Chapter

Essential Oils of *Thymbra capitata* and *Thymus hyemalis* and Their Uses Based on Their Bioactivity

Ana Belén Sabater-Jara, María Pina Funes, María Angeles Pedreño and Sarai Belchí-Navarro

Abstract

Essential oils (EO) are volatile compounds produced by the secondary metabolism of aromatic plants. They are complex mixtures whose main components are synthesized by the mevalonic acid and the methyl erythritol phosphate pathways, which lead to the biosynthesis of terpenes, and the shikimic acid pathway, responsible for the biosynthesis of phenylpropanoid compounds. In nature, EOs are stored in the aerial parts of the plant, being of vital importance for their survival due to their antimicrobial properties. In addition, EOs provide protection against herbivores to the aromatic plants and allow them to repel or attract insects because of their strong fragrance, as well as compete with other plants of the same environment. Humans have exploited the properties of their EOs since ancient times, being used as medicinal remedies, among other uses. Currently, aromatic plants are used in pharmaceutical and food industries. One of the most commonly used aromatic plants is thyme. Thyme is a perennial aromatic plant, taxonomically belonging to the genera *Thymus* and *Thymbra*, belonging to the family Lamiaceae. These plants are very abundant in the Mediterranean Region. In this review, we focus on the study of the properties and use of EOs of *Thymbra capitata* (L) Cav. and *Thymus hyemalis* Lange., whose EOs are rich in phenolic monoterpenes. These compounds are responsible for their antioxidant, anti-inflammatory, anticarcinogenic, antibacterial, antifungal, and antiparasitic properties.

Keywords: essential oil, *Thymus hyemalis*, *Thymbra capitata*, aromatic plant, antioxidant, antimicrobial, carvacrol, thymol

1. Introduction

Essential oils (EOs) are volatile odorous compounds, are liquids at room temperature, and are produced by aromatic plants, as a result of their secondary metabolism [1, 2].

In nature, the EOs are stored in the secretory cells, cavities, channels, epidermal cells and trichomes of all the aerial organs of the plants, since they are of vital importance for plant survival, due to their antifungal, antibacterial, and antiviral activities. Also, they provide plants protection against herbivores and allow plants to compete with other plants, acting as allelopathic compounds. In addition, they are involved in pollination, attracting insects which favor the dispersal of seeds and pollen [3].

On the other hand, aromatic plants which produce these EOs have been used since ancient times to treat diseases, due to their healing properties. In fact, there are studies that claim that already in Ancient Egypt (2000 BC), these compounds were used as medicinal remedies, beauty products, and in religious rituals. Likewise, Hippocrates (460–377 BC), the father of medicine, studied and documented the properties of 300 aromatic plants, confirming the use of EOs in Ancient Greece. The Romans also showed great interest in the fragrance and properties of the EOs. Dioscorides, a Greek physician and botanist, described in Ancient Rome more than 500 aromatic plants and their EOs, in his book *De Materia Medica* [4]. In the tenth century, the Arabs also began to extract the EOs and use them in medicine. The use of EOs began to expand due to their pleasant fragrances, and at the end of the twelfth century, they began to be used in Europe. Their popularity was such that when the bubonic plague reached England in the mid-fourteenth century, it was ordered to burn aromatic plants in the streets, to fight the infection. At the beginning of the eighteenth century, EOs were already used to treat many diseases.

In the nineteenth century, EO composition was investigated [5]. It is now known that the major components of EOs are synthesized from three biosynthetic pathways: the mevalonic acid pathway, active in the cytosol, and the methyl erythritol phosphate pathway, active in the chloroplast, both of which lead to the biosynthesis of terpenes. A third route is the shikimic acid pathway, responsible for the biosynthesis of phenylpropanoid compounds [6]. EOs of terpene nature are synthesized from isopentenyl pyrophosphate and its isomer dimethylallyl pyrophosphate, which give rise to geranyl diphosphate, precursor of monoterpenes, and farnesyl diphosphate, precursor of sesquiterpenes, as shown in **Figure 1**. Among the numerous compounds present in the EOs derived from this biosynthetic pathway, thymol and carvacrol (isomers) stand out due to the numerous properties that are granted to them. As seen in **Figure 2**, both are phenolic monoterpenes synthesized from p-cymene, whose precursor is γ-terpinene [7].

Thymol and carvacrol are usually found in thyme EOs. On the other hand, compounds such as alcohols, aldehydes, ketones, esters, and, less frequently, carboxylic acids, as well as aromatic compounds such as phenolic ethers and aromatic esters are also present, although in a significantly lower proportion than the previous ones [6]. The properties of the EOs are mainly attributed to the major compounds, thymol and carvacrol; however it has been observed that these compounds can interact with the minority compounds, causing synergistic or antagonistic effects, thus influencing the properties of the EO [8]. In addition, the chemical composition varies according to environmental and genetic factors, influencing the phenological stage in which the harvest is made on the quality and quantity of the EO [9, 10]. The high variability occurs even within the same species, there being different major compounds among the specimens of the populations, which gives rise to the existence of different chemotypes [11].

