Journal of Medicinal Plants Research Vol. 5(24), pp. 5773-5778, 30 October, 2011
Available online at http://www.academicjournals.org/JMPR
ISSN 1996-0875 ©2011 Academic Journals

Full Length Research Paper

Chemical composition and antioxidant activity of essential oils and solvent extracts of *Thymus capitatus* (L.) Hoffmanns and link from Morocco

El Mokhtar El Ouariachi[1], Julien Paolini[2]*, Abdelhamid Bouyanzer[1], Pierre Tomi[2], Belkheir Hammouti[1], Rachid Salghi[3], Lhou Majidi[4] and Jean Costa[2]

[1]LCAE-URAC18, Faculté des Sciences, Oujda, Morocco.
[2]Université de Corse, UMR CNRS 6134, Lab. de Chimie des Produits Naturels, Corte, France.
[3]E N S A, Equipe Génie de l'Environnement et de Biotechnologie, Agadir, Morocco.
[4]Université My Ismail, Laboratoire des Substances Naturelles, Synthèse et Dynamique Moléculaire, Errachidia, Morocco.

Accepted 24 June, 2011

Analysis of the chemical composition of *Thymus capitatus* essential oil from Morocco was carried out using gas chromatography (GC) and gas chromatography-mass spectral (GC-MS). The oil was dominated by *p*-cymene, carvacrol, geranyl acetate and borneol. The amounts of total phenolics and flavonoids in the solvent extracts were determined spectrometrically. The antioxidant activity of essential oil and extracts was evaluated using free radical scavenging. The 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging activity increased in the order ethyl acetate > diethyl ether > essential oil. A relationship was observed between the antioxidant activity potential and total phenolic and flavonoid levels of the extracts. Finally, extracts and essential oils of *T. capitatus* showed antioxidant activity, and therefore it could be used as a natural preservative ingredient in food and/or pharmaceutical industries.

Key words: *Thymus capitatus*, essential oil, solvent extract, antioxidant activities, phenolic compounds, gas chromatography-mass spectral, 1,1-diphenyl-2-picrylhydrazyl.

INTRODUCTION

It is well-known that free radicals cause oxidation of unpreserved aliments rich in unsaturated fatty acids (Li et al., 2008). The naturally occurring antioxidants of medicinal plants such as polyphenols and flavonoids exhibit a high ability to donate hydrogen from phenolic hydroxy groups, thereby forming stable free radicals, which do not initiate or propagate further oxidation of lipids (Fecka et al., 2007; Gülçin et al., 2004; Havsteen, 2002). Thus, the preservative effect of many aromatic plants has increased the interest in finding secondary metabolites with antioxidant properties for use in foods to replace synthetic antioxidants (Amrutha and Bhaskar, 2010). Moreover, the secondary metabolites with phenolic hydroxyl groups have benefits to the human health in playing an important role in neutralizing free radicals, which can cause several disorders of immune system and gene expression (Halliwell, 1995; Pourmorad et al., 2006; Safaei-Ghomi et al., 2009; Sharma and Bhat, 2009).

The scavenging of reactive oxygen species (ROS) is a possible mechanism of action for antioxidant compounds (Havsteen, 2002). ROS may be the causative factor involved in many human degenerative diseases, and antioxidants are known to have some degree of preventive and therapeutic effects on these disorders. Small molecular weight antioxidants are considered possible protection agents that reduce oxidative damage in the

*Corresponding author. E-mail: paolini@univ-corse.fr. Tel: (33) 04 95 45 01 93. Fax: (33) 04 95 45 01 62.

human body when the internal enzymatic mechanisms fail or are inadequately efficient (Halliwell, 1995).

