The EOs obtained from *J. phoenicea* showed that the scale leaves collected from the trees growing at altitude I the most active oils among the three altitudes against the growth of *A. tumefaciens, E. carotovora , C. fascians* and *P. solanacearum* with MIC 680, 580, 520 and 450 mg/L, respectively. However, the scale leaves collected from the trees growing at altitude III showed the lowest activity with MIC of 940, 670, 735 and 650 mg /L, respectively.

According to Fekih, *et al*. (2014), the EO of *P. halepensis* showed good inhibitory effects on some tested microorganisms including *Lysteria monocytagenes, Klebsiella pneumonia*, *Enterococcus faecalis* and *Acinetobacter baumanii* with inhibition zones 10, 10, 9, 9.5 mm, respectively.

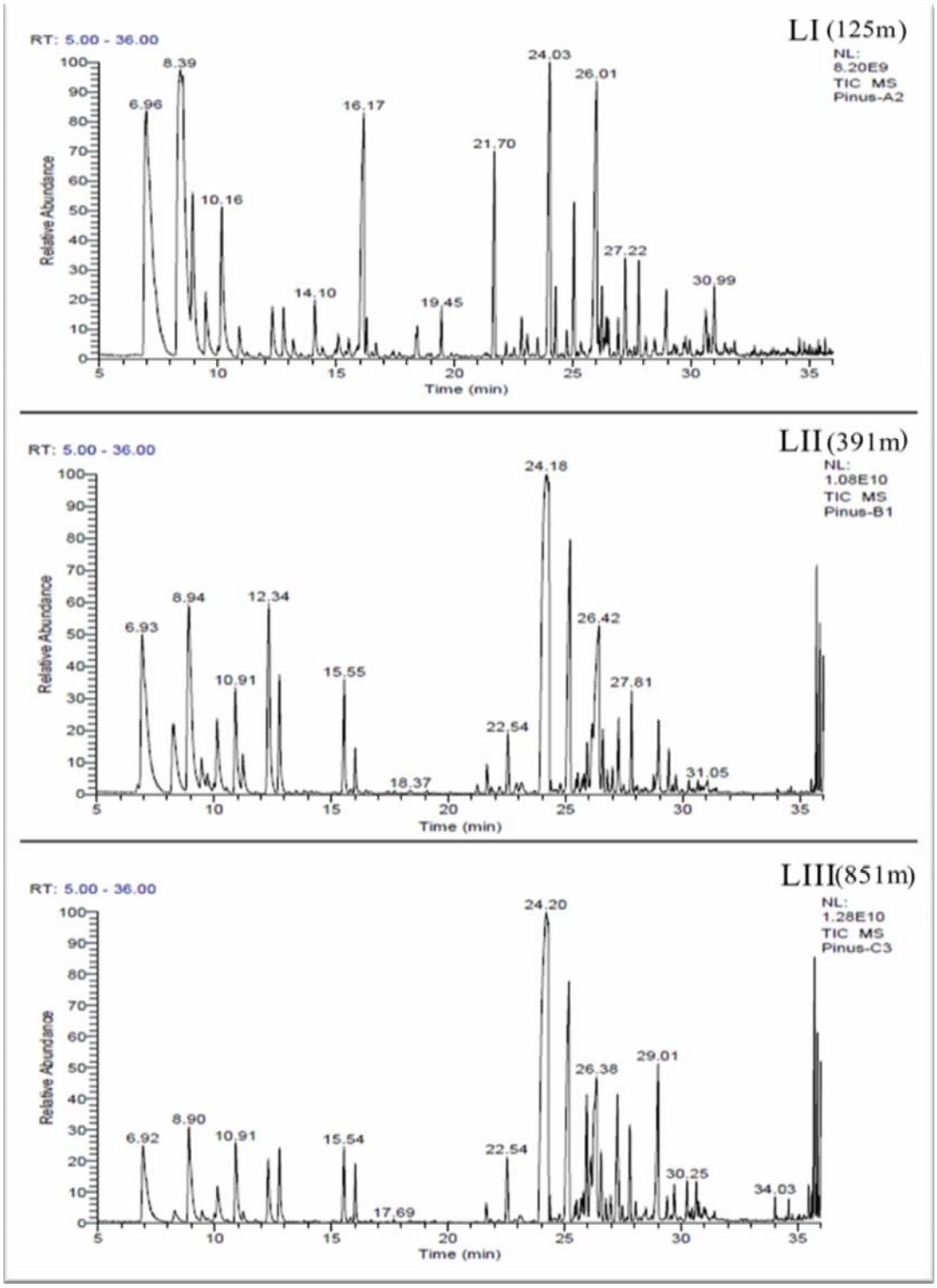

Figure 1. GC/MS chromatograms of essential oils of *P. halepensis,* needles at different altitudes in AL-Jabal AL-Akhdar region

