Research Article

Chemical Composition and Antioxidant Activity of Essential Oils of *Thymus ciliatus* ssp. *coloratus* from Annaba-Algeria

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ABSTRACT

Thymus ciliatus is a plant widely used in traditional medicine, for her therapeutic proprieties attributed essentially to essential oil. The aim of this work is to determine the differences in chemical composition and antioxidant activity of essential oil at different harvest period. The composition of essential oil was analyzed by GC-MS was showed that essential oil obtained at vegetative, flowering, after flowering stage was characterized with thymol (33. 39%), carvacrol (30.85%) and carvacrol (31.37%) respectively, their essential oils was screened for antioxidant activity using DPPH assay, and reducing power, the essential oil obtained after flowering showed the best antioxidant than vegetative and flowering stage with IC_{50} to 438 ± 9.07µg / ml, 580 ± 1.15 µg / ml, and 590 ± 8.62µg/ml respectively, We have also found that the essential oil after flowering (1.001) which shows high activity by reducing power assay.

Keywords: *Thymus ciliatus* ssp. *coloratus*, Essential oil, GC/MS, DPPH, reducing power.

INTRODUCTION

The Mediterranean flora is well known for its abundance of aromatic plants, with an estimated 49% of genera containing aromatic species in this climate zone Members of the Lamiaceae from this region have attracted a great deal of attention due to the diversity of monoterpenes produced by different[1]. The genus Thymus is one of the largest and economically most important genera within the Lamiaceae (= Labiatae) family, they include 215 species widespread all around the Mediterranean region[2]. In Algerian flora, there are 12 Thymus species from which 9 are endemic, among which ***Thymus ciliatus*** (Desf.) Benth, which is an endemic species from North Africa. This species includes three subspecies: ssp. eu-ciliatus Maire, ssp. *coloratus* (Boiss. et Reut.) Batt. and ssp. munbyanus (Boiss. et Reut.) Batt.[3] In North African folk medicine, Thymus plants are used as remedies in various diseases e.g. bronchitis, pulmonary infection, flu, cough and some gastrointestinal disorders[4].

Therefore, several EOs of the Thymus species have been studied to investigate their chemical composition and antimicrobial activities[5].These products are of particular interest, because no bacterial resistance or adaptation has been described, and low or insignificant side effects have been found both in the essential oils and whole extracts.[6] He most species of thymus contain phenolic monoterpenes, thymol and/or carvacrol.[4] Therefore, various species studied for their antioxidant activities 789. Recently, interest has increased considerably in finding naturally occurring antioxidants for use in foods or medicinal materials to replace synthetic antioxidants, which are being restricted due to their carcinogenicity.[10] Furthermore, natural substance possessed of antioxidant activity present a very important socio-economic interest in the field biopharmacological of research. Several laboratories around the world have turned to the search for substances bioactive and recovery.[11]

The aim of this work was to evaluate the chemical composition of Algerian ***Thymus ciliatus* subsp. *coloratus*** essential oils, isolated by hydrodistillation from the aerial parts of plants collected during different phases, and to determine in which way this would affect the corresponding oils antioxidant.

MATERIALS AND METHODS

Plant Material

The aerial part (leaves, stems) of *T. ciliatus* was harvested at vegetative, flowering, and after flowering stage of development from Annaba, Algeria. The plants collected were identified by the Biological Vegetable Laboratory. Plant samples were dried in the share and conserved for future use.

Essential Oil Isolation

The100g of the air-dried leaves, stems were submitted for 3 hours to hydrodistillation using a Clevenger type-apparatus according to the method recommended in the European Pharmacopoeia.[12] The essential oils were dried over anhydrous sodium sulphate and then stored at 4 °C.

Essential Oil Analysis

The essential oil was analysed by GC/MS Agilent HP Agilent MSD 5973 mass spectrophotometer and coupled with a Agilent HP 6800 plus gas chromatograph, equipped with hp-5MS column: l30 m x 0.25 mm, 0.25 µm film thickness. Initially, the oven temperature was 60 °C which was held for 8 min, followed by an increase in temperature to 250 at 2 °C/min and this was held isothermally for 10 min. The injector and detector temperature were 250 °C and 280° C respectively.

