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Research Article

Effects of Cuts and Different Phenological Stages on Antibacterial and Antioxidant Activities and Chemical Attributes of Garden Thyme (*Thymus vulgaris* L.) Essential Oil

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Abstract: Garden thyme is an aromatic plant with various applications in the pharmaceutical, food, and hygienic-cosmetic industries around the world. In this research, field-cultivated plants were harvested in two cuts and three consecutive stages (pre-flowering, flowering, and post-flowering). The essential oil percentage and compositions were identified in the essential oil samples. The antibacterial activity of the essential oil was measured against *Escherichia coli* and *Staphylococcus aureus*. The antioxidant activity, total phenol and flavonoid contents, chain-breaking activity, and IC_{50} were recorded. The highest essential oil percentage (2.56%) was obtained from the pre-flowering stage. The results of GC/MS revealed that *p*-cymene, *γ*-terpinene, thymol, and carvacrol were the most important constituents of the studied essential oil. The lowest antibacterial activity was recorded by the second cut at the pre-flowering stage. The highest antibacterial activity against *E. coli* and *S. aureus* were recorded by the second cut during the flowering stage and the first cut during the flowering stage, respectively. The lowest MIC was 15.75 µg mL^{-1} related to the second cut during the flowering stage. The plants had the highest total phenol (16.64 mg GAE g^{-1} DM) and total flavonoid contents (2.88 mg QE g^{-1} DM) at the pre-flowering stage. The highest antioxidant activity (IC_{50} = 134.05 µg mL^{-1}) was observed at the pre-flowering stage. It can be said that phenological stages and cuts can affect essential oil antibacterial and antioxidant activities, as well as its chemical characteristics.

Kesimlerin ve Farklı Fenolojik Aşamaların Bahçe Kekiği (*Thymus vulgaris* L.) Uçucu Yağının Antibakteriyel ve Antioksidan Aktiviteleri ve Kimyasal Özellikleri Üzerine Etkileri

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Anahtar Kelimeler

Antibakteriyel aktivite,
Antioksidan aktivite,

Öz: Bahçe kekiği, dünya çapında ilaç, gıda ve hijyenik-kozmetik endüstrilerinde çeşitli uygulamalara sahip aromatik bir bitkidir. Bu araştırmada, tarlada yetiştirilen bitkiler iki kesimde ve birbirini takip eden üç aşamada (çiçeklenme öncesi, çiçeklenme ve çiçeklenme sonrası) hasat edilmiştir. Uçucu yağ örneklerinde uçucu yağ yüzdesi ve bileşimleri belirlendi. Uçucu yağın antibakteriyel aktivitesi *Escherichia coli* ve *Staphylococcus aureus*'a karşı ölçülmüştür. Antioksidan aktivite, toplam fenol ve flavonoid içerikleri, zincir kırma aktivitesi ve IC_{50} kaydedildi. En yüksek uçucu yağ yüzdesi (%2.56) çiçeklenme öncesi dönemden elde edilmiştir. GC/MS sonuçları, çalışılan uçucu yağın en önemli bileşenlerinin *p*-cymene, *γ*-terpinen, timol ve karvakrol olduğunu ortaya koydu. En düşük

Kesmek,
Esans,
Bahçe kekiği,
Fenolojik dönemler.

antibakteriyel aktivite, çiçeklenme öncesi aşamadaki ikinci kesimde kaydedilmiştir. E. coli ve S. aureus'a karşı en yüksek antibakteriyel aktivite, sırasıyla çiçeklenme döneminde ikinci kesimde ve çiçeklenme döneminde ilk kesimde kaydedilmiştir. En düşük MIC, çiçeklenme döneminde ikinci kesime bağlı olarak 15.75 µg mL^{-1} idi. Bitkiler, çiçeklenme öncesi aşamada en yüksek toplam fenol (16.64 mg GAE g^{-1} DM) ve toplam flavonoid içeriğine (2.88 mg QE g^{-1} DM) sahipti. En yüksek antioksidan aktivite (IC_{50} = 134.05 µg mL^{-1}) çiçeklenme öncesi aşamada gözlenmiştir. Fenolojik aşamaların ve kesimlerin uçucu yağın kimyasal özelliklerinin yanı sıra antibakteriyel ve antioksidan aktivitelerini de etkileyebileceği söylenebilir.

1. Introduction

Garden thyme (*Thymus vulgaris* L.) is a medicinal and aromatic plant species from the family Lamiaceae. The latest findings show that the essential oil of this plant species contains over 80 compounds, most of which have antioxidant and antimicrobial activities (Wesolowska & Jadczak, 2019). Phenol compounds like thymol (44-60%) and carvacrol (2.2-4.%) are among the main components of its essential oil (Pasqua et al., 2005).

The composition of the essential oil of aromatic plants is profoundly influenced by genetics, ecology, technology, cultivation techniques, harvesting methods, the storage conditions of the raw material, and processing methods. Thus, the wild and domesticated types of similar plant species can exhibit very different chemical components and characteristics (Miladi et al., 2013).

The results of a study in New Zealand on the effect of seasonal variations on the percentage and components of garden thyme essential oil revealed that the highest essential oil yield (22.8 L ha^{-1}) was obtained in December after the termination of the flowering period. The components were also very variable during the vegetative period. The highest contents of thymol and carvacrol (37%) were observed at the post-flowering stage in summer. As one of the most important components, *p*-cymene constituted 40-50% of the essential oil in winter and early spring, but it was reduced to 21% in January (McGimpsey et al., 1994). The constituents of the shoot essential oils of *T. kotschyanus* and *T. pubescens* were studied at the full flowering stage in Behshahr, Iran. The main components in the essential oil of *T. kotschyanus* were reported to include pulegone (18.7%), thymol (14.17%), 1,8-cineole (9%), piperitenone (6.3%), and carvacrol (37%). The essential oil of *T. pubescens* was found to mainly compose of 32.1% carvacrol, 19.1% thymol, 14.6% α -terpineol, and 6.1% p-cymene (Morteza-Semnani et al., 2006).

Escherichia coli is a common pathogenic bacterium in humans and animals that is mostly responsible for mild to severe diseases depending on the serotype. *E. coli* is transmitted by food and excretory pathways and has been isolated from various foodstuffs (Kordali et al., 2008). *Staphylococcus aureus* is the most common pathogen of skin infections (Bensouilah and Buck, 2006). Both bacteria are resistant to conventional synthetic antibiotics, complicating the treatment of the diseases caused by them. This inherent or acquired resistance, which has been shown in various pathogens, has so far been among the most important treatment problems. This has drawn the attention of researchers to alternative treatments, especially natural components that possess antibacterial properties. As such, the essential oil of medicinal and aromatic plant species has been proposed as a possible alternative (Kordali et al., 2008).

Various studies have reported the potential of using plant essential oils as a preservative in food industries against pathogenic and decaying microorganisms since they are more advocated by consumers owing to their natural compounds (Rojas-Gra et al., 2007). Although some main components of essential oils act similar to industrial antibiotics, they are very unlikely to be available as medication or food preservatives since a limited number of bacterial strains have been researched and even they have shown different sensitivities. Thus, to use these compounds as a medicine or food preservative, they need to be tested against a more diverse set of bacterial strains and species to prove their effectiveness (Fournomiti et al., 2015).

Few studies have addressed the effect of Shirazi thyme essential oil on preventing the toxicity of important bacteria in foodstuffs. In a study, for example, different rates of the essential oil of this plant species were applied to *Staphylococcus aureus* to check its impacts on the production of alpha-

hemolysin and enterotoxin C under in vitro conditions. The results revealed its inhibitory effect on its toxicity (Parsaeimehr et al., 2010).

