Portuguese *Thymbra* and *Thymus* Species Volatiles: Chemical Composition and Biological Activities

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Abstract: *Thymbra capitata* and *Thymus* species are commonly known in Portugal as thyme and they are currently used as culinary herbs, as well as for ornamental, aromatizing and traditional medicinal purposes. The present work reports on the state of the art on the information available on the taxonomy, ethnobotany, cell and molecular biology of the Portuguese representatives of these genera and on the chemotaxonomy and antibacterial, antifungal and antioxidant activities of their essential oils and other volatile-containing extracts.

Key Words: Lamiaceae, Labiateae, *Thymbra*, *Thymus*, volatiles, essential oil, biological activity, Portugal.

INTRODUCTION

The word thyme is a general name for more than three hundred *Thymus* species, hybrids, varieties and ecotypes, all of which are small perennial herbs native to Europe and Asia. The word *Thymus* has two possible derivations: from the Greek word *thyo* meaning scent, cleanse or fumigate, or from the word *thymon* for courage. Thyme was a symbol of grace and elegance for the Greeks; they used the expression "smell as thyme" as a symbol of pleasure. There are many traditions related to the tonic character of these plants. The Egyptians used it in embalmment. The Roman soldiers used to take a bath in water with thyme to provide vigour. In the Age of Chivalry, thyme motifs were embroidered on knights' scarves to give courage before a jousting tournament. Thyme sprigs were thought to offer protection against the plague, and were also burned indoors to cleanse the air. In the past thyme was also associated with death, as the plant used to be planted on graves in Wales, and carried at the funerals of the members of the Oddfellows (British secret benevolent society). Moreover, an old superstition believes that planting a bed of thyme in the garden would bring fairies to the home, or enable one to catch sight of them. Thyme oil was used as an antiseptic during World War I. Still today, thyme is used in the embalmment liquids, protects paper from mould, and is used to preserve anatomy and botany specimens [1-4].

Teas from several species of thyme are a traditional remedy for gastro-intestinal complaints and the oils were once taken to expel intestinal parasites, particularly hookworm. Thyme also has antispasmodic properties, which make it an effective remedy for the sore throats, irritable coughs, and bronchitis. Thyme mouthwashes are also used against gum infections. Externally it is applied to clean the skin against acne.

Thyme from several origins is traditionally included in sausages, meat loaf, terrines and stuffing mixtures, both for its preservative qualities as well as its savoury taste. Due to its wide applications, from poultry, shellfish, hunt meat, meat, fish, fruit salads to sweets, it is an important herb in southern French, Greek, Creole and Cajun cuisines. The honey from thyme flowers is largely appreciated for its delicacy [1, 2, 5].

In addition to the plant applications, thyme oils are also used in flavour and food industries, mainly in the manufacture of perfumes and cosmetics, or for flavouring chocolates, toothpastes, mouthwashes, and cough medicines [1-4].

As an ornamental, several species of thyme are used in rock gardens, along walks and borders, mainly because they are aromatic evergreen shrubs that do not need any special care. As an aromatizer, thyme is a common ingredient from sachets and *potpourris* [1, 2, 4].

Thymus species have thus a huge economic importance, being understandable the interest shared by a high number of researchers on several aspects of this genus as well as the existing large number of monographs on *Thymus*: Pharmacopoeias (European, British, French, German, Portuguese and Swiss, among others), European Scientific Cooperative on Phytotherapy (ESCOP), German Commission E Monographs and World Health Organization (WHO).

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1381-6128/08 $55.00+.00

As stated above, thyme commercial products include the fresh or dried herb, different plant extracts (essential oils and oleoresins) and landscape plants. Among these products essential oils are particularly important, namely those from *Th. mastichina*, *Th. zygis*, *Th. vulgaris* and *Thymbra capitata*, and several oil specifications are known, some from each producing country or international ones, such as International Organization for Standardization (ISO, Essential oils section), Association Française de Normalisation (AFNOR), Fragrance Materials Association of the United States Standards (FMA) and USA Food Chemical Codex (FCC).

Given the importance of thyme species as ornamental plants, culinary herbs, flavouring agents or herbal medicines, the aim of this work was to gather the information of the chemical composition, common uses and biological activities of Portuguese thyme species essential oils and volatiles. As the several existing studies have either addressed the essential oils (obtained by hydro-, steam- or drydistillation or by expression, according to the European Parmacopoeia [6] and ISO rules) or volatiles isolated by other extraction techniques (such as distillation-extraction or supercritical CO_2 extraction), and they both allow the characterization of a plant volatile fraction, they will all be considered here. Under the common Portuguese thyme name, both *Thymbra capitata* and *Thymus* species are included, thus they will all be treated in the present work. In addition, the chemistry of the essential oils and volatiles is directly correlated with their biological activities and thus all factors that influence volatiles chemical composition will more or less influence their biological activity, so they will also be covered in some detail.

THYME SPECIES OCCURRING IN PORTUGAL

Eleven species of *Thymus*, totalizing fourteen *taxa*, occur in Portugal. These taxa belong to five of the eight sections of this genus [7]: Sect. *Mastichina* (Mill.) Benth., Sect. *Micantes* Velen., Sect. *Pseudothymbra* Benth., Sect. *Serpyllum* (Mill.) Benth. [subsect. *Alternantes* Klover and subsect. *Pseudomarginati* (H. Braun & Borbás) Jalas] and Sect. *Thymus* [subsect. *Thymus* and subsect. *Thymastra* R. Morales], Table **1**.

Thymbra capitata (L.) Cav. [= *Thymus capitatus* Hoffms. et Link., *Thymus creticus* Brot., *Corydothymus capitatus* Rechenb. f., *Satureja capitata* L.] is also usually known in Portugal under the Portuguese thyme name, "tomilho". This species from the genus *Thymbra* was included by Franco [8] in the genus *Thymus*, because of the great similarities between these plants.

The Portuguese Flora of Coutinho [9] considers thirteen *Thymus* species, four of which, *Th. ciliatus*, *Th. hirtus*, *Th. serpyllum* and *Th. vulgaris*, do not occur in Portugal, unless cultivated.

Thymus is a genus with a high hybridization rate. The species that produce more hybrids are *Th. mastichina*, *Th. vulgaris* and *Th. zygis*, which are also the most frequent and widespread throughout Mediterranean Region [10]. From over fifty hybrids pointed out to Iberian Peninsula [3] five of them occur in Portugal, Table **2**. The chemical composition of three Portuguese hybrids, *Thymus mastichina* x *Th. villosus* ssp. *villosus* [11], *Thymus* x *mourae* [12, 13] and *Thymus* x *viciosoi* [12, 14] was very helpful to realize that they were of hybrid origin.

In the North of Portugal, *Thymus mastichina* subsp. *mastichina* and *Thymus zygis* subsp. *zygis* are often closely associated. It is thus natural that the hybrid *Thymus mastichina* (L.) L. subsp. *mastichina* x *Thymus zygis zygis* Loefl. ex L. subsp. *zygis* can also occur in Portugal. This hybrid is not easily distinguishable from the former progenitor [10].

PORTUGUESE THYME USES AND ETHNOBOTANY

In 1949, Vasconcellos [15] depicted not only the botany description, but also the distribution, chemical composition, applications, culture conditions and collection of the cultivated *Th. vulgaris* and of the spontaneous *Th. serpyllum*. According to the botany description and distribution referred by the author, the latter species should nowadays be classified as *Th. pulegoides* and *Th. praecox* Opiz subsp. *ligusticus*, Table **1**. Vasconcellos [15] stated the applications of this species as antiseptic, carminative, spasmolytic, emmenagogue, tonic and expectorant.

Fernandes Costa [16] also mentioned the use of *Th. serpyllum* in home medicine. Later on Feijão [17] described again *Th. vulgaris* and *Th. serpyllum*. Based on the distribution and synonymy referred by the author, the later species should be not only *Th. pulegoides* and *Th. praecox* Opiz subsp. *ligusticus*, but also *Th. caespititius* (in Azores). According to Feijão [17], in Portugal, apart from its current use as a spice, the infusion of the flowers from *Th. vulgaris* or *Th. serpyllum* (10-20:1000) or from the dry plant (20-40:1000) are used internally as tonic, stomachic, emmenagogue and spasmolytic, and externally as antiseptic, rubefacient and parasiticide (in baths, lotions or frictions). The "dust" from the dry plant was used like snuff, against nosebleed, and in a mixture with honey against strong coughs.

In S. Miguel (Azores archipelago) there is reference to the use of *Th. caespititius* to subdue cough and hoarseness and of the plant infuse for stomachache treatment [18]. In Madeira archipelago, an infuse of the whole plant of *Th. caespititius* is used for apoplexy, whereas the infuse of the whole plant of *Th. vulgaris* is used as a sleep inducer, the tea for relieving headache, and baths as uterine stimulating during childbirth [19].

Ribeiro *et al*. [20] described the use of *Th. zygis*, *Th. mastichina* and *Th. pulegioides* as teas for medicinal purposes (as digestive tonic, against colds and sore throat), and for cosmetic use in creams, skin and hair lotions. Monjardino [21] referred the use of *Th. capitatus* (= *T. capitata*) infusion against respiratory infections and as stimulant for the heart and blood circulation. Salgueiro [22] mentioned the internal use of *Th. vulgaris*, as infusions, for the tonic, expectorant, astringent and antiseptic properties and also the external use in toothaches, insect stings, falling hair and cutaneous eruptions.

As condiment, although there are recent references to the use of cultivated *Thymus vulgaris* [22, 23], or *Th. zygis*, *Th. mastichina* and *Th. pulegioides* [20], to season food, other studies indicate that thyme is among the herbs and spices

Table 1. *Thymbra* and *Thymus* Species Occurring in Portugal [Adapted from 3, 36, 37, 104-106]

Thymbra and Thymus	Species	Portuguese Common Name
Genus Thymbra		
	T. capitata (L.) Cav. [=*Th. capitatus* Hoffms. et Link., *Th. creticus* Brot., *Corydothymus capitatus* Rechenb. f., *Satureja capitata* L.]	Tomilho de Creta, tomilho de Dioscórides, tomilho cabeçudo
Genus Thymus		
Sect. Mastichina	*Th. mastichina* (L.) L. subsp. *mastichina* ♣	Bela-luz, sal-puro, manjerona-brava, manjerona de Espanha, cabeças-de-homem, amor-de-Deus
	Th. mastichina (L.) L. subsp. *donyanae* R. Morales ♣ [=*Th. donyanae* sensu auct. lusit., non Willd.]	
	Th. albicans Hoffm. & Link ♣ [=*Th. tomentosus*]	Tomilho alvadio
Sect. Micantes	*Th. caespititius* Brot. *	MP: Tormentelo, serpol-do-monte; M: alecrim da serra, hisopo; A: erva-úrsula
Sect. Pseudothymbra	*Th. lotocephalus* G. López & R. Morales ♦ [=*Th. cephalotus* Hoffmanns. & Link]	Erva-ursa, tomilho-cabeçudo
	Th. villosus L. subsp. *villosus* ♦	Tomilho-peludo, azeitoneira
	Th. villosus L. subsp. *lusitanicus* (Boiss.) Coutinho ♣ [=*Th. lusitanicus* Boiss.]	Pimenteira, erva-santa
Sect. Serpyllum		
Sbusect. Alternantes	*Th. pulegoides* L. [=*Th. ovatus* Mill., *Th. serpyllum* L. var. *ovatus* (Mill.) Briq., *Th. serpyllum* L. subsp. *ovatus* (Mill.) Coutinho]	Serpol, serpão, serpilho, serpil
Sbusect. Pseudomarginati	*Th. praecox* Opiz subsp. *ligusticus* (Briq.) Paiva & Salgueiro [=*Th. serpyllum* L. var. *ligustricus* Briq., *Th. serpyllum* L. subsp. *ligustricus* (Briq.) Coutinho]	Serpol, serpão, serpilho, serpil
Sect. Thymus		
Subsect. Thymus	*Th carnosus* Boiss. ♣	Tomilho-das-praias
	Th. zygis Loefl. ex L. subsp. *zygis* ♣	Serpão-do-monte, tomilhinha
	Th. zygis Loefl. ex L. subsp. *sylvestris* (Hoffm. & Link) Brot. ex Coutinho ♣ [=*Th. sylvestris* Hoffmanns. & Link, *Th. sylvestris* var. *intermedius* Coutinho]	Erva-de-Santa-Maria, marganiça, sargacinha, erva-santa, erva-das-azeitonas, serpão, tomilho-branco
Subsect. Thymastra	*Th. capitellatus* Hoffmanns. & Link ♦	Tomilho-do-mato, erva-ursa
	Th. camphoratus Hoffmanns. & Link ♦	Tomilho-do-mar

♣ Iberian endemism; * Iberian, Madeira and Azores endemism; ♦ Portuguese endemism; MP: Mainland Portugal; M: Madeira, A: Azores.

used in less than 0.5% of Portuguese recipes when compared with garlic (79%), onion (65%) and bay leaf (48%) [24].

The aromatic role of thyme is also expressed in the reference to the use of *Th. mastichina* to scent the traditional *S. João* fires [20].