Nowadays, many of the active ingredients used in the development of both traditional and modern drugs are extracted from plant species [12]. For the extraction of the EO, the technique most often used is the hydrodistillation, which consists of submerging fragments of the aromatic plant in boiling water, and so, the volatile compounds are dragged with the vapor, arriving at a condenser which separates them, and thus, the EO is obtained. Other conventional techniques such as steam distillation or extraction with volatile solvents as well as hydrodistillation by microwaves or extraction by supercritical fluids can be used [4]. After extracting, EOs can be analyzed by gas chromatography and mass spectrophotometry (GC–MS). GC allows the separation of the components of a complex mixture from the EO, and the MS serves for the identification of the individual components, already separated [13].

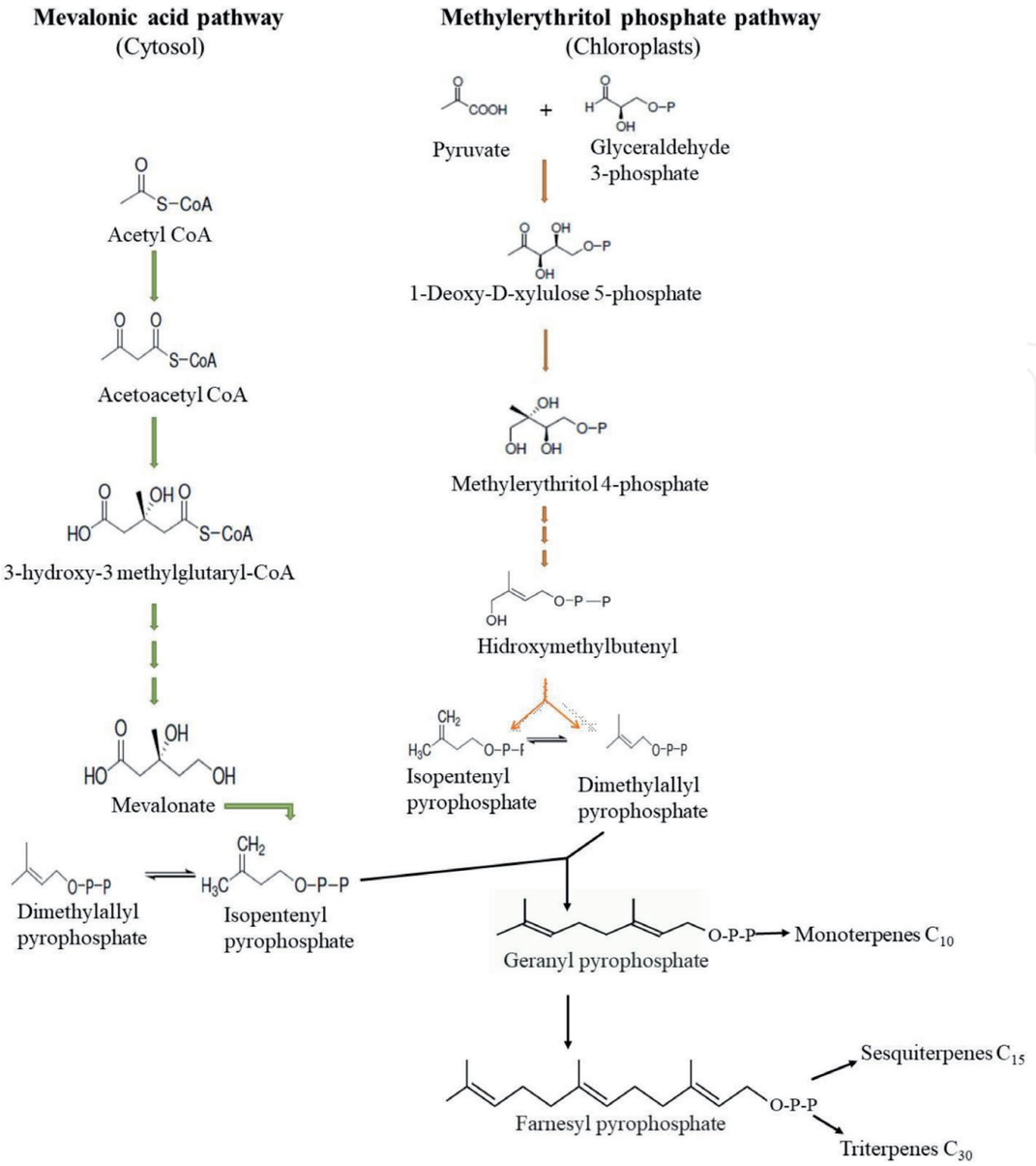

Figure 1.
Biosynthetic pathways responsible for the biosynthesis of the compounds present in the EOs.

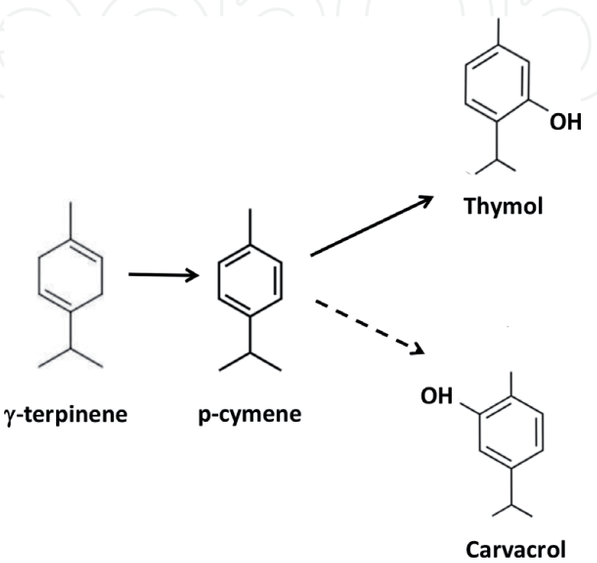

Figure 2.
Thymol and carvacrol chemical structure and their precursors.

