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Review

Plants belonging to the genus *Thymus* as antibacterial agents: From farm to pharmacy

Seyed Mohammad Nabavi [a,b], Anna Marchese [c], Morteza Izadi [b], Valeria Curti [d], Maria Daglia [d,*], Seyed Fazel Nabavi [a,b]

[a] Applied Biotechnology Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran
[b] Health Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran
[c] Microbiology Unit-DISC, University of Genoa, Italy
[d] Department of Drug Sciences, Medicinal Chemistry and Pharmaceutical Technology Section, University of Pavia, Italy

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ABSTRACT

In traditional medicine, plants have been used since ancient times for the prevention and/or protection against infectious diseases. In recent years, the use of herbal medicines and food supplements containing botanical ingredients, as alternative therapy for infectious diseases, has been intensified due to their high content of antimicrobial agents such as polyphenols, i.e. flavonoids, tannins, and alkaloids. Plants from the genus *Thymus* are important medicinal herbs, which are known to contain antimicrobial agents, and are rich in different active substances such as thymol, carvacrol, *p*-cymene and terpinene. In this review, we summarise the available literature data about the *in vitro* antibacterial effects of the main plants belonging to the genus *Thymus*. We also provide information about cultivation, chemical composition of the essential oils obtained from these plants, and their use for medicinal purposes.

Contents

1. Introduction

Infectious diseases are a leading cause of morbidity and mortality worldwide, especially in developing countries (World Health Organization, 2010). The World Health Organization (WHO) has estimated that 55 million people died worldwide in 2011, and infectious diseases were responsible for one-third of all deaths. This situation is aggravated by the increasing number of disease-causing microorganisms resistant to antibiotic therapy, which are able to recover and survive after antibiotic drug exposure through their ability to acquire and transmit resistance. Therefore, antibiotic resistance has become a public health problem of increasing magnitude, and the discovery and development of novel

* Corresponding author at: Department of Drug Sciences, Medicinal Chemistry and Pharmaceutical Technology Section, University of Pavia, Via Taramelli 12, 27100 Pavia, Italy. Tel.: +39 0382 987388; fax: +39 0382422975.
E-mail address: maria.daglia@unipv.it (M. Daglia).

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antimicrobial agents to address this problem is an important priority (Högberg, Heddini, & Cars, 2010).

Numerous scientific reports have shown that plants have a high potential to synthesize different antimicrobial substances (Daglia, 2011) which act as plant defence mechanisms and protect them against abiotic (UV radiation, drought, high or low temperatures, excessive soil salinity) and biotic stresses (i.e. microorganisms, insects, and herbivores). Plant-derived antimicrobial agents can be classified into phenolics and polyphenolics, terpenoids, alkaloids, lectins, polypeptides, and polyacetylenes (Simoes, Bennett, & Rosa, 2009). Based on traditional use, these extracts obtained from plants are usually the first treatments recommended, due to their multi-target antimicrobial actions and low adverse effects (Simoes et al., 2009). In fact, in traditional medicine, some plants such as bearberry, cranberry, lemon balm, garlic, tee tree and thyme have been used to treat different conditions such as infectious diseases, inflammation, etc. The search of natural products for their healing potential is an idea coming from ancient times which has been taken up and developed in recent years (Nabavi, Daglia, Moghaddam, Habtemariam, & Nabavi, 2014; Nabavi et al., 2013, 2012).

The genus *Thymus* L. (belonging to the family Lamiaceae) consists of 928 species, native to Europe, and grown in the Mediterranean basin and northern Europe, as well as other parts of the world such as Asia, South America, and Australia (Morales, 2002). *Thymus vulgaris* L. (thyme) is known as one of the most important species, and is used in the food, cosmetic and pharmaceutical industries (Stahl-Biskup & Sáez, 2003), with a large number of studies providing evidence for its antimicrobial (especially antibacterial) effects under *in vitro* conditions (Asbaghian, Shafaghat, Zarea, Kasimov, & Salimi, 2011; Rota, Herrera, Martínez, Sotomayor, & Jordán, 2008; Ulukanli, Cigremis, & Ilcim, 2011). Many other species of the genus *Thymus* have also been studied for their composition and antibacterial activity (Rota et al., 2008). The present paper is aimed to critically review the scientific reports which demonstrated the *in vitro* antibacterial effects of essential oils and extracts obtained from plants belonging to the genus *Thymus*. In addition, we discuss their cultivation, chemical composition, and traditional uses of these medicinal plants (Table 1).

Table 1
Chemical composition of *Thymus* genus plant essential oils and extracts.