Analyses were carried out using helium as carrier gas at a flow rate of 0.5 ml/min, at a split ratio of 1:20 Mass spectra were obtained by electron ionization (EI) at 70 eV, using a spectral range of 34 at 450 m/z. Relative percentages of the different constituents was calculated automatically from the peak area of total ion chromatograms. Constituents of the oil were identified by comparison of their retention indices relative to a homologous n-alkane series and matching their mass spectra with those of reference compounds in Wiley 7n.1 libraries[13].

Antioxidant Activity

The antioxidant activity of the essential oil of *T.ciliatus* has been determined by the different test systems namely DPPH and reducing power.

DPPH Assay

In this assay, antioxidant activity of essential oils was evaluated by measuring the bleaching of the purple-colored methanol solution of DPPH.[14] The antioxidant activity of thyme oils was measured in terms of hydrogen-donating of radical-scavenging ability, using the stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) as a reagent. The antioxidant activity of the EO was estimated using a protocol reported by Sharififar.[15] Fifty microlitres of various concentrations of the sample in methanol (both essential oil and control substance) were added to 5 ml of 0.004 % methanolic solution of DPPH.

Absorbance measurements were read at 517 nm, after 30 min of incubation time at room temperature. Absorption of a blank sample containing the same amount of methanol and DPPH solution acted as the negative control. All determinations were performed in triplicate. The percentage inhibition of the DPPH radical by the samples was calculated according to the formula: I% = 100 X ($A_{Control} - A_{sample}$) / $A_{Control}$[16].

Reducing Power

The reducing power was determined according to the method of Oyaizu[17]. Each extract (0.2–1.0 mg/ml) in methanol and water (2.5 ml) was mixed with 2.5 ml of 200 mM sodium phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide and the mixture was incubated at 50 C for 20 min. Then, 2.5 ml of 10% trichloroacetic acid were added, and the mixture was centrifuged at 200 g (MSE Mistral 2000, London, UK) for 10 min. The upper layer (2.5 ml) was mixed with 2.5 ml of deionized water and 0.5 ml of 0.1% ferric chloride. Finally the absorbance was measured at 700 nm against a blank. Ac ascorbique was used as a control.

RESULTS AND DISCUSSION

Chemical Composition

The essential oils extracted by hydrodistillation from the dried aerial parts of *T. ciliatus,* collected at different developmental stages, ranged from 0.54% to 1.2% (w/w) with a maximum obtained during the post-flowering phase (Table 1). In fact more rapporteurs suggest that the plant accumulates the most EO at flowering stage.[18,19]

Analyses of *T.ciliatus* EOs obtained at vegetative, floraison, and after floraison period by GC/MS was identified 50, 54, 55 constituents respectively; representing 98,62 – 99,5 – 99,28%, of the total oil at the vegetative, flowering and post-flowering phases (Table1).

The essential oil composition of T. *ciliatus* obtained at vegetative stage was characterized by high percentage of thymol (33.39%), carvacrol (24.16%) as the main compound, followed by γ-terpinene (08.89%), *p*-cimene (8.25%), α-terpinolene (7.36%).

However the essential oil composition of T. *ciliatus* obtained at flowering stage was characterized by high percentage of thymol (29.52%), carvacrol (30.85%) as the main compound, followed by *p*-cimène (8.45%), α-terpinolène (7.71%) and γ-terpinène (05.58%), nevertheless the essential oil composition of T. *ciliatus* obtained at after flowering stage was dominated by high percentage of thymol (21.02%), carvacrol(31.37%), followed by γ-terpinène (10.95%),*p*-cimène (8.71%), α-terpinolène (6.11%) and α-pinène (5.18%).

