Chapter 7

Thymus Plants: A Review—Micropropagation, Molecular and Antifungal Activity

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Additional information is available at the end of the chapter

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Abstract

Medicinal and aromatic plants are important sources for plant secondary metabolites. The genetic manipulation of plants associated with in vitro plant regeneration systems facilitates efforts to engineer secondary product metabolic pathways. The fungal infections have been increasing in recent years due to several factors, namely, the increased incidence of high-risk patients, particularly immunocompromised hosts. Aromatic plants have been empirically used as antimicrobial agents, but the mechanisms of action are still unknown. Thyme has a great interest due to the possibility of its use in different applications, in medicine, in the cosmetic industry, or as food additives. Several studies have shown that thyme oils possess antimicrobial activity. Increasingly, plant breeding has taken advantage of molecular biology developments in order to genotype the species of interest to accelerate their selection. These approaches consist in choosing desired genotypes based on molecular markers or the knowledge of the genes involved in a particular trait. The in vitro culture techniques can be used to multiply plants selected after molecular and antifungal studies. The course of the investigation and the current state in relation to micropropagation, molecular studies, and antifungal action of the *Thymus* genus plants will be presented.

Keywords: *Thymus*, micropropagation, molecular, antifungal

1. Introduction

The genus *Thymus* L. belongs to the Lamiaceae family and consists of over 400 species of herbaceous annuals and perennial plants that are extensively used, for medicinal and non-medicinal purposes. These plants are widely distributed throughout the Old World [1, 2] and

have been used for many centuries in traditional medicine due to their antiseptic, carminative, antiviral, and antioxidant properties [3]. *Thymus* species are also interesting as a source of pentacyclic triterpenoids with several properties, as anti-inflammatory, hepatoprotective, antimicrobial, anti-HIV-1 activity, antiulcer, gastroprotective, hypoglycemic, antihyperlipidemic activity, and specific cytotoxicity against a variety of tumor cell lines [4–6]. By all this it becomes increasingly important to know the bioactive components of the *Thymus*.

Furthermore, interests focusing mainly on few selected chemotypes for the cosmetic and food industries, among others, lead to the loss of other species in nature, such as *Thymus cariensis* Hub.-Mor. & Jalas, *Thymus cilicicus* Boiss. & Balansa, *Thymus sipyleus* Boiss., *Thymus pulvinatus* Čelak., and *Thymus cherlerioides* Vis. [7]. These species should be preserved to make available the access to a wide range of genetic diversity. On the other hand, as the plant has a low propagation rate in nature, a suitable method to obtain a high number of plants is needed [8].

2. *Thymus* main active compounds

Among the *Lamiaceae* family, the genus *Thymus* is one of the most studied genera, due to the use of these plants as a remedy in folk medicine and as a condiment, mainly in the Mediterranean zone [1]. High bioactivity (e.g., antioxidant, anti-inflammatory, antiproliferative, and antimicrobial effects) is linked to the considerable content in phytochemicals of different *Thymus* species. Volatile compounds are extracted in essential oils (EOs), while nonvolatile compounds are found in alcoholic and aqueous extracts obtained by maceration, decoction, or infusion [9].

Noticeable interest was given to the study of EO in different *Thymus* species. High intraspecific chemical polymorphism was reported, especially in *Thymus vulgaris* L., which was shown to have a polymorphic variation in monoterpene production [10, 11]. Phenolic monoterpenes, such as carvacrol and thymol (**Figure 1**, compounds 1 and 2), seem to explain the important biological activity of the majority of *Thymus* spp. essential oils characterized by phenolic chemotypes. However, the presence of other terpenes in the EO results in the enhancement or reduction of EO bioactivity due to synergistic or antagonistic effects [12]. In the *Lamiaceae* family, EOs are produced in the glandular trichomes and stored in their cavities [13]. Several enzymes are involved in the biosynthetic pathways of terpenes, such as the terpene synthases enzyme class and the cytochrome P450s that are involved in the core terpene molecule functionalization [14].

Carvacrol and thymol have *p*-cymene as common precursor (**Figure 1**, compound 5). Carvacrol is frequent in some Lamiaceae aromatic plant genera like *Thymus*, *Origanum*, and *Satureja* [15], while thymol is mainly frequent in *Thymus* plants. Phenolic chemotypes are characteristic of *T. vulgaris* [10], *Thymus hyemalis* Lange [16], *Thymus capitatus* L. [17], *Thymus zygis* L. [18], and some populations of *Thymus pulegioides* L. [19]. Thymol's antibacterial effect is enhanced by the presence of carvacrol in the essential oil [12]. Both thymol and carvacrol have important antifungal effect; they act by causing damage in the cell membrane and interacting with ergosterol [18, 20–22]. This mechanism was also proved with other monoterpenes, namely, linalool (**Figure 1**, compound 3), which acts also by interfering with biofilm formation and stability [23].

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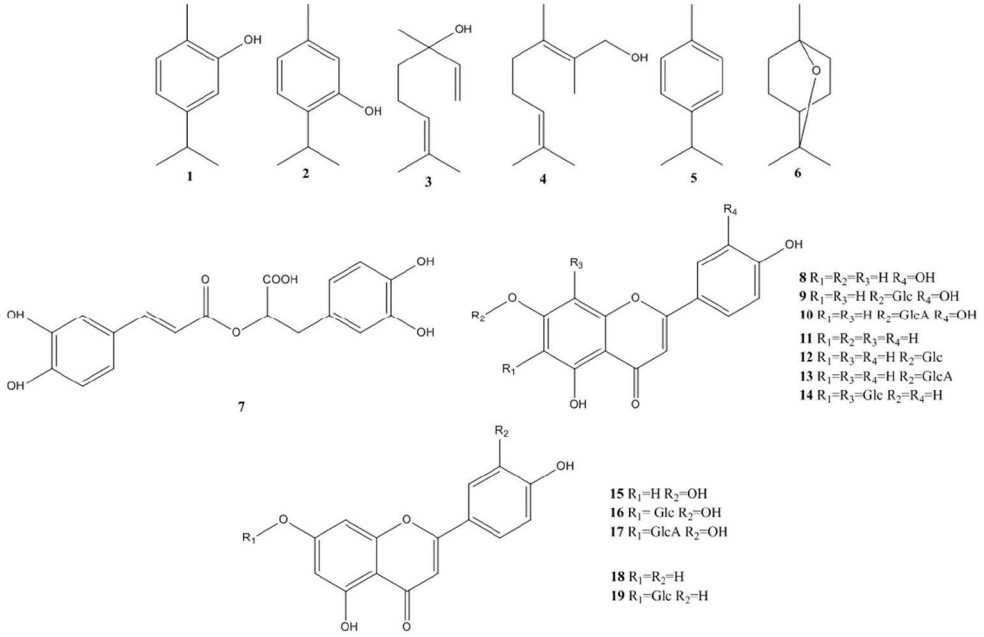

Figure 1. Chemical structure of main active compounds in *Thymus* spp. (1) carvacrol, (2) thymol, (3) linalool, (4) geraniol, (5) *p*-cymene, (6) eucalyptol, (7) rosmarinic acid, (8) luteolin, (9) luteolin-*O*-glucoside, (10) luteolin-*O*-glucuronide, (11) apigenin, (12) apigenin-7-*O*-glucoside, (13) apigenin-7-*O*-glucuronide, (14) apigenin-6,8-di-*C*-glucoside, (15) eriodictyol, (16) eriodictyol-*O*-glucoside, (17) eriodictyol-*O*-glucuronide, (18) naringenin, (19) naringenin-7-*O*-glucoside. Glc, glucoside unit; GlcA, glucuronide unit.

