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# Review on ethnobotany, phytochemical, molecular and pharmacological activity of *Thymus daenensis* Celak.

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ABSTRACT

*Thymus daenensis* Celak. is identified as an endemic popular medicinal and flavoring herb, contains a wide range of medicinally bioactive and aroma profile, including phenolic compounds, flavonoids and high amounts of terpenoids. Essential oil of this herb is very rich in monoterpenes (such as thymol and carvacrol) and sesquiterpenes (like $\beta$-caryophyllene and $\beta$ -caryophyllene oxide). In addition, phenolic constituents containing high amounts of phenolic acids (syringic, gallic, vanillic, caffeic, chlorogenic, rosmarinic, and cinnamic acids), and flavonoids (quercitrin, apigenin, luteolin, naringenin, and rutin) were reported to possess the highest antioxidant activity.

Following studied in ethnopharmacological of this herb indicated antimicrobial, antioxidant, insecticidal and cytotoxic effects. Moreover, numerous studies have been published on the composition of the herb's essential oil under abiotic stress. Due to its high concentration of thymol, the plant's essential oil possesses high antimicrobial and antioxidant activities on human pathogenic strains. They are also very interesting view point as potential sources of novel antimicrobial compounds to serve as alternatives to treat infectious diseases. However, there are no comprehensive studies on thorough review of this species from Iran. Thus,the present review was conducted to discuss aroma profile, phenolic components and biological activity studies performed so far.

## 1. Introduction

The genus *Thymus* L. belonging to the botanical family of Lamiaceae, and consists of about 250 species of small shrubs and herbaceous perennials in the world. The center of the genus has been identified in Mediterranean region (Cronquist, 1988). *Thymus vulgaris* L. one of the most important herb has considered as an economically and commercial herb, native to Southern Europe, and with a worldwide distribution (Hosseinzadeh et al., 2015). It is widely consumed for antitussive, antibroncholitic, antispasmodic, anthelmintic, carminative, and diuretic properties (Miraj and Kiani, 2016; Nabissi et al., 2018). *Thymus*, with the common Persian name of "Avishan or Azorbe," consists of 14 species which are found wild in many regions of Iran, some of which are endemic; *T. carmanicus* Jalas, *Thymus daenensis* Celak subsp. *daenensis* Celak, *T. daenensis* Celak subsp. *lancifolius* (Celak.) Jalas, *Thymus persicus* (Roniger ex Reach. F.) and *Thymus* trautvetteri Klokov and Desj-Shost (Rechinger, 1963–1998; Mozaffarian, 2008).

*T. daenensis* as an aromatic and medicinal plants, commonly known as denaian thyme is one of the most famous and economically important flavoring and culinary herbs in Iran (Mozaffarian, 2008; Jamzad, 2009), This specie distributed in a wide range of mountainous and rangelands. The high altitudes in Zagros and some parts of the Alborz mountain ranges are the locations where this plant is most abundant (Bahreininejad et al., 2013; Ghaemi Pirbalouti et al., 2015). This genus is known as robust and dense shrubs presenting a robust root system of extreme importance because of their effect on soil stabilization and prevention of water erosion in mountainous and sharp slope regions (Khoshsokhan et al., 2014).

The species is frequently used as a carminative, expectorant, antiviral, antibacterial and antifungal agent among other medical applications (Ghasemi Pirbalouti et al., 2014 Ghasemi Pirbalouti et al., 2009). In spite of the fact that there is various valuable chemotype, such as flavonoids, phenolic, and anthocyanins have been isolated from thyme, undoubtedly the essential oil and aroma profile are one of the most famous natural product which play key role in the main biological and antioxidant activities (Rustaiee et al., 2011; Emami Bistgani et al., 2017a; Emami Bistgani et al., 2017b; Emami Bistgani et al., 2018; Emami Bistgani et al., 2019; Ghasemi Pirbalouti et al., 2014; Ghasemi

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Pirbalouti et al., 2013; Sefidkon et al., 2005).

Various chemical polymorphisms can be regarded as important characteristics of *T. daenensis*. The chemical composition of essential oils is affected by several factors including climatic, environmental stress, seasonal, geographic conditions, phenological cycle, harvest period, storage conditions, drying methods, elicitation, and distillation technique (Tohidi et al., 2019; Zarshenas and Krenn, 2015; Dehghani Mashkani et al., 2018; Rowshan et al., 2013; Akbarinia et al., 2008; Rustaiee et al., 2010). The current review is based on the information and data in literature about *T. daenensis* from Iran, to verify the essential oil components, phenolic constituents and biological activities, by focusing on their antimicrobial and antioxidant properties.

## 2. Botany and anatomy

*T. daenensis* is small shrubs and herbaceous perennials consists of the narrow leaves and purple flowers. The height plant is 15–30 cm and straight, with or without axial leaflets. The leaves 10–20 x 2–5 mm linear and narrow, without petiole leaves and 2–3 prominent marginal veins; the leaves have also numerous red glands on both surfaces. The inflorescences is terminal, sometimes elongate, calyx is tubular or lanceolate and 3–4.5 mm, long; corolla is red colour with 5–6 mm long. The time of full flowering is in summer (Jamzad, 2009). The characteristics of leaf anatomical are composed of epidermis, trichomes, cortex, and the presence of collenchyma, spongy and ladder parenchyma layers in the leaf blade. Stem is commonly composed of parenchyma, collenchyma, phelloderm, endoderm, xylem and phloem tissue. The leaves, stem, and calyx bear numerous glandular and non-glandular trichomes. Glandular hairs are generally peltate and capitates hairs. The epidermis is consists of also a thin cuticle covering externally. Diacytic stomata distributed predominantly on the abaxial side and its worth mentioning that the stomata frequency on the abaxial leaf surface is more than adaxial leaf surface in *T. daenensis* plants (Sharifi Ashour Abadi et al., 2019).

