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Original Article

Study of Chemical Composition, Antibacterial and Antioxidant Activity of Thyme Leaves and Stems Essential Oil

Ali Mehrabi[1,2], Razzagh Mahmoudi[3*], Hajar Khedmati Morasa[1], Shaghayegh Mosavi[4], Masoud Kazeminia[5], Fatemeh Attaran Rezaei[6], Saeed Shahsavari[7] and Roghayeh Vahidi[7]

[1]Food Hygiene and Safety, School of Public Health, Qazvin University of Medical Sciences, Qazvin, Iran
[2]School of Health, Qazvin University of Medical Sciences, Qazvin, Iran
[3]Medical Microbiology Research Center, Qazvin University of Medical Sciences, Qazvin, Iran
[4]Faculty of Medical Sciences, Qazvin University of Medical Sciences, Qazvin, Iran
[5]Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran
[6]Department of Microbiology, Faculty of Medical Sciences, Qazvin University of Medical Sciences, Qazvin, Iran
[7]Health Productions Safety Research Center, Qazvin University of Medical Sciences, Qazvin, Iran

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ABSTRACT

Given the increase in drug resistance of microorganisms and the tendency in using medicinal plants, the present study was carried out to investigate the chemical compounds of Thymol as a constituent of essential oil and antimicrobial activity against several pathogenic bacteria and its antioxidant activity. In this study, the chemical compounds of Thymol extracted from the two parts of the plant together (the leaves and stems) were performed by making use of gas chromatography and the other gas chromatography linked to the mass spectrograph and its antibacterial activity against *Ecoli*, *Klebsiella*, *Pseudomonas*, *Staphylococcus*, *Salmonella Paratyphi B*, *Salmonella Typhi* typhus and *Enterococci* through Microdilution and the minimum inhibitory concentration (MIC), and the minimum bactericidal concentration (MBC) were investigated. The antioxidant activity of essential oils was measured through the ability of essential oil compounds in decolorizing diphenyl picrilhydrazyl (DPPH) free radicals. Chemical analysis of thyme essential oil resulted in the identification of 23 (83.68%) compounds, which Thymol with 25.30% was considered as the main part of the essential oil compound. The highest inhibitory effect was imposed on *Klebsiella*, *Escherichia coli*, and *Staphylococcus aureus*, and the largest inhibitory zone diameter of essential oil appeared against growth *S. paratyphi B*. Concerning the results, total phenol was equal to 114.3 mg of gallic acid.g and IC_{50} of thyme was considered to be 49.94 µM/ml. The achieved results from this study declared that thyme essential oil had a suitable inhibitory effect against pathogenic bacteria and also possessed antioxidant properties. In other words, thyme was considered as an alternative to synthetic drugs and food additives.

INTRODUCTION

Nowadays, infectious pathogens and their resistance to various antibiotics are known as major challenges in medical science, and subsequently, the production of new antibiotics is increasing daily. Hence, researchers attempt to select herbal alternatives that, while having antibacterial effects, have no side effects caused by chemical drugs [1]. For this alarming situation, the identification and evaluation of adequate procedures to control pathogens and ensure the safety of food products have become one of the biggest challenges today [2]. The active plant ingredients used for medicinal purposes help microbial control agents, especially bacteria [3]. Many plants are used due to their antimicrobial properties, which are as a result of the compounds synthesized in the secondary metabolism of the plant. These products are known through their active ingredients, such as phenolic compounds, which are one part of the Essential oil (EO) [4]. Herbal EOs are

*Corresponding author: Medical Microbiology Research Center, Qazvin University of Medical Sciences, Qazvin, Iran
Email Address: r.mahmodi@yahoo.com

