Mechanism of action and bioactivities of *Cinnamomum zeylanicum* essential oil against some pathogenic microbes

Eman W. Elgammal[a], Abd El Nasser G. El Gendy[b], Abd El-Basset A. Elgamal[a]

Departments of [a]Chemistry of Natural and Microbial Products, [b]Medicinal and Aromatic Plants Research, National Research Centre, Giza, Egypt

Correspondence to Eman W. Elgammal, PhD, Department of Chemistry of Natural and Microbial Products, National Research Centre, Dokki 12311, Giza, Egypt. Tel: 3283749; fax: +202-33370931; e-mail: emanelgammal50@yahoo.com

Received: 14 December 2019
Accepted: 28 February 2020
Published: 30 June 2020

Egyptian Pharmaceutical Journal 2020, 19:162–171

Background and objective
The emergence of multiple drug-resistant pathogenic microorganisms leads to the search for new, safe, and effective therapeutic substances. Essential oils (EOs) of the cinnamon species are known for their biological antimicrobial and antioxidant properties. Therefore, in our study, the EO extracted from the bark of wild *Cinnamomum zeylanicum* grown in the green mountains of Oman was investigated to study both the aspects.
Materials and methods
Chemical composition of the EO was analyzed by gas chromatography–mass spectrometry, and then its antimicrobial potential was tested against different Gram-positive, Gram-negative bacteria and fungi. The minimum inhibitory concentration (MIC) was detected and a further in-depth study on the antimicrobial mode of action of the EO at the MIC concentration was studied, in addition to the determination of the antioxidant potential of the studied EO.
Results and conclusion
Thirty compounds were identified, and the major constituents were cinnamaldehyde (81.78%), bornyl acetate (5.33%), and cinnamyl acetate (2.82%). The antimicrobial results showed a highly significant activity against all tested microbes. The MIC of our EO ranged from 3.3 μl/ml for Gram-positive bacteria and fungi to 10 μl/ml for Gram-negative ones. The mode of action of EO at MIC concentration showed that it had a strong effect on cell viability, permeability of cell membrane, and leakage of cell constituents such as DNA, RNA, and protein from the cell membrane to outside with the lethal percent reaching 99.99%. Moreover, it was observed that the bactericidal and fungicidal effects act in a dose- and time-dependent manner and they depend on the culture condition. Finally, the EO had a strong antioxidant effect with IC_{50} equaling 2.3 mg. Cinnamon essential oil has a great potential as a useful agent help in combating resistant microorganisms coupled with healing of wounds according to its antioxidant activity.

Keywords:
antimicrobial activities, *Cinnamomum zeylanicum*, essential oils, minimum inhibitory concentration, mode of action and antioxidant effect

Egypt Pharmaceut J 19:162–171

1687-4315

Introduction

During the last decades of the twentieth century, there was an increase in the incidence of drug-resistant pathogens to common antibiotics, which necessitated extensive research to find new therapeutic agents from other natural resources such as medicinal plants to treat human infectious diseases [1]. So, many plants were investigated for the production of new natural products. These natural products have the ability to inhibit the growth of pathogenic microorganisms, without harming the host, indicating that they will be good alternatives for synthetic antibiotics [2].

Cinnamon is a plant, belonging to the Lauraceae family. It has shown many biological applications due to its antiseptic, analgesic, antispasmodic, astringent, aphrodisiac, carminative, parasiticide, insecticidal, and hemostatic properties [3]. Furthermore, it is exhibited to have antimicrobial efficacy in its essential oils (EOS) obtained from the branches and barks [4] because of the presence of trans-cinnamaldehyde which have antimicrobial activity against a wide range of microbial pathogens, with various spectrums of activity [5]. Moreover, oils and extracts from cinnamon possess a distinct antioxidant activity, which is attributed to the presence of phenolic and polyphenolic substances. These previous effects coupled with antioxidant activity could be helpful in the treatment of microbial infection and promoting wounds to heal [6].

Since the antimicrobial pattern of the EOs is partly dependent on their mechanism of action, our research

discussed this point to measure that effect on cell membrane disruption, which occurs by passing any EO components through the plasma membrane causing the leakage of cell constituents [7]. Furthermore, there are many measures for detecting the mode of action such as observing changes in cell morphology using transmission electron microscopy [8], measuring ATP synthesis, change of intracellular pH [9], measuring oxygen consumption [10], and determination of antimicrobial action kinetics [11]. Detailed information on how these compounds work to achieve their antimicrobial effect is still to be explored.

This study aimed to analyze the chemical composition of the EO obtained from wild *Cinnamomum zeylanicum* bark collected from the green mountains of Oman and assess its inhibitory effect on the growth and survival of some pathogenic bacteria and fungi, in addition to studying its mode of action with different methods.

Materials and methods

Collection, extraction, and identification of essential oil

Plant material collection and extraction of essential oil

The bark of *C. zeylanicum* plant was collected from the green mountain of Oman. The plant samples were identified in the herbarium of the Faculty of Science, Mansoura University, Egypt.

The EO of the plant bark was obtained from the entire plant by hydrodistillation using a modified Clevenger-type apparatus for 3 h according to the method of Guenther [12]. The oily layer obtained was separated and dried with anhydrous sodium sulfate. All the EOs were kept in sealed airtight glass vials covered with an aluminum foil and maintained at 4°C until further analysis.

Gas chromatography–mass spectrometry analysis

The gas chromatography–mass spectrometry (GC-MS) analysis of the EO samples was carried out using GC-MS instrument stands at the Department of Medicinal and Aromatic Plants Research, National Research Center with the following specifications. Instrument: a TRACE GC Ultra Gas Chromatograph (Thermo Scientific Corp., Miami, CA, USA), coupled with a Thermo mass spectrometer detector (ISQ Single Quadrupole Mass Spectrometer; Model ISQ Spectrometer, Thermo Scientific Corp., Miami, CA, USA). The GC-MS system was equipped with a TR-5 MS column (30 m×0.32 mm i.d., 0.25 µm film thickness). Analyses were carried out using helium as carrier gas at a flow rate of 1.3 ml/min and a split ratio of 1 : 10 using the following temperature program: 60°C for 1 min; rising at 4.0°C/min to 240°C and held for 1 min. The injector and detector were held at 200°C. Diluted samples (1 : 10 hexane, v/v) of 1 µl of the mixtures were always injected. Mass spectra were obtained by electron ionization at 70 eV, using a spectral range of m/z 40–450.