Linalool and geraniol (**Figure 1**, compounds 3 and 4, respectively) are monoterpene alcohols, which characterize several chemotypes of *Thymus* species such as *Thymus pulegioides*, *T. x. citriodorus* (Pers.) Schreb., *Thymus algeriensis* Boiss. & Reut., and some populations of *T. vulgaris* [11, 19, 24, 25]. Eucalyptol, or 1,8-cineole (**Figure 1**, compounds 6), a monoterpene cyclic ether monoterpene that is mainly present in *Eucalyptus* genus, is the major component of *Thymus mastichina* (L.) L. EO [26] and was also reported in populations of *T. algeriensis*, *Thymus hirtus* Raf., and *Thymus glabrescens* [27, 28].

Although more interest has been given to study thyme essential oils, nonvolatile extracts contain highly active secondary metabolites which are mainly phenolic compounds. Aromatic amino acids L-phenylalanine and L-tyrosine, produced by the shikimic acid pathway, are the precursors of the biosynthesis of polyphenols, namely, phenolic acids and flavonoids [29, 30]. Rosmarinic acid and luteolin (**Figure 1**, compounds 7 and 8, respectively) are the main frequent phenolic compounds found in thyme plants and which are related to their extracts' biological activity. Rosmarinic acid, caffeic acid esterified with 3,4-dihydroxyphenyllactic acid, is the most abundant phenolic acid in several *Thymus* species: *T. vulgaris*, *T. pulegioides*, *T. zygis*, *T. mastichina*, *T. capitatus*, *Thymus longicaulis* C. Presl, and *T. x. citriodorus* [9, 31–36].

From the class of flavones, luteolin and apigenin (**Figure 1**, compounds 8 and 11, respectively) are the most important in *Thymus*. Luteolin is frequently present also in fruits and other

Lamiaceae genera like *Mentha*. Its glycosylated derivatives such as luteolin-*O*-glucoside (**Figure 1**, compound 9) are also frequent in thyme species. Apigenin and its derivatives (**Figure 1**) are reported in some species such as *T. x. citriodorus*, *T. vulgaris*, and *Thymus herba-barona* Loisel. [37]. Eriodictyol and naringenin (**Figure 1**, compounds 15 and 18) are flavanones which were described as well as their glucoside and glucuronide derivatives (**Figure 1**, compounds 16 and 19 and 17, respectively) in some *Thymus* species such as *T. x. citriodorus* and *T. vulgaris* [34].

3. Micropropagation of *Thymus*

Advances in biotechnological approaches provide a set of techniques that contribute to solving problems of extinction or genetic erosion in particular of plants. Alternatives for fast multiplication, like "in vitro micropropagation" that enables propagation of plants under controlled environmental conditions, can help in multiplying selected plants after molecular and antifungal studies or subjected to excessive demand by the people.

Furthermore, it was also possible to develop techniques that allow the maintenance of germplasm for a long time, like "cryopreservation" that make available long-term storage of *Thymus* germplasm at ultra-low temperatures [8].

Species	Achievements	Growth regulators or others	References
Thymus mastichina	Propagation of thyme from mature field-grown plants	Nodal segments—MS + 0.1 mg L^{-1} BAP Roots—MS + 1 mg L^{-1} NAA	[40]
Thymus vulgaris and *Thymus longicaulis*	In vitro propagation protocol	Shoots, semisolid MS + 1 mg L^{-1} KN and 0.3 mg L^{-1} GA3; roots, MS + 0.05 mg L^{-1} 2,4-D	[41]
Thymus lotocephalus	Propagation protocol using seeds as explants	MS BAP induce high % of hyperhidric shots	[42]
Thymus caespititius	Shoots with high proliferation capacity	MS + 0.4 mg L^{-1} BA + 0.1 mg L^{-1} IBA	[43]
Thymus persicus	System for regeneration via direct organogenesis	Shoot, 8.9 μM BAP + 2.7 μM NAA; roots, 1/2 MS + 2.5 μM IBA	[44]
Thymus hyemalis	Regeneration of plants through somatic embryogenesis	MS + 4.44 μM BAP, 0.54 μM NAA, and 4.65 μM KIN	[45]
Thymus daenensis	Colloidal silver nanoparticles reduce hyperhydricity	2.5 mg L^{-1}AgNPs (silver nanoparticles)	[46]
Thymus moroderi	Propagation disinfection processDouble phase culture system	growth regulators did not improve the morphogenic response	[47]
Thymus persicus	Callus induction and micropropagation	Callus, MS + 2.0 mg L^{-1} NAA and 0.5 mg L^{-1} KN Shoot, MS + 2.0 mg L^{-1} BAP + 1.0 mg L^{-1} NAA	[48]

Table 1. Approaches in *Thymus* plant micropropagation.

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Optimizations for micropropagation process involve the use of different growth regulators like cytokinins, auxins, or gibberellic acid for the induction of multiple shoots. The first works in *Thymus* micropropagation are from Furmanowa and Olszowska dated from 1980 [38] to 1992 [39]. Since that date, a lot of works for the micropropagation of different species of *Thymus* (**Table 1**), as well as the cryopreservation of the same plants, were developed (**Figure 2**).

Figure 2. *Thymus caespititius* multiple shoots produced. (**A**) Outgrowth of the axillary buds maintained on MS medium with 0.4 mg L^{-1} BA and 0.1 mg L^{-1} IBA. (**B**) Detail of spontaneous root formation in shoot cultures (bars = 1 cm) [43].

Cryopreservation procedures, PVS2 vitrification, encapsulation—vitrification and droplet—and freezing methods showed to be effective to induce cryotolerance and long-term conservation of thyme shoot tips obtained from in vitro propagated plantlets [49]: Different *Thymus* species have already been studied, *Thymus moroderi* [50], *T. vulgaris* and *T. longicaulis* [2], *T. cariensis* and *T. vulgaris* [41], and *Thymus lotocephalus* [51].

4. Genetic analysis

Researchers, from different areas, use the genetic analysis of plants as the basis of their work in order to identify and to characterize plant materials in nature, to detect genetic diversity or the genetic homogeneity, and to select plants with desired compounds.

The pool of genetic variation in plants, namely, the medicinal and the aromatic ones, serves as the base for plant breeding as well as for selection. Molecular markers are very useful in breeding program allowing germplasm screening independent to the developmental stage of the plants and/or environmental factors [52].

Applications of DNA methods, with different purposes, in *Thymus* genus are few when compared with other economically important plant species. A few reports are available about genetic characterization of species of *Thymus* genus (**Table 2**), using different molecular markers, being the techniques of random amplified polymorphic DNA markers (RAPD) and

inter-simple sequence repeat (ISSR) the most commonly used. Amplified fragment length polymorphisms (AFLPs) and microsatellites are also utilized for genetic analysis and genetic relationships in *Thymus* species [56, 63].