Perpetual exposure to invasive factors results in the production of more free radicals, cell degradation, and in the long run, senescence and other organic disorders. Reactive oxygen species (ROS), nitrogen, and sulfur contain free radicals that cause various diseases including neural disorders, cancer, cardiovascular diseases, cataract, rheumatism, ulcer, and atherosclerosis. Antioxidants can neutralize free radicals and protect cell molecules including proteins, lipids, carbohydrates, and nucleic acids (Martins et al., 2015). These antioxidants are also used to preserve food quality for a longer time. Presently, there is a dispute on the use of synthetic antioxidants, so it is desirable to replace them with natural antioxidants (Delgado et al., 2014). Flavonoids are a group of pigments in plants that are responsible for the color of flowers and fruits and many of their biological properties, such as their antioxidant activities (Tripoli et al., 2007). Owing to its high thymol content, garden thyme has higher antioxidant activity than other thyme species so that its IC50 has been shown to be 59.159 μg mL^{-1} (Gedikoğlu et al., 2019).

In addition to assessing the antioxidant activity and other compounds in the essential oil of garden thyme, the present research aimed to measure the antibacterial activity of its leaf essential oil against *E. coli* and *S. aureus* during harvest at different phenological stages and at two cuts under the ecological conditions of Urmia, Iran.

2. Material and Methods

The seeds of garden thyme from the landrace of Deutsche Welle, Germany were sown in sowing trays on a substrate composed of perlite and peat moss under greenhouse conditions in February. For better acclimation, the seedlings were transferred to pots containing a mixture of soil, sand, and manure and were kept outdoors for one week. In mid-June, the seedlings were planted at the main farm in 2 × 3 m^2 plots spaced by 50 × 30 cm^2 in the research farm of the Faculty of Plant Production and Genetic Engineering, Department of Agriculture, Urmia University, Iran. The experiment was conducted as split plots based on a randomized complete block design with three replications. The main factor was assigned to harvest at different phenological stages (before, during, and after flowering) and the sub-plot to the cut (first and second cut). The plants were established in the first year and were harvested in the second year. After harvesting, the plant samples were dried at room temperature away from sunlight in 7 d. Then, they were refrigerated at 4 °C in paper pockets until the laboratory assays.

2.1. Essential oil distillation

The plant samples were separately distilled with a Clevenger for 3 h. Their essential oil was collected, and they were, then, measured and recorded in percentage (g/100gDW). After that, the essential oils were dried with water-free Na_2SO_4 (Sigma-Aldrich) and were stored in glass vials at 4°C.

2.2. Determination of essential oil components by GC/MS

The essential oil samples were examined by an Agilent 7890A gas chromatograph (US) equipped with an Agilent 5975C mass detector and the HP Chemstation software in Microsoft Windows, a split/splitless mode injector, and an HP-5 MS capillary column with a height of 30 m, an internal diameter of 0.25 mm, and a thickness of 0.25 mm (Agilent Co., US). The oven temperature was initially kept at 80 °C for 3 min, then increased to 180 °C at a rate of 8 °C min^{-1}, and kept at that temperature for 10 min. Helium was used as carrier gas. The flow rate of the carrier gas was 1 mL min^{-1} and the electron impact was 70 eV. The injection valve in the split mode had a ratio of 500:1 and a mass range for 40-500 mass bar^{-1}. To identify the components, the Wily 2007 and NIST 2005 mass references were used. The temperature of the injection valve was set at 250 °C. Data were analyzed by the Chemstation software package in Microsoft Windows.

2.3. Antibacterial activity

The antimicrobial susceptibility of the essential oil against *E. coli* and *S. aureus* were determined by the following two methods.

i. Broth microdilution to determine MIC and MBC
ii. Agar well diffusion

Distilled water was used as the solvent control and gentamicin (Sigma, US) was employed as the standard antibiotic. Each assay was performed in three replications.

2.3.1. Broth microdilution

The method is used to determine the minimum inhibitory concentration (MIC) or the maximum bactericidal concentration (MBC) of essential oils against bacteria. In summary, base-2 consecutive dilutions were prepared from the essential oils separately in round-bottom 96-well microplates (from dilutes 1:2 to 1:4096) containing the Mueller Hinton broth medium (Merc, Germany) and 5% dimethyl sulfoxide. Similar dilutions of distilled water, enrofloxacin, and dimethyl sulfoxide in the Mueller Hinton broth medium were prepared as the negative control, antibiotic control, and solvent control, respectively. It should be noted that the final volume of the wells had been adjusted at 180 µL until this stage. At the final step, 20 µL of the suspension of each bacterium equivalent to 0.5 McFarland standard (1-2 × 108 CFU mL^{-1}) was added to all wells. Also, in each row, one well was allocated to the culture medium control and one well to the bacteria growth control. After the bacteria were added, the microplates were placed in an oven in aerobic conditions at 37 °C for 24 h. To prepare the bacteria suspensions, 24 hours before preparing the foregoing dilutions, the bacteria were separately cultured in the TSB broth medium under aerobic conditions at 37 °C for 24 h. Then, the cultures were centrifuged at 2500 rpm at 4 °C for 15 min and the resulting sediment was suspended twice in a sterile physiological serum and its density was adjusted at 0.5 McFarland standard to be added to the wells (the final amount of bacteria inoculated into each group was 105 CFU/well).

To estimate MBC, 10 µL of the content of a well before and a well after the well in which MIC was observed was cultured linearly on an agar TSA medium.

2.3.2. Agar well diffusion

In the present study, the antibacterial activity of the essential oil was checked by the agar well diffusion method too for which plates with a diameter of 8 cm were first prepared from Mueller-Hinton agar culture and 6 mm wells were created with a sterile punch. After 24 h culture of the bacteria, a suspension was prepared similar to the previous method (0.5 McFarland) and linear culture was performed by a sterile swab on the plate. Finally, base-2 dilutions of essential oils (100, 200, 400 and 800 ppm) were prepared by the aforementioned method, 50 µL of each dilution was added into the wells, and the plates were placed in an oven under aerobic conditions at 37 °C for 24 h after which the diameter of the growth inhibition zone was measured with a ruler in mm (Qaiyami, 2007).

2.4. Antioxidant Activity

2.4.1. Measurement of DPPH radical scavenging percentage

The capability of essential oil in donating hydrogen atoms or electrons was measured by the extent of bleaching or reducing absorbance of purple DPPH solution in methanol (Brand-Williams et al., 1995). In this method, 2,2-diphenyl-1-picrylhydrazyl (DPPH) was used as a stable radical composition and reagent for which 100 µL of different essential oil solutions (diluted to 1:500) was added to 2 mL of 0.004% DPPH in methanol. After 30 min of incubation at room temperature and in darkness, the absorbance of the samples was read against blank at 517 nm. The spectrophotometer was zeroed with methanol. DPPH free radical inhibition percentage was calculated by the following equation:

$$I\% = \frac{A_{blank} - A_{sample}}{A_{blank}} \times 100 \quad (1)$$

Then, to estimate IC50 (expressing the effective concentration of essential with the potency of 50% DPPH inhibition), five different rates of essential oil were prepared and after they were added to the DPPH solution similar to what described above, their absorbance was read with a spectrophotometer and was calculated by the diagram.