Interest in EOs has skyrocketed in recent times. The demand for "natural" products increases year after year, and aromatic plants and EOs are becoming part of daily life. Likewise, more and more people are investigating the use of compounds obtained from plant extracts in medicine, such is the case of the EOs of many aromatic plants such as lavender (*Lavandula angustifolia*), eucalyptus (*Eucalyptus globulus*), or mint (*Mentha piperita*), which are being investigated for their neuroprotective effects [14]. Others such as EOs from oregano (*Origanum spp*.) are studied for their antioxidant and antibacterial activities [15].

It is estimated that more than 250,000 hectares are currently used to produce about 250 different plant extracts and, so on, different EOs, so they have a high socioeconomic importance in the places where they are produced, being generally rural areas in developing countries. These EOs are often used in the food industry as well as in other products of daily use such as bath gels, soaps, detergents, oral care products and body lotions. They are also widely used in aromatherapy (International Trade Center 2014). This justifies that, on a global level, 45,000 tons of EOs are produced annually, which implies an investment of more than 600 million euros, according to a study carried out by the Ministry of Agriculture of France. The main exporters are China, the USA, Brazil, EU countries, India, and Indonesia, and the largest imports are Switzerland, the USA, EU countries, Japan, and Canada [16].

In the industry of the EOs, one of the aromatic plants with greater use is thyme. Thyme is a small shrub and perennial aromatic plant, belonging taxonomically to the genera *Thymus* and *Thymbra*, of the family *Lamiaceae*, which includes 220 genera with plants such as mint, peppermint, basil, oregano, or pennyroyal, known throughout the world [17]. Thyme is very abundant in the Mediterranean Region. In the Iberian Peninsula, there is a high number of endemism, and it is common to find them in groups of thickets commonly known as "tomillares" [18]. Spain is one of the main suppliers of thyme worldwide [19], being the provinces with higher production Almeria, Murcia, and Granada, although it is also important in other areas of Andalusia, Castilla-La Mancha, and other provinces of interior, as Teruel [20].

Within the Region of Murcia, we found several species of thyme, two of them being of special relevance, both for their properties and for their environmental situation [18]: (1) *Thymbra capitata* (L.) Cav., commonly known as the Andalusian thyme, has a compact and stiff, fairly branched shrubby appearance, with pink flowers arranged in pineapple-shaped heads and leaves that are linear, glandular, and fleshy-looking, with a flat margin (**Figure 3**) [21]. (2) *Thymus hyemalis* Lange, commonly known as purple thyme or winter thyme, since the flowering stage

Figure 3.
T. capitata *inflorescence, characteristical disposal, and leaf morphology.*

Figure 4.
T. hyemalis *inflorescence (left), characteristical disposal, and leaf morphology (right).*

occurs in this season of the year, is a much branched woody shrub whose flowers have a pink corolla and a calyx with ciliated teeth, with leaves that are of small size, linear, and with revolute margin [22] (**Figure 4**).

This work focuses on these two species, due to the fact that *T. capitata*, a species of Mediterranean distribution, is found in the Region of Murcia in a retrograde situation and *T. hyemalis* is an endemic species from southeastern Spain, being mainly found in Murcia and Almería [18]. In addition, the properties and the possible uses of their EOs are the aims of this review.

2. Bioactive compounds of *T. capitata* EO

According to several studies, the EO of *T. capitata* is characterized by its high chemical homogeneity. Russo et al. [23], in an experiment carried out with wild populations of *T. capitata* in Calabria (Italy), observed that all the collected specimens, despite having grown under different environmental conditions, had a very similar chemical composition and all the specimens were of chemotype carvacrol (81.5–78.4%). A relatively high percentage of *p*-cymene, γ-terpinene, and β-caryophyllene were also found in these specimens. In addition, it has been observed that the percentages of *p*-cymene and γ-terpinene decreased when the percentage of carvacrol increased, which indicated that both compounds were its precursors [24]. Saija et al. [25], studying the chemical composition of this EO found that, all the wild specimens of *T. capitata* analyzed were of chemotype carvacrol. These results agree with studies conducted by Miguel et al. [9, 26, 27], where the major component was carvacrol, regardless of both the part of the plant used and the state of development. Likewise, Tuttolomondo et al. [28], in Sicily (Italy), found 38 compounds, being the most representative α-pinene, myrcene, α-terpinene, *p*-cymene, γ-terpinene, borneol, β-caryophyllene and carvacrol (67.4–79.5%), being the 13 biotypes studied of carvacrol chemotype. These results suggest that there is no polymorphism in the EO of *T. capitata*. However, other studies are contradictory to the results mentioned above, showing the existence

of three different chemotypes for *T. capitata*. In this sense, Miceli et al. [29] found 75 components and the majority being carvacrol and thymol, which, in all cases, constituted more than 50% of the composition of EO, followed by γ-terpinene, borneol, and *p*-cymene, when the chemical composition of the EO of *T. capitata* specimens were analyzed in flowering stage. The analysis of the compounds found in this EO revealed that there was a direct correlation between myrcene, α-terpinene, and γ-terpinene, whose concentrations decreased as the thymol concentration increased. An inverse relationship between linalool and myrcene was also observed. Thus, the analysis of the compounds presents in the EO of the specimens studied revealed that there were three distinct chemotypes: thymol, carvacrol, and thymol/carvacrol, the most common being those of chemotype thymol. For the first two chemotypes, a negative correlation was observed between thymol and carvacrol, so when one of the components was majority, the other was at low concentration. The thymol /carvacrol chemotype resulted from the crossing between the specimens with the two previous chemotypes. In short, independently of the chemotype, it was observed that the content of monoterpenes reached 78% of the total of compounds present in the EO of *T. capitata* [29].

