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Original Article

Antibacterial activity of ajwain oil on multidrug-resistant Enterobacteriaceae isolated from human faeces

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Abstract

The aims of this study were to evaluate the effects of ajwain oil on multidrug-resistant (MDR) Enterobacteriaceae isolated from human faeces, the mode of action, and the active components. The studies also include the methicillin-resistant *Staphylococcus aureus* (MRSA). The ajwain oil showed broad spectrum and strong antibacterial activity against all the bacteria tested. The ajwain oil contained at least nine chemical compounds, and thymol was shown to be the most active component against all tested bacteria. Time-kill assay showed bactericidal effect and over 2 log reduction of MRSA and MDR Enterobacteriaceae after exposure to the ajwain oil for 30 and 10 minutes, respectively (1x, 2x, and 4x MIC). The scanning electron micrograph of MRSA and MDR *Escherichia coli* demonstrated bacterial cellular destruction after one-hour exposure to the 1x MIC of ajwain oil. Our results suggest that the application of ajwain oil may have potential for control and treatment of antibiotic resistant bacteria.

Keywords: ajwain oil, thymol, antibacterial activity, multidrug-resistant bacteria

1. Introduction

Infectious disease caused by antibiotic resistant bacteria has become a major global healthcare problem (World Health Organization, 2020). The most common resistant bacterial strains causing important community acquired infections are methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Staphylococcus aureus* (VRSE), vancomycin-resistant Enterococcus (VRE) and multidrug-resistant (MDR) Enterobacteriaceae (Walther, Tedin, & Lubke-Becker, 2017). The resistant bacterial strains have been a worldwide concern as they are highly prevalent and can potentially cause death in developing countries where the high cost of antimicrobial agents makes them unaffordable to many people. Currently, the occurrence of multidrug resistant bacteria has considerably reduced the efficacy of antibiotic arsenal and, consequently, increased the frequency of therapeutic failure. Because of undesirable side effect of some synthetic compounds, it is necessary to explore new compounds with fewer side effects (Tillotson & Zinner,

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2017). Various types of plant have been used as traditional treatments for many health problems. Several bioactive plants with activity against multidrug resistant bacteria and their ability to synergize the activity of antibiotics have been reported (Abreu *et al*., 2017; Fankam, Kuete, Voukeng, Kuiate, & Pages, 2011). For example, extract of *Piper betel* was evaluated against MDR bacteria. *Pseudomonas aeruginosa*, *Escherichia coli*, and *Klebsiella pneumonia*, and showed minimal inhibitory concentration (MIC) ranging from 0.312-0.625 mg/mL (Assis *et al*., 2018).

Ajwain (*Trachyspermum ammi* (Linn.) Sprague) belonging to Apiaceae family, is a medicinal plant with several biological effect including analgesic, anti-inflammatory, anxiolytic and antispasmodic activities. Its seed has been widely used as a food flavouring agent and spice. Moreover, the seeds have several therapeutic effects and have been used for treatments of digestion, flatulence, colic, diarrhoea, and dyspepsia (Hejazian, Bagheri, & Safari, 2014). The seeds contain essential oil. The major component of this oil is thymol (35-60%), other components include α-pinene, *p*-cymene, limonene, which mainly contributes to its therapeutic properties (Bairwa, Sodha, & Rajawat, 2012; Moein *et al*., 2015). The ajwain oil exhibits antimicrobial (Hosseinkhani *et al*., 2016; Kaur & Arora, 2009), antiviral (Roy, Chaurvedi, & Chowdhary, 2015), antifungal (Sharifzadeh, Khosravi, Shokri, & Sharafi, 2015), antitussive, antioxidant (Goswami & Chatterjee, 2014; Singh & Ahmad, 2017) and antitumor activities (Singh & Kale, 2010). There were previous reports on antimicrobial activity of the ajwain oil against MRSA and its synergistic effect with antibiotics (Vazirian, Hamidian, Noorollah, Manayi, & Samadi, 2019). It was also noted that thymol has antimicrobial activity against MRSA (Tohidpour, Sattari, Omidbaigi, Yadegar, & Nazemi, 2010).

The mode of infection of the Enterobaceriaceae is faecal-oral route transmission. Even though there were several reports about antibacterial activity of ajwain oil against these bacteria, including the MDR strains isolated from food (Gunasegaran *et al*., 2011), samples from faeces that came from patients have not been previously reported. Therefore, the samples from faeces were included in this study. The 14 tested MDR strains have been proved to the resist and to ceftazidime, the third generation of cephalosporin (Kiddee *et al*., 2019).

