Vietnam Journal of Science and Technology ***57*** *(3B) (2019) 127-136*
doi:10.15625/2525-2518/57/3B/14494

VOLATILE COMPOSITION, ANTIOXIDANT PROPERTY AND ANTIMICROBIAL ACTIVITIES AGAINST FOOD-BORNE BACTERIA OF VIETNAMESE THYME (*Thymus vulgaris* L.) ESSENTIAL OIL

Cung Thi To Quynh[1, 2, *], Vu Thu Trang[1]

[1]*School of Biotechnology and Food Technology, Hanoi University of Science and Technology, No 1 Dai Co Viet, Ha Noi*

[2]*Institute of Research and Development of Natural Products, Hanoi University of Science and Technology, No 1 Dai Co Viet, Ha Noi*

[*]Email: *quynh.cungthito@hust.edu.vn*

Received: 14 October 2019; Accepted for publication: 4 November 2019

Abstract. The essential oil (EO) obtained from the leaves of thyme (*Thymus vulgaris* L.) grown in Vietnam was found to contain thymol (39.79 %), cymene (17.33 %), and *γ*-terpinene (13.45 %) as the main volatile components. The antimicrobial activities of this oil were screened against several food-borne bacteria and fungi species. Significant growth inhibition effects against food-borne bacteria *Escherichia coli (E. coli), Staphylococcus aureus (S. aureus), Bacillus cereus (B. cereus),* and *Salmonella* Typhimurium were observed using the standard disc diffusion method. Thyme EO exhibited the antibacterial effect against all the tested pathogenic strains. The inhibition zones were 23.3 ± 0.4 mm, 24.7 ± 0.4 mm, 29.0 ± 0.7 mm, 32 ± 0.7 mm in diameter against *B. cereus, E. coli, S.* Typhimurium and *S. aureus,* respectively. The minimum inhibitory concentration (MIC) determined by the micro-dilution method in MHB liquid medium was 1.56 µl/mL. The bactericidal concentrations (MBC) was 3.13 µl/mL for three isolates from *B. subtilis, E. coli,* and *S. aureus*, while the MBC tested for *S.* Typhimurium was 1.56 µl/mL. The antifungal properties of the thyme EO were also determined in this study against three important pathogenic fungi (*Candida albicans, Rhizoctonia solani* and *Fusarium oxysporum)* with the inhibition zones ranging approximately from 23.20 ± 0.06 to 44.10 ± 0.03 mm. On the other hands, the results also showed the antioxidant activity of Vietnamese thyme EO and suggested that thyme EO can be applied in food industries as natural flavoring preservatives/additives to control food spoilage and food-borne bacteria and fungi.

Keywords: *Thymus vulgaris* L., thyme essential oil, antibacterial activity, antifungal activity.

Classification numbers: 1.2.1, 1.4.6.

1. INTRODUCTION

Microbial food safety is an increasing public health concern worldwide. Each year, as many as 600 million, or almost one in 10 people in the world, fall ill after consuming some form of

contaminated food. Of these, 420,000 people die, including 125,000 children under 5 years of age as stated in the World Health Organization's estimates on the global burden of food-borne diseases. Since ancient times, commercial antimicrobial agents have been applied as a way to manage food deterioration or contamination. Nowadays, user concerns towards synthetic preservatives have resulted in increasing focus on various natural antimicrobials such as essential oils. Aromatic and medicinal plant essential oils and their components demonstrate antibacterial, antifungal, and food preservative activities against a wide range of microbial pathogens [1].

Thyme (*Thymus vulgaris* L.) is a flowering, aromatic perennial evergreen herbs in the mint family Lamiaceae, originating from the Western Mediterranean to southern Italy but has now acclimated all over the world. In Vietnam, thyme has been used for many years as a food additive and traditional folk medicine. This herb is mainly grown in Da Lat and Sa Pa, sold both fresh and dried but is able to retain its flavor upon drying better than many other herbs. The fresh form is more flavorful, but also less convenient due to short storage life being rarely more than a week. Many pharmacological studies demonstrated that *Thymus vulgaris* L. possesses antimicrobial functions, and thymol, a major compound in its essential oil, is an antiseptic ingredient in various commercially produced mouthwashes such as Listerine [2, 3].

