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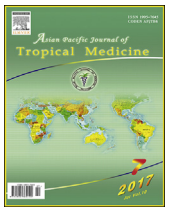

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Chemical analysis and *in vitro* antimicrobial effects and mechanism of action of *Trachyspermum copticum* essential oil against *Escherichia coli*

Wei Huang[1,2], Jian-Qing Wang[1], Hai-Yan Song[1], Qian Zhang[1], Guang-Fa Liu[1✉]

[1]*Tianjin University of Science & Technology, Tianjin 300222, People's Republic of China*

[2]*China Packaging Research & Test Center, Tianjin 300457, People's Republic of China*

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ABSTRACT

Objective: To find a natural plant essential oil (EO) with excellent antimicrobial effects on food-borne bacteria and to explore the mechanism of its antimicrobial function against *Escherichia coli* (*E. coli*).

Methods: The antimicrobial activity of seven EOs against Gram-negative *E. coli* ATCC 8739 and Gram-positive *Staphylococcus aureus* ATCC 6538 was investigated using agar disk diffusion method, and the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of each EO was determined using the broth dilution method. The chemical composition of the *Trachyspermum copticum* (*T. copticum*) EO was analyzed using gas chromatography–mass spectrometry (GC/MS). In order to explore the mechanism of the antimicrobial action, 1 MIC and 2 MIC of *T. copticum* EO was added to a suspension of *E. coli*, the growth curve and the scanning electron microscopy (SEM) analysis of *E. coli*, and the release of cell constituents and protein and potassium ions from the bacterial cell were measured.

Results: The *T. copticum* EO had the best antimicrobial activity against the test bacteria, and 10 compounds accounting for 94.57% of the total oil were identified, with the major components being thymol (46.22%), *p*-cymene (19.03%), and γ-terpinene (22.41%). The addition of 1 MIC that *T. copticum* EO significantly inhibited the growth of *E. coli* and increased the release of cell constituents and protein and potassium ions from the bacterial cells. Scanning electron micrographs showed that *T. copticum* EO caused most of the *E. coli* cell membranes to collapse and rupture, leading to cell death.

Conclusions: These results indicate that *T. copticum* EO is a good natural antimicrobial agent for food-borne pathogens.

1. Introduction

Bacteria in foods may cause many foodborne diseases [1]. Food safety incidents and foodborne diseases often occur and cause widespread concern, although people having making many efforts in developing food processing technologies and storage measures [2,3]. Excessive growth of microorganisms is the main cause of food spoilage and foodborne disease [4]. For example, pork is popular with Chinese consumers because of its good flavor and high nutritional value [5]. *Escherichia coli* (*E. coli*) is the main food-borne microorganisms which causes the corruption of pork, and has been found at the meat processing and its sale stages. In 2006, an event of foodborne diseases occurred in spinach in the USA which caused by *E. coli* 0157:H7, and resulted in 3 deaths and 276 cases of illness [6]. Many synthetic chemicals have been used to control food spoilage and the growth of pathogenic bacteria, but these chemicals have unwanted potential effects such as carcinogenicity, teratogenicity, and toxicity, and have a long degradation time [7].

First author: Wei Huang, Tianjin University of Science & Technology, Tianjin 300222; China Packaging Research & Test Center, Tianjin 300457, People's Republic of China.
E-mail: huangwei@packagetest.net
✉Corresponding author. Guang-Fa Liu, Tianjin University of Science & Technology, Tianjin 300222, People's Republic of China.
E-mail: liuguangfa@tust.edu.cn
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There is an urgent need for effective and safe measures to control food-borne disease and food decay.

In recent years, essential oils (EOs) from natural plants have been used to inhibit the growth of pathogenic microorganisms [8–12], and many EOs have been studied for their effectiveness in food preservation applications [13,14]. The seeds of *Trachyspermum copticum* (*T. copticum*) are widely used as an aromatic herb and spice both in food and in traditional medicine in India, Eastern Asia, Iran and Egypt. The EO from *T. copticum* fruit has been researched previously for its antimicrobial and antioxidant activity [15,16]. However, to date there is a very limited amount of knowledge on the mechanism of antimicrobial activity of *T. copticum* against *E. coli* and other food-borne pathogens.