Ethnobotanical studies performed in Portugal showed that thyme species are not, usually, among the most commonly (top 10) referred plant species, although they have aromatic, medicinal, cosmetic and seasoning use. It is important to stress, as mentioned by Santos [25], that whereas in some studies there is a clear predominance to reference to the use of species from mountains, woods and fields, other studies are more rich in species from rural and/or urban environment (cultivated in small rural properties, yards and home-gardens), or that may be easily acquired in more or less specialized shops. Thyme species used are thus variable, Table **3**, and reflect local social and environmental characteristics. For instance, although usually thyme is not one of the main referred species, *Th. pulegioides* was mentioned for its cooking use in 26 out of the 35 interviews in Serra do Açor [26]. On the other hand, *Th. camphoratus* medicinal use was

Table 2. Hybrids within *Thymus* Genus Occurring in Portugal [Adapted from 10, 11, 37]

Thymus Hydrids	Progenitors
Th. x *brachychaetus* (Willk.) Coutinho	*Th. mastichina* (L.) L. subsp. *mastichina* x *Th. caespititius* Brot.
Th. mastichina x *Th. villosus* ssp. *villosus*	*Thymus mastichina* x *Th. villosus* ssp. *villosus*
Th. x *mourae* Paiva & Salgueiro	*Th. mastichina* (L.) L. subsp. *donyanae* R. Morales x *Th. lotocephalus* G. López & R. Morales
Th. x *ramonianus* Paiva & Salgueiro	*Th. camphoratus* Hoffmanns. & Link x *Th. mastichina* (L.) L. subsp. *mastichina*
Th. x *viciosoi* (Pau) R. Morales	*Th. pulegioides* L. x *Th. zygis* L. subsp. *zygis*
Th. x *welwitschii* Boiss.	*Th. carnosus* x *Th. mastichina* (L.) L. subsp. *mastichina*

Table 3. Reference to the Use of Thyme Species in Portuguese Ethnobotanical Studies

Study Region[1]	Methodology[2]	Total No of Species Evaluated[3]	Thyme Species Mentioned	Observations on the Uses	Ref.
Parque Natural da Serra da Estrela	51 inqs	113	*Th. mastichina*	Medicinal purposes	[107]
			Th. serpyllum	Cooking purposes	
Reserva Natural da Serra da Malcata	200 inqs	73	*Th. vulgaris*	Cooking purposes	[108]
Parque Natural do Douro Internacional	198 inqs	104	*Th. mastichina*	Medicinal and cooking purposes	[30]
			Th. zygis	Cooking purposes	
Parque Natural da Serra de S. Mamede	37 invs	165 (150 med)	*Th. mastichina*	Medicinal, cooking and aromatic purposes	[109, 110]
Parque Natural do Douro Internacional	22 invs	96	*Th. mastichina*	Cooking purposes	[31]
Serra do Açor	35 invs	140 (124 med)	*Th. pulegioides*	Cultivated for medicinal and cooking purposes	[26]
Parque Natural do Vale do Guadiana	83 inqs	118	*Th. mastichina*	Medicinal and cooking purposes	[32]
			Th. vulgaris	Medicinal and cooking purposes	
Parque Natural da Arrábida	72 invs	176	*T. capitata*	Medicinal and cooking purposes	[28]
			Th. capitellatus	Medicinal and aromatic purposes	
			Th. mastichina	Medicinal and cooking purposes	
Parque Natural de Sintra-Cascais	178 inqs 28 invs	203	*Th. pulegioides* (from inqs)	Cooking purposes (from inqs)	[33]
			Th. spp.	Medicinal and cooking purposes	
Parque Natural do Douro Internacional	24 ins	99	*Th. mastichina*	Medicinal and cooking purposes	[111]
Península de Setúbal	102 invs	253	*T. capitata*	Cooking purposes	[25]
			Th. capitellatus	Medicinal and cooking purposes	
			Th. carnosus	Medicinal and cooking purposes	
			Th. pulegioides	Cooking purposes	
			Th. spp.	Medicinal and cooking purposes	
Parque Natural da Serra da Estrela	(?) inqs	54	n.m.	n.m.	[112]

(Table 3) contd….

Study Region[1]	Methodology[2]	Total No of Species Evaluated[3]	Thyme Species Mentioned	Observations on the Uses	Ref.
Parque Natural de Montesinho	110 invs obs-part	364 (166 med)	*Th. mastichina*	Medicinal and cooking purposes	[34]
			Th. pulegioides	Medicinal and cooking purposes	
			Th. vulgaris	Cooking purposes	
			Th. zygis sp. *zygis*	Medicinal and cooking purposes	
Ilha da Madeira (concelho de Santana; freguesia da Ilha)	16 invs	77	*Th. vulgaris*	Cultivated for medicinal and cooking purposes	[29]
Reserva Natural do Sapal de Castro Marim e Vila Real de Santo António	19 invs	52 (38 med)	*Th. mastichina*	Medicinal and cooking purposes	[113]
Sudoeste Algarvio	49 invs	173 (164 med)	*Th. capitatus* (= *T. capitata*)	Medicinal and cooking purposes	[27]
			Th. camphoratus	Medicinal and cooking purposes	

[1] The name of the study region was kept in Portuguese; [2] interviews (invs), inquiries (inqs), observation-participation (obs-part); [3] in brackets the number of medicinal plants (med), if mentioned; n.m.: no thyme species mentioned.

mentioned not as main species but to be used in medicinal *Lithodora diffusa* syrups together with other species, in studies in the Sudoeste Algarvio [27].

From the medicinal point of view, ethnobotanical studies show that the use of thyme species is mainly devoted to the treatment of circulatory and digestive problems, and for general blood purification. It is frequent the use as tea, either alone or in mixture with other plants, to lower the blood-pressure and nerves [26], to alleviate toothache, stomach, intestine problems, whooping cough (pertussis), colds, to regulate the menstrual cycle [25, 28, 29] and as syrup with sugar or honey to alleviate cough [25]. The plant can also be boiled with wine to lessen constipation [29].

Ethnobotanical studies show also that, apart from the general aromatizing role in perfumes and soaps, thyme species are used in home-cosmetic preparations mainly to improve hair-health [25]. For cooking purposes, thyme is currently used as spice, mostly to season olives, which is even depicted in some Portuguese thyme species common names, Table **1** ("azeitoneira", "erva-das-azeitonas"), as well as, to season snails, soups, salads, meat, hunt meat and as salt substitute [25, 28, 30-34].

THE VOLATILE OILS OF PORTUGUESE *THYMBRA CAPITATA* AND *THYMUS* SPECIES

Thyme essential oils have been studied by several researchers, as can be seen from the important exhaustive review from Stahl-Biskup [35].

Fernandes Costa [36] performed the first researches on the essential oils from Portuguese thyme species, Table **4**. Since then, and with the improvement of analytical techniques, other researchers devoted their attention to the volatiles from these two genus, mainly the extensive work performed by Salgueiro [37] on the essential oils from mainland Portuguese thyme species and later studies with thyme from Azores and Madeira archipelagos, Table **4** (and references therein). Recent studies on the composition of the volatiles of Portuguese *Thymbra* and *Thymus* species evaluated for their biological activity, considered in the section "Biological activity", were not included in Table **4** (see Tables **5-9**), but confirm the data there presented.

On the opposite of *Thymus* species oils, those of *Thymbra capitata* show a great chemical homogeneity characterized by high carvacrol relative amounts (60-77%), thus being typical phenolic thyme oils, Table **4**.

In general, the oxygen-containing monoterpene fraction is clearly dominant in the Portuguese *Thymus* species oils, 1,8-cineole, borneol, linalool, thymol and carvacrol, being common main components, Table **4**. Moreover, the number of monoterpene components in the oils is almost always higher than that of sesquiterpenes, exception made for *Th. caespititius* oils, in which the proportion may be different. The oil of this species is also the only to show the oxygen-containing sesquiterpene dihydroagarofuran. The sesquiterpene fraction is usually not higher than 15%, although in the oils of *Th. caespititius*, *Th. camphoratus* and *Th. lotocephalus* this value can be, occasionally, somewhat higher. Phenylpropanoids, although widely present in other families, are only seldom found in *Thymus* oils, as, for instance, in amounts ≤0.3%, in *Th. albicans*, *Th. carnosus* and *Th. mastichina*.

In some cases linalyl acetate and linalool were found to be the main components in thyme oils, such as in *Th. lotocephalus*. The high amounts of linalool in this case, were proven to be a distillation artefact resulting from the high amounts of linalyl acetate [38].

As mentioned above, although the essential oils of *Th. albicans, Th. camphoratus, Th. capitellatus, Th. lotocephalus* and *Th. mastichina* showed different qualitative and quantitative compositions, they have in common 1,8-cineole as one of their major components. This is in agreement with literature data, which refers that Iberian thyme oils are characterized by high levels of 1,8-cineole [39]. According to Stahl-Biskup [35], 1,8-cineole is the seventh main constituent of

Table 4. Studies on the Volatile Oils of *Thymbra capitata* and *Thymus* Species Grown in Portugal. Species are Arranged in Alphabetical Order. Unless Otherwise Specified, Volatiles were Extracted by Hydrodistillation and/or Distillation-Extraction. Studies Where Essential oil Analysis is Accompanied by Biological Activity Determination were not Considered in this Table (See Tables 5-9). Unless Otherwise Specified the Minimum and Maximum Range Values Correspond to the Analysis of Different Populations

***Thymbra* and *Thymus* Species**	**Main Components of the Oil (≥5%)**	**Reference**
Genus Thymbra		
T. capitata	Presence of carvacrol and thymol in the phenolic fraction	[114]
	Presence of α-pinene and α-caryophyllene in the non-phenolic fraction	[115]
	Carvacrol 69%, γ-terpinene 6%, *p*-cymene 6%	[116]
	Carvacrol 68-72%, *p*-cymene 11-13%	[117]
	Carvacrol 60-64%, γ-terpinene 9-10%, *p*-cymene 6-8%	[37]
	Carvacrol 51-77%, thymol 9-21%, *p*-cymene 5-11%, γ-terpinene 2-9%	[42]
Genus Thymus		
Th. albicans	Only a reference to the thymol content	[118]
	1,8-Cineole 29-43%, linalool 3-22%, α-terpineol 6-10%, borneol 2-8%, α-pinene 4-5%, α-pinene 4-6%, camphene 1-5%	[37, 119]
	1,8-Cineole 42-68%, linalool 0.4-37%, α-terpineol 4-5%, β-pinene 3-5%	[45]
Th. caespititius	Alcohols 60%, mainly α-terpineol; 10% esters	[16, 36]
	NW Portugal; α-Terpineol 31-41%, *p*-cymene 6-9%, γ-terpinene 4-7%, T-cadinol 6-9%	[37]
	Pico, Azores: Carvacrol 36%, thymol 16%, carvacryl acetate 8%, *p*-cymene 7%, α-terpineol 5%	[120]
	São Jorge: Thymol 1-58%, carvacrol 1-52%, α-terpineol t-68%, sabinene 0.1-40%, *p*-cymene 1-9%, γ-terpinene 1-8%, terpinen-4-ol 1-6%, carcacryl acetate t-5%	[51]
	Pico: Carvacrol 45-57%, thymol 1-12%, carcacryl acetate 2-17%, *p*-cymene 4-6%, α-terpineol 0.4-5% Faial: Carvacrol 51-54%, *p*-cymene 5-10%, α-terpineol 5-12%, thymol 0.1-5%, carcacryl acetate 3-5% Graciosa: Carvacrol 3-35%, α-terpineol 15-37%, T-cadinol 4-11%, *p*-cymene 3-10%, γ-terpinene 4-8%	[43]
	Corvo, Flores, São Miguel: Carvacrol 41-65%, carcacryl acetate 2-24%, *p*-cymene 4-19%, γ-terpinene 1-6%, T-cadinol n.d.-15%, γ-cadinene n.d.-5% Terceira: Thymol 35-51%, thymyl acetate 10-19%, *p*-cymene 10-14%, γ-terpinene 2-5%, α-terpineol 1-8%, Madeira: α-Terpineol 33-37%, sabinene 16-18%, β-myrcene 7-10%, γ-terpinene 4-6%, terpinen-4-ol 3-5%, *epi*-guaiol 3-5%	[52]
Th. camphoratus	Reference to the probable presence of carvacrol	[16, 36]
	Reference to the probable presence of carvacrol	[118]
	1,8-Cineole + limonene 20%, borneol + α-terpineol + bornyl acetate 16%, camphene 10%, terpinen-4-ol 10%, camphor 9%	[121]
	Terpinen-4-ol 29%, γ-terpinene 12%, α-terpinene 7%, *p*-cymene 7%, borneol 6%, camphor 5%	[122]
	1,8-Cineole 1-36%, borneol 1-35%, linalool 1-26%, linalyl acetate 0.3-13%, *trans*-sabinene hydrate 0.1-11%, terpinen-4-ol 1-10%, α-pinene 1-11%, sabinene 1-5%, camphene 0.2-14%, camphor 0.3-10%, β-caryophyllene oxide 1-6%	[37, 123, 124]
Th. capitellatus	1,8-Cineole 30%; 30% alcohols, mainly borneol	[36]
	Reference to the presence of carvacrol	[118]
	Linalool 32%, linalyl acetate 9%, α-terpineol 9%, 1,8-cineole 8%, borneol 6%	[125]
	1,8-Cineole 25-59%, borneol 1-21%, linalool 1-14%, camphene 1-11%, α-pinene 2-8%, linalyl acetate 0.1-8%	[37, 123]

(Table 4) contd….

Thymbra and Thymus Species	Main Components of the Oil (≥5%)	Reference
	Flowers: 1,8-cineole 50%, borneol 7%, α-pinene 5%, Vegetative phase: 1,8-cineole 56%, α-pinene 8%	[38]
Th. carnosus	Reference to the probable presence of carvacrol	[118]
	Reference to the probable presence of carvacrol	[16]
	Borneol 15-32%, camphene 3-13%, *trans*-sabinene hydrate 2-17%, *cis*-sabinene hydrate 3-11%, terpinen-4-ol 4-12%, α-pinene 2-7%, linalool t-26%, bornyl acetate 2-7%	[37, 126]
	Borneol 11-31%, terpinen-4-ol 4-19%, camphene 6-18%, bornyl acetate 0.3-13%, *cis*-sabinene hydrate 2-14%, α-pinene 2-9%, γ-terpinene 2-8%, *trans*-sabinene hydrate 1-8%,	[50]
Th. lotocephalus	1,8-Cineole 60%, 12% alcohols	[16, 36]
	Reference to large amounts of 1,8-cineole, camphor, linalool, linalyl acetate and α-pinene	[123]
	Flowers: linalyl acetate 23%, linalool 11%, 1,8-cineole 10%, α-pinene 7%, α-terpineol 5% Vegetative phase: 1,8-cineole 24%, α-pinene 9%, linalool 6%, linalyl acetate 5%, viridiflorol 5%	[38]
	1,8-Cineole 7-13%, linalool 4-17%, geranyl acetate 0.2-15%, intermedeol 1-10%, α-pinene 5-9%, viridiflorol 2-7%	[37, 127]
	1,8-Cineole 10-19%, linalyl acetate 6-14%, linalool 4-12%	[105]
Th. mastichina* subsp. *donyanae	1,8-Cineole 38%, borneol 15%, camphene 5%, α-terpineol 5%	[37, 119]
Th. mastichina* subsp. *mastichina	1,8-Cineole 58%, linalool, α-terpineol, α-pinene	[36]
	Linalool 69-73%, 1,8-cineole <15%	[128, 129]
	Linalool 64-80%, 1,8-cineole 7-19%	[130]
	Linalool 70%, 1,8-cineole 3%	[131]
	Linalool 77%, 1,8-cineole 7%	[132]
	Flowers: 1,8-cineole 48%, limonene 10%, α-pinene 6%, β-pinene 6%, limonene 10%, α-terpineol 6% Leaves: 1,8-cineole 54%, limonene 11%, α-terpineol 6%	[43]
	1,8-Cineole 15-66%, α-pinene 2-6%, β-pinene 2-6%, α-terpineol 1-9%, sabinene 1-5%, borneol 1-5%, camphene 0.2-5%, camphor 0.1-6%, linalool 1-45%	[37, 119]
	1,8-Cineole 45-59%, camphor 6-9%, α-pinene 5-7%, camphene 4-6%, borneol 4-6%, β-pinene 2-5%	[49]
	1,8-Cineole 64%, α-terpineol 6%, β-pinene 5%	[57]
	1,8-Cineole 10-69%, α-pinene 1-5%, camphene 0.2-6%, camphor 0.1-11%, linalool 1-74%	[46]
Th. pulegoides	Reference to carvacrol, thymol and pinene	[15]
	Reference to the reduced amount of thymol and carvacrol	[16]
	Carvacrol 82-86%, *p*-cymene 7%	[117]
	Thymol 18-45%, carvacrol 2-31%, geranyl acetate t-34%, geraniol t-33%, γ-terpinene 4-11%, *p*-cymene 3-11%, 3-octanone 1-5%, β-caryophyllene 1-5%	[14, 37, 133]
Th. villosus* subsp. *lusitanicus	Linalool 9-25%, geranyl acetate 12-24%, geraniol 7-18%, terpinen-4-ol 5-15%, γ-terpinene 1-6%	[37, 127]
Th. villosus* subsp. *villosus	Alcohols 37%, 28% esters, 5% phenols	[36]
	Reference to the presence of carvacrol	[118]
	p-Cymene 11-40%, borneol 2-23%, camphor 3-14%, linalool 2-12%, γ-terpinene 2-11%, 1,8-cineole 0.1-12%, α-pinene 3-9%, camphene 1-9%, α-terpineol 0.2-17%, myrcene 0.3-13%	[37, 123, 134]

(Table 4) contd….

