Archives of Microbiology (2021) 203:1529–1537
https://doi.org/10.1007/s00203-020-02138-w

ORIGINAL PAPER

Antibacterial, anti-biofilm and anti-quorum sensing activities of *Artemisia dracunculus* essential oil (EO): a study against *Salmonella enterica* serovar Typhimurium and *Staphylococcus aureus*

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Received: 18 September 2020 / Revised: 19 November 2020 / Accepted: 3 December 2020 / Published online: 5 January 2021

Abstract

The study evaluates the effect of *Artemisia dracunculus* essential oil (EO) on two pathogenic bacteria *Salmonella enterica* serovar Typhimurium and *Staphylococcus aureus* and Vero cell line. To evaluating the anti-biofilm potential of the EO, a microtiter-plate test (MtP) and scanning electron microscopy (SEM) were performed. The quorum-sensing inhibitory properties were examined by QS-related gene expression at sub-MIC concentrations of *Artemisia dracunculus* EO. MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, a tetrazole) test was used to determine the cytotoxicity potential of the EO against the Vero cell line and finally, the major components of the EOs were determined using Gas chromatography–mass spectrometry (GC–MS) analysis. The minimum inhibitory concentration (MIC) of the tested EO against *S.* Typhimurium and *S. aureus* were 2.5 and 1.25 µl/ml, respectively. In addition, the minimum bactericidal concentration was 5 and 2.5 µl/ml for *S.* Typhimurium and *S. aureus*, respectively. Both MtP and SEM showed an acceptable inhibitory and disruption effect of the EO on the biofilm formation at Sub-MIC concentrations. Significant downregulation of *luxS*, *pfs,* and *hld* genes by treatment with MIC/2 concentration of *A. dracunculus* EO was observed. The IC50 value of *A. dracunculus* EO against Vero cells was 20 µl/ml. The main detected compound using GC–MS was estragole (methyl chavicol or tarragon) (64.94%). Anti-biofilm, QSI activity, and non-toxicity of *A. dracunculus* EO reported for the first time in this study propose the use of these plant compounds as alternatives to antibiotics and chemical additives.

Keywords *Salmonella enterica* serovar Typhimurium · *Staphylococcus aureus* · *Artemisia dracunculus* · Essential oil · biofilm · Quorum sensing · Non-toxicity

Introduction

Herbal essential oils (EO) are also known as volatile oils and most of them well known for their antimicrobial activities. They contain many bioactive compounds with destructive effects on different parts of microbial pathogens as well as food spoiling bacteria. This property makes them attractive to be used for therapeutic and industrial purposes (Tongnuanchan and Benjakul 2014).

Plant essential oils also play a significant role in the control of bacterial biofilm production (Alni et al. 2020; Sharifi et al. 2018b) and cell–cell communication processes, commonly called quorum sensing (QS) (Sharifi et al. 2018a). Biofilms are specialized bacterial communities that create an extracellular matrix to adhere to biotic or abiotic surfaces and play a fundamental role in the survival of bacteria under adverse environmental conditions and pathogenesis of pathogenic bacteria (Aumeeruddy-Elalfi et al. 2018; O'Toole et al. 2000).

QS system, which operates by autoinducer (AI), modifies gene expression in response to population density. This system is associated with bacterial biofilm formation and antibiotic resistance, as well as bacterial proliferation in infectious tissue/catheters, and foods (Cortese et al. 2018). Therefore, QS inhibition (QSI) is considered a good strategy to control bacterial infection and ensure food safety. Besides, according to the relation between QS activity and biofilm formation, most anti-QS agents exhibit anti-biofilm properties (Hammer and Bassler 2003).

Communicated by Erko Stackebrandt.

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Lux and *pfs* are the two most important genes involved in the QS system in *S.* Typhimurium and other Gram-negative bacteria. Furthermore, previous studies have tested the expression level of these genes as an indicator of QS activity (Kim et al. 2016). Since the RNAIII transcript produced by the *hld* gene acts as a functional molecule in *the S. aureus* QS system (Yarwood and Schlievert 2003), previous studies such as Sharifi et al. (2018a, b) have used the expression level of this gene to study the QS system activity in *S. aureus* bacterium (Sharifi et al. 2018b).