Beyond the genetic characterization of different species, the knowledge of genetic diversity within species is necessary for any improvement of cultivars and biodiversity maintenance and restoration [64]. Yousefi et al. [54] studied ecotypes grown in different parts of Iran using ISSRs and verified that the accessions were relatively grouped according to their location and conclude that ISSRs provided a powerful and reliable molecular tool for detecting genetic variation and relationships. A similar study was done by Rahimmalek et al. [55] with the purpose to assess the genetic diversity of *Thymus daenensis* Celak accessions toward the conservation of the endangered aromatic species. Solyman and Alkowni [59] studied genetic diversities of five Palestinian *Thymus* species using RAPD markers and concluded that it could be useful tools for identifying *Thymus* species in any putative breeding programs that will be carried in the country. A study, with AFLPs, in *Thymus*, section *Serpyllum* [63], demonstrated that this markers could be suitable for complex genetic relationships analysis, including frequent interspecies hybridization events. Recently Karaca et al. [56] present the first report of genomic microsatellite markers for the genus *Thymus*, used in 48 individuals representing 9 species and subspecies of *Thymus*.

The overexploitation of wild plants for commercial purposes (and consequent decreased of populations) associated to the increasing demand for secondary metabolites has intensified the application of biotechnological methods to propagate and reproduce high-yielding plants under controlled growing conditions and/or to obtain homogenous and stable genotypes. Other application of molecular markers in this genus is the analysis of the reliability of the in vitro propagation regarding the genetic homogeneity, most of times associated to the phytochemical productivity of the produced plantlets, as the experiment reported by Bakhtiar et al. [48] using RAPDs. In this work RAPD profiles confirmed the homogeneity and high-performance liquid chromatography (HPLC) confirmed the phytochemical productivity of the in vitro regenerated plants.

Mendes et al. [53], using in vitro genotypes, characterized *Thymus caespititius* terpene synthase 2 (*Tctps2*) gene and identified other terpene synthase genes responsible for the chemical polymorphism observed in *T. caespititius* essential oils.

Another genetic approach is the analysis of the chemical and the genetic relationships among species as the study described by [58] that also determinate the correlation between these two sets of data, the essential-oil composition and genetic variability of six populations of *Thymus*. RAPD markers allowed a perfect distinction between the six species, based on their distinctive genetic background: however, they did not show identical clustering with the volatile-oil profiles [58]. Contrary Echeverrigaray et al. [60], also based in RAPD profiles, observed that the cultivars (*T. vulgaris* L.) could be divided into two clusters, which coincided with results obtained by oil GS-MS analysis. Chemical and genetic differences of four *Thymus* species were studied by Pluhár et al. [61] in order to determine whether molecular characters (RAPDs) and essential oil components could be used as taxonomic markers and obtained a partial correlation between molecular and chemical assessments. In

Species	Achievements	Markers	References
Thymus caespititius	Identification of terpene synthase genes in *Lamiaceae*	TPS gene	[53]
Thymus daenensis *Thymus kotschyanus* *Thymus vulgaris*	Assessment of genetic diversity and relationships	ISSRs	[54]
Thymus daenensis subsp. *daenensis*	Assessment of genetic diversity and geographic differentiation	ISSRs	[55]
Thymus cilicicus *Thymus revolutus* *Thymus cherlerioides* *Thymus leucotrichus* *Thymus zygioides* *Thymus sipyleus* *Thymus longicaulis*	Development of 23 microsatellite primer pairs for *Thymus* genus and assessment of genetic diversity and relationships of 48 samples representing nine species and subspecies of the genus *Thymus*	Microsatellites	[56]
Thymus persicus	Confirmation of genetic homogeneity in in vitro regenerated plants	RAPDs	[48]
Thymus kotschyanus	Assessment of genetic diversity of wild populations	RAPDs	[57]
Thymus daenensis *Thymus fallax* *Thymus fedtschenkoi* *Thymus migricus* *Thymus vulgaris*	Assessment of genetic diversity and chemical polymorphism of *Thymus* species	RAPDs	[58]
Thymus syriacus *Thymus fruticosus* *Thymus incanus* *Thymus majorana* *Thymus capitatus*	Assessment of thyme genetic diversity in Palestine	RAPDs	[59]
Thymus vulgaris	Correlation between the chemical and genetic relationships among commercial *Thymus* cultivars	RAPDs	[60]
Thymus glabrescens *Thymus pannonicus* *Thymus praecox* *Thymus pulegioides*	Essential oil composition and molecular analysis	RAPDs	[61]
Thymus caramanicus	Assessment of genetic and chemical variability	ISRRs	[62]
Thymus pulegioides *Thymus glabrescens* *Thymus marschallianus* *Thymus pannonicus* *Thymus balcanus* *Thymus moesiacus* *Thymus praecox*	Assessment of genetic diversity and relationships among species of the genus *Thymus* L. (section *Serpyllum*)	AFLPs	[56]

Table 2. Employment of molecular markers in genetic characterization of *Thymus* genus plants.

Thymus caramanicus Jalas, Hadian et al. [62] assessed the genetic (using ISSRs) and chemical variability and observed a relationship between genetic and chemical variability and geographic distribution.

5. Fungal infections

Fungal infections are a serious problem of public health concern and have been increasing in recent years due to several factors given increased international travel, immigration, changing climate conditions, and the increased incidence of high-risk patients [65]. Invasive mycoses are especially problematic for immunocompromised individuals and patients in intensive care units, and some conditions can predispose as organ transplantation, the use of drugs in treatments as corticosteroids and antineoplastics, and complex surgical procedure acts [66, 67]. Other cases may be found in patients suffering from diabetes mellitus, patients with human immunodeficiency virus infection, patients with neoplasias after receiving chemotherapy, patients with transplantation surgeries, or those with prolonged antibiotherapy [68].

Oral and vulvovaginal candidiases caused by *Candida albicans* are the most common fungal diseases [69]. Invasive fungal diseases can be less frequent but much more severe. Other mycotic diseases or complications associated included asthma, bronchopulmonary and invasive aspergillosis, pneumocystosis, meningeal cryptococcosis, mucormycoses, or invasive candidiasis [70]. Deaths related with fungal infections are mostly associated with *Cryptococcus*, *Candida*, *Aspergillus*, and *Pneumocystis* [71].

Aromatic plants have been empirically used as antimicrobial compounds, but the mechanisms of action are still under study [72]. Inhibitory action of aromatic plants possibly includes cytoplasm granulation, cytoplasmic membrane lesion, and inhibition and/or inactivation of extracellular and intercellular enzymes [72, 73] and might be due to different compounds, including phenolics, terpenoids, and alkaloids. These compounds together or independently use different levels of antifungal effect ending with mycelium germination inhibition [73]. Also, it is described that plant lytic enzymes act in the fungal cell wall causing breakage of β-1,6 [72]glycan, β-1,3 glycan, and chitin polymers [74]. The antimicrobial activity of the aqueous extracts could be due to the anionic components such as chlorides, thiocyanate, nitrate, and sulfates besides other water-soluble constituents which are naturally occurring in the plant material [75].