Thymbra and Thymus Species	Main Components of the Oil (≥5%)	Reference
Th. zygis* subsp. *sylvestris	1,8-Cineole 12-26%, phenols 11-16%	[36]
	Thymol 16-40%, *p*-cymene 5-28%, γ-terpinene 4-13%	[117]
	Thymol 4-25%, carvacrol 1-23%, *p*-cymene 10-22%, γ-terpinene 4-17%, linalool 3-30%, geranyl acetate t-21%, geraniol t-21%, 1,8-cineole 1-12%	[37, 135-139]
	Hydrocarbon monoterpenes 29%, terpene phenols 22%, terpenic alcohols 21%, terpenic esters 19%, sesquiterpenes 5% * Hydrocarbon monoterpenes 5-11%, terpene phenols 12-29%, terpenic alcohols 11-26%, terpenic esters 4-10%, sesquiterpenes 1-3%	[55]
Th. zygis* subsp. *zygis	Presence of phenols 22%, among which thymol	[36]
	p-Cymene 21-45%, thymol 12-42%, γ-terpinene 10-13%	[14, 117]
	p-Cymene 5-30%, carvacrol 0.3-41%, thymol 0.2-34%, γ-terpinene 1-24%, geranyl acetate 0.1-44%, geraniol 0.1-25%	[37]
	Thymol 3-25%, *p*-cymene 5-30%, geraniol 2-30%, geranyl acetate 7-30%, γ-terpinene 7-21% (seasonal variation)	[48]**

* Supercritical CO_2 extraction, ** although originally identified as *Th. zygis* subsp. *sylvestris* the correct assignment should be *Th. zygis* subsp. *zygis*, t (traces): <0.05%, n.d.: not detected.

the oils of ca. 70% of *Thymus* species after thymol, carvacrol, linalool, *p*-cymene, γ-terpinene and borneol. Notwithstanding the chemical variability of each species oils, *Th. carnosus* with the high amounts of borneol, *Th. pulegioides* with thymol/carvacrol, *Th. villosus* subsp. *lusitanicus* with linalool, *Th. villosus* subsp. *villosus* and *Th. zygis* subsp. *zygis* with *p*-cymene, thymol and carvacrol and *Th. zygis* subsp. *sylvestris* with thymol also fit within this main group, Table **4**.

As with any general conclusion, *Th. caespititius* oils, namely those from NW mainland Portugal plants, constitute an exception that is mainly characterized by high amounts of α-terpineol. This component belongs to what Stahl-Biskup [35] defined as a second group of components (α-terpenyl acetate, α-terpineol, geranyl acetate, camphor, citral, linalyl acetate, myrcene and terpinen-4-ol), that occur much less frequently in *Thymus* oils, and only in a few species, but when present they attain relatively high amounts. It is nevertheless important to stress, that the studies performed on *Th. caespititius* oils, from plants collected in Azores archipelago (Table **4** and references therein), showed a major chemical polymorphism and that in addition to α-terpineol, the main oil components were sabinene, thymol and carvacrol.

In some studies [38], it was shown that the oils isolated from the leaves and from the flowers were markedly different showing that the volatiles composition will depend on the developmental stage of the plants and on the amount of flowers present in the plant material used to isolate the essential oil. This should be kept in mind when studies on the occurrence of chemotypes are performed as well as on an industrial level, since this factor may affect both the yield and volatile composition.

Chemical Polymorphism of Thymus Species

As previously mentioned, the eleven Portuguese *Thymus* species are included in five sections of the genus *Thymus*, Table **1**. The morphological characterization of these sections is sometimes difficult, but the studies on the chemical polymorphism of Portuguese *Thymus* species have shown that the analysis of their essential oils may help in the characterization of some sections, in the identification of species, to document natural hybrids and in the study of variations within taxa.

From the analysis of Table **4**, it is clear that there is a large chemical polymorphism for the large majority of Portuguese *Thymus taxa*. Only *Th. mastichina* subsp. *donyanae*, a rare plant that grows in a restricted area from Portugal, and *Th. caespititius* growing in the mainland NW Portugal, showed no chemical polymorphism. Nevertheless, as stressed before, despite the large chemical polymorphism, several *taxa* show similar chemotypes, such as 1,8-cineole, geranyl acetate/geraniol and thymol, whereas other *taxa* show quite specific chemotypes.

The *taxa* of the section *Mastichina* (*Th. mastichina* subsp. *mastichina*, *Th. mastichina* subsp. *donyanae* and *Th. albicans*), although showing chemical polymorphism for *Th. mastichina* subsp. *mastichina* and *Th. albicans*, are characterized by being 1,8-cineole type oils, Table **4**. Moreover, this chemical affinity is in agreement with the morphological resemblance between the species of this section [37].

The section *Micantes* represented only by *Th. caespititius* shows, in the mainland Portugal, an essential oil markedly different from all other sections, characterized by high α-terpineol content, and by having a number of sesquiterpenes larger then that of monoterpenes. Nevertheless, the essential oils isolated from populations grown on Azorean islands showed remarkable chemical polymorphism (α-terpineol, sabinene, carvacrol and thymol chemotypes) and those from Madeira populations showed to be similar to those from the mainland (α-terpineol type), Table **4**.

The large chemical polymorphism of species from the section *Pseudothymbra* (*Th. lotocephalus*, *Th. villosus* subsp.

villosus and *Th. villosus* subsp. *lusitanicus*) does not allow a general characterization of this section. Nevertheless, it should be mentioned that the three taxa are morphologically quite similar, and two of them, *Th. villosus* subsp. *villosus* and *Th. villosus* subsp. *lusitanicus*, having also a similar geographic distribution, possess essential oils with remarkable differences: *Th. villosus* subsp. *villosus* possesses *p*-cymene type oils, whereas *Th. villosus* subsp. *lusitanicus* shows geranyl acetate/geraniol or linalool type oils.

The section *Serpyllum* shows, among others, Table **4**, and as some taxa of the section *Thymus*, thymol type oils, and is characterized by the presence of 3-octanone in a considerable amount [37].

Section *Thymus* shows also a broad polymorphism. The morphology and the chemical profile of the oils of the *taxa* of each subsection do not allow a clear separation between them: both *Th. zygis* subsp. *zygis* and *Th. zygis* subsp. *sylvestris* show mainly, thymol type oils, along with carvacrol and *p*-cymene types. Nevertheless, *Th. carnosus* that also belongs to the same *Thymus* subsection, shows borneol rich oils, Table **4**. Oils with high borneol contents are found in some populations of the *taxa* from *Thymastra* subsection. *Th. carnosus* can not be included in this subsection, since it shows morphological features similar to those of the *taxa* from *Thymus* subsection.

The reason for such chemical polymorphism may reflect a genetic diversity probably relating to the heterogeneity of Portuguese environmental conditions such as humidity degree, thermic amplitude and soil type. In fact, some of these species have special ecological niches that are very sensitive to climatic changes that will certainly affect not only the composition, but also the oil yield. Moreover, apart from several other physiological factors [40] we can not forget that the essential oils are produced by special secretory structures that are not evenly distributed over the aerial parts of the plant (sometimes the flowers posses a higher number of different types of trichomes), and that their secretory stage will depend on the stage of development of the plant material as well as on the essential oil seasonal variation.

Although the common chemotypes do not show any immediate taxonomic value, the industries may choose which ones have potential commercial interest, namely those with high levels of thymol and/or carvacrol, and the knowledge of the seasonal variation of the oil is also important since the most interesting compound and the oil yield can peak over a very short period of time.

Volatiles Secretory Structures

Plant volatiles are accumulated in specialized, external or internal, secretory structures that are diversely distributed over the plant body. The type and location of these structures is, mostly, characteristic of the plant family. In some cases, the differences found in the composition of essential oils obtained from diverse plant parts can be partly explained by the existence of distinct secretory structures that are heterogeneously distributed over the plant body as well as to ontogenic variations [40].

Only a few studies exist on the indumentum of Portuguese *Thymbra capitata* and *Thymus* species. Studies on *Thymbra capitata* indumentum show Lamiaceae-like trichome types [41, 42], similar to those found on the *Thymus* studied species. The studies performed on the indumentum of the hybrid *Thymus mastichina* x *Th. villosus* ssp. *villosus* [11], *Th. mastichina* [43], *Th. caespititius* [44] and *Th. lotocephalus* (Fig. **1**), showed similar nonglandular and glandular trichome types in the different species. The nonglandular trichomes may be conical unicellular and/or multicellular bent point-shaped-type. The glandular trichomes are of two main types, usually found in the Lamiaceae: peltate and two types of capitate (subtype I and II). Peltate trichomes consist of a short stalk cell and a large head with a variable even-number of cells (12-14) arranged in two concentrical rings (the inner ring with four cells and the outer ring with eight to ten cells). The secreted material accumulates in the subcuticular space and it is released after cuticle rupture, following a fragility line on the medium region of the glandular head. Both capitate trichomes subtypes (I and II) are composed of a stalk, a short neck and a unicellular head cell, differing only in the size of the latter one: subtype I trichomes have a larger ovoid head cell than subtype II. The secretion accumulates in a small subcuticular space.

Although the type of trichomes observed in the different *Thymus* species was similar, their distribution over the plant body differed, showing, in some cases, differences in the distribution and frequency of the glandular peltate trichomes [11, 43] which may partly explain the different oil composition and yield found when different aerial plant parts were analysed separately, as in *Th. lotocephalus* [38], *Th. albicans* [45] and *Th. mastichina* [46].

In other cases, as for instance in *Th. caespititius*, although the glandular and nonglandular trichomes were of similar type and showed identical distribution, both on the vegetative and reproductive organs: calyx, petiole, corolla and leaf, and the essential oil yields from the populations analyzed were similar (0.7% and 0.6%, respectively from Pico and S. Jorge), the oil composition of the three populations analyzed showed marked differences. The oil from the Pico population was carvacrol-rich (57%), whereas the oil from a population of S. Jorge possessed a high content in sabinene (74%), and another population was thymol/sabinene-rich (30 and 27%, respectively) [44, 47]. These results show that, at least in some cases, the chemical polymorphism does not seem to be correlated with secretory structures ontogeny or with their type and distribution, and may be probably due to genetic variability and/or edaphic factors.

Seasonal Variation

There are several examples of the existence of a seasonal variation in the yield and essential oil composition for several plant species [40], which has obvious agronomic and economic implications and may also determine, if not taken into consideration, to assume the existence of distinct chemotypes.

Moldão-Martins *et al.* [48] showed that the essential oil yield of *Thymus zygis* L. subsp. *sylvestris* attained a maximum at the flowering stage. Likewise, the oil composition also showed marked seasonal differences. Having in mind the oil yield and the acceptability of the consumer, that fa-

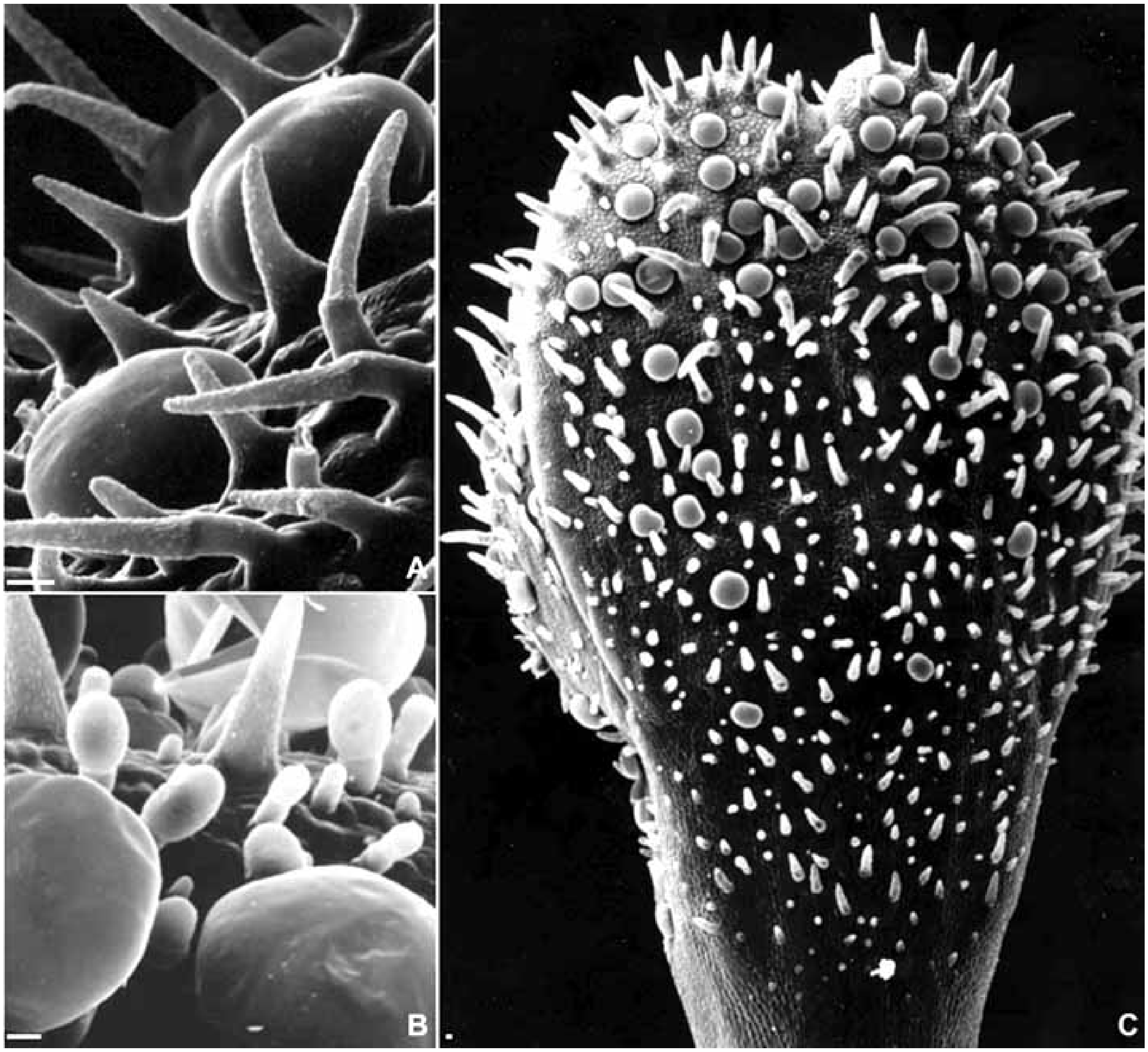

Fig. (1). Scanning electron micrographs of the indumentum of *Th. lotocephalus* (**A**) and *Th. caespititius* (**B** and **C**) showing distribution, density and types of nonglandular and glandular trichomes. Bar=10µm.

vour the presence of geraniol and geranyl acetate, the authors indicated the post-flowering period as the most proper time of harvest.

