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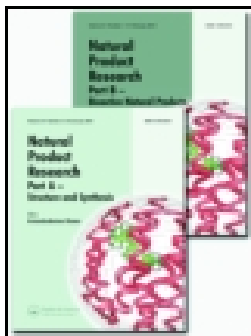
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Thymus catharinae Camarda essential oil: β -cyclodextrin inclusion complexes, evaluation of antimicrobial activity

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ABSTRACT

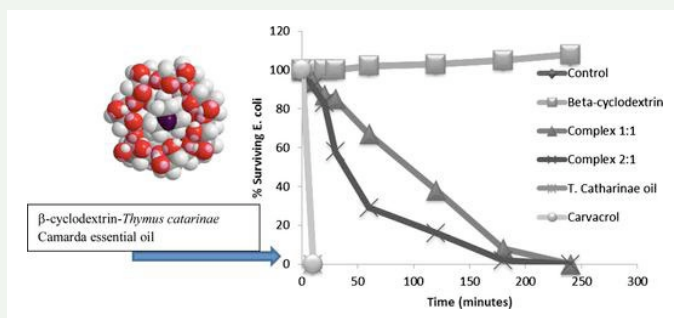
An efficient antimicrobial activity was evidenced in a complex β -cyclodextrin–essential oil of *Thymus catharinae* Camarda (carvacrol chemotype). The release of carvacrol with respect to the antimicrobial activity was calculated as function of time. The β CD-complex of the bioactive agent was obtained by a simple, efficient and non-expensive method without purification of the carvacrol chemotype essential oil. According to the starting stoichiometry of β -cyclodextrin with respect to carvacrol, two inclusion complexes were produced, 1:1 and 2:1, respectively. The results demonstrate that, although the antimicrobial activity of the essential oil of *T. catharinae* Camarda is remarkable but acts too quickly in some types of application, its inclusion in a bio-matrix allows a slower release and improves its effectiveness.

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Thymus catharinae Camarda; essential oil; carvacrol; antimicrobial; *E. coli*; *C. albicans*; cyclodextrin inclusion complex



1. Introduction

Essential oils isolated from plants are well known in ethno-medicine because of their different bioactivities, *i.e.* antimicrobial (Edris 2007; Bakkali et al. 2008; Nerio et al. 2010; Verma et al. 2010; Cruz-Galvez et al. 2013).

The essential oils of *Thymus* species are used for their aromatic, medicinal and agricultural properties (Maggi et al. 2014). Among the others, they find application as culinary herb,

flavour, as food preservatives, scent, natural antioxidant, natural insecticides and antibacterial (Vardar-Ünlü et al. 2003; Usai et al. 2010; Maissa & Walid 2015).

Among the *Thymus* species, *Thymus catharinae* Camarda (Camarda 2003) is of particular interest. It is a pulvinate, woody suffrutescent aromatic plant and until 2003 was referred to as *T. herba-barona* Loisel and considered as a Sardinian and Corsican endemism (Camarda & Valsecchi 1990).

In 2003, botanical studies (Camarda 2003) indicated that this species differs from *T. herba-barona* Loisel and must be considered as a Sardinian endemism; it grows in mountain and sub-mountain zones between 800 and 2000 m and prefers schistose and granitic substrates. Sardinian *T. herba-barona* has been already studied with respect to its phytochemical profile. The composition of its oil has a seasonal variability and possess antimicrobial activity. (Corticchiato et al. 1998; Cosentino et al. 1999; Juliano et al. 2000; Usai et al. 2003). According to previously reported studies, *T. catharinae* Camarda is moderately rich in essential oil. This oil has expectorant and antiseptic properties, it is used in folk medicine as diaphoretic, sedative, antiseptic and anti-mycotic (Atzei 2003).

It is well known that essential oils of Mediterranean area are a source of bioactive compounds with interesting properties (Araújo et al. 2003; Bakkali et al. 2008; Gholivand et al. 2013) and particularly Sardinia is a privileged setting where *Thymus* genus is present with only two species.

It is known that some essential oils have good biological activities but, in general, they are volatile and tend to deteriorate depending on the temperature. Furthermore, they are poorly soluble in water limiting their use in biological systems. For practical application they should be used at concentration much higher than that in which they are active.