Thymus specie	Compound	Reference
Thymus caucasicum	1,8-Cineole (21.5%) Thymol (12.6%) β-Phenyl alcohol Nerol	Asbaghian et al. (2011)
Thymus kotschyanus	Carvacrol (24.4%) β-Caryophyllene (14.5%) Terpinene (12.4%) α-Phellandrene (10.8%) *p*-Cymene (9.8%)	Asbaghian et al. (2011)
Thymus vulgaris	Thymol (43.8%) *p*-Cymene (15.2%)	Asbaghian et al. (2011)
Thymus capitatus *Thymus caramanicus* and *Thymus fallax*	Carvacrol (>80%) Carvacrol (from 50% to 70%)	Eftekhar et al. (2009)
Thymus algeriensis	Borneol (23.4%) Linalool (8.9%) Carvacrol (7.8%) b-Caryophyllene (6.4%)	Ait-Ouazzou et al. (2011)

2. Genus *Thymus* plant cultivation

Cultivation of genus *Thymus* plants is considered as an interesting possibility for providing additional agricultural revenue for farmers, due to their wide use in the food, cosmetic, and pharmaceutical industries. Genus *Thymus* plants are small aromatic perennial herbaceous plants that grow in well-drained calcareous soil and need full sun to develop to their full potential. Seeds, cuttings, and root division are the most common methods for their propagation (Nicola, Fontana, & Hoeberechts, 2002). Among the genus *Thymus* plants, the most common is *T. vulgaris* L., whose seeds grow within 1–2 weeks at 10–30 °C. Seeds germination is usually accelerated by sunlight. In temperate or warm climates, the best time period for thyme seed propagation is March, at the end of winter. Thyme is also easily propagated from cuttings during the spring (Iapichino, Arnone, Bertolini, & Amico Roxas, 2006). Moreover, when thyme starts to become woody and produces fewer leaves, after three or four years, the plant is separated using root division and replanted. Yields of *T. vulgaris* for fresh herb production are about 5–6 t/ha, depending on the environmental conditions and soil fertilisation. Thyme should not be fertilised heavily, because over-fertilised plants tend to show weak growth. A basal fertiliser application containing nitrogen (which is given after each harvest to promote new shoot growth during the growing season), phosphorus, potassium and sulphur should be applied annually, as it influences the final concentrations of the plant's main components and therefore the quality of the essential oils, which are generally obtained by steam distillation. Some studies show that there is a close correlation between increasing fertiliser and thyme production. (Baranauskiene, Venskutonis, Viškelis, & Dambrauskiene, 2003).

Besides fertilisation, other agronomical factors, such as soil type, salinity, planting density, time of harvesting, and irrigation have a great effect on the phytomass production, and the quality and quantity of essential oils. For example, in *Thymus maroccanus* Ball, high salinity causes a significant reduction in germination and in the fresh and dry masses of both shoots and roots. Comparison between the compositions of the essential oils produced in normal conditions or under salt stress, showed that chemical compositions were similar, suggesting that *T. maroccanus* is a moderately salt tolerant species (Belaqziz, Romane, & Abbad, 2009). Ezz El-Din, Aziz, Hendawy, and Omer (2009) reached similar conclusions through a study on *T. vulgaris*. Sotomayor, Martínezm, García, and Jordán (2004) evaluated the influence of irrigation on phytomass production and essential oil composition of *Thymus zygis* subsp. *gracilis* (Boiss.) R. Morales. They tested three different watering levels corresponding to 63%, 44%, and 30% of the local potential evapotranspiration and found that irrigation at intermediate level was optimal for maximum plant dry matter production and essential oil yield. Moreover, the essential oils produced at the lower and intermediate levels of irrigation were richer in thymol. The influence of plant space and time of harvesting on *T. vulgaris* plant growth and essential oil quality was evaluated by Hassanali, Darab, Ali, and Nazari (2004). Planting space showed a significant effect on plant diameter, and had a very significant effect on yields of dry and fresh herbage, and content of thymol and carvacrol, while the oil content was not affected. Time of harvest had a significant effect on all the above reported parameters, especially those related to plant growth.

3. Chemical composition of essential oils obtained from genus *Thymus* plants

The main chemical classes of the compounds occurring in the essential oils, obtained from the plants belonging to the genus

Thymus, are terpenes, terpene alcohols, phenolic derivatives, aldehydes, ketones, ethers, and esters (Hudaib, Speroni, Di Pietra, & Cavrini, 2002; Lee, Umano, Shibamoto, & Lee, 2005). The essential oil chemical composition varies depending on the species and chemotype considered. As regards the genus *Thymus* plants, there are different chemotypes, depending on the dominant component of the essential oil such as thymol, carvacrol, linalool, geraniol, thujanol, α-terpineol, borneol, and *p*-cymene (see Fig. 1) (Schmidt et al., 2012; Thompson, Chalchat, Michet, LInhart, & Ehelers, 2003). Asbaghian et al. (2011) compared the composition of the essential oils obtained from *Thymus caucasicum*, *Thymus kotschyanus*, and *T. vulgaris*, and found that *T. caucasicum*'s main compound is 1,8-cineole (21.5%), followed by thymol (12.6%), β-phenyl alcohol, and nerol (see Fig. 2). Carvacrol is not present. Differently, the major compounds of *T. kotschyanus* are carvacrol (24.4%), β-caryophyllene (14.5%), γ-terpinene (12.4%), α-phellandrene (10.8%) and *p*-cymene (9.8%) (see Fig. 3). Finally, in *T. vulgaris* the most represented compounds were found to be thymol (43.8%) and *p*-cymene (15.2%) (Asbaghian et al., 2011).

