Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2014, 6(10):57-63

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Seasonal chemical compositions and antifungal activities of Tunisian *Lupinus pilosus* Murr. volatiles

Hamdi Djemni[a], Guido Flamini[b]*, Mohamed Gorcii[c], Rachid Chemli[d] and Zine Mighri[a]

[a]*Research Unit 13 ES 63, Applied Chemical- Environment, Faculty of Sciences of Monastir, Monastir, Tunisia*
[b]*Dipartimento di Farmacia, Via Bonanno, Pisa, Italy*
[c]*Laboratoire de Microbiologie, CHU Fattouma BOURGUIBA, Avenue Farhat Hached, Monastir, Tunisie*
[d]*Laboratoire de Pharmacognosie, Faculté de Pharmacie de Monastir, Avenue Avicenne, Monastir, Tunisie*

ABSTRACT

The chemical composition and antifungal activities of the volatile fractions from leaves, roots and flowers of Lupinus pilosus Murr. harvested in winter and spring were studied. The yields of volatiles ranged from 0.01 to 0.04%. Those in spring were higher than in winter. The GC and GC-MS analysis permitted us to identify 83 compounds. The harvest season and the studied organ affected qualitatively and quantitatively the percentage of the different constituents. Spathulenol (1.0 to 8.7%), globulol (2.3 to 8.7%), α -bisabolol (7.0 to 8.8%) and nonanal (2.8 to 16.3%) were the major compounds of the studied volatile fractions. Non- terpene derivatives (14.3 to 72.5%) and oxygenated sesquiterpenes (6.4 to 33.4%) were the most represented chemical classes. The antifungal properties depended on the studied organ and the harvest season. The fraction obtained from roots harvested in winter showed moderate antifungal activity against C. glabrata and C. krusei (ΦIZ= 10 mm).

Key words: *Lupinus pilosus* Murr ; chemical composition ; volatile fractions ; seasonal variation ; antifungal activity

---

INTRODUCTION

Lupinus is a large genus in the legumes family (Fabaceae). The number of species in this genus is not well defined and it is estimated to be greater than 1000 [1]. This genus is grouped in species "Old World" growing in the Mediterranean region and North Africa and the species “new world” growing in America. Species "Old World" are represented by only 12 annual species with chromosome numbers ranging from 2n = 32 to 42. These species are divided into two distinct groups: species with smooth seeds and those with rough seeds. *Lupinus pilosus* Murr. belongs to the latter group [2, 3, 4].

L. pilosus Murr. = *L. varius* Batt. et Trab = *L. hispanicus* = *L. microanthus* = *L. hirsutus* (Fabaceae) grow wild in TUNISIA in sandy uncultivated and cultivated soils [5]. However, most genotypes of *L. pilosus* grow naturally on calcareous soils [6]. This tolerance appears to be related to the ability of these plants to exclude bicarbonate or prevent its transport to the leaves [7].

This species is used by farmers to enrich the soil with nitrogen [8]. The feed value of this species is noted by Lemordant [9]. The seeds of *L. pilosus*, recognized as a good source of glucomannan, help to normalize blood glucose levels and relieve the stress of pancreas [10]. *Lupinus pilosus* seeds are also used for liver disorders, hemorrhoids and eczema [11, 12]. However, the varieties that are rich in alkaloids are toxic and bitter [5].

Previous studies on this species have shown the presence of several quinolizidines alkaloids, such as (−)-13β-hydroxymultiflorine and (−)-13α-hydroxymultiflorine, among the others [13, 14]. The antioxidant activities of the

aerial parts and roots essential oils of *Lupinus pilosus* have been previously evaluated by our team during different phases of development [15].

The seasonal variations in chemical composition and antifungal activity of the volatile fractions of *Lupinus pilosus* Murr. have never been previously published. The compositions of the volatile fraction were analyzed by gas chromatography coupled with mass spectrometry (GC-MS). In this research, we studied the chemical compositions of volatiles and their antifungal activities from leaves, roots and flowers of *Lupinus pilosus* growing wild in Tunisia and collected in two different seasons.