To overcome these limitations, ideally the essential oil should be entrapped in such way that it is protected from degradation and slowly released. Between the various available compounds potentially useful in this respect, cyclodextrins are of great interest. Cyclodextrins are cyclic oligosaccharides consisting of six α -cyclodextrin, seven β -cyclodextrin, eight γ -cyclodextrin or more glucopyranose units linked by α -(1,4) bonds. They are produced as a result of intramolecular transglycosylation reaction from degradation of starch by cyclodextrin glucanotransferase (CGTase) enzyme (Szejtli 1998). β -Cyclodextrin is the most accessible, due to its lower price and its availability.

Moreover, all toxicity studies have demonstrated that orally administered cyclodextrins are practically non-toxic due to lack of absorption from the gastrointestinal tract (Irie & Uekama 1997). Inclusion complexes consist of steric and electrostatic interaction between the torus-like macro ring of β -cyclodextrin (β CD) (host) and the active agent (guest) that is suitable for the lipophilic cavity of the bio-matrix.

In previous papers, we described the essential oil composition of Sardinian *T. catharinae* Camarda (ex *T. herba-barona* Loisel) and its antibacterial activity; moreover, in that study we reported the predominance of carvacrol (66%).

In the present paper, we describe the preparation of two inclusion complexes with β -CD and *T. catharinae* essential oil. Moreover, we have investigated the antimicrobial effect over as function of the time.

The interest of these complexes is that they maintain the antimicrobial efficacy of the product included, but they avoid its volatilization into the environment and modulate its action, since the active components are released from the inclusion complex in a prolonged time and produce long-term effects. When included in β CD, *T. catharinae* essential oil is well

preserved and is conveniently transformed into an easily manageable powder. The powders resulting from this inclusion can find application in industrial sectors related to agriculture (insecticides, herbicides and repellents) food, cosmetic and pharmaceutical industries, and in human and veterinary medicine.

2. Results and discussion

In order to make the methodology of controlled release more valuable, and to overcome problems related to purification of the bioactive agent from the other components of the essential oil, we decide to prepare an inclusion complex by using β CD and a carvacrol chemotype *T. catharinae* essential oil (Table 1) (Yan et al. 2005; de Carvalho & da Fonseca 2006).

^1H NMR spectroscopy, which has proved to be a good diagnostic tool in the study and characterisation of β CD inclusion complexes, was used to verify the true formation of the β CD-carvacrol inclusion complex and to measure the host-guest molar ratio. All ^1H NMR analyses were carried out in deuterated water. Since the β CD-carvacrol complex has a reasonable solubility in water (>2 mM at 20°C) while carvacrol is insoluble in water, formation of the inclusion complex in water assures the absence of free carvacrol in the mixture. We carried out inclusion of the carvacrol into the β CD in water at 60°C . At our conditions, the other components of the essential oil do not form inclusion complex with β CD, but they might still be present in the environment as supramolecular complex or aggregates (Duan et al. 2005).

Significant highfield shift for H_3 and H_5 β CD proton resonance in the β CD-carvacrol complex with respect to the free β CD have been measured (Mulinacci et al. 1996; Locci et al. 2004). According to the starting stoichiometry of β CD respect to carvacrol, main constituent of the essential oil, two inclusion complexes have been achieved in ratio 2:1 and 1:1, respectively (Schneider et al. 1998). Chemical shift variation of H_3 and H_5 of β CD protons indicate the formation of a true inclusion complex. In fact, the entry of the apolar guest (carvacrol) into the lipophilic cavity of the host (β CD) induces a significant shielding to high-field of H_3 and H_5 as reported as below: ($\Delta\delta$ represents the chemical shift differences in ppm between the two states. Negative values indicate shift to high field). H_1 : β CD = 5.049, β CD-carvacrol complex = 4.988, $\Delta\delta = -0.061$; H_2 : β CD = 3.629, β CD-carvacrol complex = 3.579, $\Delta\delta = -0.050$; H_3 : β CD = 3.944, β CD-carvacrol complex = 3.854, $\Delta\delta = -0.090$; H_4 : β CD = 3.564, β CD-carvacrol complex = 3.520, $\Delta\delta = -0.044$; H_5 : β CD = 3.841, β CD-carvacrol complex = 3.685, $\Delta\delta = -0.156$; H_6 : β CD = 3.857, β CD-carvacrol complex = 3.755, $\Delta\delta = -0.102$.