For *Th. mastichina* subsp. *mastichina,* Miguel *et al.* [49] showed that the harvesting dates influenced oil yield and chemical composition, more than the different growing media conditions assayed, although higher oil yield was obtained with the fertilized growing material.

Miguel *et al.* [50] also found a high variability on the chemical composition and oil yield in *Th. carnosus* oils, depending not just on the collection site and plant part used but also on the harvesting period. According to the authors, such variability may reflect not only genetic diversity and heterogeneity in environmental conditions but also a differential secretory capacity that depends on the developmental stage of the plant material.

Chiral Compounds Evaluation

Although the chemical polymorphism is widespread in Portuguese *Thymus* oils, the enantiomeric composition of these oils has not been studied in much detail. This is understandable in some cases, due to the high amounts of the achiral terpene phenolic compounds, thymol and carvacrol and of the symmetrical oxygen-containing monoterpene, 1,8-cineole.

Since the oils isolated from several populations of *Th. caespititius* collected in some Azorean islands showed chemical polymorphism, studies were performed on the enantiomeric distribution of the chiral compounds present in high relative amount in the oils, such as sabinene, terpinen-4-ol and α-terpineol [51, 52]. The results showed that, although the oils were dominated by the same major components, they possessed a clear enantiomeric polymorphism. Whereas in some oils (-)-sabinene, (-)-terpinen-4-ol and (+)-α-terpineol were the main enantiomers in other cases the opposite ratio was found.

The enantioselective analysis of *Thymus* oils may be an important tool not only for authenticity control but also in understanding the different biological activity behaviour of some oils that possess similar chemical composition.

Molecular Markers and Chemical Polymorphism

Given the high chemical variability from Portuguese *Thymus* oils and since no correlation was found between the chemical compositions of the oils and morphological and/or environmental factors, a new approach is currently being explored in order to evaluate the possible relationship between chemical polymorphism and genetic variability.

The molecular analysis of *Th. caespititius* individuals, collected during the flowering period on the islands of Pico, S. Jorge and Terceira (Azores), did not provide the same clustering as that obtained with the essential oil composition and no straight correlation between chemical and molecular assessments could be found [53, 54]. The plants clustered according to their genetic profiles, and plants with different chemotypes were clustered in the same molecular group. In view of these preliminary results, other molecular methodologies should be explored in order to fully determine the influence of both environmental and genetic factors on volatiles composition.

Other Volatiles-Containing Extracts

In addition to essential oils, that is, the volatile aromatic portion of a plant extracted by distillation or expression, other types of extracts also contain volatile components. Since several parameters can affect the volatile composition and sensory character, as much as its biological activity, this is also an important factor to have in mind from both the academic and industrial point of view.

Supercritical CO_2 extraction (SFE) is nowadays frequently used as an alternative way of obtaining a volatile fraction. Studies by Moldão-Martins *et al.* [55, 56] showed that the SFE extraction procedures affected the *Th. zygis* extracts composition and sensorial attributes. Depending on the SFE extraction conditions, the extract composition was similar to the essential oil aroma profile [55], or completely different [56]. Nevertheless, SFE always produced a higher yield of heavier compounds, such as alkanes, waxy esters, carotenoids and traces of chlorophylls.

Sensorial Evaluation of Thymus Extracts

Sensory analysis is an important parameter in quality control as well as on the evaluation of the customer preferences and acceptability, which is, in its turn, dependent on the incorporation level of the extract in the matrix and of the chemical composition. Only two studies have been performed on the sensory evaluation of commercial sunflower and olive oil incorporated with either SFE extract or essential oil, respectively, from Portuguese *Thymus* species.

The sensorial evaluation of the *Th. zygis* SFE aromatic extracts, incorporated into commercial sunflower oil [56], gave preference to extracts with higher levels of terpene phenols and geranyl acetate. Extracts with higher amounts of *p*-cymene and γ-terpinene were not accepted or badly scored. The detection threshold was 0.001 mg/kg of sunflower oil with a preferred incorporation level of 0.02 mg/kg. For *Th. mastichina* essential oil, incorporated into olive oil, the detection threshold was 0.0009 mg/kg of olive oil with a preferred incorporation level of 0.005 mg/kg [57].

BIOLOGICAL ACTIVITY OF PORTUGUESE THYME OILS

Antiacetylcholinesterase

Acetylcholinesterase (AChE) is a human enzyme that exists in multiple isoforms and that hydrolyzes the acetylcholine neurotransmitter, thereby terminating signal transmission at the synapses. Any acetylcholinesterase inhibitor, that is any compound that shows antiacetylcholinesterase activity, will increase the level as well as the extent of action of the acetylcholine neurotransmitter. Due to its possible relevance in the treatment of Alzheimer's disease, several types of plant extracts are now being assayed for their antiacetylcholinesterase activity [58-60].

Only one carvacrol rich (56%) essential oil, of the Portuguese wild thyme, *Th. serpyllum* (collected in Beira Interior so probably *Th. pulegioides* is meant, see Table **1** and the subtitle "Thyme species occurring in Portugal"), as been assayed for antiacetylcholinesterase capacity [61]; carvacrol showed an IC_{50}=115µg/ml and *Th. serpyllum* oil an IC_{50}= 190µg/ml. Ethanol and water extracts were also comparatively assayed and the essential oil showed the highest antiacetylcholinesterase inhibitory activity.

Antibacterial

Thyme species essential oils have been known to possess antibacterial properties for long, Gram-positive bacteria being, generally, more susceptible than Gram-negative ones [4 and references therein].

The antibacterial activity of Portuguese *Thymus* essential oils was first reported for *Th. mastichina* (L) L. subsp *mastichina* and *Th. albicans* Hofmanns & Link [62]. The antibacterial activity of the essential oils, isolated from the leaves, of these species was tested against two Gram-positive bacteria (*Staphylococcus aureus* and *Listeria monocytogenes*) and one Gram-negative bacterium (*Salmonella* sp.). The strains of either *S. aureus* or *L. monocytogenes* were more susceptible to *Th. albicans* essential oils than to *Th. mastichina* essential oil. By contrast *Salmonella* sp. was more susceptible to *Th. mastichina* essential oil. These differences may be explained by the different composition of both essential oils. *Th. albicans* possessed 1,8-cineole and borneol rich oils whereas *Th. mastichina* possessed camphor rich oils, Table **5**.

The essential oils isolated from the flowering aerial parts of *Th. mastichina* (L) L. subsp. *mastichina* (collected at two different geographic regions; Algarve and Estremadura), *Th. camphoratus* and *Th. lotocephalus* (collected at Algarve) were evaluated against three Gram-negative bacteria (*Escherichia coli, Proteus mirabilis* and *Salmonella* sp.), two Gram-positive bacteria (*S. aureus* and *L. monocytogenes*) and the yeast *Candida albicans* [63], Table **5**. The microorganisms tested showed different susceptibility to the *Thymus* essential oils assayed. *E. coli* was more susceptible to *Th. mastichina* leaf oil from both regions, whereas *P. mirabilis* was more vulnerable to *Th. lotocephalus* flower essential oil. *C. albicans* was more susceptible to Estremadura *Th. mastichina* leaf essential oil. Gram-positive bacteria demonstrated different vulnerability: *S. aureus* was more susceptible to *Th. mastichina* leaf essential oil whereas *L. monocytogenes* EGD was more susceptible to *Th. lotocephalus* flower oil. The different susceptibility may be explained both by strain susceptibility and by the different oil composition, Table **5**.

In this study, Faleiro *et al.* [63] also assessed the antimicrobial activity of the pure main oil components (1,8-cineole, linalool and linalool/1,8-cineole). 1,8-Cineole was almost inactive to all microorganisms tested and linalool showed a low antimicrobial capacity, which correlated to the little antimicrobial performance of the tested oils, Table **5**.

The possibility of using essential oils on the control of *L. monocytogenes*, an important food borne pathogen, was evaluated by testing *Thymbra capitata* (= *Th. capitatus*) and *Origanum vulgare* L (Cav.) essential oils [64]. In this study forty one *L. monocytogenes* strains isolated from different food origins were used. The antilisterial activity of *T. capitata* essential oil was higher than that of *Origanum vulgare* and than that of the antibiotic chloramphenicol. The MIC value (Minimum Inhibitory Concentration, the concentration at which no listerial growth is observed) determined for a set of 5 *L. monocytogenes* strains varied between 0.05 and 0.20 μL/mL and the minimum bactericidal concentration (MBC, the minimum concentration at which no recovery of viable cells was registered) was 0.30 μL/mL for the strains of *L. monocytogenes* tested. The MIC value for carvacrol (the major component of *T. capitata* oil) ranged from 0.15 to 0.015 μL/mL. The MBC value was 0.25 μL/mL for all 5 *L. monocytogenes* tested. The susceptibility of the *L. monocytogenes* strains to carvacrol was only slightly higher than that of *T. capitata* oil.

A set of 13 strains of *L. monocytogenes* from the previous group of forty one strains were also tested for their vulnerability to essential oils isolated from different *Thymus* species grown in Algeria [65] and the maximum value of the inhibition zone achieved was 14mm. The antilisterial activity of these essential oils was similar to that previously determined for Portuguese *Thymus* endemic species [63].

In 1991, Crespo [*in* 4] classified thyme essential oils in two main groups: a) thymol and carvacrol rich oils with major antimicrobial activities and b) non-phenolic oxygenated monoterpenes- or even sesquiterpene hydrocarbons-rich oils, showing lower antimicrobial activities than the former group.

The results from the assessment of the antimicrobial activity of Portuguese thyme oils, Table **5**, support this view; the non-phenolic essential oils of *Th. albicans*, *Th. camphoratus*, *Th. lotocephalus* and *Th. mastichina* were only weakly active or almost inactive. Stronger antibacterial activity was observed for typical carvacrol rich oils from *T. capitata*. The essential oil from this species may constitute a promising tool for controlling food borne pathogens.

Antifungal

Fungal infections have been increasing in recent years, particularly in patients with impaired immunity from the use of cytotoxic drugs, immunosuppressive therapy, in intensive care units and following treatment with broad-spectrum antibiotics. Recurrent mucosal, cutaneous or nail infections can be severe in debilitated or immunocompromised patients [66, 67]. Considering the importance of fungal infections and the difficulties encountered in their treatment, as well as the increase in resistance to antifungals [68], many scientists have recently paid attention to extracts and biologically active compounds isolated from plant species used in herbal medicines. A wide variety of essential oils are known to possess

Table 5. Antibacterial Activity of Portuguese *Thymbra capitata* and *Thymus* Species Essential Oils [Adapted from 62-64]

Test Organism*		***Thymus* and *Thymbra* Species and Main Essential Oil Component(s)****							
		Th. albicans	***Th. camphoratus***	***Th. lotocephalus***	***Th. mastichina* ssp. *mastichina***				***Thymbra capitata***
		1,8-Cineole / Borneol	**1,8-Cineole**	**Linalyl Acetate / 1,8-Cineole / Linalool**	**Camphor**	**1,8-Cineole**	**Linalool / 1,8-Cineole**	**Linalool**	**Carvacrol**
Gram-positive bacteria									
Staphylococcus aureus	{1}	++	-	+	++	+ to ++	+	++	
Listeria monocytogenes	{3}, {1}, {41}	++	-	+	+	-		-	++ to +++
Gram-negative bacteria									
Escherichia colli	{1}		-			- to +	-	+	
Proteus mirabilis	{1}		+	++		-	-	++	
Salmonella sp.	{1}	+	+	+	+ to ++	-	-	+	

* nº of assayed strains in curly brackets: from references [62-64]; Inhibition area: (-) <7mm, (+) 7-10mm, (++) 11-16mm, (+++) >16mm. ** The antibiotic chloramphenicol was used as a positive control showing inhibition areas >16mm [64].

antifungal activity [69]. The lipophilic character of the hydrocarbon skeleton of the essential oil constituents as well as the hydrophilic character of their functional groups is of the main importance in the antimicrobial action of the essential oils. Therefore, a rank of activity has been proposed as follows: phenols > aldehydes > ketones > alcohols > esters > hydrocarbons [70].

Several *in vitro* and *in vivo* screenings have shown that volatile oils containing phenolic structures, such as carvacrol and thymol, are highly active against a broad spectrum of plant, animal and human pathogenic fungi [71-73]. The importance of the hydroxyl group has been confirmed and the relative position of the hydroxyl group on the phenolic ring does not appear to strongly influence the degree of the activity [74]. Low activity was observed with components containing only an aromatic ring with alkyl substituents as in *p*-cymene.

Several studies have shown that thyme oils possess antifungal activity, those of the phenol type (thymol and carvacrol) being the most active [4 and references therein]. The limited occurrence of these phenols in nature is one of the reasons why *Thymus* oils containing thymol and carvacrol have been of great interest for some time.

Several studies with Portuguese *Thymus* oils, Table **6**, show an important antifungal activity against *Candida* (clinical strains isolated from recurrent cases of vulvovaginal candidosis and ATCC type strains), *Aspergillus* strains (clinical strains isolated from bronchial secretions and ATCC and CECT type strains) and dermatophyte strains (clinical strains isolated from nails and skin). The antifungal activity of the oils and their main compounds were evaluated by determining the minimal inhibitory concentration (MIC) and the minimal lethal concentration (MLC), according the NCCLS protocol M27-A and M38-A [75, 76] for yeasts and filamentous fungi, respectively [67, 77-79].

