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GC/MS Analysis and Antioxidant Activity of the Essential Oil leaves *Thymus numidicus* Poiret. *growing in Mila*

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Abstract

Essential oil components of the leaves of *Thymus numidicus* growing in Mila have been studied by gas chromatography mass spectrometry GC/MS to afford 60 Sixty compounds representing 94.0 % of the total oil and mainly represented by oxygenated monoterpenes,. The main constituents of the essential oil from the leaves were thymol (41.2%), *β*-cymene (12.9%), chlorocresol (11.2%), *β*-linalool (10.7%) and methyl thymol ether (3.3%). The antioxidant activity was evaluated by spectroscopic method, used for that free radical compound (DPPH) and in comparison with vitamin C as a standard. The essential oil showed a moderate activity against free radical compound (DPPH) 17.4% at 1M.

1. Introduction

The genus *Thymus* L., belonging to the Lamiaceae family represented by around 350 species of perennial, aromatic herbs and subshrubs predominantly found in Mediterranean region, Asia, Southern Europe and North Africa [1], and comprised in Algeria over 12 species [2].

Thymus species are considered as medicinal plants due to their pharmacological and biological properties. In native medicine, flowering parts and leaves of *Thymus* species have been extensively used as herbal tea, tonic, carminative, antitussive and antiseptic, as well as for treating colds [1,3,4,5]. Recent studies have shown that, thyme have strong antibacterial, antifungal, antiviral, antiparasitic [6,7,8,9], sedative ,antispasmodic [10,11], antioxidant [8,12,13] and antiaflatoxinogenic [14] activities.

Thymus species as well as many other aromatic plants biosynthesize variable amounts of volatile compounds known as essential oil; therefore, chemical classification of *Thymus* species was based on the main essential oil components and their chemical polymorphism, moreover, numerous chemotypes have been defined, such as carvacrol and thymol, γ-terpineol, thujone, geraniol, linalool and others [15]. Hence the main objectives of this study were:

(i) determine the chemical composition of hydrodistillated oils of the leaves of *Thymus numidicus* Poiret. (End. W. Alg. Tun.) growing in Mila - Algeria by gas chromatography/mass spectrometry (GC/MS).

(ii) Evaluate the antioxidant capacity of the plant essential oils.

2. Material and Methods

2.1 Plant material

The Leaves of *Thymus numidicus* Poiret. were collected at the end of April 2012 (flowering stage) from Grarem wilaya Mila (North Eastern Algeria). The plant was identified by Pr. Zellagui Amar, Oum El Bouaghi University. A voucher specimen was deposited at Laboratory of Biomolecules and Plant Breeding, Life Science and Nature Department under the code number ZA 140.

2.2 Extraction

Essential oils were obtained by hydrodistillation of 100 g of dried leaves using a Clevenger-type apparatus for 3 h. The oil was stored in sealed vials protected from the light at +4°C before analyses. The oil sample was subsequently analyzed by GC-MS.

2.3 Gas chromatography/mass spectrometry (GC/MS)

Analyses were performed with a Varian CP-3800 gas chromatograph equipped with a DB-5 capillary column (30m × 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 0.2 µL (10% n-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 by computer matching against commercial (NIST 98 and ADAMS) and homemade library mass spectra built up from pure substances and components of known oils and MS literature data [16].

2.4 Antioxidant activity

The capacity of essential oil extracted from *Thymus numidicus* Poiret. leaves to reduce the radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) was assessed using the method of Masuda *et al.* [17] modified in the laboratory. 15 µl of the essential oil at different concentrations was added to 1500 µl of a DPPH ethanolic solution. The mixture was shaken vigorously and left standing at room temperature for 05 min in the dark. The absorbance of the resulting solution was then measured at 517 nm. The normal purple color of DPPH will turn into yellow when its singlet electron is paired with a hydrogen atom coming from a potential antioxidant. The scavenging activity of essential oil was evaluated according to the formula:

DPPH scavenging effect (%) = $[(A_0 - A_1)/A_0] \times 100$

Where: A_0 is the absorbance of the control at 05 min, and A_1 is the absorbance of the sample at different times. All samples were analyzed in three replications.

3. Results and Discussion

The composition and percentage of the compounds are summarized in Table 1. They are listed by order of their retention times. The oil yield was 0.8 % (w/w) based on the dried weight which means that the organs are a potential oil source. Sixty compounds were identified in the essential oil, representing 94.0% of the total oil. The essential oils were dominated by a large amount of oxygenated monoterpenes (12.4%), monoterpene hydrocarbons (2.6%) and sesquiterpenes hydrocarbons (2.3%), while the oxygenated sesquiterpenes (0.9%) contents were very low.