Small but significant chemical shift variations have been observed for the other protons, we do not exclude an interaction with the minor components of the essential oil and the external wall of β CD torus. Furthermore, it would be noticed the significant H_6 proton shielding to high-field whose value is comparable to that of H_3 and H_5 . In such a way, a strong interaction between the guest and H_6 proton which point to the small rim of the β CD should be considered.

The stoichiometry of the inclusion complex (host-guest 2:1 and 1:1) was determined by integration of the proton signals in the ^1H NMR spectra. Although the ^1H NMR spectra of the two complexes of different host-guest ratio shown very similar chemical shift, different release of carvacrol can be observed both in 2:1 and 1:1 β CD-carvacrol complexes, respectively.

Table 1. Chemical composition of essential oil from *T. catharinae* Camarda collected in Punta Balestreri.

Compounds	KI	%
3-Heptanone	880	2.2
α -Thujene	931	0.3
α -Pinene	939	0.1
6-Methyl-3-heptanone	941	0.5
Camphene	953	0.2
3-Octanone	986	3.6
Myrcene	991	0.4
3-Octanol	993	2.8
α -Terpinene	1018	0.6
p-Cymene	1028	2.4
Limonene	1031	0.1
1-8-Cineole	1033	3.0
γ -Terpinene	1062	5.3
cis-Sabinene hydrate	1068	0.2
3-Nonanone	1089	0.4
3-Nonanol	1090	0.3
Linalool	1098	3.2
Borneol	1165	2.5
Terpinen-4-ol	1177	0.9
Linalyl propionate	1221	0.3
cis-Carveol	1229	0.4
Thymol methyl ether	1235	0.2
Pulegone	1237	0.5
Thymol	1290	1.4
Carvacrol	1297	66
Dihydro carveol acetate	1305	0.1
Carvacrol acetate	1371	0.3
β -Caryophyllene	1418	1.1
epi-a-Cadinol	1640	0.1
α -Cadinol	1653	0.1
Total		99.5

Table 2. Antimicrobial activity (M.I.C.s in mg/mL) of commercial carvacrol and *T. catharinae* Camarda oil.

	<i>Escherichia coli</i> (mg/mL)	<i>Candida albicans</i> (mg/mL)
β -CD	>15	>15
Carvacrol	0.250	0.125
o.e. <i>Thymus catharinae</i>	0.250	0.250
β -CD-oil 1:1	>15	>15
β -CD-oil 2:1	>15	>15
β -CD-carvacrol 2:1	>15	>15

It should be taken into account that a large excess of essential oil with respect to β CD (4:1 equivalents) do not improve the ratio guest–host and the yield of the β CD-carvacrol.

The complex β CD/essential oil is stable at the solid state; equimolar host–guest ratio was observed for complex 1:1 even after 6 months storage at room temperature in a closed dark bottle. Previous studies demonstrated that the essential oil of *T. herba-barona* is effective in microbicidal activity used in these assays in very short times. Carvacrol has also a very quick microbicidal activity (Juliano et al. 2000). In order to compare the antimicrobial activities of the essential oil of *T. catharinae* Camarda and the commercial carvacrol, we carried out some test against *Escherichia coli* and *Candida albicans* and the results are reported in Table 2.

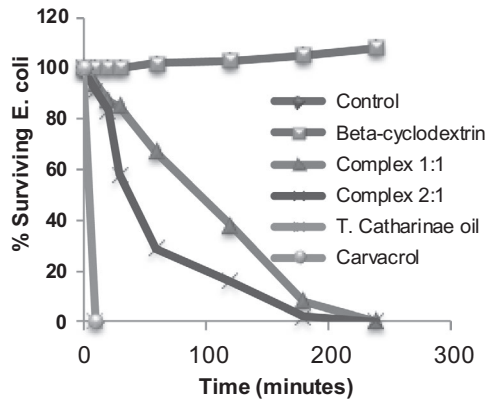


Figure 1. Percentages of surviving *E. coli* after different exposure time to β CD-oil complex, *T. catharinae* Camarda and commercial carvacrol (see text for concentrations; *T. catharinae* Camarda oil and carvacrol lines are superimposed)