The aim of the present work was to examine the EOs from seven plants to identify one that has the strongest antimicrobial effect, and then to analyze the chemical composition of the selected EO using gas chromatography–mass spectrometry (GC/MS). In addition, the mechanism of the antimicrobial activity of the EO against *E. coli* was investigated by measuring the growth curve, imaging with scanning electron microscopy (SEM), and measuring the release of cell constituents, intracellular protein, and potassium ions.

2. Material and methods

2.1. Essential oils and chemicals

2.1.1. Plant material

The seeds of *T. copticum* were collected after ripening from a farm in India.

2.1.2. Essential oils

The *T. copticum* EO was extracted by steam distillation from the seeds of *T. copticum* after crushing, and was dried with anhydrous sodium sulfate. The oil yield was approximately 3.3%. The *Litsea cubeba* (*L. cubeba*), clove (*Syringa oblata* Lindl), humifuse euphorbia herb, nutmeg, laurel leaf (*Laurus nobilis* L.) and thymifolious euphorbia (*Euphorbia thymifolia* L.) EOs were purchased from Jiangxi Hengcheng Natural Spices Oil Co. Ltd. (Ji'an, China). The fruit of *L. cubeba*, clove and nutmeg, the whole plant of humifuse euphorbia herb and thymifolious euphorbia, and the laurel leaf was distilled. All the EOs were stored at 4 °C before use.

2.1.3. Chemicals

Nutrient broth and nutrient agar were purchased from Beijing Aoboxing Biotechnology Co. Ltd. (Beijing, China). Sodium chloride (spectral purity) was purchased from Tianjin Guangfu Fine Chemical Research Institute (Tianjin, China). Potassium phosphate monobasic was purchased from Tianjin Liyuan Chemical Co. Ltd (Tianjin, China). Sodium hydroxide was purchased from Tianjin Fuqi Chemical Co. Ltd, and the Coomassie Brilliant Blue kit was purchased from Nanjing Jian Cheng Bioengineering Institute (Nanjing, China).

2.2. Antimicrobial activity

2.2.1. Microbial strains and cultures

Two food-borne bacterial microorganisms were used to evaluate the antimicrobial activity of the EOs: Gram-positive *Staphylococcus aureus* (*S. aureus*) ATCC 6538, and the Gram-negative *E. coli* ATCC 8739 (Strain Collection Center, Tianjin University of Science & Technology, Tianjin, China). The two strains were maintained on slants of nutrient agar (Aoboxing, Beijing, China) at 4 °C.

Active cultures were prepared by transferring a loop of cells from the nutrient agar slants of each strain into 100 mL of broth medium, which was incubated at 37 °C for 24 h. The turbidity of the cell suspension was adjusted to the required concentration of 1×10^8 CFU/mL measuring the value of the optical density of the bacterial suspension at 600 nm (OD_{600}).

2.2.2. Agar disk diffusion assay

The antimicrobial activity of the EOs against *S. aureus* and *E. coli* was tested by the method of agar disk diffusion assay described previously [17], with some modifications. In order to evaluate effectively the antimicrobial activity of the EOs, bacteria in the logarithmic growth phase were used. The nutrient agar was sterilized in an autoclave and approximately 15 mL was poured into 90 mm petri dishes before they were solidified. After solidification of the agar, 100 μL of fresh inoculum suspension (approximately 1.0×10^8 CFU/mL) was spread on the surface of each agar plate and covered with a sterile blank filter disk (6 mm diameter) containing 10 μL of the EO. The inoculated plates were incubated at 37 °C for 24 h. The diameter of the zone of inhibition of bacterial growth was measured and used to evaluate the antimicrobial activity of the EOs. Agar disk diffusion assay of each EO against one bacterial microorganism was performed in triplicate.