A number of reports on the essential oil composition of thyme leaves have been published. K. Chetehouna *et al.* [4] identified and categorized the volatile compounds in thyme essential oil into 6 main groups: monoterpene, sesquiterpene, and oxygen derivatives composed of monoterpenic alcohol, monoterpenic phenol and monoterpenic ether. In addition, regardless of any variations in temperature and duration of the conducted experiments, the three main identified volatile components always were thymol, *p*-cymene and γ-terpinene. In other studies, essential oils from Romania [5], Brazil [6] and India [7] were found to be quite rich in thymol (30.82 %; 44.7 % and 61.63 %, respectively). Additionally, *p*-cymene was reported as one of the main components in Romanian and Indian essential oils (30.53 % and 11.28 %, respectively) but only played a minor role in the Brazilian sample (accounting for only 0.1 %). Finally, γ-terpinene appeared only in EOs from Brazil and India, and was absent in Romanian essential oils.

A number of studies have focused on the chemical characteristics and antimicrobial activities of EOs from natural flora in a specific region or country. However, research about essential oil composition and the antimicrobial activities of EO from Vietnamese *Thymus vulgaris* L. has not been previously reported yet. The purpose of this study was to determine the EO chemical composition and also to investigate the antimicrobial activities of *Thymus vulgaris* L. essential oil against several food-borne, pathogenic bacteria and fungi.

2. MATERIALS AND METHODS

2.1. Materials, reagents, bacterial strains and culture conditions

Fresh leaves of Vietnamese thyme were collected from the cultivated plants during April and May 2018 in Dai Yen village, Doi Can, Hanoi. The samples were collected and preserved according to the method of Trang *et al.* [8]. Plants were confirmed as *Thymus vulgaris* L. at the Department of Plant, Faculty of Agronomy, Vietnam National University of Agriculture. Mueller-Hinton broth (MHB), commercial standard discs (φ = 6 mm) and other discs were purchased from Becton, Dickinson and Company (New Jersey, USA). DPPH and MeOH were imported from Sigma-Aldrich, USA (Sigma-Aldrich, St. Louis, MO, USA). All other reagents

were of the highest commercial grade available. Milli-Q water or sterilized water was used in all procedures.

Seven food-related species: *Escherichia coli ATCC* 25923™ (*E.coli*), *Bacillus cereus* ATCC 13061™ (*B. cereus*), *Staphylococcus aureus* sub sp. ATCC 25022™ (*S. aureus*), *Salmonella Typhimurium* ATCC 14028 (*S.* Typhimurium), *Candida albicans* ATCC 10231 (*C. albicans*), *Rhizoctonia solani*, *Fusarium oxysporum* obtained from the American Type Culture Collection were used in this study. All strains were grown on MHB agar plates supplemented with 1.4 % agar and incubated at 37 ^{o}C under aerobic conditions for 24 hrs.

2.2. Essential oil preparation

One hundred g of fresh thyme leaves were cut into 1 cm pieces and then transferred immediately into a Clevenger-type apparatus, to which 400 mL of deionized water was added. The essential oil was obtained by distillation for 3 hrs according to the Vietnamese standard TCVN 7039:2002, its physical properties were determined according to TCVN 8450:2010. The essential oils were preserved in a sealed vial at 4 ^{o}C.