In this sense, the experiments carried out by [10] confirmed the existence of these three chemotypes, which supports the hypothesis that *T. capitata* has a high polymorphism in the EO composition. To carry out these experiments, specimens grown in areas at different temperatures and degrees of humidity were used. As a result of this experiment, it was observed that those of carvacrol chemotype only appeared under conditions of high temperatures and low humidity. On the other hand, an experiment was carried out in which nine specimens were used, collected from three different areas, to later be cultivated under the same controlled climatic conditions. The results showed that the specimens maintained the chemotype that they originally presented, which is determined genetically, and did not change in the absence of climatic variations. These data suggest that the chemical composition of the EO is determined by the genetic endowment of the specimen and the different chemotypes are distributed according to the environmental conditions of the area in which they are cultivated [30].

Finally, in relation to other components found in smaller proportion (such as geraniol, camphor, or β-caryophyllene, among others), there is a high variability between populations and even within the same population [24, 31]. This variability can influence the bioactivity of *T. capitata* EO, which does not only depend on the majority component but also depends on the synergistic and antagonistic interactions that occur among all the phenolic and non-phenolic components [9, 25–27].

3. Bioactive compounds of *T. hyemalis* EO

In a study conducted by [32], it was observed that *T. hyemalis* EO had a high heterogeneity, there being three different chemotypes: thymol, thymol/linalool, and carvacrol. The main components for the thymol chemotype were thymol (43%) followed by p-cymene (16%) and γ-terpinene (8.4%). For the thymol/linalool chemotype, the major compounds were linalool (16.6%), thymol (16%), γ-terpinene (9.8%), 1–8-cineol (5.4%), borneol (4.7%), and verbenone (4.8%). Finally, the carvacrol chemotype was characterized by a majority composition of carvacrol (40.1%), *p*-cymene (19.8%), borneol (5.0%), and thymol (2.9%).

The variability in the chemical composition of *T. hyemalis* EO may be related to seasonal variations [33, 34] as well as to the edapho-climatic factors [35].

One of the studies that supports the previous statement were carried out by Jordán et al. [36], where it was observed that, in the case of thymol chemotype, the

synthesis of this major compound occurred during the flowering/fruit ripening stage. The precursors of thymol, γ-terpinene, and *p*-cymene (**Figure 1**) were at their maximum concentration during the flowering stage. Therefore, between the stage of flowering and that of the beginning of fruit maturation, the composition of the EO of *T. hyemalis* reached its highest quality. This phenological stage coincides with winter, being recommendable to harvest the specimens in this season of the year. However, according to this study, it is also possible to obtain a high thymol content in the *T. hyemalis* EO during the spring season, but to achieve this, it is necessary to increase the irrigation, a condition that is not always achieved in arid and semiarid regions. In contrast to these results, Cabo et al. [33] proposed that August was the best time to harvest because the *T. hyemalis* EO contained a high concentration of 1,8-cineol in specimens collected during different phases of the vegetative cycle of *T. hyemalis*. These results seem to indicate the existence of a 1,8-cineol chemotype, with a very low concentration of thymol and carvacrol.

Finally, similar to the results of *T. capitata,* it should be noted that although the phenolic compounds, thymol and carvacrol, are mainly responsible for the bioactivity of the EO, the existence of synergistic or antagonistic effects between these phenolic components and other minor compounds (alcohols, other terpenoids, ketones, etc.) of *T. hyemalis* EO has been observed, which are essential for the quality of this EO [32].

4. Bioactivity of *T. capitata* EO

4.1 Antioxidant activity

It has been observed that the EO of *T. capitata* shows a potent antioxidant activity due to its high content of phenols (thymol or carvacrol) [37]. This statement is supported by Aazza et al. [38], when compared with the antioxidant activity of several thyme species. The results showed a higher antioxidant activity in EO rich in phenolic monoterpenes, like those of *Thymus caespititius* Brot. and *T. capitata*.

This antioxidant capacity has been widely researched in order to prevent lipid oxidation during the storage of vegetable oils for culinary use, such as olive or sunflower. Likewise, Miguel et al. [26] showed that *T. capitata* EO, rich in carvacrol, avoided the lipid oxidation of sunflower oil and even turned out to be a more potent antioxidant than butylated hydroxytoluene (BHT), a synthetic antioxidant commonly used in the food industry. In addition, it has been seen that by isolating the carvacrol from the EO, this one by itself showed an antioxidant activity like EO, indicating an absence of synergistic or antagonistic effects due to the interaction between the different components of the EO. However, when the antioxidant activity of the EO of *T. capitata* was tested on the lipid oxidation of olive oil, it was observed that the EO was less potent than BHT [27, 39]. This low antioxidant capacity is also evident in the studies conducted by Saavedra et al. [40], in which it was observed that *T. capitata* EO did not help to avoid the oxidation of olive oil during storage and even increased the peroxidation levels, which indicated a greater number of oxidation products.