2.2.3. Testing of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The values of MIC and MBC of EOs were tested by the method described by Roya Moghimi *et al.* [18] with some modifications. The EOs were sequentially diluted in glass tubes containing 10 mL of nutrient broth (NB) medium to produce a series of EO concentrations covering the range 0.025–16 μL/mL. Bacterial suspension was added to the tubes and the final concentration of *E. coli* adjusted to 1×10^8 CFU/mL. Those tubes were incubated at 37 °C for 24 h, and the lowest concentration of EO that gave rise to no significant turbidity was MIC. To determine the MBC, 100 mL of the broth from the tubes with no significant turbidity was plated onto nutritional agar and incubated at 37 °C for 24 h. The MBC was the lowest concentration that killed all the bacteria. Each assay was performed in triplicate.

2.3. GC/MS analysis of the *T. copticum* EO

The *T. copticum* EO was analyzed using GC/MS (VARIAN 4000MS, USA) equipped with a VF-5ms chromatographic column (30 m × 0.025 mm, thickness 0.25 μm). The inlet temperature was 300 °C, and split-mode injection was used (split ratio 20:1). The injection volume was 0.2 μL. Helium (purity 99.999%) was used as the carrier gas.

The initial oven temperature was 60 °C. The temperature was increased to 200 °C at a rate of 5 °C/min, and then increased to 300 °C at a rate of 10 °C/min, and held for 3 min. The mass spectrometry conditions were as follow: ion source temperature was 220 °C, transmission line temperature was 280 °C, scanning mode was full/SIS, and scanning range from 43 to 500 amu. The

compounds constituent of the EOs were identified by comparing the spectra in library spectra (NIST05).

2.4. Antimicrobial mechanism of T. copticum EO against E. coli

2.4.1. Growth curve assay

The influence of the *T. copticum* EO on the growth of *E. coli* was determined using the method described previously [19,20], with some modifications. Suspensions of *E. coli* were incubated in broth medium for 24 h at 37 °C. The suspensions were then diluted 1/100 in fresh broth and incubated at 37 °C in an air bath thermostat oscillator (120 rpm) until the bacterial concentration was approximately 1×10^8 CFU/mL. A series of aliquots of the suspensions in the logarithmic growth phase were added to 1 MIC and 2 MIC of the selected EOs. For the blank an equivalent amount of distilled water was added instead. The suspension samples were incubated in an air bath thermostat oscillator (120 rpm) at 37 °C for 24 h. The optical density of the bacterial suspension at 600 nm (OD_{600}) was monitored using a UV–visible spectrophotometer (UV-2700, Shimadzu Corporation, Japan) over a period of 20 h.

2.4.2. Scanning electron microscopy (SEM) analysis

In order to explore the effects of the EOs on the morphology of *E. coli*, SEM analysis was carried out using the method described by Han *et al.* [21], with some modifications. The EOs were added to working cultures at three different concentrations [0 (control), 1 MIC, and 2 MIC]. After being incubating for 1 h at 37 °C, 2 mL of the bacterial suspensions were removed from each tube and centrifuged for 10 min at 3 000 rpm. The collected cells were then washed twice with 50% water/alcohol solution; centrifugation (3 000 rpm for 10 min) was performed during each cleaning process. Next, 20 μL of suspension was spread on a cover glass (1 cm × 1 cm) and 100 μL of 2.5% glutaraldehyde (pentanedial) (v/v) in phosphate buffered saline was added to the coverslip (after the ethanol had volatilized and dried).

2.4.3. Measurement of cell constituents released

The release of cell constituents into the supernatant was measured using the method described by Mélanie Turgis *et al.* [22] with some modifications. *E. coli* were inoculated in nutrient broth medium until the OD_{600} of the bacterial suspension reached 0.1–0.2 (the exponential growth phase). Cells were collected by centrifugation (4 000 rpm for 15 min), washed three times with sterile normal saline (0.85% NaCl), and then resuspended in the initial volume of sterile normal saline. Aliquots (25 mL) of the bacterial suspensions were incubated at 37 °C in an air bath thermostat oscillator (CHA-SA, Jintan Prius Machinery Co., Ltd, Changzhou, China) for 1 h in the selected EO at three different concentrations [0 (control), 1 MIC, and 2 MIC]. The mixture was centrifuged for 15 min at 4 000 rpm and the concentration of cell constituents in 3 mL of the supernatant was measured using a UV–visible spectrophotometer (UV-2700, Shimadzu Corporation, Japan) at 260 nm.