2. Materials and Methods

2.1 Preparation of ajwain oil

Ajwain dried seeds were purchased from Thai herbal drugstore in Bangkok in January 2018. The seeds were authenticated at Faculty of Science, Maejo University, Chiang Mai, Thailand (specimen voucher No. MJU 2017-007). The dried seeds were blended using a kitchen blender, and the essential oil was extracted by hydrodistillation. The oil was stored in an amber glass bottle at -20°C before use.

2.2 Bacterial strains and culture conditions

Bacillus cereus DMST 5040, *Listeria monocytogenes* DMST 17303, *Staphylococcus aureus* DMST 8840, Methicillin-resistant *Staphylococcus aureus* (MRSA) DMST 20651, *Escherichia coli* DMST 4212, *Pseudomonas aeruginosa* DMST 4739, and *Salmonella* Typhi DMST 5784 were obtained from the Department of Medical Science of Thailand (DMST), Ministry of Public Health. Fourteen strains of MDR Enterobacteriaceae (i.e., *E. coli* 1-5, *Enterobacter cloacae* 1-4 and *K. pneumonia* 1-5) were recovered from human faecal samples and identified using RapID ONE™ (Remel, USA) (Kiddee et al., 2019). They were sub-cultured onto Brain Heart Infusion Agar (BHA) and incubated at 37°C for 24 h before testing and stored in 10% (v/v) Tween 20 at -80°C.

2.3 Agar disc diffusion assay

The assay was performed according to the standard Kirby-Bauer method (Bauer, Kirby, Sherris, & Jurek M., 1966). Briefly, the bacteria were adjusted to 0.5 McFarland standard turbidity and spread onto BHA. Ten µL of crude ajwain oil was impregnated on 6 mm diameter filter paper disc (Macherey-Nagel, Germany) and placed onto the inoculated BHA. The plates were then incubated at 37°C for 24 h before measuring the zone of inhibition. Tetracycline (30 µg/disc, Oxoid, UK) was used as a control for bacteria derived from DMST. Imipenem (10 µg/disc, Oxoid, UK) and ceftazidime (30 µg/disc, Oxoid, UK) were used as controls for the MDR Enterobacteriaceae. The tests were performed in triplicates.

The data were expressed as means ± standard error (SE) and analyzed using SPSS 16.0 for windows. To test any significant differences between the means, one-way analysis of variance (ANOVA) and Duncan's multiple comparisons were carried out. Differences between the means at the 95% confidence level were considered significant.

2.4 Minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC)

The modified MIC and MBC were performed as described in a previous report (National Committee for Clinical Laboratory Standards, 2003, Llagas, Santiago, & Ramos, 2014). Briefly, the ajwain oil was prepared up to 64 mg/mL with 10% DMSO and 2-fold diluted in the range of 0.006 to 64 mg/mL with Brain Heart Infusion Broth (BHB) in a 96-well microtiter plate format. Ten percent DMSO without the ajwain oil was used as a control. The volume of the oil samples and the control was 50 µL. The tested bacterial strains were adjusted to 0.5 McFarland standard turbidity followed by a 1:100 dilution in BHB. The bacterial suspension of 50 µL was added into each well. The microtiter plates were tapped at 4 corners to ensure the well-mixed solution. The plates were then incubated at 37°C for 24 h. The MIC is defined as the lowest concentration of essential oil that showed no visible growth of bacteria. The MBC was tested by culturing 10 µL of each of the clear well on BHA at 37°C for 24 h. Here, the lowest concentration of essential oil that showed no growth of bacteria on BHA plate was defined as the MBC.