The essential oils and extracts of many Thymus species (Lamiaceae family) native to Mediterranean basin are widely used in pharmaceutical, cosmetic and perfume industry, and for flavouring and preservation of several food products (Bauer et al., 1997). The essential oils of *Thymus* species are rich sources of phenolic monoterpenes such as thymol and carvacrol (Karousou et al., 2005; Miceli et al., 2006). Several studies have been published on biological properties of *Thymus capitatus* (L.) Hoffmanns and Link as antibacterial (Akrout et al., 2010; Cosentino et al., 1999; Usai et al., 2010), antifungal (Goren et al., 2003), antioxidant (Biondi et al., 2006; Bounatirou et al., 2007; Dorman et al., 2004; Harmandar et al., 2006; Mkaddem et al., 2010) and antiviral activities (Salah-Fatnassi et al., 2010). The essential oils of *T. capitatus* (Syn. *Coridothymus capitatus* (L.) Rchb.f., *Satureja capitata* L. and *Thymbra capitata* (L.) Cav) have been investigated by many researchers who reported the chemical variability of essential oils from this species (Akrout et al., 2010; Bentes et al., 2009; Biondi et al., 2006; Bounatirou et al., 2007, 2010; Cosentino et al., 1999; Goren et al., 2003; Karouso et al., 2005; Miceli et al., 2006; Mkaddem et al., 2010; Napoli et al., 2010; Ibraliu et al., 2011). The objective of the present investigation was to get a knowledge on chemical composition and antioxidant activity of *T. capitatus* from Morocco for a possible valorization of essential oil and solvent extracts.

MATERIALS AND METHODS

Plant material

The aerial parts of *T. capitatus* were harvested in February 2009 (full bloom) from Al Hoceima (three samples), Morocco. Voucher specimens were deposited in the herbarium of Mohamed 1st University, Oujda, Morocco. The species is a dwarf shrub of 20 to150 cm height with ascending to erect woody branches bearing axillary leaf clusters. Leaves are sessile, linear, and acute. Inflorescences (purplish-pink colour) are oblong-conical: the calyx measures approximately 5 mm and the corolla up to 10 mm (Tutin et al., 1968-1993).

Essential oil isolation

The air-dried leaves of the studied plant were submitted for 4 h to hydrodistillation using a Clevenger type-apparatus according to the method recommended in the European Pharmacopoeia (Council of Europe, 1996). The essential oil yield was 0.5% (v/w). The obtained essential oils were dried over anhydrous sodium sulphate and then stored in sealed glass vials at 4 to 5°C prior to analysis.

Preparation of the extracts

Boiling water extracts (100 ml) of plant samples obtained under reflux conditions (hydrodistillation process) were extracted three times (3 × 20 ml) with organic solvents (diethyl ether and ethyl acetate). Water extract residues were then extracted by boiling acidified water (2 N HCl) prior to liquid–liquid extraction. The diethyl ether and ethyl acetate extracts were filtered and concentrated under vacuum to obtain two extracts in yields of 0.16 and 0.27% (w/w), respectively. The organic solvent extracts were dried over anhydrous sodium sulfate and then stored in sealed glass vials at 4 to 5°C prior to analysis. Each extraction was performed in triplicate.

GC and GC–MS analysis

Analysis was carried out using a Perkin-Elmer Autosystem XL GC apparatus (Waltham, MA, USA) and a Perkin-Elmer turbo mass detector (quadrupole) coupled to a Perkin–Elmer Autosystem XL equipped with a dual flame ionization detection (FID) system and the fused-silica capillary columns (60 m × 0.22 mm I.D., film thickness 0.25 ml) Rtx-1 (polydimethylsiloxane) and Rtx-wax (polyethyleneglycol). In previous studies (Paolini et al., 2005, 2007), the complementarity of these two analytical techniques (GC-FID and GC-MS) with two chromatographic columns (apolar and polar) have been demonstrated for the identification and quantification of volatile components in complex mixture. The oven temperature was programmed from 60 to 230°C at 2°C/min and then held isothermally at 230°C for 35 min. Injector and FID temperatures were maintained at 280°C and MS source temperature at 150°C. Samples were injected in the split mode (1/50) using helium as a carrier gas (1 ml/min) and 0.2 µl injection volume of pure oil. Retention indices (RI) of compounds were determined relative to the retention times of a series of n-alkanes (C5–C30) (Restek, Lisses, France) with linear interpolation using the Van den Dool and Kratz equation and software from Perkin–Elmer. Electron ionization mass spectra (energy ionization: 70 eV) were acquired over the mass range 35 to 350 Da. Identification of individual components was based on: (i) comparison of calculated RI, on polar and apolar columns, with those of authentic compounds or literature data (Konig et al., 2001); and (ii) computer matching with commercial mass spectral libraries and comparison of mass spectra with those of our own library of authentic compounds or literature data (Adams, 20001; Konig et al., 2001).

