

Assessment of the antioxidant ability of *Thymus albicans*, *Th. mastichina*, *Th. camphoratus* and *Th. carnosus* essential oils by TBARS and Micellar Model systems

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ABSTRACT: The essential oils isolated by hydrodistillation from different populations of *Thymus albicans*, *Th. mastichina*, *Th. carnosus* and *Th. camphoratus*, collected during the vegetative phase were analysed by GC and GM-MS and used in antioxidant activity evaluation using the TBARS and micellar model systems. *Th. albicans* oils were 1,8-cineole-rich as well as two of the oils from *Th. mastichina* populations, the third one being linalool rich. *trans*-Sabinene hydrate, borneol and linalool in similar amounts and 1,8-cineole were the main components of the oils from each of the three populations from *Th. camphoratus*. *Th. carnosus* oils were mainly constituted by borneol. All essential oils revealed some degree of antioxidant activity either in the presence or absence of ABAP, the micellar model system showing higher antioxidant indexes than those obtained by the TBARS method.

KEY WORDS: *Thymus albicans*, *Thymus mastichina*, *Thymus camphoratus*, *Thymus carnosus*, essential oils, antioxidant activity

Introduction

The antioxidant food additives butylated hydroxyanisol (BHA), butylated hydroxytoluene (BHT) and propyl gallate have been considered as possessing some toxicological effects [1-3]. This fact, together with the demand of the consumers has increased the interest for the use and research of natural compounds obtained from different sources. The essential oils or their main compounds isolated from several aromatic plants of different origins have been the target of many studies, aiming at using them as potential antioxidants [4-16]. Nevertheless, the great complexity of the essential oils composition along with the different methods that can be used for detection of antioxidant activity makes problematical the comparison of the results.

The present study gives continuity to previous work on the evaluation of the antioxidant capacity of the essential oils isolated from the Iberian Peninsula endemic species of the *Thymus* genus. The essential oils of some of these species reveal to possess a great chemical diversity [17-19]. Our preceding work using different methodologies and essential oils isolated from *Thymus mastichina*, *Th. albicans*, *Th. carnosus*, *Th. camphoratus* and *Th. caespititius* and *Thymbra capitata* demonstrated their relative antioxidant ability when compared to compounds generally used in food industry as antioxidant additives [20-22]. In this work the antioxidant activities of the essential oils of three populations of each species of *Th. mastichina*, *Th. albicans*, *Th. carnosus* and *Th. camphoratus*, collected in different regions of Portugal, was evaluated and compared with those of BHT, BHA and α -tocopherol. The antioxidant activity of the essential oils was evaluated and compared, by measuring the formation of primary (hydroperoxydienes) and secondary (malonaldehyde) components of the oxidative process of a lipid matrix, using TBARS and the micelar model system, respectively.

Material and Methods

Plant material. The aerial parts of *Th. albicans* Hoffmanns. & Link. (*Ta*) collected in Loulé (Ll), Ludo (Ld) and Quinta do Marim (QM); *Thymus mastichina* (L.) L. subsp. *mastichina* (*Tm*) collected in Mértola (M), Vila Real de Santo António (VA) and Sesimbra (S); *Th. camphoratus* Hoffmanns. & Link (*Tcm*) collected in Cabo de S. Vicente (CV), Aljezur (A) and Lagos (L) and *Th. carnosus* Boiss. (*Tcr*) collected in Tróia (T), Alcácer do Sal (AS) and Sines (S), during the vegetative phase (February 2003) were used for isolation of the essential oils. For each species, the

collective sample was constituted by a mixture of 12-15 individual plants.

Essential oil isolation procedure. The essential oils of each collective sample were isolated from fresh plant material (100g) by hydrodistillation, for four hours, using a Clevenger-type apparatus [23].

Gas chromatography. Gas chromatography analyses were performed using a Perkin Elmer 8700 gas chromatograph equipped with two FIDs, a data handling system and a vaporising injector port into which two columns of different polarities were installed: a DB-1 fused-silica column (30m x 0.25mm i. d., film thickness 0.25 µm; J & W Scientific Inc., Rancho Cordova, CA, USA) and a DB-17HT fused-silica column (30m x 0.25mm i. d., film thickness 0.15 µm; J & W Scientific Inc.). Oven temperature was programmed, 45-175°C, at 3°C/min, subsequently at 15°C up to 300°C, and then held isothermal for 10 min; injector and detector temperatures, 280°C and 290°C, respectively; carrier gas, hydrogen, adjusted to a linear velocity of 30cm/s. The samples were injected using split sampling technique, ratio 1:50. The percentage composition of the oils was computed by the normalisation method from the GC peak areas, calculated as mean values of two injections from each oil, without using correction factors.