Artemisia dracunculus L., known as Tarragon belongs to the Asteraceae family is an aromatic plant, spice, and natural food preservative. The *A. dracunculus* EO and its major components can be used as safe natural compounds in the food industry as a replacement for synthetic preservatives and additives (Obolskiy et al. 2011). Researches have shown that this plant has various pharmacological activity including carminative, digestive, anti-inflammatory, antioxidant, antipyretic, antiseptic, antispasmodic, antiparasitic, antimicrobial, anthelmintic, and fungicidal effects (Mumivand et al. 2017; Obolskiy et al. 2011).

Accordingly, the first aim of the present study was to determine the antimicrobial effect of *A. dracunculus* EO against *S.* Typhimurium and *S. aureus* as major human pathogens and cause of food poisoning with challenging treatment (Bintsis 2017; Taylor and Unakal 2019). Then, anti-biofilm and QSI activity of the plant EO were evaluated against both tested organisms by assessment of QS contributing genes. MTT assay was designed to check the cytotoxicity potential of the EO, and major bioactive components of the EO were determined to find the relation between observed effects and the EO compounds.

Materials and methods

Bacterial strains and essential oil

Staphylococcus aureus ATCC 25923 and *Salmonella enterica* serovar Typhimurium ATCC 14028 were obtained from Persian Type Culture Collection (PTCC), Tehran, Iran. Iranian *A. dracunculus* was prepared from the Medicinal Plants and Drugs Research Institute (MPDR) of the Shahid Beheshti University of Iran. The essential oil extraction procedure was performed by a Clevenger apparatus for 4 h according to the standard protocol (El Gendy et al. 2015).

MIC and MBC determination

MIC and MBC for *A. dracunculus* EO were determined by the microdilution broth method as described previously (Duarte et al. 2015). Briefly, twofold serial dilutions of EO (from 20 to 0.15 µl/ml) prepared in 96-well plates in Müeller–Hinton broth (MHB) in 2% of Dimethyl sulfoxide (DMSO) were used to increase the solubility. Bacterial suspensions were prepared with a turbidity of 0.5 McFarland, diluted in MHB, and added to each well to yield a final concentration of 5×10^5 CFU/ml per well (MHB–DMSO (1%) was used as negative control). The plates were incubated at 37 °C for 48 h under aerobic conditions. After the incubation period, the growth was visually assessed. The MIC was defined as the lowest concentration of EO without visible growth. From the wells without visible growth, 10 µl was plated on the tryptic soy agar (TSA) and after incubation, the number of colonies was counted. The MBC was defined as the lowest compound concentration which caused the death of 99.9% of the bacterial inoculum. The tests were repeated three times independently.

Inhibition of biofilm formation

Inhibition and disruption effects of the *A. dracunculus* EO on *S.* Typhimurium and *S. aureus* biofilms were tested by MtP assay. In detail, for the assembly of the plates, sterile 96-well polystyrene plates were filled with 100 µl of MHB containing MIC/2, MIC/4, and MIC/8 concentrations of the EO (6 wells were considered for each concentration). Next, 100 µl of the bacterial culture with ~ 1.5×10^5 CFU/ml concentration (by three times 1/10 dilution preparation from turbidity equals 0.5 McFarland) was added to each well. The negative control contained medium-DMSO (1%) and bacteria and the positive controls contained medium-DMSO (1%) with bacteria and gentamycin (0.1 mg/ml). After 24 h incubation at 37 °C, the plates were washed with physiological saline three times (200 µl/well) to remove the loosely adhered and air-dried cells. After that, the adhered cells of the biofilm were fixed for 15 min by the addition of 200 µl of methanol. Next, methanol was removed and 200 µl of a 0.1% Safranin dye was added for 15 min. The plates were washed with physiological saline and air-dried again, after which 100 µl of ethanol (96%) was added to each well which was left shaking for 5 min, and optical density values (OD) were measured using a microplate reader (ELx808, BioTek, USA) at 490 nm. Each assay was repeated three times, and the data are presented as the mean ± SD (standard deviation). As a measure of efficacy, the mean ODs of treated wells were compared with those of negative control (without EO) (Sharifi et al. 2018b).