2.5 Thin layer Chromatography (TLC) bioautography

The active compounds in the ajwain oil that inhibited tested bacteria were identified by TLC bioautography and further analyzed by Gas Chromatography-

Mass Spectrometry (GC-MS). The procedures were performed as described previously (Lomarat, Phanthong, Wongsariya, Chomnawang, & Bunyapraphatsara, 2013) with modifications. Two silica gel plates, Silica Gel 60 F254 (MERCK, Germany), were prepared and used in the same conditions. 1 µL of the ajwain oil was applied onto the TLC plates. Chemicals used for TLC separation were AR-grade; toluene (Fisher Scientific, UK) and ethyl acetate (RCI Labscan Limited, Thailand). Separation of the ajwain oil was achieved using toluene: ethyl acetate, 93:7, as a mobile phase. The separation was performed in a saturated chamber. To remove the solvent, the TLC plates were dried in a fume hood for 15 min. The separated compounds on the first TLC plate were visualized under UV lamp (Upland, USA, 254 nanometer), the spots were marked by pencil, and the retention values (R_f) were measured. The second TLC plate was overlaid with molten BHA seeded with bacteria (0.5 McFarland). This TLC plate was incubated in a moist chamber at 37°C for 24 h and then sprayed with 1% Thiazolyl blue tetrazolium bromide (AppliChem, Spain) prior to an incubation at room temperature for 10 min. Clear spots appearing against purple background suggested the presence of antibacterial agent. The second TLC plate was compared in parallel with the first plate; the spots on the first plate with R_f values corresponding to those with the antibacterial zone were scraped off. The active compound was eluted from silica gel plate with dichloromethane (J.T. Baker, USA) and analyzed by GC-MS.

2.6 GC-MS analysis

The crude ajwain oil and active compounds from TLC plate were analysed by GC-MS as described previously (Wongkattiya, Sanguansermsri, Fraser, & Sanguansermsri, 2019) with slight modifications. The GC-MS analysis was performed using Agilent 6890 Plus, USA, with an HP-5MSI column (0.25 mm x 30 m x 0.25 µm) (Agilent 19091S-433) and detector (Hewlett Packard 5973, USA). The oven temperature was set to the flowing conditions: 60°C (0-3 min); 60-200°C (3°C/min); 200-280°C (15°C/min); 280°C (5 min), carrier gas was helium with the flow rate of 1 mL/min and injection volume was 1 µL. Five µL of the ajwain oil was diluted with 495 µL dichloromethane. As for the TLC-derived active compounds, they were eluted with 200 µL of dichloromethane, and filtered using 0.2 µm filter membrane before injection. MS was connected to GC through a transfer line with the temperature set to 150°C; the ion source temperature was 230°C. Kovat indices were calculated using a homologue series of C_9 – C_{20} n-alkanes injected using the same conditions. Identification of the compounds was confirmed by Wiley 7 and Nist 05 Library as well as comparison of Kovats indices with literature data (National Institute of Standards and Technology, 2018).

2.7 Time-kill assay

S. aureus DMST 8840, MRSA DMST 20651, *E. coli* DMST 4212 and MDR Enterobacteriaceae (*E. coli* -1) were subjected to the time-kill assay (Hashim, Almasaudi, Azhar, Al Jaouni, & Harakeh, 2017). Bacterial suspension was adjusted to a turbidity which was equivalent to 0.5 McFarland standard in normal saline solution. The ajwain oil was added to the bacterial suspension to the final concentrations ranging from 0.5, 1, 2, and 4 times of the MIC values and incubated at 37°C in a reciprocal shaker set to 150 rpm. Viable counting was performed in triplicates from initial time point to 60 min on BHA. After incubating the BHA plates at 37°C for 24 h, viable colonies were observed.

2.8 Scanning electron microscope (SEM)

MRSA DMST 20651 and MDR *E. coli* -1 were selected to study cell morphology using SEM. The bacterial cells were cultured in Brain Heart Infusion broth (BHB) at 37°C for 24 h, and the cells were washed with normal saline solution (NSS) and centrifuged at 5,000 rpm for 10 min twice. The washed bacterial cells were re-suspended in NSS to reach the 0.5 McFarland turbidity. The cell suspension was exposed to the essential oil in a final concentration of MIC value, and the suspension was mixed on a reciprocal shaker (150 rpm; 1 h). The treated cells were collected by centrifugation at 5,000 rpm for 10 min and washed with NSS then centrifuged at 5,000 rpm for 10 min twice. Non-treated cells were used as control. The bacterial cells were fixed with 2.5% glutaraldehyde for 15 minutes and 1% osmium tetroxide for 15 minutes. The fixed cells were dehydrated by ethanol series (30%, 50%, 70%, 80%, 90%, and 100%; 5 min for each concentration) before drying with critical point drying (Polaron, CPD7501, UK). After critical point drying, the cells were coated with gold (Joel JFC-1200, Japan). The cells were examined by SEM (Joel JSM-6610LV, Japan).