The essential oil from *T.ciliatus* showed a high content of oxygenated monoterpenes at the vegetative (62.58%), flowering (66.89%), and post-flowering phases (56.69 %), and low contents of monoterpene hydrocarbons (29.68%), (26.42%), (36.29%) respectively the vegetative, flowering, and post-flowering phases, followed by the sesquiterpene hydrocarbons (04.1%), (03.72%), (04.11%) respectively and oxygenated sesquiterpenes detected in the oil samples collected only during both vegetative, flowering phases in average (0.3%).

The comparison of our results with those of the literature showed that the chemical composition of the endemic species *Thymus ciliatus* is markedly different from that of other region from Algeria. Generally the North Algerian *T.ciliatus* EO is characterized by a chemotype Thymol[20-,22], while Algerian West- by carvacrol chemotype[20,23]. A chemical composition quite different from that the oil of the same species (subspecies unspecified) collected in Morocco showed that there is a variability in the chemical composition, besides the EO *T.ciliatus* Azrou is represented by the chemotype (Thymol, β-ocimene) with respectively rate (44.20 and 25.8%), and the Imilchil is Thymol (17.30%) and Carvacrol (26.20%)[21,24].

According to several authors, variations in the chemical composition of essential oils from the qualitative and quantitative stand point are due to, environmental factors[4,25], the part of the plant used, the period of the vegetative cycle during which the harvest has been made[7,26,27] and also genetic factors.[28]

Antioxidant and Radical Scavenging Activity

Concentration DPPH is widely used to evaluate antioxidant capacity and changes colour from purple to yellow upon acceptance of electrons/hydrogens, thus

indicating scavenging activity. the lower IC_{50} value reflects better protective action[29].

As can be seen from the Table 2, free radical scavenging effect of the samples exhibited a dose-dependent increase.

The weakest radical scavenging activity was exhibited by the essential oil obtained after flowering and determined as à 91.735%, followed by flowering, and vegetative stages, the average is 88.21 et 73.92 % respectively at the concentration 1000 µg/ml.

The radical scavenging activity of ascorbic acid remains higher than that EOs tested with an IC_{50} of 2.48 ± 0.02µg/ml.

However, among these three thyme EOs, the EO after flowering has the highest antioxidant activity with IC_{50} rate to 438 ± 9,07µg/ml, the flowering EO also has an important antioxidant with IC_{50} of approximately 580 ± 1.15 µg/ml, followed by essential oil vegetative phase with IC_{50} equal to 590 ± 8.62µg/ml.

The strong antioxidant activities in Algeria EOs Thyme species with large amount of carvacrol and thymol has been reported.[4] The DPPH scavenging activity EOS was be attributed to the presence of phenolic compounds such as thymol or carvacrol.[8,9,30] But the antioxidant activity of EOs showed lower antioxidant activity than reported by Amarti[31], who found a way antioxidant potential, with IC50 about 74.025 µg/ml, of *T.ciliatus* EO from Morocco. These results imply that non-phenolic contents could be also responsible for this activity. Furthermore, some researchers show that some essential oils rich in nonphenolic compounds also have antioxidant potentials.[32]

The effect of antioxidants on DPPH radical scavenging was thought to be due to their hydrogen-donating ability. DPPH radical is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule[10].