Disruption of the performed biofilm

MtP test was applied to study the effects of the EO on the performed biofilm or biofilm disruption. At first, 24 h biofilms were allowed to establish in 96-well microtiter plates as described above. After the incubation period, the supernatants (loosely adhered and air-dried cells) were

discarded and replaced with 100 µl of MHB containing MIC/2, MIC/4, and MIC/8 concentrations of the *A. dracunculus* EO (six wells were allocated for each concentration). Negative control wells contained medium-DMSO with bacteria. After 24 h incubation at 37 °C, the plates were washed (three times) with physiological saline and air-dried. The adhered cells were fixed with methanol for 15 min, and then, the biofilm cells were treated with 200 µl Safranin. Next, the plates were filled with 100 µl ethanol (96%), the biofilm cells were disrupted for 5 min, and the OD of the plate was recorded at 490 nm. The biofilm disruption potential was measured by a comparison of the mean OD of the treated group versus the mean OD of the negative group (without EO) (Sharifi et al. 2018b).

Scanning electronic microscopy of biofilm cells

Electronic microscopy was also used to observe the effects of the EO on the bacterial biofilm. To do this, the biofilm of the tested bacteria was prepared in six-well plates (each well contained a glass coverslip at this time). The control wells contained medium-DMSO (1%) with bacteria, and the treated biofilm groups contained medium-DMSO (1%) with MIC/2 concentration (1.25 and 0.625 µl/ml for *S.* Typhimurium and *S. aureus*, respectively) of the EO and bacteria. After 24 h of incubation at 37 °C, the samples were fixed in 2.5% buffered glutaraldehyde for 3 h followed by dehydration in graded ethanol. The samples were then dried at room temperature and glued onto stubs. At last, the processed samples were sputter-coated with gold and examined in a JEOL JSM-840 SEM operating at an accelerating voltage of 15 kV.

Effect of the EO on the bacterial quorum sensing

The QSI activity of the EO was examined through the analysis of gene expression involved in the QS system of tested bacteria. Using quantitative real-time RT-PCR, the level expression of *luxS* and *pfs* for *S.* Typhimurium and *hld* for *S. aureus* in biofilm growth was evaluated and compared with negative control (bacterial culture without EO).

RNA extraction and cDNA synthesis

For total RNA extraction, the tested bacterial strains were grown with and without the EO in six-well polystyrene tissue culture plates containing MHB medium with DMSO and incubated at 37 °C for 24 h. Then the plates were washed with deionized water to remove the unattached cells and then the biofilm cells were scraped and immediately processed for RNA extraction using a commercial RNA extraction and purification kit (SinaClon, Iran) according to the manufacturer's instructions.

The quality and quantity of the extracted RNA were cheeked by agarose gel electrophoresis and confirmed by measuring the absorbance at 260/280 nm using a Nanodrop spectrophotometer (ND-1000, Thermo Fisher Scientific, USA). The extracted RNAs were stored at − 70 °C until further experiments. After that, the purified RNAs were reverse transcribed to cDNA using a commercial cDNA synthesis kit according to the manufacturer's instructions (Takara, Japan), and the obtained cDNA was stored at − 70 °C until used as DNA templates in the real-time RT-PCR reactions.

Quantitative real-time RT-PCR

The real-time PCR assay was carried out using a commercial SYBR Green master mix (Amplicon, Denmark) and previously described pairs of primers listed in Table 1. The reactions were conducted in a Corbett Life Science Rotor-Gene 6000 Cycler (Qiagen, Germany), and for both tested bacteria to normalize the expression of target genes, the *16S rRNA* housekeeping gene was considered as an internal control. The efficacy of the real-time PCR was calculated by the following formula: $E = 10^{(-1/slop)} - 1$. After the optimization

Table 1 Primers were used for the quantitative real-time RT-PCR assay

Bacteria	Genes	Sequence (5′-3′)	Annealing temperature	Reference
S. aureus	*hld*	F-TCACGGAGTGGCCAAAATTT	56 °C	(Kolar et al. 2013)
		R-GACGCGCATTTGTTATCATCA		
	16S rRNA	F-AGCCGACCTGAGAGGGTGA	59 °C	(Koprivnjak et al. 2006)
		R-TCTGGACCGTGTCTCAGTTCC		
S. Typhimurium	*luxS*	F-TCACGGAGTGGCCAAAATTT	56 °C	(Li and Lee 2017)
		R-GACGCGCATTTGTTATCATCA		
	pfs	F-GGAAGAAGAAGTTACGCTGC	56 °C	Present study
		R-GATTTCAGCAACGCCACTTC		
	16S rRNA	F-CGGGGAGGAAGGTGTTGTG	55 °C	(Brunelle et al. 2015)
		R-GAGCCCGGGGATTTCACATC		

and qualification of standards curves, the main reaction was performed and negative control was included in each run. In addition, the specificity of the real-time PCR was checked by gel electrophoresis for products as well as the post-PCR melting-curve analysis performed. All the samples were analyzed in triplicate and finally, relative gene expression was calculated by the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen 2001).