3. Results and Discussion

3.1 Antibacterial activity

The ajwain oil had no colour and the yield was 0.86% (volume/dry weight). The antibacterial activities were demonstrated by zone of inhibition, MIC, and MBC values as shown in Tables 1 and 2. Agar disc diffusion assay indicated that the ajwain oil had strong, broad-spectrum antibacterial effects against both Gram-positive and Gram-negative bacteria and was more potent than tetracycline. The results showed the antibacterial effect against MDR Enterobacteriaceae, MRSA DMST 20651 with MIC/MBC value of 0.5/1 mg/mL. The MIC/MBC ratio indicated a bactericidal effect of the ajwain oil on almost all tested bacteria. It exhibited strongest antibacterial effect against tested MDR *E. coli* and *E. cloacae* with the zone of inhibition ranging from 19-29 mm. The ajwain oil also inhibited the growth of all tested MDR Enterobacteriaceae with MIC/MBC ranging from 4-16 mg/mL. Many researchers have reported the effects of the ajwain oil in inhibiting various bacteria. For example, the study from India (Patil, Maknikar, Wankhade, Ukesh, & Rai, 2016) showed that the ajwain oil had powerful, broad-spectrum antibacterial effects against several bacterial strains (*Streptococcus mutans*, *S. pyogenes*, *Proteus vulgaris*, *E. coli*, *K. pneumoniae*, *S.* Typhi, *S.* Paratyphi, *Enterobacter aerogenes*, and *Pseudomonas aeruginosa*). Antibacterial effect of the ajwain oil against MRSA and the synergistic effect with gentamicin was observed (Vazirian *et al*., 2019).

Table 1. Agar disc diffusion assay, MICs and MBCs of the ajwain oil against Gram positive, Gram negative, and MRSA

Bacteria	Inhibition zone (mm)		Ajwain oil	
	Tetracycline (30 µg)	Ajwain oil	MIC (mg/mL)	MBC (mg/mL)
Bacillus cereus DMST 5040	26.8 ± 0.6^{b}	70.0 ± 8.0^{a}	1	1
Listeria monocytogenes DMST 17303	32.1 ± 0.2^{b}	77.0 ± 3.0^{a}	1	2
Staphylococcus aureus DMST 8840	29.0 ± 0.4^{b}	70.0 ± 10.0^{a}	4	4
MRSA DMST 20651	7.0 ± 1.0^{b}	69.0 ± 3.0^{a}	0.5	1
Escherichia coli DMST 4212	22.3 ± 0.9^{b}	57.0 ± 7.0^{a}	2	4
Pseudomonas aeruginosa DMST 4739	17.8 ± 0.2^{b}	27.0 ± 3.0^{a}	8	8
Salmonella Typhi DMST 5784	21.0 ± 0.4^{b}	79.0 ± 1.0^{a}	4	4

Statistical analysis was carried out using ANOVA and Duncan's multiple comparisons. Different letters of inhibition zone in the same row indicate significant difference ($p < 0.05$). All tests were performed in triplicate.

Table 2. Agar disc diffusion assay, MICs and MBCs of the ajwain oil against MDR Enterobacteriaceae

	Inhibition zone (mm)			Ajwain oil	
	Ceftazidime (30 µg)	Imipenem (10 µg)	Ajwain oil	MIC (mg/mL)	MBC (mg/mL)
E. coli-1	17.0 ± 3.0^{b}	27.0 ± 2.0^{a}	24.0 ± 1.0^{a}	4	4
E. coli-2	9.0 ± 4.0^{b}	26.0 ± 1.0^{a}	30.0 ± 2.0^{a}	4	4
E. coli-3	17.0 ± 3.0^{b}	27.7 ± 0.6^{a}	22.0 ± 6.0^{ab}	4	4
E. coli-4	7.0 ± 1.0^{c}	26.3 ± 0.6^{b}	30.0 ± 3.0^{a}	8	8
E. coli-5	17.0 ± 5.0^{b}	27.0 ± 2.0^{a}	25.0 ± 4.0^{a}	8	8
E. cloacae-1	7.0 ± 1.0^{b}	23.0 ± 3.0^{a}	26.0 ± 2.0^{a}	4	4
E. cloacae-2	9.0 ± 1.0^{b}	20.0 ± 2.0^{a}	22.0 ± 1.0^{a}	4	4
E. cloacae-3	9.0 ± 2.0^{b}	22.3 ± 0.6^{a}	22.0 ± 0.2^{a}	4	4
E. cloacae-4	7.0 ± 1.0^{b}	23.0 ± 2.0^{a}	19.0 ± 5.0^{a}	4	4
K. pneumoniae-1	6.0 ± 0.1^{c}	27.0 ± 2.0^{a}	15.0 ± 2.0^{b}	4	8
K. pneumoniae-2	6.3 ± 0.6^{c}	27.0 ± 2.0^{a}	14.0 ± 1.0^{b}	4	8
K. pneumoniae-3	13.0 ± 1.0^{b}	25.0 ± 2.0^{a}	16.0 ± 3.0^{b}	4	16
K. pneumoniae-4	6.0 ± 0.1^{c}	33.0 ± 2.0^{a}	24.0 ± 2.0^{b}	4	8
K. pneumoniae-5	12.0 ± 2.0^{b}	24.0 ± 4.0^{a}	16.0 ± 2.0^{b}	4	16