comprised of complex mixtures of volatile compounds produced by living organisms obtained through physical methods such as extraction and distillation of all plants, or parts of the plant [5]. There is no certain reason for the formation of these EOs. Still, these compounds are generally affected by the main metabolic processes of plants, especially under stress that is not chemically homogenous and is often observed in different forms with terpenes origin [6]. The antimicrobial properties of EOs are popular for many years. Besides antimicrobial properties, EOs also have antifungal, antiviral, anti-parasitic effects [7]. The thymus is one of the most important items of the Lamiaceae family and is one of the most famous items belonging to EOs. It is observed typically in the Mediterranean, Asia, Southern Europe, and North Africa and has 300-400 species. In Iran, 18 stable and aromatic species grow in different parts of the country [8]. Phytochemical analysis of thyme species confirms the existence of phenolic compounds such as Thymol, carvacrol, thymonine, caffeic acid, and rosemarinic acid, terpenoids, phallaenoids and saponins [9]. Thymol and carvacrol are the main constituents of the EOs in the lamiaceae family. The two compounds are very similar in chemical terms, and the only difference between them is the position of the hydroxyl group. Thymol and carvacrol are highly effective antimicrobial components available in EOs. Their antimicrobial effect is because of their permeability of the cell membrane, which is able to collide with the cations on the surface of the membrane and then hamper the vital activities [9]. In other words, the mechanism of the antibacterial effect of herbal EOs is ralted in their hydrophobic properties, which leads to the penetration of these substances into the phospholipids of the bacterial membrane and a disruption in their structure and an increase in permeability. This eventuates to the withdrawal and leakage of ion and other cellular contents, which will ultimately lead to the death of the bacteria [10]. The EO of this plant also exhibits different biological activities, such as antioxidant properties [8]. Nowadays, using new preservative methods such as the use of bacteriocins and herbal EOs particularly are shining in the food industry [11]. Lately, the use of herbal EOs and plant extracts is pretty considered in order to expand food additives for preventing the growth of foodborne pathogens or postponing the invasion of food spoilage agents [12]. Uninterrupted use of chemical drugs results in the formation of highly resistant microbes that are under no or less influence of chemical drugs [13]. While many medicinal plants have a lot of positive effects, they will entail fewer harms and side effects compared to chemical drugs [14]. This is why many researchers prefer to study the antibacterial effects of plant extracts [15]. The most popular pathogenic microorganisms can be named as *Ecoli*, *Klebsiella*, *Pseudomonas*, *Staphylococcus*, and *Enterococci*. Food poisoning posed by *S. aureus* (*S. aureus*) and *Escherichia coli* (*E. coli*) is one of the most remarkable diseases. A lot of money is spent annually on controlling and treating the diseases caused by them. In the United States, *Salmonella typhimorium* (*S. typhimorium*) and *S. aureus* are regarded as the first and second leading causes of foodborne illness [16], respectively. Moreover, medicinal plants are supposed as valuable sources of natural antioxidants, such as some terpenoids and phenolic compounds. They potentially are an appropriate alternative to synthetic antioxidants in terms of reducing oxidative stress [17]. The antioxidant is a substance that is present in low concentrations compared to the oxidizable substrate and can significantly delay or prevent the oxidation of that substrate [18]. Therefore, in this study, the chemical compounds and antioxidant properties and antibacterial effect of thyme plant EO against *Ecoli*, *klebsiella*, *Pseudomonas*, *Staphylococcus*, *S. paratyphi B* (*S. paratyphi B*), *S. typhi* typhus and *enterococci* were investigated.

MATERIAL AND METHODS

Plant material

The studied thyme plant was collected (5 kg) from Qazvin city (Latitude: 36° 16' 7.57" N, Longitude: 50° 00' 14.76" E) of Iran in 2019.

EO extraction

The dried form of the plant was ground to isolated the EO and then, hundred gram powdered plant material was subjected to hydro-distillation (1000 ml distillated water) for 5 h using a Clevenger-type apparatus [18]. The EO was dried by sodium sulfate (Merck Co. Germany) and then stored in a dark-colored vials at 4 ± 1 °C in a refrigerator [19].

Analysis of EO

GC-MS condition

In order to decompose the Chemical Constitution of EOs, first, the isolated EO sample of the plant was injected into the gas chromatography-mass spectrometry (GCMS) (Agilent Technologies, Palo Alto, CA, USA), and the most appropriate column temperature programming was obtained for complete separation of EO compounds. The EO was then injected into the GCMS connected to the mass spectrograph, and the mass spectrum of the compounds was achieved. In the present study, the GCMS was applied along with HP5MS capillary column with a length of 30m, an internal diameter of 0.25 mm and internal layer thickness of 0.25 µm with a column temperature programming initially at 70 ºC with 2 minutes stop at this temperature, and then with an increase in the temperature to 220 ºC and rate of 15 ºC per minute and an increase in the column temperature to 300 ºC for 2 minutes. The injection chamber temperature was 290ºC, and helium gas was used as the carrier gas at a flow rate of 0.8 mm/min. The mass spectrograph was 220ºC, which was used by the Agilent5973 (Palo Alto, CA, USA) model with ionization energy of 70 E/V and EI indicator and ionization source temperature [20]. Yield was calculated as percent of the ratio between extracted EO and the plant dry weight. The yield was calculated using the following equation, where W1 is the mass of the extracted oil (g) and W2 is the plant dried biomass (g) [21].