EXPERIMENTAL SECTION

2.1. Equipment

2.1.1. Plant material

Lupinus pilosus Murr. ssp. digitatus (Forsk.) Mayor has been identified according to the flora of TUNISIA [5] by Pr. Fethia HARZALLAH SKHIRI. A reference specimen (H10) was deposited at the Faculty of Sciences of Monastir, TUNISIA. Wild plants were harvested in winter (December 2012) and in spring (April 2013) from the governorate of Monastir (latitude 35 ° 46'0" N, longitude 10 ° 59'0" E) in the coastal region of TUNISIA, with a sub- humid climate. The average values of maximum and minimum temperatures (° C) for December 2012 and April 2013 were respectively 18.8 and 9.7 (average 14.25) and 22.9 and 14.7 (average 18.8). The averages of humidity and rainfall for December 2012 and April 2013 were respectively 62% and 1.4 mm; 69 mm and 23.9%. The fresh plants, previously separated in leaves, roots and flowers, were dried in the shade and at room temperature.

2.1.2. Biological material

Tests were performed on following pathogenic fungi: *Candida albicans* (ATCC 90028), *Candida glabrata* (ATCC 90030), *Candida krusei* (ATCC 6258) and *Candida parapsilosis* (ATCC 22019).

These strains are maintained by subculture and stored in the laboratory of Parasitology-Mycology at the University Hospital Fattouma Bourguiba Monastir, TUNISIA.

2.2. Methods

2.2.1. Hydrodistillation

Each sample was ground and subjected to hydrodistillation for 4 hours. The volatile compounds were extracted with hexane.

After drying the extract over anhydrous sodium sulfate, the solvent was evaporated and the volatiles were kept in a refrigerator until study.

The yields of volatile fractions were calculated as follows: FV / PM × 100 with VF = mass of the volatile fraction and PM = mass of dry plant material.

2.2.2. GC-FID and GC-MS analysis

The GC-FID analyses were accomplished with a HP-5890 Series II instrument equipped with HP-WAX and HP-5 capillary columns (both 30 m x 0.25 mm, 0.25 μm film thickness), working with the following temperature program: 60°C for 10 min, ramp of 5°C/min up to 220°C; injector and detector temperatures 250°C; carrier gas helium (2 ml/min); detector dual FID; split ratio 1:30; injection of 0.5 μl). The identification of the components was performed, for both columns, by comparison of their retention times with those of pure authentic samples and by means of their linear retention indices (l.r.i.) relative to the series of *n*-hydrocarbons.

GC-EIMS 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: injector and transfer line temperatures 220 and 240°C respectively; oven temperature programmed from 60°C 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. Identification of the constituents 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 [16, 17] and home-made library mass spectra built up from pure substances and components of known oils and MS literature data [18, 19, 20, 21, 22]. Moreover, the molecular weights of all the identified substances were confirmed by GC-CIMS, using MeOH as CI ionizing gas.

2.2.3. Antifungal activity of *Lupinus pilosus* Murr .volatile fractions

The diffusion method on solid medium (Sabouraud Chloamphenicol) was used. The fungal inoculums were prepared by sterile physiological water NaCl 9 ‰, from a culture of 24 hours. The inoculum was adjusted to a value of 1 McFarland using a densitometer (Bio Merieux). In a second step, we flooded the surface of Sabouraud medium with 2-3 ml of fungal inoculum. Then, the excess was removed using a sterile Pasteur pipette. The dishes were dried by incubation in the oven for 15 minutes at 37 ° C. Disks of n° 3-sterile Whatman paper 6 mm in diameter were used. Each one was impregnated with 20 µL of test samples, which corresponds to 1.2 mg/disc of tested volatiles. Then, the impregnated discs were placed on the surface of Sabouraud medium in the presence of control disks impregnated only by solubilizing solvent extract and a positive control disc (Fluconazole).

After incubating Petri dishes at 37 °C for 18 h, we proceed to measure the diameters of the clear inhibition zones (ϕIZ) surrounding the discs [23].

RESULTS AND DISCUSSION

3.1. Volatiles yields

The yields of volatile fractions obtained from the *Lupinus pilosus* Murr. leaves, roots and flowers harvested in winter and spring are summarized in Table 1. The yields depended on the studied organ and the harvest season. The yields ranged from 0.01 to 0.04%. This is in agreement with the researches of Clara Grosso et al. who found that the yields of aerial parts essential oils of nine populations of the legume *Pterospartum tridentatum* were below to 0.05% [24]. Our study showed, also, that the yields of volatile fractions of leaves and roots harvested in spring (flowering stage) are higher than those harvested in winter. This is in harmony with the results found by Merghache et al. who showed that the yield of the aerial parts essential oil of *Ruta chalepensis* harvested in April (flowering stage) is greater than that of aerial parts collected during other months [25]. Li et al. studied the yields of leaves essential oils of *Cinnamomum cassia* in different stages of development. These researchers found a correlation between the yields of essential oils and the cell oils density of studied leaves. Their results showed that two years old leaves have the highest density of oil cells, which coincides with the most important oil yield (2.12%) [26]. During the development of the plant, the biosynthesis of secondary metabolites can be affected by temperature, moisture, soil nature, wind, harvest time and altitude [27].