2.3. Assay for antimicrobial activity

Using the filter paper disc diffusion method on MHB agar plate, the bacterial growth inhibition by EO was assessed. Sterile standard discs (φ= 6 mm) containing essential oils were placed on the MHB agar plates previously spread with 0.1 mL of bacterial suspension (cell density = 10^6 CFU/mL) in MHB liquid medium. The plates were kept in the refrigerator for 4 hrs for the diffusion of EO in the plate and incubated at 37 ^{o}C for 24 hrs under aerobic conditions. The diameters of the zones of inhibition were measured and recorded in millimeters and the values were given as the average of three replicates at least. The inhibition zones were measured and recorded in millimeters. An inhibition zone greater than 6 mm is defined as antibacterial active [8].

The minimum inhibitory and bactericidal concentrations (MICs and MBCs) were determined by microbroth dilution assay in 96-well microtitre plates, according to the previous report [9]. EO is dissolved in sterile water containing 0.5% Tween 80 to achieve the final concentration ranging from 0.39 µl/mL to 50 µl/mL. Each well (200 µl) contained 20 µL of sample; and 20 µl of bacterial suspension (10^6 CFU/mL) and 160 µl of MHB medium. After incubation at 37 ^{o}C for 24 hrs, the optical density was measured at 600 nm using an Elisa reader (Bio-rad Model 680, Japan). The MIC was determined as the lowest concentrations showing no growth. The MBCs were determined by spreading 10 µl of the culture on MHB agar plate, followed by inoculation at 37 ^{o}C for 24 hrs. The lowest concentration with no visible growth was defined as the MBC, indicating death of 99.5% of the original inoculums. All tests were performed in triplicates.

2.4. Preliminary evaluation of antioxidant evaluation with 1,1-Diphenyl-2-Picrylhydrazyl (DPPH)

DPPH measurements were used based on the method of Brand-Williams and co-workers and Lee and co-workers [10, 11], in which the determination of antioxidant potency is based on the scavenging activity of the stable DPPH free radical. DPPH was mixed with methanol to form a 0.25 µM solution. Trolox mixed in methanol 80 % with concentrations of 31.25 –500 µg/mL, using as the positive control. To conduct the experiment, the sample was dissolved in methanol

80 %. Then, 180 µL of the prepared DPPH solution was added to 120 µL of the sample solution. The resultant was shaken, and stored in darkness at 30 ◦C for 30 min. The pigment solution was obtained by adding 180 µL of 80 % MeOH solution to 120 µL of the sample solution (120 µL). For the blank solution, instead of the sample, 120 µL of 80 % MeOH solution was used. The absorbances of the solutions were measured at 517 nm. Each experiment was repeated 3 times to calculate the average. The percentage of DPPH radical scavenging was calculated according to the following formula: DPPH (%) = $[OD_b-(OD_s-OD_c)]$ /OD_b , where OD_b is the optical density of the blank sample; OD_s is the optical density of sample; OD_c is the optical density of pigment; and IC50 value was calculated by the graph of % inhibition.

2.5. GC-MS analysis

Volatile compounds of the EO were analyzed by GC-MS QP 2010 (Shimazu, Japan) equipped with a flame-ionization detector (FID). An DB-5 capillary column (30 m × 0.25 mm i.d., and 0.25 µm) was used. The oven temperature was maintained at 60 ^oC for 4 min, increased to 230 ^oC at the rate of 3 ^oC / min and then held for 15 min. The injector and detector temperatures were set at 200 ^oC and 230 ^oC, respectively. Helium was used as the carrier gas at a flow rate of 1.5 mL/min. Each compound was identified by the agreement of mass spectrum with those of the authentic compound in the GC-MS library (Willey, Chemstation).

3. RESULTS AND DISCUSSION

3.1. Volatile components in the essential oil from thyme

After 3 hrs of distillation, the EO of thyme leaves with deep yellow color and typical flavor was obtained. Its physical properties were determined according to the respective Vietnamese standards. The result was shown in Table 1.

Table 1. Physical properties of EO from thyme leaves.