EO yield (%) = W1 / W2 × 100

Evaluation of antibacterial activity of EO

To evaluate the antibacterial activity of EO, Gram-positive and Gram-positive pathogenic bacteria were of paramount importance in causing infections and food poisoning (*Klebsiella* bacteria, *S. aureus*, *P. aeruginosa*, *S. paratyphi B*, *Salmonella typhi* (*S. typhi*), *E. coli*, *Enterococci*), which were prepared and used as active culture from the Microbiology Laboratory of Qazvin University of Medical Sciences. Disk diffusion test was applied to evaluate the antimicrobial impacts of the desired EO on *E. coli*, *S. aureus*, *P. aeruginosa*, *S. paratyphi B*, *S. typhi*, *Klebsiella*, and *Enterococci*. So, a bacterial suspension equal to the 0.5 McFarland standard was prepared from each of the studied bacteria in the solution of sterile physiological serum, and then 100 µl of bacterial suspension was grown in Mueller Hinton Agar (MHA) medium (Merck Co., Darmstadt, Germany). Then, it was placed on the culture medium of the raw antibiotic disk with a diameter of 6 mm (Oxoid). Then, 20 µl of concentrations of 100%, 80%, 50%, 25%, 12.5%, 6.25% of thyme EO was poured on the raw antibiotic disk, and the plates were heated in the oven at 37ºC for 24 hours [1]. Antibiogram ampicillin, amoxicillin, azithromycin, erythromycin, penicillin, tetracycline, vancomycin, and chloramphenicol were applied. All tests were conducted in three replications, and average results were obtained. The inhibition zone was measured and evaluated in millimeters.

Minimal inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC)

The basis for determining the MIC and MBC was taken place regarding the inhibition of bacterial growth by the tested material in such a way that no turbidity should be observed visually in the desired pipes after completion of the heating period. MIC and MBC were assigned for the desired microorganisms through Microdilution. 96 well plates round bottom (Made in korea brand SPL) was used in this study with a volume of 300 µl. to determine the MIC of EO on the bacterium, 20 µl of EO with the percentage in question and 160 µl of the broth medium were added to each well. Then 20 µl of bacterial suspension was added to each well (the studied bacterial suspension was set to 0.5 McFarland standard). The well was also observed as a positive control containing 180 µl of the nutrient broth medium along with 20 µl of bacteria. One well was prepared, which only encompassed the broth medium. A well, containing 20 µl of EO and 180 µl of broth medium, was prepared to control possible contamination. In the following, the microplates were homogenized for 20 seconds (300 rpm). The microplates were heated for 24 hours at 37 ºC, and after heating, turbidity or non-turbidity was observed visually in the wells [18,22]. The concentration of the first well without turbidity, which was the outcome of bacterial growth, was regarded as MIC. To measure MBC, sterile swabs were impregnated with the contents of the turbid-free wells under completely sterile conditions near the flame and were cultured on the surface of the TSB medium through area method. The plate was kept at a temperature of 37 ºC for 24-48 hours and then examined in terms of bacterial growth. The minimum

concentration that inhibited the growth of 99.9% of bacteria (MBC) would be taken into consideration. The effect of thyme EO in concentrations of 100%, 80%, 50%, 25%, 12.5% and 6.25% was examined. It was worth considering that positive and negative control was regarded in each series of tests, and each of the mentioned stages.

Evaluation of diphenylpicrylhydrazyl (DPPH)

The antioxidant activity of thyme EO was measured based on the hydrogenating ability of the EO by decolorizing the purple-colored methanol solution of DPPH stable radical. In this spectroscopic assessment, the DPPH stable radical was used as a reagent agent. 50 µl of different concentrations of EO was added to 5 ml of 0.004% methanolic solution of DPPH. The mixture was shaken vigorously and left standing at room temperature for 30 min in the dark. The absorbance was then measured at 517 nm. Decreasing of DPPH solution absorbance indicates an increase of DPPH radical scavenging activity. The amount of methanolic extract necessary to decrease the absorbance of DPPH by 50 % (IC50) was calculated using the following equation [18].

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

Where "$A_{control}$" is the absorbance of the control reaction (absorbance of DPPH solution) and "A_{blank} " is the absorbance of the blank solution (absorbance of the EO solution) and "A_{sample} " is the absorbance of the test compound (absorbance of the EO with DPPH).

The measurement of total phenolic materials was also carried out using the folin ciocalteu and gallic acid reagent as standard. 0.01 ml of the EO was transferred to Erlenmeyer, and 46 ml of distilled water and 1 ml of ciocalteu reagent were added to it. The contents of Erlenmeyer flask were mixed with high intensity. After 3 minutes, 3 ml of 2% sodium carbonate solution was added, and the mixture was placed on a shaking plate with medium intensity for 2 hours, and its absorption was read at 760 nm. The above steps were performed for standard solutions of gallic acid, and then one standard curve was achieved [18].

