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Comparison of antibacterial effects and fumigant toxicity of essential oils extracted from different plants

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ABSTRACT

The chemical composition, antibacterial and insecticidal potential of Anise, Peppermint, Clove, Cinnamon, Pepper, Citronella and Camphor essential oils (EOs) extracted by steam distillation from seven different plant species were investigated. GC–MS analysis revealed that terpenes and phenols were the main components of EOs. The different kinds of EOs exerted appreciable antibacterial action against Gram-positive (*B. subtilis* and *S. aureus*) as well as Gram-negative (*E. coli* and *S. typhimurium*) bacteria. Among EOs, cinnamon EO exhibited the highest antibacterial effect for all the bacterial strains with the lowest MIC ranging from 0.125 to 0.25 mg/mL, the largest inhibition zone and the strongest inhibition of bacterial growth, followed by clove EO. The moderate inhibitory effects were observed for remaining EOs. On the other hand, cinnamon EO, clove EO, and anise EO exhibited significant fumigant toxicity against *Sitophilus oryzae* at 7.69 μL/L air concentration after 24 and 48 h exposure. Furthermore, the single component analysis showed the LC_{50} values of 2.90 for eugenol, 5.45 for cinnamaldehyde, and 5.42 μL/L air for estragole extracted from selected EOs (clove, cinnamon, anise), respectively. The results will pave a way for utilization of plant derived EOs as natural food preservatives to counteract food spoilage and pest's managements.

1. Introduction

Food spoilage and food poisoning caused by microbial infection during the harvesting, processing, transportation and storage of foods present an enormous threat to consumers and the development of food industries. The use of chemically synthesized additives in foods to prevent the food spoilage and pathogenic bacteria has been controversial because of their potential to cause respiratory diseases or other health risks (Fleming-Jones and Smith, 2003). Meanwhile, the continuous application of pharmaceutical antibiotics also caused the emergence of bacterial resistance problems in the past decade. With the increasing concern of safety for the addition of synthetic foods preservatives, the use of natural materials as viable alternative antibacterial agents has become an interesting researchable aspect worldwide. On the other hand, the rice weevil, *Sitophilus oryzae* L. (Coleoptera: Curculionidae), known as the most destructive pest in stored grains, causes huge loss in the quantity and quality of food and the incidences of foodborne diseases occur every year (Haddi et al., 2015; Herrera et al., 2015). At present, the recurrent and extensive application of chemical-based methods to combat the pests such as phosphine (PH3) led to increased insect resistance in packed food products (Kljajic and Peric, 2006; Ren et al., 2008). Moreover, some chemical fumigants are being prohibited due to their contribution for environmental pollution and harm to human health. Therefore, it is essential to develop novel and safe strategies for identification and characterization of natural plant based antibacterial agents as well as fumigants for their direct application in food industries.

Among the plant derived materials, plant essential oil is an important volatile secondary metabolite in plants, which is well known for its high volatility, low residual generation, and very rare resistance problems (Dippel et al., 2014). Plant EOs contain two main components, terpenes and aromatic compounds (Nouri-Ganbalani et al., 2016), which are commercially used as medicines, flavoring agents in many foods, flavor enhancers and insecticides in spices. In the past decade, the antibacterial, antifungal, antioxidant and insecticidal activities of plant EOs have attracted widespread attention (Celia et al., 2013; Smeriglio et al., 2017; Santos et al., 2018). Many reports have pointed out that the plant essential oils have contact and fumigation toxicity (Stefanazzi et al., 2011), repellent activities (Nenaah, 2014; Nerio et al., 2010), anti-feeding activity (Stefanazzi et al., 2011), and

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development and growth inhibitory activity (Waliwitiya et al., 2009) against insects. Meanwhile, many plant EOs have also been investigated for their inhibitory effects on the growth of many bacteria (*Bacillus cereus*, *Staphylococcus aureus* and *Staphylococcus epidermidis*) and fungi (*Candida albicans*, *Cryptococcus neoformans* and *Trichophyton mentagrophytes*) (Burt, 2004; Salgueiro et al., 2010). Generally, EOs harbor broad spectrum in activity due to the presence of complex chemicals as well as diverse mode of action (Dasgupta, 2016). Considering the excellent antibacterial and insecticidal functions, EOs have the high potential to be developed as novel natural additives to increase the food safety and enhance shelf-life of various foods. Nevertheless, after careful consideration of previous literature analysis, it can be concluded that comprehensive evaluation of antibacterial activity and fumigation toxicity of different plant EOs were rarely investigated. In the lieu of above gaps in knowledge, our research hypothesis can be of great interests for food industries as well as for human health concern.

Briefly, we identified the main chemical components of the EOs of the seven different plant species through GC–MS analysis. The antibacterial effects of different plant EOs on food-borne spoilage and pathogenic bacteria were compared, besides, fumigation toxicity of these EOs against *S. oryzae*. On the basis of these antibacterial and insecticidal activities, the obtained data would eventually promote the utilization of selected EOs as an environment friendly industrial preservative.