Our results showed also that the flowers volatile fraction yield was the highest (0.04%).

Table 1. Seasonal variation in yields of leaves, roots and flowers volatile fractions of *Lupinus pilosus* Murr.

	Leaves (%)		Roots (%)		Flowers (%)
	Winter	Spring	Winter	Spring	Spring
Yield	0.01	0.03	0.02	0.03	0.04

3.2. GC-FID and GC-MS analyses

3.2.1. Constituents of leaves, roots and flowers volatiles fractions of *Lupinus pilosus* Murr .

The constituents of the leaves, roots and flowers volatile fractions of *Lupinus pilosus* Murr. collected during the winter and spring seasons are reported in Table 2.

The volatile fractions of leaves collected during the winter and spring are composed by 33 and 41 chemicals, respectively. The volatile fractions of roots harvested in winter and spring contained 43 and 35 compounds, respectively. Furthermore, 40 compounds were identified in the volatile fraction of flowers collected in spring.

The volatile fractions of roots harvested in both seasons and that of leaves harvested in spring contained sesquiterpene hydrocarbons as major constituents. Non-terpene derivatives represented the major chemical class of the volatile fraction of leaves harvested in winter and that of flowers (see Table 3).

Among the 83 identified compounds, only a few constituents are in common to all the five studied volatile fractions: nonanal, β-caryophyllene, viridiflorene, spathulenol, viridiflorol and hexahydrofarnesyl acetone.

The constituents of the leaves and roots varied according to the season of harvest. However, during the same season, the chemical compositions of the volatile fractions varied depending on the studied organ.

As shown in Table 2, nonanal (12.2%) and spathulenol (8.7 %) were the major volatile compounds of leaves harvested during winter and spring, respectively. Nonanal (16.3 %) was also the main volatile constituent of the flowers harvested in spring. Globulol (8.7 %) and α -bisabolol (8.8%) were the major volatile compounds of roots harvested in the winter and spring seasons, respectively.

Table 2. Chemical composition (%) of leaves, roots and flowers volatile fractions of *Lupinus pilosus* Murr. during the winter and spring seasons

