Phytomedicine 18 (2010) 40–45

Contents lists available at ScienceDirect

Phytomedicine

journal homepage: www.elsevier.de/phymed

Reversing β-lactam antibiotic resistance of *Staphylococcus aureus* with galangin from *Alpinia officinarum* Hance and synergism with ceftazidime

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ARTICLE INFO

Keywords:
Alpinia officinarum Hance
Galangin
Quercetin
Baicalein
The synergism with ceftazidime
Penicillin-resistant *Staphylococcus aureus*

ABSTRACT

The purpose of this investigation was to extract and identify the bioactive phytochemicals from smaller galanga (*Alpinia officinarum* Hance). The antibacterial, synergy effects and primary mechanism of action of galangin and ceftazidime against *S. aureus* DMST 20651 are also investigated by minimum inhibitory concentration (MIC), checkerboard, killing curve determinations, enzyme assay and electronmicroscopy method. The rhizomes chloroform extract of this plant showed that these compounds were galangin, kaempferide and kaempferide-3-*O*-β-D-glucoside, which had not been previously reported in this species. Synergistic FIC indices were observed in the combination of test flavonoids (galangin, quercetin and baicalein) and all selected β-lactams (methicillin, ampicillin, amoxicillin, cloxacillin, penicillin G and ceftazidime) (FIC index, <0.02–0.11). The combination of ceftazidime at 5 μg/ml and 5 μg/ml of test flavonoids (galangin, quercetin and baicalein) exhibited synergistic effect by reduced the cfu/ml of this strain to 1×10^3 over 6 and throughout 24 h. Galangin showed marked inhibitory activity against penicillinase and β-lactamase. Electronmicroscopy clearly showed that the combination of galangin and ceftazidime caused damage to the ultrastructures of the cells of this strain. It was concluded that galangin, quercetin and baicalein exhibited the potential to reverse bacterial resistance to β-lactam antibiotics against penicillin-resistant *S. aureus* (PRSA). This may involve three mechanisms of action that galangin inhibit protein synthesis and effect on PBP 2a, interact with penicillinase and cause cytoplasmic membrane damage. These findings lead us to develop a new generation of phytopharmaceuticals that may use galangin, quercetin and baicalein in combination with ceftazidime to treat PRSA that currently almost untreatable microorganism. The anti-PRSA activity and mode of action of galangin is reported for the first time. These *in vitro* results have to be still confirmed in an animal test or in humans.

Introduction

Bacterial resistance to β-lactam antibiotics is a global problem. Today around 90–95% and 70–80% of *Staphylococcus aureus* (*S. aureus*) strains are resistant to penicillin, methicillin around the word and in most of the Asian countries (Casal et al. 2005; Chambers 2001) Strains of β-lactam-resistant *S. aureus* including methicillin-resistant *S. aureus* (MRSA) now pose serious problem to hospitalized patients, and their care providers (Mulligan et al. 1993). Antibiotics available for the treatment of MRSA infection are fairly toxic and their use is frequently associated with unwanted side-effects (Brumfitt and Hamilton-Miller 1989). Novel antibiotics and/or new generation of phytopharmaceuticals approaches that can reverse the resistance to well tried agents which have lost their original effectiveness or enable their use to treat diseases instead of synthetic drugs alone are research objectives of far reaching importance (Reading and Cole 1977; Wagner and Ulrich-Merzenich 2009).

Smaller galanga (*Alpinia officinarum* Hance) is a pungent and aromatic rhizome, which is a member of the ginger family (Zingiberaceae). The rhizome is cultivated in India, Vietnam, Southern China and Thailand because of its use as a spice and as a traditional medicine for several purposes such as treatment for pyogenic diseases (infectious acne, carbuncles, sty, pyoderma, pustular impetigo in Thailand), ring worm, venereal diseases, carminative, abdominal discomfort (Athamaprasangsa et al. 1994). The chemical and pharmacological studies of the rhizomes of small galanga have three groups of important chemical constituents, flavonoids, glycosides and diarylheptanoids. It has been reported that smaller galanga has biological activities, including antitumor, antiulcer, antibacterial, and antifungal properties (Itokawa et al.

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doi:10.1016/j.phymed.2010.09.003

1985; Newman et al. 2003; Ly et al. 2003; Matsuda et al. 2006). The purpose of this investigation was to separate and identify the bioactive compounds from the rhizome of smaller galanga. We have also investigated the *in-vitro* activity of galangin, a major bioactive constituent isolated from smaller galanga, and other test flavonoids (quercetin and baicalein) against β-lactam-resistant *S. aureus* when used alone and in combination with β-lactam antibiotics.

Materials and methods

General experimental procedures

The UV spectra were obtained with a Hewlett Packard 8452A diode array UV–vis spectrophotometer, whereas the IR spectra were measured with a Perkin-Elmer FT-IR 2000 spectrophotometer (by a KBr disk method). The ^{1}H and ^{13}C NMR spectra were recorded with a Bruker DRX 400 spectrometer in CD3OD solution and chemical shifts are expressed in δ (ppm) with reference to the solvent signals. Silica gel 60 (70–230 mesh) and silica gel 60 PF 254 were used for column chromatography and preparative thin-layer chromatography, respectively. Solvents of technical grade were used for chromatographic purposes.