## 3. Ethnobotany and traditional usage of *T. daenensis*

Medicinal plants have been applied in traditional drug systems for many years (Nickavar et al., 2005, Sefidkon et al., 2004) . The therapeutic properties of these plants are remarkable (Ghasemi Pirbalouti, 2009; Dadashpour et al., 2011a,b). Due to the adverse effects related to drugs the search for new drugs from natural origin has obtained impetus in recent years. In this regard, different species of the genus thyme have always been in the focus, specifically in the Mediterranean region and Asian countries including Iran and Turkey. The aerial parts and aroma profile of this species are commonly used as a medicinal herb. *T. daenensis* is commonly used as flavoring agents and herbal tea, (condiments and spices) and medicinal purposes (Stahl- Biskup and Saez, 2002). In traditional medicine, infusion and decoction of leaves and flowers of denaian thyme could be used in production of tonic, carminative, digestive, antispasmodic, anti-inflammatory, expectorant and for the treatment of colds (Nickavar et al., 2005; Ghasemi Pirbalouti, 2009).

## 4. Phytochemical composition

### 4.1. Essential oil

Essential oil and aroma profile in this genus and also herb determine the specific taste and nice smell of the plants (Tohidi et al., 2019; Sefidkon et al., 2001). Furthermore, the volatile components creates biological potentials, have considerably improved their value in areas such as food, cosmetic industries and pharmaceutical (Pavela et al., 2018). Some literature indicated that the location of collection, climate, time and kinds of harvesting, the mode of drying and preserving, storage condition, environmental stress as well as nutrient management profoundly affect on aroma profile of *T. daenensis* (Dehghani Mashkani et al., 2018; Rowshan et al., 2013; Ghasemi Pirbalouti et al., 2013; Ghasemi Pirbalouti et al., 2014, Emami Bistgani et al., 2016; Emami Bistgani et al., 2017a,b; Emami Bistgani et al., 2018; Emami Bistgani et al., 2019).

The essential oil and extracts from the aerial parts of *T. daenensis* contain mainly monoterpenes, sesquiterpenes, phenolic compounds and flavonoids (Ghasemi Pirbalouti et al., 2011a). The high concentration of monoterpenes, especially the phenolic isomers compounds like carvacrol and thymol, which are responsible for the typical aroma profile and antioxidant activity. Essential oil of *T. daenensis* also have various other active compounds which are present in lower or higher amounts, including γ-terpinene, p-cymene, borneol, geraniol, and linalool (Emami Bistgani et al., 2017b) (Fig. 1). Due to the high percentage of thymol, *Thymus* species can be considered as a great foundation of this valuable constituents. (Golparvar et al., 2015, Emami Bistgani et al., 2018).

Askari and Sefidkon (2003) indicated that the aerial parts of *T. daenensis* at full flowering stage, were steam distilled for 45 min to obtain essential oil in 1.1% (w/w). Thirteen aroma profile was identified by GC and GC-MS. The major essential oil components were thymol (49.7%), carvacrol (15.2%), p-cymene (6.4%), γ-terpinene (5.4%) and 1, 8–cineole + limonene (3.2%).

The highest concentration of thymol (82%), as well as carvacrol (3.90%), was reported in *T. daenensis* under well watered and moderate drought stress condition (Emami Bistgani et al., 2017b). The second most abundant constituents from phenolic chemotype essential oil are p-cymene and *B*-Caryophyllene. Ghasemi Pirbalouti et al. (2013) identified (carvacrol and thymol) along with their precursors (p-cymene and γ-terpinene) were the main compounds of *T. daenensis* essential oil (Table 1). In general, samples secondary metabolites, are depend on the environmental conditions, water availability in the soil, level irrigation, climatic conditions, genetic variability and cultivation place as well as drying temperature and extraction mode.

#### 4.1.1. *The effect of drought tress on essential oil and aroma profile of Thymus daenensis*

Agricultural crop production is frequently affected by various stressful environmental factors such as drought stress (Hayat et al., 2012). Drought stress is considered to be one of the most important abiotic factors limiting growth, adversely affect growth and crop production (Yang et al., 2009). Drought stress has a considerable impact on crop productivity and metabolism activities of plant species. Secondary metabolites or active compounds of plants can be changed by water deficit (Vosoughi et al., 2018). For aromatic and medicinal crops, drought may cause significant changes in their metabolites yield and compositions (Bettaieb et al., 2009). The concentration of aroma profile is the most main quality index in herbs, spices and medicinal plants under water stress condition. Water deficiency often changes aroma