Identification of essential oil constituents

Identification of the chemical constituents of the EO was deconvoluted using AMDIS software (www.amdis.net) and identified by its retention indices (relative to *n*-alkanes C_8–C_{22}), mass spectrum matching to authentic standards (when available, Wiley spectral library collection and NSIT library database).

Evaluation of antimicrobial potential and mode of action of essential oil

Test microorganisms

Antimicrobial tests were studied using Gram-positive bacteria such as *Bacillus subtilis* ATCC6633, *Bacillus cereus* ATCC6629 and *Staphylococcus aureus* ATCC29213, Gram-negative bacteria such as *Escherichia coli* ATCC25922, *Salmonella typhimurium* ATCC14028, *Klebsiella pneumonia* ATCC13883, and *Proteus vulgaris* (isolate) as well as the fungi *Candida albicans* ATCC10231 and *Aspergillus niger* obtained from Chemistry of Natural and Microbial Products, National Research Center. Bacteria and fungi were kept at 4°C on Nutrient Agar slants and Sabouraud dextrose agar slants, respectively.

Inoculum preparation

Bacteria and fungi inocula were obtained from overnight cultures grown on nutrient agar and Sabouraud dextrose agar slants, respectively. The cultures were incubated at 37°C for bacteria and 30°C for fungi. A loopful of cells was transferred into sterile saline solution (NaCl 0.85% w/v) to provide a final concentration of ~10^6 count forming units per ml adjusted according to the turbidity of 0.5 McFarland scale tube at 600 nm.

Antimicrobial activity of essential oil

Antimicrobial activities of the EO were carried out by agar well diffusion method using the macrodilution approach [13]. The culture media were prepared for the tested microbes as mentioned above. After sterilization, these media were poured in 9 cm Petri dishes containing 100 µl of different inocula prepared as mentioned above. After solidification of the media,

10 mm wells were made and loaded with 100 µl of crude EO and diluted EO which ranged from 5 to 50 µl/ml. Then the inoculated plates were incubated for 24 h at 37°C for bacteria and for 2 days at 30°C for fungi. After the incubation time, antimicrobial activities were detected by measuring the inhibition zone diameter expressed in millimeters (mm) against ciprofloxacin and ketoconazole (0.5 mg/ml) as standards for antibacterial and antifungal activities, respectively. Negative controls were prepared using DMSO used for diluting the EO.

Minimal inhibitory concentration of essential oil

Minimum inhibitory concentrations (MICs) were assessed by serial broth dilution method [14]. The inocula were freshly prepared as mentioned above. The EO was first dissolved in DMSO and then different concentrations were prepared by diluting the samples in the respective media. The concentrations ranges were from 3 to 30 µl/ml. Each one ml from the above concentrations received 0.1 ml of the inoculum to reach 10^6 CFU/ml for our tested microbes. Triplicate tests were performed, and the average was taken as the final reading comparing negative and positive controls.

Kinetic study and mode of action of essential oil

Viability of microbial cells: the antimicrobial effect of EO on the viability of some pathogens was evaluated applying two different methods as given below.

Kill time analysis at MIC of cinnamon essential oil (CEO) using ordinary culture conditions: *B. subtilis* ATCC6633, *E. coli* ATCC25922, and *C. albicans* ATCC10231 were selected as examples for Gram-positive, Gram-negative bacteria, and fungi, respectively. EO at MIC concentration was added to the cultures (10^5 CFU/ml) in nutrient broth medium for bacteria and Sabouraud medium for fungi to reach a final volume of 5 ml. Cultures in broth with DMSO only was considered as positive control. The cultures were incubated at 37°C for 6 h at 150 rpm, then samples were taken every 2 h, serially diluted in saline, spread on the surface of the respective media, and then incubated at 37°C for 24 h. Subsequently, the colonies were counted in terms of CFU/ml [15].

Kill time analysis using different concentrations of CEO: kill-kinetics study using the previous organisms was performed to analyze the kill time of CEO using different concentrations of MIC. Cinnamon oil was diluted into tubes with phosphate buffered saline at 1×MI, 2×MIC, and 3×MIC concentrations. Then, the tubes were inoculated with freshly prepared bacterial suspension in order to maintain the initial bacterial concentration of 10^5CFU/ml and incubated at 37°C. Samples were removed at 0, 0.5, 1, and 2 h of incubation, and diluted serially. Viable counts were determined by plating the diluted aliquots on respective agar plates and incubated for 24 h at respective temperature. As a control, the bacterial suspension in sterile phosphate buffer solution with DMSO was also tested [16].

Integrity of the cell membrane: the cell membrane integrity of the same organisms was determined by quantification of cellular materials released into the extracellular medium, especially DNA, RNA, and protein. EO at the MIC concentration was added to 5 ml of the microbial inoculum (10^5 CFU/ml) in a sterilized medium (nutrient broth for bacteria and Sabouraud for fungi) and then incubated at 150 rpm, 37°C for 6 h. Then the samples were collected after 2, 4, and 6 h of incubation and centrifuged at 4000 rpm for 10 min, and the supernatant was collected for the determination of leakage of intracellular content by measuring the concentration of DNA, RNA, and protein using NanoDrop [17]. EO without bacteria in the culture media was used as the negative control. The untreated cultures were served as positive controls.

Permeability of the cell membrane: the cell membrane permeability in terms of relative electrical conductivity (EC) was performed [18]. Our tested microbes were cultured in broth media and incubated at 37°C for 10 h, then, the suspensions were centrifuged, and the pellets were washed with 5% glucose until its EC became close to that of 5% glucose. CEO at MIC concentration was added to 5% glucose (L_1), and to the isotonic bacterial solution, and incubated at 37°C for 2 h, then the EC was measured every 30 min (L_2). The control was killed bacteria in 5% glucose (L_0). The permeability of the bacterial membrane was calculated as follows:

$$\text{Relative conductivity}\% = 100 \times (L_2 - L_1/L_0)$$

Evaluation of antioxidant potential by 2,2'-diphenyl-1-picrylhydrazyl free radical scavenging assay

The antioxidant activity of CEO was measured in terms of hydrogen-donating or radical-scavenging ability with some modifications [19]. The tested EO was diluted in methanol (0.25–5 mg) and a volume of 100 µl of it was put into a test tube containing 900 µl of 2,2'-diphenyl-1-picrylhydrazyl (DPPH) of 0.1 mmol/l in methanol. Shaken vigorously and kept in dark for 30 min at room temperature. The control was prepared as above without the EO and methanol was used for zero adjustment. The decrease in absorbance was measured at 517 nm.