Thymus species with a high content of carvacrol, include *Thymus capitatus*, *Thymus caramanicus*, *Thymus fallax*, and *T. maroccanus.* The first one is a species growing wild in the Mediterranean area that produces essential oil with a higher concentration of carvacrol (>80%). *T. caramanicus* and *T. fallax* are endemic to Iran. The carvacrol content of the essential oils obtained from these species ranges from 50% to 70% (Eftekhar, Nariman, Yousefzadi, Hadiand, & Ebrahimi, 2009).

T. maroccanus is an endemic plant in Morocco. In a recent article, Belaqziz et al. (2013) compared the chemical composition of the essential oils obtained from different parts of this species. The content of carvacrol is 84.9, 60.8, and 33.0% in inflorescence, stem, and leaf essential oil, respectively.

The chemical profile of the essential oil of *Thymus algeriensis* is rather different in comparison with the other reported above, because it is rich in borneol (23.4%), linalool (8.9%), carvacrol (7.8%) and β-caryophyllene (6.4%) (Ait-Ouazzou et al., 2011).

Thymol, carvacrol, γ-terpinene and *p*-cymene are the major compounds of genus *Thymus* plants which are responsible for its

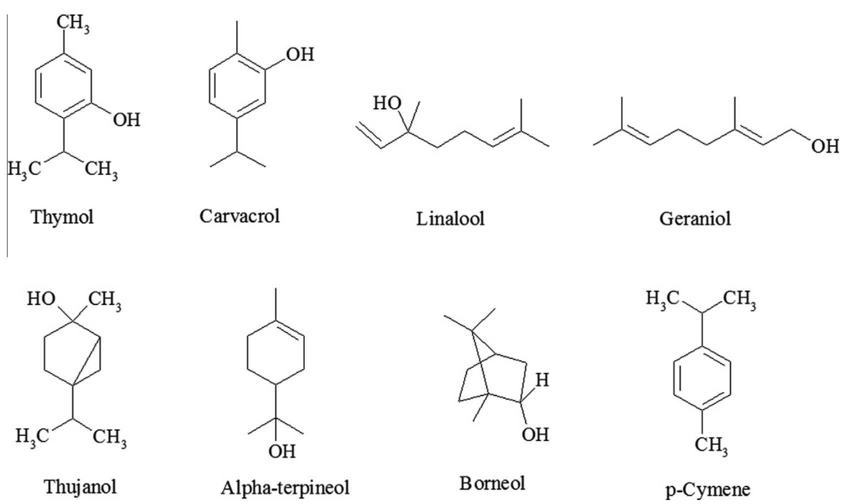

Fig. 1. Chemical structure of the main substances occurring in genus *Thymus* plants: thymol, carvacrol, linalool, geraniol, thujanol, α-terpineol, borneol, and *p*-cymene.

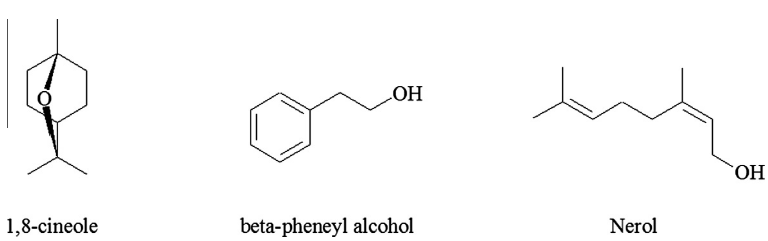

Fig. 2. *Thymus caucasicum* main compounds: 1,8-cineole, β-phenyl alcohol, and nerol.

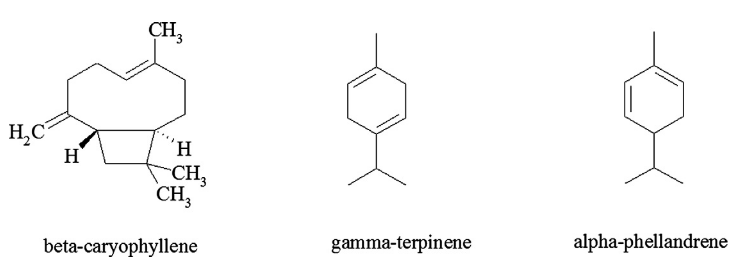

Fig. 3. *Thymus kotschyanus* main compounds: β-caryophyllene, γ-terpinene, and α-phellandrene.

antibacterial effects (Bagamboula, Uyttendaele, & Debevere, 2004; Sato, Krist, & Buchbauer, 2007).

3.1. Thymol

Thymol (2-isopropyl-5-methylphenol, Fig. 1) is a common antiseptic substance which is commonly found as a white crystalline compound in essential oil and extracts of thyme, and is responsible for its pleasant aromatic odour (Wattanasatcha, Rengpipat, & Wanichwecharungruang, 2012). Thymol is a natural monoterpene phenol derivative of cymene, and an isomer of carvacrol (Darre, Kollanoor-Johny, Venkitanarayanan, & Upadhyaya, 2014). It is commonly used in dental preparations to reduce malodor-producing bacteria (Botelho et al., 2007). It has also been reported that thymol reduces bacterial resistance to some antibiotics such as penicillin (Gallucci, Casero, Oliva, Zygadlo, & Demo, 2006). In addition, thymol possesses different biological and pharmacological properties such as antimutagenic, antitumor, antioxidant, anti-inflammatory, etc. (Bhalla, Gupta, & Jaitak, 2013; Deb, Parimala, Saravana Devi, & Chakraborty, 2011).