Properties	Density at 20 ^oC (g/mL)	Acid index (mg KOH/g)	Ester index (mg KOH/g)
Values	0.887	4.675	4.203

The relative peak area of each compound to the total peak area (%) is expressed in Table 2. Agreement of the mass spectra in the GS-MS library enabled 26 compounds to be identified as the volatiles in the essential oil of thyme leaves. The results showed that thymol (39.79 %) was the principal component; cymene and *γ*-terpinene were the second and third major compounds in this essential oil, accounting for 13.45 % and 17.33 % of total peak area, respectively. These findings are quite in agreement with those of the previous study in which thymol was the major component of EO of Thyme leaves from Romania [5], Brazil [6] and India [7], accounting for 30.82 %, 44.7 % and 61.63 % of total peak area, respectively. On the other hand, some authors also reported cymene as the major compound of the EO from Romania and India with 30.53 % and 11.28 % of total peak area; however this component only played a minor role (0.1%) in the EO obtained from Brazil thyme leaves. Furthermore, γ-terpinene proportions in thyme essential oil of Viet Nam, Brazil and India were quite high (13.45 %), but were absent in Romanian leaves. The different contents in the EO thyme leaves may reflect variations due to origin, geographical location, etc.

Table 2. Volatile compounds identified in the essential oil obtained from thyme leaves.

No.	Compounds	Relative area* (%)
1	2 – hydroxyl isobutyric acid	0.41
2	*α* - thujene	0.44
3	*α* – pinene	5.02
4	camphene	0.29
5	*β* – pinene	0.43
6	1 – octen – 3 – ol	1.06
7	*β* – myrcene	1.62
8	*α* – ocimene	0.85
9	*δ* – 2– carene	1.35
10	cymene	17.33
11	limonene	0.66
12	*1,8* – cineole	0.90
13	*γ*-terpinene	13.45
14	*(Z)* – *β* – terpineol	1.07
15	linalool	3.87
16	camphor	0.51
17	borneol	1.21
18	4 – terpineol	0.59
19	*(Z)* - thymol methyl ether	0.49
20	*(E)* - thymol methyl ether	0.23
21	thymol	39.79
22	carvacrol	1.94
23	junipene	0.42
24	*β* – caryophyllene	4.17
25	germacrene *D*	0.36
26	caryophyllene oxide	0.41

* Peak area relative to the total peak area (%) on GC-MS with DB-5 column.

3.2. Antimicrobial activity of Vietnamese thyme EO against food-borne bacteria and fungi

Vietnamese thyme EO had been found to have a good antimicrobial activity against various bacteria and fungi (Table 3). Thyme EO showed the antibacterial effect against all the tested pathogenic strains, with the inhibition zones being 23.3 ± 0.4 mm, 24.7 ± 0.4 mm, 29.0 ± 0.7 mm, 32 ± 0.7 mm in diameter against *B. cereus, E. coli, S.* Typhimurium, and *S. aureus,*

respectively (Table 3). Similar results were also obtained with Serbia thyme samples against *B. cereus*, *E. coli*, *S.* Typhimurium and *S. aureus* by Boskovica *et al*. [12]. The Vietnamese thyme EO was found in this study (Table 2) to be a variable mixture of 26 active compounds. The most dominant of all identified compounds of thyme EO were thymol (39.79 %), cymene (17.33 %) and γ-terpinene (13.34 %). The composition and active ingredients of EO are greatly influenced by genotype, environmental factors including geographical conditions, nature of soil, temperature, season of collection and harvesting plant, and more importantly, the oil extraction procedure. Although differences in components were found in Vietnamese Thyme EO upon comparison with Serbian Thyme EO (thymol (50.48 %), followed by p-cymene (24.79 %), linalool (4.69 %), the strong antimicrobial activities against pathogenic bacteria were very similar. From these results, thyme EO showed potential activities against both Gram positive and Gram negative tested bacteria. Thymol (2-isopropyl-5-methylphenol) is a monoterpene phenol, and was found to be the most effective against both Gram negative and Gram positive bacteria [13]. Thymol-rich essential oils have been evaluated for their possible benefits in medical applications. In the field of food technology, Mexican Thyme (0.1 %) and Mexican lime (*Citrus aurantifolia*) (0.5 %) oil reduced disease incidence in papaya fruit [14], extended the storage life of banana by up to 28 days and reduced fungal disease incidence in banana [15]. The antimicrobial activity of cymene was also used as a novel means of controlling *Escherichia coli* O157:H7 in un-pasteurised apple juice [16]. The results in this study indicated that Vietnamese Thyme EO could be applied as an antimicrobial reagent in food preservation. Several authors have been investigating the mechanism of antimicrobial activities of EO against pathogenic bacteria. EO and their components showed activities against a variety of targets, particularly the membrane and cytoplasm, and even changed the morphology of the cells, which enhanced its incorporation into the cell membrane, resulting in degradation of the cell [3]. García-Salinas *et al.* [17] also cited bacterial membrane disruption as the bactericidal mechanism exerted by Thymol in the EOs.