Free radical scavenging activity of the tested EO was calculated according to the following formula:

$$\%\text{Free radical scavenging} = \left[\left(A_{control} - A_{sample}\right)/A_{control}\right] \times 100$$

where A sample is the absorbance of DPPH solution with EO and A control is the absorbance of DPPH solution in methanol without EO. Each assay was carried out in triplicate.

Results

Chemical constituents of the essential oil

In our research, 30 components in CEO were extracted from the bark and are presented in Table 1. The main components are cinnamaldehyde (81.78%), bornyl acetate (5.38%), and cinnamyl acetate (2.82%).

Antimicrobial activity

In-vitro antimicrobial potential of CEO was assessed by agar well diffusion method (Table 2). The results showed that the plant EO was significantly effective against all tested microbes. The EO fully inhibited the growth of all tested organisms except *A. niger* which is partially inhibited giving an inhibition zone of 33 mm.

The data obtained from Table 3 demonstrate microbial susceptibility in relation to oil concentration. It depends on the growth ability of microorganisms under the effect of different concentrations of EO indicating by growth inhibition zone. The values of oil concentrations ranged between 5 and 50 µl/ml. It was noticed that the sensitivity of microorganisms increased with the increment of oil concentration. Different effective concentrations of CEO against the assayed organisms were found.

Table 1 Relative percentage of volatile compounds in cinnamon essential oil bark

No.	Name of compound	Class	Formula	RT (min)	%
1	α-Pinene	mh	$C_{10}H_{16}$	4.7	0.27
2	Camphene	mh	$C_{10}H_{16}$	5.15	0.34
3	β-Pinene	mh	$C_{10}H_{16}$	5.89	0.32
4	1,8-Cineole	om	$C_{10}H_{18}O$	7.59	1.75
5	Linalool oxide cis	om	$C_{10}H_{18}O_2$	8.93	0.08
6	Linalool oxide trans	om	$C_{10}H_{18}O_2$	9.54	0.1
7	l-Linalool	om	$C_{10}H_{18}O$	10.09	0.13
8	Fenchyl alcohol	om	$C_{10}H_{18}O$	10.92	0.06
9	Camphor	om	$C_{10}H_{16}O$	12.09	0.07
10	Lis-sabinene hydrate	om	$C_{10}H_{18}O$	12.37	0.11
11	Endo-borneol	om	$C_{10}H_{18}O$	13.09	1.15
12	Terpinene-4-ol	om	$C_{10}H_{18}O$	13.38	0.3
13	α-Terpineol	om	$C_{10}H_{18}O$	14.08	0.76
14	α-Fenchyl acetate	om	$C_{12}H_{20}O_2$	15.29	0.07
15	Vinyl benzaldehyde	nt	C_9H_8O	15.43	0.62
16	Bornyl acetate	om	$C_{12}H_{20}O_2$	17.60	5.38
17	Cinnamaldehyde	nt	C_9H_8O	17.93	81.78
18	α-Copaene	sh	$C_{15}H_{24}$	21.19	0.35
19	Cinnamyl acetate	nt	$C_{11}H_{12}O_2$	25.05	2.82
20	α-Muurolene	sh	$C_{15}H_{24}$	26.32	0.17
21	Calamenene	sh	$C_{15}H_{22}$	27.32	0.19
22	Cinnamic acid	nt	$C_9H_8O_2$	28.42	0.10
23	β-Cedrene	sh	$C_{15}H_{24}$	29.45	0.11
24	Caryophyllene oxide	os	$C_{15}H_{24}O$	29.63	0.67
25	Isoaromadendrene epoxide	os	$C_{15}H_{24}O$	30.48	0.1
26	Cubenol	os	$C_{15}H_{26}O$	31.40	0.49
27	α-Cadinol	os	$C_{15}H_{26}O$	32.11	0.32
28	δ-Cadinol	os	$C_{15}H_{26}O$	32.20	0.20
29	Veridiflorol	os	$C_{15}H_{26}O$	32.53	0.15
30	Globulol	os	$C_{15}H_{26}O$	32.66	0.13
	Total identified			99.09	
	Monoterpene hydrocarbons (MH)			0.93	
	Oxygenated monoterpenes (OM)			9.96	
	Sesquiterpene hydrocarbons (SH)			0.82	
	Oxygenated sesquiterpenes (OS)			2.06	
	Non-terpenes (NT)			85.32	

Table 2 Antimicrobial activity of cinnamon essential oil and standard references (0.5 mg/ml) expressed as inhibition diameter zones in millimeters

Treatment type	Inhibition zone diameter (mm)								
	Gram positive			Gram negative				Fungi	
	Bacillus subtilis	*Staphylococcus aureus*	*Bacillus cereus*	*Klebsiella pneumoniae*	*Escherichia coli*	*Salmonella typhimurium*	*Proteus vulgaris*	*Aspergillus niger*	*Candida albicans*
Crude oil	NG	NG	NG	NG	NG	NG	NG	33±2.5	NG
CFL	16±2.0	15±0.5	16±1.0	19±1.5	18±2.0	19±1.5	18±0.5	–	–
KET	–	–	–	–	–	–	–	ND	16±1.0

CFL, ciprofloxacin; KET, ketoconazole; ND, not detected; NG, no growth.

Table 3 Microbial susceptibility toward oil concentrations

Test organisms	Inhibition zone (mm) at different concentrations (µl/ml)			
	5	10	20	50
Gram positive				
Bacillus subtilis	14±1	29±2	35±3	NG
Staphylococcus aureus	17±2	20±2	NG	NG
Bacillus cereus	15±1	25±3	NG	NG
Gram negative				
Klebsiella pneumoniae	0	6±1	NG	NG
Escherichia coli	0	7±1	NG	NG
Salmonella typhimurium	0	7±1	18±1	NG
Proteus vulgaris	6±1	15±2	32±2	NG
Fungi				
Aspergillus niger	16±2	20±3	21±2	26±3
Candida albicans	14±2	20±3	30±1	NG

NG, no growth.

Table 4 Minimum inhibitory concentration (µl/ml) of cinnamon essential oil against pathological organisms

Treatment type	EO concentration (µl/ml)								
	Gram positive			Gram negative				Fungi	
	Bacillus subtilis	*Staphylococcus aureus*	*Bacillus cereus*	*Klebsiella pneumoniae*	*Escherichia coli*	*Salmonella typhimurium*	*Proteus vulgaris*	*Aspergillus niger*	*Candida albicans*
Crude oil	3.3	3.3	3.3	10	10	10	5	3.3	3.3
CFL	12	13	12	14	13	14	14	–	–
KET	–	–	–	–	–	–	–	10	12

CFL, ciprofloxacin; KET, ketoconazole.