2.4.4. Measurement of intracellular protein released

Protein release tests were performed by testing the protein content of the bacterial suspension supernatant. The bacterial suspension supernatant was prepared in the same way as for the supernatant for measuring the release of cell constituents. The amount of protein in the supernatants was tested by the Coomassie Brilliant Blue method [23] and a UV–visible spectrophotometer at 595 nm. The amount of released protein from *E. coli* was calculated by a standard curve of bovine serum albumin.

2.4.5. Measurement of potassium ions released

The potassium ions released from bacterial was measured using the method as described by Miao *et al.* [24], with some modifications. Cultures were grown in nutrient broth medium and incubated in an air bath thermostat oscillator (CHA-SA, Jintan Prius Machinery Co. Ltd, Changzhou, China) at 120 rpm and 37 °C until the OD_{600} of the bacterial suspension reached 0.1–0.2 (exponential growth phase). The bacterial suspension (525 mL) was then collected and centrifuged for 15 min at 4 000 rpm, washed three times with sterile normal saline solution (0.85% NaCl, spectral purity, and ultrapure water), resuspended in 525 mL of nutrient broth culture, and divided into 25 mL aliquots. The suspension aliquots were incubated at 37 °C in an air bath thermostat oscillator with the EOs at three different concentrations [0 (control), 1 MIC, and 2 MIC]. The cell suspensions were centrifuged for 15 min at 4 000 rpm at time intervals of 0, 1, 2, 3, and 4 h, and 1 mL supernatant was collected. The 1 mL sample was diluted ×10 with ultrapure water. The potassium ion concentration of the supernatant was measured using an atomic absorption spectrophotometer (PinAAcle 900T, PerkinElmer, USA).

2.5. Statistical analysis

Each experiment test was conducted in triplicate, and the mean value was calculated. The statistical analysis software SPSS with version of 17.0 was used for the data analysis. Data were analyzed using the one way ANOVA, with a level of significance of $P < 0.05$.

3. Results

3.1. Antimicrobial activity of the EOs against E. coli

The antimicrobial activity of the EOs was evaluated by measuring the diameter of the inhibition zone, the MIC, and the MBC. As a preliminary procedure for screening the antimicrobial activity, agar disk diffusion assays were undertaken to determine the diameter of the inhibition zone.

The agar disk diffusion assay showed that the inhibition zone diameter [including the diameter (6 mm) of the paper disk] of the seven EOs, *L. cubeba*, clove, humifuse euphorbia herb, nutmeg, laurel leaf, thymifolious euphorbia and *T. copticum* against *E. coli* was 12, 35, 15, 10, 37, 10 and 78 mm respectively, and against *S. aureus* was, 46, 30, 48, 6, 40, 20 and 76 mm respectively, with the range 6–78 mm. The *T. copticum* EO showed the strongest antimicrobial activity against the tested microorganisms, the inhibition zones for *E. coli* and *S. aureus* being 78 and 76 mm, respectively, which were significantly better than the other six EOs ($P < 0.05$). The EOs of clove and laurel leaf were also active against *E. coli* and *S. aureus*, with inhibition zones of >30 mm. The nutmeg EO showed the

Table 1
Chemical composition of the EO from *T. copticum*.

Component	Compound	CAS number	Area percentage (%)
1	α-pinene	80-56-8	0.34
2	β-pinene	127-91-3	3.23
3	Myrcene	123-35-3	0.54
4	*p*-cymene	99-87-6	19.03
5	γ-terpinene	99-85-4	22.41
6	α-terpineol	98-55-5	0.15
7	Carvacrol	499-75-2	0.08
8	Thymol	89-83-8	46.22
9	Terpinyl acetate	80-26-2	2.15
10	Eugenol	97-53-0	0.42

weakest antimicrobial activity, with an inhibition zone diameter of <10 mm.