Thyme essential oils or some of their constituents are indeed effective against a large variety of fungi, particularly the oils with high amounts of thymol and/or carvacrol, such as those from *Th. zygis* subsp. *zygis* (thymol type), *Th. pulegioides* (thymol/carvacrol type) and *Thymbra capitata* (carvacrol type), Table **6**. For these oils MIC values ranged from 0.08 to 0.64 μL/mL. In the majority of cases MIC and MLC values were similar, indicating fungicidal activity [67, 77, 78]. It is difficult to attribute the activity of a complex mixture to a particular compound. Nevertheless, it is reasonable to associate the activity of these oils with the presence of carvacrol and thymol. These compounds were found to be the most active compounds with MIC values ranging from 0.04 to 0.32 μL/mL.

Some Portuguese thyme species producing non-phenolic essential oils, like *Th. mastichina* subsp. *mastichina* and *Th. capitellatus* (three different chemotypes) were also evaluated against the same strains [77, 79]. These oils, Table **7**, showed only a modest antifungal activity. This may reflect the reduced antifungal activity of their major compounds, mainly 1,8-cineole and borneol [77, 79]. Nevertheless, the three chemotypes of *Th. capitellatus* (1,8-cineole, 1,8-cineole/borneol and 1,8-cineole/linalyl acetate/linalool) showed activity against dermatophyte strains [79], being the 1,8-cineole/linalyl acetate/linalool chemotype the more active, with MIC values of 0.32-0.64 μL/mL. The highest activity of this chemotype can be correlated with the content of linalyl acetate, Table **7**. For *Candida* and *Aspergillus* strains the three chemotypes showed low activity.

The studies of Faleiro *et al.* [62, 63] showed also that *Th. mastichina* ssp. *mastichina* linalool / 1,8-cineole- and linalool- chemotypes were more effective against *Candida albicans* than the 1,8-cineole chemotype or than *Th. camphoratus* 1,8-cineole chemotype.

Most antifungals act as germ tube and hypha formation inhibitors. The effect of essential oils upon germ tube formation, an important virulence factor, was also studied. Subinhibitory concentrations of *T. capitata*, *Th. zygis* and *Th. mastichina* oils were able to inhibit germ tube formation [77, 78]. Curiously, a greater germ tube inhibition was found with *Th. mastichina* and 1,8-cineole.

Considering the large number of different chemical compounds present in the essential oils, it is most likely that their antifungal properties are not attributable to one specific mechanism, but to other targets in the cell [80]. To clarify the mechanism of these oils action on yeasts and filamentous fungi flow-cytometric studies of plasma membrane integrity were performed, using PI (propidium iodide) as a nucleic acid stain that only penetrates cells that are already dead or have severe lesions of the plasma membrane [81]. The results showed that the oils of *Th. pulegioides*, *Th. zygis* and *T. capitata* act by primary lesion of the plasma membrane [67, 77, 82]. On *Candida* they act as fungicidal, promoting severe lesion on the plasma membrane, as PI could penetrate most of the yeast cells after 5 min at the MLC values [67]. This suggests that the fungicidal effect results from direct damage to the cell membrane rather than from metabolic impairment leading to secondary plasma membrane damage.

The essential oil of *Th. pulegioides* therefore induces considerable impairment of *Candida albicans* and *Trichophyton rubrum* biosynthesis of ergosterol [67], the predominant sterol in fungi cells, that plays an important role in membrane fluidity, permeability and on the activity of many membrane-bound enzymes [74].

Considering that essential oils are complex mixtures of numerous molecules, the effect of different combinations of the same major compounds from Portuguese *Thymus* oils (thymol/carvacrol; thymol/*p*-cymene; thymol/1,8-cineole; carvacrol/*p*-cymene; carvacrol/I,8-cineole; *p*-cymene/1,8-cineole) was assayed with *Candida albicans* and *C. krusei* according to the checkerboard method [77]. No antagonistic effect was detected when assaying the different combinations of the compounds tested, and in terms of synergistic activity, the most synergic combinations were thymol/1,8-cineole and thymol/*p*-cymene.

Taking in account the information available on the antifungal effect of thyme oils, they appear as promising agents against pathogenic fungi to humans. Particularly phenol-rich oils are broad-spectrum agents that inhibit not only dermatophytes, *Aspergillus* and *Candida* species, but also fluconazole-resistent *C. albicans* isolates, and *C. krusei* and *C. glabrata*, which are intrinsically resistant to fluconazole or whose resistance is easily inducible [67].

Table 6. Antifungal Activity of the Phenolic Portuguese Thyme Essential Oils and of Some Main Oil Components [Adapted from 67, 77-79]. The Positive Controls, Fluconazole and Amphotericin B, Showed MIC Values Ranging from <1 to ≥128µg/mL and 1-4µg/mL, Respectively [67, 78, 79]

Essential Oils or their Components	*Thymus zygis*		*Thymus pulegioides*		*Thymbra capitata*		Thymol		Carvacrol		*p*-Cymene		γ-Terpinene	
Organisms	**MIC (µL/mL)**	**MLC (µL/mL)**	**MIC (µL/mL)**	**MLC (µL/mL)**	**MIC (µL/mL)**	**MLC (µL/mL)**	**MIC (µL/mL)**	**MLC (µL/mL)**	**MIC (µL/mL)**	**MLC (µL/mL)**	**MIC (µL/mL)**	**MLC (µL/mL)**	**MIC (µL/mL)**	**MLC (µL/mL)**
Candida albicans ATCC 10231	0.16 – 0.32	0.32	0.64	0.64	0.32	0.32 - 0.64	0.16	0.32	0.16	0.16 - 0.32	5.0 - 10.0	>10.0	2.5 - 5.0	5.0 - 10.0
C. albicans H37	0.16 - 0.32	0.32	0.32	0.32 - 0.64	0.16 - 0.32	0.32	0.16	0.32	0.16	0.16 - 0.32	5.0	>10.0	5.0	5.0 - 10.0
C. albicans M1	0.32	0.32	0.32 - 0.64	0.64	0.32	0.32	0.16	0.32	0.16	0.32	5.0 - 10.0	>10.0	2.5 - 5.0	5.0 - 10.0
C. tropicalis ATCC 13803	0.16 - 0.32	0.32	0.32 - 0.64	0.32 - 0.64	0.32	0.32 - 0.64	0.16 - 0.32	0.32	0.16	0.16 - 0.32	5.0 - 10.0	>10.0	10 - 20	≥20
C. tropicalis H18	0.32	0.32	0.64	0.64	0.32	0.32 - 0.64	0.16 - 0.32	0.32	0.16	0.16 - 0.32	5.0 - 10.0	>10.0	10 - 20	10 - 20
C. glabrata H16	0.32	0.32	0.32 - 0.64	0.32 - 0.64	0.32	0.32 - 0.64	0.16	0.32	0.16	0.32	5.0 - 10.0	>10.0	5.0	10
C. glabrata H30	0.32	0.32	0.64	0.64	0.32	0.32 - 0.64	0.32	0.32	0.16	0.32	10.0	>20.0	5.0 - 10.0	20
C. krusei ATCC 6258			0.32 - 0.64	0.32 - 0.64			0.16 - 0.32	0.32	0.16	0.16 - 0.32	5.0 - 10.0	10.0	5.0 - 10.0	10
C. krusei H9	0.16 - 0.32	0.32	0.32 - 0.64	0.32 - 0.64	0.32	0.32	0.16 - 0.32	0.32	0.16	0.16 - 0.32	5.0 - 10.0	10.0	5.0 - 10.0	10
C. guilermondii MAT 23	0.16	0.16	0.32	0.16	0.16 - 0.32	0.32	0.16	0.32	0.08 - 0.16	0.16	5.0	10.0	1.25 - 2.5	2.5 - 5.0
C. parapsilosis ATCC 90018	0.32	0.32	0.64	0.32	0.32	0.32 - 0.64	0.32	0.16	0.16	0.16 - 0.32	2.5	>20.0	5.0	20
Epidermophyton floccosum FF9			0.16	0.16	0.08	0.16	0.16	0.32	0.08	0.08	5.0	5.0	2.5	2.5
Trichophyton rubrum FF5			0.32	0.32	0.16	0.16	0.16	0.16	0.08	0.08	1.25	1.25 - 2.5	5.0	5.0
T. mentagrophytes FF7			0.16	0.32	0.08	0.16	0.16	0.16	0.04	0.08	5.0	>5.0	10.0	10.0
Microsporum canis FF1			0.16	0.16 - 0.32	0.08	0.16	0.08	0.16 - 0.32	0.04	0.08	2.5	2.5	5.0	5.0
M. gypseum FF3			0.16	0.32	0.08	0.16 - 0.32	0.16	0.16	0.04	0.08 - 0.16	10.0	>10.0	10.0	10.0
Aspergillus niger ATCC 16404			0.32	0.16	0.16	0.16 - 0.32	0.16	0.32	0.16	0.16 - 0.32	>20	>20	>20	>20
A. niger CETC 2574			0.32	0.16	0.16	0.16	0.16	0.64	0.16	0.16 - 0.32	>20	>20	20	>20
A. niger F01			0.32	0.16	0.16	0.16 - 0.32	0.16	0.64	0.16	0.32	>20	>20	>20	>20
A. fumigatus ATCC 46645			0.16	0.16	0.16	0.32	0.16	0.64	0.16	0.32	>20	>20	20	>20
A. fumigatus CETC 2071			0.16	0.16	0.16	0.32	0.16	0.64	0.16	0.32	>20	>20	20	20
A. fumigatus F05			0.16	0.16	0.32	0.32 - 0.64	0.16	0.64	0.16	0.32	>20	>20	10 - 20	>20
A. fumigatus F07			0.16	0.16	0.32	0.64	0.16	0.64	0.16	0.16	>20	>20	20	>20
A. fumigatus F17			0.16	0.16	0.32	0.32 - 0.64	0.16	0.64	0.16	0.32	>20	>20	20	>20
A. flavus F44			0.32	0.32	0.32	0.32	0.32	0.64	0.32	0.32	>20	>20	20	20

Antioxidant

The use of antioxidants in food industry is important as they inhibit oxidation, one of the major causes of chemical spoilage, preventing rancidity and/or deterioration of the nutritional quality, colour, flavour and texture of foods [83]. The antioxidant properties of different plant extracts, essential oils and pure compounds can be evaluated using a quite diverse number of *in vitro* assays. Antioxidant assays in foods and biological systems can be divided into two groups: a) those that evaluate lipid peroxidation, and b) those that measure free radical scavenging ability [84].

Several works have demonstrated the antioxidant capacity of *Thymus* species oils either by preventing lipid peroxidation or by scavenging free radicals [65, 85-94].

The antioxidant activity of Portuguese thyme species has been measured mainly by evaluating their ability for preventing lipid peroxidation, Table **8**.

Table 7. Antifungal Activity of the Non-Phenolic Portuguese Thyme Essential Oils and of Some Main Oil Components [Adapted from 77, 79]. The Positive Controls, Fluconazole and Amphotericin B, Showed MIC Values Ranging from <1 to ≥128µg/mL and 1-4µg/mL, Respectively [67, 78, 79]

Essential Oils or their Components	*Thymus mastichina*		*Thymus capitellatus* (1,8-Cineole Chemotype)		*Thymus capitellatus* (1,8-Cineole/Linalyl Acetate/Linalool Chemotype)		*Thymus capitellatus* (1,8- Cineole/ Borneol Chemotype)		1,8-Cineole		Borneol		Linalool		Linalyl Acetate	
Organisms	**MIC (µL/mL)**	**MLC (µL/mL)**	**MIC (µL/mL)**	**MLC (µL/mL)**	**MIC (µL/mL)**	**MLC (µL/mL)**	**MIC (µL/mL)**	**MLC (µL/mL)**	**MIC (µL/mL)**	**MLC (µL/mL)**	**MIC (µL/mL)**	**MLC (µL/mL)**	**MIC (µL/mL)**	**MLC (µL/mL)**	**MIC (µL/mL)**	**MLC (µL/mL)**
Candida albicans ATCC 10231	2.5	2.5	2.5 - 5.0	2.5 - 5.0	2.5	2.5 - 5.0	2.5 - 5.0	2.5 - 5.0								
C. albicans H37	1.25 - 2.5	2.5	1.25 - 2.5	2.5	1.25	1.25	1.25 - 2.5	1.25 - 2.5								
C. albicans M1	2.5	5.0	2.5 - 5.0	2.5 - 5.0	2.5	5.0	2.5 - 5.0	2.5 - 5.0								
C. tropicalis ATCC 13803	2.5 - 5.0	5.0	5.0	5.0	2.5 - 5.0	5.0	2.5 - 5.0	5.0								
C. tropicalis H18	5.0 - 10.0	5.0	5.0	5.0	2.5 - 5.0	2.5 - 5.0	2.5 - 5.0	2.5 - 5.0								
C. glabrata H16	1.25 - 2.5	5.0	2.5	2.5 - 5.0	1.25 - 2.5	2.5	1.25 - 2.5	1.25 - 2.5								
C. glabrata H30	2.5	5.0	5.0	5.0	2.5	5.0	2.5 - 5.0	5.0								
C. krusei H9	1.25 - 2.5	2.5	2.5 - 5.0	5.0	2.5 - 5.0	2.5 - 5.0	1.25 - 2.5	2.5								
C. guilermondii MAT 23	1.25	1.25	2.5	2.5	2.5	2.5	2.5	2.5								
C. parapsilosis ATCC 90018	2.5 - 5.0	5.0	2.5 - 5.0	5.0	2.5	2.5 - 5.0	5.0	5.0								
Epidermophyton floccosum			0.64	0.64	0.32	0.32	0.64	0.64	5.0	5.0	2.5	2.5 - 5.0	1.25 - 2.5	2.5	0.32	0.32
Trichophyton rubrum			0.64	1.25	0.64	1.25	1.25	1.25	2.5 - 5.0	2.5 - 5.0	2.5	2.5 - 5.0	1.25	1.25	0.32-0.64	0.32-0.64
T. mentagrophytes			0.64	0.64	0.64	0.64	0.64	0.64 -1.25	5.0	5.0	2.5	2.5 - 5.0	1.25	2.5	0.64	0.64
Microsporum canis			0.64	0.64	0.64	0.64-1.25	1.3	1.25	5.0	5.0	2.5	2.5 - 5.0	2.5	2.5	0.32-0.64	0.32-0.64
M. gypseum			1.25	1.25	0.64	0.64-1.25	0.6	1.25	10.0	10.0	2.5	2.5 - 5.0	2.5	2.5	0.64	0.64
Aspergillus niger ATCC 16404			5.0	5.0 - 10.0	2.5	20.0	5.0	20.0								
A. niger CETC 2574			5.0	5.0 - 10.0	2.5	20.0	5.0	20.0								
A. niger F01			5.0	10.0	2.5	10.0	5.0	10.0								
A. fumigatus ATCC 46645			2.5 - 5.0	10.0	2.5	10.0	2.5	10.0								
A. fumigatus CETC 2071			2.5 - 5.0	5.0 - 10.0	2.5	10.0	2.5 - 5.0	10.0								
A. fumigatus F05			5.0	10.0	2.5	10.0	2.5 - 5.0	10.0								
A. fumigatus F07			5.0	20.0	2.5	10.0	2.5 - 5.0	10.0								
A. fumigatus F17			5.0	10.0	2.5	10.0	2.5	10.0								
A. flavus F44			10.0	≥20	5.0	≥20.0	10.0	10.0-20.0								

Carvacrol, the main component of *T. capitata* essential oil, has been considered responsible for preventing lipid olive oil peroxidation, which as been supported by the peroxidation inhibition percentages determined for the oil and pure compound, 69% and 66%, respectively [95].