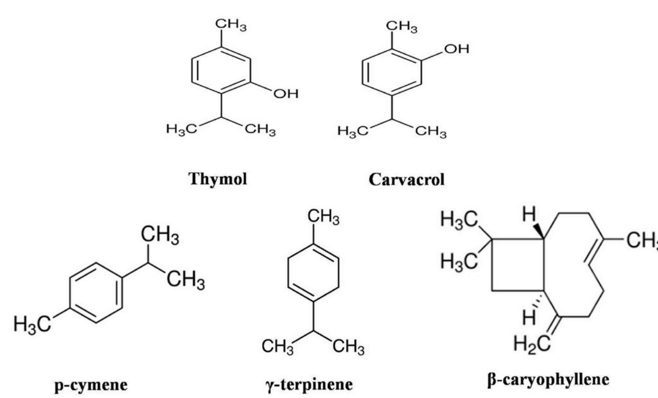


**Fig. 1.** Main aroma profile identified in *T.daenensis* (Emami Bistgani et al., 2017b, 2018).

**Table 1**
Essential oil chemotypes of *T. daenensis* from different location of Iran.

| location | Main constituents (%) | Essential oil yields (%) | References |
|---|---|---|---|
| ChaharmahalvaBakhtiari | Thymol (80.4), Carvacrol, *β*-Caryophyllene | 1.63–2.17 | Emami Bistgani et al. (2017b) |
| ChaharmahalvaBakhtiari | Thymol (72.1), *p*-Cymene | 1.8 | Abousaber et al. (2012) |
| ChaharmahalvaBakhtiari | Thymol (33.96–67.77), Carvacrol, *γ*-Terpinene | 0.85–1.16 | Ghasemi Pirbalouti et al. (2013) |
| Damavand | Thymol (53.6), Carvacrol, *p*-Cymene | 1.1 | Abousaber et al. (2012) |
| Fars | Thymol (20–60.55), Carvacrol, *p*-Cymene | 1.1–1.9 | Kashkooli and Saharkhiz (2014) |
| Fars | Thymol (52.2), Carvacrol, *β*-Caryophyllene | – | Karami-Osboo et al. (2015) |
| Fars | Geraniol (66.8), Geranyl acetate, *β*-Caryophyllene | – | Sabahi et al. (2013) |
| Hamadan | Thymol (73.9), Carvacrol, *p*-Cymene | 0.7 | Sajjadi and Khatamsaz (2003) |
| Hamadan | Thymol (74.7), *p*-Cymene, Caryophyllene | 2.4 | Nickavar et al. (2005) |
| Hamedan | Thymol (46.3–77.7), Carvacrol, *p*-Cymene | 2.8 | Rustaiee et al. (2011) |
| Ilam | Carvacrol (76.8), Linalool, Trans caryophyllene | – | Saidi (2014) |
| Isfahan | Thymol (51.3–78.3), *p*-Cymene, *γ*-Terpinene | 3.9 | Barazandeh and Bagherzadeh (2007) |
| Isfahan | Thymol (66.83), Carvacrol, *ρ*-Cymene | 2.66 | Bahreininejad et al. (2013) |
| Isfahan | *γ*-Terpinene (42.35), Thymol, *α*-Thujene | – | Golparvar et al. (2015) |
| Kohgiluyeh and Boyer Ahmad | Thymol (39.91), Carvacrol, Linalool | – | Jaberi et al. (2015) |
| Kohgiluyeh and Boyer Ahmad | Thymol (35.5), Carvacrol, *p*-Cymene | 1.8 | Rustaiee et al. (2011) |
| Lorestan | Geraniol (34.9–37.2), Geranyl acetate, Geranial | – | Hashemi et al. (2010) |
| Lorestan | *α*-Terpineol (13.18), Carvacrol, cis-Sabinene hydrate | 0.7 | Safaei-Ghomi et al. (2009) |
| Lorestan | Carvacrol (52.3), Thymol, *γ*-Terpinene | 2 | Amiri (2012) |
| Lorestan | *Thymol (45.2), Carvacrol, p-Cymene* | 2.8 | Rustaiee et al. (2011) |
| Tehran | Thymol (64.8), α-Terpinene, *p*-Cymene | 5.1 | Nabigol and Morshedi (2011) |
| Tehran | Thymol (70.1–73.4), *γ*-Terpinene, E–caryophyllene | 1.77–2.28 | Nikkhah et al. (2014) |
| West Azerbaijan | Thymol (70.8), Carvacrol | 1 | Abousaber et al. (2012) |

(−): Not presented (based on Tohidi et al., 2019)

profile in plant cells (Morshedloo et al., 2017). Result of a study conducted by Emami Bistgani et al. (2017a,b) indicate that essential oil concentration of thyme was maximized when plants were grown under severe stress condition. It was also reported by Ghasemi Pirbalouti et al. (2014) essential oils of thyme were considerably increased under severe stress; they also indicated that the percentages of carvacrol, α-pinene, β-myrcene and B-caryophyllene in essential oils were higher in stressed plants than in well watered plants, whereas thymol percentage decreased under water deficit irrigation.

Severe water stress influenced the concentrations of α-terpinene, p-cymene, 1,8-cineole, α-terpinene, thymol, carvacrol and B-caryophyllene in *T. daenensis*. The responses of main identified aroma profile to implication of water deficit irrigation treatments were not similar however, changes in concentration followed almost the same trends in both years of experiment. As depicted in (Fig. 1) thymol is one of the main components in thyme essential oil and it reduced significantly when severe drought stress was imposed (Emami Bistgani et al., 2017b).

Overall, the stimulation of essential oil content may be due to higher terpene production under water stress condition. It is crystal clear that in this situation allocation of carbon to plant growth has been reduced and leads to a balance between plant growth and defense capability (Bahreininejad et al., 2013). Furthermore, elevated concentration of secondary metabolites prohibited oxidation of the cells membrane under water stress condition (Hernandez et al., 2004).

#### *4.1.2. The effect of organic and inorganic sources of nutrients on essential oil and aroma profile of T. daenensis*

Nowadays, there is a dispute in Iran to increase the essential oil yield of *T. daenensis* through improvement of agricultural practices without increasing the cultivation area. Plant nutrients are the chemical elements to the nourishment of plant health and secondary metabolites including the terpenoid components and phenolic compounds (Pandey et al., 2015; Patel et al., 2015). Furthermore, it has been illustrated that medicinal and aromatic plants application of fertilizers could enhance effectively the essential oil yield and concentration (Aziz et al., 2010; Jabbari et al., 2011).