3.2. Carvacrol

Carvacrol (5-isopropyl-2-methylphenol, Fig. 1) is a monoterpenoid phenol, which is responsible for oregano's (*Origanum vulgare*) warm odour (De Martino, De Feo, Formisano, Mignola, & Senatore, 2009). Carvacrol is mainly found in some plant species such as thyme, oregano, wild bergamot, as well as pepperwort (Singh & Chittenden, 2010).

Carvacrol shows a wide range of biological activities such as antibacterial, antifungal, antioxidant, anticancer, etc. (Chami, Chami, Bennis, Trouillas, & Remmal, 2004; Koparal & Zeytinoglu, 2003; Xu, Zhou, Ji, Pei, & Xu, 2008; Yanishlieva, Marinova, Gordon, & Raneva, 1999). Due to its pleasant odour and antimicrobial activity, carvacrol is used as a food additive for the prevention of bacterial growth and contamination (Oussalah, Caillet, Saucier, & Lacroix, 2007). Numerous reports have shown that carvacrol has a potent protective role against bacteria, fungi, yeasts, mites and also insects (Altintas et al., 2013; Mancini et al., 2014; Nostro et al., 2009) Carvacrol is hydrophobic in nature and, due to the free hydroxyl group in the chemical skeleton as well as delocalized electron system, affects the cytoplasmic membrane of bacteria and in this way exerts its antibacterial effects (De Sousa et al., 2012). It has also been reported that carvacrol possess antibacterial effects through inhibition of ATPase activity and induction of Hsp60 (Burt et al., 2007).

3.3. p-Cymene

p-Cymene (Fig. 1) is the biological precursor of carvacrol. It is a natural alkyl-substituted aromatic hydrocarbon which is found in different plant species such as thyme and cumin (Eaton, 1997; Juven, Kanner, Schved, & Weisslowicz, 1994). Structurally, *p*-Cymene contains a benzene ring which is substituted with a methyl group as well as an isopropyl group, and is known as the most important hydrophobic antibacterial agent of thyme (Eaton, 1997). It has been reported that *o*-cymene (which contains ortho-substituted alkyl groups) and *m*-cymene (which contains meta-substituted alkyl groups) are two geometric isomers of *p*-cymene (Romanenko & Tkachev, 2006).

3.4. γ-Terpinene

Terpinenes are isomeric hydrocarbons which have differences in the location of their carbon-carbon double bonds in their chemical skeletons (Clarke, 2008). γ-Terpinene (see Fig. 3) has a similar chemical structure to α-phellandrene (Marzec, Reva, Fausto, & Proniewicz, 2011). γ-Terpinene is a well-known natural product, used in perfume and flavouring, and is widely found in different medicinal and aromatic plants (Foti & Ingold, 2003). In fact, γ-terpinene is one of the major chemical components of thyme essential oils which possess potent biological activities, especially antioxidant and antibacterial properties (Hazzit, Baaliouamer, Veríssimo, Faleiro, & Miguel, 2009). It has been also reported in tangerine flavours (Minh et al., 2002). γ-Terpinene is rapidly oxidised into the alkyl benzene *p*-cymene (Salah & Matoussi, 2005).

4. The genus *Thymus* in traditional medicine

Essential oils and extracts from *Thymus* plants exhibit different biological properties such as antioxidant, antibacterial, antifungal, antiviral activities, cytotoxicity, antiparasitic and so on (AitMBarek et al., 2007; Jukić & Miloš, 2005; Rasooli, Rezaei, & Allameh, 2006; Santoro et al., 2007; ŠegvićKlarić, Kosalec, Mastelić, Pieckova, & Pepeljnak, 2007). In traditional medicine, herbal medicines belonging to the genus *Thymus* are employed for internal use in the oral treatment of coughs, upper respiratory infections, acute and chronic bronchitis, whooping cough, and catarrh (Basch, Ulbricht, Hammerness, Bevins, & Sollars, 2004). Their use in respiratory system disorders is due to their antitussive, expectorant, antioxidant, anti-inflammatory, and antimicrobial properties. The expectorant properties, in particular, are mainly attributed to the presence of saponins (Keyhanmanesh & Boskabady, 2012). Moreover, *T. vulgaris* is used to treat dyspepsia and other gastrointestinal disorders, because of its carminative effect and spasmolytic activity, which seems to be due to thymol and flavones (Keyhanmanesh & Boskabady, 2012). It is also used as an anthelminthic in cases of intestinal parasitic infections and in urinary tract infections (Van Den Broucke & Lemli, 1983), and also to quit smoking due to its anti-smoking properties (Carlini, Rodrigues, Mendes, Tabach, & Gianfratti, 2006).

Thyme is also employed for external use as a mouthwash in gargles to treat laryngitis, as anti-acne and anti-stomatitis agents, and in the topical treatment of minor injuries. Recent data suggest that thyme essential oil, and its main component carvacrol, show anti-inflammatory effects that have been attributed to the inhibition of inflammatory edema and leukocyte migration (Fachini-Queiroz et al., 2012).