Gas chromatography-mass spectrometry. Gas chromatography-mass spectrometry unit consisted of a Carlo Erba 6000 Vega gas chromatograph, equipped with a DB-1 fused-silica column (30m x 0.25mm i. d., film thickness 0.25 µm; J & W Scientific Inc.), and interfaced with a Finnigan MAT 800 Ion Trap Detector (ITD; software version 4.1). Oven temperature was as above; transfer line temperature, 280°C; ion trap temperature, 220°C; carrier gas, helium, adjusted to a linear velocity of 30cm/s; splitting ratio, 1:40; ionisation energy, 70 eV; ionisation current, 60 µA; scan range, 40-300u; scan time, 1 s. The identity of the components was assigned by comparison of their retention indices, relative to C₉-C₁₇ *n*-alkanes, and GC-MS spectra with corresponding data of components of reference oils, laboratory-synthesized components and commercial available standards from an home-made library.

Antioxidant activity measurement

TBARS assay: Two sets of experiments based on a modified thiobarbituric acid (TBA) reactive species assay (TBARS) were used to measure the antioxidant ability of the sample (essential oils or tested substances): without (1) and with (2) a lipid peroxidation inducer. In both cases egg yolk

homogenate was used as lipid-rich media obtained as described by Dorman *et al.* [24], that is, an aliquot of yolk material was made up to a concentration of 10% (w/v) in KCl (1.15%, w/v). The yolk was then homogenized for 30s followed by ultrasonication for further 5min. For set (1) of TBARS assay, 500µl of the homogenate and 100µl of sample, solubilized in methanol, were added to a test tube and made up to 1ml with distilled water, followed by addition of 1.5ml 20% acetic acid (pH 3.5) and 1.5ml 0.8% (w/v) TBA in 1.1% (w/v) sodium dodecyl sulphate (SDS). Each essential oil and tested substance was assayed in the concentrations of 1000, 800, 640, 320 and 160mg.l⁻¹. This mixture was stirred in a vortex, and heated at 95°C for 60min. After cooling, at room temperature, 5ml butan-1-ol was added to each tube, stirred and centrifuged at 3000rpm for 10min. The absorbance of the supernatant was measured at 532nm using a spectrophotometer Shimadzu 160-UV. All the values are expressed as antioxidant index (AI%), whereby the control is completely peroxidized and each oil and tested substance demonstrated a comparative percentage of antioxidant protection. The AI% was calculated using the formula: $(1-t/c) \times 100$, c being the absorbance value of the fully oxidized control and t, the absorbance of the tested sample.^{4,5} For set (2) of TBARS assay, 50µl of 2,2'-azobis-(2-amidinopropane) dihydrochloride (ABAP) (0.07M) was added to induce lipid peroxidation, soon after the addition of sample, the remaining procedure being as reported above.

Micellar model system: The samples were analysed according to the method of Ruberto *et al.* and Fogliano *et al.* [7,25] with slight modifications. Fifteen millilitres of the micellar suspension of linoleic acid were prepared with 0.1 M solution of SDS set in aqueous 0.01 M phosphate buffer (pH 7.4) and linoleic acid to a concentration of 0.026M. The micellar suspension was stirred in a reactor at 50°C. After equilibration for 10min, 75µl of 0.07M ABAP in water was added and peroxidation was monitored at 234nm. After 20min, 75µl of the sample (32mg.ml⁻¹) dissolved in methanol was added and the kinetics was monitored for the following 20min. The buffered SDS solution was used as a blank. The ratio between the slope of the linear plot of absorbance versus time after and before the addition of the sample gave the antioxidant index (AI%), $AI\% = [1 - (S_{inh}/S_{ABAP})] \times 100$, where S_{inh} and S_{ABAP} are the slopes after and before the addition of the sample solution respectively.

Results

Composition of the essential oils

From the four species studied, *Th. albicans* afforded the highest oil yields (2-4%), whereas *Th. carnosus* showed the lowest ones (0.4-1%) (Table 1).

In total, one hundred compounds could be identified, representing 81-99% of the total oils, which are listed in Table 1 in order of their elution on a DB-1 column. Monoterpenes were dominant in all oils (68-95%), sesquiterpenes constituting the second main fraction, in variable amounts (2-20%).

The oils from two of the *Th. albicans* populations showed high relative amount of 1,8-cineole (49% in both cases), linalool being the second main component (12-14%). The third population from this species was equally rich in 1,8-cineole and limonene (28% for both), borneol being, in this case, the third main component (12%). 1,8-Cineole (49-61%) was also the main component of two of the three *Th. mastichina* populations studied, whereas the third one was linalool rich (40%).