Determination of total phenolic contents

Total phenolic contents of the extracts were determined by using Folin–Ciocalteu reagent according to the method previously reported by Slinkard and Singleton (1977), using caffeic acid as a standard, and as modified by Li et al. (2008). 200 µl of the diluted solution extract was mixed with 1 ml of Folin–Ciocalteu (diluted in distillated water) and the volumetric flask was vigorously shaken. After 4 min, 800 µl of Na_2CO_3 (75 mg/ml) solution was added and the mixture was allowed to stand for 45 min at room temperature. At the end of the incubation, the absorbance was measured at 760 nm. The same procedure was also applied to the standard solutions of caffeic acid, and a standard curve was obtained. The concentrations of phenolic compounds expressed as µg caffeic acid equivalent per mg of extract were calculated according to the standard caffeic acid graph. All experiments were carried out in triplicate, and caffeic acid equivalent values were reported as X (average) ± SD (standard deviation) of triplicates.

Antioxidant activity

The free radical-scavenging activities of essential oil and solvent extracts were measured using 1,1-diphenyl-2-picrylhydrazyl (DPPH) as described by Hatano et al. (1988); antioxidants react with the stable free radical DPPH (deep violet color) and convert it to

1,1-diphenyl-2-picrylhydrazine with discoloration. Various concentrations (0.1 ml) of the oil (0.5 to 5.00 mg/ml), the diethyl ether and ethyl acetate extract (33 to 150 mg/L) in ethanol and water were added to 3.9 ml of a DPPH radical solution in ethanol (the final concentration of DPPH was 0.05 mM). The mixture was strongly shaken and left to stand at room temperature for 30 min in the dark. The absorbance was measured at 517 nm against a blank. The radical-scavenging activity was expressed as percentage of inhibition (I%) according to the following formula (Brand-Williams et al., 1995):

$$I(\%) = 100^* (A_{control} - A_{sample})/A_{control}$$

Where $A_{control}$ is the absorbance of the control reaction and A_{sample} is the absorbance of the test compound. The sample concentration providing 50% inhibition (IC_{50}) was calculated from the graph of inhibition percentage against sample concentration. Tests were carried out in triplicate. Ascorbic acid was used as a positive control.

Determination of total flavonoids contents

Total flavonoid contents were determined using the Dowd method as adapted by Arvouet-Grand et al. (1994). 1 ml of 2% aluminium trichloride ($AlCl_3$) in methanol was mixed with the same volume of extracts (200 µg). The absorption at 430 nm was measured after 10 min against a blank sample consisting of 1 ml methanol without $AlCl_3$. The concentrations of flavonoid compounds expressed as µg quercetin equivalent per mg of extract were calculated according to the standard quercetin graph. All experiments were carried out in triplicate, and quercetin equivalent values were reported as X ± SD of triplicates.

RESULTS AND DISCUSSION

Chemical composition of essential oils

The analysis of the essential oil of *T. capitatus* from Morocco was carried out using GC and GC-MS. The oil composition was characterized by 32 constituents, which accounted for 91.2% of the total oil (Table 1). The *T. capitatus* oil was dominated by aromatic components (9, 21, 22, 24 and 25) with carvacrol 24 as main compound followed by oxygenated monoterpene hydrocarbons (33.3%). The main compounds of the essential oil were *p*-cymene 9 (18.9%), carvacrol 24 (13.4%), geranyl acetate 27 (12.2%) and borneol 17 (10.2%).

Various chemical profiles of essential oils (thymol, cavacrol or thymol/carvacrol as main components) have been reported according to geographical origins of *T. capitatus* (Karouso et al., 2005; Miceli et al., 2006). For instance, carvacrol (62 to 83%) followed by para-cymene (5 to 17%) were identified as major compounds of *T. capitatus* oils from Tunisia (Akrout et al., 2010; Bounatirou et al., 2007, 2010). The Albanian (Ibraliu et al., 2011), Sicilian (Biondi et al., 2006; Napoli et al., 2010) and Portuguese oils (Bentes et al., 2009) were also characterised by high amount of carvacrol. It should be noted that carvacrol was scarcely represented in our Moroccan oil in comparison with oils of others geographical origins. A recent study reported the presence of thymol (89%) as major component of essential oil from Tunisia (Mkaddem et al., 2010). Sardinian *T. capitatus* oils (Cosentino et al., 1999) was dominated by thymol (29.3%) and *p*-cymene (26.4%) followed by carvacrol (10.8%). Finally, the Turkish oil (Goren et al., 2003) is characterized by high amount of carvacrol (35.6%) in addition with p-cymene (26.4%) and thymol (18.6%).