In the antioxidant assays one should never neglect the role played by the substrates. As previously shown by Miguel *et al*. [95], sunflower oil is difficult to stabilise due to both high linoleic acid and α-tocopherol contents. This may explain the lower peroxidation inhibition percentage of *T.*

capitata essential oil (50%) and carvacrol (46%) in sunflower oil when compared with that in olive oil. In addition, butylated hydroxytoluene (BHT), used as control, showed lower peroxidation inhibition percentage (20%) than those of the essential oil and carvacrol, Table **8**. According to Miguel *et al.* [95], the hindrance of the two tertiary butyl substituents of BHT can explain the weak reaction with free radicals, being more relevant for polyunsaturated fatty acids, such as linoleic acid of sunflower oil. These results were supported by those of Miguel *et al.* [96], with an extended assay to 57 days, using other sunflower oil. It is now well known that the antioxidant activity is dependent on several factors such as medium polarity, temperature, oxidation conditions, physical state and type of substrate [97].

On the other hand, other *T. capitata* essential oil showed weak capacity for preventing lipid peroxidation both in peanut oil and sunflower oil [98]. After 78 days of storage at 60ºC in the dark, the peroxide content of peanut oil and sunflower oil, with added *T. capitata* oil, was almost similar to that found with the control (without added essential oil). This weak capacity of *T. capitata* oil for preventing peroxidation in both lipid substrates was unexpected and in contradiction with the previous works. According to Miguel *et al.* [98] these results could be due to the different nature of the lipid substrate and/or essential oil composition, in spite of its carvacrol richness, that is, in addition to carvacrol other oil components may contribute synergistically or antagonistically to the oil antioxidant activity.

Miguel *et al.* [98] showed that peanut oil or sunflower oil, to which *Th. mastichina* essential oil was added, had lower acid value than the control, after 78 days of storage. The authors stressed, therefore, the importance of using several methods for evaluating lipid oxidation as different methods provide different antioxidant results.

Using the TBARS method (thiobarbituric acid reactive species), that measures malonaldehyde, one of the secondary lipid peroxidation products formed, after reacting with thiobarbituric acid (TBA), Faleiro *et al.* [64] determined that *T. capitata* oil showed antioxidant ability (82%) mainly at high concentrations (1000 mg/L), comparable to those of

Table 8. Methods and Lipid Substrates Used in the Determination of Antioxidant Activity of the Essential Oils Isolated from Thyme Portuguese Species

***Thymus* and *Thymbra* Species**	**Antioxidant Assay Method**	**Reference**
***Thymbra* genus**		
T. capitata	Peroxide value in olive oil	[95]
	Peroxide value in peanut and sunflower oil	[96]
	Acid value in peanut and sunflower oil	[98]
	TBARS with and without AAPH	[64]
	Micellar model system	[64]
***Thymus* genus**		
Th. albicans	Peroxide value in sunflower oil	[96]
Th. caespititius	TBARS with and without AAPH	[102]
Th. camphoratus	Peroxide value in peanut and sunflower oil	[98]
	Acid value in peanut and sunflower oil	[98]
	TBARS with and without AAPH	[102]
Th. carnosus	Peroxide value in sunflower oil	[96]
Th. mastichina		
1,8-Cineole chemotype	Peroxide value in sunflower oil	[96]
	Peroxide value in peanut and sunflower oil	[98]
	Acid value in peanut and sunflower oil	[98]
	TBARS with and without AAPH	[102]
Linalool chemotype	Peroxide value in sunflower oil	[96]
Th. serpyllum*	β-Carotene-linoleic acid (spectrophotometry)	[61]

*probably *Th. pulegioides* as explained in the text, see Table **1**.

BHT (86%) and BHA (80%). The antioxidant activity of the essential oil was not modified when the radical inducer 2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH) was added to the system (84%). Using the micellar model system method, that indicates the formation of primary components (hydroperoxydienes) of the oxidative process of a lipid, Faleiro *et al.* [64] also concluded that *T. capitata* essential oil possessed a remarkable activity (96% at 160 mg/L). Based on a comparison of *T. capitata* essential oil antioxidant indices obtained with both methods, the authors suggested that the essential oil would act preferentially reducing the formation of hydroperoxydienes, that is, by preventing the primary oxidation. This assumption is supported by the higher antioxidant indices detected using the micellar model system method when compared with those obtained by the TBARS method.

Th. serpyllum (probably *Th. pulegioides* as mentioned above, see Table **1**) is another carvacrol-rich essential oil, which Mata *et al.* [61] reported as possessing antioxidant activity evaluated by β-carotene-linoleic acid (IC_{50}=96.9μg/mL).

Although the antioxidant activity is generally attributed to phenolic compounds such as carvacrol [99, 100], other oil components may also possess some antioxidant activity. For instance, *Th. albicans* and *Th. mastichina* essential oils showed some ability for preventing sunflower oil lipid peroxidation [96]. 1,8-Cineole is the dominant component of *Th. albicans* essential oils (68%) as well as of one of *Th. mastichina* chemotypes (45%). Their corresponding antioxidant activity, after 57 days of storage at 60ºC in the dark, expressed as percentages of inhibitions were 48% and 49%, respectively. 1,8-Cineole and carvacrol, assayed under the same conditions, showed similar antioxidant activity, 50% and 55%, respectively, whereas only 20% was attained with BHT.

Th. mastichina linalool chemotype also possessed antioxidant activity (59%). Nevertheless, linalool only showed a lipid peroxidation inhibition of 49%, lower than the linalool rich *Th. mastichina* essential oil. According to Ruberto and Baratta [101], allylic alcohols seem to possess some antioxidant activity when measured by TBARS method and by spectrophotometric determination of the rate of conjugated diene formation. Nevertheless, the authors obtained an unexpected pro-oxidant activity with linalool, a tertiary allylic alcohol. This pro-oxidant activity was not recorded by Miguel *et al.* [96], in spite of the lower activity of the pure compound when compared with the essential oil.

Table 9. Antioxidant Activity and Major Components of Some *Th. albicans*, *Th. camphoratus*, *Th. carnosus* and *Th. mastichina* Oil Chemotypes, as well as of Standard Components [Adapted from 103]

Thyme Species	Essential Oil Main Components (%)	TBARS		Micellar Model System
		Without AAPH	With AAPH	
		Antioxidant Index (%) at 1000mg/L		Antioxidant Index (%) at 160mg/L
Th. albicans	1,8-Cineole 49%, Linalool 14%, Borneol 3%	69	66	3
	1,8-Cineole 49%, Linalool 12%, Borneol 4%	66	41	36
	1,8-Cineole 28%, Borneol 12%, Linalool 0.3%	45	40	47
Th. camphoratus	*trans*-Sabinene hydrate 20%, 1,8-Cineole 4%, Borneol 4%, Linalool 1%	69	60	36
	Borneol 13%, Linalool 11%, 1,8-Cineole 4%, *trans*-Sabinene hydrate 2%	43	43	52
	1,8-Cineole 33%, *trans*-Sabinene hydrate 1%, Borneol 5%, Linalool 5%	35	49	67
Th. carnosus	Borneol 23%, *trans*-Sabinene hydrate 14%	72	57	39
	Borneol 38%, *trans*-Sabinene hydrate 7%	72	53	68
	Borneol 23%, *trans*-Sabinene hydrate 16%	76	74	55
Th. mastichina	1,8-Cineole 61%, Linalool 1%	59	46	49
	1,8-Cineole 49%, Linalool 1%	73	63	37
	Linalool 40%, 1,8-Cineole 10%	79	53	19
BHA		80	96	84
BHT		86	90	84
α-Tocopherol		65	93	76

AAPH, 2,2'-azobis(2-amidinopropane) dihydrochloride; BHA, butylated hydroxyanisole; BHT, butylated hydroxytoluene.

Other non-phenolic oils have also shown some antioxidant activity: *Th. caespititius* (76%) > *Th. camphoratus* (52 %) > *Th. mastichina* (39%) when evaluated by the TBARS method [102].

As previously mentioned, there is a large chemical polymorphism for the majority of Portuguese *Thymus* taxa. Miguel *et al.* [103] assayed the antioxidant activity of diverse chemotypes of *Th. albicans*, *Th. camphoratus*, *Th. carnosus* and *Th. mastichina* collected at different regions of Portugal and compared with those of BHT, BHA and α-tocopherol, Table **9**.

With the TBARS method and in the absence of the radical inducer AAPH, the antioxidant activities of *Th. carnosus* oils did not differ considerably, independent on the relative amount of *trans*-sabinene hydrate and borneol. These results contrast with those obtained with *Th. camphoratus* essential oils, Table **9**, where the high content of 1,8-cineole (33%) seemed to be responsible for the lower antioxidant activity (35%). Similar results were reached for *Th. mastichina* essential oils: the lower the percentage of 1,8-cineole the higher antioxidant activity. Nevertheless, this behaviour was not so clear for *Th. albicans* essential oils, Table **9**. Generally the presence of AAPH decreased the antioxidant activities, with the exception of the controls and *Th. camphoratus* oils. With the micellar model system, it was also evident that the chemical composition of the oils was responsible for the differences observed in the antioxidant activities, Table **9**. In addition, the antioxidant capacity was different when evaluated by each of the methods. Miguel *et al.* [103] concluded on the importance of the nature and relative amount of the essential components in the antioxidant activity and also on the evaluation method chosen.

The Portuguese thyme species studied so far showed antioxidant capacity, namely for preventing lipid peroxidation. Nevertheless, their chemical polymorphism forces to investigate deeply the synergism and antagonism promoted by the oils components.

CONCLUSION

In the early 1940s the chemical variation of thyme species volatiles attracted the attention of the first Portuguese researchers of this field. Since then, several aspects of Portuguese thyme species botany, taxonomy, ethnobotany, phytochemistry, pharmacology, molecular biology, among others, have been the focus of continuous research. With the present work we aimed at a comprehensive report on the scientific achievements obtained until now on the chemistry and biological activities of Portuguese thyme essential oils and volatiles, as well as to show that many doors are still open that worth exploring in future works.

ACKNOWLEDGEMENTS

The authors should like to express their gratitude to MSc Joana Camejo-Rodrigues for the precious help in gathering most of the information on the ethnobotanical uses of Portuguese thyme species.

ABBREVIATIONS

MIC = Minimum inhibitory concentration

MBC = Minimum bactericidal concentration

MLC = Minimal lethal concentration

IC_{50} = Half maximal (50%) inhibitory concentration)

TBA = Thiobarbituric acid

TBARS = Thiobarbituric acid reactive species

BHT = Butylated hydroxytoluene

BHA = Butylated hydroxyanisole

AAPH = 2,2'-azobis(2-amidinopropane) dihydrochloride

invs = Interviews

inqs = Inquiries

obs-part = Observation-participation

n.m. = Not mentioned

SFE = Supercritical CO_2 extraction

t = Traces

n.d. = Not detected

REFERENCES

References 140-142 are related articles recently published.

[1] Kruger A. An illustrated guide to herbs their medicine and magic. Dragon's World, London 1992.

[2] Bremnes L. Plantas aromáticas culinárias, medicinais e cosméticas. Editora Civilização, Lisboa 1993.

[3] Morales R. The history, botany and taxonomy of the genus. In: Stahl-Biskup E, Sáez F, Eds. Medicinal and Aromatic Plants – industrial profiles, vol. 17. Taylor & Francis, London 2002; 1-43.

[4] Zarzuelo A, Crespo E. The medicinal and non-medicinal uses of thyme. In: Stahl-Biskup E, Sáez F, Eds. Medicinal and Aromatic Plants – industrial profiles, vol. 17. Taylor & Francis, London 2002; 263-92.

[5] Simon JE, Chadwick AF, Craker LE. Herbs (1971-1980). An indexed bibliography. Elsevier. Amsterdam 1984.

[6] Council of Europe (COE) - European Directorate for the Quality of Medicines. European Pharmacopoeia 6th ed. Strasbourg, 2007.

[7] Morales Valverde R. Synopsis of the genus *Thymus* L. in the Mediterranean area. Lagascalia 1997; 19: 249-62.

[8] Franco JA. Nova Flora de Portugal (Continente e Açores), Vol. II. Sociedade Astória Lda. Lisboa 1984.

[9] Coutinho AX. Flora de Portugal (Plantas vasculares), 2nd ed, Bertrand, Lisboa 1933.

[10] Morales Valverde R. Híbridos de *Thymus* L. (Labiatae) en la Peninsula Ibérica. Anales Jard Bot Madrid 1995; 53: 199-211.

[11] Cotrim HC, Ascensão L, Barroso JG, Figueiredo AC, Pais MS. Study of the indument and analysis of the essential oil from the leaves of *Thymus mastichina* x *T. villosus* ssp. *villosus*. XXVIII Reunião Anual da Sociedade Portuguesa de Microscopia Electrónica e Biologia Celular, Lisboa 1993; 68.

[12] Paiva J, Salgueiro L. Novedades corológicas, taxonómicas e nomenclaturales en tomillos portugueses (*Thymus* L. *Labiatae*). Anales Jard Bot Madrid 1994; 52: 114-7.

[13] Salgueiro LR, Vila R, Tomàs X, Tomi F, Cañigueral S, Paiva J, *et al*. Essential oil composition and variability of *Thymus lotocephalus* and *Thymus* x *mourae*. Biochem Syst Ecol 2000; 28: 457-70.

[14] Salgueiro LR, Proença da Cunha A. Chemotaxonomic characterization of a *Thymus* hybrid from Portugal. Flavour Fragr J 1993; 8: 325-30

[15] Vasconcellos JC. Plantas medicinais e aromáticas, Direcção geral dos Serviços Agrícolas, Lisboa, Portugal 1949; 150-3.