2. Material and methods

2.1. Plant materials

Seven different species of plant materials such as Anise (*Pimpinella anisum*), Peppermint (*Mentha haplocalyx*), Clove (*Syzygium aromaticum*), Cinnamon (*Cinnamomum zeylanicum*), Pepper (*Zanthoxylum bungeanum*), Citronella (*Cymbopogon nardus*), and Camphor (*Cinnamomum camphora*) were procured from the local market of Hefei city, China. The obtained raw materials were dried at 40 °C and powered for further extraction.

2.2. Extraction of essential oils

The EOs were extracted by hydro distillation in a Clevenger-type apparatus for 3 h from all kind of grounded powder according to the previously reported method (Hamdaoui et al., 2018). The water residues were removed from the oil extracts by using anhydrous sodium sulfate. The obtained EOs were preserved at 4 °C until next use.

2.3. Gas chromatography–mass spectrometry analysis of essential oils

The obtained EOs were subjected for chemical composition characterization via gas chromatography–mass spectrometry (GC–MS) (Agilent GC-MS 7890) using a DB-5MS column (30 m × 0.25 mm × 0.25 μm). The experimental conditions were as followed: helium was used as a carrier gas (13 psi), oven temperature was programmed to 50 °C for 2 min and raised to 260 °C at a rate of 5 °C/min and held at this temperature for 10 min and the injector temperature was set at 250 °C. The mass spectra were recorded at 70 eV with the mass range of 42–350 *m/z* with the ion source temperature of 280 °C (Polatoglu et al., 2016). The major constituents of essential oils were identified by comparing their relative retention times with those of authentic samples and matching their mass spectral peaks available with mass spectral library (NIST 14 Mass Spectral and Wiley Registry™ of Mass Spectral Data) or with published data in the literature (Vasantha-Srinivasan et al., 2016).

2.4. Antibacterial activity

2.4.1. Bacterial strains

To determine the antibacterial effects of EOs, two kinds of bacteria were used in this study such as Gram-negative strains: *Escherichia coli* and *Salmonella typhimurium,* and Gram-positive strains: *Bacillus subtilis* and *Staphylococcus aureus*. The test strains were incubated in the Luria Broth medium (Beijing Land Bridge Technology Co., Ltd., China) at 37 °C for 16 h to obtain the active cultures (Ma et al., 2018)

2.4.2. Agar diffusion method for antibacterial activity

The antimicrobial activity of the tested EOs were evaluated by agar diffusion method as described previously (Mostafa et al., 2018). Plant EOs were dissolved in ethanol to different concentrations (6.25 mg/mL, 12.50 mg/mL, 25.00 mg/mL, 50.00 mg/mL, 100.00 mg/mL, 200.00 mg/mL, 400.00 mg/mL and 800.00 mg/mL) and filtered by a 0.45 μm microporous filter. 10 mL of Nutrient Agar (Beijing Luqiao Science and Technology Co., Ltd.) was added to each Petri dishes. After solidification, 100 μL of the test bacterial suspension (10^6 CFU/mL) was spread to the Petri plates. Then after, sterile filter paper discs (6.0-mm diameter, 1.0-mm thickness) containing 10 μL of each plant EO was placed on the surface of seeded Petri plates. The Petri plates were kept in a refrigerator at 4 °C for 2 h to allow the diffusion of plant essential oil followed by incubation at 37 °C for 24 h. The filter papers loaded with 10 μL of ethanol was used as control in each plate during this assay. The inhibition zones were evaluated by measuring the diameter of clear inhibition zone around each paper disc using a vernier caliper which was recorded as an indication of antibacterial activity (Li et al., 2018).

2.4.3. Minimal inhibitory concentration (MIC)

The lowest concentration of EOs without visual growth of bacteria for incubating for 24 h was considered as MIC. MIC was determined according to the previously reported method with slight modification (Zhang et al., 2016). Initially, the stock solution of each EO was dissolved in ethanol to obtain the concentration of 8 mg/mL. Subsequently, two-fold serial dilutions of EOs were prepared in sterile autoclaved tryptone soy broth (TSB) medium to obtain a final concentration ranging from 0.0625 to 8 mg/mL. Finally, 100 μL of bacterial suspension was added individually to each tube. All the tubes were placed in a rotary shaker (ZHWY-200B) at 37 °C and 180 rpm for 24 h to observe the growth of the bacteria.

2.4.4. Growth curves

The anti-bacterial effects of EOs were investigated through growth curve determination. For this, four bacteria were treated with the MIC and 1/2 MIC concentrations, respectively. Then after, the cultured broths (Erlenmeyer flask, 100 mL) supplemented with different EOs were incubated in a rotary shaker (ZHWY-200B) (180 rpm) for 24 h at 37 °C followed by absorbance measurement at 600 nm by using micro plate reader (Infinite 200 PRO, Austria) after every 2 h intervals (Zhang et al., 2017).

2.5. Insecticidal activity against Sitophilus oryzae

2.5.1. Insects culturing

The *Sitophilus oryzae* used in the experiment was collected from Hefei grain storage, Hefei, China and maintained without exposure to any insecticides over many generations. The insects were reared on whole rice and kept in the glass container with the controlled conditions (27 ± 1 °C, 70 ± 5% relative humidity, 12:12 h light:dark cycle). All the bioassays were conducted under the constant environmental conditions.