Table 3. Antimicrobial activity of Vietnamese thyme essential oil against food-borne bacteria.

No.	Bacteria and fungi strains	Gram	Average diameter of inhibition zone (mm)* used 5 µl of EO after three trials
1	*B. cereus*	+	23.3 ±0.4
2	*S. aureus*	+	32.0 ±0.7
3	*E. coli*	-	24.7 ±0.4
4	*S.* Typhimurium	-	29.0 ±0.7
5	*Candida albicans*		44.1 ± 0.1
6	*Rhizoctonia solani*		32.3 ± 0.1
7	*Fusarium oxysporum*		23.2 ± 0.1

* Diameter of each disc was 6 mm and the inhibition circle representing more than 6 mm is defined as anti-bacterial active; Values are the mean of triplicates at least.

Results of antifungal activity of the thyme EO are shown in Table 3. The essential oil also showed antifungal activities against three important pathogenic fungi such as *Candida albicans*, *Rhizoctonia solani* and *Fusarium oxysporum* (Table 3) with the inhibition zones ranging from 23.2 to 44.1 mm. The strongest antimicrobial activity was found against *Candida albicans* with

inhibition zones of about 44.1 mm in diameter, followed by *Rhizoctonia solani (*32.3 ± 0.1 mm). *Candida albicans* is the most common human fungal pathogens, ranked as the fourth-greatest cause of nosocomial bloodstream infections, while *Fusarium oxysporum* is responsible for skin invasion [18]. Similar results of the thyme EO's antifungal activity against *C albicans* isolated from patients with denture stomatitis were also reported [19].

Curiosity about essential oils that can act as antimicrobial agents is growing because of the broad range of activities, natural origins, and generally recognized as safe (GRAS) status of essential oils [20]. The results of this study clarified that the addition of natural antimicrobial substances such as Vietnamese thyme EO provides a potential new route to ensure safety and extend the shelf-life of food.

3.3. Killing effects of EO against pathogenic bacteria

Vietnamese thyme EO exhibited inhibition activities and bactericidal activities against all tested pathogenic strains (Table 4). To determine the bactericidal activity of this EO against pathogenic bacteria, killing experiments were performed in the presence of 2 x MIC to 64 × MIC of thyme EO. The bactericidal effects of thyme EO, a reduction of more than $10^{4\text{-}5}$ CFU mL^{-1} of culturable cells, were mainly observed after 24 hrs of incubation for all isolates at a concentration of 100 µl/mL. The bactericidal behavior of this EO did not differ significantly in all isolates. The MIC and MBC values of thyme EO against *Bacillus cereus, Staphylococcus aureus* and *Escherichia coli* were 1.56 µl/mL and 3.13 µl/mL, respectively. In most cases, considering the ratio MBC to MIC was < 4, the majority of EO were bactericidal against the tested strains. The results indicated that Vietnamese thyme EO showed strong inhibition and bactericidal effect against food-borne bacteria.

Table 4. Killing ability of EO of thyme against food-borne bacteria.