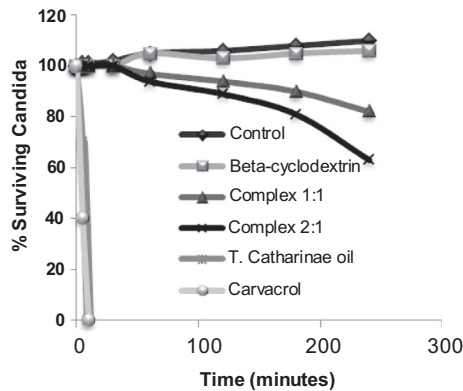


Figure 2. Percentages of surviving *C. albicans* after different exposure time to β CD-oil complex (see text for concentrations)

The β CD/essential oil inclusion complex showed a very slow killing kinetics vs. *E. coli* and, to a lesser extent, *C. albicans* (Figures 1 and 2). It is noteworthy that, with both microorganisms, killing curve relative to complex 2:1 has a higher slope, showing a greater rate of killing. This behaviour may be related to a quicker delivery of carvacrol from 2:1 complex compared to 1:1 complex.

3. Experimental

3.1. Plant material

T. catharinae Camarda was collected at flowering stage (July 2013) in North Sardinia (Punta Balestreri).

M. Usai identified the analysed plants. Voucher specimens have been deposited at the Herbarium SASSA of the Dipartimento di Chimica e Farmacia, University of Sassari under a collective number (no. 1077 bis) for all *T. catharinae* Camarda samples.

3.2. Oil distillation and yield

The plant material was submitted for 4 h to hydrodistillation using a Clevenger-type apparatus; the reached yield was 0.44% (w/w). The oil was dried over anhydrous sodium sulphate and stored at $-20\text{ }^{\circ}\text{C}$ until analysed.

3.3. Oil analyses

3.3.1. Gas chromatography

Three replicates of each sample were analysed by using a Hewlett-Packard Model 5890A GC equipped with a flame ionisation detector and fitted with a $60\text{ m} \times 0.25\text{ mm}$, thickness $0.25\text{ }\mu\text{m}$ AT-5 fused silica capillary column (Alltech). Injection port and detector temperature were $280\text{ }^{\circ}\text{C}$. The column temperature was programmed from $50\text{ }^{\circ}\text{C}$ to $135\text{ }^{\circ}\text{C}$ at $5\text{ }^{\circ}\text{C}/\text{min}$ (1 min), $5\text{ }^{\circ}\text{C}/\text{min}$ up $225\text{ }^{\circ}\text{C}$ (5 min), $5\text{ }^{\circ}\text{C}/\text{min}$ up $260\text{ }^{\circ}\text{C}$ and held for 10 min. The samples ($0.1\text{ }\mu\text{L}$ each), generally analysed without dilution (using 2,6-dimethylphenol as internal standard) were injected using a split/splitless automatic injector HP 7673 and using helium as the carrier gas. The data reported in Table 1 are the average of three GC injections. The quantitation of each compound was expressed as absolute weight percentage using internal standard and response factors (RFs). The detector RFs were determined for key components relative to 2,6-dimethylphenol and assigned to other components on the basis of functional group and/or structural similarity.

3.3.2. Gas chromatography/Mass spectrometry

MS analyses were carried out with a Agilent Technologies model 7820A connected with a MS detector 5977E MSD (Agilent), and using the same conditions and column described above. The column was connected with the ion source of the mass spectrometer. Mass units were monitored from 10 to 900 at 70 eV. The identification of compounds was based on comparison of their retention times with those of authentic samples and/or by comparison of their mass spectra with those of published data (Nist Library Mass spectra) or on the interpretation of the EI-fragmentation of the molecules.

3.4. Preparation of the inclusion complex

βCD (CAVAMAX $^{\circ}$ 7 PHARMA) was obtained from Wacker – Chemie Italia SpA. Commercial carvacrol (technical grade) was purchased from Sigma-Aldrich and used as received. $^1\text{H-NMR}$ spectra of βCD complex were recorded in D_2O solution with a Varian VXR 5000 spectrometer at 299.94 MHz. Chemical shifts are given in ppm (δ) which were measured relative to the peak of the solvent D_2O (4.74 ppm). Multiplicity is indicated by d (doublet), t (triplet), m (multiplet) or dd (double of doublets). All ^1H NMR spectra were recorded with a 5 mm tube in D_2O , without degassing.