[16] Fernandes Costa A. Elementos da flora aromática. Junta de Investigações Científicas no Ultramar 1975.

[17] Feijão RD'O. Medicina pelas plantas. 7th ed, Livraria Progresso Editora, Lisboa, Portugal 1979; 278-9.

[18] Braga T, Pontes G. Plantas usadas na medicina popular. 2nd ed, Edição Amigos dos Açores, Ponta Delgada, S. Miguel, Açores 2006; 43.
[19] Riviera D, Obón C. The ethnopharmacology of Madeira and Porto Santo islands, a review. J Ethnopharmacol 1995; 46: 73-93.
[20] Ribeiro JA, Monteiro AM, Silva MLF. Etnobotânica. Plantas bravias comestíveis, condimentares e medicinais. Azevedo J, Ed. Mirandela, Portugal 2000.
[21] Monjardino JR. Plantas medicinais e aromáticas do Parque Natural de Sintra-Cascais. Instituto da Conservação da Natureza (ICN) 2002.
[22] Salgueiro J. Ervas, usos e saberes. Plantas medicinais do Alentejo e outros produtos naturais. Edições Colibri/Marca-ADL, Lisboa, Portugal 2004.
[23] Frazão-Moreira A, Fernandes MM. (Org.) Plantas e saberes. No limiar da etnobotânica em Portugal, Edições Colibri / Instituto de Estudos de Literatura Tradicional, Lisboa, Portugal 2006.
[24] Dias LS, Dias AS. Herbs and spices in traditional recipes of Alentejo (Portugal). Proceedings of the IVth International Congress of Ethnobotany (ICEB 2005) 2006; 69-72.
[25] Santos S. Plantas medicinais da Península de Setúbal. Contributo para o conhecimento da sua relevância etnobotânica. Relatório de Estágio final de Licenciatura em Biologia. Faculdade de Ciências da Universidade de Lisboa 2004.
[26] Camejo-Rodrigues JS. Plantas medicinais da Serra do Açor. Eição ICN/APPSA, Portugal 2002.
[27] Camejo-Rodrigues JS. Plantas e usos medicinais populares, Concelhos de Aljezur, Lagos e Vila do Bispo. Associação Aflosul, Bordeira 2007.
[28] Novais MH. Plantas Aromáticas e/ou Medicinais no Parque Natural da Arrábida. Trabalho de Fim de Curso. Universidade de Évora 2002.
[29] Sequeira M, Fontinha S, Freitas F, Ramos L, Mateus MG. Plantas e usos tradicionais nas memórias de hoje. Freguesia da Ilha. Edição Casa do Povo da Ilha / Parque Natural da Madeira, Portugal 2006.
[30] Santos C. As plantas aromáticas e medicinais no Parque Natural do Douro Internacional – Miranda do Douro. Mogadouro, Parque Natural do Douro Internacional, ICN 2000.
[31] Fernandes J. Plantas Aromáticas e Medicinais no Parque Natural do Douro Internacional. Relatório de Estágio. Parque Natural do Douro Internacional, ICN 2001.
[32] Melo C. Estudo etnobotânico, Parque Natural do Vale do Guadiana. Relatório de Projecto do Curso de Engenharia Agro-Florestal. Escola Superior Agrária de Beja 2002.
[33] Sommer MR. Um estudo sobre a flora aromática e medicinal utilizada pela população residente na área do Parque Natural de Sintra-Cascais e zonas envolventes. Relatório de trabalho de fim de curso de Engenharia Agronómica. Universidade Técnica de Lisboa, Instituto Superior de Agronomia 2003.
[34] Carvalho AMP. Etnobotánica del Parque Natural de Montesinho. Plantas, tradición y saber popular en un territorio del nordeste de Portugal. Tesis Doctoral. Universidad Autónoma de Madrid. UAM Ediciones 2005.
[35] Stahl-Biskup E. Essential oil chemistry of the genus *Thymus* – a global view. In: Stahl-Biskup E, Sáez F, Eds. Medicinal and Aromatic Plants – industrial profiles, Taylor & Francis, London 2002; Vol. 17: 75-124.
[36] Fernandes Costa A. Espécies Peninsulares do género *Thymus* L. Bol Fac Far Coimbra 1945; 5: 6-134.
[37] Salgueiro LR. Os tomilhos portugueses e os seus óleos essenciais. *Dissertação de Doutoramento*, Universidade de Coimbra 1994.
[38] Figueiredo AC, Barroso JG, Pedro LG, Pais MS, Scheffer JJC. The essential oils of two endemic Portuguese thyme species: *Thymus capitellatus* Hoffmanns. & Link and *T. lotocephalus* G. López & R. Morales. Flavour Fragr J 1993; 8: 53-7.
[39] Adzet T, Granger R, Passet J, San Martin R. Le polymorphisme chimique dans le genre *Thymus*: sa signification taxonomique. Biochem Syst Ecol 1977; 5: 269-72.
[40] Figueiredo AC, Barroso JG, Pedro LG, Scheffer JJC. Factors affecting secondary metabolites production in plants: volatile components and essential oils. Flavour Fragr J 2008; 23: 213-26.
[41] Rodrigues LS, Monteiro P, Moldão Martins M, Monteiro A, Póvoa O, Teixeira G. Biodiversity studies on Portuguese *Thymbra capitata*. Acta Hortic 2006; 723: 127-32.
[42] Rodrigues LS, Vasconcelos T, Forte P, Monteiro A, Rocha R, Teixeira G, *et al*. Caracterização química e micromorfológica de populações Portuguesas de *Thymbra capitata*. Actas do II Colóquio Nacional de Plantas Aromáticas e Medicinais, Associação Portuguesa de Horticultura 2007; 52-8.
[43] Silva JAT, Figueiredo AC, Barroso JG, Pedro LG, Ascensão L. Estudo dos tricomas glandulares e do óleo essencial de *Thymus mastichina* L. Actas do 1º Colóquio Nacional de Plantas Aromáticas e Medicinais, Associação Portuguesa de Horticultura 1996; 229-32.
[44] Pereira SI, Santos PAG, Barroso JG, Pedro LG, Figueiredo AC, Salgueiro LR, *et al*. Morphology and distribution of glandular trichomes and essential oil composition of three populations of *Thymus caespititius* Brot. from the Azores. XXXIV Reunião Anual da Sociedade Portuguesa de Microscopia Electrónica e Biologia Celular. Lisboa 1999; 63.
[45] Miguel MG, Duarte F, Venâncio F, Tavares RJ. Composition of the essential oils from Portuguese *Thymus albicans* collected at different regions of Ria Formosa (Algarve). J Essent Oil Res 2004; 16: 308-11.
[46] Miguel MG, Duarte F, Venâncio F, Tavares RJ. Comparison of the main components of the essential oils from flowers and leaves of *Thymus mastichina* (L.) L. subsp. *mastichina* collected at different regions of Portugal. J Essent Oil Res 2004; 16: 323-7.
[47] Pereira SI, Santos PAG, Barroso JG, Pedro LG, Figueiredo AC, Salgueiro LR, *et al*. Chemical polymorphism of the essential oils from populations of *Thymus caespititius* grown on the islands Pico, Faial and Graciosa (Azores). Phytochem Anal 2003; 14: 228-31.
[48] Moldão-Martins M, Bernardo-Gil MG, Beirão-da-Costa ML, Rouzet M. Seasonal variation in yield and composition of *Thymus zygis* L. subsp. *sylvestris* essential oil. Flavour Fragr J 1999; 14: 177-82.
[49] Miguel MG, Guerrero C, Rodrigues H, Brito H, Duarte F, Venâncio F, *et al*. Essential oils of Portuguese *Thymus mastichina* (L.) L. subsp. *mastichina* grown on different substrates and harvested on different dates. J Hortic Sci Biotech 2003; 78: 355-8.
[50] Miguel MG, Duarte J, Figueiredo AC, Barroso JG, Pedro LG. *Thymus carnosus* Boiss.: effect of harvesting period, collection site and type of plant material on essential oil composition. J Essent Oil Res 2005, 17: 422-6.
[51] Pereira SI, Santos PAG, Barroso JG, Pedro LG, Figueiredo AC, Salgueiro LR, *et al*. Chemical polymorphism of the essential oils from populations of *Thymus caespititius* grown on the island S. Jorge (Azores). Phytochemistry 2000; 55: 241-6.
[52] Santos PAG, Barroso JG, Figueiredo AC, Pedro LG, Salgueiro LR, Fontinha SS, *et al*. Chemical polymorphism of populations of *Thymus caespititius* grown on the islands Corvo, Flores, São Miguel and Terceira (Azores) and on Madeira assessed by analysis of their essential oils. Plant Sci 2005; 169: 1112-7.
[53] Trindade H, Costa MM, Lima AS, Pedro LG, Figueiredo AC, Barroso JG. Chemical polymorphism and genetic diversity of *Thymus caespititius*: is there a correlation? 38th International Symposium on Essential Oils, Graz 2007; 66.
[54] Trindade H, Costa MM, Lima AS, Pedro LG, Figueiredo AC, Barroso JG. Genetic diversity and chemical polymorphism of *Thymus caespititius* from Pico, São Jorge and Terceira islands (Azores). Biochem Syst Ecol 2008; (in press).
[55] Moldão-Martins M, Palavra A, Beirão-da-Costa ML, Bernardo-Gil MG. Supercritical CO_2 extraction of *Thymus zygis* L. subsp. *sylvestris* aroma. J Supercrit Fluids 2000; 18: 25-34.
[56] Moldão-Martins M, Bernardo-Gil MG, Beirão da Costa ML. Sensory and chemical evaluation of *Thymus zygis* L. essential oil and compressed CO_2 extracts. Eur Food Res Technol 2002; 214: 207-11.
[57] Moldão-Martins M, Beirão-da-Costa S, Neves C, Cavaleiro C, Salgueiro L, Beirão-da-Costa ML. Olive oil flavoured by the essential oils of *Mentha* x *piperita* and *Thymus mastichina*. Food Qual Prefer 2004; 15: 447-52.
[58] Oh MH, Houghton PJ, Whang WK, Cho JH. Screening of Korean herbal medicines used to improve cognitive function for anti-cholinesterase activity. Phytomedicine 2004; 11: 544-8.
[59] Tiwari S, Singh A. Piscicidal and anti-acetylcholinesterase activity of *Euphorbia royleana* stem bark extracts against freshwater common predatory fish *Channa punctatus*. Environ Toxicol Phar 2004; 18: 47-53.
[60] Lee KY, Yoon JS, Kim, ES, Kang SY, Kim YC. Anti-acetylcholinesterase and anti-amnesic activities of a pregnane gly-

coside, cynatroside B, from *Cynanchum atratum*. Plant Med 2005; 71: 7-11.

[61] Mata AT, Proença C, Ferreira AR, Serralheiro MLM, Nogueira JMF, Araújo MEM. Antioxidant and antiacetylcholinesterase activities of five plants used as Portuguese food spices. Food Chem 2007; 103: 778-86.

[62] Faleiro ML, Miguel G, Guerrero C, Brito J. Antimicrobial activity of essential oils of *Rosmarinus officinalis* L., *Thymus mastichina* (L.) ssp. *mastichina* and *Thymus albicans* Hoffmanns. & Link. Acta Hortic 1999; 501: 45-8.

[63] Faleiro ML, Miguel MG, Ladeiro F, Venâncio F, Tavares R, Brito JC, *et al*. Antimicrobial activity of essential oils isolated from Portuguese endemic species of *Thymus*. Lett Appl Microbiol 2003; 36: 35-40.

[64] Faleiro L, Miguel G, Gomes S, Costa L, Venâncio F, Teixeira A, *et al*. Antibacterial and antioxidant activities of essential oils isolated from *Thymbra capitata* L. (Cav.) and *Origanum vulgare* L. J Agr Food Chem 2005; 53: 8162-8.

[65] Hazzit M, Baaliouamer A, Faleiro ML, Miguel MG. Composition of the essential oils of *Thymus* and *Origanum* species from Algeria and their antioxidant and antimicrobial activities. J Agr Food Chem 2006; 54: 6314-21.

[66] Eggimann P, Garbino J, Pittet D. Management of *Candida* species infections in critically ill patients. Lancet Infect Dis 2003; 3: 772-85.

[67] Pinto E, Pina-Váz C, Salgueiro L, Gonçalves MJ, Oliveira S, Cavaleiro C, *et al*. Antifungal activity of the essential oil of *Thymus pulegioides* on *Candida*, *Aspergillus* and dermatophyte species. J Med Microbiol 2006; 55: 1367-73.

[68] Rapp RP. Changing strategies for the management of invasive fungal infections. Pharmacotherapy 2004; 24: 4-28.

[69] Bakkali F, Averbeck S, Averbeck D, Idaomar M. Biological effects of essential oils – review. Food Chem Toxicol 2008; 46: 446-75.

[70] Kalemba D, Kunicka A. Antibacterial and antifungal properties of essential oils. Curr Med Chem 2003; 10: 813-29.

[71] Couladis M, Tzakou O, Kujundzic S, Sokovic, Mimica-Dukic N. Chemical analysis and antifungal activity of *Thymus striatus*. Phytother Res 2004; 18: 40-2.

[72] Giordani R, Regli P, Kaloustian J, Mikaïl C, Abou L, Portugal H. Antifungal effect of various essential oils against *Candida albicans*. Potentiation of antifungal action of amphotericin B by essential oil from *Thymus vulgaris*. Phytother Res 2004; 18: 990-5.

[73] Sokovic M, Griensven L. Antimicrobial activity of essential oils and their components against the three major pathogens of the cultivated button mushroom, *Agaricus bisporus*. Eur J Plant Pathol 2006; 116: 211-24.

[74] Koroch A, Juliani R, Zygadlo J. Bioactivity of essential oils and their components. In: Flavours and Fragrances - chemistry, bioprocessing and sustainability. Berger RG, Ed. Springer 2007; 87-90.

[75] National Committee for Clinical Laboratory Standards. Reference method for broth dilution antifungal susceptibility testing of yeasts: Approved standard M27-A. Wayne, PA; USA 1997; Vol. 27.

[76] National Committee for Clinical Laboratory Standards. Reference method for broth dilution antifungal susceptibility testing of filamentous fungi: Approved standard M38-A. Wayne, PA; USA 2002; Vol. 22.

[77] Pina-Vaz C, Rodrigues AG, Pinto E, Costa-de-Oliveira S, Salgueiro L, Cavaleiro C, *et al*. Antifungal activity of *Thymus* oils and their major compounds. J Eur Acad Dermatol 2004; 18: 73-8.