2.4.2. Measurement of total phenol content.

Total phenol was measured by Folin-Ciocalteu reagent using Oki et al. (2002)'s procedure. To this end, 1 mL of the Folin-Ciocalteu reagent (diluted to the ratio of 1:10) was added to 120 µL of essential oil solution (diluted to the ratio of 1:500). After 3 min, 0.3 mL of 2% sodium carbonate was added to the solution. The resulting solution was incubated at room temperature for 30 min and then, its absorbance was read at 750 nm with a spectrophotometer (Biowave, WPSA 2100, UK). The total phenol content was measured by using a standard gallic acid curve and expressed in mg gallic acid equivalent (GAE)/g dry matter (DM). The spectrophotometer was zeroed with methanol.

2.4.3. Measurement of total flavonoid content

The procedure described in Serra Bonvehí et al. (2001) was used to measure total flavonoid content. To this end, 260 µL of essential oil solution (diluted to 1:500) was mixed with 0.3 mL of 5% sodium nitrite and 3 mL of distilled water. After 5 min, 0.3 mL of 5% aluminium chloride was added. After 6 min, 2 mL of 1M sodium hydroxide solution was added to the solution and finally, the volume of the 10-mL volumetric flask containing the solution was adjusted with methanol. The absorbance was immediately read at 510 nm with a spectrophotometer against a control sample. The flavonoid content was calculated by the standard quercetin curve and expressed in quercetin equivalent (EC) per g DM.

2.4.4. Measurement of chain-breaking activity

Chain-breaking rate activity was measured using the DPPH reagent by Brand-Williams et al. (1995)'s procedure with slight modifications. The reaction rate was estimated by the following equation:

$$Abs^{-3} - Abs_0^{-3} = 03kt \quad (2)$$

in which *Abs* denotes absorbance over time, Abs_0 denotes absorbance at time 0, *t* denotes time in minutes, and k denotes chain-breaking rate.

2.5. Statistical Analysis

Data were statistically analyzed by the SAS software package. Means of the samples were compared by Duncan's test at the $P < 0.05$ level. The relationships between the recorded parameters were also checked by Pearson's test. MS-Excel was used to draw the graphs. All measurements were replicated three times and the values were reported as mean ± SD.

3. Results and Discussion

3.1. Essential oil percentage and compositions

Essential oil efficiency was found to be 40.20%, 1.2%, and 1.21% for the three phenological periods including pre-flowering, at flowering, and post-flowering in the first cut and 2.91%, 2.15%, and 1.76% for the three phenological periods in the second cut, respectively. Based on the results of the analysis of variance (ANOVA; Table 1), the main effect of the cut and phenological period was

significant ($P < 0.01$) on essential oil efficiency, but their interactive effect was revealed to be insignificant for this trait.

Table 1. The results of analysis of variance for the essential oil percentage based on a split-plot design over time

Sources of variations	df	Means of squares
		Essential oil percentage
Block	2	0.005^{ns}
Phenological stage	2	1.85^{**}
Error *a*	4	0.02
Cut	1	12.03^{**}
Phenological stage × cut	2	0.017^{ns}
Experimental error	6	0.03
Coefficients of variations (%)		8.21

ns, *, and ** show insignificance and significance at the $P < 0.05$ and $P < 0.01$ levels, respectively.

Essential oil efficiency was higher in the second cut (2.28%) than in the first (1.6%; Table 2). The highest essential oil efficiency (2.56%) was observed at the pre-flowering period, then it declined to 1.78% during flowering reaching its minimum value (1.49%) at the post-flowering period. It implies that as the plant approached maturity, its essential oil efficiency was decreased (Table 2). These findings are consistent with the results of other studies on garden thyme and other plants as to achieving the highest essential oil efficiency at the pre-flowering period. In a study on garden thyme, Salehi et al. (2014) found that the highest essential oil efficiency was 2.42% for the flower initiation period and as the plant approached the end of flowering, its essential oil percentage declined. A research on *Satureja sahendica* Bornm. revealed that the highest essential oil efficiency (3.30%) was at the early-flowering period and the lowest (1.65%) was at the late-flowering period among all phenological periods (Sefidkon & Akbari-nia, 2009). In a study on the hybrid *Thymus* × *citriodorus*, the highest and lowest essential oil was observed at the pre-flowering (2%) and post-flowering (1.3%) periods, respectively (Toncer et al., 2017). On the other hand, our findings are not consistent with the results of Zantar et al. (2015) on wild thyme, Ghasemi et al. (2016) on *Echinophora cinerea*, and Morshedloo et al. (2018) on *Origanum vulgare* subsp. *gracile*. They found that the studied plant species produced the highest essential oil percentage at the full flowering period. The inconsistency can be related to the differences in plant species and vegetative conditions.

Table 2. The comparison of means for the simple effects of phenological stage and cut on the recorded traits

Treatment	**Essential oil percentage**
Phenological stage	
Pre-flowering	2.56 ± 0.15 a
Flowering	1.78 ± 0.18 b
Post-flowering	1.49 ± 0.14 c
Cut	
First cut	1.60 ± 0.15 b
Second cut	2.28 ± 0.17 a

Means in each column with similar letter(s) did not differ statistically at the $P < 0.05$ level based on Duncan's test

The profile of the chemical compounds, which was determined by GC/MS, is presented in Table 3. The percentage of the compounds in the samples was determined based on the normalization of the curve peak and listed in order of their retention index on the HP-5MS column (Figure 1). A total of 25 compounds were detected of which 20 compounds were observed during and after flowering in the first cut and 19 compounds in the next cuts. The amount of oxygen-containing monoterpenes was higher in the first cut than in the second cut. With respect to the phenological periods, the highest value was 87.13% related to the harvest during flowering whereas the amount of hydrocarbonic monoterpenes was higher in the second cut than in the first cut and its highest amount (35.06%) among different phenological periods was related to the pre-flowering period.

Table 3. The chemical composition of the essential oils and the antibacterial activity of some constituents at different phenological stages. For the essential oil of *T. vulgaris*, the GC signal was observed and identified. The main components included p-cymene (3.37-18.04%), γ-terpinene (4.88-16.61%), thymol (43-63.18%), and carvacrol (4.73-17.84%).