The main constituents of the essential oil were found to be, thymol (41.2%), *β*-cymene (12.9%), chlorocresol (11.2%), *β*-linalool (10.7%) and methyl thymol ether (3.3%) and carvacrol (2.8%) and some other compounds were only present in minor amounts. In total, essential oil composition of *Thymus numidicus* Poiret. was

considered as a rich source of oxygenated Monoterpenes

The predominance of phenolic compounds is in agreement with previous results reported from *Thymus numidicus* Poiret. essential oil from Constantine (North-Eastern of Algeria) [18] which was mainly represented with thymol (68.2%), carvacrol (16.9%) and linalool (11.5%). Whereas, *T.numidicus* (poiret) essential oil from Tizi Ouzou (North of Algeria) [19] was mainly represented with thymol (51.0%) followed by carvacrol (9.4%), linalool (3.3%), thymol-methyl-ether (3.2%) and *iso*-caryophyllene (2.7%); in the same of *Thymus numidicus* Poiret. essential oil from Berrahal (area located 500 km East of Algiers) during the flowering stage, which was characterized by thymol (66.31%) as the major component, followed by linalool (8.61%), *P*-cymene (6.20%), γ-terpinene (6.12%) and carvacrol (4.31%) and *Thymus numidicus* Poiret. from Souk aharas (East of Algeria) that the major components were thymol (57.20%), linalool (9.26%), γ-terpinene (9.19%) and *p*-cymene (7.55%) [20].

Table 1. *Composition of the leaves essential oil of Thymus numidicus* Poiret. growing in Mila

Pic	Chemical constituents	T_R	%
1	α-Thujene	4.535	0.2
2	Cyclofenchene	4.683	0.4
3	α -Phellandrene	4.935	0.1
4	α Pinene	5.078	0.1
5	Pentyl vinyl ketone	5.928	0.5
6	β-Myrcene	6.452	0.6
7	β -Pinene	6.760	0.4
8	1,3-Cyclohexadiene, 1-methyl-4-(1-methylethyl)-	7.219	0.3
9	**β -Cymene**	7.334	**12.9**
10	D-Limonene	7.917	0.2
11	c-Terpinen	8.781	0.2
12	Bicyclo[3.1.0]hexan-2-ol, 2-methyl-5-(1-methylethyl)-, (1.alpha.,2.alpha.,5.alpha.)-	8.958	0.7
13	Octanol	9.433	0.1
14	Octyl formate	9.604	0.1
15	1-Nonen-3-ol	9.715	0.1
16	5-Isopropyl-2-methylbicyclo[3.1.0]hexan-2-ol	10.187	0.1
17	**β -Linalool**	10.440	**10.7**
18	α-Campholenal	10.953	0.1
19	cis-Verbenol	12.063	0.1
20	Borneol	12.827	0.1
21	α-Terpieol	13.407	0.6
22	**Thymol methyl ether**	16.069	**3.3**
23	(1R,2R,3S,5R)-(-)-2,3-Pinanediol	16.636	0.2
24	(+)-3-Carene, 2-(acetylmethyl)-	17.662	0.1
25	2-Chlorooctane	18.276	0.1
26	**Thymol**	18.968	**41.2**
27	**Carvacrol**	19.201	**2.8**
28	**Chlorocresol**	20.278	**11.2**
29	cis-Verbenol	21.834	0.1
30	Copaene	22.589	0.1
31	β -Bourbonene	22.850	0.1
32	β -Caryophyllene	24.201	0.9
33	Germacrene D	24.661	0.1
34	β -cis-Ocimene	25.558	0.1
35	β -Farnesene	26.216	0.1
36	α-Amorphene	26.635	0.2

37	4aH-cycloprop[e]azulen-4a-ol, decahydro-1,1,4,7-tetramethyl-	27.035	0.1
38	Ylangene	27.289	0.1
39	Ethanol, 2-(2-chloroethoxy)-1-phenyl-	27.397	0.1
40	α-Methylcyclohexene	28.191	0.6
41	α-Cubebene	28.523	0.3
42	α-Zingiberene	28.657	0.1
43	α-Caryophyllene=Humulene	29.512	0.2
44	Spathulenol	30.160	0.1
45	Caryophyllene oxide	30.277	0.3
46	Ethyl iso-allocholate	30.515	0.1
47	3,4-Dimethyl-2,5-diprop-2-enyl-2,5-dihydrothiophene 1,1-dioxide	31.558	0.1
48	δ-Cadinol, (-)-	32.739	0.1
49	Cubenol	32.893	0.1
50	alpha-Cadinol	33.187	0.1
51	Patchoulane	34.194	0.1
52	3-Ethoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris(trimethylsiloxy)tetrasiloxane	36.433	0.3
53	Ledol	36.626	0.1
54	1-Cyclohexene-1-butanol, 2,6,6-trimethyl-	38.894	0.1
55	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	40.430	0.1
56	Hexahydrofarnesyl acetone	40.779	0.1
57	Cyclopropane, 2-methylene-1-pentyl-1-trimethylsilyl-	42.199	0.1
58	Stearic acid	44.857	0.3
59	Cycloisolongifolene, 8,9-dehydro-9-formyl-	45.548	0.3
60	Diazoprogesterone	46.709	0.1
	94.0		

Table 2. *Classification of the constituents of the Thymus numidicus* Poiret.