5. Essential oil obtained from genus *Thymus* plants as a food preservative

To stop or reduce the rate of spoilage that can cause a reduction in food quality, edibility and nutritional value, important food preservation methods include preventing the growth of bacteria, yeasts, fungi, and other micro-organisms, retarding the oxidation of fats which can cause rancidity, and inhibiting natural discolouration, which can occur during food preparation. The essential oils from aromatic plants, especially from Thymus plants, can be exploited as food antimicrobial additives, due to their bactericidal or bacteriostatic activity against foodborne pathogens (e.g. *Listeria monocytogenes*, *Salmonella typhimurium*, *Escherichia coli*, *Shigella dysenteria*, *Bacillus cereus*, and *Staphylococcus aureus*). *Thymus* essential oils are generally used to preserve meat and meat products (which are highly prone to microbial contamination) because their aroma does not adversely affect the organoleptic properties of these types of foods (Burt, 2004; Chorianopoulos et al., 2004; Nunes Barbosa et al., 2009).

6. Antibacterial activity of genus *Thymus* plants

One of the most well-established properties of the essential oils and extracts obtained from genus *Thymus* plants is represented by

Table 2
Antibacterial activity of *Thymus* genus plants.

Thymus specie	Antibacterial activity	Reference
Thymus vulgaris	Antibacterial activity is registered against 14 clinical isolates of methicillin-resistant *S. aureus* (MRSA) and other standard bacterial strains (*B. cereus* ATCC 9634, *E. coli* ATCC 3428, *K. pneumoniae* ATCC 13883, *S. aureus* ATCC 25922, and *S. aureus* ATCC 33592)	Tohidpour et al. (2010)
	Strong antibacterial activity is registered against 120 strains of *Staphylococcus*, *Enterococcus*, *Escherichia*, and *Pseudomonas* genera	Sienkiewicz et al. (2011)
	Linalool seems to be active only against Gram positive bacteria	Abu-Darwis et al. (2012)
Thymus vulgaris and *Thymus serpyllum*	The essential oils show greater activity than chloroformic extracts	Abu-Darwis et al. (2012)
	Antibacterial activity of 19 isolated compounds from the tested essential oils, is registered against clinical and standard strains of *S. aureus*, *E. coli*, and *P. aeruginosa*	
	Clinical and standard strains of *S. aureus* show high sensitivity to thymol, carvon, β-caryophyllene, β-pinene, camphor, camphene, limonene, p-cymene, 1,4-cineol, α-pinene, menthone, myrcene, α-terpinene, γ-terpinene, linalool, and carvacrol	Abu-Darwis et al. (2012)
	Clinical and standard strains of *E. coli* show lower sensitivity to camphene, β-pinene, thymol, α-terpinene, 1,4-cineol, γ-terpinene and carvacrol, but are susceptible to limonene. Clinical and standard strains of *P. aeruginosa* are resistant to most of the tested components, with the exception of β-pinene, thymol, γ-terpinene, and α-terpinene	Abu-Darwis et al. (2012)
	Thymol and carvacrol show antibacterial activity against some strains of verocytotoxigenic *E. coli*	Rivas et al. (2010)
Thymus leucotrichius and the essential oil prepared starting from *Thymus syriacus*	Antibacterial activity of the methanol and acetone extracts is registered against some strains of verocytotoxigenic *E. coli*	Al-Mariri and Safi (2014), Ulukanli et al. (2011)
Thymus vulgaris	Antibacterial activity is registered against *S. pyogenes*	Sfeir et al. (2013)
	Antibacterial activity is registered against *Helicobacter pylori*	Esmaeili et al. (2012)
Thymus capitatus	Antibacterial activity is registered against skin microorganism, such as *S. epidermidis* and *P. acnes*	Usai et al. (2010)
	Ethanol leaf extracts inhibits *S. aureus*	Usai et al. (2010)
	The essential oil shows higher activity than the extracts against *E. coli*, *E. aerogenes*, *S. aureus*, and *P. aeruginosa*	Usai et al. (2010)
Thymus caramanicus	Essential oil inhibits the growth of *H. pylori*	Eftekhar et al. (2009)
Thymus maroccanus	The antibacterial effect is significantly greater than the antimicrobial activity of tetracycline, used as positive control against *B. subtilis*, *S. aureus*, and Salmonella species	Belaqziz et al. (2013)
	Antimicrobial activity is exerted via the damages of the cell wall (permeabilization of membranes, loss of ions, leakage of macromolecules, and lysis)	Bakkali, Averbeck, Averbeck, and Idaomar (2008)
	Essential oil can induce a membrane disruption facilitating the uptake of antibacterial agents	Fadli et al. (2011)
Thymus numidicus	Essential oil was active against many *P. aeruginosa* strains	Dorman and Deans (2000)

its antibacterial activity (Table 2). In fact, the first article showing evidence for this antimicrobial property was published in the 60's (Li, Prescott, Chi, & Martino, 1963). Since then, and especially over the past 20 years, a large number of *Thymus* essential oils, extracts, and their isolated compounds have been studied for the antimicrobial activity. These products are of particular interest, because no bacterial resistance or adaptation has been described, and low or insignificant side effects have been found both for the essential oils and whole extracts.