Minimum inhibitory concentration

Table 4 shows the evaluation of MIC of CEO against the pathological strains. The MIC values of the oil ranged from 3 to 30 µl/ml. Gram-positive bacteria and fungi were found to be more sensitive than Gram-negative ones. Also, it is noticed that Gram-positive bacteria and fungi have similar sensitivity toward EOs giving the smallest MIC (3.3 µl/ml) value compared with Gram-negative ones (5–10 µl/ml).

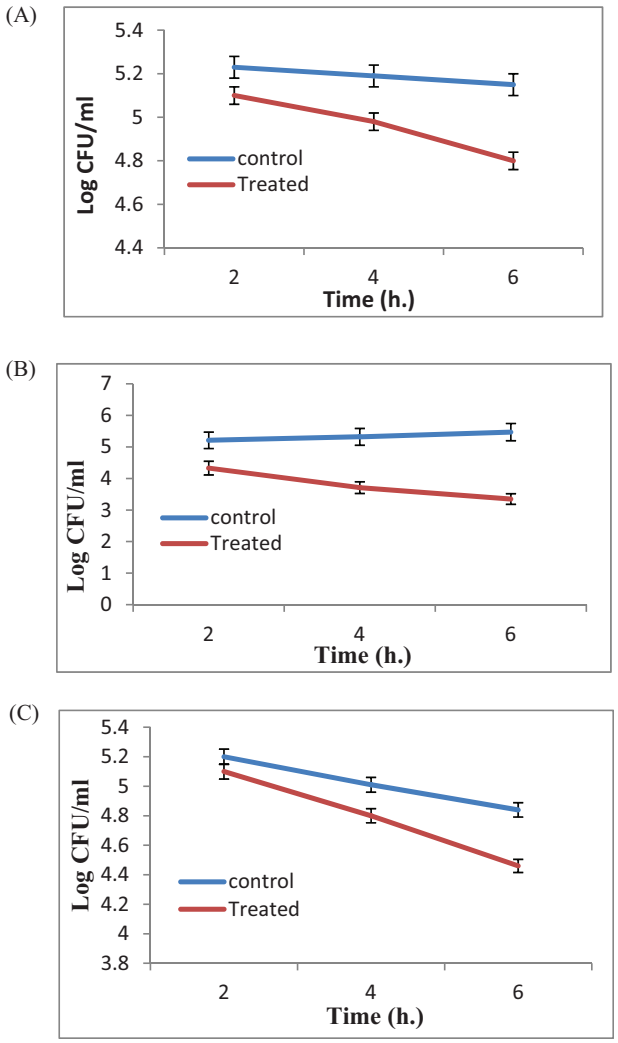
Mechanism of action of cinnamon essential oil

Three organisms (*B. subtilis* ATCC6633, *E. coli* ATCC25922, and *C. albicans* ATCC10231) were selected for a further in-depth study of the antimicrobial mode of action of EO. The mode of action of EO against the investigated microbes was studied by several assays, including time-kill analysis, quantity of biomolecules released, and cell membrane permeability.

Study of kill time assay applying ordinary culture conditions

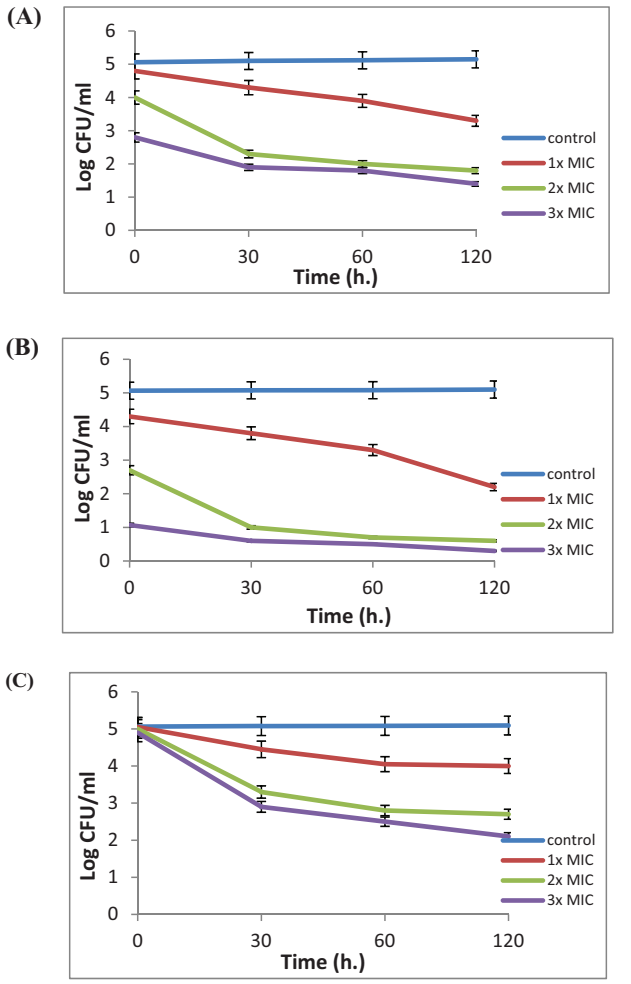
The relationship between microbial cell viability and kill time analyses of CEO under the effect of ordinary culture conditions was investigated for determining the bactericidal or fungicidal effect of the EO. As shown in Fig. 1, EO at the MIC (10 µl/ml for *E. coli* and 3.33 µl/ml for *B. subtilis* and *C. albicans*) concentration showed an increased reduction of viable cell count along with increasing incubation time for all organisms used

Figure 1



Time-kill curves in control and treated samples with cinnamon essential oil at minimum inhibitory concentration of (a) *Bacillus subtilis* ATCC6633, (b) *Escherichia coli* ATCC25922, and (c) *Candida albicans* ATCC10231.

Figure 2



Time-kill curves in control and treated samples with cinnamon essential oil at different concentrations of minimum inhibitory concentrations of (a) *Bacillus subtilis* ATCC6633, (b) *Escherichia coli* ATCC25922, and (c) *Candida albicans* ATCC10231.

compared with the control (without EO). However, the EO had positive results against the three tested microbes during the tested time, but the reduction rate was not significant (0.35–2.1 log) and did not reach the total kill point during our incubation time.