6. Antifungal activity

Only limited numbers of new antifungal drugs were developed in recent years, and there are only small numbers of drugs available for their treatment [68]. Toxicity and drug resistance have become an increasing problem. The resistance to antifungal drugs, the high costs associated with treatment, and the fungistatic activity of most of the antifungal drugs are problems

making their treatment difficult and expensive [67]. So, alternatives for treating invasive fungal infections are necessary [67].

The spread of multidrug-resistant strains of fungi is a medical problem worldwide, and the reduced number of drugs available led to a search for therapeutic substitutes, namely, among aromatic and medicinal plants and compounds isolated from them used for their antifungal [76, 77].

Numerous molecules obtained from the natural environment are investigated and described in bibliography with antimycotic activity. Several extracts are investigated for antifungal activities like crude extracts or isolated constituents as essential oils, saponins, terpenoids, alkaloids, phenolic compounds, peptides, and proteins [78, 79].

The in vitro evaluation methods of antifungal activity can be divided into diffusion methods and dilution methods. The diffusion methods yielding to inhibition diameters are used mostly for the qualitative screening. Examples are the agar overlay technique [80]. Dilution methods offer more quantitative results regarding the action of essential oils. Serial dilutions are used for detecting the minimal inhibitory concentration (MIC in mg ml^{-1}) of an essential oil in a liquid medium its antimicrobial properties and rank it among the most potent essential oils in this respect [81].

A wide range of aromatic and medicinal plants with therapeutic properties have been explored and used for the extraction of essential oils all over the world due to their antimicrobial capacity against fungal pathogens [79]. Several studies have shown that thyme oils possess antimicrobial activity [82–84].

Thymus spp., most of them possessing a large quantity of phenolic monoterpenes, revealed activity against fungi [83].

Medicinal plants have the capacity to inhibit the growth of a wide range of opportunistic or pathogenic microorganisms due to the presence of essential oils [85]. Essential oils are natural, volatile liquid, complex compounds characterized by rarely colored and a strong odor, soluble in lipid and organic solvents [85]. About 60% of essential oils show antifungal activity [84].

The essential oils and their components have been used broadly against molds [85]. The essential oil extracts from many plants have shown their considerable antifungal activity against the wide range of fungal pathogens [73].

Thyme essential oils are apparently among the greatest inhibitors of fungal microorganisms because of the presence of the phenolic compounds such as thymol as main components which might disrupt the fungal cell membrane [85]. Another component that appears to show antimicrobial activity is terpene hydrocarbons (γ-terpinene) [86]. *p*-Cymene is a compound of essential oil that does not show antibacterial efficacy when used alone which suggest a synergistic effect of the compounds [86, 87].

Thyme essential oils may in the future represent a new source of natural antiseptics with applications in industry of pharmaceutics and food [86]. The essential oils have the ability

to penetrate and disrupt the fungal cell wall and cytoplasmic membranes, permeabilizing them and finally causing damage to mitochondrial membranes [88].

Variability in essential oil compounds might be linked to differences in concentration and amount recovered based on several factors, including species of plant used, method of extraction, solvents, and extraction time, which in turn may differ in their antifungal potency [68, 89]. Differences can also be attributed to raw materials used (dried or fresh), types of soils used for cultivation, the harvesting time in the year, or differences in oil extraction techniques [90].

These oils have been used in folk medicine in different communities for patients suffering from mycotic infections [91].

Differences in essential oil compounds might be related to variability in the dried or fresh materials used, to the harvesting time in the year, to types of soils used for cultivation, or to differences in oil extraction techniques. *T. vulgaris* has medicinal properties and is widely used in traditional medicine for its expectorant, antispasmodic, antibronchiolitic, antitussive, anthelmintic, carminative, and diuretic properties [92].

Many studies have shown that thyme (*T. vulgaris*) has antifungal activities and have suggested their integration into pharmaceutical preparations in the treatment of candidiasis [72, 86, 93]. Previous studies showed fungistatic activity of the essential oils of *T. vulgaris* as carvacrol, *p*-cymene, and thymol [94]. The antifungal mechanism of action by which thymol or carvacrol acts is actually not well understood, although the antifungal activity is probably because thymol is lipophilic and together with carvacrol can act in the fatty acyl chains of membrane lipid bilayers, and alters the fluidity and permeability of cell membranes [95]. Other mechanisms have been theorized as damaged of membrane and cell wall with disruption associated with morphological,

Thymus plant	**Inhibited microorganisms**	**References**
Thymus vulgaris	*Candida albicans*	[72, 86, 94, 99, 100]
Thymus vulgaris	*Aspergillus flavus*	[92]
Thymus vulgaris	*Aspergillus ochraceus*	[96]
Thymus eriocalyx *Thymus x-porlock*	*Aspergillus niger*	[98]
Thymus vulgaris	*Penicillium chrysogenum*	[96]
Thymus serpyllum	Dermatophytes	[101]
Thymus schimperi	*Penicillium chrysogenum*	[66]
Thymus schimperi	*Verticillium* sp.	[66]
Thymus schimperi	*Aspergillus tubingensis*	[66]
Thymus schimperi	*Aspergillus minutus*	[66]
Thymus schimperi	*Beauveria bassiana*	[66]
Thymus schimperi	*Microsporum gypseum*	[66]

Table 3. Effect of *Thymus* plants on the fungal microorganisms.

deformation, deterioration, collapse, and of the conidia and/or hyphae [96]. Thymol and carvacrol have a stronger antifungal capacity, indicating more susceptibility of *Aspergillus* spp. than that of *Penicillium* spp. [97]. Thyme essential oil showed its capabilities of inhibiting fungal development causing leakage of the cytoplasm of *Aspergillus flavus* and was responsible for degenerative alterations in hyphae alterations, which appeared degraded or with the complete absence of conidia [92]. A previous report showed capabilities of inhibiting aflatoxin production [92].

Thymus essential oil causes irreversible damage to cell wall, cell membrane, and cellular organelles which affects *Aspergillus niger* growth and morphology [98].

The fungal activity of *Thymus* plants has been summarized in **Table 3**.

7. Conclusions

Thymus plants have been playing, in recent years, an increasingly important role in the intense study being targeted to run through the different areas of biotechnology. So here we intend to present the latest developments involving this plant in such diverse areas as active compound analysis and their effect on antifungal activity, genetic analysis, micropropagation, and finally cryopreservation techniques, one of the most recent methodologies to plant preservation.

The utilization of new plant breeding technologies will be the future in aromatic and medicinal plant manipulation and production, in order to obtain a multitude of valuable characteristics like increased nutrient and metabolite production and resistance to different stresses. Next-generation sequencing (NGS) technology and the associated bioinformatics tools will allow general profiling of RNA expression in plant species with limited molecular genetics studies as the majority of aromatic and medicinal plants.