Each of the oils from the *Th. camphoratus* populations was dominated by different components, Table 1. *trans*-Sabinene hydrate (20%) was the main component from *TcmCV* oil, borneol (13%) and linalool (11%) dominated *TcmA* oil and 1,8-cineole (33%) was the major compound in *TcmL* oil. *Th. carnosus* oils were mainly constituted by borneol (23-38%), *cis*-sabinene hydrate being the second main component ranging from 7% to 16%.

Antioxidant activity

TBARS assay - without and with ABAP

The essential oils isolated from *Th. albicans* collected at different locations showed a diverse capacity of prevent lipid oxidation (Fig. 1A and B), the oil from *TaQM* showing the lowest antioxidant capacity regardless the concentrations tested. The highest antioxidant indexes were detected in the oils from *TaLl* and *TaLd*, only at concentrations of 1000mg l⁻¹ (69% ± 1 and 66% ± 3, respectively), which were relatively similar to those of α -tocopherol (65% ± 3) at the same concentration.

With the exception of the lower antioxidant index of *TaLd* oils, the presence of the radical inducer ABAP did not prevent the antioxidant capacity of the other *Th. albicans* oils (Fig. 1B). Nevertheless, the antioxidant indexes of *Th. albicans* oils were significantly lower than those of

BHT, BHA and α -tocopherol (Fig. 1).

In general, the antioxidant activity, in the absence of ABAP, of the essential oils of *Th. mastichina* increased over the concentration range tested (160-1000 mg l⁻¹) (Fig. 1A). The highest differences in the antioxidant activities of these oils were observed at extreme tested concentrations, that is, at 160 mg l⁻¹, the *TmM* oil showed the lowest activity (20% \pm 5), whereas *TmS* oil possessed the highest one (42% \pm 8). Similarly for the highest concentration tested (1000mg l⁻¹), *TmM* oil showed an antioxidant index of 59% \pm 7 and *TmS* oil presented an inhibition percentage of 79% \pm 4. At 1000mg l⁻¹, the *TmVA* and *TmS* oils showed higher ability to inhibit the lipid oxidation than α -tocopherol and were within the same range of activity of BHA.

In comparison with the assays without ABAP (Fig. 1A), the presence of the radical inducer reduced the ability of *Th. mastichina* oils to prevent oxidation (Fig. 1B), particularly at the concentrations of 160, 800 and 1000mg l⁻¹.

From the *Th. camphoratus* oils, the *TcmCV* oil showed the highest antioxidant activity (Fig. 1A), similar to that of the α -tocopherol, regardless the concentrations tested. The remaining *Th. camphoratus* oils, *TcmA* and *TcmL*, showed similar but much lower antioxidant indexes than that of *TcmCV* oil.

The presence of ABAP reduced in about 10% the antioxidant ability of *TcmCV* oils (Fig. 1B), whereas for the *TcmA* and *TcmL* oils the opposite was seen, higher antioxidant indexes being registered in the presence of the radical inducer at 160 and 320mg l⁻¹ for *TcmA* oils and at 800 and 1000mg l⁻¹ for *TcmL* oils.

Th. carnosus oils showed higher degree of similarity in antioxidant capacity among themselves than the remaining *Thymus* oils studied (Fig. 1A). In contrast with *TcrAS* and *TcrS* oils the *TcrT* oils showed similar degree of antioxidant capacity independently of the test concentration used and also antioxidant indexes comparable to those of α -tocopherol and BHA.

Interestingly, in the presence of ABAP, *TcrT* oils and also *TcrAS* oils showed lower antioxidant capacity compared to the experiment without ABAP, whereas that effect was not so pronounced with *TcrS* oils (Fig. 1B).

α -Tocopherol, BHA and BHT showed different behaviours in the presence of the radical inducer ABAP and in its absence. BHA and α -tocopherol improved their antioxidant ability in the presence of ABAP, whereas BHT showed no major difference in activity either in the absence or presence of

this radical inducer.

Micellar model system

Since with the TBARS assay all samples tested showed, even at the lowest concentration tested, some degree of antioxidant capacity even in the presence of ABAP, the micellar model system was performed at only one concentration (160mgL⁻¹) and in the presence of ABAP.

BHT, BHA and α -tocopherol showed the highest antioxidant capacities (84%, 84% and 76%, respectively), (Fig. 2). *Th. camphoratus* and *Th. carnosus* oils showed the highest antioxidant indexes ($\geq 36\%$) in contrast to some of *Th. mastichina* and *Th. albicans* oils that only reached antioxidant indexes of 19% and 3%, respectively. *TcmL* and *TcrS* oils showed the highest capacity for preventing peroxidation of linoleic acid (67% and 68%, respectively).