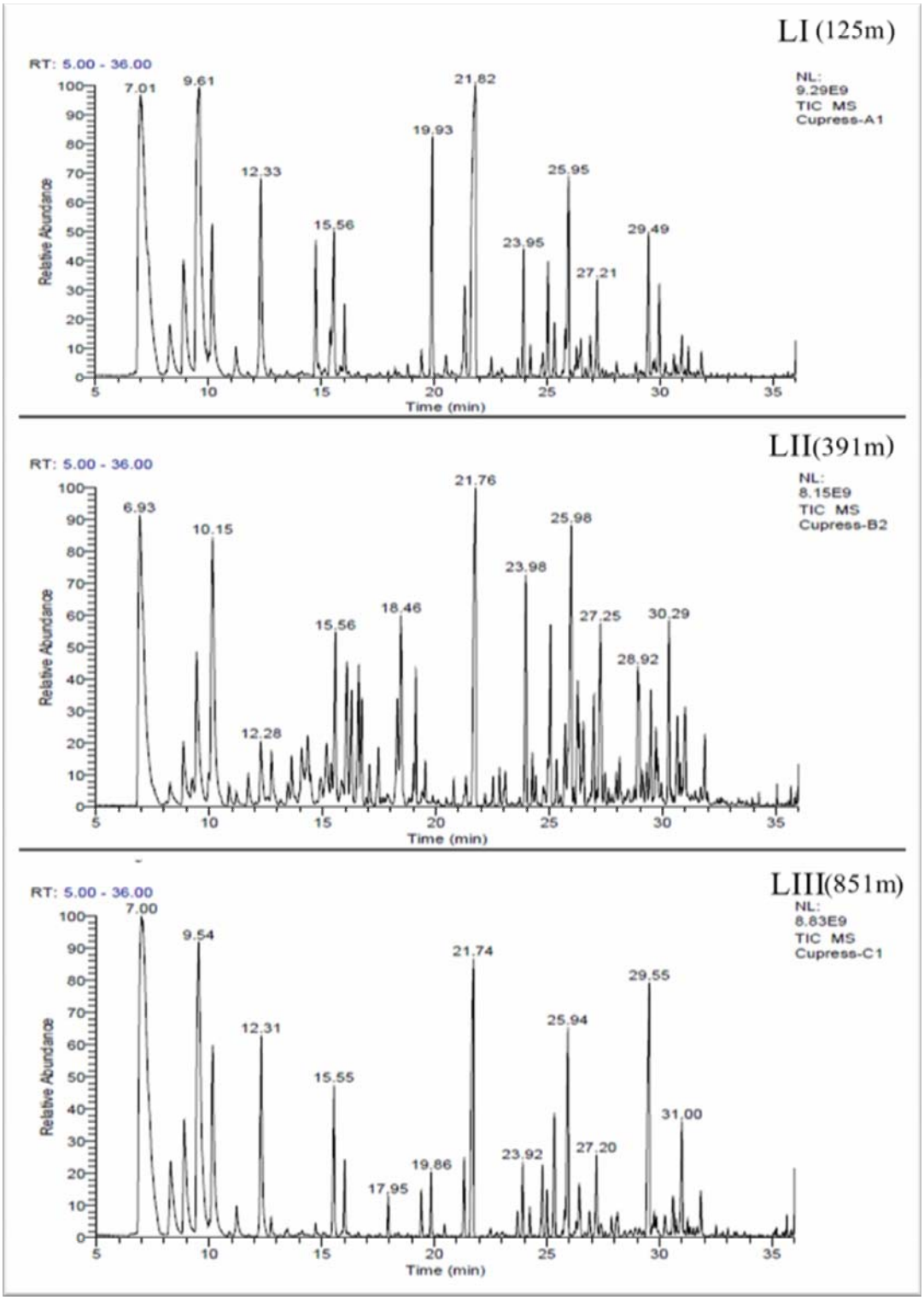

Figure 2. GC/MS chromatograms of essential oils of *C. sempervirens* leaves at different altitudes in AL-Jabal AL-Akhdar region

