Industrial Crops & Products 109 (2017) 358–366

Contents lists available at ScienceDirect

Industrial Crops & Products

journal homepage: www.elsevier.com/locate/indcrop

Dodartia orientalis L. essential oil exerts antibacterial activity by mechanisms of disrupting cell structure and resisting biofilm

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ARTICLE INFO

Keywords:
Dodartia orientalis L
Essential oil
Antibacterial activity
Antibacterial mechanism
Antibiofilm mechanism
GC/MS analysis

ABSTRACT

Concerns about the use of synthetic preservatives during food processing and storage have been raised due to their potential side effects on human health and the environment. The antibacterial activities, mechanisms, and chemical compositions of the essential oil of *Dodartia orientalis* L. which has been long consuming as traditional Chinese medicine against foodborne and spoilage bacteria were investigated as a potential substitute for synthetic preservatives for the first time. *Dodartia orientalis* L. essential oil (DoEO) exhibited significant antibacterial activities against three foodborne pathogens (*Staphylococcus aureus*, *Escherichia coli*, and *Salmonella enteritidis*). The minimum inhibition concentrations (MIC) of DoEO were 0.5, 1, and 2 μL/mL while the minimum bactericide concentrations (MBC) were 1, 2 and 4 μL/mL against the strains of *S. aureus*, *E. coli*, and *S. enteritidis*, respectively. Alkaline phosphatase (AKP) leak assay revealed that DoEO caused the destruction of structure and function of cell wall. Nucleic acids leak, potassium ions leak, and propidium iodide (PI) assays showed that DoEO aroused the stimulating release of intracellular nucleic acids and potassium ions by damaging membrane integrity. Scanning electron microscopy (SEM) observation further verified that DoEO could exert bactericidal activity via disrupting cell walls and membranes. On the other hand, XTT and crystal violet (CV) experiments indicated that DoEO was able to prevent fresh biofilm formation and destroy preformed biofilm through decreasing biofilm biomass and biofilm cellular activity. Bacterial surface hydrophobicity assay revealed that DoEO was capable of reducing the adhesive capacity of the strains, belonging to its antibiofilm mechanisms. Furthermore, the main chemical compositions of DoEO were identified by gas chromatography–mass spectrometry analysis (GC–MS). Therein, antibacterial components were classified and analyzed. All results above confirmed that the antibacterial activity of DoEO was mainly aroused by disrupting cell structure and resisting biofilm, which indicated that DoEO was expected to be an efficient functional ingredient with great potential applications in food preservative.

1. Introduction

Food spoilage caused by pathogens have been threating food safety, and the phenomenon of food poisoning originated from pathogenic microbes is becoming more serious and widespread (Shan et al., 2007). Nowadays, the high incidence of foodborne disease makes the bacterial food contamination of great concern (Lee and Je, 2013). What's worse, food spoilage has brought huge economic losses in the food industry (Runyoro et al., 2010). Therefore, the popularity of antibacterial agents is inevitable. However, most chemical bactericides and their by-produthcts may increase the risk of human organs hypofunction, and cannot be degraded effectively or lack of environmental compatibility (Gabriel et al., 2015). Meanwhile, the emergence and spread of antibiotic resistance are challenging public health (Aria et al., 2015), resulting in the urgent demands of new antibiotic classes against pathogenic bacteria (Fisher and Phillips, 2008). Consequently, the shortage of new antibiotics has provoked researchers much attention on the usage of natural ingredients, such as essential oils (EOs) extracted from

Abbreviations: AKP, Alkaline phosphatase; CV, Crystal violet; DIZ, Diameter of inhibition zone; DoEO, Dodartia orientalis L. essential oil; GC–MS, Gas chromatography–mass spectrometry analysis; MATH, Microbial adhesion to hydrocarbons; MBC, Minimum bactericide concentration; MIC, Minimum inhibition concentration; PBS, Phosphate buffered saline; PI, Propidium iodide; RI, Retention indices; SEM, Scanning electron microscopy; XTT, 2, 3-bis (2-methyloxy 4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide

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http://dx.doi.org/10.1016/j.indcrop.2017.08.058
Received 31 March 2017; Received in revised form 28 August 2017; Accepted 30 August 2017

traditional medicinal herbs with antimicrobial properties (Benjilali et al., 1986).

Previous studies have demonstrated the EOs exert many biological properties such as antineoplastic, antiphlogistic, antiviral, antifungal (Burt, 2004), and antibacterial effects (Benjilali et al., 1986). Especially, among these investigations, the antibacterial activity of EOs is most notable for its potential medical property of decreasing the bacterial resistance (Stefanakis et al., 2013). Moreover, EOs have been certified as safe substances, which guarantee their security for applications in various fields (Lanciotti et al., 2004). Such as in the field of food safety, EOs play vital roles in food protection by killing pathogens and removing bacterial contamination (Djenane et al., 2012). All above materials have proved that EOs possess extensive applications in the food industry even though the hygiene conditions have been improving (Benjilali et al., 1986; Burt, 2004).

Dodartia orientalis L. is the sole specie of *Dodartia* belonging to the *Scrophulariaceae* family, which primarily distributes in Xinjiang Uygur Autonomous Region and Gansu Province of China, and other countries including Kazakhstan and Uzbekistan also have its habitats (Xu et al., 2016). *D. orientalis* has been long consuming as traditional Chinese medicine (TCM) for the treatment of bronchitis, allergic rhinitis, and urticarial in the past (Xu et al., 2016). Heretofore, phytochemical researches have demonstrated the existence of monoterpenoids, sesquiterpenes, triterpenoids, and iridoids in *D. orientalis* (Umarova and Gorovit, 1988; Maksudov et al., 1995; Furukawa et al., 2013). However, the EO of *D. orientalis*, which is abundant in the clear aromatic flavor herb, has not been in-depth investigated. Moreover, the antibacterial activity and antibacterial mechanism of the EO still remain unclear. Therefore, the investigations on the antibacterial mechanisms and chemical compositions of DoEO can provide practical and scientific guidance for its potential applications in the fields of medicine, perfume, and food industry.