Discussion

Composition of the essential oils

Although some 1,8-cineole/linalol and linalol chemotypes can occur on *Th. albicans* oils, the present study is in accordance with previous studies on that *Th. albicans* are mainly characterized by 1,8-cineole type oils [21]. The chemical heterogeneity already reported for *Th. mastichina* taxon [21] is perceptible again in the present study in the presence of 1,8-cineole- and linalool-rich oils. The chemical and morphological resemblance of *Th. mastichina* and *Th. albicans* supports its inclusion in the same section *Mastichina*.

Borneol, 1,8-cineole and linalool are among the main components usually found in *Th. camphoratus* oils [17,22]. The chemical polymorphism of this species is also noticeable in the present study, each population being dominated by different components. *Th. carnosus* oils are usually borneol-rich,[21] which is in accordance with the results of the present study.

Antioxidant activity

The degree of lipid oxidation should be evaluated by more than one method since it occurs through several steps, originating several types of products. In view of this, in the present work two methods were chosen: a) the thiobarbituric acid reactive species (TBARS) assay, that measures the extent of lipid degradation by spectrophotometric evaluation, at 532nm, of the pink pigment

produced through reaction of thiobarbituric acid (TBA) with malonaldehyde (MDA), one of the secondary lipid peroxidation products and b) the micellar model system, based on the spectrophotometric determination, at 234 nm, of the rate of conjugated diene formation from linoleic acid, that is, the formation of primary components (hydroperoxydienes) of the oxidative process of a lipid.

In what concerns the TBARS assay, the results showed that almost all essential oils possessed antioxidant activity. Previous studies, [22] using the same methodology and different *Th. mastichina* and *Th. camphoratus* oils in diverse test concentrations, also reported antioxidant ability of these oils, the latter species oils showing higher antioxidant activity than *Th. mastichina* oils, either in the absence or in the presence of radical inducer ABAP.

Previous studies have shown also a positive antioxidant activity from other *Th. mastichina*, *Th. albicans* and *Th. carnosus* oils, collected at different locations and using a different procedure of antioxidant capacity evaluation, the periodic determination of peroxides values of the oils stored at 60°C [21].

The results of the present work confirm the difficulty in comparing the antioxidant activities of the essential oils tested and relate them with the essential oil chemical composition. Considering the main component of the oil as sole responsible for the biological activity is obviously wrong since, for instance, all the 1,8-cineole-rich *Th. mastichina*, *Th. albicans* and *Th. camphoratus* oils showed, independently on the concentrations tested, major differences in their antioxidant capability, either in the absence or presence of ABAP. The essential oils are complex mixtures in which there is a conjugation of synergistic and antagonistic effects responsible for the different results obtained. This differential response has also been reported by other authors [13] that demonstrated that the crude oil isolated from *Curcuma zedoaria* possessed, in general terms, lower antioxidant activity than one of the fractions obtained from the essential oil after fractionation by column chromatography eluted sequentially with various solvents.

The results obtained with the micellar model system support the above conclusions with the TBARS assay. Once again comparing the 1,8-cineole-rich oils from *Th. mastichina*, *Th. albicans* and *Th. camphoratus*, significant different antioxidant indexes were obtained. On the other hand, with the exception of *TaL1* oil, all the remaining oils showed higher antioxidant activity by the micellar model system than that detected by the TBARS assay. Using other antioxidant evaluation

methodologies and oils, some authors [9] detected weak ability of fennel and coriander essential oils for scavenging the ABTS radical cation, whereas other authors [5,7] found high activity with the same species oils, by the TBARS assay.

The different behaviour of α -tocopherol in the presence or absence of the radical inducer ABAP was also already detected and explained elsewhere [5,22].

In conclusion, the antioxidant capability of an essential oil depends, not only on the nature and relative amount of the essential oil components but also on the chosen method for antioxidant determination. Nevertheless, despite these difficulties several approaches should be used in conjunction in order to evaluate the potential of the essential oils as a resource of natural antioxidants for food industry.

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Table 1. Percentage composition of the essential oils isolated by hydrodistillation from the aerial parts of *Thymus albicans*, *Th. mastichina*, *Th. camphoratus* and *Th. carnosus*.