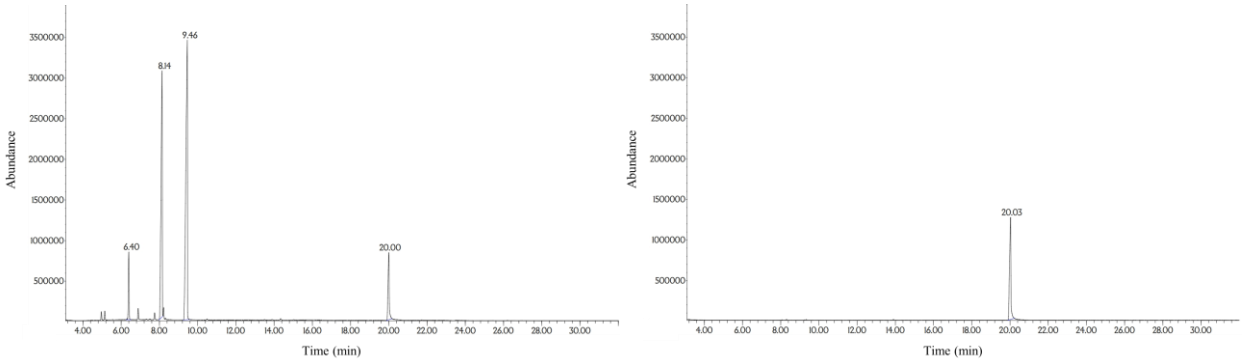
Statistical analysis was carried out using ANOVA and Duncan's multiple comparisons. Different letters of inhibition zone in the same row indicate significant difference ($p < 0.05$). All tests were performed in triplicate.

3.2 GC-MS analysis of composition of the ajwain oil

The chemical composition of the ajwain oil was analyzed by GC-MS. The result is shown in Table 3 and the chromatogram is shown in Figure 1a. The oil was identified and consisted of 9 components (Table 3). In the previous study, γ-terpinene, *p*-cymene and thymol were reported to be the major constituents of the ajwain oil (Chung, Khanh, Lee, & Ahmed, 2007; Howyzeh, Noori, & Vahid, 2018; Moein *et al*., 2015; Patil *et al*., 2016; Vitali *et al*., 2016). In this study, major components were γ-terpinene (47.56%) followed by *p*-cymene (33.98%) and thymol (9.15%) and the three components comprised over 90% of the essential oil. Other reports showed the similar results (Howyzeh *et al*., 2018; Kedia, Prakash, Mishra, Dwivedy, & Dubey, 2015; Moein *et al*., 2015). The different concentrations of γ-terpinene, *p*-cymene and thymol probably arise because γ-terpinene is a precursor of *p*-cymene and thymol (Poulose & Croteau, 1987). The differences of chemical constituents may be due to the harvesting time, genetic variability, seasonal influence and geographic region (Duarte, Naves, Santos, Seraphin, & Ferri, 2009; Howyzeh *et al*., 2018; Vitali *et al*., 2016).