No	Constituents	l.r.i. HP-5	l.r.i. HP-WAX	Leaves (%)		Roots (%)		Flowers(%)
				Winter	Spring	Winter	Spring	Spring
1	Furfural	834	1438					0.4
2	*(E)*-2-hexenal	856	1209	1.7	0.8	0.7		0.6
3	2-heptanone	891	1186			0.6		
4	Heptanal	901	1179	6.2	1.8			4.3
5	Benzaldehyde	963	1493	0.6		0.7		1.4
6	1-octen-3-ol	980	1390			0.9		
7	6-methyl-5-hepten-2-one	987	1340					0.4
8	2-pentyl furan	993	1241	1.8		5.4	1.6	1.5
9	*n*-decane	1000	1000					0.9
10	Octanal	1002	1278	4.9	1.9			4.7
11	*trans*-2-(2-pentenyl) furan	1005	-			1.3		
12	Limonene	1032	1198			0.6	5.1	
13	1,8-cineole	1034	1209	0.6		1.4		0.4
14	Phenylacetaldehyde	1045	1618	1.3	1.1			
15	*(E)*-2-octenal	1063	1343	0.6		1.2		
16	1-octanol	1071	1560	0.7	0.6	1.3		1.2
17	*(E,E)*-3,5-octadien-2-one	1095	1521			0.9		
18	Linalool	1101	1560		0.7	3.4		
19	Nonanal	1104	1382	12.2	5.4	3.9	2.8	16.3
20	*cis-p*-menth-2-en-1-ol	1123	1642			0.6		
21	*trans-p*-menth-2-en-1-ol	1142	1649			0.6		
22	*(E)*-2-nonenal	1162	1444			0.9		0.5
23	2-methoxy-3-(1-methylpropyl) pyrazine	1176	1511			1.9	0.8	
24	*n*-dodecane	1200	1200				0.7	1.5
25	Safranal	1201	1598	2.1	1.4			
26	Decanal	1206	1481	1.1		1.1	0.7	0.9
27	β-cyclocitral	1222	1636	2.0	1.5			
28	cumin aldehyde	1241	1765	0.8	0.6			
29	Carvone	1244	1742	1.3	1.2	3.7		
30	*(E)*-2-decenal	1263	1592					0.6
31	acide nonanoique	1276	2204			0.8		
32	*(E)*-anethole	1285	1816		1.8	0.9	2.9	
33	Carvacrol	1301	2219			2.6		
34	Undecanal	1307	1649	1.2				2.0
35	*p*-vinylguaiacol	1310	2179	10.7	3.7			2.1
36	*(E,E)*-2,4-decadienal	1316	1706			1.0	1.0	
37	α- terpinyl acetate	1351	1695		1.6	5.2	1.0	
38	*(E)*-β-damascenone	1383	1593	3.1	3.3		2.3	
39	*n*-tetradecane	1400	1400	0.8			1.7	3.1
40	α-dihydroionone	1411	-		1.4			
41	β-caryophyllene	1419	1598	5.2	2.8	2.1	3.0	0.4
42	*trans*-α-bergamotene	1437	1782		0.6		0.9	
43	Aromadendrene	1440	1649		0.6	0.5		
44	*(E)*-geranylacetone	1454	1842	1.3	1.5		1.6	
45	Alloaromadendrene	1461	1622		0.7	0.6	0.7	
46	2-methyltetradecane	1465	-	0.8	1.2		1.3	3.9
47	β-chamigrene	1472	1755		5.2		7.1	
48	γ-himachalene	1480	1698		2.3		2.7	
49	*(E)*-β-ionone	1487	1930	7.8	4.7		3.8	1.3
50	Valencene	1493	1717		1.3		1.8	
51	Viridiflorene	1495	1715	1.0	0.6	2.3	0.8	0.5
52	β-bisabolene	1509	1708		1.8		3.2	
53	*trans*-γ-cadinene	1514	1750			0.5		
54	δ-cadinene	1524	1732			1.7	0.8	
55	*(E)*-nerolidol	1564	2006		0.9		1.1	
56	Ledol	1567	2008	0.6		0.7		
57	Spathulenol	1577	2136	1.1	8.7	5.3	3.3	1.0
58	Caryophyllene oxide	1582	1966		7.3		7.3	
59	Globulol	1584	2055	7.1		8.7		2.3
60	Viridiflorol	1591	2104	2.0	0.5	5.4	0.7	1.0
61	Guaiol	1597	2101	1.1		3.2		0.8
62	*n*-hexadecane	1600	1600				2.1	3.5
63	Tetradecanal	1615	1915					0.7
64	1,10-di-*epi*-cubenol	1616	2045			0.5		
65	*epi*-10-γ-eudesmol	1622	2111	0.8		3.3	0.8	
66	caryophylla-4(14),8(15)-dien-5-ol	1637	2292	1.0	1.8		3.7	
67	T-cadinol	1641	2162		0.7	2.9		0.9
68	β-eudesmol	1650	2240			0.6		
69	α-cadinol	1654	2188		0.8	2.8		0.4
70	α-bisabolol	1684	2237		7.0		8.8	

71	*epi*-α-bisabolol	1686	2252		2.6		5.2	
72	*n*-heptadecane	1700	1700					1.3
73	Pentadecanal	1716	2013		0.7	0.7	1.3	1.2
74	*n*-octadecane	1800	1800				1.1	2.6
75	Hexadecanal	1814	2112					1.1
76	Hexahydrofarnesylacetone	1843	2118	5.9	8.2	0.8	7.5	11.8
77	*n*-nonadecane	1900	1900					2.7
78	Farnesylacetone	1920	2359		1.4			
79	methyl hexadecanoate	1925	2205	1.5	0.6			0.7
80	Hexadecanoic acide	1961	2933			7.1		
81	ethyl hexadecanoate	1994	2251					6.7
82	*n*-eicosane	2000	2000					3.7
83	Octadecanal	2014	2402					2.0
Total				90.9	93.3	91.3	91.2	93.3

The nitrogen derivative, 2-methoxy-3-(1- methylpropyl) pyrazine, characterized only the volatile constituents of the roots.