Bacteria strains	Gram	MIC (µl/mL)	MBC (µl/mL)
B. cereus	+	1.56	3.13
S. aureus	+	1.56	3.13
E. coli	-	1.56	3.13
S. Typhimurium	-	1.56	1.56

In another study, Deans *et al.* [21] found that the susceptibility of Gram-positive and Gram-negative bacteria to plant volatile oils had little influence on growth inhibition. The structure of the Gram- positive bacteria cell wall allows hydrophobic molecules to easily penetrate the cells and act on both the cell wall and within the cytoplasm. It is often reported that Gram-negative bacteria are more resistant to plant-based essential oils because of the differing structures of the cell walls of Gram-negative and Gram positive bacteria [22]. However, Vietnamese thyme EO did not show much difference in antimicrobial activity between Gram-positive and Gram-negative food-borne bacteria. The difference in the antimicrobial activities of EO depended on the composition of EO. Similar results were also obtained for Iranian thyme (MIC from 1.9 to 3.6 µg/mL). However, Vietnamese Thyme EO showed stronger antimicrobial activities against both Gram-positive and Gram-negative bacteria [19].

The results obtained in this study strongly indicated that Vietnamese thyme might be the candidate to develop natural antibiotics and disinfectants to control infective agents in food. The bacteria studied above are considered to be the most common pathogen causing outbreaks of food poisoning and food-spoilage [23, 24]. Thus, thyme EO could be used as a natural source for food preservative to reduce, substitute, or avoid chemical preservatives.

3.4. Preliminary evaluation of antioxidant activity of thyme EO

The free radical inhibition percentage of thyme EO and the control sample were shown in Fig. 1. When the EO sample was prepared at a concentration from 1563 to 2500 µg/mL, the IC_{50} of the tested sample was 1020.05 µg/mL This results were similar with the study cited as [25], in which the antioxidant activity of thyme EO at a range of concentration from 60 to 2500 µg/mL was reported with the value of IC_{50} at 1377 ± 1.70 µg/mL. In another study [26], T. Kulisic *et al.* reported that IC_{50} of thyme EO was 300 µg/mL, lower than that of our result.

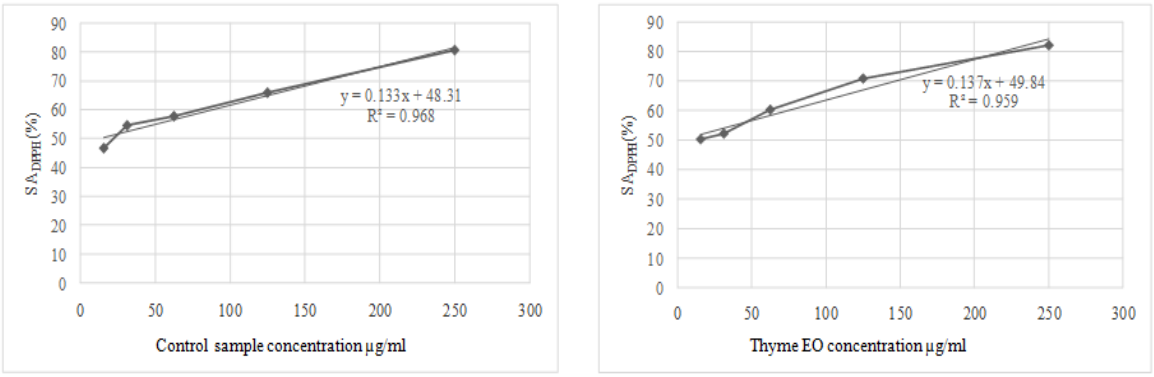

Figure 1. The free radical inhibition percentages of Thyme EO and control samples.