As far as *T. vulgaris* is concerned, Tohidpour, Sattari, Omidbaigi, Yadegar, and Nazemi (2010) determined the antibacterial activity of a thyme essential oil against 14 clinical isolates of methicillin-resistant *S. aureus* (MRSA) and other standard bacterial strains (*B. cereus* ATCC 9634, *E. coli* ATCC 3428, *Klebsiella pneumoniae* ATCC 13883, *S. aureus* ATCC 25922, and *S. aureus* ATCC 33592). To determine the antibacterial activity the authors used the disk diffusion and agar dilution methods. The results showed that the most sensitive microorganism was *E. coli*, with a minimum inhibitory concentration (MIC) value of 9.25 μg/ml), while *K. pneumoniae* resulted to be relatively insensitive with a higher MIC (55.50 μg/ml). As regards MRSA clinical strains, the MIC values ranged from 18.5 to 37.0 μg/ml. Interestingly, in some cases the inhibition zones registered for the analysed essential oil are similar to those obtained from standard antibiotic disk diffusion studies (tetracycline, ciprofloxacin, gentamicin, norfloxacin, trimethoprim–sulfamethoxazole, ticarcillin, carbenicillin, vancomycin, methicillin, and chloramphenicol). The authors also studied the chemical composition of this essential oil. Due to the fact that thymol was the most abundant substance (48.1%), followed by *p*-cymene (15.6%) and γ-terpinene (15.4%), they concluded that the registered antibacterial activities could be associated with the presence of thymol (Tohidpour et al., 2010). Later, Sienkiewicz, Lysakowska, Ciecwierz, Denys, and Kowalczyk (2011) showed that *T. vulgaris* essential oil exhibited extremely strong activity against 120 strains of *Staphylococcus*, *Enterococcus*, *Escherichia*, and *Pseudomonas* genera, isolated from patients with infections of the oral cavity, respiratory, genitourinary tracts, and from the hospital environment and clinical staff. The antibacterial activity was tested using the agar diffusion method. *Staphylococcus* strains were found to be the most sensitive, while *Pseudomonas* bacteria were found the most resistant.

Schmidt et al. (2012) studied the antibacterial activity of thyme essential oils belonging to 4 different chemotypes: geraniol (whose geranyl acetate content corresponds to 21.8%), 4-thujanol/terpinel-4-ol (whose main compound is cis-sabinene-hydrate (32.7%)), thymol (thymol and *p*-cymene concentrations of 38.8% and 24.0%, respectively) and linalool (68.5%) (Sienkiewicz, Łysakowska, Denys, & Kowalczyk, 2012). The most effective essential oil was that obtained from the thymol chemotype, followed by the linalool chemotype. These results are partially in agreement with other research showing that thymol possesses high activity against both gram positive and gram negative bacteria. On the contrary, linalool seems only to be active against gram positive bacteria (Abu-Darwis, Al-Ramamneh, Kyslychenko, & Karpiuk, 2012). So, the finding of linalool activity against *Pseudomonas aeruginosa*, registered by Schmidt et al. (2012), seems to be contradictory with the findings that linalool alone is not active against *P. aeruginosa.* The authors concluded that a positive synergism among the substances occurring in the linalool chemotype essential oil could explain the excellent antibacterial activity of the essential oil obtained from this chemotype.

Abu-Darwis et al. (2012) tested the antibacterial activity of the essential oils and chloroformic extracts produced with dried powdered leaves from *T. vulgaris* and *Thymus serpyllum*, which are the only *Thymus* species allowed in food supplements as reported in the BELFRIT list (http://www.trovanorme.salute.gov.it/norme/renderNormsanPdf?anno=0&codLeg=48636&parte=3&serie=), derived from the joint research effort and adopted in Europe by France, Belgium and Italy. Overall, the essential oils showed a greater activity than chloroformic extracts. Moreover, in the same study they reported also the antibacterial activity (registered as inhibition zone diameter) of 19 isolated compounds from the tested essential oils, against clinical and standard strains of *S. aureus*, *E. coli*, and *P. aeruginosa*. All the tested strains were resistant to linallyl acetate, bornyl acetate and 1,8-cineol. On the contrary, clinical and standard strains of *S. aureus* showed high sensitivity to thymol, carvon, β-caryophyllene, β-pinene, camphor, camphene, limonene, *p*-cymene, 1,4-cineol, α-pinene, menthone, myrcene, α-terpinene, γ-terpinene, linalool, and carvacrol, in that order. Clinical and standard strains of *E. coli* showed lower sensitivity to camphene, β-pinene, thymol, α-terpinene, 1,4-cineol, γ-terpinene and carvacrol, but were susceptible to limonene. Finally, clinical and standard strains of *P. aeruginosa* were found to be resistant to most of the tested components, with the exception of β-pinene, thymol, γ-terpinene, and α-terpinen (Abu-Darwis et al., 2012).