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References

[1] Morales, R. The history, botany and taxonomy of the genus Thymus. In: Stahl-Biskup, E. & Sáez, F. (eds). Thyme: The Genus Thymus. London: CRC Press Taylor & Francis Group; 2002. pp. 1–43. ISBN 0203216857

[2] Ozudogru, E. A., Kaya, E. & Kirdok, E. Development of protocols for short-, medium- and long-term conservation of thyme. Acta Horticulturae. 2011; 918: 43–50. DOI: 10.17660/ActaHortic.2011.918.3

[3] Stahl-Biskup, E. Thyme as a herbal drug pharmacopoeias and other product characteristics. In: Stahl-Biskup E. & Sáez F. (eds). Thyme: The Genus Thymus. London: CRC Press Taylor & Francis Group; 2002. pp. 293–316. ISBN 0203216857

[4] Huang, M. T., Ho, C. T., Wang, Z. Y., Ferraro, T., Lou, Y. R., Stauber, K., et al. Inhibition of skin tumorigenesis by rosemary and its constituents carnosol and ursolic acid. Cancer Research. 1994; 54(3): 701–708.

[5] Liu, J. Pharmacology of oleanolic acid and ursolic acid. Journal of Ethnopharmacology. 1995; 49: 57–68.

[6] Liu, J.Oleanolic acid and ursolic acid: research perspectives. Journal of Ethnopharmacology. 2005; 100: 92–94.

[7] Rey, C. & Sáez, F. Field culture, in vitro culture and selection of Thymus. In: Stahl-Biskup E. & Sáez F. (eds) Thyme, the Genus Thymus. London: Taylor & Francis; 2002. pp. 177–196.

[8] Shabnum, S. & Wagay, M. G. Micropropagation of different species of Thymus. Journal of Research & Development. 2011; 11: 71–81. ISSN 0972-5407

[9] Martins, N., Barros, L., Santos-Buelga, C., Silva, S., Henriques, M. & Ferreira, I. C. F. R. Decoction, infusion and hydroalcoholic extract of cultivated thyme: antioxidant and antibacterial activities, and phenolic characterisation. Food Chemistry. 2015; 167: 131–137.

[10] Thompson, J. D., Chalchat, J. C., Michet, A., Linhart, Y. B. & Ehlers, B. Qualitative and quantitative variation in monoterpene co-occurrence and composition in the essential oil of *Thymus vulgaris* chemotypes. Journal of Chemical Ecology. 2003; 29: 859–880.

[11] Kaloustian, J., Abou, L., Mikail, C., Amiot, M. J. & Portugal, H. Southern French thyme oils: chromatographic study of chemotypes. Journal of the Science of Food and Agriculture. 2005; 85: 2437–2444.

[12] Cosentino, S., Tuberoso, C. I. G., Pisano, B., Satta, M., Mascia, V., Arzedi, E. & Palmas, F. In-vitro antimicrobial activity and chemical composition of Sardinian Thymus essential oils. Letters in Applied Microbiology. 1999; 29: 130–135.

[13] Turner, G., Gershenzon, J., Nielson, E. E., Froehlich, J. E. & Croteau, R. Limonene synthase, the enzyme responsible for monoterpene biosynthesis in peppermint, is localized to leucoplasts of oil gland secretory cells. Plant Physiology. 1999; 120: 879–886.

[14] Degenhardt, J., Kollner, T. G. & Gershenzon, J. Monoterpene and sesquiterpene synthases and the origin of terpene skeletal diversity in plants. Phytochemistry. 2009; 70: 1621–1637.

[15] De Vincenzi, M., Stammati, A., De Vincenzi, A. & Silano, M. Constituents of aromatic plants: carvacrol. Fitoterapia. 2004; 75: 801–804.

[16] Rota, M. C., Herrera, A., Martinez, R. M., Sotomayor, J. A. & Jordan, M. J. Antimicrobial activity and chemical composition of *Thymus vulgaris*, *Thymus zygis* and *Thymus hyemalis* essential oils. Food Control. 2008; 19: 681–687.

[17] Bounatirou, S., Smiti, S., Miguel, M. G., Faleiro, L., Rejeb, M. N., Neffati, M., Costa, M. M., Figueiredoe, A. C., Barroso, J. G. & Pedro, L. G. Chemical composition, antioxidant and antibacterial activities of the essential oils isolated from Tunisian *Thymus capitatus* Hoff. et Link. Food Chemistry. 2007; 105: 146–155.

[18] Pina-Vaz, C., Rodrigues, A. G., Pinto, E., Costa-De-Oliveira, S., Tavares, C., Satgueiro, L., Cavaleiro, C., Goncalves, M. J. & Martinez-De-Oliveira, J. Antifungal activity of *Thymus* oils and their major compounds. Journal of the European Academy of Dermatology and Venereology. 2004; 18: 73–78.

[19] Loziene, K., Vaiciuniene, J. & Venskutonis, P. R. Chemical composition of the essential oil of different varieties of thyme (*Thymus pulegioides*) growing wild in Lithuania. Biochemical Systematics and Ecology. 2003; 31: 249–259.

[20] Lima, I. O., Pereira, F. D., De Oliveira, W. A., Lima, E. D., Menezes, E. A., Cunha, F. A. & Diniz, M. D. F. M. Antifungal activity and mode of action of carvacrol against *Candida albicans* strains. Journal of Essential Oil Research. 2013; 25: 138–142.

[21] Chavan, P. S. & Tupe, S. G. Antifungal activity and mechanism of action of carvacrol and thymol against vineyard and wine spoilage yeasts. Food Control. 2014; 46: 115–120.

[22] Nobrega, R. O., Teixeira, A. P., Oliveira, W. A., Lima, E. O. & Lima, I. O. Investigation of the antifungal activity of carvacrol against strains of *Cryptococcus neoformans*. Pharmaceutical biology. 2016; 54: 2591–2596.

[23] Hsu, C. C., Lai, W. L., Chuang, K. C., Lee, M. H. & Tsai, Y. C. The inhibitory activity of linalool against the filamentous growth and biofilm formation in *Candida albicans*. Medical Mycology. 2013; 51: 473–482.

[24] Houmani, Z., Azzoudj, S., Naxakis, G. & Skoula, M. The essential oil composition of Algerian Zaâtar: *Origanum* spp. and *Thymus* spp. Journal of Herbs Spices & Medicinal Plants. 2002; 9: 275–280.

[25] Omidbaigi, R., Sefidkon, F. & Hejazi, M. Essential oil composition of *Thymus x citriodorus* L. cultivated in Iran. Flavour and Fragrance Journal. 2005; 20: 237–238.

[26] Salgueiro, L. R., Vila, R., Tomas, X., Canigueral, S., Dacunha, A. P. & Adzet, T. Composition and variability of the essential oils of *Thymus* species from Section *Mastichina* from Portugal. Biochemical Systematics and Ecology. 1997; 25: 659–672.

[27] Elhadj Ali, I. B., Zaouali, Y., Bejaoui, A. & Boussaid, M. Variation of the chemical composition of essential oils in Tunisian populations of *Thymus algeriensis* Boiss. et Reut. (*Lamiaceae*) and implication for conservation. Chemistry and Biodiversity. 2010; 7: 1276–1289.

[28] Fatma, G., Mouna, B., Mondher, M. & Ahmed, L. In-vitro assessment of antioxidant and antimicrobial activities of methanol extracts and essential oil of *Thymus hirtus* sp. *algeriensis*. Lipids in Health and Disease. 2014; 13: 114.