No.	Components	RI	%						Antibacterial Activity	
			Cut1			Cut2				
			Pre-flowering stage	Flowering stage	Post-flowering stage	Pre-flowering stage	Flowering stage	Post-flowering stage	Species of bacteria	References
1	α -Thujen	927	0.55	-	0.40	0.84	0.82	0.76		
2	α -Pinene	931	0.55	-	0.51	0.56	0.55	0.51	*Campylobacter jejuni*	(Kovač et al.,2015)
3	1-Octen-3-ol	975	0.51	0.51	0.49	0.83	0.75	0.79		
4	β -Myrcene	989	1.05	-	0.91	1.18	1.08	1.00		
5	α -Terpinene	1015	1.70	0.38	1.15	2.04	1.35	1.13		
6	***P*-Cymene**	**1026**	**7.20**	**3.37**	**10.55**	**12.54**	**17.86**	**18.04**	*M. tuberculosis*	(Andrade et al., 2015)
7	Limonene	1028	0.42	-	0.37	0.46	0.44	-		
8	1,8-cineole	1032	-	0.46	0.89	0.59	0.48	0.54		
9	**γ -Terpinene**	**1060**	**13.78**	**4.88**	**7.55**	**16.61**	**10.60**	**8.46**	*S. aureus*	(Cristani et al., 2007)
10	Trans-sabinene hydrate	1068	0.60	0.63	0.68	0.7	0.76	0.57		
11	Linalool	1099	1.75	1.78	2.03	2.10	1.88	2.10	*Streptococcus mutans*	(Park et al.,2012)
12	Camphor	1148	-	-	-	-	0.43	-		
13	Borneol	1168	1.23	0.60	0.71	0.81	1.07	1.15		
14	Terpineol-4	1180	0.73	0.68	0.66	0.73	0.64	0.86		
15	α -Terpineol	1194	-	0.20	-	-	-	-	*E. coli&Staph. aureus*	(Cosentino et al.,1999)
16	Thymol, methyl ether	1235	-	1.23	0.68	1.12	-	1.05		
17	Carvacrol methyl ether	1245	0.88	1.13	0.75	0.89	0.62	0.86		
18	**Thymol**	**1295**	**55.14**	**62.12**	**63.18**	**51.10**	**43.00**	**44.41**	*E. coli&Staph. aureus*	(Kavoosi et al., 2013)
19	Borneol, acetate	1297	0.45	0.46	0.54	0.58	0.85	1.015		
20	**Carvacrol**	**1303**	**9.91**	**17.84**	**5.85**	**4.73**	**14.77**	**14.03**	*E. coli&Staph. aureus*	(Mahboubi& Kazempour.,2011)
21	Trans-caryophyllene	1425	1.84	2.45	1.64	1.27	1.57	1.54	*Staph. aureus*	(Dahham et al.,2015)
22	β-bisabolen	1509	-	0.23	-	-	-	-		
23	Delta-Cadinene	1527	-	0.33	-	-	-	-		
24	Caryophyllene oxide	1590	0.87	0.51	0.46	-	-	0.52		
25	α -cadinol	1645	0.38	0.22	-	-	-	-		
Monoterpene hydrocarbons			25.76	**9.14**	21.93	**35.06**	33.45	30.69		
Oxygenated monoterpenes			70.69	**87.13**	75.97	**63.35**	64.5	66.585		
Sesquiterpene hydrocarbons			1.84	**3.01**	1.64	**1.27**	1.57	1.54		
Oxygenated sesquiterpenes			1.25	0.73	0.46	-	-	0.52		
sum			**99.54**	**100.01**	**100**	**99.68**	**99.52**	**99.335**		

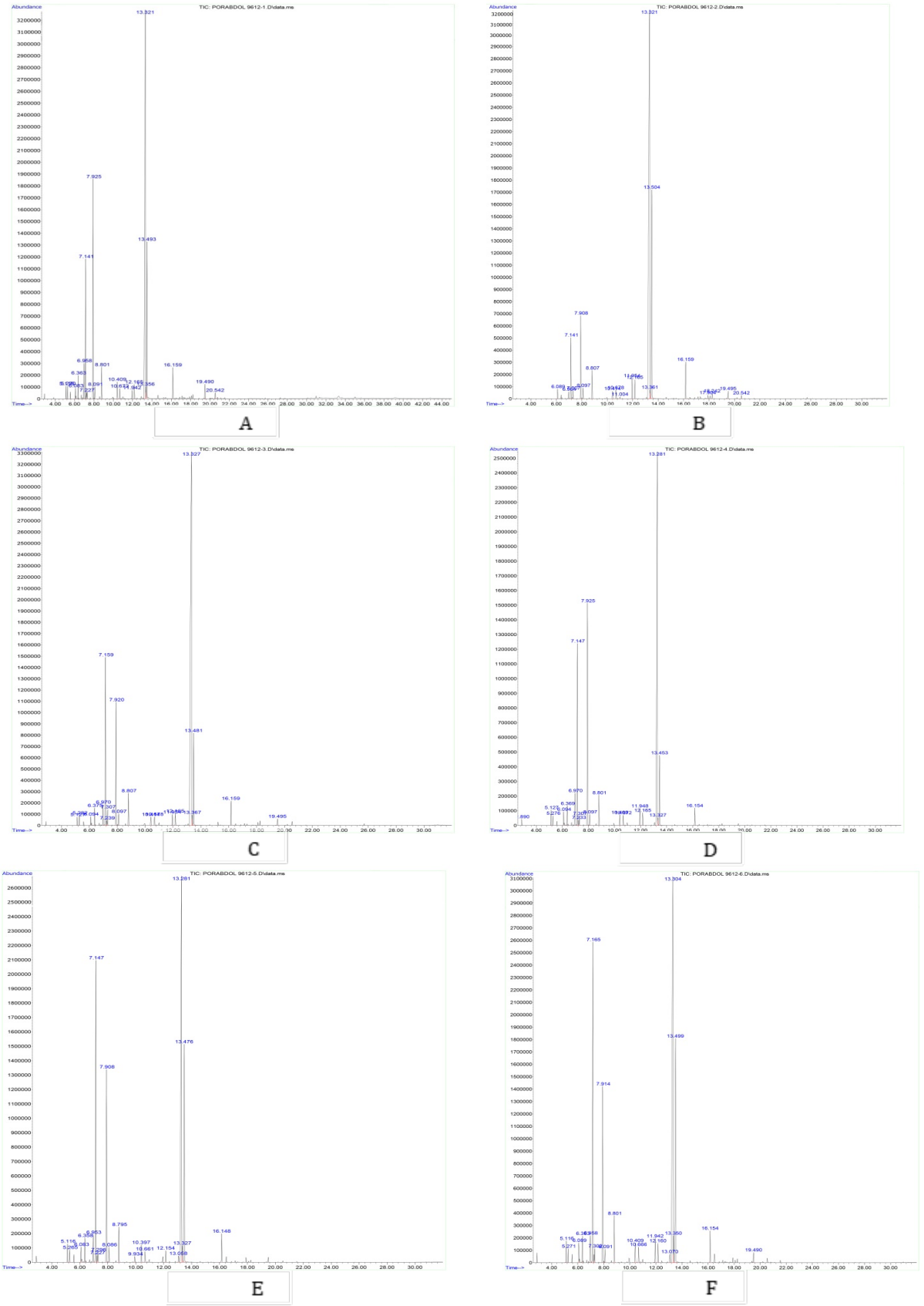

Figure 1. GC/MC chromatograms of *T. vulgaris* analyzed in GC/MC (Agilent, US) at the pre-flowering first cut (A), flowering first cut (B), post – flowering first cut (C), pre-flowering second cut (D), flowering second cut (E) and post – flowering second cut (F), using a capillary column (MS-5) attached to a mass detector.

The lowest amount of hydrocarbonic monoterpenes was obtained from the harvest during flowering in the second cut whilst these plants exhibited the highest amounts of oxygen-containing monoterpenes and sesquiterpenes too. The abundance of hydrocarbonic monoterpenes at the pre-flowering stage is reasonable since monoterpenes like c-terpinene and p-cymene are regarded as the biogenetic precursors of terpene phenol carvacrol (Casiglia et al., 2015). The main components of the essential oil were thymol (43-63.18%), *p*-cymene (3.37-18.04%), carvacrol (4.73-17.84%), and *γ*-terpinene (4.88-16.61%). The other components were observed in lower values in ranges smaller than 11.79-14.70%. The highest thymol content was related to the pre-flowering and post-flowering periods in the first cut, the highest *p*-cymene content to the post-flowering period in the second cut, the highest carvacrol content to the flowering period in the first cut, and the highest *γ*-terpinene to the pre-flowering period in the second cut. These results are partially in agreement with the results of Rota et al. (2008) who found that thymol, carvacrol, and *γ*-terpinene were the main components of the essential oil of *T. vulgaris*. However, our results are inconsistent with the results of Sartoratto et al. (2004) in their study on garden thyme in Brazil so that, according to their findings, geranyl was the most abundant component (21.8%), and *γ*-terpinene and thymol constituted 2.6% and 17.5% of the essential oil, respectively – much lower than our observations in the present study. This may point to the impact of geography or environment on the components of *T. vulgaris* essential oil. These differences can also be ascribed to the differences in chemotypes. On the other hand, a study on *Thymus* × *citriodorus* showed that the most abundant components on its essential oil were terpinolene, α-terpineol, linalool, bornyl acetate, and borneol (Toncer et al., 2017). Based on the results, it seems that the cut and phenological stage can influence the amount and type of essential oil components.

