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ORIGINAL ARTICLE

Antimicrobial, antibiofilm and antitumor activities of essential oil of *Agastache rugosa* from Xinjiang, China

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Abstract In the study, we evaluated chemical composition and antimicrobial, antibiofilm, and antitumor activities of essential oils from dried leaf essential oil of leaf and flower of *Agastache rugosa* for the first time. Essential oil of leaf and flower was evaluated with GC and GC–MS methods, and the essential oil of flower revealed the presence of 21 components, whose major compounds were pulegone (34.1%), estragole (29.5%), and p-Menthan-3-one (19.2%). 26 components from essential oil of leaf were identified, the major compounds were p-Menthan-3-one (48.8%) and estragole (20.8%). At the same time, essential oil of leaf, there is a very effective antimicrobial activity with MIC ranging from 9.4 to 42 μg ml^{-1} and potential antibiofilm, antitumor activities for essential oils of flower and leaf essential oil of leaf. The study highlighted the diversity in two different parts of *A. rugosa* grown in Xinjiang region and other places, which have different active constituents. Our results showed that this native plant may be a good candidate for further biological and pharmacological investigations.

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1. Introduction

Agastache rugosa is an edible plant, which belongs to Lamiaceae family and widely grows in China. The cultivated plant is used as a treatment for people suffering from anxiety, nausea, and bacterial infections. The herb is known under many different names, such as Korean Mint, purple giant hyssop,

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Indiana mint, and the wrinkled giant hyssop (Hudaberdi and Pan, 2004). As one of the 50 fundamental herbs, *A. rugosa* is known as huò xiāng, which is reported to have antifungal, antibacterial, carminative, and antipyretic properties (Liu, 1993). As an antifungal agents, this plant is used against Trichophyton species (Shin, 2004). It is reported that *A. rugosa* have antioxidant activity (Oha et al., 2006), antimicrobial activity (Kim, 2008) and anti-HIV integrase action (Kim et al., 1999). Essential oils are natural compounds with their components gaining increasing interest because of their relatively safe status, their wide acceptance by consumers and their exploration for potential functional use (Ormancey et al., 2001; Sawamura, 2000; Gianni et al., 2005). Actually, the essential oil shows a great promise as a new prototype from which antifungal agents may be developed (Yoon et al., 1994; Bidlack et al., 2000; Faleiro et al., 2003; Shin and Kang, 2003). In a previous report, the main component of the essential oil of *A. rugosa* is estragole (Shin and Kang, 2003), which has antifungal activity (Shin, 2004). In order to develop native herbal medicine resource and realize complementary and alternative for advantage resources, our research group cultivated the *A. rugosa* in Xinjiang for the last two years. However, to the best of our knowledge, the constituents of essential oils isolated from the dried flower and leaf of *A. rugosa* which grows in Xinjiang region, and its activity have never been evaluated and studied. As we known, the multi-drug resistance bacteria and biofilm development are ubiquitous phenomena with several deleterious medical and economic consequences. The ability to form biofilms is the important factor in pathogenesis of most microorganisms. Thus, the present study was to search for new antimicrobials agents, especially from native herbal plants. In this study, we aim to evaluate essential oil constituents of *A. rugosa*, which have many pharmacological activities and been cultivated in Xinjiang of China and to investigate the antimicrobial, antibiofilm and cytotoxic activities of essential oil of leaf and flower from *A. rugosa*. The current investigation was to find whether there is diversity in two different parts of *A. rugosa* grown in Xinjiang and other places. At the same time, we initiated this study to find an efficiency and low toxicity natural medicines, which will apply to treatment of human diseases, lower resistance rates and benefit of mankind, for further research.

2. Material and methods

2.1. Plant material

The aerial part of *A. rugosa* was collected in September 2010 from Liyu mountain in Urumqi city of Xinjiang, China, which was identified by Traditional Chinese Medicine Ethnical Herbs Specimen Museum, Yonghe Li. A voucher specimen (No. TCMEHSM 2010-352) was deposited in the museum of this institute.

2.2. Essential oil isolation

The flower and leaf of examined plants were separated and dried in shadow at room temperature, and submitted 100 g to hydrodistillation with 1 L of distilled water in a Clevenger-type apparatus for 6 h. At the end of each distillation the oils were collected, dried with anhydrous sodium sulfate prior to analyses, measured, transferred to glass flasks and stored at 4 °C until the moment of analysis.

2.3. Gas chromatography and gas chromatography–mass spectrometry

GC was carried out using a Agilent 6890 N GC-FID system, equipped with a flame ionization detector (FID) on a Agilent capillary column, HP-5 (30 m × 0.32 mm; film thickness 0.25 μm). The column temperature was programed from 40 °C to 250 °C for 5 °C/min. The column temperatures of injector and detector were fixed to 250 °C. Helium was used as the carrier, flow rate 1.0 ml/min.

The GC–MS analysis was carried out using a Agilent 6890 N GC-FID system, equipped with a flame ionization detector (FID) on a Agilent capillary column, HP-5 (30 m × 0.32 mm; film thickness 0.25 μm). The column temperature was programed from 40 °C to 250 °C at a rate of 5 °C/min. The column temperatures of injector and detector were 250 °C. Helium was used as the carrier, flow rate 1.0 ml/min. Split ratio was 1:100. The GC–MS analysis was done in the EI mode at 70 eV, inlet temperature was 200 °C and transfer line temperature was 250 °C. The temperature program was the same with that of the GC analysis. The injected volume was 0.2 μl.

2.4. Identification of components

The identification of components and peak was done by comparison of their retention time with respect to the n-alkane series (C6-C22) internal standards under the identical experimental conditions. The mass spectra and relative Retention Index (RI) were compared with those of commercial (NIST 05 and NIST 05 s). The relative amounts of individual components were calculated based on GC integrator peak areas without using correction factors.

2.5. Test organisms

Organisms contain *Staphylococcus aureus* (ATCC 25923, positive control: Penicillin) and *Escherichia coli* (ATCC 25922, positive control: Gentamycin Sulfate Injection) were used for the study. The organisms were maintained by serial subculturing every month on nutrient agar slants and incubating at 37 °C for 18–24 h.

The cultures were stored under refrigerated condition. The antifungal activity of the oil was tested against *Candida albicans* (ATCC 10231, positive control: Fluconazole).

2.6. Determination of minimum inhibitory concentrations (MICs)

The inhibition effect of EOF and essential oil of leaf on bacterial growth was determined, with a broth microdilution susceptibility test, as recommended by CLSI (2014) and described in experiment technique of medical microbiology (Guan et al., 2006). The oils were added aseptically to sterile melted Mueller Hinton Broth medium to produce the concentration range of 5.25–336 μg/ml for EOF and range of 4.72–302 μg/ml for essential oil of leaf. The bacterial plates were incubated at 37 °C.