Study of kill time assay applying different concentrations of cinnamon essential oil

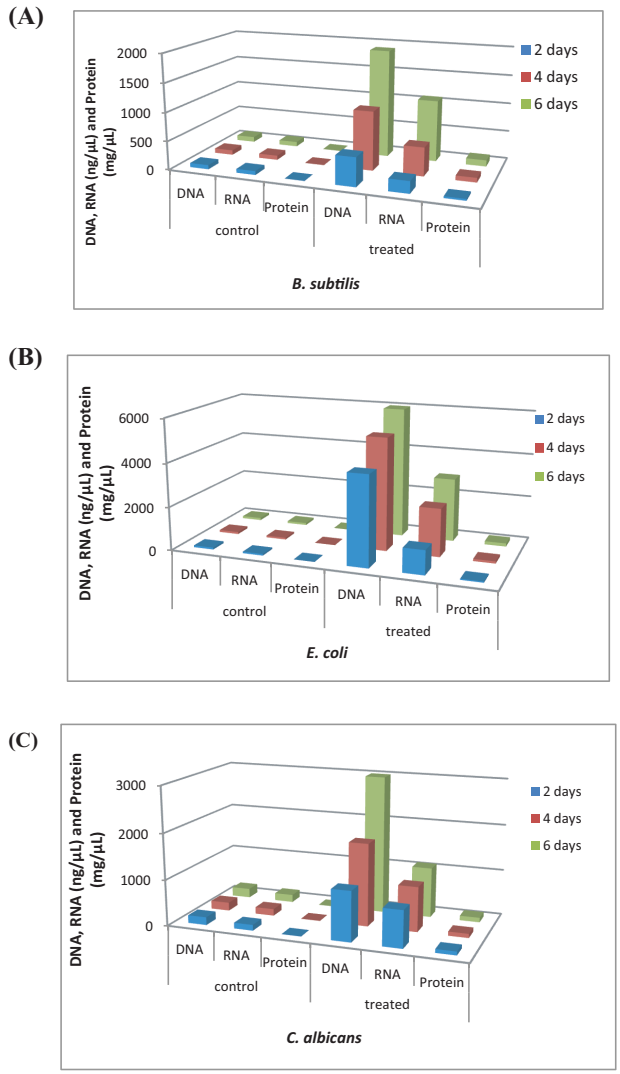
From the previous experiment, it was concluded that the ordinary culture condition was effective and enhanced cell viability; therefore, in the following test to confirm that effect, we neglected this factor and used instead phosphate buffered saline.

Time-kill curves at 1×MIC, 2×MIC, and 3×MIC concentrations of CEO for the same microorganisms were cultured in phosphate buffer saline and incubated at 37°C for 2 h as shown in Fig. 2. The concentration effect relationship has shown that EO at higher MIC values had bactericidal and fungicidal effects and the reduction value was greater than or equal to 3 log CFU/ml.

Effects of cinnamon essential oil on cell release

The cell constituents' leakage under the effect of CEO at MIC concentration was investigated to give further insight into the mode of action of our EO against the three tested microbial examples. In this experiment, nucleic acid and protein leakage were studied in time-dependent test (Fig. 3). It was observed that the amounts of released DNA, RNA, and protein of all tested microbes increased with the time. The maximum concentration of leaked DNA, RNA, and protein (after subtracting the negative control) in cell suspensions was (1900, 6000, and 3000 ng/µl), (1080, 2920, and 1090 ng/µl), and (110, 150 and 100 mg/µl), respectively for treated *B. subtilis*, *E. coli*, and *C. albicans* compared with the untreated controls (91, 105, and

Figure 3



Quantity of biomolecules released by (a) *Bacillus subtilis* ATCC6633, (b) *Escherichia coli* ATCC25922, and (c) *Candida albicans* ATCC10231 treated with cinnamon essential oil.

200 ng/µl), (82, 95, and 165 ng/µl), and (1.6, 3.2, and 4 mg/µl), respectively after 6 h.

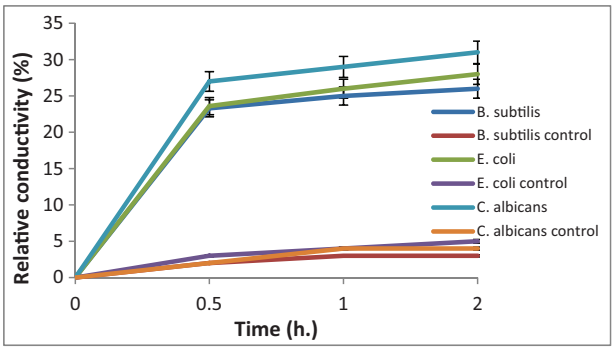
Effects of cinnamon essential oil on cell permeability

Cell membrane permeability was measured in terms of EC as shown in Fig. 4. The cells treated with CEO at MIC concentration showed high EC compared with the control indicating the effect of CEO on membrane permeability. The increased EC is considered as a result of leakage of intracellular electrolytes. The maximum relative conductivity of 26.1, 28.2, and 31.0 was reached after 2 h.

Evaluation of antioxidant potential

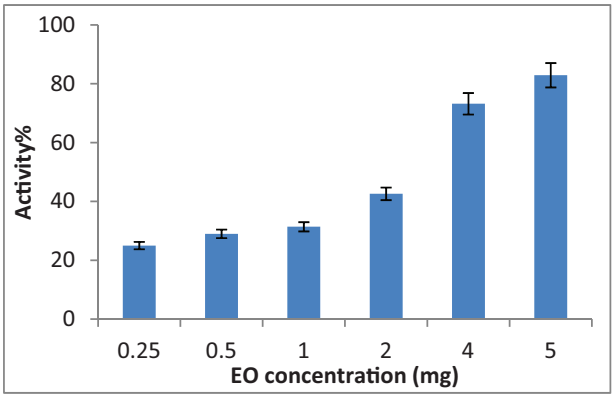
Radical scavenging activity of CEO using DPPH is illustrated in Fig. 5 which showed that the antioxidant potential (25±1.4–82.9±2.1%) is dependent on the concentration with IC_{50} reaching 2.3 mg.

Figure 4



Effect of cinnamon essential oil at minimum inhibitory concentration on cell membrane permeability of *Bacillus subtilis* ATCC6633, *Escherichia coli* ATCC25922, and *Candida albicans* ATCC10231.

Figure 5



Antioxidant activity of cinnamon essential oil using 2,2'-diphenyl-1-picrylhydrazyl.