Table 1: Chemical Composition of the Essential Oil of *T.ciliatus*

Components	RT(min)	Vegetative	Flowering	Post-flowering
isovaleric acide	**4,36**	0,02	0,02	0,05
α-thujene	**9,84**	0,78	0,45	0,88
α-pinène	**10,24**	1,41	1,63	5,18
Camphene	**11,05**	0,56	0,31	0,62
Verbenene	**11,39**	-	-	0,05
β-pinene	**12,75**	0,19	0,15	0,26
1octen 3ol	**13,12**	1,49	1, 6	1, 6
3octanone	**13,51**	0,14	0,17	0,17
β-myrcene	**13,70**	0,85	0,70	1,22
3-octanol	**14,102**	0,22	0,24	0,22
α-phellandrene	**14,57**	0,16	0,09	0,19
3-carene	**14,96**	0,04	0,03	0,06
α-terpinene	**15,46**	1,08	0,63	1,42
p-cymene	**16,17**	8,25	8,45	8,71
Limonene	**16,32**	0,50	0,68	1,07
Eucalyptol	**16,45**	0,13	0,12	0,09
β-ocimene	**17,72**	0,05	0,05	0,04
ï-terpinene	**18,54**	8,86	5,58	10,97
Linalool oxide	**19,37**	0,12	0,12	0,13
1-octen-3-ol	**19,99**	-	0,1	-
α-terpinolene	**21,72**	7,36	7,71	6,11
β-phellandrene	**22,82**	0,05	0,05	0,05
Trans-pinocarveol	**23,97**	0,11	0,02	0,10
Mentha-1,4,8-drienne	**24,46**	0,10	0,10	-
Z-citral	**24,45**	-	-	0,04
Borneol	**25,99**	2,26	2,98	1,78
Terpinene-4-ol	**26,82**	1,55	2,41	1,03
p-cymen-ol	**27,37**	-	0,15	0,17
α-terpinolene	**27,76**	-	0,08	0,04
b fenchyl alcohol	**28,63**	0,11	-	-
cis-dihydrocarvone	**29,13**	0,06	0,22	0,14
Methyl thymol ether	**30,92**	-	0,04	0,04

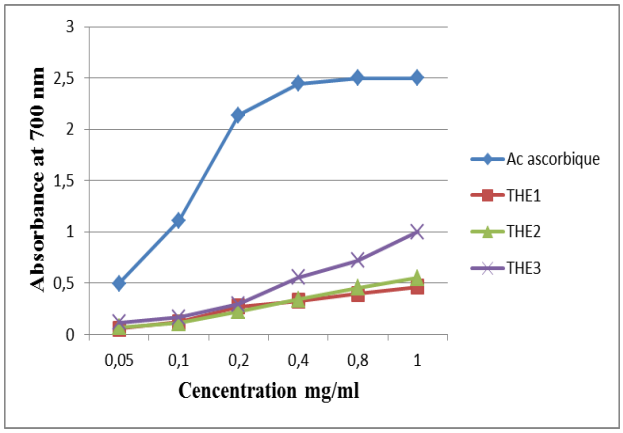
carvacrol methyl ether	**31,53**	0,07	-	0,03
3-carene	**31,86**	-	0,04	0,04
Carvone	**33,13**	-	0,05	0,03
Thymol	**35,63**	33,39	29,52	21,02
Carvacrol	**36,62**	24,16	30,85	31,37
Eugenol	**39,30**	0,04	0,14	0,03
α-copaene	**40,32**	0,21	0,15	0,25
β-bourbonene	**40,90**	0,29	0,29	0,34
caryophyllene	**43,03**	0,23	0,29	0,32
β-cubebene	**43,67**	0,15	0,13	0,14
aromadendrene	**45,56**	0,07	0,10	0,12
α-amorphene	**46,60**	0,39	0,31	0,40
germacrene d	**46,86**	0,61	0,23	0,28
β-selinene	**47,14**	0,06	0,07	0,07
α-cubebene	**47,70**	0,27	0,18	0,25
α-muurolene	**48,07**	0,13	0,12	0,14
β-bisabolene	**48,62**	0,11	0,11	0,06
ï-cadinene	**48,87**	0,33	0,28	0,42
Delta cadinene	**49,50**	1,04	1,02	1,02
α-cadinene	**50,24**	-	0,04	0,05
Calacorene	**50,54**	-	0,15	0,03
Ledene	**53,31**	-	0,08	0,05
Apiol	**55,29**	0,04	-	-
α-cadinol	**56,89**	0,28	0,30	0,14
dehydroromadendrene	**58,60**	0,12	0,13	0,11
cetanol	**68,73**	0,05	0,04	0,08
β-fernesene	**80,61**	0,09	0,04	0,06
Cuminol	**92,51**	0,04	-	-
Total		**98,62**	**99,50**	**99,28**
Groups				
Monoterpene hydrocarbons		**29,68**	**26,42**	**36,29**
Oxygenataed monoterpenes		**62,58**	**66,89**	**56,69**
Sesquiterpene hydrocarbons		**04,1**	**3,72**	**4,11**
Oxygenataed sesquiterpenes		**0,32**	**0,3**	-
Others		**1,94**	**2,17**	**2,19**