MTT assay

The toxicity of the tested EO against eukaryotic cells was measured using an MTT test which was based on the cleavage of the MTT, tetrazolium salt, by viable cells as reported previously (Quassinti et al. 2013). Briefly, the Vero cells (prepared from National Cell Bank of Iran (NCBI) in Pasteur Institute of Iran) were cultured in RPMI-1640 medium with 10% fetal bovine serum (FBS) and penicillin–streptomycin at 37 °C, in humidified air containing 5% CO2 (all materials prepared from Gibco, UK). Cells with a density of about 2×10^4–2×10^5 per well were plated in 96-well plates and cultured at 37 °C in the presence of 5% CO2. After 24 h incubation, samples in quadruplicate were exposed to different concentrations of *A. dracunculus* EO (0.16–40 µl/ml) with DMSO (1%). The plates were incubated for a further 24 h under the mentioned incubation conditions. Then, 10 µl of MTT dye solution (5 mg/ml in phosphate-buffered saline) was added to each well. The plates were incubated for an additional 4 h at 37 °C. After aspirating the medium, the formed formazan crystals were solubilized in 100 µl DMSO. The extent of MTT reduction was measured spectrophotometrically at 490 nm using a microplate reader (ELx808, BioTek, USA). This experiment was conducted in triplicate. The cytotoxicity potential was calculated by comparing the treated cells with the control group (only treated with 1% DMSO). The cytotoxicity effect was expressed as the concentration of compound inhibiting cell growth by 50% (IC50).

GC–MS analysis

The main components of the *A. dracunculus* EO were determined by gas chromatography–mass spectrometry (GC–MS) analysis according to the standard procedure (Hites 2016).

Statistical analysis

The statistical calculations were performed using GraphPad Prism 4 software (GraphPad Software, San Diego, CA, USA). All the experiments were performed in triplicate and repeated three times, and the data expressed as the mean ± SD. For evaluation of the anti-biofilm potential, a Student's *t* test was used. A *p* value of less than 0.05 was statistically significant.

Results

Antibacterial activity

In vitro bacteriostatic and bactericidal properties of the EO were evaluated against *S.* Typhimurium and *S. aureus* bacteria. Results of MIC showed that the *A. dracunculus* EO could prevent the growth of tested bacteria. The MICs of the EO against planktonic cells of *S.* Typhimurium and *S. aureus* bacteria were 2.5 and 1.25 µl/ml, respectively. In addition, the *A. dracunculus* EO exhibited bactericidal properties, and the MBC values were 5 and 2.5 µl/ml for *S.* Typhimurium and *S. aureus*, respectively.

Anti-biofilm activity

MtP test

The results of the MtP assay showed that the EO of *A. dracunculus* significantly prevented the biofilm formation by *S.* Typhimurium ($P<0.001$) in MIC/2 (1.25 µl/ml) and MIC/4 (0.625 µl/ml) concentration. Besides, in the presence of the EO, biofilm formation decreased in a dose-dependent manner. In addition, the *A. dracunculus* EO caused the significant disruption of the formed biofilm of *S.* Typhimurium at the MIC/2 and MIC/4 concentrations. In the case of *S. aureus*, the results indicated that the EO had good potential to inhibit biofilm formation and disrupt preformed biofilms of this pathogen. The EO was able to significantly inhibit the biofilm formation at MIC/2 (0.625 µl/ml) and MIC/4 (0.312 µl/ml) and disrupted the biofilm at MIC/2 concentrations (Fig. 1).

SEM observation

Microscopic picture analyses showed that the tested EO inhibited the biofilm formation for both tested bacteria, and by treatment with MIC/2 (1.25 and 0.625 µl/ml for *S.* Typhimurium and *S.* aureus, respectively) of *A. dracunculus* EO the concentration of attached cells was significantly reduced (Fig. 2).