3.2.2. Seasonal variation

As shown in Table 2, the chemical composition of the volatile fractions varied significantly depending on the season. In the leaves volatile fractions, the non- terpene derivatives subjected to major variations were *p*-vinyl guaiacol, nonanal and heptanal (10.7%, 12.2% and 6.2% in winter and 3.7%, 5.4% and 1.8% in the spring, respectively). For oxygenated sesquiterpenes, this trend was observed for spathulenol and caryophyllene (8.7 % and 7.3 % in spring and 1.1 % and 0% in winter, respectively). In case of sesquiterpene hydrocarbons, β-chamigrene, was the compound interested by this behavior (5.2 % in spring and 0% in winter). The percentages of non-terpene derivatives were higher in winter (46.1%) than in spring (17.8%). On the contrary, oxygenated sesquiterpenes were higher in spring (30.3%) than in winter (13.7%) (Table 3).

In the volatile fractions of roots, the main variability among non-terpene derivatives was noted for hexadecanoic acid and 2- pentyl furan (7.1% and 5.4% in winter and 0% and 1.6% in spring, respectively). Among oxygenated sesquiterpenes, this was observed for α-bisabolol, globulol, caryophyllene oxide and viridiflorol (0%, 8.7%, 0% and 5.4% in winter and 8.8%, 0%, 7.3% and 0.7% in spring, respectively). The percentages of the apocarotenoid hexahydrofarnesylacetone also varied significantly, passing from 7.5% in spring to 0.8% in winter. The percentages of non-terpene derivatives and oxygenated monoterpenes were higher in winter (28.5%, 17.5%) than in spring (14.3%, 1.0%), while those of sesquiterpene hydrocarbons were higher in spring (21.0%) than in winter (7.7%) (Table 3).

Table 3. Constituents of leaves, roots and flowers volatile fractions of *Lupinus pilosus* Murr. harvested during the winter and spring seasons

	Leaves **(%)**		Roots **(%)**		Flowers **(%)**
	Winter	Spring	Winter	Spring	Spring
Monoterpene hydrocarbons	0.0	0.0	0.6	5.1	0.0
Oxygenated monoterpenes	2.7	4.1	17.5	1.0	0.4
Sesquiterpene hydrocarbons	6.2	15.9	7.7	21.0	0.9
Oxygenated sesquiterpenes	13.7	30.3	33.4	30.9	6.4
Apocarotenoids	22.2	23.4	0.8	15.2	13.1
Phenylpropanoids	0.0	1.8	0.9	2.9	0.0
Nitrogen derivatives	0.0	0.0	1.9	0.8	0.0
Non-terpene derivatives	46.1	17.8	28.5	14.3	72.5

3.3. Seasonal variations of antifungal activities of leaves, roots and flowers volatile fractions of *Lupinus pilosus* Murr.

Seasonal variations of antifungal activities of leaves, roots and flowers volatile fractions of *Lupinus pilosus* Murr. are shown in Table 4. It can be noted that the antifungal activity was dependent both on the studied organ and the harvest season. The diameters of the inhibition zones (ΦIZ) ranged from 6 to 10 mm. The volatile fraction of roots harvested in winter was the most active especially against *C. krusei* and *C. glabrata* with an inhibition zone diameter of 10 mm. This fraction showed antifungal activity illustrated by ΦIZ=10mm against *C. glabrata* comparable to that of fluconazole (2 $mg.mL^{-1}$) at a concentration of 60 $mg.mL^{-1}$. It is interesting to note that this active fraction is the richest in terpenes (59.2%). Indeed, these compounds include monoterpenes, sesquiterpenes and their oxygenated derivatives, known active antimicrobial agents [28]. It can also be noted that this fraction is particularly rich in potentially antifungal agents such as globulol (8.7 %) [29], linalool (3.4%) [30, 31], T-cadinol (2.9%) and α-cadinol (2.8%) [32], 2-methoxy-3-(1-methylpropyl) pyrazine (1.9%) [33, 34] and 1,8-cineole (1.4%) [35].