Plant material, β-lactam antibiotics and bacterial strains sources

The fresh rhizomes of smaller galanga were digged from Saengduan Konekratoke's paddy field located in Chokchai District, Nakhonratchasima Province in July and December 2007, June and November 2008. The plant specimen has been deposited at the National Herbarium after it was identified by Dr. Paul J. Grote, School of Biology, Institute of Science, Suranaree University of Technology, Nakhon Ratchasima Province. The rhizomes of smaller galanga was separated from the stems, washed thoroughly, and dried in an oven at 50 °C for three days. The dried samples were then ground to powder. Quercetin and baicalein were obtained from Indofine Chemical Company (USA). Ceftazidime, methicillin, ampicillin, amoxicillin, cloxacillin, penicillin G (benzylpenicillin), penicillinase (β-lactamase) and clavulanic acid were obtained from Sigma (Sigma–Aldrich, UK). Mueller-Hinton broth was obtained from Oxoid (Basingstoke, UK). Seven clinical isolates of penicillins-resistant *S. aureus* DMST 20651-655, 20661-2 (PRSA), were obtained from Department of Medical Sciences, Ministry of Public Health, Thailand. *S. aureus* ATCC 29213, positive control, was purchased from American Type Culture Collection (ATCC).

Extraction and isolation

The 2 kg of dried powder of rhizomes of smaller galangal were extracted consecutively with hexane, chloroform and methanol by soxhlet extraction apparatus. The extracted solutions were then filtered. The filtrates were concentrated by evaporation under reduced pressure to afford 13.79 g of hexane crude extract as dark yellow oil, 22.38 g of chloroform crude extract as dark yellow slush, and 30.12 g of methanol crude extract as dark brown gum.

The chloroform crude extract was separated using a column packed with hexane slurry of silica gel. Then, was dissolved in chloroform and loaded onto the column. Four major fractions (I 3.24 g, II 5.76 g, III 9.72 g and IV 13.93 g) were separated by TLC.

A portion of fraction III (1.0085 g) was further separated by preparative TLC to give crude compound **1** (0.0105 g), which was recrystallized from chloroform–methanol mixed solvents to obtain pure compound **1** (0.0007 g).

Fraction IV (13.93 g) was further separated using a column with hexane, then by preparative TLC to afford three fractions (C 0.0109 g, D 0.0127 g and E 0.0155 g). Fraction C and D were further purified by preparative TLC to give crude compounds **2** and **3** which

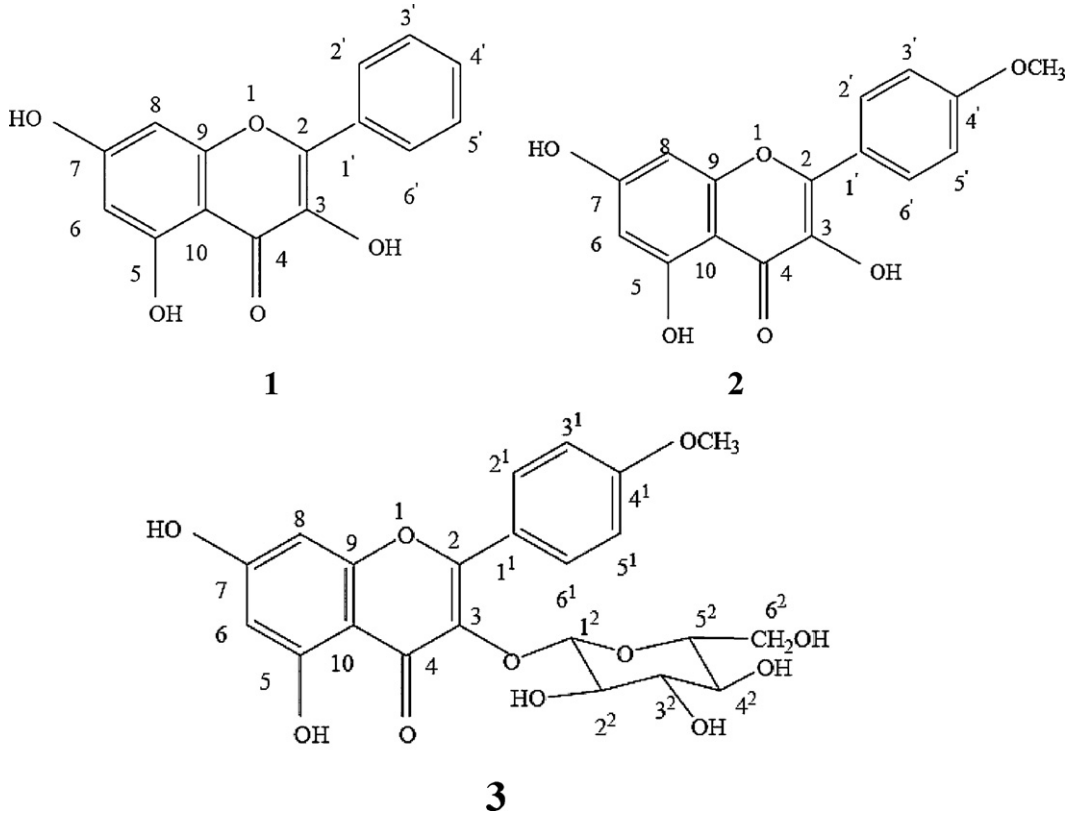

Fig. 1. Structure of compounds **1**, **2** and **3**.