Component	Peak area, %
Oxygenated monoterpenes	12.4
Monoterpene hydrocarbons	2.6
Oxygenated sesquiterpenes	0.9
Hydrocarbons Sesquiterpene	2.3
Aromatics	70.4

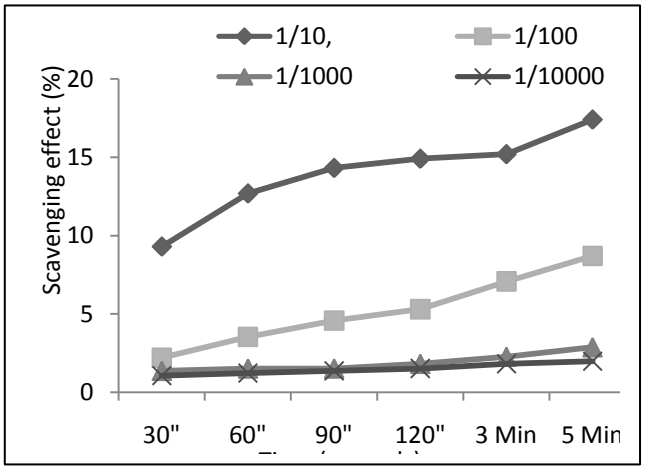

Figure 1. *DPPH radical scavenging activity of essential oil at different times.*

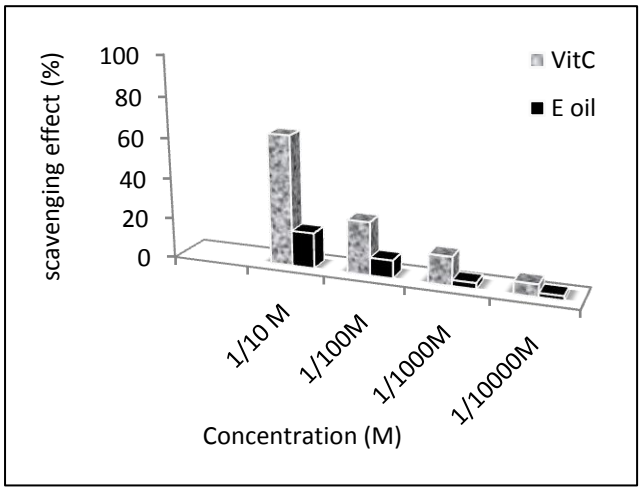

Figure 2. *DPPH radical scavenging activity of essential oil and Vitamin C.*

The antioxidant activity of *Thymus numidicus* Poiret. essential oil was measured by using DPPH assay. The scavenging activity of the essential oil was tested at concentrations of 10^{-1}M, 10^{-2}M, 10^{-3}M and 10^{-4}M; Vitamin C was used as a standard. It has been showed that the radical scavenging capacity of the tested essential oil increased in a concentration and time dependent manner (figure 1). The highest DPPH radical scavenging activity (%) was shown by essential oil at 10^{-1} M (17.4 %), which was lower than the antioxidant activity of the standard vitamin C (Figure 2). Similar results were reported in a study of antioxidant effect of essential oils of *Thymus linearis* and *Thymus serpyllum* by different *in-vitro* tests [21].

There are a few reports about the antioxidant activity of *Thymus* essential oils [21,22,23] showed that essential oils containing high amounts of thymol and carvacrol were reported to possess the highest antioxidant activity [24,25,26] and the principal active compounds of these oils are principally carvacrol, thymol, citral, eugenol, 1–8 cineole, limonene, pinene, linalool and their precursors [27].

4. Conclusion

Our results demonstrate the high chemical variability of constituents found in genus *Thymus* in general and especially in *Thymus numidicus* Poiret. species and stresses the importance of investigations dealing with the chemistry of separate plant organs. our results showed that thymol was the main phenolic compound of the leaves of *Thymus numidicus* Poiret. essential oil collected from Grarem - Mila (Eastern Algerian) in flowering stage and possess a moderate antioxidant activity.

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