Table 4. Seasonal variation of antifungal activities of leaves, roots and flowers volatile fractions of *Lupinus pilosus* Murr. (60 mg.mL^{-1})

	Leaves (mm)		Roots (mm)		Flowers (mm)	Flu[a] (2mg.mL^{-1})
	Winter	Spring	Winter	Spring	Spring	
C. krusei (ATCC 90028)	8 ± 0	7 ± 0	10 ± 0	6.5 ± 0	8 ± 0	28 ± 2
C. glabrata (ATCC 90028)	6 ± 0	6 ± 0	10 ± 0	8 ± 0	6 ± 0	11 ± 0
C.parapsilosis (ATCC 90028)	8.5 ± 0	7 ± 0	6 ± 0	6 ± 0	6 ± 0	28 ± 2
C. albicans (ATCC 90028)	7 ± 0	8.5 ± 0	8 ± 0	6 ± 0	8 ± 0	21 ± 1

The data are displayed with mean standard deviation of two replications after 18h of incubation at 37°C.
[a]Flu: Fluconazole

The mechanism of action of terpenoids is not fully understood, but it is assumed that these lipophilic compounds could damage the cell membrane [36]. Highly lipophilic compounds of essential oils can easily pass through the plasma membrane to induce biological responses [28]. Meroterpenoids isolated from another legume species, *Psoralea glandulosa*, were also endowed with antifungal activity against eight species of yeasts [37].

A study of methanol and ethanol extracts of *Vicia faba* (fabaceae) revealed activity against *Candida albicans* and *Candida maltose*. The yeasts showed more sensitivity to methanolic extracts than ethanolic ones [38].

CONCLUSION

Our results showed that the different percentages of *L. pilosus* constituents, as well as their antifungal activities were function of the collecting season (abiotic factors) and of the different biosynthesis performed by each organ.

Nonanal (12%) and spathulenol (8.7%) were the major volatile constituents of leaves harvested during winter and spring, respectively. On the contrary, globulol (8.7%) and α-bisabolol (8.8%) were the major volatile constituents of roots harvested in winter and spring, respectively. The chemical study showed also that nonanal (16.3%) was the major constituent of the volatile fraction of the flowers.

The seasonal chemical variation can be explained by seasonal changes in temperature, humidity and rainfall, as well as the different stages of plant development and metabolism.

The fraction obtained from roots harvested in winter was endowed with moderately antifungal properties against *C. glabrata* and *C. krusei* (ΦIZ = 10 mm).

REFERENCES

[1] BS Kurlovich, FL Soddard, P Earnshow. Potential and problems of Lupinus polyphllus lindl. Domestication. In "Lupinus for Health and Wealth" Proceedings of the 12th International Lupin Conference, 14-18 Sept. 2008", Palta J.A. and Berger J.B. eds, International Lupin Association, Canterbury, New Zealand, **2008**, 304-307.
[2] B Naganowska; B Wolko; E Sliwinska; Z Kaczmarek. *Ann. Bot.*, **2003**, 92(3), 349-355.
[3] M Wink, F Merino, E Käss. Lupin an ancient crop for the new millennium: Proceedings of the 9th International Lupin Conference Klink/ Muritz, Germany, 20-24 June, E van, M Wink, S Weissmann, P Römer eds, International Lupin Association, Canterbury, New Zealand, **1999**, 278-286.
[4] JS Gladstones. Present situation and potential of Mediterranean/ African Lupinus for crop production, In "proceedings of the third international Lupin Conference, UNIP, La Rochelle, France", **1984**, 18-37.
[5] G Pottier-Alapetite. Flore de la Tunisie ANGIOSPERMES-DICOTYLEDONES Apétales, dialypétales, Imp. Off. Rep. Tunisienne, Tunisia, **1979**, 542 p.
[6] PF White, *Aust. J. Agric. Res.*, **1990**, 41(5), 871-890.
[7] JD Brand; CT Tang; RD Graham, *Plant and Soil*, **2000**, 224(2), 207–215.
[8] V Tackholm. Students flora of Egypt, 2nd edition, Cairo University Press, Cairo, **1974**, 225 p.
[9] E Le Floc'h. Contribution à une étude ethnobotanique de la flore Tunisienne, Imprimerie officielle de la République Tunisienne, **1983**, 402 p.
[10] T Hozumi; M Yoshida; Y Ishida; H Mimoto; J Sawa; K Doi, *Endocrine Journal*, **1995**, 42(2), 187 p.
[11] KHC Baser, G Honda, W Miki. Herb drugs and herbalists in Turkey, Studia Culturae Islamicae. 27, Institute for the Study of Languages and Cultures of Asia and Africa, Tokyo, **1986**, 299 p.
[12] T Baytop. Therapy with medicinal plants in Turkey (past and present), 2nd edition, Nobel Tip Kitabevleri, Istanbul, **1999**, 211 p.
[13] OB Abdel-Halim; AA El-Gammal; H Abdel-Fattah; K Takeya, *Phytochemistry*, **1999**, 51(1), 5-9.
[14] MH Mohamed; HA Hassanean, *Phytochemistry*, **1997**, 46(2), 365-369.
[15] F Bel Haj Khether; MA Mahjoub; AN Helal; Z Mighri, *Tunisian Journal of Medicinal Plants and Natural Products*, **2009**, 2, 50-59.