Statistical analyses

Data analysis was performed through statistical software of SPSS 23. Variance analysis was used to examine the significant differences in the obtained results, and Duncan test was found useful for multiple comparisons. The whole test steps were repeated three times, and the results were provided on average.

RESULTS AND DISCUSSION

EO yield and composition

The evaluation of the results of this study showed that the yield of the EO was 0.71 (v/w) based on dry weight. In study of Mohammadzadeh *et al*., showed that the yield of the EO before flowering was 0.43 % (v/w) and its after flowering yield was 0.2 % (v/w) based on dry weight [23]. In study of Borugă *et al*., showed that the isolation yield was 1.25% (v/w) [24]. In study of Fadil *et al*., showed that EO yield was 1.9%, 2.3% and 0.68% for *T. vulgaris L*., *R. officinalis* and *M. communis*, respectively [25]. In study of Khoshsokhan *et al*., showed that the yield of the Thymus EO was 2.5% in mazandaran city [17]. The chemical analysis of the EO showed that the EO contained a complex mixture of several components (Table 1). As could be observed, generally, 23 compounds (83.68%) were identified. The main compounds forming studied thyme were *Thymol* (25.30%), δ-2- carene (8.825%) and carvacrol (8.43%), respectively. In another study the presence of 45 compounds in Thymus essential oil such as carvacrol (52.4%), γ-trpynen (12.1%) and *Thymol* (10.4) was proved [8]. In study of Faldi *et al*., Forty-six constituents, which represented 97.47%, 98.88% and 99.96% of the total of *T. vulgaris L*., *R. officinalis* and *M. communis* L EOs, respectively, were identified. Thymol (37.54%), *p*-cymene (14.49%), γ-terpinene (11.15%), linalool and carvacrol (4.71% and 4.62%, respectively) were major components of *T. vulgaris* L. EO [25]. The herbal essential oils compounds widely changed based on the geographical area of plant growth, plant variety, age of the plant while preparing the essential oil, and the method of drying and extracting the essential oil [26]. In another study, the main compound in *T. daenensis* and *T. vulgaris* was Thymol with percentage of 43.8 for the former and 45.1 for the latter. The dominate compounds in *T. migricus*, *T. eriocalyx*, *T. serpylum*, *Zataria multiflora* and *T. kotschyanus* were linalool (41.8%), *geraniol* (61.8%), *para cymene* (23.8%), *carvacrol* (57.7%), and *Pulegone* (37.2%), respectively [27]. In another study the presence of 25 compounds for *T. vulgaris* essential oil such as *Thymol* (55.3%; 50.53%), *p-cymene* (11.2%;

11.79%), was proved [28]. One study conducted by Borugă *et al*. in Romania indicated that the most prevalent identified compounds in thyme essential oil were attributed to p-cymene (8.41%) and *γ-terpinene* (30.90%) and *Thymol* (47.59%) respectively [24].

Antibacterial activity

Moreover, the results of the antimicrobial effect of EO were exhibited in table 2. Based on the results of essential oil obtained from thyme in concentrations of 25%, 12.5% and 6.25% imposed no inhibitory effect on the studied microorganisms. All the studied bacteria were under the influence of inhibition in 100% concentration, and then the zone was formed. Also, at 50% concentration, the EO had an inhibitory effect only on *E. coli*, which was significantly different from the effect of the same concentration on the inhibition of other pathogens. The results also indicated that a direct relationship was observed between the concentration of EO and the inhibition zone of bacteria growth. It was understood from table 2 that the maximum inhibitory zone diameter at 100% concentration was attributed to *S. paratyphi B* and the minimum inhibitory zone diameter at 50% concentration to *E. coli.* The results of the analysis of variance test illustrated that there was a significant difference between the inhibitory zone diameters at different concentrations of extracted EO against the studied bacteria ($P< 0.05$). In other words, different concentrations were considered to have a significant effect on the inhibitory zone diameters. In the studies carried out inside and outside the country, it was observed that the antimicrobial and antifungal effects of several medicinal plants such as lavender, Zataria multiflora boiss, thymus, rosemary, nigella seed, sagebrush were studied and evaluated positive [8].