Discussion

EOs of cinnamon bark have been used for thousands of years, in natural therapy, alternative medicine, and in pharmaceutical applications [20]. So, it is necessary to investigate the role of this plant EO in the treatment of common infectious diseases to overcome the emergence of antibiotic-resistant microorganisms [2]. The composition of CEO is greatly influenced by the method of extraction, species of the plant, plant parts, development stage, plant age, geographic origin, and time of harvest [21] Our results showed that the main components are cinnamaldehyde (81.78%), bornyl acetate (5.38%), and cinnamyl acetate (2.82%). It has been validated by [22] that transcinnamaldehyde (86.16%) was the major component of CEO of the bark. Furthermore [23], observed that the major component of CEO extracted from the leaf was cinnamaldehyde which possessed inhibitory activity against some microorganisms by the inhibition of cell enzyme synthesis, disruption of the cell wall structure resulting in shortage in the

cytoplasm, cytoplasmic granulation, cytoplasm acidity, and depletion of intracellular ATP collect [24]. And this is in agreement with our obtained results with CEO which had a significant inhibitory effect on all tested microbes.

About the antimicrobial activity, our tested EO was significantly effective against all tested microbes. These findings are in agreement with the results of [25,26], who reported that CEO was effective against 10 examined bacteria including *S. aureus* and *E. coli*. Furthermore [27] obtained the same result with some Gram-negative, Gram-positive bacteria, fungi, and yeasts which were completely inhibited by cinnamon bark oil. This is because the main component of CEO is cinnamaldehyde, which has been proven to be effective substance against some Gram-negative and Gram-positive bacteria including *Pseudomonas*, *clostridium*, and yeasts like *Candida* strains [28,29]; moreover, the presence of terpenes in CEO is believed to accumulate in bacterial cell membrane and cause a loss of the membrane integrity, cell lysis, and death [30].

The microbial susceptibility test showed that Gram-positive bacteria and fungi were more sensitive than Gram-negative ones in low concentrations. In that context, the study by [31] showed different effective concentrations of CEO against *S. aureus.* Also [24] obtained the same results with CEO against the tested microbes.

The MIC test showed that Gram-positive bacteria and fungi were more sensitive than Gram-negative ones. This is in agreement with [24] who tested the CEO of leaves against *E. coli*, *P. aeruginosa*, *S. aureus*, and *S. enterica* and found that the EO exhibited marked inhibitory effect toward all assayed bacteria. And the smallest MIC (1.25 µl/ml) was against *S. aureus* (Gram positive) while the highest one was against *P. aeruginosa* (Gram negative), while [20] reported that the oil of *C. zeylanicum* and C. *cassia* were more effective against *E. coli* more than *P. aeruginos and S. aureus.*

Study of kill time assay applying ordinary culture conditions demonstrated that the reduction rate was not significant (0.35–2.1 log) and did not reach the total kill point during our incubation time; this is may be due to the presence of culture medium which not only can protect the microbial cells from the action of EO but also can activate or decrease the EO activity [32]. Overall, the reduction of microbial growth is concentration dependent or time dependent on the applied EO [33,34]. In this context [35] studied the effect of *C. burmannii*'s EO on the growth of *E. coli*, *C. albicans*, and *S. aureus* and found that the growth reduction was increased over the time interval compared with the control. Also [36] applied 0.05% of oregano EO against meat microbial flora cultured in broth for several days at 37°C and they obtained a 2 log decrease in viable cell count and the total kill point was not achieved. Furthermore [37] found that the CEO decreased the viable cell count of the tested bacteria (*E. coli*, *Listeria monocytogenes*, *Pseudomonas erogenous*, *Staphylococcus aureus*, and *Enterobacter aerogenes*) cultured in nutrient broth from 2 to 5 log after 24 h. of incubation compared with the control using concentrations of 10, 20, and 30 µl/ml.

On the other hand, the concentration effect relationship has demonstrated that EO at higher MIC values had bactericidal and fungicidal effects because the reduction value was greater than or equal to 3 log CFU/ml [38]. The concentration of 3xMIC (30 µl/ml for E. coli and 10 µl/ml for *C. albicans*, *B. subtilus*) was found to be the most effective ones after half an hour. of incubation and beyond that time the reduction rate in viable cell count was not significant. The reduction reached about 3, 3.6, and 4.7 log with a lethal percent of 99.87, 99.97, and 99.99% for *C. albicans*, *B. subtilus*, and *E. coli*, respectively, after 2 h. This indicates that it had bactericidal effect [38] acting in a dose-dependent manner [33,39]. This is in agreement with [40] who reported that cinnamon bark oil had a bactericidal effect against *S. aureus* with 3.6 log10 reduction in bacterial cell count at a concentration of 4×MIC after 24 h of incubation time. this suggests that the primary mechanism of action of CEO is cell wall lysis [41] or weakening of the cell wall leading to irreversible damage to the cytoplasmic membrane due to osmotic pressure [42], or the EO penetrated the cells causing complete lysis and cellular death [43].

On the other hand, the results showed that Gram-negative bacteria is more resistant than Gram-positive bacteria and fungi, the reduction in viable cells of *E. coli* reached 2.2 log at 10 µl/ml, while it reached 1.4 and 2.0 log at a concentration of 10 µl/ml for *B subtilis* and *C. albicans*, respectively, after 120 min of incubation. The MIC for E. coli was three times more than that of *B. subtilis* and *C albicans* and this confirmed our previous obtained results of MIC test.

Effects of CEO on cell release revealed that the amount of released DNA, RNA, and protein of all tested organisms increased with time [22]. The high

amount of the released biomolecules after exposure to CEO indicated that the cytoplasmic membrane was damaged leading to cell lysis and death [22]. That is in agreement with [44], who measured the concentrations of released protein, DNA, and RNA from three strains of *Mycobacterium tuberculosis* (IS310, IS53, and H37Rv) and found the protein concentration ranges (5×10^4-107×10^4 ng/µl for untreated strains and 37.9×10^4–1200.9×10^4 ng/µl in treated strains) were higher than that of released DNA (70 at MIC –506 ng/µl at MBC) and RNA (20 at MIC –151.6 ng/µl at MBC). From our results, there were some differences between the microorganisms which could be related to the differences in plasma membrane and cell wall structure [45]. Our data indicated that, however, *E. coli* is more resistant to the EO than *B. subtilis* and *C. albicans* but it released nucleic acid and protein more than of the other ones; this may be due to the different action of EO on cell damage. This is in accordance with [46] who treated *E. coli* APL 87/1 and *B. subtilis* APL 87/35 with clove and oregano EOs and found that *E .coli* was damaged by forming holes on the cell surface, whereas *B. subtilis* damage was just on the cell surface contributing to the malformation. Furthermore, the cell membrane integrity indicated by cell permeability may affect cell metabolism because of the fact that it is integral with the maintenance of cell energy, metabolic regulation, transport of solutes, and control of turgor pressure; so, any slight change in cell integrity may lead to cell death [47]. This is in agreement with [48] who observed that an increase in EC positively is proportional to plant extract concentration.