Antioxidant activity, total phenolics and flavonoid contents

Free radical-scavenging capacity of the essential oil and solvent extracts were measured by DPPH method (Table 2). Both extracts of *T. capitatus* exhibited potential antioxidant activity; the ethyl acetate extract and the diethyl ether scavenged 50% DPPH free radical at the concentration of 1.5 and 1.92 µg/ml, respectively. Conversely, the antiradical activity of essential oil was weak (IC_{50}: 103 µg/ml) in comparison with ascorbic acid (IC_{50}: 0.97 µg/ml). Thus, the DPPH scavenging effect increased in the order of essential oil < diethyl ether extract < ethyl acetate extract < ascorbic acid. As shown in Table 2, free radical scavenging activity also increased with increasing concentration of solvent extracts. At higher concentrations, the antioxidant activity of extracts was closer to the scavenging effect of ascorbic acid. For .instance, at 2.0 µg/ml, the scavenging activity of ascorbic acid was about 82%, and ethyl acetate extract solution of 2.5 µg/ml had a scavenging activity of 73%. These results may be due to hydroxyl groups existing in the chemical structure of phenolic compounds from *T. capitatus* extracts that can provide the necessary component as a radical scavenger (Das and Pereira, 1990; Matkowski, 2008; Shimoi et al., 1996). Indeed, a highly positive relationship between phenolic compounds and antioxidant activity were reported in this study. For instance the ethyl acetate extract, which contain higher amount of phenolic compounds (225.6 µg/mg) than diethyl ether (150.4 µg/mg), also exhibited a greater antioxidant activity (Table 3). Similarly, the ethyl acetate extract was found to be richer in flavonoids (39.7 µg/mg) than the diethyl ether extract (22.7 µg/mg).

Moreover, the antioxidant activity of essential oil could be attributed to its relatively high content of carvacrol. Indeed, several studies (Biondi et al., 2006; Bounatirou et al., 2007; Dorman et al., 2004; Harmandar et al., 2006; Mkaddem et al., 2010) have been published on antioxidant activities of phenol rich oils from *T. capitatus*. On the basis of the results of this work, *T. capitatus* can be used as easily accessible source of natural antioxidants and as a possible food supplement or in pharmaceutical applications.

ACKNOWLEDGEMENTS

The authors are indebted to the Délégation Régionale à la Recherche et à la Technologie de Corse (DRRT), the

Table 1. Chemical composition of essential oil from *T. capitatus*.

N°[a]	Components	RI *l*[b]	RI a[c]	RI *p*[d]	%[e]	Identification
1	α-Thujene	932	922	1031	0.6 ± 0.1	GC, GC-MS
2	α-Pinene	936	930	1029	2.9 ± 1.2	GC, GC-MS
3	Camphene	950	943	1070	3.2 ±1.8	GC, GC-MS
4	Oct-1-en-3-ol	962	961	1449	1.2 ± 0.3	GC, GC-MS
5	Sabinene	973	965	1125	0.5 ± 0.3	GC, GC-MS
6	β-Pinene	978	970	1114	1.2 ± 0.2	GC, GC-MS
7	Myrcene	987	981	1162	1.8 ± 0.5	GC, GC-MS
8	α-Terpinene	1013	1010	1181	0.6 ± 0.2	GC, GC-MS
9	*p*-Cymene	1015	1015	1272	18.9 ± 3.1	GC, GC-MS
10	Limonene	1025	1022	1203	1.1 ± 0.4	GC, GC-MS
11	1,8-Cineole	1024	1022	1208	1.0 ± 0.3	GC, GC-MS
12	γ-Terpinene	1051	1050	1247	2.5 ± 1.0	GC, GC-MS
13	*trans*-Sabinene hydrate	1053	1054	1463	0.8 ± 0.3	GC, GC-MS
14	Linalol	1086	1085	1545	1.5 ± 0.5	GC, GC-MS
15	Camphor	1123	1122	1511	1.2 ± 0.4	GC, GC-MS
16	*trans*-Pino-carveol	1126	1124	1648	0.7 ± 0.1	GC, GC-MS
17	Borneol	1150	1153	1631	10.2 ± 2.0	GC, GC-MS
18	Terpinen-4-ol	1164	1163	1597	0.9 ± 0.4	GC, GC-MS
19	α-Terpineol	1176	1174	1688	0.6 ± 0.1	GC, GC-MS
20	Myrtenol	1178	1180	1781	0.4 ± 0.3	GC, GC-MS
21	Thymyl methyl ether	1215	1217	1766	1.2 ± 0.5	GC, GC-MS
22	Carvacryl methyl ether	1226	1227	1559	1.1 ± 0.2	GC, GC-MS
23	Bornyl acetate	1270	1270	1578	1.2 ± 0.3	GC, GC-MS
24	Thymol	1267	1273	2168	1.5 ± 0.6	GC, GC-MS
25	Carvacrol	1278	1285	2193	13.4 ± 1.5	GC, GC-MS
26	α-Terpinyl acetate	1335	1335	1670	2.6 ± 0.8	GC, GC-MS
27	Geranyl acetate	1362	1365	1753	12.2 ± 1.7	GC, GC-MS
28	(E)-β-caryophyllene	1421	1418	1595	2.9 ± 0.7	GC, GC-MS
29	δ-Selinene	1493	1503	1697	0.3 ± 0.1	GC, GC-MS
30	Germacrene D-4-ol	1571	1566	2035	0.5 ± 0.1	GC, GC-MS
31	Caryophyllene oxide	1578	1570	1965	1.9 ± 0.4	GC, GC-MS
32	α-Bisabolol	1659	1669	2200	0.6 ± 0.2	GC, GC-MS
	Total identified				**91.2**	
	Monoterpene hydrocarbons				14.4	
	Aromatic components				36.1	
	Oxygenated Monoterpenes				33.3	
	Sesquiterpene hydrocarbons				3.2	
	Oxygenated Sesquiterpenes				3.0	
	Others components				1.2	