3.2. Antibacterial Activity

The results of ANOVA indicated that the interactive effect of cuts and phenological stage at harvest time was significant ($P < 0.01$) on the diameter of the zone of bacterial growth inhibition. Based on the comparison of means (Table 4), the highest diameter of the inhibition zone was the second cut during flowering (19.9 mm) for *E. coli* and the first cut during flowering (18.2 mm) for *S. aureus*. The results revealed that the plant had the lowest antibacterial activity among the studied essential oils before flowering at the second cut in which the diameter of the inhibition zone was 15.2 mm for *E. coli* and 13.2 mm for *S. aureus* (Table 4). Nedorostova et al. (2009) reported the antimicrobial activity of the essential oil of three plant species – *T. vulgaris*, *T. pulegioides*, and *T. serpyllum* – in the vapor phase against *E. coli* and *S. aureus*. In addition, a high level of antifungal activity has been reported for the essential oil of thyme isolated at the flowering stage (Saoud et al., 2013). The results indicate that the lowest MIC was 15.75 μg mL^{-1} related to the second cut during flowering and the highest was 125 μg mL^{-1} related to the second cut before flowering. These results are consistent with the findings as to the diameter of the growth inhibition zone.

Table 4. The antibacterial activity of the *T. vulgaris* essential oil at different phenological stages against *Staphylococcus aureus* and *Escherichia coli*. Gentamicin (Sigma, US) was used as the control medicine. The DMSO solvent (dimethyl sulfoxide) was 10% with no antibacterial activity.

Cut	Phenological stage	*S. aureus* ATCC 29213			*E. coli* ATCC35218		
		MIC (μg mL^{-1})	MBC (μg mL^{-1})	Inhibition zone (mm)	MIC (μg mL^{-1})	MBC (μg mL^{-1})	Inhibition zone (mm)
First	Pre-flowering	62.5	123	17.2 ± 0.06 b	62.5	125	16.3 ± 0.11 d
	Flowering	31.25	62.5	18.2 ± 0.17 a	31.25	62.5	19.4 ± 0.15 b
	Post-flowering	62.5	125	17.26 ± 0.2 b	125	250	17.2 ± 0.09 c
second	Pre-flowering	125	250	13.2 ± 0.11 d	125	250	15.2 ± 0.08 e
	Flowering	15.75	31.5	17.13 ± 0.08 b	15.75	31.5	19.9 ± 0.09 a
	Post-flowering	62.5	125	15.13 ± 0.15 c	62.5	125	16.4 ± 0.11 d
	Gentamycin	17.5	35	22	15	30	28

Means in each column with similar letter(s) did not differ statistically at the $P < 0.05$ level based on Duncan's test.

The antibacterial activity of carvacrol and p-cymene was examined against *Vibrio colerae*. Carvacrol showed a high inhibitory effect on *V. colerae* whereas p-cymene did not. Nonetheless, the presence of p-cymene reinforced the inhibitory activity of carvacrol when they were applied together. The synergy of p-cymene and carvacrol may justify their combined application to suppress *V. cholera* and other pathogens in foodstuffs (Mitropoulou et al., 2015). In another study on the antibacterial activities of various essential oils, including p-cymene, thymol, and carvacrol, against *Mycobacterium tuberculosis* and *M. bovis*, p-cymene showed the lowest antibacterial activity, but thymol and carvacrol were found to be the most active terpenes (Andrade-Ochoa et al., 2015). An interesting finding as to the relationship between the phytochemical analysis by GC and bioactivity on the bacteria of *Streptococcus mutans* showed that the bacteria was influenced by the high percentage of menthol in *M. arvensis* (over 70% of the total composition of the essential oil) and the high percentage of phenol monoterpenes such as carvacrol in *T. capitatus* (over 65% of the total composition of the essential oil) and thymol in *T. vulgaris* (over 25% of the total composition of the essential oil), which had a MIC of 8 μL mL^{-1} (Tardugno et al., 2018). The mechanism by which menthol and other phenolic isomers of thymol and carvacrol influence oral bacteria is associated with their performance in inducing disorder in membranes, which leads to cell leakage (Gursoy et al., 2009; Franz, 2010; Oyanagi et al., 2012; Freires et al., 2015; Kouidhi et al., 2015). Other components of essentials oils, e.g. α-pinene, γ-terpinene, linalool, α-terpineol, and trans-caryophyllene, have also been subject to extensive research (Table 3).

The results showed that the essential oil of garden thyme had an effective inhibitory impact on the growth of both tested bacteria (Figure 2). This finding corroborates similar reports as to the antibacterial activity of plant essential oils against Gram-positive and Gram-negative bacteria (Roldán et al., 2010; Soković et al., 2007). We observed that *E. coli* (a Gram-negative bacteria) was more sensitive to the antibacterial activity of the garden thyme essential oil than *S. aureus* (a Gram-positive bacteria). Fatma et al. (2014) report that despite the possession of an external layer composed of hydrophobic compounds around their cell wall, which acts as a barrier against permeability, the Gram-negative bacteria are usually more sensitive to the essential oil of *Thymus hirtus* sp. There is seemingly a relationship between the low antibacterial activity of thyme essential oil and the deficiency of oxygen-containing monoterpenes. Also, the low molecular weight of the essential oil compounds seems to allow them to penetrate across the internal membrane of Gram-negative bacteria (Pattnaik et al., 1997). The results of our screening prove the potential of the garden thyme essential oil to be used as an active ingredient in medications and food preservatives in the treatment of plant and animal disease and remove food-rotting microorganisms.

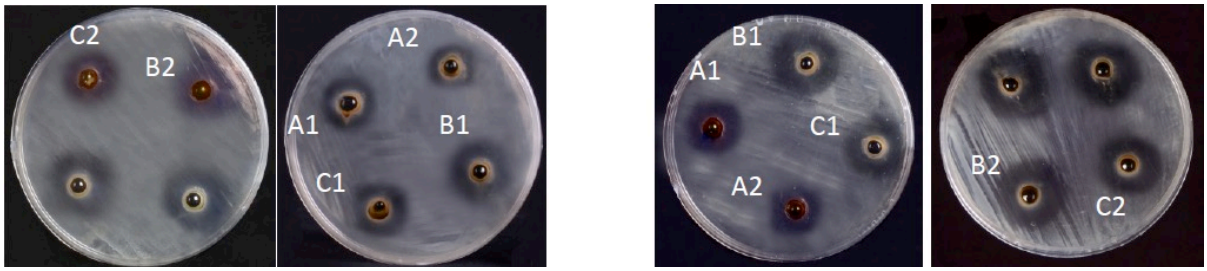

Figure 2. The inhibitory effect of the garden thyme essential oil on *Escherichia coli* and *Staphylococcus aureus* at different cuts and phenological stages: A = pre-flowering stage, B = flowering stage; C = post-flowering stage; 1 = first cut; 2 = second cut.

3.3. Antioxidant activity

The assay of the antioxidant activities of the *T. vulgaris* essential oil at a rate of 3.68 μg mL^{-1} provided the following results.

Based on the results of ANOVA (Table 5), the main effects of cut and phenological stage were significant ($P < 0.01$) on total phenol content, but their interaction was not. Among the phenological stages (Table 7), the highest total phenol content was obtained at the pre-flowering stage (16.64 mg GAE g^{-1} DM) and the lowest at the post-flowering stage (8.88 mg GAE g^{-1} DM).