QS inhibitory (QSI) activity

The effect of sub-MIC concentrations of *A. dracunculus* EO on the expression of QS-related genes was measured in biofilm form bacteria. Our results showed that the MIC/2 concentration of *A. dracunculus* EO caused a significant down-regulation of three investigated genes ($P<0.05$). Treatment

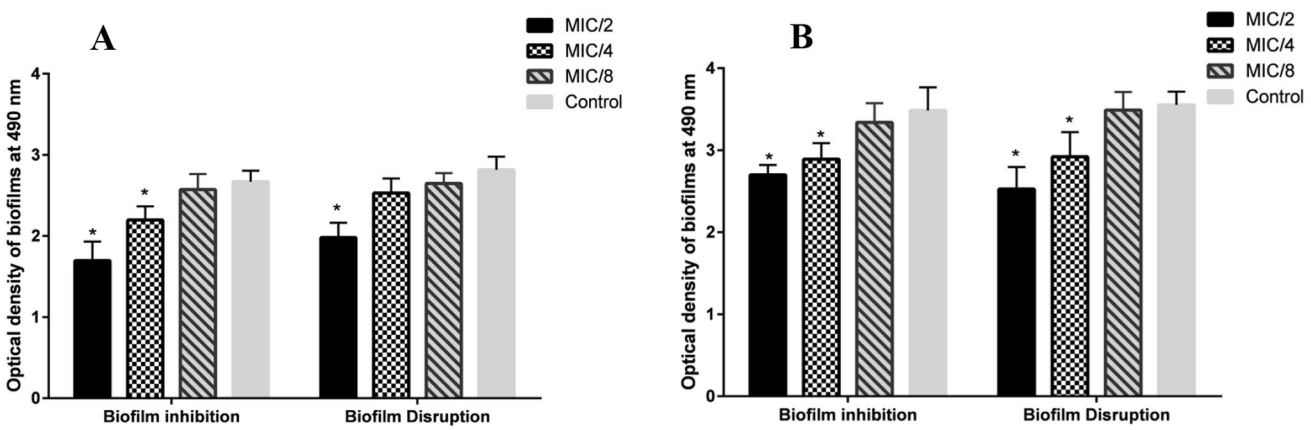

Fig. 1 Biofilm inhibition and disruption potential of the *A. dracunculus* EO on *S. aureus* (**a**) and *S.* Typhimurium (**b**) and at different concentrations. The error bars represent the standard deviation of three replicates (*$P < 0.05$)