Componentes	IR	<i>Thymus</i>											
		Section <i>Mastichina</i>						Section <i>Thymus</i>					
		<i>Th. albicans</i>			<i>Th. mastichina</i>			<i>Th. camphoratus</i>			<i>Th. carnosus</i>		
		<i>TaLi</i>	<i>TaLd</i>	<i>TaQM</i>	<i>TmM</i>	<i>TmVA</i>	<i>TmS</i>	<i>TcmCV</i>	<i>TcmA</i>	<i>TcmL</i>	<i>TcrT</i>	<i>TcrAS</i>	<i>TcrS</i>
Tricyclene	921	0.1	t	0.2	0.2	0.1	0.1	0.1	0.2	0.2	0.4	0.4	0.4
α -Thujene	924	t	1.0	0.2	0.3	0.2	0.1	0.1	t	t	3.4	1.4	2.6
α -Pinene	930	2.2	1.6	4.2	3.6	3.9	1.5	2.6	5.4	8.1	4.7	6.7	3.3
Camphene	938	0.8	1.6	5.0	4.2	4.5	3.9	1.0	4.2	3.6	11.6	11.5	11.1
Sabinene	958	2.0	2.2	2.3	0.8	1.0	0.4	5.3	0.7	1.5	1.9	1.2	1.7
3-Octanone	961						t	t		t	t	t	1.9
β -Pinene	963	3.3	3.4	3.9	3.3	3.3	0.9	0.4	0.4	1.0	2.1	2.0	0.1
Dehydro 1,8-Cineole	973	0.1	0.1		0.1	0.1	0.3	0.3					
3-Octanol	974						t						
Myrcene	975	0.8	0.9	1.2	0.5	0.7	0.2	0.3	0.6	0.4	0.6	0.3	0.4
α -Phellandrene	995				0.1	0.1		0.1	0.2	0.2	0.1	t	0.1
<i>o</i> -Cymene	1000					0.4	0.3						
α -Terpinene	1002	0.1	0.1	0.2	0.4	0.3	0.2	1.1	0.1	t	1.0	0.3	1.5
<i>p</i> -Cimene	1003	0.2	0.2	0.4	0.9	0.4	0.3	1.2	0.4	0.3	1.1	0.5	0.9
1.8-Cineole	1005	49.3	49.3	27.5	61.0	49.4	9.6	3.8	3.8	33.0			
β -Felandrene	1005							t	t	t	0.3	0.2	0.4
Limonene	1009	0.4	t	27.5	t	t	0.8	0.7	1.1	1.0	1.9	1.7	0.8
<i>cis</i> - β -Ocimene	1017	0.1	0.1		0.2	0.1	0.1	t	0.3	0.1	0.2	0.2	0.1
<i>trans</i> - β -Ocimene	1027	0.9	0.7	0.3	0.8	0.6	0.8	0.9	1.8	1.3	1.5	1.8	0.6
γ -Terpinene	1035	0.2	0.2	0.4	0.7	0.6	0.2	2.2	0.3	0.4	2.0	0.6	2.8
<i>trans</i>-Sabinene hydrate	1037	0.9	0.8	0.2	0.2	0.2	0.3	19.6	1.6	1.2	7.0	4.7	8.4
<i>cis</i> -Linalol oxyde	1045	0.1	0.1				0.4	t	0.4	0.2			
<i>trans</i> -Linalol oxyde	1059	0.1	t				0.4	t	0.3	0.2			
Terpinolene	1064	0.1	0.1	0.1	0.2	0.2		0.6	0.3		0.5	0.2	0.7
<i>cis</i>-Sabinene hydrate	1066	0.2	0.3	0.1	0.1	0.1	0.1	2.3	0.5	0.5	13.6	7.3	16.4
Linalol	1074	14.0	11.8	0.3	1.1	0.5	39.7	0.9	11.4	4.7	0.7	0.4	0.8
Isovaleric acid isoamyl ester	1080	t	t										
<i>cis</i> -rose oxyde	1083	0.1	t										
Octen-3-yl acetate	1086				0.2	0.1	0.1						0.1
α -Campholenal	1088	0.1	0.1	0.1	0.2	0.1	0.1	0.5	0.4	0.5	0.1		0.1
<i>trans</i> - <i>p</i> -2-Menthen-1-ol	1095	0.1	t	0.1	0.1	0.1	t	0.7	0.3	0.1	0.6	0.2	0.6
Camphor	1095	0.5	2.1	1.2	5.3	6.9	6.5	2.2	6.1	4.8	0.9	0.6	0.9
<i>trans</i> -Pinocarveol	1106	0.2	0.2	0.2	0.3	0.3	0.2	0.3	0.5	0.4	0.2	0.2	0.3
<i>cis</i> - <i>p</i> -2-Menthen-1-ol	1110				0.1	0.1							0.3
<i>cis</i> -Verbenol	1110	0.1	t	0.1				0.6	0.3	0.3	0.3	0.2	
<i>trans</i> -Verbenol	1114	0.2	0.2	0.1	0.2	0.2	0.2	1.8	2.0	2.0	1.6	1.4	1.5
Pinocarvone	1121	0.2	0.2	0.2	0.2	0.2	0.1		0.2	0.2	0.2	0.2	0.2
δ -Terpineol	1134	t	0.1		t			t	t				
Borneol	1134	3.1	3.7	11.7	3.8	3.2	3.0	4.0	12.7	4.7	23.0	38.3	23.7
Terpinen-4-ol	1148	1.0	0.6	1.4	1.5	1.5	0.3	7.8	0.8	0.8	6.2	2.1	0.1
Myrtenal	1153	0.3	0.6	0.2	0.1	0.1	0.1	0.1	0.4	0.3	0.1	0.2	0.1
α -Terpineol	1159	4.3	4.5	3.5	2.3	2.6	0.7	1.7	1.8	0.6	0.5	0.4	0.5
Verbenone	1164							0.5	0.5	0.2			
Myrtenol	1168	0.4	0.3	0.2	0.2	0.1	0.1				0.3	0.2	0.2
<i>n</i> -Decanal	1180							0.3	0.2	0.1			
<i>trans</i> -Carveol	1189	0.1	t		0.1	0.1	t	0.3	0.2	0.2	0.1		
Bornyl formate	1199	0.1	0.1	0.3	0.1	0.1	0.1				0.2	0.1	0.1