4. CONCLUSION

The growing tendency for replacing synthetic additives with natural ones has brought about great interest in the evaluation of antimicrobial properties of the plant products because of their relatively safe status, wide acceptance by consumers, and their exploitation for potential multipurpose functional use. To the best of our knowledge, this is the first report on the chemical composition, antioxidant and antimicrobial activities of Vietnamese thyme (*Thymus vulgaris* L.) EO. Vietnamese Thyme EO contained 26 individual components with three main bioactive compounds, which are thymol (39.79 %), *p*-cymene (17.33 %) and *γ*-terpinene (13.45 %). Data presented in this study revealed strong *in vitro* antimicrobial activity of Vietnamese *Thymus vulgaris* EO against food pathogen and food borne bacteria including Gram – positive, Gram – negative, and fungi with the inhibition zone ranging from 23.3 to 44.1 mm; low MIC and MBC values (1.56 µl/mL and 3.13 µl/mL). The results of this study also showed the antioxidant activity of Vietnamese thyme EO and suggested that this EO could be used as a natural source for food preservative to reduce, substitute, or avoid chemical preservatives.

REFERENCES

1. Gormez A., Bozari S., Yanmis D., Gulluce M., Agar G., Sahin F. - The use of essential oils of *Origanum rotundifolium* as antimicrobial agent against plant pathogenic bacteria, J. Essent. Oil Bear. Plants **19** (2016) 656–663.
2. Gavahian M., Farahnaky A., Javidnia K., Majzoobi M. - Comparison of ohmic-assisted hydrodistillation with traditional hydrodistillation for the extraction of essential oils from Thymus vulgaris L, Innovative Food Science & Emerging Technologies **14** (2012) 85-91.
3. Saharkhiz M. J., Kamyab A. A., Kazerani N. K., Zomorodian K., Pakshir K., and Rahimi M. J. - Chemical compositions and antimicrobial activities of *Thymus vulgaris* L. essential oils at different harvest stages, Jundishapur J. Microbiol **8** (1) (2015) e13720.
4. Chetehouna K., Courty L., Lemee L., Garo J. P. - Experimental determination of volatile organic compounds emitted by *Thymus vulgaris*, Modelling, Monitoring and Management of Forest Fires **3** (2012) 189-198.
5. Nikolić M., Glamočlija J., Ferreira I. C. F. R., Calhelha R. C. - Chemical composition, antimicrobial, antioxidant and antitumor activity of *Thymus serpyllum* L., *Thymus algeriensis* Boiss. and Reut and *Thymus vulgaris* L. essential oils, Industrial Crops and Products **52** (2014) 183-190.
6. Alexandre P., Godoy R. - Chemical composition of *Thymus vulgaris* L. (thyme) essential oil from the Rio de Janeiro State (Brazil), J. Serb. Chem. Soc. **73** (3) (2008) 307–310.
7. Syamasundar K.V., Srinivasulu B., Stephen A., Ramesh S. and Rao R. R. - Chemical composition of volatile oil of *Thymus vulgaris* L. from Western Ghats of India, Journal of Spices and Aromatic Crops **17** (3) (2008) 255–258.
8. Trang V. T, Van N. T, Quynh C. T. T. - Antimicrobial activities of essential oil of *Perilla frutescens* against food-borne bacteria. Journal of Science and Technology, Vietnam Academy of Science and Technology **51** (6A) (2013) 35-41.
9. Nakhaei M., Khaje-Karamoddin M., and Ramezani M. - Inhibition of *Helicobacter pylori* growth *in vitro* by Saffron (*Crocus sativus* L.), Iran Journal of Basic Medical Science **11** (2008) 91-96.
10. Brand-Williams W., Cuvelier M. E., Berset C. - Use of a free radical method to evaluate antioxidant activity, Lwt-Food Sci. Technol. **28** (1995) 25–30.
11. Lee J., Durst R. W., Wrolstad R. E. - Determination of total monomeric anthocyanin pigment content of fruit juices, beverages, natural colorants, and wines by the pH differential method: Collaborative study. J. AOAC Int. **88** (2005) 1269–1278.
12. Boskovica M., Zdravkovica N., Ivanovica J., Janjica J., Djordjevica J., Starcevica M., Baltica M. Z. - Antimicrobial activity of Thyme (*Tymus vulgaris*) and Oregano (*Origanum vulgare*) essential oils against some food-borne microorganisms, Procedia Food Science **5** (2015) 18-21.
13. Marchese A., Orhan I. E., Daglia M., Barbieri R., Lorenzo A. D., Nabavi S. F., Gortzi O., Izadi M., Nabavi S. M. - Antibacterial and antifungal activities of thymol: A brief review of the literature, Food Chemistry **210** (2016) 402–414.
14. Borugă O., Jianu C., Mişcă C., Goleţ I. - Thymus vulgaris essential oil: chemical composition and antimicrobial activity, Journal of medicine and life **7** (3) (2014) 56-60.