Table 1 Chemical composition of thyme EO

Number	Composition name	R.T*	%
1	α-thujene	5.79	1.06
2	α-pinene	6.01	1.34
3	β-pinene	6.15	1.85
4	β-myrcene	6.28	2.22
5	α- phellandrene	6.56	2.66
6	δ-2- carene	6.67	8.82
7	D- limonene	7.29	1.54
8	β- phellandrene	7.45	2.76
9	p -cymene	8.86	1.38
10	γ- terpinene	9.25	2.01
11	terpineol	10.23	1.16
12	Thymol	11.97	25.30
13	Carvacrol	12.08	8.43
14	α-terpinyl acetate	12.72	2.24
15	Geranyl acetate	13.16	4.89
16	E-caryophyllene	13.26	1.11
17	Cyclohexene, 1-methyl-4-(5-methyl-1-Methylene-4-hexenyl)	13.81	1.44
18	Spathulenol	15.51	1.04
19	Germacrone	15.66	1.47
20	n-hexadecanol	16.03	1.28
21	Benzyl cinnamate	16.37	1.49
Total	-	-	-

*R.T:Retention time (minute)

Ghasemi *et al*. studied the antimicrobial effect of eight native medicinal plants in Iran on *E. coli* bacteria, among which thymus daenensis celak was the most effective plant [29]. After investigating antimicrobial properties of thyme EO, bayberry and chamomile, Ramazanpour *et al*. perceived that Zataria multiflora boiss EO was the most important inhibition for the growth of *E. coli* bacteria and

showed the most bactericidal effect among the three Eos [30]. In the present study, this EO had an inhibitory effect on *E. coli* up to a concentration of 50%. In a study conducted by Fadil *et al*. results showed that T. vulgaris L., R. officinalis L. and M.communis L. EOs alone or combined are effective against *S. typhimurium* [25]. One study evaluated the antibacterial effect of a number of medicinal plants, such as thyme, rosemary, and oregano, against *P. savastanoi* (*P. savastanoi*). Its results indicated that thyme had the most antibacterial effect [31]. According to one study by Nozohour *et al*., the results of a microbial susceptibility test for standard tetracycline and amikacin antibiotics and a disc impregnated with oregano EO illustrated that the created inhibitory zone diameter by this compound was larger than the 4 tested isolates and this difference was significant in the case of *E. coli* and *Klebsiella pneumoniae* (*K. pneumoniae*) [30]. No antibacterial action from sage EOs against *E. coli* was observed, in concentrations of 1,000 mg/mL or even as high as above 8,000 mg/mL for both *E. coli* and *K. pneumonia* [32]. In the study of Mohammadpour *et al*., The antimicrobial effect of three kinds of thyme on *S. aureus* and *E. coli* bacteria was investigated, and the highest effect was reported on *E. coli* [33]. In the present study, only *E. coli* was sensitive to up to 50% concentration of EO. One study conducted by Borugă *et al*., showed that, thyme EO was effective in all concentration on the *S. aureus*, *S. typhimurium*, *Klebsiella* and *E. coli*, and *Enterococci faecalis* (*E. faecalis)* and *Pseudomonas* bacteria. The study by Borugă *et al*. was in line with the current study in terms of inhibitory effect on all concentrations of 100% and 80% and no other concentrations, which was due to the difference in concentrations in use [24]. Thyme inhibitory effect against *S. aureus*, *Pseudomonas, Klebsiella*, and *E. coli* were seen and reported in other studies [34]. Another study investigated the effect of thyme essential oil on *S. typhimurium*, and it was recognized that the highest compound of thyme was related to Thymol (37.54%). Then, after comparing the EOs activity with amoxicillin and cefotaxime control antibiotics, it was understood that EOs in question had strong antibacterial activity against *S. typhimurium* [25]. This study was not in line with the current study in terms of inhibitory effect on *S. paratyphi B* and *typhi*. One study conducted by Gedikoğlu *et al*., showed that, the maximum effect was found against *S.aureus* ATCC9144 (24–35 mm), and the minimum activity was shown against *S. typhimurium* ATCC 14028 (11–14.5 mm) and Overall, both EOs showed a higher inhibition effect against Gr (+) bacteria in comparison with Gr (−) bacteria; this could be due to the difference in the wall type for Gr (+) and Gr (−) bacteria [28].

The results of the study of antibiotics effect on the inhibitory zone diameter (in mm) also exhibited the lack of growth of bacterial strains by disc diffusion test (Table 3), which indicated that investigated *P. aeruginosa* and *Enterococci* were resistant to ampicillin, while the concentration of 100% and 80% of EOs constrained their growth and led to the formation of zones and was seen with a significant difference. Also, *Klebsiella* and *P. aeruginosa* and *Enterococci* were resistant to amoxicillin, while the 100% and 80% concentration of EOs inhibited their growth. *S. aureus* and *S. paratyphi B* were resistant to azithromycin, which was significantly different from other antibiotics. These two pathogens were inhibited only at 100% concentration of EO. Three pathogens, including *S. aureus*, *P. aeruginosa*, *S. paratyphi B*, were resistant to erythromycin. Penicillin only imposed an inhibitory effect on *S. paratyphi*, in which case, there was a significant difference in the resistance of this pathogen against other antibiotics ($P<0.05$). Only this pathogen was inhibited against all antibiotics investigated in this study. The highest inhibitory zone diameter was created by azithromycin against *Klebsiella* (23.5 ± 1.5) and *S. paratyphi* (24±1). The results declared that a significant difference was cleared between the different concentrations of thyme EO and the inhibitory zone diameter of each of the studied bacteria in comparison with antibiotics ($P<0.05$). Figure 1 also displayed the effect of different concentrations of EOs on *P. aeruginosa* and *S. typhi*.