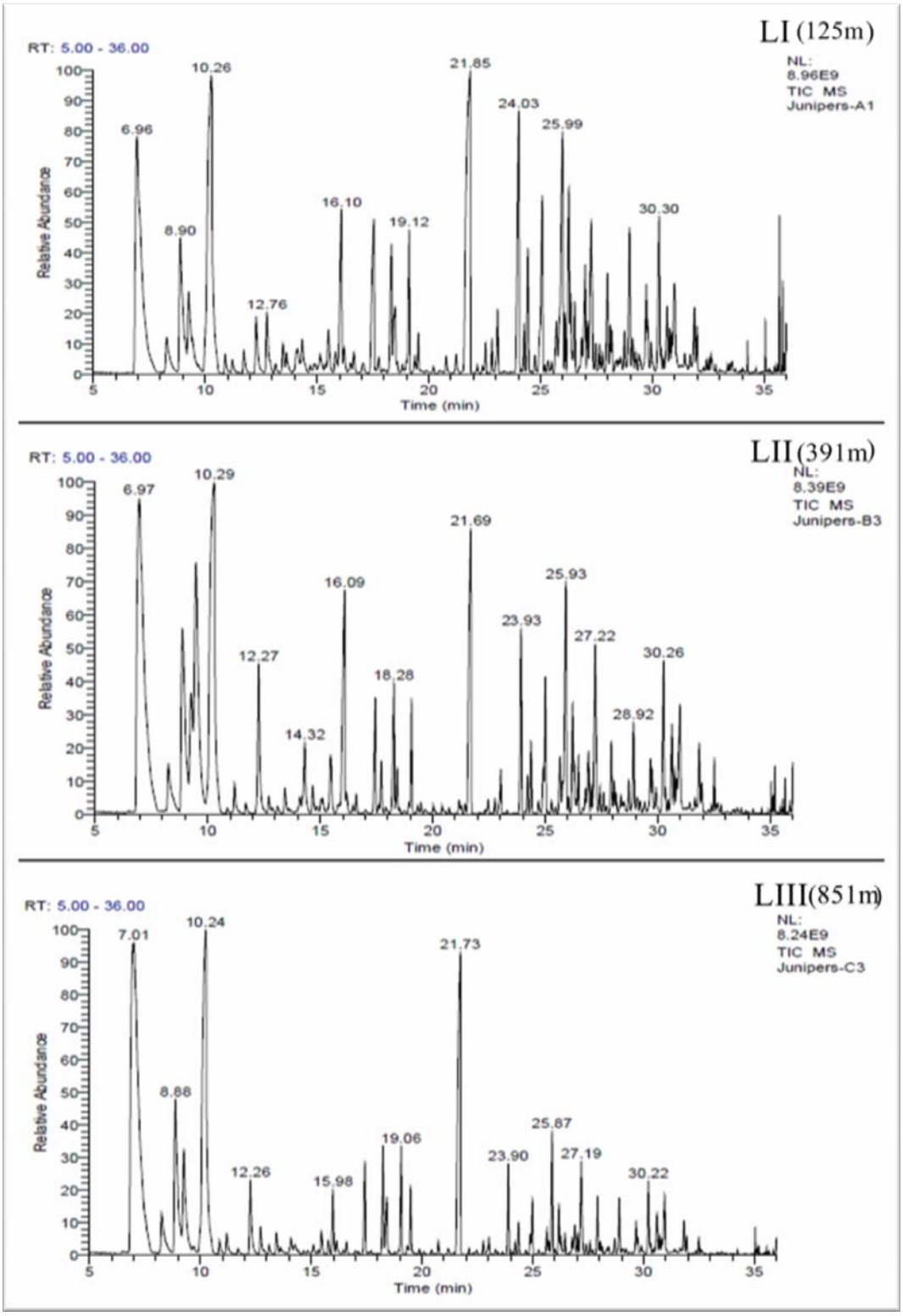

Figure 3. GC/MS chromatograms of essential oils of *J. phoenicea* leaves at different altitudes in AL-Jabal AL-Akhdar region

Table 3. Components of the essential oils from scale leaves of *C. sempervirens* at different altitudes in AL-Jabal AL-Akhdar region