Based on the results of agar disk diffusion assay, the EOs of clove, laurel leaf and *T. copticum* were selected for further analysis of their antimicrobial activity. The MIC values of these three EOs against *E. coli* were 0.5, 0.5 and 0.25 μL/mL respectively, and against *S. aureus* were 2, 0.5, and 0.125 μL/mL respectively. The MBC values of the above three EOs against *E. coli* were 0.5, 0.5 and 0.4 μL/mL respectively, and against *S. aureus* were 2, 1, and 0.5 μL/mL respectively. Overall, the *T. copticum* EO had the best antimicrobial activity against the bacterial strains tested. It had both the lowest MIC and the lowest MBC for both *E. coli* and *S. aureus*.

3.2. Chemical composition of T. copticum EO

The chemical composition of the *T. copticum* EO was analyzed using GC/MS. Ten components were identified, accounting for 94.57% of the total oil. The main components are listed in Table 1. The major components were thymol (46.22%), *p*-cymene (19.03%), γ-terpinene (22.41%), β-pinene (3.23%), and terpinyl acetate (2.15%).

3.3. Antimicrobial mechanism of T. copticum EO against E. coli

3.3.1. Effect of on growth curve of E. coli

The effect of *T. copticum* EO on the growth of *E. coli* is shown in Figure 1. The *E. coli* in the control (no EO treatment) showed rapid growth, with the highest OD_{600} value being reached about 2.0 after being incubated in an air bath thermostat oscillator for 14 h. In contrast, the OD_{600} value showed a horizontal trend at both the low concentration (1 MIC, 0.25 μL/mL) and the high concentration (2 MIC, 0.5 μL/mL) of *T. copticum* EO, indicating that the growth of *E. coli* was significantly inhibited by the *T. copticum* EO at even the lower concentration.

3.3.2. SEM analysis

Morphological changes in the bacterial membrane can be observed in SEM images. The SEM images of *E. coli* cells with and without (control) treatment with the *T. copticum* EO were observed by scanning electron microscopy (magnification ×10 000). In the control group, the untreated *E. coli* cells show the typical rod-shaped features, and the cell surface is regular and smooth. In contrast, after 60 min of treatment with the *T. copticum* EO at 1 MIC (0.25 μL/mL) there are damages to most of the *E. coli* cell membranes, and in some cases the membrane has ruptured. Even greater detrimental effects to the *E. coli* cell membrane were seen after 60 min of treatment with *T. copticum* EO at 2 MIC (0.5 μL/mL), almost all the cells show collapse and disruption of the membrane.

3.3.3. Release of materials that absorb light at 260 nm

The results obtained when *E. coli* was treated with different concentrations of the *T. copticum* EO for 0 h, 1, and 2 h are shown in Figure 2. It can be seen that the release of cell constituents increased, compared with the control group, with time and with the concentration of the EO. After 2 h of treatment, the maximum OD_{260} observed for the *E. coli* cells treated with 2 MIC EO was 0.850, and that for cells treated with 1 MIC EO