Table 2 Mean inhibitory zone diameters of bacteria EOs studied in disc diffusion test (Mean±SD)

Concentration	*Enterococci*	*E. coli*	*S. typhi*	*S. paratyphi B*	*P. aeruginosa*	*S. aureus*	*Klebsiella*
100%	9.5 ± 0.5	13 ± 1	15.5 ± 1.5	17 ± 1	10.66 ± 1.75	9.5 ±1.5	16.5±1.5
80%	6.5 ± 1.5	11 ± 1	_	_	7 ± 1	_	11±1

50%	_	5 ± 1	_	_	_	_	_
25%	_	_	_	_	_	_	_
12.5%	_	_	_	_	_	_	_
6.25%	_	_	_	_	_	_	_
Significance level	(P< 0.05)	(P< 0.05)	(P< 0.05)	(P< 0.05)	(P< 0.05)	(P< 0.05)	(P< 0.05)
Total	2.66 ± 4.01	2.58 ± 5.9	2.58 ± 5.96	2.83 ± 6.52	2.94 ± 4.47	1.58 ± 3.67	4.58±6.89

_: Lack of Inhibitory Zone

Table 3 Mean results of antibiogram test of the inhibitory zone of different antibiotic disks (Mean±SD)

Antibiotics	*Enterococc*	*E. coli*	*S. typhi*	*S. paratyphi B*	*P. aeruginosa*	*S. aureus*	*Klebsiella*
Ampicillin	_	_	9.5 ± 1.5	14± 1	_	22 ± 1	1.66 ± 4.72
Amoxicillin	_	_	16 ± 1	20± 1	_	8 ± 1	_
Azithromycin	14.66 ± 0.57	21.5 ± 1.5	24 ± 1	_	12 ± 1.5	_	23.5 ± 1.5
Erythromycin	15.5 ± 1.5	12 ±2	11.5 ± 2.5	_	_	_	6 ± 1
Penicillin	_	_	5.5 ± 1.5	_	_	_	_
Tetracycline	_	15 ± 1	22± 1	22.33 ± 2.08	5.5 ± 0.5	10 ± 1	_
Vancomycin	14.5 ± 1.5	6.5 ± 1.5	5± 5	_	_	17.5 ± 1.5	_
Chloramphenicol	_	7± 2	4± 4	20 ± 2	_	_	10 ± 1
Significance level	(P < 0.05)	(P < 0.05)	(P < 0.05)	(P < 0.05)	(P < 0.05)	(P < 0.05)	(P < 0.05)
Total	5.58 ± 7.39	7.75 ± 7.66	12.18 ± 7.72	9.54 ± 10.04	2.25 ± 4.38	7.18 ± 8.42	27.6 ± 8.11

_: Lack of Inhibitory Zone

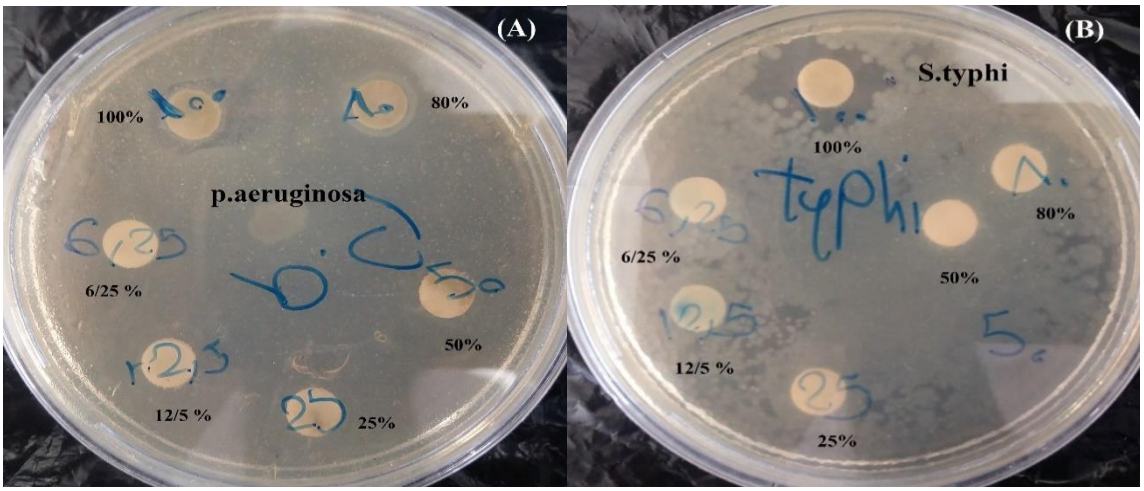

Fig. 1 Effect of different concentrations of EO on (A): *P. aeruginosa* and (B): *S. typhi*.