Results in relation to the *T. daenensis* delineate that the highest essential oil content (2.4–3.33 (mL/100 g dry matter) was recorded with the combined fertilizers in the first and second year of the experiment respectively. On the other hand, the lowest essential oil content was observed in samples treated with vermicompost (1.41 mL/100 g dry matter) and with no fertilizers (1.72 mL/100 g dry matter) in 2014 and 2015, respectively (Emami Bistgani et al., 2018). As a matter of fact, the chemical profiles of essential oils was improved by the application of fertilizers over the control conditions. The phenolic monoterpene thymol was the dominant essential oil component in plants subjected to all treatments (57.2–76.8%) (Fig. 2). It seems that the combined fertilizers have positive effects on thymol due to the effect of combinations various minerals nutrient which affect the secondary metabolism of plants (Pandey and Patra, 2015; Pandey et al., 2015). The highest amount of this component was achieved in combined fertilizer treatments reaching percentage levels of 72.7 and 76.8% in the first and second year, respectively. In general, these minerals will positively affect cellular metabolism and biomass production. As a consequence, an enhanced vegetative growth along with an increase of glandular trichomes is obtained (Patel et al., 2015; Pandey and Patra, 2015;Pandey et al., 2015; Pandey et al., 2016 ). Interestingly, in the first year, the highest percentages of the monoterpenes p-cymene(5%), α-terpinene (1.98%), γ-terpinene (3.68%), 1,8-cineole (2.6%) and carvacrol (4.0%), were obtained in plants treated with cow manure, whereas in the second year the highest amount of α-terpinene, γ-terpinene, 1,8-cineole and carvacrol were obtained in plants treated with combined fertilizers. This increase was of 9.93%, 45%, 38% and 17%, respectively, compared with the control plants. The results of Safaei et al. (2017) showed that the highest essential oil yield (3.88%) and thymol (86.68%) were obtained by application of 40 kg N, 32 kg P and 32 kg K combined with 25 ton/ha manure, but the highest carvacrol percentage (10.11%) obtained by application of 25 ton/ha manure. Overall, it seems that application of combined fertilizers in *T. daenensis* improves the quality and quantity of essential oil. Studies demonstrated that the combination of cow manure and chemical fertilizers is the most appropriated method to improve the soil properties and the economic productivity of this crop.

#### *4.1.3. The effect of elicitation on essential oil and aroma profile of Thymus daenensis*

Elicitors are used to alter biosynthesis of secondary metabolites in medicinal plants. Elicitors can be classified on the basis of their 'nature' (biotic or abiotic) elicitors like salicylic acid and chitosan. In a study conducted by Ghasemi Pirbalouti et al. (2014) indicated that the effect

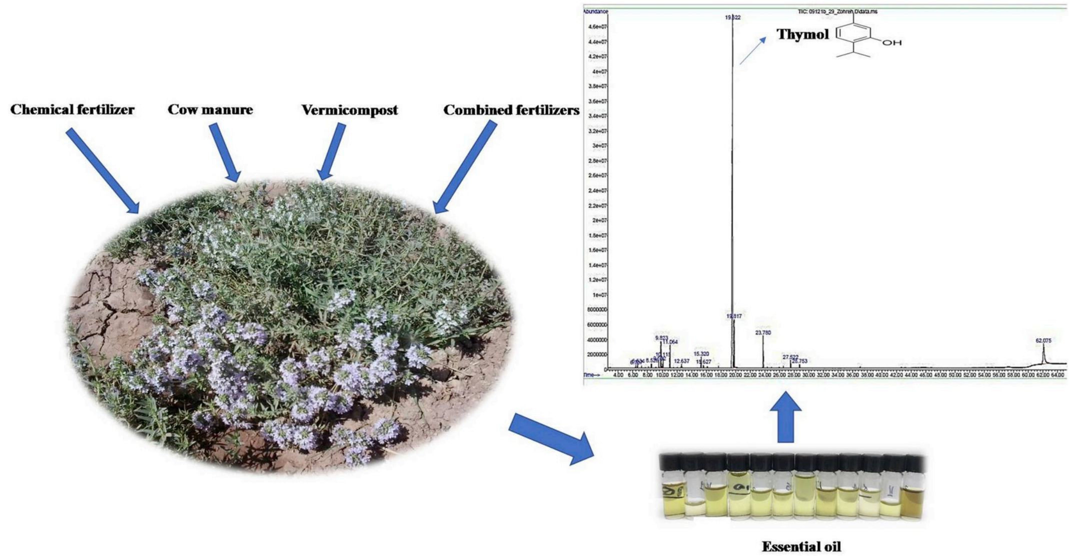


**Fig. 2.** Aroma profile identified by GC–MS under different fertilizer on *T.daenensis* in 2014 and 2015 (Emami Bistgani et al., 2018).

of foliar application of salicylic acid on oil content of *T. daenensis* was influenced by irrigation regime. The highest oil content (3.3% v/w) was achieved by foliar spray of 1.5 M salicylic acid under reduced irrigation. The lowest oil content (2.0% v/w) was achieved when salicylic acid was not applied under normal irrigation.

They also indicated that salicylic acid application significantly increased carvacrol, α-thujene, α-pinene and p-cymene, while decreased thymol and β-caryophyllene contents in thyme essential oil. Salicylic acid may convert thymol to its isomer (carvacrol), and is thought to play an important signaling role in the activation of various plant defense responses, such as the biosynthesis of special secondary metabolites, which function as phytoalexins in plants (Sirvent and Gibson, 2002; Kang et al., 2004). It should be noted that salicylic acid can act as an important endogenous signaling molecule in plant defense. It also regulates several plant physiological processes and is essential for the expression of some defense genes (Rodas-Junco et al., 2013).

Emami Bistgani et al. (2017b) indicated that elicitation of thyme plants enhanced the concentrations of the compounds where plants reached their highest level when sprayed with 400 $\mu L\,L^{-1}$ chitosan. In 2014, elicitation of plants with 400 $\mu L\,L^{-1}$ chitosan resulted in highest increase in the thymol content (79.4%), whereas established plants in 2015 reached their highest thymol level (82.5%) when sprayed with only 200 $\mu L\,L^{-1}$ chitosan. It seems that chitosan might be the synthesis of the de-novo enzyme by the processes involving transcription and translation of the particular genes in biosynthetic pathway. Furthermore, increased activity of the enzyme can also be attributed to induce different signaling pathways and stimulate various physiological phenomena in plants (Sinha et al., 2011).

#### 4.1.4. The effect of storage conditions on the essential oil composition of T. daenensis

Medicinal and aromatic plants are most sensitive to storage conditions that effect on biological deterioration. Thus, carful drying is essential to achieve a high quality product. As a matter of fact; drying method and storage condition increases the shelf life of plants by slowing microorganism growth and by preventing biochemical reactions and metabolism process that can alter organoleptic characteristics (Díaz–Maroto et al., 2003; Hamrouni–Sellami et al., 2012).