To prepare inclusion complex βCD , analytic grade, was dissolved as a 16 mM solution in degassed distilled water under an inert atmosphere at $70\text{ }^{\circ}\text{C}$ for 1 h. Then, one equivalent of essential oil of *T. catharinae* Camarda (one equivalent respect to carvacrol contents) was added in one pot and the reaction mixture was heated at $60\text{ }^{\circ}\text{C}$ under stirring for 12 h. At different time, an aliquot of the mixture was analysed by ^1H NMR (in D_2O) in order to check reaction completeness. In a few hours, the solution became opalescent, turned milky and

a precipitate was observed in the reaction mixture. After 12 h at 60 °C, the precipitate was filtered off the solution at room temperature to obtain the inclusion complex as a pale yellow solid. A 2:1 molecular ratio β CD-carvacrol was detected by ^1H NMR spectrum of the solid (70% yield). Interestingly, an equimolar ratio host–guest was calculated in the precipitate (90% yield) by ^1H NMR when two equivalent of essential oil of *T. catharinae* Camarda (two equivalent respect to carvacrol contents) and one equivalent of β CD was used as starting stoichiometry. In both preparations (2:1 and 1:1, respectively), the inclusion complex has been characterised by NMR analysis.

3.5. Characterisation of inclusion complex

^1H NMR (unbuffered D_2O , δ): 1.19 (d, 6H, $J = 7.2$ Hz, $-\text{CH}_3$); 2.12 (bs, 3H, $-\text{CH}_3$); 2.75 (m, 1H, $-\text{CH}$); 3.52 (t, $J = 9.0$ Hz, 7H, $\beta\text{CD}-\text{H}_4$); 3.57 (dd, $J = 3.3, 9.0$ Hz, 7H, $\beta\text{CD}-\text{H}_2$); 3.68 (d, 7H, $J = 10.0$ Hz, $\beta\text{CD}-\text{H}_5$); 3.75 (s, 14H, $\beta\text{CD}-\text{H}_6$); 3.85 (t, $J = 10.0$ Hz, $\beta\text{CD}-\text{H}_3$); 5.99 (d, $J = 3.3, 7\text{H}$, $\beta\text{CD}-\text{H}_1$); 6.57 (s, 1H, aromatic); 6.60 (d, $J = 6.0$ Hz, 1H, aromatic); 6.92 (d, $J = 6.0$ Hz, 1H, aromatic).

3.6. Antimicrobial activity of carvacrol and essential oil

The antimicrobial activities of commercial carvacrol and of *T. catharinae* essential oil were previously evaluated as minimum inhibitory concentration (MIC) against *E. coli* ATCC 25922 and *C. albicans* ATCC 10231 by an agar dilution technique (Barry 1986).

Carvacrol and *Thymus* oil solutions were prepared at 10% w/vol in PEG 200 (Sigma) and subjected, in Petri plates, to twofold serial dilutions (2–0.0039 mg/mL) in molten Mueller Hinton Agar (Oxoid) or Sabouraud Dextrose Agar (Oxoid) for *E. coli* and *C. albicans*, respectively. Plates were then seeded with about 1×10^5 bacteria or 10^3 – 10^4 conidia and incubated at 35 °C for 24 h, for bacteria, or at 25 °C for 4 days, for yeasts. MICs were recorded as the lowest concentration which completely inhibit bacterial growth.

3.7. Killing time

Antimicrobial activities of the complexes β -cyclodextrin-essential oil were characterised by a 'killing time' assay, i.e. by evaluating the contact time required by a suitable complex amount to kill, in a liquid medium, a standardised microbial inoculum. The test was performed with the above-mentioned strains of *E. coli* and *C. albicans*. Microorganisms in the logarithmic phase of growth were centrifuged, washed in phosphate buffered saline (PBS, pH 7.3; Oxoid) and resuspended at the density of 5×10^5 – 1×10^6 cfu/mL in appropriate volumes of PBS containing aliquots of each complex corresponding to inhibitory concentrations of carvacrol (for *E. coli*, 85.5 mg of 1:1 complex in 10 mL and 165.2 mg of 2:1 complex in the same buffer volume, corresponding to 1 mg/mL of carvacrol; for *C. albicans*, 42.8 mg of 1:1 complex in 10 mL and 82.6 mg of 2:1 complex in 10 mL, both corresponding to 0.5 mg/mL of carvacrol). Control tubes (bacteria suspended in PBS and in PBS + cyclodextrin) were included in each assay. At time zero and after 30, 60, 120, 180 and 240 min of incubation of the mixtures at 37 °C, 0.5 mL were removed, subjected to serial 10-fold dilutions in PBS and inoculated in Tryptone Soya Agar or Sabouraud Dextrose Agar (for *E. coli* and *C. albicans*, respectively). The number of viable microorganisms at each time was evaluated counting colonies after incubation for 24 h at 35 °C or for 4 days at 25 °C.