Compound Name	Altitude (m)					
	Level I (125m)		Level II (391m)		Level III (851m)	
	RT(min)	(%)	RT(min)	(%)	RT(min)	(%)
α-pinene	6.98	15.65	6.97	17.69	6.97	21.03
α-myrcene	8.91	4.52	8.86	1.66	8.90	4.08
3-carene	-	-	9.53	11.93	9.54	12.41
Limonene	10.17	3.93	10.6	5.48	10.16	5.59
Sylvestrene	-	-	10.14	3.29	-	-
γ-terpinene)	11.22	0.46	11.22	0.61	11.20	0.57
Terpinolene	12.30	1.81	12.32	5.49	12.32	5.47
Linalool	12.77	1.22	12.75	1.26	12.75	1.78
terpinen-4-ol	15.90	20.82	15.56	3.13	15.55	2.93
α-terpineol	8.91	4.52	8.86	1.66	8.90	4.08
α-terpineol	16.30	7.49	16.05	2.06	16.00	0.91
2-isopropyl-4-methylanisole	-	-	-	-	17.95	0.63
bornyl acetate	19.43	0.48	19.43	0.60	19.41	0.36
α-terpinyl acetate)	21.79	8.78	21.75	7.91	21.70	7.09
Cedrene	-	-	-	-	23.68	0.67
Caryophyllene	23.95	1.40	23.97	3.47	23.92	1.30
Cubebene	24.26	0.68	24.25	0.47	24.24	0.81
α-cubebene	24.80	0.49	24.79	0.35	24.78	0.27
α-caryophyllene	25.03	1.01	25.05	2.48	25.00	1.03
epi-bicyclosesquiphellandrene	25.34	1.24	26.26	1.82	25.34	2.15
germacrene D)	25.95	3.90	25.98	5.87	25.94	4.06
α-muurolene	27.24	2.32	27.21	1.72	27.21	2.67
2-(4α,8-Dimethyl-1,2,3,4,4α,5,6,7-octahydro-naphthalen-2-yl)-prop-2-en-1-ol	-	-	28.09	0.52	28.07	0.36
Cedrol	29.49	2.43	29.49	1.55	29.54	6.50
humulane-1,6-dien-3-ol	29.97	1.47	29.99	1.54	29.95	0.47
Cubenol	30.26	0.53	30.29	2.77	30.24	0.43
Cadino	-	-	-	-	30.61	1.13
tau.-muurolol	30.66	1.40	30.63	0.73	30.61	0.76
α-cadinol	30.99	0.90	31.00	2.02	31.01	3.01
α-muurolene)	36.29	1.42	36.29	2.27	36.30	3.00
manoyl oxide	-	-	36.28	0.98	36.29	2.56
Dehydroabietane	-	-	-	-	36.83	1.31
Cembrene	37.06	0.59	37.07	1.57	37.07	2.39
Totarol	-	-	38.65	2.13	38.64	1.75
TOTAL		84.94%		93.37%		99.48%

The EOs of *P. halepensis* showed good inhibitory effects as related to their oxygenated monoterpenes, such as linalool and terpinene-4-ol. It can be noted that an increase in activity of EOs obtained from the trees growing at altitude I (MIC lower) than those growing at the other altitudes. The previous observation might be attributed to the effect of presence of the monoterpene hydrocarbon and oxygenated monoterpenes (α-pinene, myrtenol, α-caryophyllene as well as other compounds) in the EOs which were high in altitude I among three altitudes.

Interestingly, the results indicated that *J. phoenicea* EOs showed maximum antibacterial activity with lowest MIC values of 680-520 mg/L against all tested bacterial strains.

However, it is difficult to attribute the antibacterial activities of a complex mixture to a single or particular constituent (Bekkali, *et al.* 2008). Many literatures reported that minor components have a critical part to play in antibacterial activity, possibly by causing a synergistic effect between other components (Bekhechi *et al.,* 2008; Ennajar *et al.,* 2009; Mazari1 *et al.,* 2010 and Fekih, *et al.,* 2014).

Table 4. Components of the essential oils from scale leaves of *J. phoenecea* at different altitudes in AL-Jabal AL-Akhdar region