Finally, CEO have good free radical scavenging property [49] that may play an important role in the prevention of some diseases such as cancer, immune system decline, and heart disease and act as anti-inflammatory agent [50]. Furthermore, the antioxidant activity coupled with antibacterial, antifungal, antiallergic, and anticarcinogenic agents [51] could be a helpful therapeutic agent in promoting wound healing [6].

Conclusion

This study has shown that CEO possesses potential antimicrobial activity against several microbes, and it has a good antioxidant activity. Cinnamaldehyde was found to be the major component of CEO which is considered as a natural antimicrobial agent. Moreover, the mechanism of action of CEO at MIC concentration demonstrated that it has bactericidal and fungicidal effects and it could penetrate the cell wall of the pathogens causing their damage and consequent release of cell constituents (DNA, RNA, and protein). Therefore, it has an adverse effect on microbial pathogens and it may be successfully applied as a promising alternative drug for treatment of infectious diseases and help in wound healing. Furthermore, the effective dose to kill microbes is very small which means CEO can be used in many other applications such as food spoilage, the limiting factor to control water contamination by bacteria or fungi.

Acknowledgements

The authors acknowledge The National Research Centre, Egypt, for supporting this work Financial support and sponsorship.

Author contribution

Eman W. Elgammal, Abd El-Nasser G. El Gendy, and Abd El-Basset A. Elgamal together proposed and designed the study. Eman Elgammal performed the microbiology part and wrote the manuscript. Abd El-Nasser G. El Gendy and Abd El-Basset A. Elgamal performed the chemistry part and collected the samples.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1 Yap PSX, Limb SHE, Huc CP, Yiap PC. Combinations of essential oils and antibiotics reduce antibiotic resistance in plasmid conferred multidrug resistant bacteria. Phytomedicine 2013; 20:710–713.

2 Sienkiewicz M, Lysakowska M, Pastuszka M, Bienias W, Kowalczyk E. The potential of use basil and rosemary essential oils as effective antibacterial agents. Molecules 2013; 18:9334–9351.

3 Gayoso CW, Lima EO, Oliveira VT, Pereira FA. Sensitivity of fungi isolated from onychomycosis to *Eugenia cariophyllata* essential oil and eugenol. Fitoterapia 2005; 76:247–249.

4 Lima IO, Oliveira RAG, Lima EO, Souza EL. Inhibitory action of some phytochemicals on yeasts potentially causing of opportunistic infections. Rev Bras Ciênc Farm 2005; 41:199–203.

5 Bassolé IHN, Juliani HR. Essential oils in combination and their antimicrobial properties. Molecules 2012; 17:3989–4006.

6 Süntar I, Küpeli AE, Nahar L, Satyajit D, Sarker SD. Wound healing and antioxidant properties: do they coexist in plants? Free Radic Antioxidant 2012; 2:1–7.

7 Shukla R, Singh P, Prakash B, Dubey NK. Antifungal, aflatoxin inhibition and antioxidant activity of Callistemon lanceolatus(Sm.) Sweet essential oil and its major component 1, 8-cineole against fungal isolates from chickpea seeds. Food Control 2012; 25:27–33.

8 Raafat D, Von Bargen K, Haas A, Sahl HG. Insights into the mode of action of chitosan as an antibacterial compound. Appl Environ Microbiol 2008; 74:3764–3773.

9 Ultee A, Kets EP, Smid EJ. Mechanisms of action of carvacrol on the food-borne pathogen *Bacillus cereus*. Appl Environ Microbiol 1999; 65:4606–4610.

10 Fitzgerald DJ, Stratford M, Gasson MJ, Ueckert J, Bos A, Narbad A. Mode of antimicrobial of vanillin against *Escherichia coli*, Lactobacillus plantarum and *Listeria innocua*. J Appl Microbiol 2004; 97:104–113.

11 Bloomfield SF. Mechanisms of action of chemical biocides. Their study and exploitation. Methods for Assessing antimicrobial activity. In: Denyer SP,

Hugo WB. Technical Series of the Society for Applied Bacteriology. Oxford: Blackwell Scientific Publications; 1991.

12 Guenther E. The essential oils book. New York, London: D. van Nostrand Comp., Vol. 3. 1961.

13 Okeke MI, Iroegbu CU, Eze EN, Okoli AS, Esimone CO. Evaluation of extracts of the root of *Landolphia owerrience* for antibacterial activity. J Ethnopharmacol 2001; 78:119–127.

14 Murray PR, Baron EJ, Pfaller MA, Tenover FC, Yolki RH. Manual of Clinical Microbiology. 6th ed. Washington, DC: American Society for Microbiology 1995. 282.

15 Joray MB, Del-Rollan MR, Ruiz GM, Palacios SM, Carpinella MC. Antibacterial activity of extracts from plants of central Argentina-isolation of an active principle from *Achyrocline satureioides*. Planta Med 2011; 77:95–100.

16 Nakayama M, Shigemune N, Tsugukuni T, Jun H, Matsushita T, Mekada Y, *et al.* Mechanism of the combined anti-bacterial effect of green tea extract and NaCl against Staphylococcus aureus and Escherichia coli O157:H7. Food Control 2012; 25:225–232.

17 Desjardins PR, Conklin DS. Nanodrop microvolume quantification of nucleic acids. J Vis Exp 2010; 22:1–5.

18 Kong M, Chen XG, Liu CS, Meng XH, Yu LJ. Antibacterial mechanism of chitosan microspheres in a solid dispersing system against E. coli. Colloids Surf Biointerfaces 2008; 65:197–202.

19 Wang M, Li J, Rangarajan M, Shao Y, LaVoie EJ, Huang TC, Ho CT. Antioxidative phenolic compounds from sage (Salvia officinalis). J Agric Food Chem 1998; 46:4869–4873.

20 Pritam DN, Hemant G, Anu G, Niharika S, Monica K, Ramalingam C. Comparison of antimicrobial activity of *Cinnamomum zeylanicum* and *Cinnamomum cassia* on food spoilage bacteria and water borne bacteria. Der Pharm Lett 2013; 5:53–59.

21 Lee HC, Cheng SS, Chang ST. Antifungal property of the essential oils and their constituents from Cinnamomum osmophloeum leaf against tree pathogenic fungi. J Sci Food Agric 2005; 85:2047–2053.

22 He J, Wu D, Zhang Q, Chen H, Li H, Han Q, *et al.* Efficacy and mechanism of cinnamon essential oil on inhibition of colletotrichum acutatum isolated from 'Hongyang' Kiwifruit. Front Microbiol 2018; 9:1288.