For *E. coli*, 'Killing time' test were also performed with commercial carvacrol (at 1 mg/mL) and with *Thymus* essential oil (a concentration of 15.4 $\mu\text{L/mL}$, corresponding to 1 mg of carvacrol/mL on the basis of previous analyses).

Conclusion

βCD allows the preparation of inclusion complex of essential oil of *T. catharinae* Camarda by a simple, efficient and non-expensive method, without any covalent or ionic bond between the bio-matrix and the guest molecule, as well as without any purification of the carvacrol. This last point, beside water solubility and toxicity free, is one of main advantage of βCD compared to other hosts.

Compared with the preparation of βCD -carvacrol complex starting from commercial (technical grade) carvacrol (Griffin et al. 2000) our preparation allows to obtain, after simple precipitation from water, two different complexes according to the starting stoichiometry of carvacrol present in the essential oil. We were able to include into the βCD only the main constituent of the essential oil. Other components of the essential oil do not form inclusion complexes with βCD , but they might still present as supramolecular complex or aggregates.

Our results demonstrate that the antimicrobial activity of essential oil of *T. catharinae* Camarda is efficiently modulated by oil complexation in a biomatrix.

Our preparation takes advantage of two key aspects: economic and environmental sustainability. In fact, we prepared the βCD -carvacrol complex in water starting from the essential oil and we can detect the formation of complex by $^1\text{H NMR}$ in D_2O without the use of an organic solvent at any preparation step.

Investigations on the inclusion of other essential oils of the Mediterranean area as well as other *T. catharinae* Camarda, carvacrol chemotype, are currently underway.

Disclosure statement

No potential conflict of interest was reported by the authors.

References

- Araújo C, Sousa MJ, Ferreira MF, Leão C. 2003. Activity of essential oils from Mediterranean *Lamiaceae* species against food spoilage yeasts. *J Food Prot.* 66:625–632.
- Atzei AD. 2003. In: Carlo Delfino editor. *Le piante nella tradizione popolare della Sardegna*. Sassari, Italy: Carlo Delfino publisher; p. 247–249
- Bakkali F, Averbeck S, Averbeck D, Domar M. 2008. Biological effects of essential oils – a review. *Food Chem Toxicol.* 46:446–475.
- Barry AL. 1986. Procedure for testing antimicrobial agents in agar media: theoretical considerations. In: Lorian V, editor. *Antibiotics in laboratory medicine*. 2nd ed. Baltimore (MD): Williams & Wilkins; p. 1–26.
- Camarda I. 2003. *Thymus catharinae* (*Lamiaceae*), *Dianthus stellaris* (*Caryophyllaceae*), *Rubus limbarae* (*Rosaceae*) species novae di Sardegna. *Parlatorea.* 6:83–93.
- Camarda I, Valsecchi F. 1990. *Thymus*. In: Carlo Delfino, editor. *Piccoli arbusti, liane e suffrutici spontanei della Sardegna*. Sassari: Delfino; p. 215–217.
- Corticchiato M, Tomi F, Bernardini AF, Casanova J. 1998. Composition and infraspecific variability of essential oil from *Thymus herba barona* Lois. *Biochem Syst Ecol.* 26:915–932.
- Cosentino S, Tuberoso CI, Pisano B, Satta M, Mascia V, Arzedi E, Palmas F. 1999. *In-vitro* antimicrobial activity and chemical composition of Sardinian *Thymus* essential oils. *Lett Appl Microbiol.* 29:130–135.