Compound Name	Altitude (m)					
	Level I (125m)		Level II (391m)		Level III (851m)	
	RT(min)	(%)	RT(min)	(%)	RT(min)	(%)
α-pinene	6.98	26.25	6.93	20.44	6.94	24.12
α-myrcene	8.87	3.81	8.88	6.67	8.89	7.88
α-thujene	9.24	1.69	9.25	3.70	9.26	3.57
γ-terpinene	11.21	0.75	10.23	16.86	10.23	19.39
Terpinolene	12.28	1.23	12.25	1.74	12.26	2.09
α-terpineol	16.00	0.97	15.98	1.91	15.99	1.98
Linalool	17.55	5.13	17.45	1.83	12.72	0.43
Piperitone	18.35	4.46	18.23	1.14	18.21	0.92
isopulegyl acetate	19.13	4.49	19.06	2.58	19.04	1.09
α-terpinyl acetate	21.85	12.06	21.73	12.43	21.73	12.14
Caryophyllene	23.98	5.19	23.93	3.42	23.89	1.38
γ-elemene	24.38	0.86	24.36	0.55	24.36	0.96
α-cubeben	-	-	24.90	0.61	24.89	0.35
germacreneD	25.94	5.02	25.92	4.62	25.68	2.08
cubebene)	26.26	2.81	26.21	0.74	26.19	1.04
α-cadinene	27.26	1.08	27.22	3.08	27.18	2.18
Elemol	27.96	1.36	27.94	1.53	27.92	1.06
caryophyllene oxide	28.93	1.18	28.92	1.15	28.90	0.58
humulene epoxide(II)	29.72	1.21	29.67	0.69	29.66	0.59
tricyclo[5.2.2.0(1,6)]undecan-3-ol, 2-methylene-6,8,8-trimethyl-	29.79	0.53	-	-	-	-
Cubenol	30.60	0.27	30.25	2.25	30.22	1.39
tau.-muurolol	-	-	30.62	1.23	30.60	0.86
Cedrol	30.95	0.63	-	-	-	-
α-cadinol	30.96	1.42	30.98	1.91	30.95	1.40
Carotol	36.80	2.44	-	-	31.94	0.41
Totarol	38.62	1.08	38.63	0.88	38.63	1.24
TOTAL		85.92%		91.08%		89.13%

Table 5. Antibacterial activity of essential oils isolated from *P. halepensis, J. phoenicea* and *C. sempervirens* trees against *A. tumefaciens*, *E. carotovora*, *C. fascians* and *P. solanacearum*

Tree species	Altitude	MIC (mg/L)			
		A. Tumefaciens	***E. carotovora***	***C. Fascians***	***P. solanacearum***
P. halepensis	I	680	485	500	390
	II	710	465	875	580
	III	755	875	945	585
C. sempervirens	I	640	895	810	410
	II	530	585	405	390
	III	750	950	760	650
J. phoenicea	I	680	580	520	450
	II	955	800	960	810
	III	940	670	735	650

Moreover, the antibacterial activity of the EO of *J. phoenicea* could be associated with major constituents such as α-pinene, δ-3-carene, α-terpineol, β-myrcene, limonene, γ -terpinene, and α-amorphene. These components have been reported to display antibacterial effects (Ennajar, *et al.,* 2009). In addition, the results of antibacterial activity of EOs isolated from *C. sempervirens* showed that the oils inhibited the growth of bacterial strains with lower MIC values from 390 to 585 mg/L for all bacteria depended on susceptibility of the tested bacteria. However, the MIC values were highest than those reported in the other studies, which showed wider inhibition zones at very low concentrations (Toroglu, 2007 and Ben Nouri, *et al,* 2015).

CONCLUSION

In this study, we noticed a significant difference in yields and chemical composition of leaves essential oils of *P. halepensis*, *C. sempervirens* and *J. phoenicea*, which existed naturally at different altitudes in Al-Jabel Al-Akhdar area, Libya. Although the main components of the all oils are common, their percentages are different based on the tree species and altitude. The results of antibacterial activities for the obtained essential oils showed distinct influences according to tree location for the same species. The MIC value of essential oils isolated from the tested tree species against the bacteria used in this study showed antibacterial potentials against Gram-positive as well as Gram-negative bacteria. The variation in the chemical composition of essential oils might be responsible for the different antibacterial activities. In conclusion, considerable qualitative and quantitative differences in yields, oil composition and antibacterial activities were detected in the studied tree species at different altitudes. These differences resulted from an adaptive process in the tested tree species to particular ecological conditions at the different altitudes.