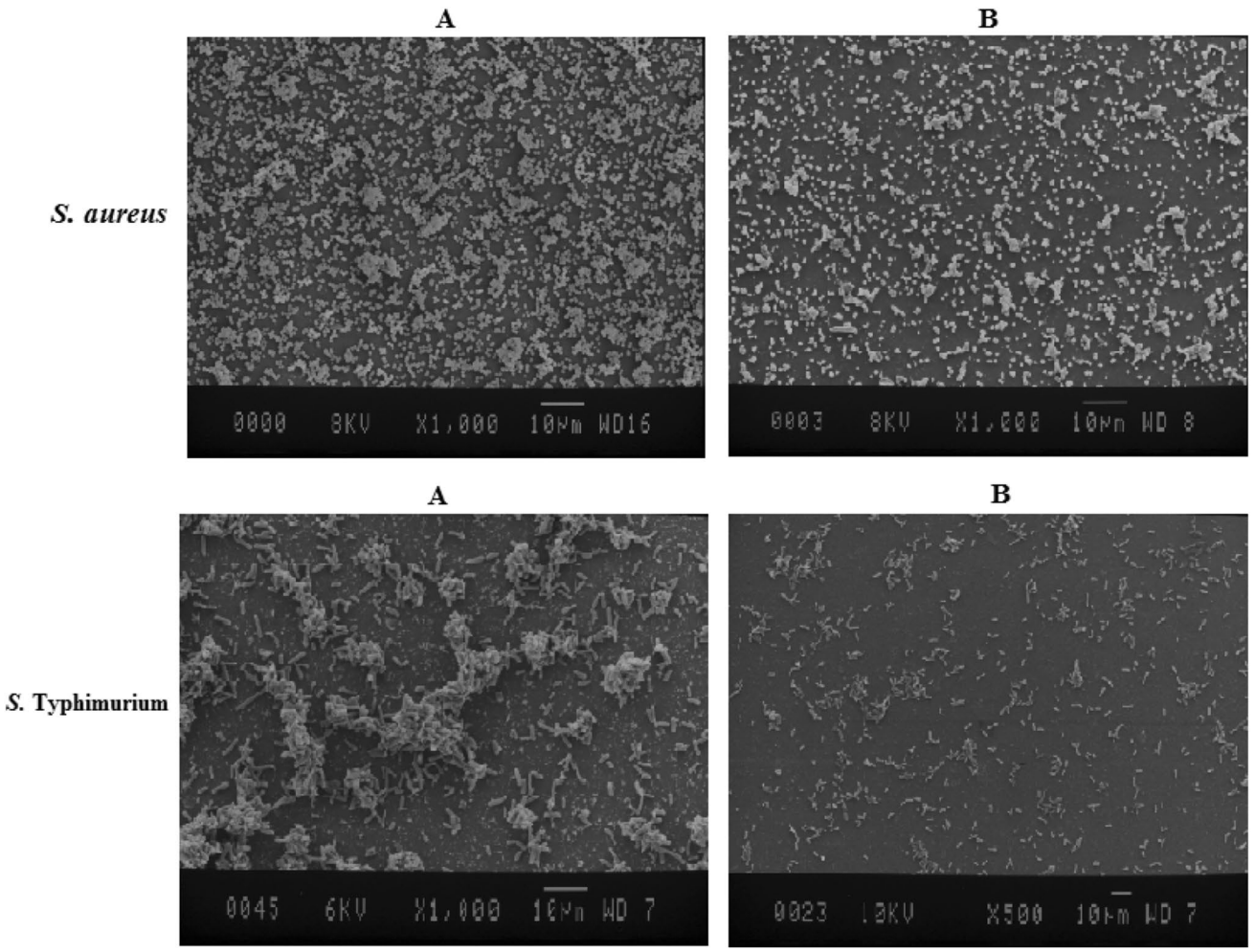

Fig. 2 SEM images of bacterial cells on the coverslips. **a** Negative control (untreated bacteria). **b** Treated with the MIC/2 concentration of the *A. dracunculus* EO

of *S.* Typhimurium biofilm with MIC/2 concentration (1.25 µl/ml) of the EO resulted in a −4.18- and −3.12-fold decrease in the expression of *luxS* and *pfs*, respectively. Similarly, after treatment of the *S. aureus* biofilm with MIC/2 concentration of EO, the expression of the *hld* gene was significantly downregulated (−5.35-fold) ($P < 0.05$) (Fig. 3).

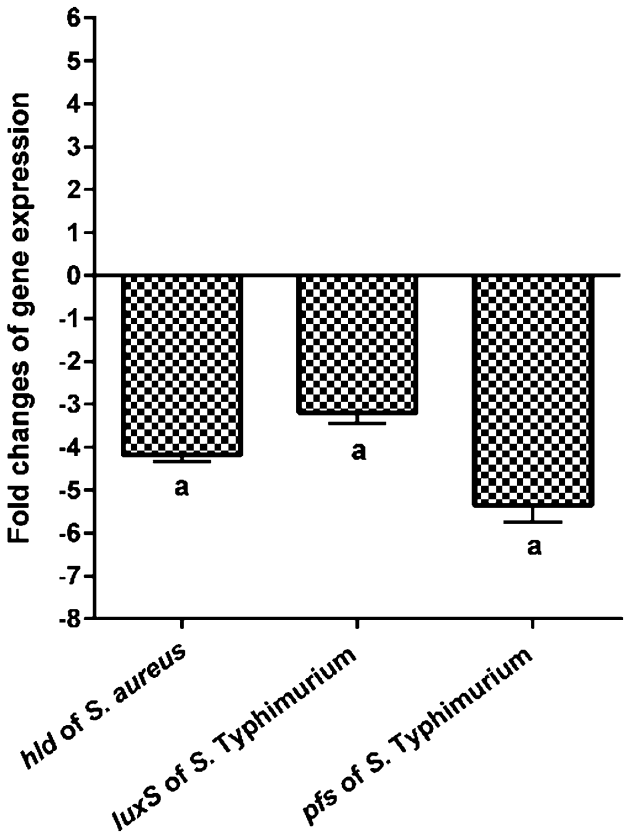

Fig. 3 Gene expression in bacterial treated with MIC/2 of *A. dracunculus* essential oil; **a** significant downregulation; **b** non-significant downregulation

Cytotoxicity assay

The results of the cytotoxicity assay showed no cytotoxic effect at MIC and MBC concentrations. Cell viability decreasing was a dose-dependent manner starting from 0.16 to 40 µl/ml. In addition, the IC50 value was 20 µl/ml (Fig. 4).