[29] Koes, R. E., Quattrocchio, F. & Mol, J. N. M. The flavonoid biosynthetic-pathway in plants – function and evolution. BioEssays. 1994; 16: 123–132.

[30] Riceevans, C. A., Miller, N. J. & Paganga, G. Structure-antioxidant activity relationships of flavonoids and phenolic acids. Free Radical Biology and Medicine. 1996; 20: 933–956.

[31] Loziene, K., Venskutonis, P. R., Sipailiene, A. & Labokas, J. Radical scavenging and antibacterial properties of the extracts from different *Thymus pulegioides* L. chemotypes. Food Chemistry. 2007; 103: 546–559.

[32] Jordan, M. J., Martinez, R. M., Martinez, C., Monino, I. & Sotomayor, J. A. Polyphenolic extract and essential oil quality of *Thymus zygis ssp gracilis* shrubs cultivated under different watering levels. Industrial Crops and Products. 2009; 29: 145–153.

[33] Jabri-Karoui, I., Bettaieb, I., Msaada, K., Hammami, M. & Marzouk, B. Research on the phenolic compounds and antioxidant activities of Tunisian *Thymus capitatus*. Journal of Functional Foods. 2012; 4: 661–669.

[34] Pereira, O. R., Peres, A. M., Silva, A. M. S., Domingues, M. R. M. & Cardoso, S. M. Simultaneous characterization and quantification of phenolic compounds in *Thymus x citriodorus* using a validated HPLC-UV and ESI-MS combined method. Food Research International. 2013; 54: 1773–1780.

[35] Delgado, T., Marinero, P., Asensio-S-Manzanera, M. C., Asensio, C., Herrero, B., Pereira, J. A. & Ramalhosa, E. Antioxidant activity of twenty wild Spanish *Thymus mastichina* L. populations and its relation with their chemical composition. LWT--Food Science and Technology. 2014; 57: 412–418.

[36] Galasso, S., Pacifico, S., Kretschmer, N., Pan, S. P., Marcianci, S., Piccolella, S., Monaco, P. & Bauer, R. Influence of seasonal variation on *Thymus longicaulis* C. Presl chemical composition and its antioxidant and anti-inflammatory properties. Phytochemistry. 2014; 107: 80–90.

[37] Pereira, O. R. & Cardoso, S. M. Overview on *Mentha* and *Thymus* polyphenols. Current Analytical Chemistry. 2013; 9: 382–396.

[38] Furmanowa, M. & Olszowska, O. *Thymus vulgaris* L. propagation through tissue culture. Acta Poloniae Pharmaceutica. 1980; 37: 243–247

[39] Furmanowa, M. & Olszowska, O. Micropropagation of Thyme (*Thymus vulgaris* L.). In: Bajaj, Y. P. S. (ed) Biotechnology in Agriculture and Forestry. Vol. 19. Berlin: Springer; 1992. pp. 230–243.

[40] Mendes, M. L. & Romano, A. In vitro cloning of *Thymus mastichina* L. field-grown plants. Acta Horticulturae. 1999; 502: 303–306.

[41] Ozudogru, E. A., Kaya, E., Kirdok, E. & Issever-Ozturk, S. In vitro propagation from young and mature explants of thyme (*Thymus vulgaris* and *T. longicaulis*) resulting in genetically stable shoots. In Vitro Cellular & Developmental Biology – Plant. 2011; 47(2): 309–320. DOI: 10.1007/s11627-011-9347-6

[42] Coelho, N., Gonçalves, S., González-Benito, M. E. & Romano, A. Establishment of an in vitro propagation protocol for *Thymus lotocephalus*, a rare aromatic species of the Algarve (Portugal). Plant Growth Regulation. 2012; 66: 69–74. DOI: 10.1007/s10725-011-9630-x

[43] Mendes, M. D., Figueiredo, A. C., Oliveira, M. M. & Trindade, H. Essential oil production in shoot cultures versus field-grown plants of *Thymus caespititius*. Plant Cell, Tissue and Organ Culture (PCTOC). 2013; 113: 341–351. DOI 10.1007/s11240-012-0276-9

[44] Bakhtiar, Z., Mirjalili, M. H., Sonboli, A., Sonboli, A., Farimani, M. M. & Ayyar, M.. In vitro propagation, genetic and phytochemical assessment of Thymus persicus — a medicinally important source of pentacyclic triterpenoids. Biologia. 2014; 69(5): 594–603. DOI: 10.2478/s11756-014-0346-z

[45] Nordine, A., Tlemcani, C. R. & Meskaoui, A. Regeneration of plants through somatic embryogenesis in *Thymus hyemalis* Lange, a potential medicinal and aromatic plant. In Vitro Cellular & Developmental Biology – Plant. 2014; 50: 19–25. DOI: 10.1007/s11627-013-9577-x

[46] Bernard, F., Navab Moghadam, N. & Mirzajani, F. The effect of colloidal silver nanoparticles on the level of lignification and hyperhydricity syndrome in *Thymus daenensis* vitro shoots: a possible involvement of bonded polyamines. In Vitro Cellular & Developmental Biology – Plant. 2015; 51(5): 546–553 DOI: 10.1007/s11627-015-9700-2.

[47] Marco-Medina, A. & Casasi, J. L. In vitro multiplication and essential oil composition of *Thymus moroderi* Pau ex Martinez, an endemic Spanish plant. Plant Cell, Tissue and Organ Culture (PCTOC). 2015; 120(1): 99–108.

[48] Bakhtiar, Z., Mirjalili, M. H. & Sonboli, A. In vitro callus induction and micropropagation of Thymus persicus (*Lamiaceae*), an endangered medicinal plant. Crop Breeding and Applied Biotechnology. 2016; 16: 48–54. DOI: 10.1590/1984-70332016v16n1a8

[49] Kaya, E. Development of efficient micropropagation and cryopreservation protocols for *Thymus* germplasm. In Vitro Biology Meeting held in collaboration with the 12th IAPB Congress, 2010, P283

[50] Marco-Medina, A., Casasi, J. L., Swennen, R., Panis, B. Cryopreservation of *Thymus moroderi* by droplet vitrification. Cryoletters. 2010; 31(1): 14–23. ISSN 0143-2044

[51] Coelho, N., González-Benito, M. E., Martín, C. & Romano, A.. Cryopreservation of *Thymus lotocephalus* shoot tips and assessment of genetic stability. Cryoletters. 2014; 35(2): 119–128.