[16] National Institute of Science and Technology (NIST) 98, Mass spectral library (NIST/EPA/NIH), National Institute of Standards and Technology, Gaithersburg, USA, **1998**.
[17] RP Adams. Identification of essential oil components by gas chromatography/mass spectroscopy, 3 rd edition, Carol Stream: Allured, IL. USA, **1995**, 456 p.
[18] E Stenhagen, S Abrahamsson, FW McLafferty. Registry of Mass spectral data, 1st edition, J. Wiley & Sons, New York, **1974**, 3136 p.
[19] Y Massada. Analysis of Essential Oils by Gas Chromatography and Mass Spectrometry, J. Wiley & Sons, New York, **1976**, 251-255.
[20] W Jennings, T Shibamoto. Qualitative Analysis of Flavor and Fragrance Volatiles by Glass Capillary Chromatography, Academic Press, New York, **1980,** 472 p.
[21] NW Davies, *J. Chromatogr.*, **1990**, 503, 1–24.
[22] AA Swigar, RM Silverstein. Monoterpenes. Aldrich Chem. Comp, Milwaukee, **1981**, 130 p.
[23] KP Lutta; C Bii; AT Akenga; WC Wanjala, *Rec. Nat. Prod.*, **2008**, 2(4), 116-127.
[24] A Clara Grosso; M Costa Monya; L Ganço; L Pereira Ana; G Teixeira; MG Lavado José; A Cristina Figueiredo; G Barroso José; G Pedro Luis, *Food Chemistry*, **2007**, 102(4), 1083-1088.
[25] S Merghache; M Hamza; B Tabti, *Algérie Afrique SCIENCE*, **2009**, 05(1), 67–81.
[26] Y-q Li; D-x Kong; R-s Huang; H-l Liang; C-g Xu; H Wu, *Industrial Crops and Products*, **2013**, 47, 92-101.
[27] ZC Gazim; ACL Amorim; AMC Hovell; CM Rezende; IA Nascimento; GA Ferreira; DAG Cortez, *Molecules*, **2010**, 15(8), 5509-5524.
[28] LK Chao, KF Hua, HY Hsu, SS Cheng, JY Liu, ST Chang, *Journal of Agricultural and Food Chemistry*, **2005**, 53(18), 7274–7278.
[29] J Aleu; JR. Hanson; RH Galán; IG Collado, *Journal of Molecular Catalysis B: Enzymatic*, **2001**, 11(4-6), 329–334.
[30] AI Hussain; F Anwar; STH Sherazi; R Przybylski, *Food Chemistry*, **2008**, 108(3), 986-995.
[31] B Prakash; Singh P; PK Mishra; NK Dubey, *International Journal of Food Microbiology*, **2012**, 153(1-2), 183–191.
[32] CL Ho; PC Liao; EI Wang; YC Su, *Natural Product Communications*, **2011**, 6 (9), 1357–60.
[33] A Bruce, D Stewart, S Verrall, RE Wheatley, *Int. Biodeterior. Biodegradation*, **2003**, 51(2), 101–108.
[34] A Bruce; S Verrall; CA Hackett; RE Wheatley, *Holzforschung*, **2004**, 58(2), 193–198.
[35] R Shukla, P Singh, B Prakash, NK Dubey, *Food Control*, **2012**, 25(1), 27–33.
[36] MM Cowan, *Clinical Microbiology Reviews*, **1999**, 12(4), 564–582.
[37] A Madrid; L Espinoza; C González; M Mellado; J Villena; R Santander; V Silva; I Montenegro, *Journal of Ethnopharmacology*, **2012**, 144(3), 809-811**.**
[38] M de las Mercedes Oliva, M N Gallucci, ME Carezzano, MS Demo, *Fighting Multidrug Resistance with Herbal Extracts, Essential Oils and their Components*, **2013**, 31-43.