Table 2: Scavenging Ability of *Thymus ciliatus* Essential Oils on DPPH Radicals (%)

T.ciliatus ssp coloratus	**Concentrations (µg/ml)**	**Scavenging ability of Thymus essential oils on DPPH radicals (%)**		
		Vegetative	**flowering**	**after flowering**
	100	8,65±0,29	10,123±3,81	28,747±3,284
	200	9,65±0,168	13,405±0,544	31,198±2,272
	400	40,45±0,821	23,17±0,695	47,449±1,648
	600	50,523±0,446	51,245±1,347	61,056±6,521
	800	58,71±5,363	73,7±2,803	80,742±0,138
	1000	73,922±0,615	88,21±0,298	91,735±1,045
Ac Ascorbique				
	2	41,835±0,548		
	4	74,546±0,274		
	6	86,951±1,054		
	8	89,917±0,593		
	10	94,859±0,362		

The Reductive Potential

The reductive potential measures the ability of a sample to act as electron donor and, therefore, reacts with free radicals converting them to more stable products and thereby terminates radical chain reactions. Generally, the reductive potential of all T. *ciliatus* essential oil samples increased steadily with increasing essential oils concentration (Figure 1), Ascorbic acid, reference antioxidant, presented a higher activity compared to EOs. The reducing power of three essential oils T.*ciliatus* (vegetative, flowering, after flowering) amounted to 0.465 ± 0.002, 0.551 ± 0.003 and 1.001 ± 0.005 at the concentration 1 mg/ml respectively. We have found that the EO after flowering (1.001) which shows high activity compared to other EOs *T.ciliatus* essential oils seem to act as electron donors.



THE1: vegattive stage, THE2: flowering, THE3: after flowering

Figure 1: Reduction Power of *Thymus ciliatus* Essential Oils

Essential oils are complex mixtures and determination of the component(s) responsible for activity is difficult. Antioxidant activity of essential oils has often been attributed to the presence of phenolic constituents, especially thymol and/or carvacrol. The reducing powers of T. oils poor in thymol or carvacrol were markedly lower than those of the essential oils rich in these two phenols. has been confirmed by Amarti.[4] This association has been confirmed for the most reporters, but other compounds also seem to play an important role to hydroxyl substitutions in the aromatic ring, which possess potent hydrogen donating abilities and show radical scavenging activity The essential oil of *T. ciliatus* could be concluded as a natural source that can be freely used in food industry as a culinary herb. Very little work has been done on studying the antioxidant properties of *T. ciliatus* EO. According to our knowledge and to this day, there is an article referring the antioxidant activity of EO studied by bleaching β-carotene method, the results according to the essential oil showed the strongest ability to inhibit the formation of radicals linoleic acid with percentage to 75.68%[20]. This antioxidant activity may be due to different mechanisms, such as prevention of chain initiation, decomposition of peroxides, prevention of continued hydrogen abstraction, free radical scavenging, reducing capacity, and binding of transition metal ion catalysts. It is thus important that for evaluating the effectiveness of antioxidants, several analytical methods and different substrates are used [7].

CONCLUSION

The genus Thymus belonging to the family Lamiaceae (Labiatae) was widely investigated especially the endemic ones of Algeria, Our data provide a scientific basis for the use of these plants as folk remedies, particularly for the prevention and treatment of diseases that are known to be caused or accelerated by oxidative stress.

These studies point out the importance of comparing and exploring the variance of essential oils composition from different stage of developpement, since this will most probably affect their potential biological activities.

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