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The antibacterial activity and action mechanism of emodin from *Polygonum cuspidatum* against *Haemophilus parasuis in vitro*

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ABSTRACT

Haemophilus parasuis is the causative agent of Glässer's disease, which leads to serious economic loss to the swine industry. Although antibiotics are widely used to control infections, outbreaks of this disease repeatedly happen. In this study, emodin from *Polygonum cuspidatum* showed potent inhibitory effect against *H. parasuis*. The minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) values of emodin were 32 and 64 μg/mL, respectively. The antibacterial kinetic curves indicated the antibacterial activity of emodin was in a concentration-dependent manner. Cell membrane permeability and flow cytometry assays proved that emodin could destroy cell membrane integrity and increase membrane permeability, and fluorescence spectra assay indicated emodin has influenced conformation of membrane protein. Under transmission electron microscopy, serious lesions of *H. parasuis* exposed to emodin (64 μg/mL) were found, including irregular cell shape, plasmolysis, ruptured cell wall and membrane and cytoplasmic vacuolation. These results suggested that emodin could be used as candidate for treating Glässer's disease.

Contents

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1. Introduction

Haemophilus parasuis, a Gram-negative pleomorphic rod, is the causative agent of Glässer's disease characterized by polyserositis syndrome, such as peritonitis, pleuritis, pericarditis, meningitis and arthritis (Oliveira and Pijoan, 2004). The disease had led to serious economic loss to the swine industry throughout the world (Aragon et al., 2012). Antibiotics are the major treatment for *H. parasuis* infection. However, after the widespread use of antimicrobials, both selection of antimicrobials and resistant bacteria appeared(Schwarz and Chaslus-Dancla, 2001). The morbidity and mortality were increased because of the failure of treating the disease caused by resistant bacteria (Kolar et al., 2010). Therefore, there has been a growing interest in developing ecologically sustainable antimicrobial agents to prevent this disease with fewer side effects.

Polygonum cuspidatum Sieb. et Zucc is widely distributed in China, Japan and Korea, even in North America. The dried root of *P. cuspidatum* is a well-known traditional Chinese medicine and officially listed in the Chinese Pharmacopoeia (China Pharmacopoeia Committee, 1999). It was also used in folk medicine in Korea and Japan (Chu et al., 2005; Shan et al., 2008). It has been traditionally used for treatment of inflammation, hepatitis, tumors, jaundice, skin burns and hyperlipemia diseases. Recent studies demonstrated that it also had antiviral, antibacterial and antifungal effects (Shan et al., 2008; Peng et al., 2013). At present, at least 67 compounds including quinones, stilbenes, flavonoids, counmarins, ligans, *etc.* have been isolated from the plant (Peng et al., 2013).

In our previous study, *P. cuspidatum* showed a strong antibacterial activity against *H. parasuis* (Li et al., 2014a). However, there are no available reports on the mechanism of antimicrobial agents against *H. parasuis* except allicin which could significantly decrease the expression levels of hhdA of hemolytic toxin, mviN and norm (Ma et al., 2014). Thus, this study is conducted to make a further exploration of antibacterial compound from *P. cuspidatum* and its mechanism of action against *H. parasuis* for the purpose of developing an alternative control method for *H. parasuis* infection.

2. Materials and methods

2.1. General reagents and microbial strain

The root of *P. cuspidatum* Sieb. et Zucc was purchased from Huimintang Pharmacy (Ya'an, China). The silica gels of thin chromatography and column chromatography were purchased from Qingdao Haiyang Chemical Co., Ltd (Qingdao, China).

Standard strain of *H. parasuis* (HS80) was provided by Prof. Sanjie Cao of the Institute of Preventive Veterinary Medicine, Sichuan Agricultural University (Ya'an, China). Tryptri Soy Btoth (TSB) and Tryptri Soy Agar (TSA) were purchased from Hangzhou Microbial Reagent Co., Ltd (Hangzhou, China).

2.2. Preparation of extracts from P. cuspidatum

Dried *P. cuspidatum* was ground to powder (about 60 mesh). The powder (200 g) was successively extracted with n-hexane, chloroform, ethyl acetate, ethanol and water, 6 h for each procedure. The extracts were dried by evaporation under reduced pressure and tested for antibacterial activity against *H. parasuis in vitro*.- The active extract, which showed the strongest antibacterial activity, was further extracted with anhydrous ether for 6 h. The antibacterial activity of the anhydrous ether extract and residuals were evaluated.

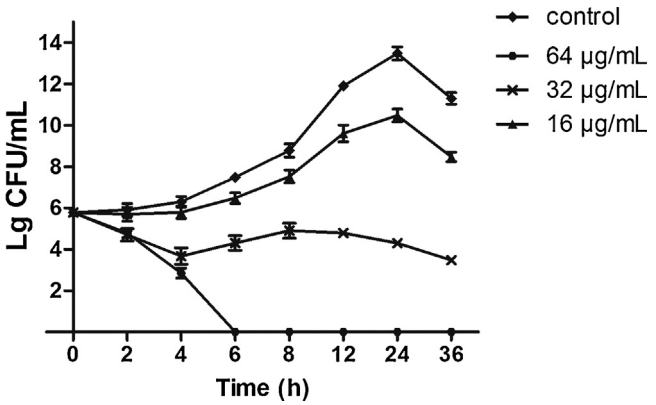

Fig. 1. The antibacterial kinetic curves of emodin against *H. parasuis*.

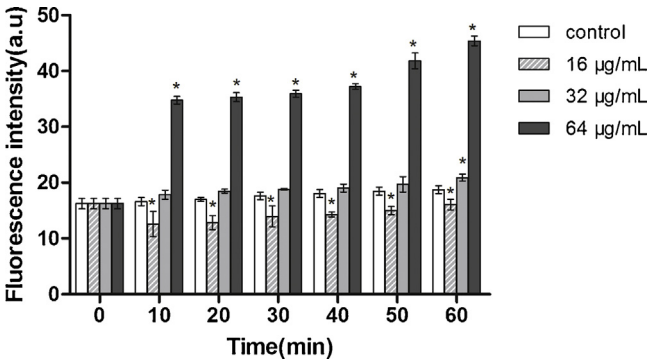

Fig. 2. NPN fluorescence of bacteria treated with emodin. Data are expressed as mean ±SD (n = 3). *p<0.05: significantly different from the control.

2.3. Isolation of anhydrous ether extract

The anhydrous ether extraction (6 g) was subjected to silica gel (400 g, 200–300 mesh) column chromatography (60 cm × 5.0 cm, id) which was eluted stepwise with petroleum ether-ethyl acetate-formic acid (10:1:0.1, 5:1:0.1, 2:1:0.1 and 1:1:0.1, v/v/v). Three fractions (I–III) were obtained based on TLC profiles (mobile phase, trichloromethane-acetone-formic acid-water, 4:3:0.5:0.2, v/v/v/v). Fraction II was repeatedly re-crystallised in acetone to yield an active constituent.