Chemical composition of the essential oil

In GC–MS analysis, 15 substances were detected. According to our results, estragole (64.94%), beta-cis-Ocimene (10.59%), trans-beta-Ocimene (10.21%), cinene (5.39%), alpha-pinene (2.74%), and methyl eugenol (2.42%) were the most detected compounds (Table 2).

Discussion

In our study, the antibacterial, anti-biofilm, and QSI properties of *A. dracunculus* EO were evaluated against two pathogens *S.* Typhimurium and *S. aureus*. Besides, the cytotoxicity and composition of *A. dracunculus* EO were determined. The results of MIC and MBC against *S.* Typhimurium were 2.5 and 5 µl/ml, respectively. Besides, the tested EO was effective on *S. aureus* and the MIC and MBC values for this bacterium were 1.25 and 2.5 µl/ml, respectively. In accordance with our results, in Raeisi et al. (2012) study, the antibacterial activity of *A. dracunculus* EO was evaluated against *E. coli* in Iranian white cheese and on culture media, and the possibility of using *A. dracunculus* EO as a natural preservative was proposed.

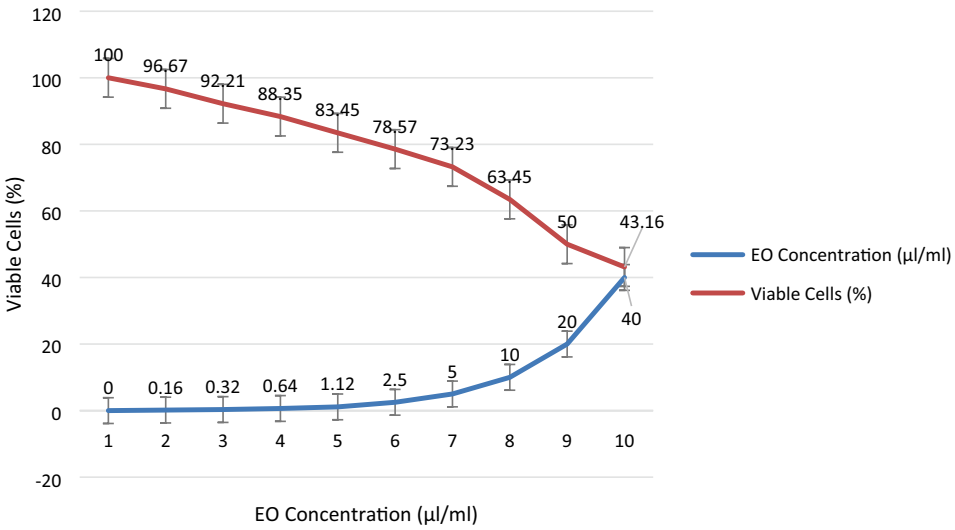

Fig. 4 Cytotoxic effects of *A. dracunculus* EO against Vero cells

Table 2 Chemical compositions, retention time, and percentage of the identified components of the *A. dracunculus* EO

Peak	Ret time	Name	%
1	7.036	alpha.-Pinene	2.745
2	7.378	Camphene	0.208
3	8.070	beta.-Pinene	0.515
4	8.444	beta.-Myrcene	0.293
5	8.769	alpha.-Phellandrene	0.119
6	9.426	Cinene	5.399
7	9.710	trans-.beta.-Ocimene	10.219
8	9.991	beta.-cis-Ocimene	10.593
9	10.904	alpha.-Terpinolene	0.295
10	11.916	2,4,6-Octatriene, 3,4-dimethyl	0.443
11	14.010	Estragole (methyl chavicol or tarragon)	64.946
12	14.923	D-Carvone	0.466
13	15.801	L-bornyl acetate	0.798
14	20.663	Methyleugenol	2.426
15	20.663	gamma.-Elemene	0.537
Total			100

As complementary for the previous studies in the second part of the present study, the anti-biofilm properties of the *A. dracunculus* EO at sub-MIC concentrations were studied against *S.* Typhimurium *and S. aureus* bacteria. As presented in Figs. 1 and 2, the EO inhibited the formation of biofilms and disrupted preformed biofilms of both tested bacteria. Based on previous reports the anti-biofilm agent may produce this effect in different ways including inhibition of bacterial growth, proliferation, and attachment (Roy et al. 2018), QSI activity (Koh et al. 2013), and the use of matrix-degrading materials (Kaplan 2010). However, the studied plant EO may have affected several bacterial pathways or components (Swamy et al. 2016).