Cumin aldehyde	1200							0.3	0.2	0.1			
Carvone	1206							0.1	0.5	0.2			
Citronelol	1210	1.0	0.5										
Geraniol	1236	1.3	0.5	0.1	0.1	0.2	0.2	0.3	0.8	0.1			
Linalyl acetate	1245						0.4	t	6.3	0.2			
<i>n</i> -Decanol	1259	0.2	0.1	0.1				0.3	0.3	0.2			
Cumin alcohol	1260							0.5	t	t			
Bornyl acetate	1265	0.1	0.1	0.6	0.2	0.2	0.2	0.1	0.6	1.9	5.6	2.7	2.8
Thymol	1275					t							
Carvacrol	1286												0.1
Eugenol	1327		0.2										0.1
α -Terpenyl acetate	1334					0.1		2.0		0.6	0.4	0.3	0.4
Citronellyl acetate	1343	0.1	0.1	0.1									
Bornyl propionate	1361	t	0.1	0.1	t	0.1					0.2		
Geranyl acetate	1370	0.2	0.1		t	0.1	t	0.2	0.9	0.3			
β -Bourbonene	1379	0.1	0.1		t	t	0.1	0.1		t			t
β -Elemene	1388	1.2	1.7	0.1	t	t	t		t				
Acetic acid decyl ester	1400							0.1	0.2	t			
α -Gurjunene	1400	0.2	0.2	t			0.1	0.1		0.1	0.1	t	
Bornyl isopentyl ester	1403												t
β -Caryophyllene	1414	0.6	0.5	0.3	0.1	0.2	0.6	1.3	0.5	0.1	0.4	0.5	0.5
<i>trans</i> - α -Bergamotene	1434							0.1	t	t			
α -Humulene	1447							t	t	t	0.1	0.1	
Bornyl butyric ester	1451				0.1							t	t
<i>allo</i> -Aromadendrene	1454	0.2	0.4	0.1		t	0.4	1.1	1.0	0.5	0.1	t	t
Geranyl propionate	1461	0.2	0.4	0.1		0.1					0.1	t	
<i>n</i> -Dodecanol	1468							0.1	t	t			
Germacrene-D	1474	0.3	0.3	0.3	t	0.1	0.1	0.8	0.3	0.3	0.1	0.2	0.1
β -Selinene	1476	0.1	0.1	0.1							0.1	0.2	
α -Selinene	1486	0.1	0.2	0.1							0.2	0.6	
Biclogermacrene	1487	0.3	0.2	0.3	0.2	0.1	0.2	0.6	0.2	0.2	0.1	0.3	
α -Murolene	1494							0.1	0.2	0.1			
Borneol 2-methyl butyric ester	1495				t	t		0.1	0.2	0.1			
Geraniol isobutyric ester	1499	0.4	0.4	0.3	0.2	0.3							
γ -Cadinene	1500	0.2	0.1	0.1			0.7	1.3	2.9	2.0			
Calamenene	1505						0.2		0.4	0.3			
δ -Cadinene	1505	0.1	0.1	0.5				0.9	0.5	0.1			
Elemol	1530				0.6	0.6	1.6	0.7		t	1.3	3.4	1.6
Geraniol butyric ester	1544	0.3	0.3	0.1	0.1								
Spathulenol	1551	0.1	0.2	0.2	t	0.1	0.1	0.3	t	0.1			
Germacrene-D-4-ol	1557	0.2	0.2	0.2				2.2	1.1	0.1			
β -Caryophyllene oxyde	1561	0.2	0.2	0.2	0.1		0.6	2.2	1.1	0.8	0.1	0.6	0.4
Globulol	1566	0.3	0.2	t		0.1	0.2	0.1	t	0.1	0.2		
Viridiflorol	1569	1.0	1.3	0.1	0.2	0.1	0.7	1.1	0.7	0.6	0.3	1.0	0.2
Ledol	1580	0.6	0.7	0.1	0.1	0.1	0.4	1.0	0.5	0.4	0.1	0.2	0.3
Geraniol isovaleryc ester	1590	0.2	0.2										
<i>epi</i> -Cubenol	1600						0.3		1.4	1.6			
T-Cadinol	1616	0.3	0.2	0.2				1.4	6.1	5.9			
δ -Cadinol	1618						1.4	0.3	t	0.2			
β -Eudesmol	1620				0.3	0.3	0.3				0.2	0.9	0.2
α -Eudesmol	1634				0.4	0.3	0.2				0.4	1.8	0.1
Intermedeol	1626	t	t	0.2	0.4	0.3		1.0	1.6	0.4			
α -Cadinol	1626	0.1	0.2	0.5				1.0	1.6	0.4			
% of Identification		96.9	97.3	98.4	96.5	85.8	81.1	86.1	90.5	91.1	99.0	98.3	90.5