[78] Salgueiro L, Cavaleiro C, Pinto E, Pina-Vaz C, Rodrigues AG, Palmeira A, *et al*. Chemical composition and antifungal activity of the essential oil of *Thymbra capitata*. Plant Med 2004; 70: 569-71.

[79] Salgueiro L, Pinto E, Gonçalves M, Pina-Vaz C, Cavaleiro C, Palmeira A, *et al*. Antifungal activity and chemical composition of the essential oil of *Thymus capitellatus.* Flavour Fragr J 2006; 21: 749-53.

[80] Carson CT, Hammer K, Riley T. *Melaleuca alternifolia* (tea tree) oil: a review of antimicrobial and other medicinal properties. Clin Microbiol Rev 2006; 19: 50-62.

[81] Pina-Vaz C, Sansonetty F, Rodrigues AG, Martinez-Oliveira J, Fonseca A, Mardh PA. Antifungal activity of ibuprofen alone and in combination with fluconazole against *Candida* species. J Med Microbiol 2000; 49: 831-40.

[82] Costa-de-Oliveira S, Gonçalves G, Pina-Vaz C, Gonçalves MJ, Salgueiro L, Cavaleiro C, *et al*. Effect of *Thymbra capitata* on *Aspergillus*: a cytometric approach. Clin Microbiol Infec 2005; 11: 126.

[83] Antolovich M, Prenzler PD, Patsalides E, McDonald S, Robards K. Methods for testing antioxidant activity. Analyst 2002; 127: 183-98.

[84] Sánchez-Moreno C. Review: Methods used to evaluate the free radical scavenging activity in foods and biological systrms. Food Sci Technol Int 2002; 8: 121-37.

[85] Teissedre PL, Waterhouse AL. Inhibition of oxidation of human low-density lipoproteins by phenolic substances in different essential oils varieties. J Agr Food Chem 2000; 9: 3801-5.

[86] Sokmen A, Gulluce M, Akpulat HA, Daferera D, Tepe B, Polissiou M, *et al*. The *in vitro* antimicrobial and antioxidant activities of the essential oils and methanol extracts of endemic *Thymus spathulifolius*. Food Chem 2004; 15: 627-34.

[87] Chericoni S, Prieto JM, Iacopini P, Morelli I. Essential oils of commonly used plants as inhibitors of peroxynitrite-induced tyrosine nitration. Fitoterapia 2005; 76: 481-3.

[88] Jukić M, Miloš M. Catalytic oxidation and antioxidant properties of thyme essential oils (*Thymus vulgarae* L.). Croat Chem Acta 2005; 78: 105-10.

[89] Kulisic T, Radonic A, Milos M. Antioxidant properties of thyme (*Thymus vulgaris* L.) and wild thyme (*Thymus serpyllum* L.) essential oils. Ital J Food Sci 2005; 17: 315-24.

[90] Kulisic T, Radonic A, Milos M. Inhibition of lard oxidation by fractions of different essential oils. Grasas Aceites 2005; 56: 284-91.

[91] Sacchetti G, Maietti S, Muzzoli M, Scaglianti M, Manfredini S, Radice M, *et al*. Comparative evaluation of 11 essential oils of different origin as functional antioxidants, antiradicals and antimicrobials in foods. Food Chem 2005; 91: 621-32.

[92] Tepe B, Sokmen M, Akpulat HA, Dafarera D, Polissiou M, Sokmen A. Antioxidative activity of the essential oils of *Thymus sipyleus* subsp. *sipyleus* var. *sipyleus* and *Thymus sipyleus* subsp. *sipyleus* var. *rosulans*. J Food Eng 2005; 66: 447-54.

[93] Bozin B, Mimica-Dukic N, Simin N, Anackov G. Characterization of the volatile composition of essential oils of some Lamiaceae species and the antimicrobial and antioxidant activities of the entire oils. J Agr Food Chem 2006; 54: 1822-8.

[94] Bounatirou S, Smiti S, Miguel MG, Faleiro L, Rejeb MN, Neffati M, *et al*. Chemical composition, antioxidant and antibacterial activities of the essential oils isolated from Tunisian *Thymus capitatus* Hoff. et Link. Food Chem 2007; 105: 146-55.

[95] Miguel MG, Figueiredo AC, Costa MM, Martins D, Barroso JG, Pedro L. Effect of the essential volatile oils isolated from *Thymbra capitata* (L.) Cav. on olive and sunflower oil. Grasas Aceites 2003; 54: 219-25.

[96] Miguel MG, Figueiredo AC, Costa MM, Martins D, Duarte J, Barroso JG, *et al*. Effect of the volatile constituents isolated from *Thymus albicans*, *Th. mastichina*, *Th. carnosus* and *Thymbra capitata*. Nahrung 2003; 6: 397-402.

[97] Laguerre M, Lecomte J, Villeneuve P. Evaluation of the ability of antioxidants to counteract lipid oxidation: existing methods, new trends and challenges. Prog Lipid Res 2007; 46: 244-82.

[98] Miguel MG, Falcato-Simões M, Figueiredo AC, Barroso JMG, Pedro LG, Carvalho LM. Evaluation of the antioxidant activity of *Thymbra capitata*, *Thymus mastichina* and *Thymus camphoratus* essential oils. J Food Lipids 2005; 12: 181-97.

[99] Madsen HL, Bertelsen G. Spices as antioxidants. Trends Food Sci Tech 1995; 6: 271-7.

[100] Bonanni A, Campanella L, Gatta T, Gregori E, Tomassetti M. Evaluation of the antioxidant and prooxidant properties of several commercial dry spices by different analytical methods. Food Chem 2007; 102: 751-8.

[101] Ruberto B, Baratta MT. Antioxidant activity of selected essential oil components in two lipid model systems. Food Chem 2000; 69: 167-74.

[102] Miguel G, Simões M, Figueiredo AC, Barroso JG, Pedro LG, Carvalho L. Composition and antioxidant activities of the essential oils of *Thymus caespititius*, *Thymus camphoratus* and *Thymus mastichina*. Food Chem 2004; 86: 183-8.

[103] Miguel MG, Costa LA, Figueiredo AC, Barroso JG, Pedro LG. Assessment of the antioxidant ability of *Thymus albicans*, *Th. mastichina*, *Th. camphoratus* and *Th. carnosus* essential oils by TBARS and Micellar Model systems. Nat Prod Commun 2007; 2: 399-406.

[104] Morales Valverde R. Taxonomia de los generos *Thymus* (excluida la sección *Serpyllum*) y *Thymbra* en la Peninsula Ibérica. Ruizia 1986; 3: 1-324.

[105] Figueiredo AC, Miguel MG, Duarte AMF, Barroso JG, Pedro LG. Essential oil composition from *Thymus lotocephalus* G. López & R. Morales collected during flowering and vegetative phases. Flavour Fragr J 2001; 16: 417-21.

[106] Fernandes FM, Carvalho LM. Portugal Botâncico de A a Z. Lidel, Portugal 2003.

[107] Dias C. Valorização do património genético de plantas aromáticas e medicinais do Parque Natural da Serra da Estrela. Relatório do Trabalho de Fim de Curso. Escola Superior Agrária, Instituto Politécnico de Castelo Branco 1999.

[108] Mesquita MF. Plantas aromáticas e/ou medicinais, inventariação e utilização. Relatório de Estágio. Reserva Natural da Serra da Malcata, Instituto da Conservação da Natureza (ICN) 2000.

[109] Camejo-Rodrigues JS. Contributo para o estudo etnobotânico das plantas medicinais e aromáticas no Parque Natural da Serra de S. Mamede. Relatório de Estágio para a Licenciatura em Biologia. Faculdade de Ciências da Universidade de Lisboa 2001.

[110] Camejo-Rodrigues J, Ascensão L, Bonet MA, Vallès J. An ethnobotanical study of medicinal and aromatic plants in the Natural Park of the "Serra de São Mamede" (Portugal). J. Ethnopharmacol 2003; 89: 199-209.

[111] Dias C. Inventariação e utilizações locais das plantas aromáticas e medicinais do Parque Natural do Douro Internacional. PNDI, Figueira de Castelo Rodrigo 2003.

[112] Oliveira ASB, Neiva RF. Plantas aromáticas e medicinais do Parque Natural da Serra da Estrela. Edição ICN, Parque Natural da Serra da Estrela, Portugal 2004.

[113] Carapeto A. Levantamento etnobotânico na Reserva Natural do Sapal de Castro Marim e Vila Real de Santo António. Relatório Final, Projecto Agro nº 800 "Rede Nacional para a Conservação e Utilização das Plantas Aromáticas e Medicinais". Reserva Natural do Sapal de Castro Marim e Vila Real de Santo António. Castro Marim 2006.

[114] Fernandes Costa A, Cardoso do Vale J. Essência de *Coridothymus capitatus* Rchb. f.: estudo da fracção fenólica. Not Farm 1942.

[115] Castello Branco MA. Determinações analíticas na essência Portuguesa de *Corydothymus capitatus*. Rchb. f. – Contribuição para o estudo da essência desfenolada. Not Farm 1952.

[116] Proença da Cunha A, Roque OR. Contribuição para o estudo anlítico do óleo essencial de *Thymus capitatus*. Bol Fac Far Coimbra 1986; 10: 31-41.

[117] Carmo MM, Frazão S, Venâncio F. Estudo da composição química de alguns tomilhos fenólicos do continente Português. Jornadas Ibéricas de Plantas Medicinais, Aromáticas e Óleos Essenciais, Lisboa 1991.

[118] Palhinha RT. Plantas aromáticas de Portugal. Brotéria 1946; 15: 97-113.

[119] Salgueiro LR, Vila R, Tomàs X, Cañigueral S, Proença da Cunha A, Adzet T. Composition and variability of essential oils of *Thymus* species from section *Mastichina* from Portugal. Biochem Syst Ecol 1997; 25: 659-72.

[120] Salgueiro LR, Vila R, Tomi F, Figueiredo AC, Barroso JG, Cañigueral S, *et al*. Variability of essential oils of *Thymus caespititius* from Portugal. Phytochemistry 1997; 45: 307-11.

[121] Velasco Negueruela A, Pérez-Alonso MJ. Aceites esenciales de tomillos ibéricos. VI. Contribución al conocimiento del aceite esencial de *Thymus camphoratus* Hoffmanns. & Link. Anales Jard Bot Madrid 1987; 43: 383-6.

[122] Adzet T, Vila R, Ibañez C, Cañigueral S. Essential oils of some Iberian *Thymus*. Plant Med 1988; 54: 369-71.

[123] Salgueiro LR. Essential oils of endemic *Thymus* species from Portugal. Flavour Fragr J 1992; 7: 159-62.

[124] Salgueiro LR, Vila R, Tomàs X, Tomi F, Cañigueral S, Casanova J, *et al*. Composition and infraspecific variability of essential oil from *Thymus camphoratus*. Phytochemistry 1997; 45: 1177-83.

[125] Velasco-Negueruela A, Pérez Alonso MªJ, Burzaco A. Aceites esenciales de tomillos ibéricos. VI. Contribución al conocimiento del aceite esencial de *Thymus capitellatus* Hoffmanns. & Link. Anales Jard Bot Madrid 1991; 49: 77-81

[126] Salgueiro LR, Vila R, Tomàs X, Tomi F, Cañigueral S, Casanova J, *et al*. Chemical polymorphism of the essential oil of *Thymus carnosus* from Portugal. Phytochemistry 1995; 38: 391-6.

[127] Salgueiro LR, Vila R, Tomàs X, Tomi F, Cañigueral S, Paiva J, *et al*. Chemotaxonomic study on *Thymus villosus* from Portugal. Biochem Syst Ecol 2000; 28: 471-82.

[128] Frazão S, Domingues AR, Costa V. Contribuição ao estudo do óleo essencial de *Thymus mastichina* (bela-luz ou manjerona brava). An Acad Bras Cienc 1971; 44: 295-7.

[129] Frazão S, Carmo MM. Differences des Huilles Essentielles de *Thymus mastichina* de la Peninsule Iberique. 3èmes Journées Scientifiques Internationales, Digne-les-Bains 1984; 89-105.

[130] Soares MIV, Tavares MCF. Determinação de linalool e cineol em oleo de majerona. Rev Port Quim 1972; 14: 96-9.

[131] Adzet T, Granger R, Passet J, San Martin R. Chimiotypes de *Thymus mastichina* L. Plant Med Phytother 1977; 11: 275-80.

[132] Gomes A, Monteiro MF, Farelo MF. Óleos de majerona – tipos e caracterização. Rev Port Quim 1980; 22: 17-28.

[133] Salgueiro LR, Neto FC, Proença da Cunha A. Contribuição para o conhecimento dos tomilhos de Trás-os-Montes (parte I). Bol Fac Far Coimbra 1990; 14: 31-8.

[134] Salgueiro LR, Proença da Cunha A, Tomàs X, Cañigueral S, Adzet T, Vila R. The essential oil of *Thymus villosus* L. ssp. *villosus* and its chemical polymorphysm. Flavour Fragr J 1997; 12: 117-22.

[135] Roque OR, Salgueiro LR. Composição do oleo essencial de *Thymus zygis* subsp. *sylvestris* (Hoffmanns. & Link) Brot. ex Coutinho da região de Souselas – Coimbra. Bol Fac Far Coimbra 1987; 11: 41-50.

[136] Salgueiro LR, Proença da Cunha A. Composição do oleo essencial de *Thymus zygis* subsp. *sylvestris* (Hoffmanns. & Link) Brot. ex Coutinho da região de centro de Portugal. Bol Fac Far Coimbra 1987; 11: 107-18.

[137] Salgueiro LR, Proença da Cunha A. Determinação de quimiotipos no *Thymus zygis* subsp. *sylvestris* (Hoffmanns. & Link) Brot. ex Coutinho da região de Eiras - Coimbra. Rev Port Farm 1989; 39: 19-27.

[138] Salgueiro LR. Pesquisa e caracterização de quimiotipos em plantas aromáticas. Bol Fac Far Coimbra 1989; 13: 75-107.

[139] Proença da Cunha A, Salgueiro LR. The chemical polymorphism of *Thymus zygis* ssp. *sylvestris* from Portugal. J Essent Oil Res 1991; 3: 409-12.

[140] Altmann KH, Memmert K. Epothilones as lead structures for new anticancer drugs--pharmacology, fermentation, and structure-activity-relationships. Prog Drug Res 2008; 66: 275-334.

[141] Fylaktakidou KC, Hadjipavlou-Litina DJ, Litinas KE, Varella EA, Nicolaides DN. Recent developments in the chemistry and in the biological applications of amidoximes. Curr Pharm Des 2008; 14(10): 1001-47.

[142] Goodman CD, McFadden GI. Fatty acid synthesis in protozoan parasites: unusual pathways and novel drug targets. Curr Pharm Des 2008; 14(9): 901-16.