Table 5. The results of analysis of variance for the antioxidant activities based on a split-plot design over time

Sources of variations	df	Means of squares				
		Total phenol	Total flavonoids	Chain-breaking	IC50	DPPH
Block	2	10.14^{*}	0.050^{ns}	4.26^{ns}	13.29^{ns}	7.95^{ns}
Phenological stage	2	90.30^{**}	2.47^{**}	2.75^{ns}	10856.44^{**}	31.62^{ns}
Error *a*	4	0.98	0.010	3.36	9.53	8.06
Cut	1	267.95^{**}	7.86^{**}	58.88^{**}	5739.85^{**}	0.19^{ns}
Phenological stage × cut	2	8.95^{ns}	0.27^{**}	27.63^{*}	718.78^{**}	74.16^{**}
Experimental error	6	4.30	0.012	3.68	16.16	6.32
Coefficients of variations (%)		16.24	5.06	10.51	2.30	6.80

ns, *, and ** show insignificance and significance at the $P < 0.05$ and $P < 0.01$ levels, respectively.

The total phenol content was higher in the second cut than in the first cut. Phenols are compounds that contain at least one hydroxyl (OH) group attached to an aromatic ring. They occur in aerial parts of the plants, e.g. flowers, leaves, seeds, fruits, stems, and also in their roots. Phenol compounds are interested in due to their appealing biological properties including antioxidant activities and free radical scavenging (Göçer et al., 2011). A research study on rosemary revealed that total phenol content was lower at the pre-flowering stage than at the fruit-bearing stage, which is inconsistent with our findings (Jordán et al., 2013).

The interaction of cut and phenological stage (Table 5) was significant for total flavonoids, IC50, and DPPH at the $P < 0.01$ level and for chain-breaking extent at the $P < 0.05$ level. Flavonoids are polyphenol compounds with a high distribution in plants and perform many functions. They may also be involved in chemical signalling, thereby contributing to physiological regulations and cell cycle inhibition (Galeotti et al., 2008). The comparison of means (Table 6) indicated that the total flavonoid content was lower in the first cut at different phenological stages and its highest value was related to the second cut at the pre-flowering stage (3.70 mg QE g^{-1} DM) and the lowest to the first cut at the post-flowering stage (1.21 mg QE g^{-1} DM). In separate studies on the alcoholic extract of garden thyme leaves, total flavonoid content was reported to be 8.56 mg QE g^{-1} DM (Nadia et al., 2013) and 36.6 mg QE g^{-1} DM (Köksal et al., 2017). The highest total flavonoid contents at the early-flowering stage were 14.94, 75.85, 8.4, and 36.87 mg QE g^{-1} DM for the extracts of *Agastache foeniculum*, *Lavandula foeniculum*, *Melissa officinalis*, and *Nepeta cataria*, respectively, which is consistent with our findings (Duda et al., 2015).

Table 6. The comparison of means for the interactive effects of phenological stage × cut on the studied traits

Phenological stage	Cut	Total flavonoid (mg QE g^{-1} DM)	Chain-breaking (%)	IC50 (μg mL^{-1})	DPPH inhibition (%)
Pre-flowering	First	2.07 ± 0.08 c	16.85 ± 2.34 c	193.37 ± 1.61 c	30.22 ± 3.07 b
	Second	3.70 ± 0.14 a	18.07 ± 1.09 bc	128.74 ± 0.98 d	38.55 ± 1.44 a
Flowering	First	1.28 ± 0.05 d	22.18 ± 0.65 a	196.51 ± 0.94 b	40.77 ± 0.69 a
	Second	2.77 ± 0.06 b	15.22 ± 0.40 c	145.54 ± 2.70 c	36.72 ± 1.39 a
Post-flowering	First	1.21 ± 0.02 d	21.11 ± 0.06 ab	241.67 ± 3.89 a	39.66 ± 0.53 a
	Second	2.05 ± 0.05 c	16 ± 0.275 c	196.12 ± 0.13 b	36 ± 0.34 a

Means in each column with similar letter(s) did not differ statistically at the $P < 0.05$ level based on Duncan's test.

The highest IC50 was observed in the first cut at the post-flowering period and the lowest in the first cut at the pre-flowering period (Table 6). This means that the antioxidant activity is higher in the first cut at the pre-flowering stage. A research study on *Echinophora cinerea* showed that the antioxidant activity was stronger at the pre-flowering stage than at the full-flowering stage (Ghasemi et al., 2016). The comparison of means showed that the first cut at the pre-flowering stage had the lowest DPPH, but it did not differ from the other treatments significantly. The highest chain-breaking activity was obtained from the first cut at the flowering stage and the lowest from the second cut at the flowering stage.

Table 7. The comparison of means for the simple effects of the phenological stage and cut on the studied traits

Treatment	Total phenol (mg GAE g^{-1} DM)	Total flavonoid (mg QE g^{-1} DM)	Chain-breaking (%)	IC50 (μg mL^{-1})
Phenological stage				
Pre-flowering	16.64 ± 2.14 a	2.89 ± 0.37 a	17.46 ± 1.88 a	134.05 ± 2.52 c
Flowering	12.76 ± 2.29 b	2.03 ± 0.34 b	18.70 ± 1.59 a	171.03 ± 11.47 b
Post-flowering	8.88 ± 1.17 c	1.63 ± 0.19 c	18.60 ± 1.15 a	218.90 ± 10.33 a
Cut				
First	8.9 ± 0.87 b	1.52 ± 0.14 b	20.04 ± 1.07 a	192.52 ± 14.85 a
Second	16.62 ± 1.64 a	2.84 ± 0.24 a	16.43 ± 0.55 b	156.80 ± 10.16 b

Means in each column with similar letter(s) did not differ statistically at the $P < 0.05$ level based on Duncan's test.

Phenol and total flavonoid contents were negatively correlated to the IC50 activity significantly ($P < 0.05$). A previous study on *T. vulgaris* (Sarikurkcu et al., 2008) revealed a close relationship between polyphenols and antioxidant activity, which can support the effectiveness of these compounds as free radical scavengers and antioxidants. Fatma et al. (2014) reported that total phenol and total flavonoid contents had a negative correlation with the DPPH assay whereas the positive correlation between a less-shown phenol compound ((+)-catechin hydrate) and the DPPH assay may support the role of trivial compounds in synergy with the main compounds in their antioxidant activity.

4. Conclusions

The results revealed the effectiveness of the thyme essential oil against the tested bacteria. All in all, the biochemical properties and the measurement of the antimicrobial activity showed that a higher percentage of aliphatic and oxygen-containing monoterpenes and mainly phenol monoterpenes such as thymol were responsible for the antioxidant activity of the studied essential oil. As well, the Gram-negative *E. coli* was more sensitive to the *T. vulgaris* essential oil than the Gram-positive *S. aureus*. The essential oil percentage was higher in the second cut than in the first cut. Also, the essential oil had stronger antioxidant activity in the second cut at the pre-flowering stage. Overall, it can be said that phenological stages and cuts can affect essential oil percentage, composition, and antioxidant and antimicrobial activities.

References

Andrade-Ochoa, S., Nevárez-Moorillón, G. V., Sánchez-Torres, L. E., Villanueva-García, M., Sánchez-Ramírez, B. E., Rodríguez-Valdez, L. M., & Rivera-Chavira, B. E. (2015). Quantitative structure-activity relationship of molecules constituent of different essential oils with antimycobacterial activity against *Mycobacterium tuberculosis* and *Mycobacterium bovis*. *BMC Complementary and Alternative Medicine, 15*(1), 332.

Bensouilah, J., & Buck, P. (2006). *Aromadermatology: aromatherapy in the treatment and care of common skin conditions*. Radcliffe Publishing.