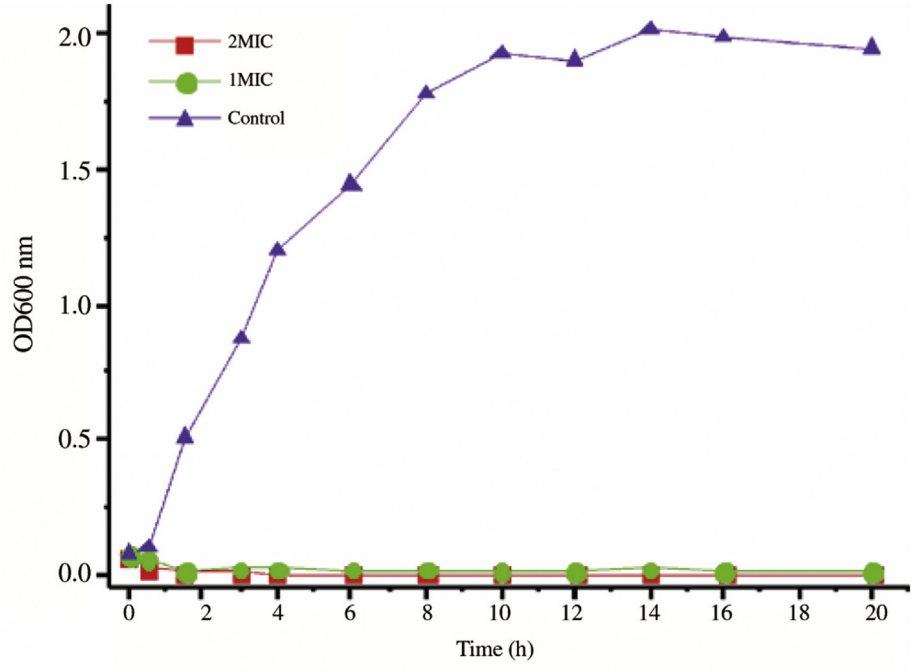

Figure 1. Growth curves for *E. coli* treated with *T. copticum* EO at different concentrations (recorded at 600 nm and 37 °C).

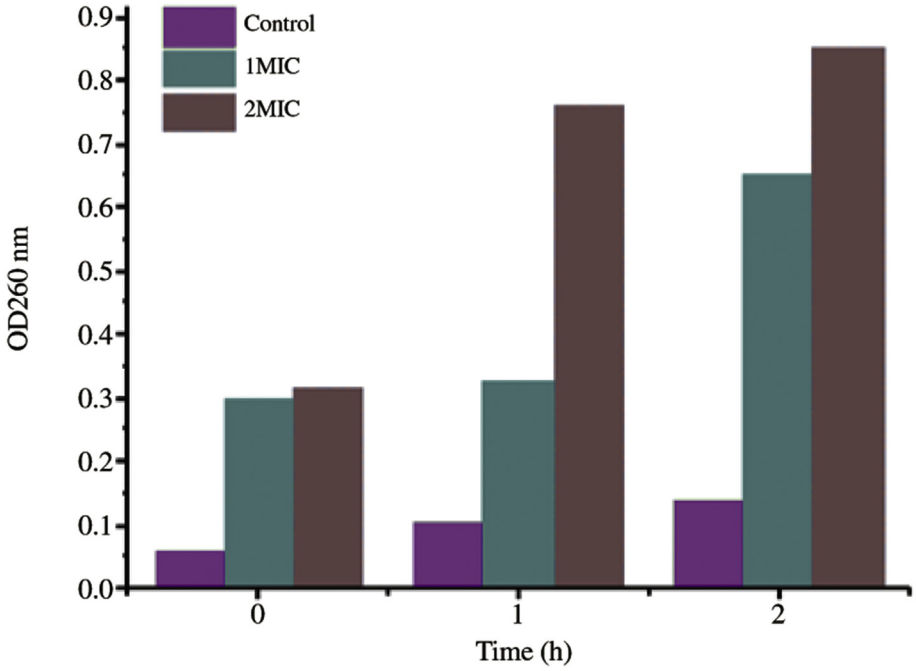

Figure 2. The effect of *T. copticum* EO on the release of cell constituents of *E. coli*.

was 0.662, which were significantly higher than the value for the control group of 0.135 ($P < 0.05$).

3.3.4. Release of protein

The degree of damage to the cell membrane due to the EO was evaluated by measuring the amount of protein in the supernatant after centrifugation. The concentration of protein in the supernatant of the *E. coli* solution treated with 1 MIC EO was much higher than that in the control. After 1 h of treatment, the OD_{595} of the control, the *E. coli* samples treated with 1 MIC EO and 2 MIC EO were 0.309, 0.318 and 0.324 respectively; and the protein release in the control group was only 1.66 mg/L, however, there were significant differences ($P < 0.05$) between the protein release of different treatment, while in the *E. coli* samples treated with 1 MIC EO and 2 MIC EO it was 3.32 and 4.43 mg/L, respectively.

3.3.5. Release of potassium ions

The amounts of potassium ions released from *E. coli* cells treated with the *T. copticum* EO at three different concentrations [0 (control), 1 MIC, and 2 MIC] were shown in Figure 3. For the control group, the extracellular concentration of potassium ions in the *E. coli* suspension increased slowly over time, reaching 0.510 mg/L after 4 h. The potassium ion concentration in the bacterial suspensions treated with 1 MIC and 2 MIC of the EO reached 1.42 and 1.54 mg/L, respectively, after 4 h.