Grouped components

Monoterpene hydrocarbons	11.2	12.1	45.9	16.2	16.4	9.8	16.6	16.0	18.1	33.3	29.0	27.5
Oxygen containing monoterpenes	79.3	77.8	48.8	77.7	67.0	63.0	51.0	53.7	58.4	61.9	59.5	57.5
Sesquiterpene hydrocarbons	3.4	3.9	1.9	0.3	0.4	2.4	6.4	6.0	3.7	1.2	1.9	0.6
Oxygen containing sesquiterpenes	2.8	3.2	1.7	2.1	1.9	5.8	11.3	14.1	10.6	2.6	7.9	2.8
Phenylpropanoids	t	0.2	t	t	t	t	t	t	t	t	t	0.1
Others	0.2	0.1	0.1	0.2	0.1	0.1	0.8	0.7	0.3	t	t	2.0
Yield (v/w)	2.0	1.6	4.0	1.0	1.3	1.1	0.5	0.7	0.8	1.0	0.4	0.4

RI = Retention index relative to C₉-C₁₇ *n*-alkanes on the DB-1 column

t = trace (<0.05 %)

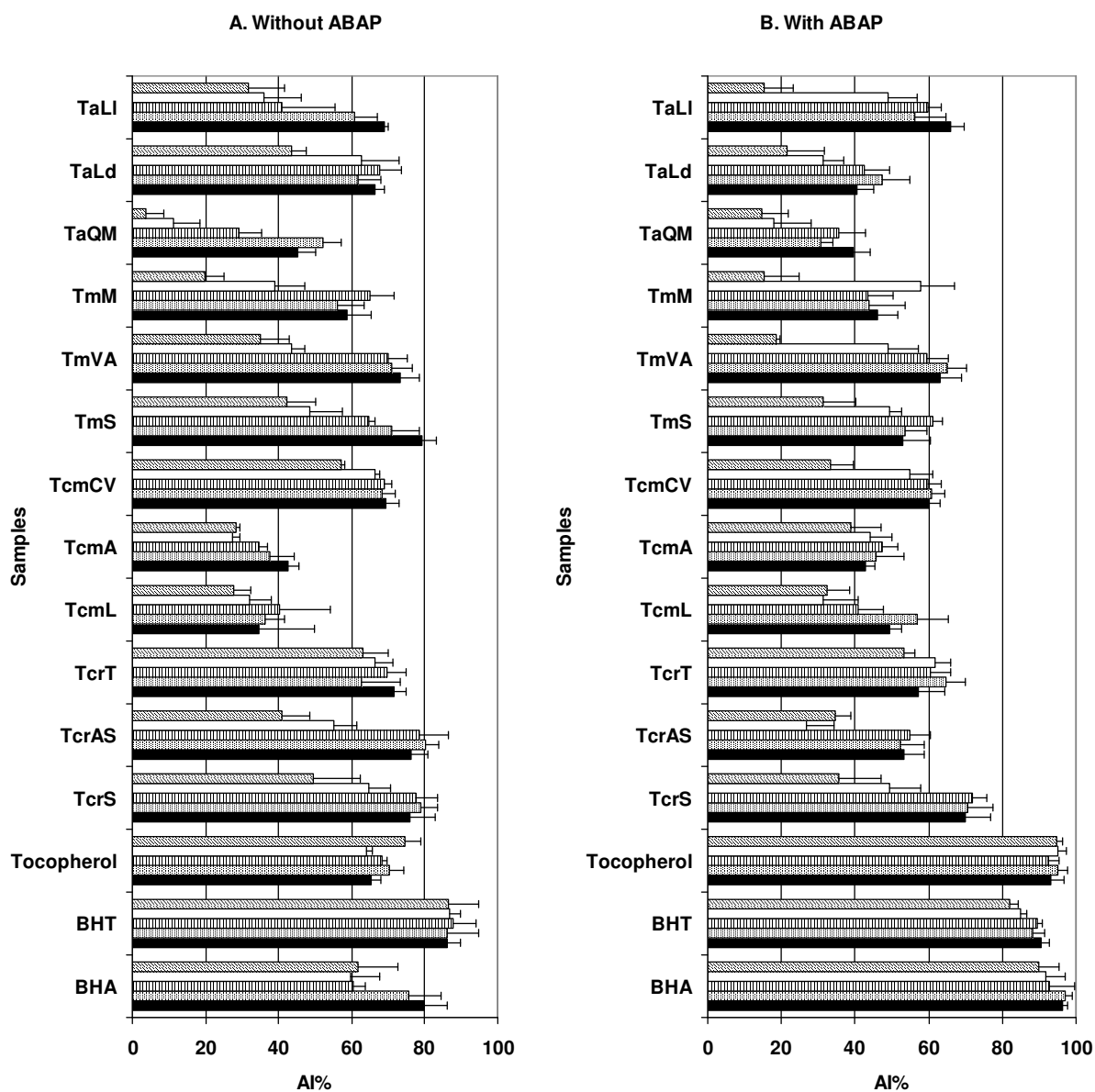