Another study conducted by Miguel et al. [9] on the lipid oxidation of peanut and sunflower oils showed a low antioxidant activity of *T. capitata* EO compared to two synthetic antioxidants, hydroxybutylanisole (BHA) and BHT, as well as a low effectiveness in elimination of free radicals compared to BHT, which contradicts the previous results found in sunflower oil. In addition, when comparing the EO of *T. capitata* with those of other species of the family Lamiaceae (*T. mastichina* and *T. camphoratus*), rich in *p*-cymene-2,3-diol, it was observed that the EO of *T. capitata*,

rich in carvacrol, had a lower antioxidant activity than those of these two species. These results could be due to the differences found at the time of harvest, since, in this study, the *T. capitata* specimens were collected in the vegetative phase, while in the studies that demonstrated an important antioxidant activity for the EO of *T. capitata*, the specimens were collected during the flowering phase.

On the other hand, Galego et al. [41] carried out a study on the antioxidant capacity of EOs extracted from *T. capitata*, *Origanum vulgare* L., *T. mastichina*, and *Calamintha baetica* Boiss & Reut. For this, the antioxidant activity was determined using modified thiobarbituric acid (TBARS), which consists of the formation of a pink pigment produced by the reaction of thiobarbituric acid with malondialdehyde, a product of lipid peroxidation. Their results indicated that *T. capitata* and *O. vulgare* had the highest antioxidant activity, like BHT and BHA, but although they were effective in eliminating free radicals, at low concentrations, they did not become as effective as BHT and BHA.

In addition, it was observed that the antioxidant capacity of the EO of *T. capitata* was higher than that of the EO of *T. mastichina* and *C. baetica*, since the composition of the EO of these species was lower in phenolic compounds than the EO of *T. capitata*.

Faleiro et al. [15] also used the TBARS method to observe the effectiveness of the EO of *T. capitata* against the lipid oxidation of the egg yolk. Their results showed that, at high concentrations, EO could be as effective as synthetic antioxidants, BHA and BHT.

It should be noted that the antioxidant activity of *T. capitata* EO has not only been investigated in the food industry. In this sense, Hortigón-Vinagre et al. [42] studied the ability of this EO to prevent the cell death of cardiomyocytes in neonatal rats treated with 4-hydroxy-2-nonenal, a compound that induces lipid peroxidation in these cells. The results showed that at low concentrations (less than 40 ppm), the EO of *T. capitata* prevented the loss of membrane potential of the mitochondria and decreased the levels of reactive oxygen species (ROS), preventing the death of cardiomyocytes. However, concentrations higher than the mentioned one caused cell death, since they were toxic for the cells. In addition, this toxicity can be used as antiproliferative activity in in vitro experiments, since the EO extracted from fruits of *T. capitata* inhibited the growth of cells isolated from cervical cancer (HeLa). Likewise, the EO extracted from flowers and fruits of this species inhibited the growth of histiocytosis cells (U937) [30] and tumor cells responsible for acute monocytic leukemia (THP-1) [37].

4.2 Antibacterial activity

The antibacterial activity of the EO of *T. capitata* as well as its main component, carvacrol, was demonstrated against *Gardnerella vaginalis* by Machado et al. [43, 44]. The EO of *T. capitata* showed a potent activity against *G. vaginalis*, which was evidenced by the low minimum inhibitory concentration (MIC) (0.16 μL/mL) and the minimum lethal concentration (MLC) (0.16–0.31 μL/mL).

This antibacterial activity of *T. capitata* EO has also been observed against *Listeria monocytogenes*, the bacteria responsible for listeriosis, in a study conducted by Faleiro et al. [15].

In addition, Delgado-Adámez et al. [30] showed that the EO extracted from both flowers and fruits of *T. capitata* had a high efficacy against *Listeria innocua* (Gram+), at concentrations higher than 0.01% (v/v), and *Escherichia coli* (Gram−), at concentrations above 0.1% (v/v). Also, Karampoula et al. [45] showed the antibacterial effectiveness of the EO of *T. capitata* in hydrosol (a complex mixture of 24 components, which came from hydrodistillation of the plant, where the

major compound was carvacrol). This antibacterial activity was studied against planktonic cells and biofilms of *Salmonella typhimurium*. The biofilms formed by the bacteria showed a slightly higher resistance to the planktonic cells, but in general, hydrosol was effective as antibacterial agent. In fact, when comparing this hydrosol with benzalkonium chloride, a commonly used synthetic antibacterial, it was observed that the hydrosol from *T. capitata* EO was much more effective as bactericide, since in order for benzalkonium chloride to show the same results as the hydrosol on planktonic cells and biofilms of *S. typhimurium*, a 200 times higher concentration was needed.

4.3 Antifungal activity

According to Salgueiro et al. [46], the EO of 22 specimens of *T. capitata* carvacrol chemotype (60–66%), with high percentages of p-cymene (6–7.5%) and γ-terpinene (8.2–9.5%), was effective as a natural antifungal agent against *Candida* spp., *Aspergillus* spp., and some species of dermatophytes (*Trichophyton rubrum*, *T. mentagrophytes*, *Microsporum canis*, and *M. gypseum*), and its effect was mainly due to the generation of lesions on the membrane surface of the microorganism.

These results agree with Palmeira-de-Oliveira et al. [47, 48], whose studies demonstrated that the EO of *T. capitata*, rich in carvacrol (75%), showed a great antifungal potential on biomass of *Candida* spp. and on preformed biofilms, since, at concentrations close to MIC (0.32 μL/mL), it caused the inhibition of its metabolism by up to 50%.