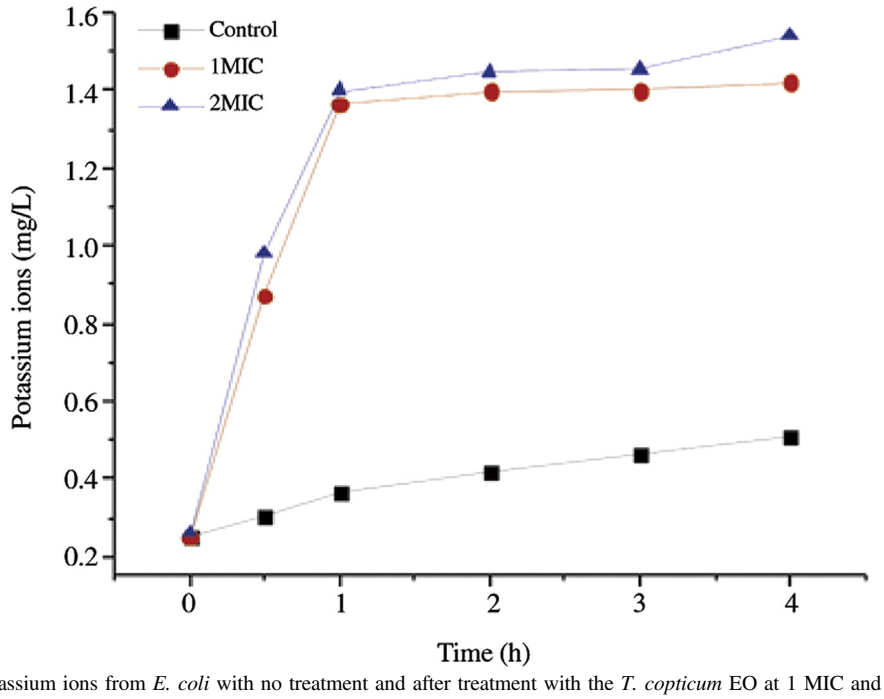

Figure 3. Release of potassium ions from *E. coli* with no treatment and after treatment with the *T. copticum* EO at 1 MIC and 2 MIC.

4. Discussion

The inhibition zone diameters were divided into the following three levels (including the disk diameter): strong inhibitory activity, inhibition zone ≥20 mm; mild inhibitory activity, inhibition zone >12 mm and <20 mm; and weak inhibitory activity, inhibition zone ≤12 mm [17].

Rasooli *et al.* [16] reported for the EO of *Carum copticum* against *Aspergillus parasiticus*, with an inhibition zone of 45 mm when 20 μL of the EO was added to the Petri dish, but there is no report in the literature of an agar disk diffusion assay of *T. copticum* EO against *E. coli* and *S. aureus*. Previous studies have shown that thymol, carvacrol, eugenol and many other phenolic compounds have clear antimicrobial activity against food-borne bacteria such as *E. coli* and *S. aureus* when used in pure form [25–28]. In addition, many EOs rich in *p*-cymene or γ-terpinene [29,30] have been shown to have relatively weak antimicrobial activity against food-borne bacteria, their antimicrobial activity being much lower than that of the *T. copticum* EO in the present study. Therefore, the excellent antimicrobial activity of the *T. copticum* EO can be attributed to its main component thymol. However, whether *p*-cymene or γ-terpinene has synergistic antimicrobial effects on thymol is worthy of further study.

Analyses of the composition of *T. copticum* EO have been reported previously. For example, Oroojalian *et al.* [8] detected 17 chemical compounds, the major ones being thymol (48.4%), *q*-cymene (21.8%), γ-terpinene (21.3%), and b-pinene (2.6%). Rasooli *et al.* [16] detected 9 compounds in the EO of *T. copticum* L., the major components being thymol (37.2%), *p*-cymene (32.3%), and γ-terpinene (27.3%). The main components of *T. copticum* EO detected in this paper are the same as those reported previously, but the amounts of the major components vary slightly. These differences in the composition of EOs of *T. copticum* could be attributed to differences in the growth environment and harvesting season.