[52] Kumar, P., Gupta, V. K., Misra, A. K., Modi, D. R. & Pandey, B. K.. Potential of molecular markers in plant biotechnology. Plant Omics Journal. 2009; 2(4): 141–162. ISSN 1836-3644

[53] Mendes, M. D., Barroso, J. G., Oliveira, M. M. & Trindade, H. Identification and characterization of a second isogene encoding γ-terpinene synthase in *Thymus caespititius*. Journal of Plant Physiology. 2014; 171: 1017–1027. DOI: 10.1016/j.jplph.2014.04.001

[54] Yousefi, V., Najaphy, A., Zebarjadi, A. & Safari, H. Molecular characterization of *Thymus* species using ISSR markers. The Journal of Animal & Plant Sciences. 2015; 25(4): 1087–1094. ISSN 1018-7081

[55] Rahimmalek, M., Bahreininejad, B., Khorrami, M. & Tabatabaei, B. E. Genetic variability and geographic differentiation in *Thymus daenensis* subsp. *daenensis*, an endangered medicinal plant, as revealed by inter simple sequence repeat (ISSR). Markers. Biochemical Genetics. 2009; 47(11–12): 831–842. DOI: 10.1007/s10528-009-9281-z

[56] Karaca, M., Ince, A. G., Aydin, A., Elmasulu, S. Y. & Turgut, K. Microsatellites for genetic and taxonomic research on thyme (*Thymus* L.). Turkish Journal of Biology. 2015; 39: 147–159. DOI: 10.3906/biy-1406-20

[57] Khoshsokhan, F., Babalar, M., Fatahi, M. & Poormeidani, A.. Assessment of genetic diversity of some wild populations of *Thymus Kotschyanus* using RAPD molecular markers. Cercetări Agronomice în Moldova. 2014; 3(159): 71–81. DOI: 10.2478/cerce-2014-0028

[58] Rustaiee, A. R., Yavari, A., Nazeri, V., Shokrpour, M., Sefidkon, F. & Rasouli, M. Genetic diversity and chemical polymorphism of some *Thymus* species. Chemistry & Biodiversity. 2013; 10: 1088–1098. DOI: 10.1002/cbdv.201200020

[59] Solyman, E. & Alkowni, R. RAPD for assessment of thymes genetic diversity in Palestine. Palestine Technical University Research Journal. 2014; 2(2): 01–08.

[60] Echeverrigaray, S., Agostini, G., Atti-Serfini, L., Paroul, N., Pauletti, G. F. & Atti dos Santos, A. C. Correlation between the chemical and genetic relationships among commercial thyme cultivars. Journal of Agricultural and Food Chemistry. 2001; 49: 4220–4223. DOI: 10.1021/jf010289j

[61] Pluhár, Z., Kocsis, M., Kuczmog, A., Csete, S., Simkó, H., Sárosi, S., Molnár, P. & Horváth, G.. Essential oil composition and preliminary molecular study of four Hungarian *Thymus* species. Acta Biologica Hungarica. 2012; 63(1): 81–96. DOI: 10.1556/ABiol.63.2012.1.7

[62] Hadian, J., Bigdeloo, M., Nazeri, V. & Khadivi-Khub, A.. Assessment of genetic and chemical variability in *Thymus caramanicus*. Molecular Biology Reports. 2014; 41(5): 3201–3210. DOI: 10.1007/s11033-014-3180-z

[63] Sostaric, I., Liber, Z., Grdisa, M., Marin, P. D., Stevanovi, Z. D. & Satovic, Z.. Genetic diversity and relationships among species of the genus *Thymus* L. (section *Serpyllum*). Flora. 2012; 207: 654–661. DOI: 10.1016/j.flora.2012.06.018.

[64] Karp, A., Edwards, K. J., Bruford, M., Funk, S., Vosman, B., Morgante, M., Seberg, O., Kremer, A., Boursot, P., Arctander, P., Tautz, D. & Hewitt, G. M. Molecular technologies for biodiversity evaluation: opportunities and challenges. Nature Biotechnology. 1997; 15(7): 625–628. DOI: 10.1038/nbt0797-625

[65] Kollipara, R., Peranteau, A. J., Nawas, Z. Y., Tong, Y., Woc-Colburn, L., Yan, A. C, Lupi, O. & Tyring, S. K. Emerging infectious diseases with cutaneous manifestations: Fungal, helminthic, protozoan and ectoparasitic infections. Journal of the American Academy of Dermatology. 2016; 75(1): 19–30. DOI: 10.1016/j.jaad.2016.04.032

[66] Pagiotti, R., Angelini, P., Rubini, A., Tirillini, B., Granetti, B. & Venanzoni, R.. Identification and characterisation of human pathogenic filamentous fungi and susceptibility to *Thymus schimperi* essential oil. Mycoses. 2011; 54(5): e364–e376. DOI: 10.1111/j.1439-0507.2010.01926.x

[67] Voltan, A. R., Quindós, G., Alarcón, K. P. M., Fusco-Almeida, A. M., Mendes-Giannini, M. J. S. & Chorilli, M. Fungal diseases: could nanostructured drug delivery systems be a novel paradigm for therapy? International Journal of Nanomedicine. 2016; 11: 3715–3730. DOI: 10.2147/IJN.S93105

[68] Labib, G. S. & Aldawsari, H. Innovation of natural essential oil-loaded Orabase for local treatment of oral candidiasis. Drug Design, Development and Therapy. 2015; 9: 3349–3359. DOI: 10.2147/DDDT.S85356

[69] Dorgan, E., Denning, D. W. & McMullan, R. Burden of fungal disease – Ireland. Journal of Medical Microbiology. 2015; 64(pt 4): 423–426.

[70] Chander, J., Stchigel, A. M., Alastruey-Izquierdo, A., et al. Fungal necrotizing fasciitis, an emerging infectious disease caused by *Apophysomyces* (Mucorales). Revista Iberoamericana de Micología. 2015; 32(2): 93–98.

[71] Brown, G. D., Denning, D. W., Gow, N. A., Levitz, S. M., Netea, M. G. & White, T. C. Hidden killers: human fungal infections. Science Translational Medicine. 2012; 4: 165rv13 DOI: 10.1126/scitranslmed.3004404.

[72] Pinto, E., Pina-Vaz, C., Salgueiro, L., Gonçalves, M. J., Costa-de-Oliveira, S., Cavaleiro, C., Palmeira, A., Rodrigues, A. & Martinez-de-Oliveira, J. Antifungal activity of the essential

oil of *Thymus pulegioides* on Candida, *Aspergillus* and dermatophyte species. Journal of Medical Microbiology. 2006; 55: 1367–1373.

[73] Cowan, M. M.. Plant products as antimicrobial agents. Clinical Microbiology Reviews. 1999; 12(4): 564–582.

[74] Brull, S. & Coote, P.. Preservative agents in foods: mode of action and microbial resistance mechanisms. International Journal of Food Microbiology. 1999; 50(1–2): 1–17. ISSN 0168–1605

[75] Darout, I., Cristy, A., Skaug, N. & Egeberg, P. Identification and quantification of some potential antimicrobial anionic components in miswak extract. Indian Journal of Pharmacology. 2000; 32: 11–14.