[a]The numbering refers to elution order on apolar column (Rtx-1),[b]RI *l* = retention indices on the apolar column of literature (König et al., 2001; National Institute of Standards and Technology, 2008), [c]RI *a* = retention indices on the apolar column (Rtx-1), [d]RI *p* = retention indices on the polar column (Rtx-Wax), [e]Relative percentages of components (%) are calculated on GC peak areas on the apolar column (Rtx-1) ; Values expressed are means of three parallel measurements of three sample locations.

Table 2. DPPH radical-scavenging of essential oil and solvent extracts from *T. capitatus*.

Sample	Antioxidant activities						
Essential oil	Essential oil concentration (µg/ml)	12.0	15.0	25.0	62.5	125.0	
	Scavenging effect on DPPH (%)	25±1.5	27±2.0	30±0.7	40±0.3	56±2.1	
	DPPH IC_{50} (µg/ml)						103.0

Table 2. Contd.

Diethyl ether	Extract concentration (µg/ml)	0.80	1.25	1.65	2.50	3.75	
	Scavenging effect on DPPH (%)	25±0.8	33±0.5	42±1.2	62±0.9	84±3.2	
	DPPH IC_{50} (µg/ml)						1.92
Ethyl acetate	Extract concentration (µg/ml)	0.80	1.25	1.65	2.50	3.75	
	Scavenging effect on DPPH (%)	32±1	44±2.1	53±1.7	73±4.2	90±3.1	
	DPPH IC_{50} (µg/ml)						1.50
Ascorbic acid	Extract concentration (µg/ml)	0.20	0.35	0.50	1.00	2.00	
	Scavenging effect on DPPH (%)	21±0.7	26±0.4	34±2.5	54±3.5	82±4.1	
	DPPH IC_{50} (µg/ml)						0.97

Values expressed are means of three parallel measurements.

Table 3. Total phenol and flavonoid content of solvent extracts from *T. capitatus*.

Solvent extract	Total polyphenol content(µg CA/mg extract)	Total flavonoid content(µg quercetin/mg extract)
Diethyl ether	150.4±9.0	22.7±1.2
Ethyle acetate	225.6±7.0	39.7±3.4

Values expressed are means ± SD of three parallel measurements.

Collectivité Territoriale de Corse (CTC) and European Community for partial financial support.

REFERENCES

Adams RP (2001). Identification of essential oils by Capillary Gas Chromatography/Mass Spectroscopy, Allured Publ. Corp., Carol Stream, IL.

Akrout A, El Jani H, Amouri S, Neffati M (2010). Screening of antiradical and antibacterial activities of essential oils of *Artemisia campestris* L., *Artemisia herb*a alba Asso, and *Thymus capitatus* Hoff. Et Link. growing wild in the southern of Tunisia. Res. Sci. Technol., 2: 29-39.