Figure 1: Antioxidant index (%) of the essential oils isolated from *Th. albicans* (Ta), *Th. mastichina* (Tm), *Th. camphoratus* (Tcm) and *Th. carnosus* (Tcr) collected at different locations (for abbreviations see experimental), plus BHA, BHT and α -tocopherol in different concentrations (■ 1000, ▨ 800, ▩ 640, □ 320 and ▤ 160 mg.l⁻¹) using TBARS assay without (A) and with (B) ABAP. Bars represent standard deviation of 3 replicas.

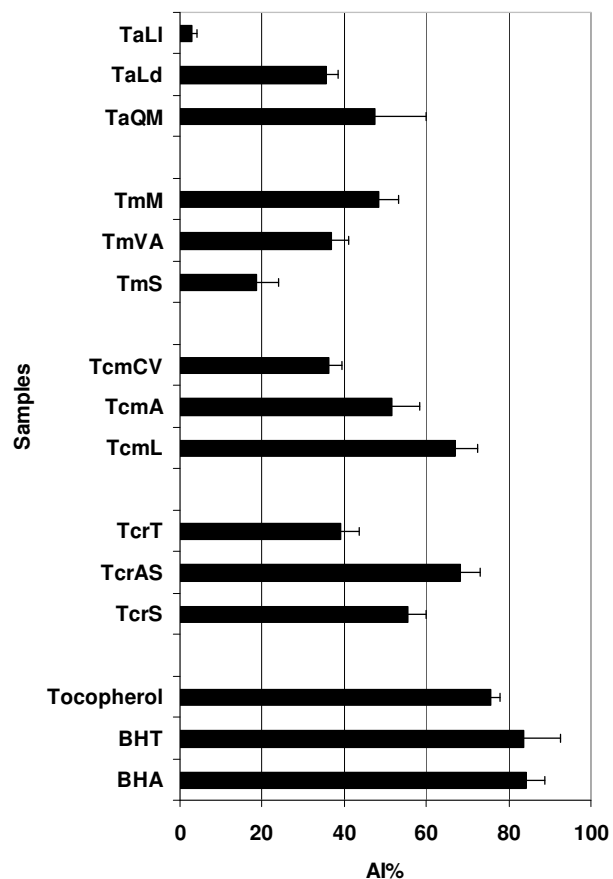


Figure 2. Antioxidant index (%) of the essential oils isolated from *Th. albicans* (*Ta*), *Th. mastichina* (*Tm*), *Th. camphoratus* (*Tcm*) and *Th. carnosus* (*Tcr*) collected in different regions of Portugal, plus BHA, BHT and α -tocopherol in the concentration of 160mg l^{-1} in the presence of ABAP, using the micellar model system. Bars represent standard deviation of 3 replicas.