Considering the mentioned aspects above, this study was executed to investigate the antibacterial activity of DoEO against foodborne pathogens (*S. aureus*, *E. coli*, and *S. enteritidis*). After evaluating the antibacterial activity of DoEO, this work further elucidated the mechanisms of DoEO against three foodborne pathogens in terms of its interaction with the cell structure including cell walls and membranes, the vital important barrier of pathogenic bacteria that serves as a defence against antibacterial agents, and biofilms which give pathogens much more resistance to antibacterial agents and also can be as a strong and effective way to spread infectious diseases. Finally, the chemical compositions of DoEO were detected and analyzed by GC–MS. Best to our knowledge, the present study focused on the antibacterial mechanisms and antibiofilm mechanism of DoEO has not been reported yet.

2. Materials and methods

2.1. Plant material and bacterial strains

The whole herb (including roots, stems, leaves, flowers, and fruits) of *D. orientalis* was harvested from Xinjiang Uygur Autonomous Region of China, in September 2015 and identified by Xinjiang Food and Drug Administration. The authentic specimens of evert plant have been stored in the National Engineering Laboratory for Druggable Gene and Protein Screening of Northeast Normal University in China.

S. aureus (ATCC 6538), *E. coli* (ATCC 35218), and *S. enteritidis* (ATCC 14028) were provided by the Microbiology Laboratory of Northeast Normal University of China and maintained in Luria-bertani (LB) agar slants at 4 °C.

2.2. Essential oil extraction

The herbs were ground and meshed. Then, the resulting powder was hydro-distilled for 4 h using a Clevenger-type apparatus (Chen et al., 2016). The essential oil was dried over anhydrous Na_2SO_4 and filtered through 0.22 μm filter membranes to remove bacterial cells. Then, the sterile essential oil was conserved in the sealed vials at −20 °C.

2.3. Antibacterial activity assessments

2.3.1. Determination of inhibition zone diameter (DIZ)

The method for measuring DIZ was based on CLSI (2012) with slight modification. Briefly, 100 μL suspension of bacteria (1×10^7 CFU/mL) cultured overnight was spread on the LB agar plate. Sterile filter paper discs (6 mm) containing 10 μL DoEO were placed on the surface of the agar plate and incubated at 37 °C for 24 h. Tetracycline (10 μg/disc, Amresco, USA) and chloramphenicol (12.5 μg/disc, Amresco, USA) were used as the positive control.

2.3.2. Determination of minimal inhibitory concentration (MIC)

The MIC was measured using broth microdilution method, as recommended by the literature (Chen et al., 2016) with slight changes. In brief, twofold dilutions in LB broth were executed in 96-well microplates to acquire serial dilutions of DoEO with final concentrations from 0.03 to 16 μL/mL, and tween 80 was used in every well to a final concentration of 2% to dissolve the DoEO. The tested strains were cultured in LB broth overnight at 37 °C to obtain suitable bacterial suspension (1×10^7 CFU/mL). A negative control including 2% tween 80 without DoEO was also observed. MIC was the lowest concentration without visible growth of bacteria.

2.3.3. Determination of minimum bactericidal concentration (MBC)

The suspension (50 μL) in above wells without visible growth was spread on LB agar plates and subcultured at 37 °C for 24 h. The surviving colonies were observed. MBC was the lowest concentration at which without any visible colony in LB agar plates.

2.4. Cell wall damage assessment

2.4.1. Leakage of alkaline phosphatase (AKP) assay

To detect the leaking of AKP, logarithmic phase cells of *S. aureus*, *E. coli*, and *S. enteritidis* (1×10^7 CFU/mL) were collected by centrifugation at 3000*g* for 15 min. The harvested bacteria were washed thrice and resuspended in phosphate buffered saline (PBS, pH 7.4). Afterwards, bacterial suspensions were exposed to different concentration of DoEO (control, MIC, and MBC) and incubated at 37 °C for 4 h. The extracellular AKP concentration was measured by a microplate reader (ThermoMK3, Shanghai, China) using the AKP kit obtained from Nanjing Jiancheng Bioengineering Institute of China. The control was not exposed to DoEO.

2.5. Membrane damage assessments

2.5.1. Leakage of nucleic acids assay

The leak of nucleic acids into bacterial suspension was evaluated by referring to the relevant literature (Zhang et al., 2015). In brief, the collected logarithmic phase cells of *S. aureus*, *E. coli*, and *S. enteritidis* (1×10^7 CFU/mL) were washed and resuspended in PBS. The suspension was incubated with different levels of DoEO (control, MIC, and MBC). After that, the mixtures were filtered through 0.22 μm filter membranes to remove bacterial cells and measured at 260 nm by the microplate reader. Samples without DoEO but with 2% tween 80 were used as controls.

2.5.2. Leakage of potassium ions assay

The leak of potassium ions into supernatant were measured according to pertinent literature (Patra and Baek, 2016). Briefly, logarithmic phase bacterial cells (1×10^7 CFU/mL) were washed and resuspended in sterile peptone water (0.1 g/100 mL). Then, the suspension was exposed to different levels of DoEO (control, MIC, and MBC). The extracellular potassium ions concentration was detected