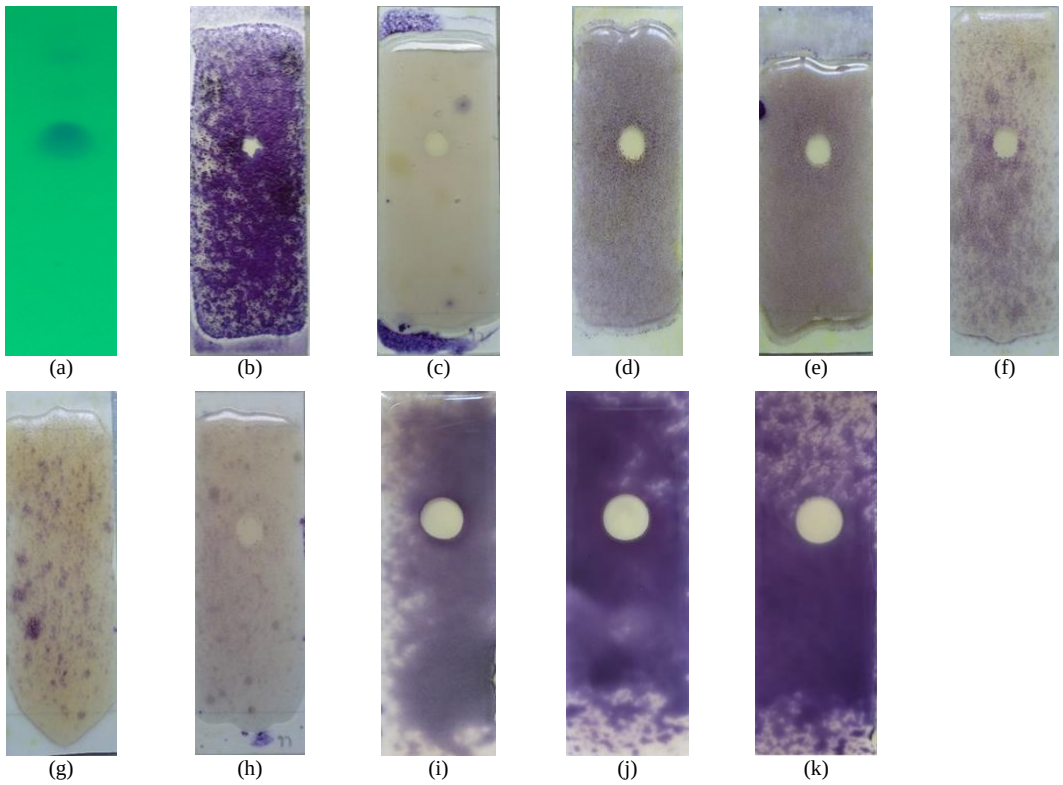
3.3 TLC-bioautography

Separation of active compound and antibacterial effect of the ajwain oil were studied in parallel on TLC plates (Figure 2). After the separation was achieved, spots on the first plate were visualized under UV light while the second plates were overlaid with bacteria for bioautography. The separation of the oil on TLC plate showed 3 major components under UV visualization at R_f value of 0.93, 0.86 and 0.66, respectively. The TLC bioautograph showed one antibacterial component with a clear zone at the same R_f value of 0.66 for all tested bacteria (Figure 2b-2f and 2h-2k) except for the *P. aeruginosa* DMST 4739 (Figure 2g). The separation of the oil on the first TLC plate at R_f of 0.66 was subjected to the GC-MS for the active component identification. According to the GC-MS chromatogram of the crude ajwain oil and the active component, the active component showed only one peak at retention time of 20.00 min which was revealed to be thymol (Figure 1b). Even though thymol did not have the highest concentration in the ajwain oil, the TLC-bioautography (Figure 2) clearly indicated that thymol was an active antibacterial constituent against tested bacteria



(a) (b)

Figure 1. GC-MS analysis of the ajwain oil (a) and active component from TLC (b)



(a) (b) (c) (d) (e) (f)

(g) (h) (i) (j) (k)

Figure 2. TLC separation of the ajwain oil on Silica Gel 60 F_{254} (a). Detection of antibacterial activity by TLC-bioautography of the ajwain oil against *B. cereus* DMST 5040 (b), *L. monocytogenes* DMST 17303 (c), *S. aureus* DMST 8840 (d), MRSA DMST 20651 (e), *E. coli* DMST 4212 (f), *P. aeruginosa* DMST 4739 (g), *S.* Typhi DMST 5784 (h), MDR *E. coli*-1 (i), MDR *E. cloacae*-4 (j), and MDR *K. pneumoniae*-2 (k)