The inhibitory effect of 100% concentration on *Salmonella typhoid* was greater than that of other concentrations (P<0 .05). Although some of the key components of EOs act in a similar way to the synthesized antibiotics [35], it is unlikely that they will be used soon in therapeutics or as food preservatives mainly because of the limited number of bacterial strains tested and the differences in their susceptibilities. The results obtained from measuring MIC and MBC of EO were observed in Table 4 on average. The findings of MIC and MBC of thyme EO on bacteria indicated that all the studied pathogens were grown at concentrations of 12.5% and 6.25%. In the present study, the MIC and MBC for thyme plant were reported as 25%. Furthermore, the most antibacterial effect of EO was found to be against *Klebsiella*, *S. aureus*, *E. coli* through determining MIC. In the other study performed by Fournomiti *et al*., results showed that the most sensitive organism was Klebsiella oxytoca (*K. oxytoca*) with a mean value of MIC of 0.9 mg/mL for oregano EOs and 8.1 mg/mL for thyme. The second most sensitive strain was Klebsiella pneumoniae (*K. pneumoniae*) with mean minimum inhibitory concentration values of 9.5 mg/mL for thyme and 73.5 mg/mL for oregano EOs. *E. coli* strains were among the most resistant to EOs antimicrobial action as the observed MIC were 24.8-28.6 mg/mL for thyme and above 125 mg/mL

for thyme and sage. Most efficient were the EOs from thyme followed by those of oregano [26]. In study of Nozohor *et al*., the results observed from MBC and MIC tests indicated that the inhibition rate of bacterial growth by EOs had a direct relationship with the amount of EO in dilutions. This was in such a way that, the increased amount of EO in each dilution led to a reduction in the number of bacterial colonies after cultivation. In dilution indicating MBC oregano EO in each isolate, none of them grew at all [36]. Some early investigations from Greece also reported an increased antibacterial action of thyme EOs with MIC values between 0.28 and 3.35 mg/mL for *E. coli* and 0.72 mg/mL for *K. pneumonia* [37]. Some researchers declared that thyme EO affected bacterial cytoplasmic membranes and ultimately led to more structural and functional degradation of bacterial membranes [38]. Proposed methods against bacteria include: membrane disruption, enzyme inhibition, reduction of lipase and coagulase activity, and reduction of proton motility [26]. Most of the studies on the effect of EOs on ulcer organisms and foodborne pathogens revealed that the effect of herbal EOs on Gram-positive bacteria was slightly Phenolic compounds, known as natural antioxidants, were one type of plant metabolite with a large number of phenolic rings. Therefore, the analysis of these compounds inferred the antioxidant activity of EOs. The results indicated that the total fennel of thyme was equal to 114.5. As previously noted, the ability to inhibit free radicals was examined by the EO of thyme studied by DPPH measurement. To this end, natural and synthetic antioxidants were compared with thyme antioxidant activity (Figure 1). Low IC_{50} was found in compounds with high antioxidant activity. Clearly, IC_{50} of essential oil was considered to be 49.94 µg/ml, and most of IC_{50} was related to Gallic acid, BHT, and Ascorbic acid as stable antioxidant compounds ($P< 0.05$). It was mentioned that the ability to scavenge radical 2,2-diphenyl-1-picrylhydrazyl DPPH (thyme essential oil on average) was IC_{50} equal to 49.94 ± 20.41 µM/ml. greater than their effect on Gram-negative bacteria. Express differently, Gram-positives were more sensitive to the antibacterial effects of EOs. The reason for less sensitivity of Gram-negative bacteria was due to the presence of an outer membrane in Gram-negative bacteria, which limited the diffusion of hydrophobic components of the EO into the lipopolysaccharide layer [7]. The time of plant harvesting was effective in their antimicrobial ability. The best time to harvest was in the 50% flowering period to achieve the highest yield of essential oils and effective thyme compounds [39]. The comparison of reported results on the antibacterial properties of different EOs was found so demanding. This was due to the difference observed in the various methods of antibacterial properties examination of EOs, the sources of their preparation, and the applied bacterial strains [40]. The herbal EOs compounds widely changed based on the geographical area of plant growth, plant variety, age of the plant while preparing the EO, and the method of drying and extracting the EO [26].