The present study used an SEM to investigate the effect of plant EO on the attachment of bacteria to the surface and also the attachment of bacteria to each other. Using SEM images, we demonstrated that MIC/2 concentration of *A. dracunculus* EO could affect the *S.* Typhimurium and *S. aureus* biofilms and the formation of micro-colonies. As presented in Fig. 3, the bacterial cells grown in an EO-free medium were well connected to each other and have formed micro-colonies, but these structures were rarely seen in EO-treated cells. These observed anti-biofilm activates may be related to the anti-adhesive properties of the EO compounds (Nostro et al. 2007), inhibition of biofilm structural compounds such as exopolysaccharide (Swamy et al. 2016), or altering the expression of biofilm-associated genes (Kim et al. 2016). Considering the role of biofilm in infection progress and food spoilage, inhibition of biofilm formation by natural antimicrobial compounds such as EOs is expected to be an alternative to antibiotics and traditional sanitizers (Orhan-Yanıkan et al. 2019).

In another part of the study, the QSI properties of the *A. dracunculus* EO were investigated against *S.* Typhimurium and *S. aureus*. To do this, the expression QS-related genes (*luxS*, *pfs* for *S.* Typhimurium, and *hld S. aureus*) was evaluated in treatment with MIC/2 of the EO versus untreated cells. The results showed significant downregulation of *luxS*, *pfs*, and *hld* (−4.18-, −3.12- and −5.35-fold, respectively) following treatment with MIC/2 concentration of EO. More importantly, it is shown that QSI agents do not impose any selection pressure; therefore, resistance to these compounds does not occur. According to this, QSI has been a novel strategy to control various bacterial infections (Koh et al. 2013). In this context, the QSI activity of some other plant species such as orange, garlic, tea tree, ginger, rosemary, and turmeric has been shown (Koh et al. 2013). The plant-derived compounds affect the bacterial QS system in three different ways, first block the signaling molecules, second degrading the signaling molecules, and third destroy the signal receptor (Koh et al. 2013).

Based on the important roles of QS in several virulence factors including bacterial toxin production and secretion, antibiotic resistance, and biofilm formation (Rutherford and Bassler 2012), *A. dracunculus* herbal EO can be used for modulation of these factors.

To evaluate the possibility of *A. dracunculus* EO compounds for human consumption, the cytotoxicity activity of the EO was evaluated against Vero cells. Our results showed the non-toxicity of the EO at sub-MIC and MBC concentrations. However, every component of the plant EO is suggested to be tested rigorously for toxicity before its use as a food additive.

The results of GC–MS showed that 64.94% of *A. dracunculus* EO was composed of estragole (methyl chavicol). It was reported that this compound possesses toxicity or mutagenic activity in rats and probably in humans (Paini et al. 2010; Phillips 1994). But according to the evidence, it is not the estragon but its metabolites activation in a dose-dependent reaction that with a very low probability can damage DNA. Therefore, the direct carcinogenic effect of the estragon was rejected (Paini et al. 2010; Rietjens et al. 2010). The results of the cytotoxicity assay on Vero cell lines showed no cytotoxic effect at MIC and MBC concentrations of the Tarragon EO. This results in parallel with reports of non-toxicity or no mutagenic activities on human make Tarragon a potential herbal drug or food additive candidate to be used against pathogenic bacteria.

According to the literature, there are variations in the concentration of chemical constituents of *A. dracunculus* EO based on harvest time, geographical situation, ground conditions, and genetic factors (Chaleshtori et al. 2013; Damjanović-Vratnica et al. 2011). This can be observed by

comparison of the main compounds of *A. dracunculus* EO in this study with the results of studies from France and Georgia in which in accordance with our results estragole was the main compound of *A. dracunculus* EO and the reverse results from Russia and Canada which showed the low-percentage estragon in the Tarragon EO (Obolskiy et al. 2011).

Conclusion

Based on the results that are reported for the first time in this study, anti-biofilm and anti-QSI activities of the *A. dracunculus* show the probable possibility of plant compounds penetration to the bacterial biofilm. This property alongside the non-toxicity of the EO makes the plant compounds potential candidates for drug or food additive development against strong biofilm-producing bacteria.

Author contributions Conceptualization: LB, Methodology: LB, MG, SHM, and LK, Investigation: LB and MG. Writing original draft preparation: SHM, Writing—review and editing: LB. Supervision: LB. Project administration: LB, MG, SHM, and LK.

Funding None.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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