In the case of biofilms, when the concentration of the EO doubled the MIC, a decrease of 80% of its metabolism was observed. Antifungal activity of the EO of *T. capitata* was also compared with the classic antifungal agents amphotericin B and fluconazole, proving to be even more effective in some of the fungi studied.

Likewise, it has been observed that carvacrol or *p*-cymene isolated from the EO of *T. capitata* by themselves showed antifungal capacity. Therefore, this EO, or its isolated components, could be used for the treatment of mucocutaneous candidiasis and dermatophytosis. In this sense, the EO of *T. capitata* could be used alone or together with other antifungal components used so far [46, 47], as in the case of the association of the EO of *T capitata* with chitosan or chitosan in hydrogel, whose antifungal activity has been demonstrated in in vitro studies. The mechanism of action of this hydrogel has been studied by confocal microscopy, observing the interaction of this formula with the cell wall of *Candida* spp. [46].

On the other hand, Russo et al. [23] observed that the EO of *T. capitata* carvacrol chemotype had an antifungal effect at a concentration of 250 ppm against *Sclerotium cepivorum*, a fungus responsible for white rot in garlic, onion, and leek crops. These authors suggested that the EO of *T. capitata* could be used as a natural antifungal, for its plant origin, not being harmful to the environment. In addition, it would be difficult to develop resistance in the fungus, due to the high chemical complexity of this oil.

4.4 Antiparasitic activity

Machado et al. [22] analyzed the antiparasitic activity of EOs rich in phenolic compounds of the species *T. capitata*, *O. virens* (Hoffmanns & Link), *T. zygis* subsp. *Sylvestris* (Hoffmanns & Link) Cout., and *Lippia graveolens* Kunth against *Giardia* spp. All EOs, including that of *T. capitata*, decreasing the viability of the parasite; altering its morphology, membrane permeability, and internal organization; and inhibiting its growth, as well as its adhesion capacity, which is essential for the parasite, to be able to bind to the intestine and not be eliminated by peristalsis. EOs blocked this adhesion from the first hours of exposure, not being more than 10% of

cells able to adhere after 7 hours of treatment. In addition, by affecting membrane permeability, they caused swelling in the cells and alterations in the cytoplasm, which ultimately leads to cell death. Therefore, these EOs could be used as an alternative treatment for giardiasis, since they are not toxic to mammalian cells.

5. Bioactivity of *T. hyemalis* EO

5.1 Antioxidant activity

Several studies have shown the antioxidant activity of *T. hyemalis* EO. In this sense, Ocaña and Reglero [49] analyzed the antioxidant properties of the EO of *T. hyemalis*, *T. zygis*, and *Thymus vulgaris* L. on a cellular model of inflammation/atherogenesis, in which human macrophages, derived from THP-1 cells, were used. These cells were incubated with EOs of the different Thymus species. The expression of inflammatory (TNF-α, IL-1B and IL-6) and anti-inflammatory (IL-10) mediators was determined. The results showed that the production of inflammatory mediators took place and the production of the anti-inflammatory mediator IL-10 increased in the presence of EOs of any of the three species of Thymus (being *T. hyemalis* the one that had less activity). This effect is due to the antioxidant capacity of these EOs, which in turn is responsible for the anti-inflammatory activity observed in this test.

On the other hand, Jennan et al. [50] compared the activity of the EO of *T. hyemalis* with that of the EO of *Thymus bleicherianus* Pomel, measuring its capacity to eliminate the free radical 1,1-diphenyl-2-picrilhydracil, observing a greater antioxidant activity in the EO of *T. bleicherianus*. The activity of EO of *T. hyemalis* was also compared with that of BHT, the synthetic compound being a more potent antioxidant. These results suggested that, although the *T. hyemalis* EO is a good antioxidant, it is not as good as the EO of other *Thymus* species.

5.2 Antibacterial activity

Rota et al. [32] conducted a study on the antimicrobial activity of EOs from several thyme species, specifically, *T. hyemalis*, *T. zygis*, and *T. vulgaris*. The EO activity was tested against the pathogenic microorganisms *E. coli*, *L. monocytogenes*, *S. typhimurium*, *Shigella flexneri*, *Shigella sonnei*, *Staphylococcus aureus*, *and Yersinia enterocolitica*. The results showed that the antimicrobial activity seemed to be related to the content of phenolic compounds, specifically thymol and carvacrol. EOs that showed the greatest antimicrobial effectiveness were *T. hyemalis* (thymol and carvacrol chemotypes, in this order), *T. zygis* (thymol), and *T. vulgaris* (thymol). These results coincided with those found by Jennan et al. [50] which suggested that *T. hyemalis* EO affected survival and inhibited the growth of bacteria Gram+ and Gram−.

Some microorganisms, such as *S. typhimurium*, *Y. enterocolitica*, *S. flexneri*, *L. monocytogenes*, and *S. aureus*, showed a high sensitivity to EOs from *T. hyemalis* (thymol and thymol/linalool chemotypes), so a high concentration of them to be effective was not necessary. In other cases, such as *E. coli*, the presence of a high concentration of carvacrol or thymol was essential to observe a potent antibacterial activity. In addition, it has been observed that the greater the richness and variety of minority components, the greater the effectiveness of EO against microorganisms [32].