- Cruz-Galvez AM, Gómez-Aldapa CA, Villagómez-Ibarra JR, Chavarría-Hernández N, Rodríguez-Baños J, Rangel-Vargas E, Castro-Rosas J. 2013. Antibacterial effect against foodborne bacteria of plants used in traditional medicine in central Mexico: studies *in vitro* and in raw beef. *Food Control*. 32:289–295.
- De Carvalho CCCR, Da Fonseca MMR. 2006. Carvone: why and how should one bother to produce this terpene. *Food Chem*. 95:413–422.
- Duan MS, Zhao N, Össurardóttir ÍB, Thorsteinsson T, Loftsson T. 2005. Cyclodextrin solubilization of the antibacterial agents triclosan and triclocarban: formation of aggregates and higher-order complexes. *Int J Pharm*. 297:213–222.
- Edris AE. 2007. Pharmaceutical and therapeutic potentials of essential oils and their individual volatile constituents: a review. *Phytother Res*. 21:308–323.
- Gholivand MB, Piryaei M, Papzan A. 2013. Evaluation effect of microwave irradiation on the amount of volatile compounds, monoterpenes and sesquiterpenoids from *Thymus kotschyanus* Boiss with four methods. *Nat Prod Res*. 27:1228–1231.
- Griffin SG, Markham JL, Leach DN. 2000. An agar dilution method for the determination of the minimum inhibitory concentration of essential oils. *J Essent Oil Res*. 12:249–255.
- Irie T, Uekama K. 1997. Pharmaceutical applications of cyclodextrins. III. Toxicological issues and safety evaluation. *J Pharm Sci*. 86:147–162.
- Juliano CA, Mattana A, Usai M. 2000. Composition and *in vitro* antimicrobial activity of the essential oil of *Thymus herba-barona* Loisel. growing wild in Sardinia. *J Essent Oil Res*. 12:516–522.
- Locci E, Lai S, Piras A, Marongiu B, Lai A. 2004. ¹³C-CPMAS and ¹H-NMR study of the inclusion complexes of β-Cyclodextrin with carvacrol, thymol, and eugenol prepared in supercritical carbon dioxide. *Chem Biodivers*. 1:1354–1366.
- Maggi F, Caprioli G, Papa F, Sagratini G, Vittori S, Kolarčík V, Mártonfi P. 2014. Intra-population chemical polymorphism in *Thymus pannonicus* All. growing in Slovakia. *Nat Prod Res*. 28:1557–1566.
- Maissa BJ, Walid H. 2015. Antifungal activity of chemically different essential oils from wild *Tunisian Thymus* spp. *Nat Prod Res*. 29:869–873
- Mulinacci N, Melani F, Vincieri FF, Mazzi G, Romani A. 1996. ¹H-NMR NOE and molecular modelling to characterize thymol and carvacrol β-cyclodextrin complexes. *Int J Pharm*. 128:81–88.
- Nerio LZ, Olivero-Verbel J, Stashenko E. 2010. Repellent activity of essential oils: a review. *Bioresour Technol*. 101:372–378.
- Schneider HJ, Hacket F, Rüdiger V, Ikeda H. 1998. NMR studies of cyclodextrins and cyclodextrin complexes. *Chem Rev*. 98:1755–1786.
- Szejtli J. 1998. Introduction and general overview of cyclodextrin chemistry. *Chem Rev*. 98:1743–1754.
- Usai M, Atzei A, Pintore G, Casanova J. 2003. Composition and variability of essential oil of Sardinian *Thymus herba-barona* Loisel. *Flavour Frag J*. 18:21–25.
- 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.
- Vardar-Ünlü G, Candan F, Sökmen A, Daferera D, Polissiou M, Sökmen M, Dönmez E, Tepe B. 2003. Antimicrobial and antioxidant activity of the essential oil and methanol extracts of *Thymus pectinatus* Fisch. et Mey. Var. *pectinatus* (*Lamiaceae*). *J Agric Food Chem*. 51:63–67.
- Verma RS, Padalia RC, Chanotiya CS, Chauhan A. 2010. Chemical investigation of the essential oil of *Thymus linearis* (Benth. ex Benth) from western Himalaya, India. *Nat Prod Res*. 24:1890–1896.
- Yan JZ, Chen G, Tong SQ, Feng YP, Sheng LQ, Lou JZ. 2005. Preparative isolation and purification of germacrene and curdione from the essential oil of the rhizomes of *Curcuma wenyujin* by high-speed counter-current chromatography. *J Chromatogr A*. 1070:207–210.