The findings of Rowshan et al. (2013) showed that phenolic constituents such as carvacrol thymol, p-cymene and γ-terpinene were the principle compounds of *T. daenensis* at different storage conditions. The evaluation of essential oil compounds at various temperatures and storage times showed that the concentration of major compounds was considerably changed during storage at room temperature. In this regard, their findings illustrated that the amount of components with a lower molecular weight reduced by prolonging the storage time especially at room temperature. This event may be due to evaporation, oxidation and other unwanted changes in aroma profile of essential oil during storage period (Baritaux et al., 1992; Mockute et al., 2005).

### 4.2. Phenolic compounds

*Thymus daenensis* is rich sources of phenolic compounds. Main examples are the phenolic monoterpenoids carvacrol and thymol that have notable antioxidant and antimicrobial properties (Ghasemi Pirbalouti et al., 2014). In addition, flavonoids such as rutin and quercetin derivatives and phenolic acids such as cinnamic, rosmarinic acids, chlorogenic acid and gallic acid (Fig. 3.) give an important contribution to the antioxidant capacity of thyme extracts (Emami Bistgani et al., 2019).

The presence of phenolic compounds has been demonstrated in the

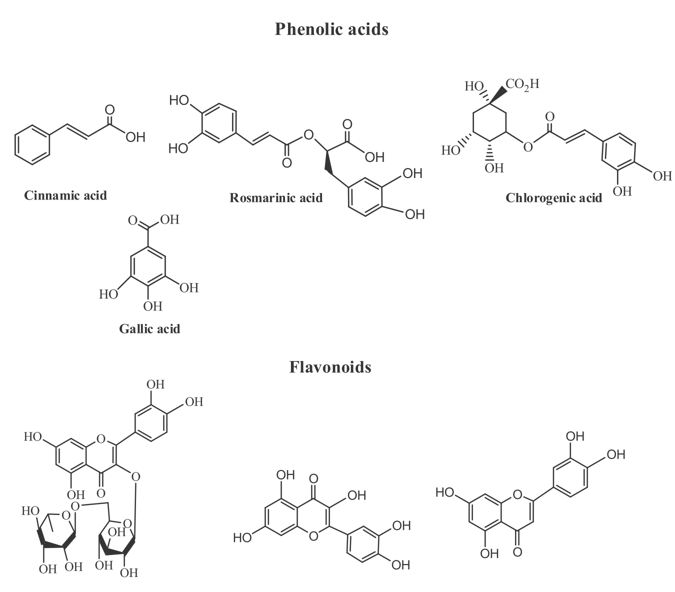


**Fig. 3.** Main phenolic compounds identified in *T.daenensis* (Emami Bistgani et al., 2019).

aerial parts of *T. daenensis* (Emami Bistgani et al., 2019). In a study conducted by (Emami Bistgani et al., 2017b, 2019) illustrated that total phenolic content of *T. daenensis* was increased when drought stress intensified. Phenolic quantities ranged from 95 to 112 mg GAE/g extraction. Interestingly, exactly the same trend of total phenolics at the moderate and severe salinity stress were reported by this author (Emami Bistgani et al., 2019). By using Folin-Ciocalteu's reagent, the phenolic content reached (295.9 ± 34.1) mg rutin equivalents/mg hydroalcoholic extract in *T. daenensis*. (Emami Bistgani et al., 2019). In another study, by using $AlCl_3$ reagent the flavonoid content was determined and the amount of the flavonoid was (35.2 ± 2.5) μgrutin/mg extract (Abousaber et al., 2012). Methanol extract contained higher amounts of total flavonoids (48.8 ± 0.7) and (16.4 ± 0.5) μg quercetin equivalents/mg extract, respectively), relative to the respective sub fractions of Polar and non-polar subfractions in *T. daenensis* (Amiri, 2012). Results of another study showed that the total phenol content of *T. vulgaris* (16.0 ± 2.2) (mg $GAEg^{-1}$dw) was much higher than that of a denaian thyme sample (14.6 ± 2.0) (mg $GAEg^{-1}$dw) (Emami Bistgani et al., 2019). In another assessment, the hydroalcoholic extract were determined the total phenol and total flavonoid contents of *T. daenensis* in range of (97.7 ± 11.6) mg GAE/gextract and (37.5 ± 2.8) mg rutin/g extract, respectively (Mirzaee et al., 2012). Alizadeh et al. (2013) indicated that harvesting time in *T. daenensis* caused a significant change in thymol and it also increased in flowering stage of harvest. They also illustrated that the highest total phenolic content was observed in flowering stage (18.97 mg GAE/g dw) than pre flowering stage (18.82 mg GAE/g dw). These findings indicate that the flowering stage of harvesting time causes increase in total phenolic content of *T. daenensis*. However, this reduction was very slight and not significant.

#### *4.2.1. The effect of salinity stress on phenolic compounds of Thymus daenensis*

Salt stress is one of the most critical factors limiting the plant production in many regions of the world including Iran (Khalvandi et al., 2019; Estaji et al., 2018). On the other hand, salinity is one of the main factors influencing physiology, biochemistry and phytochemistry in many herbs namely marigold (*Calendula officinalis* L.) (Khalid et al., 2010), apple mint (*Mentha suaveolens* Ehrh.) (Kasrati et al., 2014), basil (*Ocimum basilicum* L.) (Tarchoune et al., 2010), (*Thymus vulgaris* and *Thymus daenensis*) (Emami Bistgani et al., 2019). It is well documented that, the biosynthesis of secondary metabolites is influenced by salinity stress (Valifard et al., 2014; Taarit et al., 2009).