Amrutha VA, Bhaskar VSC (2010). Antioxidative and Antimicrobial Activity of Methanol and Chloroform Extracts of Gmelina *Arborea Roxb*. Int. J. Biotechnol. Biochem., 6: 139-144.

Arvouet-Grand A, Vennat B, Pourrat A, Legret P (1994). Standardisation d'un extrait de propolis et identification des principaux constituants. J. Pharma. Belg., 49: 462-468.

Bauer KD, Garbe H, Surburg H (1997). Common fragrance and flavor materials. Weinheim, Germany: Wiley-VCH Verlag.

Bentes J, Miguel MG, Monteiro I, Costa M, Figueired AC, Barroso JG, Pedro LG (2009). Antioxidant activities of the essential oils and extracts of Portuguese *Thymbra capitata* and *Thymus mastichina*. Ital. J. Food Sci., 21: 183-195.

Biondi D, Cianci P, Geraci C, Ruberto G, Piattelli M (2006). Antimicrobial and chemical composition of essential oils from Sicilian aromatic plants. Flavour Frag. J., 8: 331-337.

Bounatirou S, Smiti S, Miguel MG, Rejeb MN, Neffati M, Costa MM, Faleiro L, Figueiredo AC, Barroso JG, Pedro LG (2007). Chemical composition, antioxidant and antibacterial activities of the essential oils isolated from Tunisian *Thymus capitatus* Hoff. Et Link. Food Chem. 105: 146-155.

Bounatirou S, Smiti S, Miguel MG, Faleiro L, Rejeb MN, Neffati M, Costa MM, Figueiredo AC, Barroso JG, Pedro LG (2010). Thermal stability of the essential oils isolated from Tunisian *Thymus capitatus* Hoff. et link.: effect on the chemical composition and the antioxidant and antibacterial activities. Acta. Aliment, 39: 299-307.

Brand-Williams W, Cuvelier ME, Berset C (1995). Use of free radical method to evaluate antioxidant activity. *Lebensm.-Wiss.* Technol., **28**: 25-30.

Cosentino S, Tuberoso CIG, Pisano B, Mascia E, Arzedi E, Palmas F (1999). *In-vitro* antimicrobial activity and chemical composition of Sardenian *Thymus* essential oils. Lett. Appl. Microbiol., 29: 130-135.

Council of Europe (1996) Pharmacopée Européenne, Maisonneuve S.A (Ed.), Sainte Ruffine, France.

Das NP, Pereira TA (1990). Effects of flavonoids on thermal autooxidation of Palm oil: structure- activity relationship. J. Am. Oil Chem. Soc., 67: 255-258.

Dorman D, Bachmayer O, Kosar M, Hiltunen R (2004). Antioxidant properties of aqueous extracts from selected lamiaceae species grown in Turkey. J. Agric. Food Chem., 52: 762-770.

Fecka I, Raj D, Krauze-Baranowska M (2007). Quantitative determination of four water-soluble compoundsin herbal drug from Lamiaceae using different chromatographic techniques, Chromatographia, 66: 87-93.

Goren AC, Bilsela G, Bilsela M, Demira H, Kocabas EE (2003). Analysis of essential oil of *Coridothymus capitatus (*L.) and its antibacterial and antifungal activity. Z. Naturfo. B., 58: 687-690.

Gülçin İ, Şat İG, Beydemir Ş, Elmastaş M, Küfrevioğlu Öİ (2004). Comparison of antioxidant activity of clove (*Eugenia caryophylata* Thunb) buds and lavender (*Lavandula stoechas* L.). Food Chem., 87: 393-400.

Halliwell B (1995). Antioxidant characterization methodology and mechanism. Biochem Pharmacol., 49: 1341-1348.

Harmandar M, Duru ME, Diri M, Ozler MA, Kivrak I (2009). The chemical composition of essential oil and antioxidant activity of various extracts of Coridothymus capitatus (L.) Reichb. (Proceedings of the First International Medicinal and Aromatic Plants Conference on Culinary Herbs, 2007) Acta. Hortic., 826: 357-362.

Hatano T, Kagawa H, Yasuhara T, Okuda T (1988). Two new flavonoids and other constituents in licore root: their relative astringency and radical scavenging affects Chem. Pharm. Bull., 36: 1090-1097.