These observations of SEM are in agreement with the growth curve assay, which showed that *T. copticum* EO could kill the *E. coli* bacteria at a concentration of 1 MIC and was even more effective at a concentration of 2 MIC. The SEM images show that the action of *T. copticum* EO against *E. coli* might attributed to the formation of pores on the cell membrane surface. The detrimental changes of the *E. coli* cell morphology could be due to lysis of the membrane caused by the EO [31], which may lead to the leakage of inner cell materials [32]. The SEM images recorded in the present study show similarities with previously reported observations on the effects of other antimicrobial agents on *E. coli* cells, such as bifidocin A [33] and cinnamon oil [27,31].

Substances absorbed at 260 nm mainly include nucleic acids, and the optical density of the supernatant at 260 nm (OD_{260}) is thus an indication of internal cell constituents release and disruption of cell membrane [20]. Other antimicrobial agents such as mustard EO [22], cold nitrogen plasma [34], clove oil [35], a nano-emulsion of thymus [18], combinations of basil and oregano EOs [2] have also been shown to increase the release of 260-nm absorbing materials from *E. coli* cells. The results of the present study show that *T. copticum* EO causes the release of the cell contents of *E. coli*, and that this phenomenon is more obviously as the concentration of the EO is increased within a certain range. These observations are consistent with the structural changes observed on the SEM images.

This increase in concentration of protein can be attributed to the damage done by the EO to the *E. coli* cell membrane [23]. A similar effect on *E. coli* has been also reported for several other antimicrobial agents, such as thymus EO and its nano-emulsion [18], flavonoids [23], sage oil and its nano-emulsion [36]. From the measurement of protein release in the present study we can also conclude that *T. copticum* EO could damage the cell membrane, leading to intracellular protein leakage and eventually to death of cell, and the damage to the cell membrane becomes more obviously with increasing concentration of the EO.

The effect of antimicrobial agents on membrane function has been evaluated previously by determining the leakage of intracellular components, such as 260 nm absorbing materials and proteins. The microbial cell membrane is damaged when the microorganism are exposed to antimicrobial agents or inappropriate growth environment, and the changes in the permeability of cell membrane could lead to the release of electrolytes from the interior of the bacterial cells, such as potassium ions [18]. The potassium release assay is a useful tool for studying the mechanism of action of antimicrobial agents against microorganism [37], and was used in the present study to examine the effect of the *T. copticum* EO on the *E. coli* cell membrane. The slowly increase in concentration of potassium ions in control group could attributed to cell division during the normal life cycle of *E. coli* [18]. The extracellular concentration of potassium ions in the two *E. coli* suspensions treated with the *T. copticum* EO was significantly higher than in the control group ($P < 0.05$), and increased over time. Many previous studies on this topic have shown that antimicrobial agents such as thymus EO nano-emulsion [18], cinnamon EO [21], antimicrobial peptide F1 [24], and nisin Z [38] significantly increased the concentration of potassium ions released from *E. coli* compared with the control group. The potassium ions release found in the present study suggests that *T. copticum* EO could alter the permeability of the cell membrane and lead to an increase in the concentration of extracellular potassium ions, and this is consistent with the results of 260 nm absorbing materials.

In conclusion, among the seven EOs studied, the EOs of *T. copticum*, clove, and laurel leaf were found to have noticeable significant antimicrobial activity against *E. coli* and *S. aureus*, with the *T. copticum* EO having the strongest antimicrobial activity against *E. coli*. The major components of the *T. copticum* EO were thymol (46.22%), *p*-cymene (19.03%), and γ-terpinene (22.41%). SEM images and the measurements of the release from the cell of intracellular constituents, intracellular protein, and potassium ions indicated that the *T. copticum* EO destroys the integrity of the *E. coli* cell membrane, resulting in these materials being released from the cell, leading to cell death. The results of the research show that *T. copticum* EO is a good natural antimicrobial agent for food-borne pathogens. However, further study is needed to evaluate this EO, for example regarding the issues of safety and toxicity, and the application in food or packaging materials, before it is commercially used in food systems.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

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