Tepe et al. [51] also investigated the in vitro antimicrobial activity of *T. hyemalis* EO (carvacrol chemotype), which turned out to be a potent bactericide at low

Microorganisms	MIC Commercially available essential oil component		
	Thymol	Carvacrol	p-cymene
Staphylococcus aureus	1.95	0.48	250.00
Bacillus cereus	0.97	0.24	250.00
Enterobacter aerogenes	0.97	1.95	250.00
Escherichia coli	1.95	0.48	250.00
Klebsiella pneumoniae	1.95	3.90	250.00
Proteus mirabilis	1.95	1.95	250.00
Pseudomonas aeruginosa	15.62	7.81	250.00
Candida albicans	0.97	0.24	15.62

Table 1.
Antibacterial and antifungal activity of the three major components found in T. hyemalis *EO [51].*

concentrations (31.2 mg/mL) against *Bacillus cereus* and *Bacillus subtilis*. In addition, it also inhibited the growth of *Enterococcus faecalis* and *S. aureus*, although at higher concentrations (62.5 mg/mL). They also studied the antimicrobial activity of isolated carvacrol, which showed a potent activity against *B. cereus*, showing inhibition of bacterial growth with a MIC of 0.24 mg/mL. It also showed activity against *E. coli*, although at higher concentrations (**Table 1**).

This activity of carvacrol was compared with the antibacterial activity of thymol, the second most important component of *T. hyemalis* EO (carvacrol chemotype). The results obtained suggested that thymol was a good bactericide, but not as much as carvacrol. However, when the isolated precursor of carvacrol (p-cymene) was used, no antimicrobial activity was observed. Also, neither the *T. hyemalis* EO nor the isolated carvacrol was effective against *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Pseudomonas fluorescens*, *and L. monocytogenes*.

5.3 Antifungal activity

Tepe et al. [51] demonstrated the antifungal activity of *T. hyemalis* EO against *C. albicans*, which inhibited its growth at a MIC of 62.50 mg/mL. This activity was also measured using carvacrol, thymol, and p-cymene, major components of *T. hyemalis* EO. Carvacrol inhibited the growth of the fungus at a MIC of 0.24 mg/mL, while p-cymene needed high concentrations to begin to inhibit fungal growth. Thymol was also a good antifungal against *C. albicans*, although not as good as carvacrol, since the MIC turned out to be 0.97 mg/mL.

6. Discussion

In general, all the data together show that *T. capitata* and *T. hyemalis* are two important sources of EOs, which have different types of bioactivity, being of great interest for human health as well as for food and cosmetics, due to their antifungal, antibacterial, and antioxidant properties.

According to the literature reviewed, the biological activity found in the EOs is clearly related to the chemical composition of them. As regards the *T. capitata* EO, there are some controversies about its homogeneity. Several studies confirm the existence of a single chemotype in this species, determined by its major component, carvacrol [9, 23–28]. However, other studies support the existence of three

chemotypes: thymol, carvacrol, and thymol/carvacrol, resulting in a crossover from the previous two [10, 29]. Regarding *T. hyemalis*, there is no doubt about the heterogeneity of its EO, being the chemotypes: thymol, thymol/linalool, and carvacrol [32].

For both species (*T. capitata* and *T. hyemalis*), the reason for these differences in the chemical composition of their EOs extracted from different specimens could be due both to the genetic endowment of the plants and to the influence of climatic and edaphic conditions of their habitat. In fact, the influence of climate on the composition of EOs has also been described for other species within the genus Thymus, such as *T. zygis* and *T. piperella*. In general, carvacrol-rich chemotypes have been associated with arid climates and high areas. However, thymol-rich chemotypes have higher water requirements than the carvacrol chemotypes [11, 52].

Likewise, the moment in which the specimens are harvested influences the composition of EOs, since it varies throughout the life cycle of these plants, affecting their bioactivities. In fact, in *T. capitata*, the highest content of phenolic monoterpenes occurs during the flowering stage, exhibiting greater antioxidant activity at this time. This coincides with what has been demonstrated for other thyme species, such as *T. vulgaris* [53]. For this reason, it is recommended to collect them during this stage, achieving the highest qualities of this EO. Regarding *T. hyemalis*, there is controversy about the best time to harvest. Extensively works support that it is better to collect the specimens in winter, between the flowering stage and the beginning stage of fruit ripening. However, other authors propose that the best month to harvest is August, since, during this month, there is a high content of 1,8-cineol in the EO. These results point to the possible existence of a 1,8-cineol chemotype, but further studies would be necessary to confirm this hypothesis. If this compound is a major component in *T. hyemalis*, the exploitation of this chemotype at an industrial level could be interesting, since it has been shown that 1,8-cineol has anti-inflammatory and analgesic properties [54]. It has also been observed that it can act as a natural insecticide in certain plant species of the *Myrtaceae* family [55].

On the other hand, the results show that in the absence of environmental variations, the different chemotypes are genetically determined. This has been observed for the EO of other medicinal plants, such as *Lupinus argenteus* Pursh and *Piper methysticum* G. Forst, whose mutations in a few genes influence the biosynthetic pathways which promote the greater or lesser accumulation of one or another compound, giving rise to different chemotypes [56, 57].

Regarding the antioxidant activity, the results indicate that it depends on the concentration. The EO of *T. capitata* (carvacrol chemotype) is effective at high concentration to avoid lipid oxidation of egg yolk and sunflower oil and may be even more effective than BHA and BHT. However, this is only possible if *T. capitata* specimens have been harvested during the flowering stage [7, 9].