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الملخص العربي

التركيب الكيميائي والنشاط المضاد للبكتيريا للزيوت الطيارة المعزولة من أوراق أنواع مختلفة من الأشجار الخشبية النامية في منطقة الجبل الأخضر بليبيا

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يهدف هذا البحث إلى دراسة الاختلافات الموجودة في التركيب الكيميائي للزيوت الطيارة المستخلصة من أوراق أشجار الصنوبر الحلبي والسرو الإيطالي والعرعر الفينيقي والموجودة على إرتفاعات مختلفة عن مستوى سطح البحر بمنطقة الجبل الأخضر بدولة ليبيا. وكذلك فإن تلك الدراسة تتناول تعريف المركبات المكونة لتلك الزيوت في أنواع الأشجار تحت الدراسة وايضا التعرف على تأثيرها المضاد للبكتريا الممرضة للنباتات وذلك لمعرفة مدى الإمكانية في استخدامها كبديل طبيعي مضاد لتلك البكتريا بدلا من استخدام المبيدات الصناعية المخلقة.

وقد أظهرت النتائج اختلافات معنوية في محتوى الأوراق الخاصة بكل نوع من الأشجار وكذلك داخل النوع الواحد النامي على أرتفاعات مختلفة حيث كان أعلى متوسط لمحصول الزيت في أوراق أشجار الصنوبر الحلبي وهو٠,٤٧٧% في حين كان أقل متوسط لمحصول الزيت بأوراق أشجار العرعر الفينيقي ٠,١٣٠%. وقد أظهرت نتائج التحليل الكروماتوغرافي باستخدام GC، GC/MS أن أوراق أشجار الصنوبر الحلبي تحتوت على ٣٥ مركب من الأنواع للزيوت الطيارة وهي تمثل ٨٩,٩٢%، ٩١,٥٦%، ٨٦,٤٤% من إجمالي المركبات الموجودة بالزيوت الطيارة المستخلصة من تلك الأشجار عند مستويات الارتفاع (I, II, III) على الترتب. وعند إجراء عملية التعرف على تلك المركبات فقد وجد أن أوراق أشجار الصنوبر الحلبي تحتوي بشكل أساسي على مركبات مثل β-Pinene, α-caryophllene , -α-terpineol, α-pinene بنسب مختلفة في النوع الواحد والنامي على إرتفاعات مختلفة مما يدلل على تأثير العوامل البيئية على نوعية وكمية محصول الزيت والمستخلص من ذلك النوع وتلك العوامل مرتبطة ارتباطا مباشرا بالإرتفاع عن سطح البحر.

وبالنسبة للسرو الايطالي فقد تم تعريف ٣٣ مركب من الزيوت الطيارة أيضا شكلت مجموعة من المركبات التركيب الكيمائي لذلك الزيت وكان من أبرز تلك المركبات terpinen-4-o1, α- terpiny acetate, α-pinene واختلفت نسب تلك المركبات وغيرها في أوراق أشجار السرو الايطالي باختلاف مستوى الارتفاع عن سطح البحر مما يدلل مرة أخرى على التأثيرات المهمة للعوامل البيئية على تركيب ونوعية الزيوت الطيارة في ذلك النوع الشجري.

وبالنسبة للعرعر الفينيقي فقد وجد أن هناك ٢٦ مركب من الزيوت الطيارة تشكل معظم المركبات في الزيت المستخلص من أوراق تلك الأشجار كان من المركبات الأساسية الموجودة في ذلك النوع مركب α-pinene، α-myrcene ، γ-terpinene، α-terpinyl acetate.

أما فيما يتعلق بالتأثير المضاد للبكتريا فقد لوحظ أن الزيوت الطيارة المستخلصة من أوراق أشجار الصنوبر

الحلبي عند مستوى الارتفاع I كان الأعلى في التأثير على أنواع البكتريا *Agrobacterium tumefaciens, Erwinia carotovora, Corynebacterium fascians* and *Pseudomonas solanacearum* بينما في حالة الزيوت الطيارة المستخلصة من أوراق السرو الإيطالي فكان أعلى تأثير مضاد للبكتريا مع الزيوت الطيارة المستخلصة عند مستوى II.

وعموما فإن نتائج هذا البحث تدلل على أهمية الظروف البيئية وتأثيرها على كمية ونوعية الزيوت الطيارة المتحصل عليها من أوراق الأشجار تحت الدراسة مما ينعكس على تأثيرها المضاد ضد البكتريا الممرضة وتتيح نتائج هذا البحث الفرصة لاختيار الزيوت المناسبة للاستخدامات المختلفة سواء من الناحية الدوائية أوالاستخدام في صناعة العطور أوصناعة حفظ الغذية حيث أن تلك الدراسة قد وفرت قاعدة بيانات خاصة بمركبات الزيوت الطيارة في أوراق الأشجار تحت الدراسة والناميه في منطقة الجبل الاخضر بدولة ليبيا.