15. Maqbool M., Ali A., and Alderson P. G. - Effect of cinnamon oil on incidence of anthracnose disease and post-harvest quality of bananas during storage, Int. J. Agric. Biol. **12** (2010) 516–520.
16. Kiskó G. and Roller S. - Carvacrol and p-cymene inactivate Escherichia coli O157:H7 in apple Juice, BMC Microbiology **5** (2005) 25-36
17. García-Salinas S., Elizondo-Castillo H., Arruebo M., Mendoza G., Irusta S. - Evaluation of the Antimicrobial Activity and Cytotoxicity of Different Components of Natural Origin Present in Essential Oils, Molecules **23** (2018) 1399.
18. Cheng S. C., Joosten Leo A. B., Kullberg B. J., Netea M. G. - Interplay between *Candida albicans* and the Mammalian Innate Host Defense, Infect Immun. **80** (4) (2012) 1304–1313.
19. Mohammadmehdi F., and Jamshid K. - *In Vitro* Antimicrobial Activity of Thymus vulgaris Essential Oil Against Major Oral Pathogens. Journal of Evidence-Based Complementary & Alternative Medicine **22** (4) (2017) 660-666
20. Pandey A. K., Kumar P., Singh P., Tripathi N. N., and Bajpai V. K. - Essential Oils: Sources of Antimicrobials and Food Preservatives, Frontier in Microbiology **7** (2017) 1-14.
21. Deans S. G., Noble R. C., Hiltunen R., Wuryani W., and Penzes L. G. - Antimicrobial and antioxidant properties of *Syzygium aromaticum* (L) Merr Perry: impact upon bacteria, fungi and fatty acid levels in ageing mice, Flavour and Fragrance Journal **10** (1995) 323–328.
22. Zhang Y., Liu X., Wang Y., Jiang P., Quek S. - Antimicrobial activity and mechanism of cinnamon essential oil against *Escherichia coli* and *Staphylococcus aureus*, Food Control **59** (2016) 282-289.
23. Saha S., Dhar T.N., Sengupta C. and Ghosh P. - Biological activities of essential oils and methanol extracts of five *Ocimum* species against pathogenic bacteria, Czech J. Food Sci. **31** (2) (2013) 194-202.
24. Joshi R.K. - Chemical Composition, *In vitro* Antimicrobial and Antioxidant Activities of the Essential Oils of *Ocimum gratissimum*, *O. sanctum* and their Major Constituents, Indian Journal of Pharmaceutical Sciences, **75** (4) (2013) 457 - 462.
25. Grespan R., Aguiar R. P., Giubilei F. N., Fuso R. R. - Hepatoprotective Effect of Pretreatment with *Thymus vulgaris* Essential Oil in Experimental Model of Acetaminophen-Induced Injury, Evid Based Complement Alternat Med, (2014) 954136. doi: 10.1155/2014/954136. Epub 2014 Feb 4.
26. Kulisic T., Radonić A., Milos M. - Antioxidant properties of thyme (*Thymus vulgaris* L.) and wild thyme (Thymus serpyllum L.) essential oils, Ital. J. Food Sci. **3** (17) (2005) 1-10.