Antimicrobial Effect of *Inula britannica* Flower Extract against Methicillin-resistant *Staphylococcus aureus*

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Introduction

The food-borne disease and nosocomial infections caused by contaminated food and drinking water have increased throughout the world [18]. One of the most common pathogens responsible for these disorders is *Staphylococcus aureus* [21]. Concomitantly, there has been an increased emergence rate of antibiotic resistance among bacterial strains, which have caused a variety of economic and industrial problems [14]. Methicillin-resistant *S. aureus* (MRSA) has a cell wall with a low affinity for β-lactam antibiotics, which reduces the effectiveness of conventional antimicrobial agents. This resistance is commonly related to the *mecA* gene encoding for a penicillin-binding protein 2a (PBP2a). PBP2a decreases the effects of β-lactam antibiotics against resistant. The expression levels of *femA*, *mecA*, and *mecRI* were required for high-level resistance in MRSA [10, 11]. On the other hand, *mecI*, the regulator genes which is located upstream of the *mecA* gene, encodes the repressor [6].

Medicinal plants and traditional medication have a long history of clinical application, and have been used as antimicrobial properties in food system. For instance, *Glycyr rhiza uralensis*, extensively used in herbal medicine, has been shown to inhibit the growth of MRSA [10], as do some compounds derived from the roots of *Erythrina variegata* [20]. A major advantage of the use of natural products as an antimicrobial agent is that they are not thought to contribute to the further development of antibiotic resistance among pathogens, as opposed to the long-term use of synthetic antibiotics [2].

*Inula* species (Asteraceae) have been used as traditional herbal medicine to treat digestive disorders, inflammation, and bronchitis [4]. Recent studies have suggested that compounds from *Inula britannica* exhibit anticaroinogenic, antioxidant, neuroprotective, and hepatoprotective activities.
[7, 17]. Also, *I. britannica* have been permitted for use in food by the KFDA (http://www.kfda.go.kr). However, there is limited information regarding its effects against MRSA. In this study, we investigated the antimicrobial effect of methanol extract of *I. britannica* flowers in a view of decreasing of cell number, changing of morphology, and resistant gene against MRSA.

**Materials and Methods**

**Plant material and extraction preparation**

*I. britannica* flowers were obtained from kyungdong herbal markets (Seoul, Korea). The voucher specimen (KU-H26) was stored in the Laboratory of Biotechnology, Konkuk University, Seoul, Korea. Dried *I. britannica* flowers were extracted following the procedure described in a previous report [7]. Ground plant material (10 g) was soaked in 100 ml methanol overnight at room temperature. The extract was filtered and concentrated under reduced pressure. The concentrates were freeze-dried to provide crude methanol extract (0.66 g). The extract was dissolved in dimethylsulfoxide (DMSO, Samchum Chemical Co., Gyeonggi, Korea) to a final concentration of 50 mg/ml, filtered through a 0.45 μm membrane filter, and then stored at 4°C until use.

Contents of total polyphenol and total flavonoid

Total polyphenol content was determined by the Folin-Ciocalteau assay [24]. A 100 μl aliquot of extracts and 2 ml of 2% aqueous sodium carbonate solution was added in a 15 ml Falcon tube. After 3 min, 100 μl of 50% Folin-Ciocalteau’s reagent (Sigma Chemical Co., St. Louis, MO, USA) was added to the mixture and allowed to stand at room temperature for 30 min. The absorbance was measured at 750 nm with a spectrophotometer (Mecasys, Daejeon, Korea). Total phenol contents were calculated on the basis of the calibration curve of gallic acid. The results were expressed as mg gallic acid equivalent (GAE) per g sample.

Total flavonoid content was measured with an aluminum chloride assay [22]. A 100 μl aliquot was added to 1.5 ml ethanol. Then, 100 μl of 10% ammonium chloride and 100 μl of 1 M potassium acetate were added with 2.8 ml of distilled water. After incubation at room temperature for 30 min, the absorbance of the mixture was measured at 415 nm. Quercetin was used to make the calibration curve. The results were expressed as mg quercetin equivalents (QE) per g sample.

The flavonoid compositions were detected by a high performance liquid chromatography (HPLC) using a Zorbax C18 column (150 × 4.60 nm, 5 mm particle size). The mobile phase was used 0.1% formic acid (solvent A) and 100% acetonitrile (solvent B) as elution profile (0-10 min, 10-20% solvent B; 10-20 min, 20% solvent B; 20-35 min 10-20% solvent B; 35-40 min, 10-20% solvent B; 40-60 min 10-20 solvent B). A UV detector wavelength was kept at 254 nm, and the flow rate was 0.8 ml/min.

**Microorganisms and culture conditions**

The methicillin-resistant *Staphylococcus aureus* (ATCC 33591, ATCC 33593, and ATCC 33594) strains obtained from the Korean Culture Collection of Microorganisms (KCCM, Seoul, Korea). All strains were stored at −20°C in tryptic soy broth (TSB, Becton Dickson Biosciences, Sparks, MD, USA) with 20% glycerol. For the antibacterial test, the inocula of the strains were grown in TSB aerobically overnight at 35°C.

**Antimicrobial activity**

The antimicrobial properties of *I. britannica* flowers extract was conducted using the disc diffusion method. Bacterial suspension (100 μl) had been spread on TSA plates, then sterile paper discs (8 mm diameter) impregnated with 50 μl of sample solution. The plates were incubated at 35°C for 24 h. The diameters of the inhibition zones were measured in millimeters excluding disc diameter. Paper discs containing DMSO without the sample solution used as controls.

The minimal inhibitory concentration (MIC) against the bacterial strains was determined using the broth microdilution method [16]. A stock solution of sample (50 mg/ml) was diluted in DMSO to give final concentrations ranging from 0.039 to 2.5 mg/ml. One hundred microliters of inocula from each bacterial suspension (about 10^5 CFU/ml) was added to each well on 96 well plate and incubated at 35°C for 24 h. Bacterial growth was evaluated by measuring absorbance at 630 nm. The MIC was determined as the lowest concentration of the extract that inhibited the growth of the test organism to an absorbance change of less than 0.05. The minimal bactericidal concentration (MBC) was determined by inoculating the surface of TSA plates with 100 μl of suspension. After incubation at 35°C for 24 h, the plates were examined for growth. The MBC was defined as...