Brand-Williams, W., Cuvelier, M. E., & Berset, C. L. W. T. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT-Food Science and Technology, 28*(1), 25-30.

Casiglia, S., Bruno, M., Scandolera, E., Senatore, F., & Senatore, F. (2019). Influence of harvesting time on composition of the essential oil of *Thymus capitatus* (L.) Hoffmanns. & Link. growing wild in northern Sicily and its activity on microorganisms affecting historical art crafts. *Arabian Journal of Chemistry*, *12*(8), 2704-2712.

Cosentino, S. C. I. G., Tuberoso, C. I. G., Pisano, B., Satta, M. L., Mascia, V., Arzedi, E., & Palmas, F. (1999). In-vitro antimicrobial activity and chemical composition of Sardinian thymus essential oils. *Letters in Applied Microbiology*, *29*(2), 130-135.

Cristani, M., D'Arrigo, M., Mandalari, G., Castelli, F., Sarpietro, M. G., Micieli, D., ... & Trombetta, D. (2007). Interaction of four monoterpenes contained in essential oils with model membranes:

implications for their antibacterial activity. *Journal of Agricultural and Food Chemistry*, *55*(15), 6300-6308.

Dahham, S. S., Tabana, Y. M., Iqbal, M. A., Ahamed, M. B., Ezzat, M. O., Majid, A. S., & Majid, A. M. (2015). The anticancer, antioxidant and antimicrobial properties of the sesquiterpene β-caryophyllene from the essential oil of *Aquilaria crassna. Molecules, 20*(7), 11808-11829.

Delgado, T., Marinero, P., Manzanera, M. C. A. S., Asensio, C., Herrero, B., Pereira, J. A., & Ramalhosa, E. (2014). Antioxidant activity of twenty wild Spanish *Thymus mastichina* L. populations and its relation with their chemical composition. *LWT-Food Science and Technology*, *57*(1), 412-418.

Di Pasqua, R., De Feo, V., Villani, F., & Mauriello, G. (2005). In vitro antimicrobial activity of essential oils from Mediterranean Apiaceae, Verbenaceae and Lamiaceae against foodborne pathogens and spoilage bacteria. *Annals of Microbiology*, *55*(2), 139-143.

Duda, S. C., Mărghitaş, L. A., Dezmirean, D., Duda, M., Mărgăoan, R., & Bobiş, O. (2015). Changes in major bioactive compounds with antioxidant activity of *Agastache foeniculum*, *Lavandula angustifolia*, *Melissa officinalis* and *Nepeta cataria*: Effect of harvest time and plant species. *Industrial Crops and Products*, *77*, 499-507.

Fatma, G., Mouna, B. F., Mondher, M., & Ahmed, L. (2014). In-vitro assessment of antioxidant and antimicrobial activities of methanol extracts and essential oil of *Thymus hirtus sp. algeriensis*. *Lipids in Health and Disease*, *13*(1), 1-12.

Fournomiti, M., Kimbaris, A., Mantzourani, I., Plessas, S., Theodoridou, I., Papaemmanouil, V., ... & Alexopoulos, A. (2015). Antimicrobial activity of essential oils of cultivated oregano (*Origanum vulgare*), sage (*Salvia officinalis*), and thyme (*Thymus vulgaris*) against clinical isolates of *Escherichia coli*, *Klebsiella oxytoca*, and *Klebsiella pneumoniae*. *Microbial Ecology in Health and Disease*, *26*(1), 23289.

Freires, I. A., Denny, C., Benso, B., De Alencar, S. M., & Rosalen, P. L. (2015). Antibacterial activity of essential oils and their isolated constituents against cariogenic bacteria: a systematic review. *Molecules*, *20*(4), 7329-7358.

Galeotti, F., Barile, E., Curir, P., Dolci, M., & Lanzotti, V. (2008). Flavonoids from carnation (*Dianthus caryophyllus*) and their antifungal activity. *Phytochemistry Letters, 1*(1), 44-48.

Gedikoğlu, A., Sökmen, M., & Çivit, A. (2019). Evaluation of *Thymus vulgaris* and *Thymbra spicata* essential oils and plant extracts for chemical composition, antioxidant, and antimicrobial properties. *Food Science & Nutrition*, *7*(5), 1704-1714.

Ghasemi Pirbalouti, A., & Gholipour, Z. (2016). Chemical composition, antimicrobial and antioxidant activities of essential oil from *Echinophora cinerea* harvested at two phenological stages. *Journal of Essential Oil Research*, *28*(6), 501-511.

Göçer, H., & Gülçin, İ. (2011). Caffeic acid phenethyl ester (CAPE): correlation of structure and antioxidant properties. *International journal of food sciences and nutrition*, *62*(8), 821-825.

Iseppi, R., Sabia, C., de Niederhäusern, S., Pellati, F., Benvenuti, S., Tardugno, R., ... & Messi, P. (2019). Antibacterial activity of *Rosmarinus officinalis* L. and *Thymus vulgaris* L. essential oils and their combination against food-borne pathogens and spoilage bacteria in ready-to-eat vegetables. *Natural Product Research*, *33*(24), 3568-3572. doi:10.1080/14786419.2017.1329730

Jordán, M. J., Lax, V., Rota, M. C., Lorán, S., & Sotomayor, J. A. (2013). Effect of the phenological stage on the chemical composition, and antimicrobial and antioxidant properties of Rosmarinus officinalis L essential oil and its polyphenolic extract. *Industrial Crops and Products*, *48*, 144-152.

Kavoosi, G., Dadfar, S. M. M., & Purfard, A. M. (2013). Mechanical, physical, antioxidant, and antimicrobial properties of gelatin films incorporated with thymol for potential use as nano wound dressing. *Journal of Food Science*, *78*(2), E244-E250.

Köksal, E., Bursal, E., Gülçin, İ., Korkmaz, M., Çağlayan, C., Gören, A. C., & Alwasel, S. H. (2017). Antioxidant activity and polyphenol content of Turkish thyme (*Thymus vulgaris*) monitored by liquid chromatography and tandem mass spectrometry. *International Journal of Food Properties*, *20*(3), 514-525.

Kordali, S., Cakir, A., Ozer, H., Cakmakci, R., Kesdek, M., & Mete, E. (2008). Antifungal, phytotoxic and insecticidal properties of essential oil isolated from Turkish Origanum acutidens and its

three components, carvacrol, thymol and p-cymene. *Bioresource Technology*, *99*(18), 8788-8795.

Kouidhi, B., Al Qurashi, Y. M. A., & Chaieb, K. (2015). Drug resistance of bacterial dental biofilm and the potential use of natural compounds as alternative for prevention and treatment. *Microbial Pathogenesis*, *80*, 39-49.

Kovač, J., Šimunović, K., Wu, Z., Klančnik, A., Bucar, F., Zhang, Q., & Možina, S. S. (2015). Antibiotic resistance modulation and modes of action of (-)-α-pinene in *Campylobacter jejuni. PloS one*, *10*(4), e0122871.

Mahboubi, M., & Kazempour, N. (2011). Chemical composition and antimicrobial activity of Satureja hortensis and *Trachyspermum copticum* essential oil. *Iranian Journal of Microbiology*, *3*(4), 194.

Martins, N., Barros, L., Santos-Buelga, C., Silva, S., Henriques, M., & Ferreira, I. C. (2015). Decoction, infusion and hydroalcoholic extract of cultivated thyme: Antioxidant and antibacterial activities, and phenolic characterisation. *Food Chemistry*, *167*, 131-137.