Havsteen BH (2002). The biochemisty and medicinal significance of the flavonoids. Pharmacol. Ther., 96: 67-202.

Karousou R, Koureas DN, Kokkini S (2005). Essential oil composition is related to the natural habitats: *Coridothymus capitatus* and *Satureja*

thymbra in NATURA 2000 sites of Crete. Phytochem., 66: 2668-2673.
Ibraliu A, Ristic M, Stefanovic ZD, Shehu J (2011). Analysis of essential oils of three wild medicinal plants in Albania. J. Med. Plant Res., 5: 58-62.
Konig WA, Hochmuth DH, Joulain D (2001). Terpenoids and Related Constituents of Essential Oils., Library of Mass Finder 2.1, Institute of Organic Chemistry, Hamburg, Germany.
Li HB, Wong CC, Cheng KW, Chen F (2008). Antioxidant properties *in vitro* and total phenolic contents in methanol extracts from medicinal plants. Food Sci. Technol., 41: 385-390.
Matkowski A (2008). Plant *in vitro* culture for the production of antioxidants - A review. Biotechnol. Adv., 26: 548-560.
Miceli A, Negro C, Tommasi L (2006). Essential oil variability in *Thymbra capitata* (L.) Cav. growing wild in Southern Apulia (Italy). Biochem. System Ecol., 34: 528-535.
Mkaddem MG, Romdhane M, Ibrahim H, Ennajar M, Lebrihi A,Mathieu F, Bouajila J (2010). Essential Oil of *Thymus capitatus* Hoff. et Link. from Matmata, Tunisia: Gas Chromatography-Mass Spectrometry Analysis and Antimicrobial and Antioxidant Activities. J. Med. Food, 13: 1500-1504.
Napoli EM, Curcuruto G, Ruberto G (2010). Screening to the essential oil composition of wild Sicilian thyme. Biochem. System. Ecol., 38: 816-822.
Paolini J, Costa J, Bernardini AF (2005) Analysis of the essential oil from aerial parts of *Eupatorium cannabinum* subsp. *corsicum* (L.) by gas chromatography with electron impact and chemical ionization mass spectrometry, J. Chromatogr. A., 1076: 170-178.
Paolini J, Costa J, Bernardini AF (2007) Analysis of the essential oil from the roots of E*upatorium cannabinum* subsp. c*orsicum* (L.) by GC, GC-MS and ^{13}C-NMR. Phytochem. Anal., 18: 235-244.
Pourmorad F, Hosseinimehr SJ, Shahabimajd N (2006). Antioxidant activity, phenol and flavonoid contents of some selected Iranian medicinal plants. Afr. J. Biotechnol., 5: 1142-1145.
Safaei-Ghomi J, Ebrahimabadi AH, Djafari-Bidgoli Z, Batooli H (2009). GC/MS analysis and *in vitro* antioxidant activity of essential oil and methanol. extracts of *Thymus caramanicus* Jalas and its main constituent carvacrol Food Chem., 115: 1524-1528.
Salah-Fatnassi KBH, Slim-Bannour A, Harzallah-Skhiri F, Mahjoub MA, Mighri Z, Chaumont JP, Aouni M (2010). Antiviral and antioxidant activities *in vitro* of essential oils of *Thymus capitatus* (L.) Hoffmans and Link from Tunisia. Acta. Bot. Gall., 157: 433-444.
Sharma OP, Bhat TK (2009). DPPH antioxidant assay revisited. Food Chem., 113: 1202-1205.
Shimoi K, Masuda S, Shen B, Furugori B, Kinae N (1996). Radioprotective effect of antioxidant plant flavonoids in mice. Mutat. Res., 350: 153-161.
Slinkard K, Singleton VL (1977). Total phenol analysis: automation and comparison with manual methods. Am. J. Enol. Vitic., 28: 49-55.
Tutin G, Heywood H, Burges H, Moore M, Valentine H, Walters M, Webb M (1968-1993). Flora Europaea, vol. 1-4, Cambridge University Press, Cambridge, Mass.
Usai M, Foddai M, Sechi B, Juliano C, Marchetti M (2010). Comparison of antibacterial activity of natural and hydroformylated essential oil of *Thymus capitatus* growing wild in North Sardinia with commercial *Thymus* essential oils. Nat. Prod. Commun., 5: 1985-1989.