Emami Bistgani et al. (2019) reported that HPLC analysis identified fourteen constituents, namely eight phenolic acids (syringic, gallic, vanillic, caffeic, chlorogenic, rosmarinic, cinnamic, and *trans*-2-hydroxycinnamic acids), five flavonoids (quercetin, apigenin, luteolin, naringenin, and rutin), and one phenolic monoterpene (thymol) under salinity stress (Fig. 4).

Emami Bistgani et al. (2019) also reported that application of 30mMNaCl did not considerably change polyphenol compounds in *T. daenensis*. As demonstrated that cinnamic acid was the major compound reaching concentration of 35% in *T. daenensis* treated with 60mMNaCl but rosmarinic acid did not significantly change under treatments (30 and 60mMNaCl) in *T. daenensis.* It is worth to mention

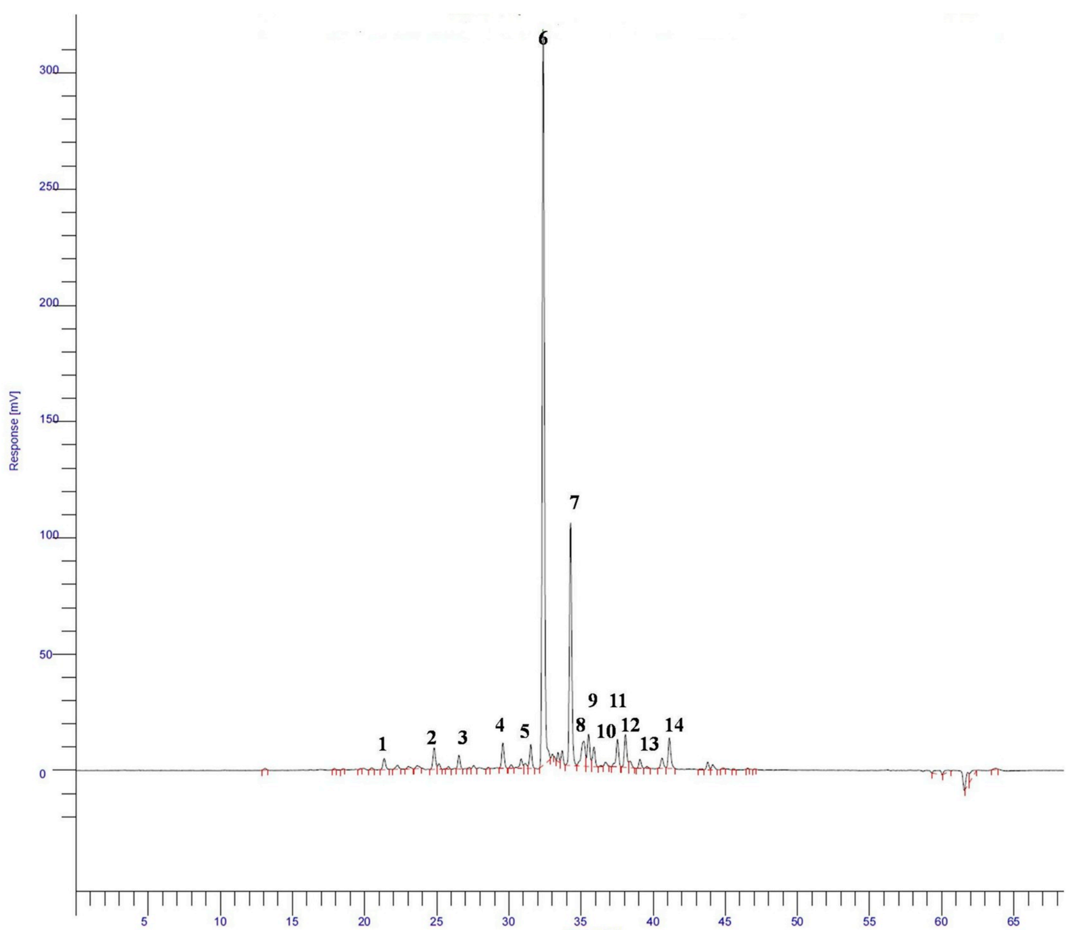


**Fig. 4.** HPLC-DAD chromatogram of *T. daenensis* extract. The peak numbers correspond to: (1) caffeic acid,(2) gallic acid, (3) syringic acid, (4) chlorogenic acid, (5) vanillic acid, (6) cinnamic acid, (7) rosmarinic acid, (8) *trans*-2-hydroxycinnamic acid, (9) rutin, (10) naringenin, (11) quercitrin, (12) luteolin, (13) apigenin and (14) thymol; they were monitored at 280 nm. In general, naringenin and rutin responded similarly to salinity stress (Emami Bistgani et al., 2019)

that the higher concentration of chlorogenic acid was obtained in (60 mM NaCl) in *T. daenensis*.

Concentrations of flavonoids, naringenin and rutin in *T. daenensis* were significantly influenced by salinity stress. In general, naringenin and rutin responded similarly to salinity stress. Treatment with 60 mM NaCl increased their production by about 16% and 10.7% respectively, in *T. daenensis*. Overall, the responses of the majority of compounds to salinity stress was not similar trend in phenolic compounds in *T. daenensis.*

The alternation of phenolic compounds under salinity stress could have been due to changes in biosynthetic pathway stress, some studies about this issue have indicated that genes responsible for the growth and development of plants expressed at a higher level concentration of salinity stress thus it cause to enhance secondary metabolites (Wang et al., 2015).

## 5. Thymol metabolic pathway

Precursors of phenolic compounds in biosynthetic pathway are (γ-terpinene and p-cymene) and these components are usually taken into consideration to define chemotypes (Fig. 5). Thompson et al. (2003) illustrated that the formation of thymol and carvacrol is thought to involve hydroxylation of γ-terpinene and p-cymene. Briefly, based on the facts obtainable in some studies, two main enzymes seem to be required to catalyze the reactions from geranyl diphosphate (GPP) to thymol and carvacrol in thyme. It also has recommended that the first step from GPP to γ-terpinene is performed by monoterpenes synthases (TPS), γ-terpinene is then transformed into thymol and carvacrol by the action of single cytochrome $P_{450}$. On the other hand; p-cymene is produced as a side product, resulting from the early release of the substrate from the enzyme active site. (Crocoll et al., 2010).