23 Singh G, Maurya S, Delampasona MP, Catalan CA. A comparison of chemical, antioxidant and antimicrobial studies of cinnamon leaf and bark volatile oils, oleoresins and their constituents. Food Chem Toxicol 2007; 45:1650–1661.

24 Trajano N, Lima EO, Travassos AE, Souza EL. Inhibitory effect of the essential oil from *Cinnamomum zeylanicum Blume* leaves on some food-related bacteria. Ciênc Tecnol Aliment 2010; 30:771–775.

25 Friedman M, Henika PR, Mandrell RE. Bactericidal activities of plant essential oils and some of their isolated constituents *Campylobacter jejuni*, Escherichia coli, *Listeria monocytogenes*, *Salmonella intrica*. J Food Prot 2002; 65:1545–1560.

26 Gupta C, Garg AP, Uniyal RC, Kumari A. Comparative analysis of the antimicrobial activity of cinnamon oil and cinnamon extract on some food born microbes. Afr J Microbiol Res 2008; 2:247–251.

27 Hadri Z, Allem R, Marin GP. Effect of essential oil of *Cinnamomum zeylanicum* on some pathogenic bacteria. Afr J Microbiol Res 2014; 8:1026–1031.

28 Shan B, Cai YZ, Brooks JD. Antibacterial properties and major bioactive components of cinnamon stick (Cinnamomum burmannii): Activity against foodborne pathogenic bacteria. Agr J Food Chem 2007; 55:5484–5490.

29 Bin Jantan I, Karim Moharam BA, Santhanam J, Jamal JA. Correlation between chemical compositionand antifungal activity of the essential oils of eight Cinnamomum. species. Pharm Biol 2008; 46:406–412.

30 Gustafson JE, Cox SD, Mann J, Markham J. Effects of tea tree oil on *Escherichia coli*. Lett Appl Microbiol 1998; 26:194–198.

31 Hoque MD, Inatsu ML, Juneja VK, Kawamoto S. J Food Sci Tech 2007; 72:9–21.

32 Faleiro ML. The mode of antibacterial action of essential oils, Science against microbial pathogens: communicating current research and technological advances Méndez-Vilas (Ed.) 2011. 1143–1156.

33 Mayaud L, Carricajo A, Zhiri A, Aubert G. Comparison of bacteriostatic and bactericidal activity of 13 essential oils against strains with varying sensitivity to antibiotics. Lett Appl Microbiol 2008; 47:167–173.

34 Pfaller MA, Sheehan DJ, Rex JH. Determination of fungicidal activities against yeasts and molds: lessons learned from bactericidal testing and the need for standardization. Clin Microbiol Rev 2004; 17:268–280.

35 Awang AFI, Muhammad Taher M, Susanti D. The mode of antimicrobial action of *Cinnamomum burmannii's* essential oil & cinnamaldehyde. J Teknol Sci Eng 2016; 78:41–47.

36 Skandamis PN, Nychas GJE. Effect of oregano essential oil on microbiological and physico-chemical attributes of minced meat stored in air and modified atmospheres. J Appl Microbiol 2001; 91:1011–1022.

37 Al-Fekaiki DF, Niamah AK, Al-Sahlan STG. Extraction and identification of essential oil from *Cinnamomum zeylanicum* barks and study the antibacterial activity. J Microbiol Biotech Food Sci 2017; 7:312–316.

38 Konaté K, Mavoungou JF, Lepengué AN. Antibacterial activity against β-lactamase producing Methicillin and Ampicillin-resistants Staphylococcus aureus: Fractional Inhibitory Concentration Index (FICI) determination. Ann Clin Microbiol Antimicrob 2012; 11:18.

39 Utchariyakiat IS, Jaturanpinyo S, Khuntayaporn PM, Chomnawang MT. Efficacy of cinnamon bark oil and cinnamaldehyde on anti-multidrug resistant Pseudomonas aeruginosa and the synergistic effects in combination with other antimicrobial agents. BMC Complement Altern Med 2016; 16:158.

40 Rohraff D, Morgan R. The evaluation of essential oils for antimicrobial activity. Student Summer Scholars. http://scholarworks.gvsu.edu/sss/124. 2014.

41 Horne DS, Holm M, Oberg C, Chao S, Young DG. Antimicrobial effects of essential oils on *Streptococcus pneumoniae*. J Essent Oil Res 2001; 13:387–392.

42 Hugo WB, Longworth R. Some aspects of the mode of action of chlorhexidine. J Pharm Pharmacol 1964; 16:655–662.

43 Sikkema J, de Bont JA, Poolman B. Interactions of cyclic hydrocarbons with biological membranes. J Biol Chem 1994; 11:8022–8028.

44 Florence MND, Ascension NM, Paul AAJ, Hervé BDP, Gaïzirène FE, Veronique PB, François–Xavier E. Effects of essential oil from *Drypetes gossweileri* S. moore stem barks on cell release and DNA synthesis of *Mycobacterium tuberculosis*. J Drug Deliv Ther 2019; 9:319–324.

45 Bouhdid S, Abrinin J, Zhiri A, Espuny MJ, Munresa A. Investigation of functional and morphological changes in *Pseudomonas aeruginosa* and *Staphylococcus aureus* cells induced by *Origanumcompactum* essential oil. J Appl Microbiol 2009; 106:1558–1568.

46 Rhayour K, Bouchikhi T, Tantaoui-Elaraki A, Sendice K, Remmal A. The mechanism of bactericidal action of oregano and clove essential oils and of their phenolic major components on *Escherichia coli* and *Bacillus subtilis*. J Essent Oil Res 2003; 15:356–362.

47 Cox SD, Mann CM, Markham JL, Gustafson JE, Warmington JR, Wyllie SG. Determining the antimicrobial actions of tea tree oil. Molecules 2001; 6:87–91.

48 Zhao Y, Chen M, Zhao Z, Yu S. The antibiotic activity and mechanisms of sugarcane (Saccharum officinarum L.) bagasse extract against food-borne pathogens. Food Chem 2015; 185:112–118.

49 Purkait S, Bhattacharya A, Bag A, Chattopadhyay RR. Antibacterial and antioxidant potential of essential oils of five species. J Food Qual Hazards Control 2018; 5:61–67.

50 Aruoma OI. Free radicals, oxidative stress, and antioxidants in human health and disease. J Am Oil Chem Soc 1998; 75:199–212.

51 Valiakos E, Marselos M, Sakellaridis N, Constantinidis T, Skaltsa H. Ethnopharmacological approach to the herbal medicines of the 'Antidotes' in NikolaosMyrepsos' dynameron. J Ethnopharmacol 2015; 163:68–82.