[76] Sharma, R., Sharma, C. L. & Kapoorl, B. Antibacterial resistance: current problems and possible solutions. Indian Journal of Medical Sciences. 2005; 59(3): 120–129. ISSN 0019–5359

[77] Moghaddam, K. M., Arfan, M., Rafique, J., Rezaee, S., Fesharaki, P. J., Gohari, A. R. & Shahverdi, A. R. The antifungal activity of Sarcococca saligna ethanol extract and its combination effect with fluconazole against different resistant *Aspergillus* species. Applied Biochemistry and Biotechnology. 2010; 162(1): 127–133. ISSN 1559–0291

[78] Edeoga, H. O., Okwu, D. E. & Mbaebie, B. O. Phytochemical constituents of some Nigerian medicinal plants. African Journal of Biotechnology. 2005; 4(7): 685–688. ISSN 1684–5315

[79] Panghal, M., Kaushal, V. & Yadav, J. P. In vitro antimicrobial activity of ten medicinal plants against clinical isolates of oral cancer cases. Annals of Clinical Microbiology and Antimicrobials. 2011; 10: 21 DOI: 10.1186/1476-0711-10-21, ISSN 14760711

[80] Janssen, A. M., Chin, N. L., Scheffer, J. J. & Baerheim Svendsen, A. Screening for antimicrobial activity of some essential oils by the agar overlay technique. Pharmaceutisch Weekblad. Scientific Edition. 1986; 8(6): 289–292.

[81] Rios, J. L., Recio, M. C. & Villar, A. Screening methods for natural products with antimicrobial activity: a review of the literature. Journal of Ethnopharmacology. 1988; 23: 127–149.

[82] Bruneton, J. Partie 3. Terpeenes et steroids. In Pharmacognosie, Phytochimie, Plantes médicinales. 4ª Édition. Paris: E´ ditions Tec&Doc & Éditions Médicales Internationals; 2009, pp. 547–934.

[83] Pina-Vaz, C., Sansonetty, F., Rodrigues, A., Costa-de-Oliveira, S., Martinez-de-Oliveira, J. & Fonseca, A. F. Cytometric approach for rapid evaluation of susceptibility of Candida strains to antifungals. Clinical Microbiology and Infection. 2001; 7: 609–618.

[84] Stahl-Biskup, E. & Sáez, F. Thyme – The Genus Thymus. London: Taylor & Francis; 2002.

[85] Mohd, S., Birhanu, D. & Tanweer, A.. Antimicrobial activity of essential oils extracted from medicinal plants against the pathogenic microorganisms: a review. Issues in Biological Sciences and Pharmaceutical Research. 2014; 2(1): 001–007.

[86] Dorman, H. J. D. & Deans, S. G. Antimicrobial agents from plants: antibacterial activity of plant volatile oils. Journal of Applied Microbiology. 2000; 88(2): 308–316.

[87] Arnal-Schnebelen, B., Hadji-Minaglou, F., Peroteau, J. F., Ribeyre, F. & de Billerbeck, V. G. Essential oils in infectious gynaecological disease: a statistical study of 658 cases. International Journal of Aromatherapy. 2004; 14(4): 192–197.

[88] Villanueva Bermejo, D., Angelov, I., Vicente, G., Stateva, R. P., Rodriguez García-Risco, M., Reglero, G., Ibañez, E. & Fornari, T. Extraction of thymol from different varieties of thyme plants using green solvents. Journal of the Science of Food and Agriculture. 2015; 95(14): 2901–2907.

[89] Wohlmuth, H., Smith, M. K., Brooks, L. O., Myers, S. P. & Leach, D. N. Essential oil composition of diploid and tetraploid clones of ginger (*Zingiber officinale* Roscoe) grown in Australia. Journal of Agricultural and Food Chemistry. 2006; 54: 1414–1419.

[90] Kalemba, D. & Kunicka, A. Antibacterial and antifungal properties of essential oils. Current Medicinal Chemistry. 2003; 10(10): 813–829.

[91] Kohiyama, C. Y., Yamamoto Ribeiro, M. M., Mossini, S. A., Bando, E., Bomfim Nda, S., Nerilo, S. B., Rocha, G. H., Grespan, R., Mikcha, J. M. & Machinski, M. Jr. Antifungal properties and inhibitory effects upon aflatoxin production of *Thymus vulgaris* L. by *Aspergillus flavus* link. Food Chemistry. 2015; 15(173): 1006–1010. DOI: 10.1016/j.foodchem.2014.10.135. Epub 2014 Nov 3.

[92] Manou, I., Bouillard, L., Devleeschouwer, M. J. & Barel, A. O.. Evaluation of the preservative properties of *Thymus vulgaris* essential oil in topically applied formulations under a challenge test. Journal of Applied Microbiology. 1998; 84(3): 368–376.

[93] Tampieri, M. P., Galuppi, R., Macchioni, F., Carelle, M. S., Falcioni, L., Cioni, P. L. & Morelli, I.. The inhibition of *Candida albicans* by selected essential oils and their major components. Mycopathologia. 2005; 159: 339–345. DOI: 10.1007/s11046-003-4790-5

[94] Ahmad, A., Khan, A., Akhtar, F., Yousuf, S., Xess, I., Khan, L. A. & Manzoor, N.. Fungicidal activity of thymol and carvacrol by disrupting ergosterol biosynthesis and membrane integrity against Candida. European Journal of Clinical Microbiology and Infectious Diseases. 2011; 30: 41–50. DOI: 10.1007/s10096-010-1050-8

[95] Sharifzadeh, A., Javan, A. J., Shokri, H., Abbaszadeh, S. & Keykhosravy, K. Evaluation of antioxidant and antifungal properties of the traditional plants against foodborne fungal pathogens. Journal of Medical Mycology. 2016; 26(1): e11–e17. DOI: 10.1016/j.mycmed.2015.11.002

[96] Markovic, T., Chatzopoulou, P., Siljegovic, J., Nikolic, M., Glamoclija, J. & Ciric, A. Chemical analysis and antimicrobial activities of the essential oils of *Satureja thymbra* L. and *Thymbra spicata* L. and their main components. Archives of Biological Sciences. 2011; 63: 457–464.

[97] Rasooli, I., Rezaei, M. B. & Allameh, A. Growth inhibition and morphological alterations of *Aspergillus niger* by essential oils from *Thymus eriocalyx* and Thymus x-porlock. Food Control. 2006; 17: 359–364.

[98] Mandras, N., Nostro, A., Roana, J., Scalas, D., Banche, G., Ghisetti, V., Del Re, S., Fucale, G., Cuffini, A. M. & Tullio, V. Liquid and vapour-phase antifungal activities of essential oils against *Candida albicans* and non-albicans Candida. BMC Complementary and Alternative Medicine. 2016; 16(1): 330. DOI: 10.1186/s12906-016-1316-5

[99] Leal, F., Matos, M., Coelho, A. C. & Pinto-Carnide, O. *In vitro* multiplication of aromatic and medicinal plants and fungicide activity. In: Dhanasekaran D., Thajuddin, N. & Panneerselvam, A. (eds). Fungicides for Plant and Animal Diseases. Croacia: Intech Open Access Publisher; 2011. pp. 119–138. ISBN 978-953-307-804-5

[100] Nardoni, S., Giovanelli, S., Pistelli, L., Mugnaini, L., Profili, G., Pisseri, F. & Mancianti, F. In vitro activity of twenty commercially available, plant-derived essential oils against selected dermatophyte species. Natural Product Communications. 2015; 10(8): 1473–1478.

[101] Lima, O. I., Oliveira, R. A. G., Lima, E. O., Porto Farias, N. M. & Souza, E. L. Antifungal activity from essential oils on Candida species. Revista Brasileira de Farmacognosia. 2006; 16: 197–201.