## 6. Molecular markers: genetic diversity

Genetic resources of native species are valuable resource in all over the world. So, their preservation and sustainable use is vital in breeding programs (Hashemifar and Rahimmalek, 2018). Breeding of plants plays a pivotal role in order to adapt to environmental stress. Breeding programs in medicinal plants have planned to increase yield of valuable compounds and elimination of unwanted compounds, for tolerance against abiotic and biotic stress. For the future, it is important to prevent the loss of genetic diversity, including the diversity within species (Carlen, 2011). There are various methods in breeding program like marker molecules. Marker molecules, known as molecular markers or genetic markers, are used to identify the position of a specific gene under investigation addressing plant genetic diversity (Hashemifar and Rahimmalek, 2018). In one study conducted by Yousefi et al. (2015), ISSR as a molecular marker has been carried to appraise genetic diversity. They also identified relationships among the accessions of *T. daenensis* and *T. kotschyanus* based on geographic distribution by using UPGMA cluster analysis and principal coordinate analysis. They classified fourteen *T. daenensis* and *T. kotschyanus* accessions in five categories. Rahimmalek et al. (2009) also performed their experiment according to the ISSR fingerprinting of the endemic accession of *T. daenensis*, they indicated that two geographically diverse groups were generated on the dendrogram. In a study conducted by Rustaiee et al. (2013) indicated that *T. daenensis* and *T. fallax*, the dendrogram of cluster analysis showed a clear separation of these two species from the other *Thymus*, revealing that. *T. daenensis* shared some genetic similarity with *T. fallax*. Talebi et al. (2015) also showed that among 79 *T. daenensis* accessions from 16 regions in Iran have genetic diversity according to SRAP markers. It was illustrated that the current scattered distribution area of the *T. daenensis* could be a result of human pressures for collection of these species that leads to limited gene flow via seed and/or pollen dispersal.

## 7. Pharmacological activities

### 7.1. Antioxidant activities

Plants have a complicated system which organized the interaction of benefit elements and cause to prevent from of free radicals. As a matter of fact, plants avoidance of continued hydrogen abstraction and free radical scavenging (Mao et al., 2006). Numerous researches have been conducted on antioxidant activity of *T. daenensis* to emphasize Iranian traditional medicine against inflammatory diseases. The essential oil and methanol extracts of *T. daenensis* were tested for this purpose. The various methods such as 2,2-diphenyl-1-picryl hydrazyl (DPPH), nitric oxide (NO) and hydroxyl (OH) radical-scavenging as well as ferric-reducing antioxidant power (FRAP) and ferric thiocyanate (FTC) were applied (Zarshenas and Krenn, 2015).

Mentioned methods were assessed in methanolic extracts have been tested. In DPPH test, the half maximal effective concentration ($EC_{50}$) was (194.24 ± 0.02) μg/mL, the extraction indicated that quercetin contains around one tenth of the activity and 25% of the one of butylated hydroxy toluene (BHT) as positive controls. There is no antioxidant activity observed by the extract at a concentration of 200 μg/mL (74%) was higher than the essential oil (35%) and BHT (42%) at the same concentrations (Sabahi et al., 2013). *T. daenensis* as an endemic species has been illustrated that the its' essential oil and methanol extraction contains antioxidant activity (Nickavar and Esbati, 2012; Amiri, 2012; Emami Bistgani et al., 2017b, Emami Bistgani et al.,2019; Emami Bistgani et al., 2018; Dadashpour et al., 2011 a,b).

In a study performed by (Emami Bistgani et al., 2019) indicated that the extracts obtained from plants treated with 90 mM NaCl exhibited the strongest antioxidant activity. It seems that polyphenol compounds in many medicinal plants contribute to trap free radicals (Bourgou et al., 2008; Bounatirou et al., 2007; Miguel et al., 2004). They also indicated

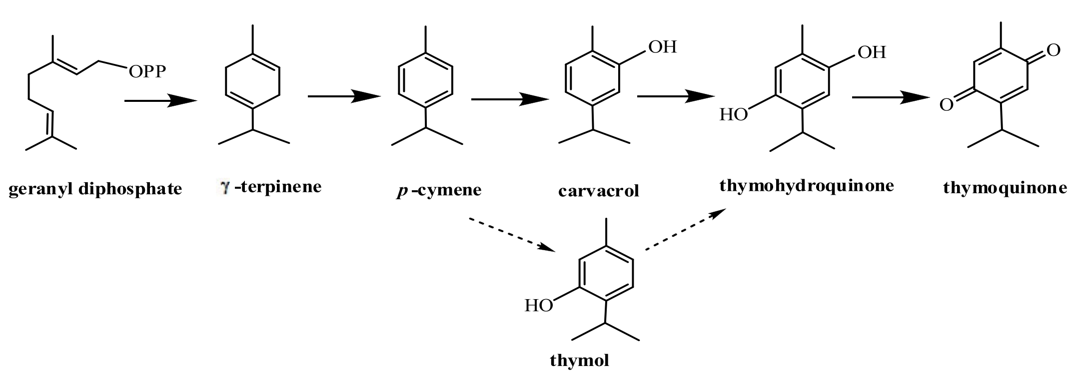


**Fig. 5.** The biosynthetic pathway of thymol/carvacrol in species of thyme (based on Botnick et al., 2012).

that thyme methanolic extracts showing the highest values of free radical scavenging activity are those with the highest content of phenolic components according to HPLC analysis. As stated by Lu and Foo (2001) thymol and carvacrol can donate single hydrogen atoms to DPPH and scavenge crystalline powder composed of stable free-radical molecules. Finally, it may be concluded that antioxidant activities of thyme extracts are influenced by phenolic constituents.