Table 3. Chemical constituents of the ajwain oil by GC-MS analysis

Peak No.	Retention time (min)	Area (%)	Kovats retention index (calculated)	Kovats retention index (tabulated)	Name
1	4.96	0.60	924	917	1-Phellandrene
2	5.14	0.67	931	939	α-pinene
3	6.40	5.57	974	978	β-pinene
4	6.89	0.92	991	992	β-Myrcene
5	7.75	0.65	1016	1017	α-Terpinene
6	8.14	33.98	1026	1026	*p*-Cymene
7	8.22	0.90	1028	1044	Sabinene
8	9.46	47.56	1061	1060	γ-Terpinene
9	20.00	9.15	1310	1306	Thymol

including antibiotic resistant bacteria MRSA. This confirmed the findings of another report that thymol was an active chemical composition that showed antibacterial effect (Moein *et al*., 2015; Nickavar, Adeli, & Nickavar, 2014). The MIC and MBC values of the oil against the tested bacteria may differ from other reports due to the different concentration of thymol in the ajwain oil (Chung *et al*., 2007; Howyzeh *et al*., 2018; Kedia *et al*., 2015; Moein *et al*., 2015; Patil *et al*., 2016; Vitali *et al*., 2016). Thymol is found in plants other than *T. ammi* such as in family Lamiaceae as well as *Thymus* spp., *Monarda* spp., *Origanum* spp., *Satureja* spp., and *Zataria* spp. These plants are found in Iran, Pakistan, Afghanistan and India. Plants from other families such as Verbenaceae and Asteraceae also were found to contain thymol (Salehi *et al*., 2018). Therefore, the oil from variety of plant families could be used as natural sources of thymol to inhibit pathogenic bacteria.

The results from TLC-bioautography were correlated with the results from the agar diffusion method except for *P. aeruginosa*. The inhibition zone presented on agar disc diffusion method but not on the TLC-bioautography. Since the agar disc diffusion assay showed the inhibition by the crude oil, the inhibition maybe due to synergistic effect of the components in the oil. With TLC separation the oil composition and inhibition may not be appeared by individual chemical component.

The antibacterial activity of terpinene and *p*-cymene were previously reported in several studies (Carson & Riley, 1995; Höferl *et al*., 2009). In this study, these substances comprised the major proportion of the ajwain oil; however, they were not found in the GC-MS analysis of the contents collected from the inhibition zone separated by TLC-bioautography.

3.4 Time-kill assay

S. aureus DMST 8840, MRSA DMST 20651, *E. coli* DMST 15537 and *E. coli*-1 were selected as representatives of susceptible and antibiotic resistant of Gram positive and Gram-negative bacteria to study for time kill. The time-kill curve of duration and viable count of each bacteria against the various concentrations of the essential oil are shown in Figure 3. The MIC value of the ajwain oil against *S. aureus* and *E. coli* showed high mortality within 30 and 10 min, respectively. Two MIC and 4 MIC values showed greater bactericidal effect against all bacterial tested which is similar to the data of time-kill curve of *E. coli* treated with thymol (Xu, Zhou, Ji, Pei, & Xu, 2018). The viable cells decreased with increases in concentration of thymol.

Although the exposure times of the oil to all tested bacteria were continued up to 12 h (data not shown), the bacterial cell survival was similar to that at 60 min. From the results, it could be concluded that MIC value showed bactericidal effect on bacterial cell survival on both antibiotic susceptible and resistant strains and the oil killed more than 99% of bacteria within 30 min.

3.5 Scanning electron microscope

The results showed severe membrane damage of MRSA (Figure 4a and 4b) and MDR *E. coli* (Figure 4c and 4d) after the oil exposure for 1 h. In the previous study, membrane permeability assay and bacterial cell morphological image showed that thymol disrupted cell membrane integrity that caused cell death (Xu, *et al*., 2018). In addition, thymol bound with genomic DNA induced mild destabilization in the DNA secondary structure then the DNA aggregated (Wang, Zhang, Zeng, Gong, & Wang, 2017).