Another investigation (Rivas et al., 2010) on the antibacterial activity of thymol and carvacrol (isolated from thyme essential oils) was carried out against some strains of verocytotoxigenic *E. coli* strains (i.e. O157:H7, O26, and O111), which are serotypes associated with public health risks worldwide. Both thymol and carvacrol showed antibacterial activity against these strains. These results were subsequently confirmed by more recent investigations, which showed the antibacterial activity against these strains with methanol and acetone extracts obtained from *Thymus leucotrichius* and the essential oil prepared from *Thymus syriacus* (Al-Mariri & Safi, 2014; Ulukanli et al., 2011). As reported above, thyme essential oils are used in respiratory system disorders. Sfeir, Lefrançois, Baudoux, Derbré, and Licznar (2013) showed the antibacterial activity of *T. vulgaris* thymol essential oil against *Streptococcus pyogenes*, which is known to play an important role in the pathogenesis of tonsillitis. The clinical strains used in this study were isolated from the pharynx of a child, following episodes of pharyngitis. The antibacterial activity was tested with the disc diffusion and broth dilution methods. Thymol and carvacrol showed antibacterial activity lower, but comparable, with that of amoxicillin, which was used as positive control.

Another potential application of thyme essential oils could derive from the results of more research showing its antibacterial activity (determined with the agar dilution method) against *Helicobacter pylori*, a gram-negative bacterium, that infects more than 50% of the worlds population (Esmaeili, Mobarez, & Tohidpour, 2012). Thyme essential oil was found to be active at concentrations of 10.8 μg/ml. Esmaeili et al. (2012) advanced a hypothesis regarding the potential mechanism of action, and reported that the antimicrobial activity is mainly attributed to the presence of some lipophilic active constituents, whose hydrophobicity enables them for rupturing cell membranes and intra-structures.

Many studies published in the last five years involve plants belonging to the genus *Thymus*, different from the most common *T. vulgaris.* For example, *T. capitatus*, which grows wild in the Mediterranean area and is rich in carvacrol, was studied for its antibacterial activity against skin microorganisms, such as *Streptococcus epidermidis* and *Propionibacterium acnes*. These results showed that the essential oil is active against the tested bacteria, even with MIC values lower than those registered for chlorhexidine, used as positive control (MIC values ranging from 0.125 to 0.5 mg/ml and 0.001 to 0.016 mg/ml for the essential oil and chlorhexidine, respectively). Also in this case the authors tested the activity of the single compounds and found that the most active substance is carvacrol (MIC values ranging from 0.062 to 0.500 mg/ml), while linalool, terpinen-4-ol, and β-myrcene resulted to be inactive. Overall, the authors suggested that the results of this investigation seem to justify the use of *T. capitatus* essential oil in cosmetics and perfumery (Usai, Foddai, Sechi, Juliano, & Marchetti, 2010).

Other research carried out on *T. capitatus*, considered both the essential oil and extracts obtained using different solvents (water, ethanol, dichloromethane and hexane). Although the essential oil showed higher activity than the extracts against the tested bacteria (*E. coli*, *Enterobacter aerogenes*, *S. aureus*, and *P. aeruginosa*), it is very interesting to note that that the ethanol leaf extracts effectively inhibited *S. aureus*. The sensitivity of bacteria to the ethanol extract of *T. capitatus* agrees with earlier results obtained by Nimri, Meqdam, and Alkofahi (1999).

Using other genus Thymus plants rich in carvacrol, Asbaghian et al. (2011) compared the antibacterial activity against gram-positive and gram-negative bacteria (determined using the broth dilution method) of three essential oils obtained from *T. kotschyanus* (carvacrol content 24.4%), *T vulgaris* (carvacrol content 3.2%), *T. caucasicum* (carvacrol not present). Despite the high content of carvacrol in the first essential oil, *T. kotschyanus* essential oil resulted to be less active than those prepared from *T. vulgaris* and *T. caucasicum*, rich in thymol and 1,8-cineolo, respectively (Asbaghian et al., 2011). Eftekhar et al. (2009) showed that *T. caramanicus* essential oil inhibited the growth of *H. pylori*. The MIC values registered for this essential oil, and carvacrol and thymol, ranged from 14 to 58 μg/ml, 20–80 μg/ml, and 100–200 μg/ml, respectively. These results showed that the anti-*H. pylori* activity of the whole essential oil was higher than that registered for each of the purified compounds, suggesting a positive synergism between the phenolic substances occurring in the tested essential oil.