This is due, in large part, to the fact that the antioxidant activity of EO (as well as the rest of activities) does not depend only on the majority component but also depends on the synergistic or antagonistic interactions of the majority component with other minority components, which according to the phenological stage will be different [58]. However, in the literature reviewed, some authors indicate the absence of these interactions because isolated carvacrol has been shown to have activity on its own [27, 39]. However, the EO of *T. capitata* did not prove to be a good antioxidant for olive oil. This is the difference between sunflower oil and olive oil. This difference can be due to the different compositions of fatty acids in both oils, being the most effective in sunflower oil (rich in linoleic) than in olive (rich in oleic) [39].

On the other hand, both EOs extracted from *T. capitata* specimens and *T. hyemalis*, at low concentrations, have antioxidant activity, which gives them

anti-inflammatory properties, which could be used for the treatment of chronic inflammatory diseases. However, at high concentrations, EOs of these species show oxidant activity, which could be toxic to the cells and so inhibit their proliferation. This effect of EOs on cell proliferation is of great interest since they could be used as potent anticancer agents [49].

Regarding their antibacterial activity, EOs have shown a potent bactericidal effect against a large number of Gram+ and Gram- species. In this sense, EO of *T. capitata* could be used for the treatment of bacterial vaginosis together with chitosan in hydrogel; this could be a good alternative to treatment with antibiotics, which usually provoke resistance. In addition, it has been observed that EOs are well tolerated by the beneficial flora, since it is not damaged [43, 44].

Likewise, both EOs from *T. capitata* and *T. hyemalis* (all the chemotypes, although the most effective is the thymol chemotype) are useful against *L. monocytogenes*, so they could be used in the food industry to avoid contamination due to this bacterium [15].

In relation to the antifungal activity, it has been demonstrated for the EO of *T. capitata* (carvacrol chemotype) against *Candida* spp., *Aspergillus* spp., and some species of dermatophytes (*Trichophyton rubrum*, *T. mentagrophytes*, *Microsporum canis*, and *M. gypseum*). This EO has also shown activity against the intestinal parasite *Giardia* spp. This fact suggests that it could be used for the treatment of giardiasis together with other compounds such as chitosan.

In addition, the *T. hyemalis* EO (carvacrol chemotype) and carvacrol by itself also showed effectiveness against *C. albicans*. However, it has not been demonstrated for its precursor, p-cymene. Although the antimicrobial activity depends on the presence of carvacrol, it is believed that p-cymene acts synergistically with carvacrol, helping to destabilize the membrane of these microorganisms.

Currently, the trade and use of thyme EOs is more focused on species such as *T. vulgaris*. This species has been widely used in aromatherapy and natural medicine for some years, as a hot poultice that relieves the pain of cystitis and renal colic, due to its analgesic properties, and in the form of vapors and inhalations for asthma and colds, among other conditions of the respiratory system. It is also used as a natural disinfectant, due to its antiseptic power. Its antimicrobial, antioxidant, and anticancer properties have been widely studied. However, the information reviewed here indicates that these two species, *T. capitata* and *T. hyemalis*, could be a very important source of economic resources, due to their properties, since they can be exploited by the pharmaceutical, food, livestock, and agricultural industries, its conservation being fundamental in the ecosystems where they are found.

Finally, in relation to the mechanism of action by which EOs have their different effects, it is not clear. It is known that all the activities mentioned are dependent on the concentration at which they are used. As we have seen throughout this work, the results vary depending on the dose of EO used. With regard to the antifungal and antibacterial activity, the EO acts by affecting the membrane permeability of the pathogen. At high concentrations, the EO denatures the proteins, whereas if the concentration is low, the enzymatic activity related to the production of energy is affected.

It has also been observed that, in the case of *Sclerotium cepivorum* Berk, in the presence of monoterpenes, its lipid composition is modified, the cell membrane is altered, and lipid peroxidation is increased, so that these compounds are toxic for the cells. Therefore, these EOs, rich in monoterpenes, could be used to treat crops affected by diseases caused by fungi, such as white rot, which would be a great economic benefit, since it would avoid large agricultural losses. However, it would be necessary to carry out further studies on the mechanism of action of the compounds present in the EOs, specifically the content of monoterpenes in both

T. capitata and *T. hyemalis*. In addition, most of the studies reviewed were carried out in in vitro experiments, so to ensure the potential of these EOs, it would be necessary to study their properties in vivo.

7. Conclusion

T. capitata and *T. hyemalis* EOs are rich in phenolic monoterpenes (carvacrol and thymol), which are associated with antioxidant, antifungal, antibacterial, or antiparasitic properties. These EOs, due to their properties, can be used in pharmaceutical, food, livestock, agricultural, and pharmaceutical industries, being a potential source of economic resources. However, climate, edaphic factors, and genetics influence the chemical composition of these EOs. The high homogeneity of *T. capitata* EOs in climates with high temperatures and low humidity (carvacrol chemotype) can be an important economic resource of easy exploitation in arid and semiarid regions.

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Conflict of interest

The authors declare that there are no conflicts of interest.

Author details

Ana Belén Sabater-Jara, María Pina Funes, María Angeles Pedreño and Sarai Belchí-Navarro*
University of Murcia, Murcia, Spain

*Address all correspondence to: saraibn@um.es

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