Antioxidant activity

Thyme antioxidant effect relative to the other three compounds (Gallic acid= 2.51 ±0.99 µM/ml, BHT= 8.52 ± 1.08 µM/ml and Ascorbic acid=2.12 ± 0.99 µM/ml), revealed that this EO was less effective in free radical's inhibition than other compounds examined in this paper (Table 4), which was statistically different ($P< 0.05$). Nasiri *et al*. stated that Thymol, carvacrol, and paracetamol, forming the most common compounds of Zataria multiflora boiss EO, and was able to inhibit DPPH free radical of thyme EO based on mean IC_{50} equal to 0.02±0.8 mg/m [41]. Zangiabadi *et al*. reported the IC_{50} of Zataria multiflora boiss EO as 0.78 ±0.03 mg/ml [42]. However, the antioxidant activity in plants is not caused only by phenolics and may also come from the presence of other antioxidant secondary metabolites such as volatile oils, carotenoids, anthocyanins and vitamins.

Table 4 (MIC) and (MBC) of EO Thyme

Enterococci	*E. coli*	*S. typhi*	*S. paratyphi B*	*P.aeruginosa*	*S. aureus*	*Klebsiella*	Nam of bacterium
50%	25%	50%	50%	50%	25%	25%	MIC
80%	50%	80%	80%	80%	50%	50%	MBC

Table 5 Comparison of the birth activity of free radical (DPPH)

Name of substance	Antioxidant activity IC_{50}(µg/ml) (Mean±SD)
Thymol EO	49.94 ± 20.41
Gallic acid	2.51 ± 0.99
BHT	8.52 ± 1.08
Ascorbic acid	2.12 ± 0.99
Significance level	(P< 0.05)
Total	15.77 ± 22.53

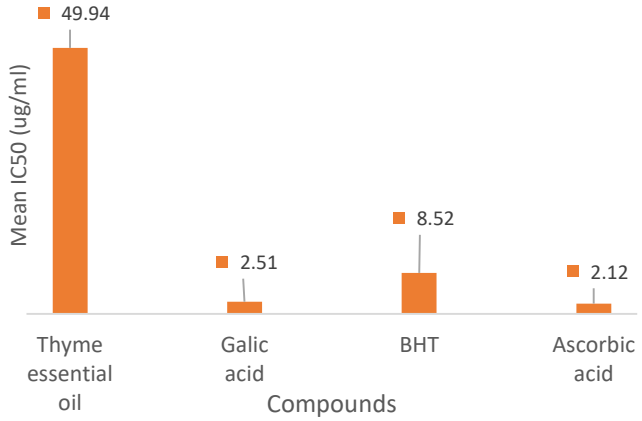

Fig. 2 Mean comparison, IC50 in Thymol EO, Gallic acid, BHT, Ascorbic acid Several studies have reported on the relationships between phenolic content and antioxidant activity in medicinal and aromatic plants [43]. Furthermore, it should be noted that antioxidant activity might be pertained to the chemical structure of phenolic compounds, as well as synergistic or antagonistic effect of compounds present in the plant extract [44]. However, it is reported that various parameters such as extraction method, pretreatment of the sample prior to the extraction, plant growth conditions and even plant age might be effective on the phenolic content and antioxidant activities in medicinal plants [45].

CONCLUSIONS

Food contamination is still an enormous public health problem, but may be better controlled by the use of natural preservatives. Since specific microbes were not mainly into consideration of many types of research, the comparative studies were not plausible between the conducted surveys. However, the results of this study showed that the positive effects of thyme EOs on pathogens were at higher concentrations. Regarding these results, it was promising to produce appropriate drugs for removing these microorganisms through this EO. Totally, the antioxidant properties of EOs were based on their compounds, the most paramount of which were phenolic compounds. Thymol EO enjoyed a high rate of Thymol and carvacrol phenolic acids. The antioxidant and radical deoxidizing properties of Thymol EO were revealed in the presence of phenolic compounds that were capable of allowing the penetration of hydrogen into free radicals. These days, one of the main issues related to pathogenic microorganisms was their increased resistance to antibiotics. Concerning the naturalness of these EOs, their detrimental effects on health and the environment or their drug resistance were considered much less to chemical preservatives and antibiotics. Despite increasing surveys in this regard, further studies are required on antimicrobial activity or chemical compounds of medicinal plants. Accordingly, more extensive researches were recommended for studying herbal EOs that may be used in preserving or increasing the preservation time of food products or as a natural antibiotic combination against pathogens.

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