T. maroccanus essential oil, commonly used in Moroccan traditional medicine, contains high levels of carvacrol. Belaqziz et al. (2013) demonstrated that its antibacterial effect (determined by the disc diffusion method) was significantly greater than the antimicrobial activity of tetracycline, used as positive control against the *Bacillus subtilis*, *S. aureus*, and *Salmonella* species. The effect of *T. maroccanus* essential oil was also studied by Fadli et al. (2011), who tried to demonstrate the mechanism of action through which the essential oil exerts its activity. Essential oils are known to confer antimicrobial activity by damaging the cell wall causing the permeabilization of membranes, loss of ions, leakage of macromolecules, and lysis (Bakkali, Averbeck, Averbeck, & Idaomar, 2008). The authors evaluated the behaviour of gram-negative bacteria (i.e. different strains of *E. aerogenes*, *E. coli*, *Salmonella enterica*), exposed to *T. maroccanus* essential oil and polymixim, which is known to disrupt the structure of bacterial cell membrane, causing damage. An influence on cell membrane permeability does not mean that a compound is an antibacterial agent. Anyway, the increase of permeability of cell membranes makes the bacterium more susceptible to antimicrobial agents, due to the fact that the higher permeability exposes the bacterium to the cytotoxic action of other compounds which can more easily penetrate the bacterial cell. Fadli et al. (2011) showed that, in the presence of increasing concentrations of *T. maroccanus* essential oil, the tested gram-negative bacteria released intracellular proteins (cytoplasmatic β-galactosidase and periplasmic β-lactamase) as well as polymyxin, in a dose-dependent manner. This result means that the essential oil can induce a membrane disruption effect facilitating the uptake of antibacterial agents.

A recent paper, published in 2014, reported that the combination of the essential oil from *Thymus glabrescens* Willd. and chloramphenicol induces a synergistic effect that leads to a significant reduction in the MIC value of the synthetic drug, thus minimising its side effects (Ilic, Kocic, Ciric, Cvetkovic, & Miladinovic, 2014). In more detail, the authors reported that the main compounds occurring in this essential oil are oxygenated monoterpenes, i.e. geraniol (22.33%), geranyl acetate (19.38%) and linalool (5.49%). Thymol concentration was found to be 13.79%. The activity of the essential oil and its isolated compounds (geraniol and thymol) was tested against 13 gram-positive and gram-negative bacteria with the broth microdilution and microdilution checkerboard methods. The results confirmed that gram-positive bacteria are more sensitive to the tested samples than gram-negative ones. Essential oil-chloramphenicol, geraniol-chloramphenicol, and thymol-chloramphenicol combinations exhibited a synergistic effect with a decrease in chloramphenicol MIC, of about 5–10 fold towards all the tested bacteria, with the exception of *S. aureus*. These results are in good agreement with those previously obtained by Rosato, Vitali, De Laurentis, Armenise, and Antonietta Milillo (2007) who tested the essential oil of *Pelargonium graveolens* and its main components (geraniol and citronellol) in combination with norfloxacin, and showed a strong synergism between the natural and synthetic compounds against *B. cereus* and *S. aureus*.

Thymus numidicus Poiret, is a plant typically endemic to North-eastern Algeria and Tunisia. Mina, Salima, Abdelghani, and Saoudi (2014) studied the antibacterial activity of the essential oil obtained from the plants harvested in the region of Annaba (Algeria) against 9 *P. aeruginosa* strains resistant to imipenem, which is a beta-lactam antibiotic, that shows a wide bacterial spectrum and high stability against beta-lactamase (Wang & Mi, 2006). The results showed that the essential oil (consisting of thymol, 77.5%, *p*-cymene, 10.1%, and γ-terpinene, 6.4%) was active against all the *P. aeruginosa* strains, despite the common resistance of gram-negative to essential oil antibacterial activity (Dorman & Deans, 2000).

7. Conclusions and recommendations

It can be concluded that the essential oils and certain extracts obtained from genus *Thymus* plants, might have a beneficial role against different types of bacterial infections. We concluded the antibacterial effect is due to their phytochemical constituents, such as thymol, carvacrol, *p*-cymene and terpinene. Despite the high antibacterial effects using *in vitro* studies, there is lack of clinical trials on the antibacterial role of genus *Thymus* plants and their active constituents against bacterial infections in humans.

Toxicity studies are required because, when overdosed, essentials oils may be toxic and can show adverse effects on human cells. In fact, a number of reports showed cytotoxicity in eukaryotic cells (Ahmed et al., 2011; Nathalie et al., 2006). So, the application of essential oils in the treatment of human infections, which is suggested by traditional medicine, may be an interesting alternative to synthetic drugs that show many side effects. Regarding high antibacterial activity, the essential oils and extracts obtained from genus *Thymus* plants can be recommended as a therapeutic multi-target strategy for the treatment of infectious diseases. In addition, because of the lack of completed clinical trials and data on the most effective dose or food–drug interaction data, we cannot make a clear conclusion about efficacy for the treatment of infections. However, genus *Thymus* plant essential oils could be used as an alternative to antibacterial drugs in the treatment of infectious diseases especially antibiotic-resistant bacterial infections.

In this review, information about thyme cultivation, its uses in traditional medicine as well as its antibacterial phytochemicals are reported to provide a better view about genus *Thymus* plants and its derived antibacterial agents.

Based on the foregoing, we recommend that future works should be focused on:

- Safety studies, carried out prior to use of *Thymus* essential oils and extracts in pharmaceutical applications.
- More studies to find the exact mechanism of the antibacterial action of *Thymus* essential oils and extracts.
- More investigations on the isolation and identification of the most active antibacterial constituents and their interactions with foods and drugs.
- Clinical trials both on experimental animals and humans, carried out to evaluate the efficacy of thymus plant essential oils and extracts in different infectious diseases and to determine the safe and efficient dosage.

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