McGimpsey, J. A., Douglas, M. H., Van Klink, J. W., Beauregard, D. A., & Perry, N. B. (1994). Seasonal variation in essential oil yield and composition from naturalized *Thymus vulgaris* L. in New Zealand. *Flavour and Fragrance Journal*, *9*(6), 347-352.

Miladi, H., Slama, R. B., Mili, D., Zouari, S., Bakhrouf, A., & Ammar, E. (2013). Essential oil of *Thymus vulgaris* L. and Rosmarinus officinalis L.: Gas chromatography-mass spectrometry analysis, cytotoxicity and antioxidant properties and antibacterial activities against foodborne pathogens. *Natural Science 05*, 729-739 doi:10.4236/ns.2013.56090

Mitropoulou, G., Fitsiou, E., Stavropoulou, E., Papavassilopoulou, E., Vamvakias, M., Pappa, A., ... & Kourkoutas, Y. (2015). Composition, antimicrobial, antioxidant, and antiproliferative activity of *Origanum dictamnus* (dittany) essential oil. *Microbial Ecology in Health and Disease*, *26*(1), 26543.

Morshedloo, M. R., Mumivand, H., Craker, L. E., & Maggi, F. (2018). Chemical composition and antioxidant activity of essential oils in *Origanum vulgare* subsp. gracile at different phenological stages and plant parts. *Journal of Food Processing and Preservation*, *42*(2), e13516.

Morteza-Semnani, K., Rostami, B., & Akbarzadeh, M. (2006). Essential oil composition of *Thymus kotschyanus* and *Thymus pubescens* from Iran. *Journal of Essential Oil Research*, *18*(3), 272-274.

Nadia, Z., & Rachid, M. (2013). Antioxidant and antibacterial activities of *Thymus vulgaris* L. *Medicinal and Aromatic Plant Research Journal*, *1*(1), 5-11.

Nedorostova, L., Kloucek, P., Kokoska, L., Stolcova, M., & Pulkrabek, J. (2009). Antimicrobial properties of selected essential oils in vapour phase against foodborne bacteria. *Food Control*, *20*(2), 157-160.

Oki, T., Masuda, M., Furuta, S., Nishiba, Y., Terahara, N., & Suda, I. (2002). Involvement of anthocyanins and other phenolic compounds in radical-scavenging activity of purple-fleshed sweet potato cultivars. *Journal of Food Science*, *67*(5), 1752-1756.

Park, S. N., Lim, Y. K., Freire, M. O., Cho, E., Jin, D., & Kook, J. K. (2012). Antimicrobial effect of linalool and α-terpineol against periodontopathic and cariogenic bacteria. *Anaerobe*, *18*(3), 369-372.

Parsaeimehr, M., Basti, A. A., Radmehr, B., Misaghi, A., Abbasifar, A., Karim, G., ... & Khanjari, A. (2010). Effect of Zataria multiflora boiss. Essential oil, nisin, and their combination on the production of enterotoxin C and α-hemolysin by Staphylococcus aureus. *Foodborne Pathogens and Disease*, *7*(3), 299-305.

Pattnaik, S., Subramanyam, V. R., Bapaji, M., & Kole, C. R. (1997). Antibacterial and antifungal activity of aromatic constituents of essential oils. *Microbios*, *89*(358), 39-46.

Qaiyami S. (2007). Macro- and microdilution methods of antimicrobial suscebtibility testing. In R. Schwalbe, L. Steele-Moore, & Goodwin A.C. (Eds), *Antimicrobial suscebtibility testing protocols* (pp. 75-81). Taylor & francis Group, New York: USA,

Rojas-Graü, M. A., Avena-Bustillos, R. J., Olsen, C., Friedman, M., Henika, P. R., Martín-Belloso, O., ... & McHugh, T. H. (2007). Effects of plant essential oils and oil compounds on mechanical, barrier and antimicrobial properties of alginate–apple puree edible films. *Journal of Food Engineering*, *81*(3), 634-641.

Roldán, L. P., Díaz, G. J., & Duringer, J. M. (2010). Composition and antibacterial activity of essential oils obtained from plants of the Lamiaceae family against pathogenic and beneficial bacteria. *Revista Colombiana de Ciencias Pecuarias*, *23*(4), 451-461.

Rota, M. C., Herrera, A., & Mart, R. M. '\inez, JA Sotomayor, and MJ Jordán. 2008. Antimicrobial activity and chemical composition of *Thymus vulgaris*, *Thymus zygis* and *Thymus hyemalis* essential oils. *Food Control*, *19*(7), 681-687. doi:https://doi.org/10.1016/j.foodcont. 2007.07.007

Salehi, S., Golparvar, A. R., & Hadipanah, A. (2014). Effect of harvest time on yield and quality of *Thymus vulgaris* L. essential oil in Isfahan province, Iran. *Agriculturae Conspectus Scientificus*, *79*(2), 115-118.

Saoud, I., Hamrouni, L., Gargouri, S., Amri, I., Hanana, M., Fezzani, T., ... & Jamoussi, B. (2013). Chemical composition, weed killer and antifungal activities of Tunisian thyme (*Thymus capitatus* Hoff. et Link.) essential oils. *Acta Alimentaria*, *42*(3), 417-427. doi:10.1556 /AAlim.42.2013.3.15

Sarikurkcu, C., Tepe, B., Daferera, D., Polissiou, M., & Harmandar, M. (2008). Studies on the antioxidant activity of the essential oil and methanol extract of Marrubium globosum subsp. globosum (lamiaceae) by three different chemical assays. *Bioresource Technology*, *99*(10), 4239-4246. doi:https://doi.org/10.1016/j.biortech.2007.08.058

Sartoratto, A., Machado, A. L. M., Delarmelina, C., Figueira, G. M., Duarte, M. C. T., & Rehder, V. L. G. (2004). Composition and antimicrobial activity of essential oils from aromatic plants used in Brazil. *Brazilian Journal of Microbiology*, *35*, 275-280. doi:10.1590/S1517-83822004000300001

Sefidkon, F., & Akbari-nia, A. (2009). Essential oil content and composition of *Satureja sahendica* Bornm. at different stages of plant growth. *Journal of Essential Oil Research*, *21*(2), 112-114.

Serra Bonvehi, J., Soliva Torrentó, M., & Centelles Lorente, E. (2001). Evaluation of polyphenolic and flavonoid compounds in honeybee-collected pollen produced in Spain. *Journal of Agricultural and Food chemistry*, *49*(4), 1848-1853.

Sokovic, M., Marin, P. D., Brkic, D., & van Griensven, L. J. (2008). Chemical composition and antibacterial activity of essential oils against human pathogenic bacteria. *Food*, *1*(2), 220-226.

Toncer, O., Karaman, S., Diraz, E., Sogut, T., & Kizil, S. (2017). Essential oil composition of *Thymus*× *citriodorus* (Pers.) Schreb. at different harvest stages. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, *45*(1), 185-189.

Tripoli, E., La Guardia, M., Giammanco, S., Di Majo, D., & Giammanco, M. (2007). Citrus flavonoids: Molecular structure, biological activity and nutritional properties: A review. *Food Chemistry*, *104*(2), 466-479.

Wesolowska, A., & Jadczak, D. (2019). Comparison of the chemical composition of essential oils isolated from two thyme (*Thymus vulgaris* L.) cultivars. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, *47*(3), 829-835.

Zantar, S., El Garrouj, D., Pagán, R., Chabi, M., Laglaoui, A., Bakkali, M., & Zerrouk, M. H. (2015). Effect of harvest time on yield, chemical composition, antimicrobial and antioxidant activities of *Thymus vulgaris* and Mentha pulegium essential oils. *European Journal of Medicinal Plants*, 69-77.