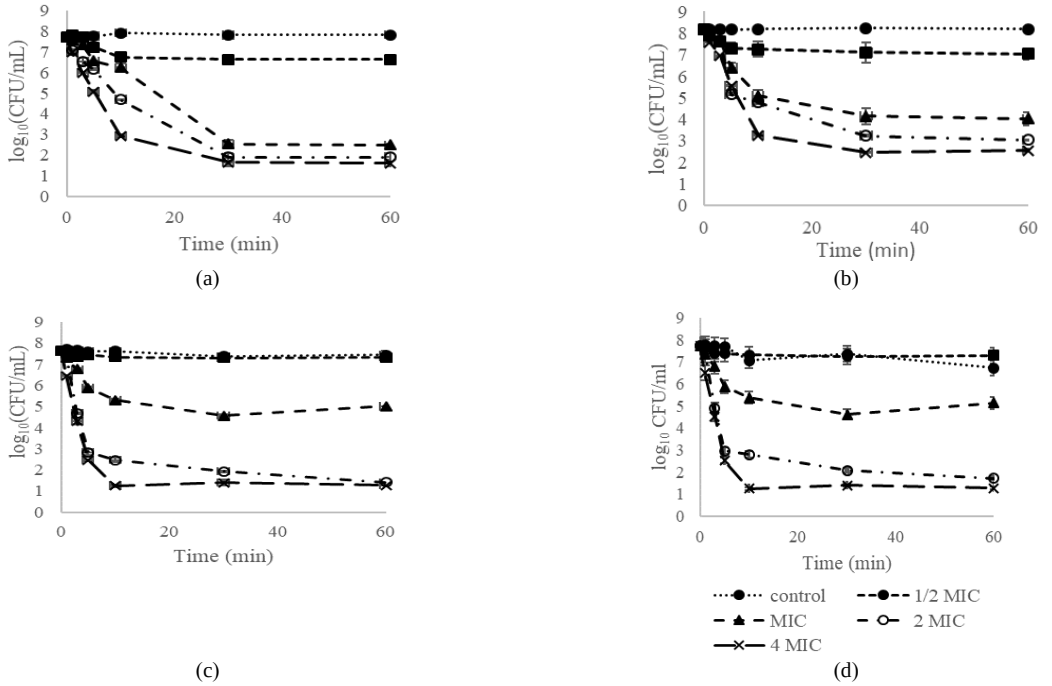
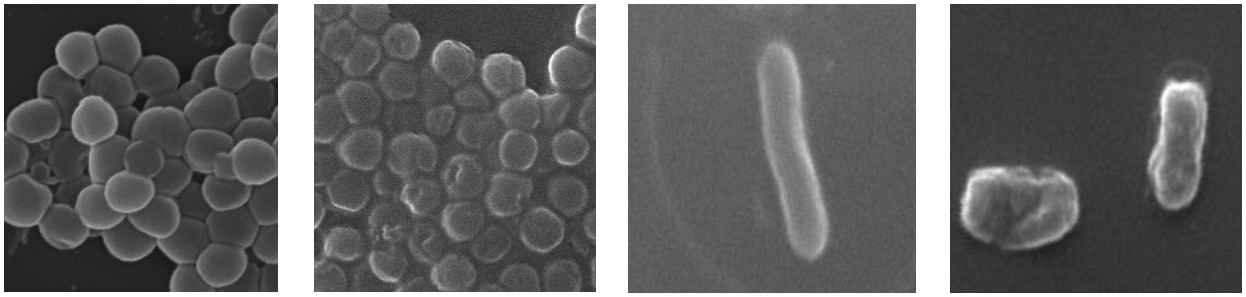


Figure 3. Time-kill curve of the ajwain oil against *S. aureus* DMST 8840 (a), MRSA DMST 20651 (b), *E. coli* DMST 4212 (c) and MDR *E. coli* -1 (d)



(a) (b) (c) (d)

Figure 4. The images from scanning electron microscope of untreated MRSA DMST 20651 (a), treated MRSA DMST 20651 (b), untreated MDR *E. coli* (c), treated MDR *E. coli* (d)

4. Conclusions

This study demonstrated an effective antibacterial activity of ajwain oil against MDR Enterobacteriaceae isolated from human faeces and also against MRSA. TLC bioautography was assessed for the antibacterial active compound, thymol. Time-kill analysis and SEM indicated that severe damage to the cell membrane of the bacterial cell was the result of the bactericidal effect. Medicinal plants or spices that contain thymol could be good sources for a natural antibacterial agent. However, further studies such as to determine how much of the compound should be used for controlling bacteria are needed in order to use this compound in practice. The present study confirmed the antimicrobial potential of this plant and provided additional information on its ability to inhibit the growth of MDR Enterobacteriaceae. Further studies are necessary for more extensive biological evaluation.

Acknowledgements

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