The biological properties of essential oils is available. The antioxidant properties of thyme essential oils have been reported and were associated with the major proportion of chemical compounds ring with an OH group (for example thymol) (Ghasemi Pirbalouti et al., 2014; Emami Bistgani et al., 2017b).

Application of different fertilizers influenced considerably the antioxidant activity of denaian thyme. Methanolic extracts from plants grown in the first year under cow manure displayed 14% higher activity against DPPH% compared with control plants. In the second year, however, combined fertilizers significantly improved the antioxidant capacity of the methanolic extracts by 10.41%. A possible factor contributing to the improvement of the antioxidant activity may be the enhancement of carvacrol and thymol production, as shown in *Lippia origanoides*(Teles et al., 2014). These essential oil constituents are generally recognized as excellent bioactive molecules.

### 7.2. Antimicrobial activity

It is well documented that substantial amounts of oxygenated monoterpenes, especially thymol and carvacrol exhibited higher antibacterial activity (Dadashpour et al., 2011a,b). As can be found from the study of Ghasemi Pirbalouti et al. (2014) the essential oils exhibited varying levels of antibacterial activities against the investigated bacteria pathogens (*B. cereus*, *L. monocytogenes*, *P. vulgaris* and *S. typhimurium*) in *T. daenensis*. The concentration ranges of MICs of the essential oils were around 0.062–0.5 mg $mL^{-1}$, with corresponding ranges of 0.125–0.5 mg $mL^{-1}$ MBCs. They also represented that thyme essential oil had higher antibacterial activity against *B. cereus* and *P. vulgaris* under normal than under reduced irrigation conditions. On the other hand, the oil extracted from stressed plants had higher antibacterial activity against *S. typhimurium* and *L. monocytogenes*. The evaluation of the activity of the essential oil components is measured in mm of the inhibition zones (IZ) for the antibacterial activity (Table 2).

Another study evaluated the bacteria such as Gram-positive, Gram-negative as well as three fungi and also antimicrobial and antifungal activity's essential oil of *T. daenensis* against them. The essential oil indicated the most prominent antibacterial activity against *Staphylococcus aureus* and antifungal activities against fungi *Aspergillus niger*, *Candida albicans* and *Saccharomyces cerevisiae* (Teimouri, 2012).

**Table 2**
Antibacterial activity of *T. daenensis*.

| Chemotype (content %) | Type of extracts | strain | IZ (mm) | References |
|---|---|---|---|---|
| N | Essential oil | *Streptococcus iniae* | 19 | Ghasemi Pirbalouti et al. (2011) |
| N | Essential oil | *Escherichia coli* | 7 | Ghasemi Pirbalouti et al. (2010) |
| | | *Bacillus cereus* | 25 | |
| | | *Listeria monocytogenes* | 16 | |
| N | Methanolic extract | *Staphylococcus aureus* | 22 | Mojab et al. (2008) |
| | | *Enterococcus faecalis* | 8 | |
| | | *M.luteus* | *18* | |
| | | *Streptococcus pyogenes* | 25 | |
| | | *Klebsiella pneumoniae* | - | |
| | | *Pseudomonas aeruginosa* | - | |
| | | *Escherichia coli* | - | |
| | | *Salmonella* spp. | - | |
| | | *methicillin-resistant Staphylococcus aureus* | *26* | |
| | | *vancomycin-resistant Enterococcus faecium* | *13* | |

N: Unknown; IZ: inhibition zones; MIC: minimum inhibitory concentrations; (−): Not presented. All literature used essential oil (based on Tohidi et al., 2019).

### 7.3. Cytotoxic effects

To evaluate the cytotoxicity of the essential oil from *T. daenensis* for human normal lymphocytes as well as on HeLa cells the 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide (MTT) was used, The $IC_{50}$ from essential oil of endemicspecies(*T. daenensis*) was much higher than the commercial thyme oil (1455 and 12.10 μg/mL, respectively) and therefore, the cytotoxicity effect of *T. daenensis* on normal lymphocytes seems to be insignificant oil. On the other hand, the essential oil of *T. daenensis* ($IC_{50} = 4.95$ μg/mL) was as valuable as the commercial thyme oil ($IC_{50} = 3.61$ μg/mL) against HeLa cell line (Dadashpour et al., 2011a,b) Thus, the difference in the activity of the two oils against normal human lymphocytes has to be confirmed.

### 7.4. Insecticidal properties

Most of the secondary metabolites of several plants including of alkaloids and terpenoids are illustrated as candidates for insecticidal properties that could be an effective methods for insect pest management. For instance, the essential oils whose repellent activities have been demonstrated, as well as the importance of the fumigant toxicity in *T. daenensis* against the first and third-instar larvae and adults of Ephestiakuehniella (Mediterranean flour moth) and Plodiainterpunctella (Indian flour moth). The evaluation of essential oil showed fumigant toxicity against both insects in adult stage (lethal concentration ($LC_{50}$) = 0.191 and 0.27 μL/Lair, respectively) while insects in larval stage were more resistant. Third instar larvae were dramatically less sensitive than first instar larvae. The repellent activity of the oil against *E. kuehniella* (repellent concentration ($RC_{50}$) = 0.69 μL/Lair) was higher than against *P. interpunctella* (RC50 = 6.88 μL/Lair) (Saeidi and Yousefi, 2013).

## 8. Conclusion

*Thymus daennesis* is well known as medicinal herb. This review emphasized on essential oil, aroma profile and phenolic compounds of the species. The diversity and various trends observed in the quantitative and qualitative of aroma profile may be due to the genetic diversity and also to the environmental conditions in geographic locations of the plant. It was also shown in the present review that the denaian thyme oils and extraction can exhibit antimicrobial properties. In conclusion, our studies indicated that *T. daenensis* has the main phenolic constituent and valuable aroma profile like thymol, thus, antioxidant and antimicrobial activity are high enough for the plant to be a new and natural source of antioxidant substances and biological activity for use as natural additives in food.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bcab.2019.101400.

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