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Antimicrobial Activity of Thai-herbal Plants against Food-borne Pathogens *E. coli*, *S. aureus* and *C. jejuni*

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Abstract

Food-borne pathogens, including *Escherichia coli, Staphylococcus aureus* and *Campylobacter jejuni*, are prophylactically prevented in livestock by using commercially available antibiotics. The European Union has recently banned the use of prophylaxis use of antibiotics in animals since 2006 while urgently considers ways to reduce food-borne pathogens. The objective of this study was to propose a screening method for identifying natural compounds with anti-bacteria activity from twenty-six Thai-herbal plants. Antimicrobial activity was evaluated by an agar diffusion method, which allowed for the determination of the minimum inhibitory concentration (MIC). The results indicated that Thai-herbs have potent antimicrobial activity against *E. coli, S. aureus and C. jejuni* at bacteria suspensions of 2.0-3.0x10^9 CFU/ml. Interestingly, *C. formosum* had the highest antimicrobial activity against the three food-borne pathogens of *E. coli, S. aureus and C. jejuni,* which were isolated from the chicken-caecum. MIC values of *C. formosum* against *E. coli, S. aureus and C. jejuni* were 3.0 mg/ml, 3.0 mg/ml and 0.3 mg/ml, respectively. Other herbal plants also had antimicrobial activity against the three food-borne pathogens in this study. The herbal plants provide not only a natural source of anti-bacterial activity, but also anti-oxidant activity and anticancer properties. The application of using Thai-herbal plants compounds by adding them in animal feed is proposed. This may be a safe means of enhancing health and production of livestock and thus benefits humans and animals. Consequently, the selection of herbal plants, for use in preventing food-borne bacterial infection, is both interesting and worthwhile for food safety.

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Keywords: antimicrobial activity; *E. coli*; *S. aureus*; *C. jejuni*; herbal plant

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1. Introduction

The World Health Organization (WHO) has defined the meaning of food-borne illness as the disease after ingestion of food or water contamination. Food contamination can occur during any time between food production consumption this is sometimes referred to "from farm to table" (Adwan et al. 2015). Major food-borne illnesses can be caused by bacteria, viruses, parasites and/or toxin through contaminated food or water (Newell et al. 2010). Common gastrointestinal symptoms include: nausea, vomiting and diarrhea. Severe cases of food-borne illness demonstrated neurologic, hepatic and renal syndromes (Mead et al. 1999). Generally, controlling or reducing the spread of food-borne pathogens is through treatment of livestock with antibiotics. Alternative methods include vaccination and bacteriophage therapy. The European Union was prohibited the use of antibiotic treatment in animal feed. EU's concerned is that antibiotic resistance of food-borne pathogens will develop causing major problems for animals and humans (Kurekci et al. 2013). Herbal plants are one choice to treat and reduce food-borne pathogens contamination. Herbal plants they have many chemical components demonstrating antibacterial activity, anti-oxidant activity and natural preservative properties (Mabona et al. 2013). Phytochemical groups are a large group of chemicals derived from herbal plants. Phytochemical groups can be classified into eight groups; carotenoids, glucosinolate/isothiocyanate, polyphenol, phytoestrogenic, phenolic/cystic compounds, saponin, phytosterol and sulfide/thiols group (Gropper and Smith. 2012). This research study focused on screening twenty-six of Thai-herbal plants for antimicrobial activity against food-borne pathogen: *E. coli, S. aureus* and *C. jejuni*.

2. Materials and Methods

2.1 Bacterial strains culture

The strains of *E. coli, S. aureus* and *C. jejuni* used in this study were isolated from caecum of chickens. In addition, the stock, bacterial strains of *E. coli* and *S. aureus* were grown in nutrient broth at 37°C, 24 hour. For *C. jejuni* it was grown in Mueller-Hinton broth at 37°C, 48 hours under microaerophilic conditions. After that, the inoculated solutions were prepared for the anti-bacteria testing. Aliquots of 150 µL of *Campylobacter* isolates containing appoximately 2-3 x 10^9 CFU/ml, was transferred into 14 mL of semi-solid brucella agar 0.75% (w/w). The inoculated medium was swirled to distribute the *Campylobacter* and held at room temperature for 30 min. The preparation was tested for the antibacterial activity by the screening test described below.

2.1. List of twenty-six Thai-herbal plants

Twenty-six herbal plants assessed are listed here and classified by phytochemical groups: 1). Carotenoids: cassod tree (*Senna siames Lam.*), taew (*Cratoxylum formosum*) and carrot (*Daucus carota*); 2). Glucosinolate/Isothiocyanate: white cabbage (*Brassica aleracea*), purple cabbage (*Brassica aleracea*) and chinese cabbage (*Lactuca sativum*); 3). Polyphenol: golden shower (*Cassia fistula*), asian pigeonwings (*Clitoria ternatea*) and mangosteen (*Garcinia mangostana*); 4). Phytoestrogenic agent: kwao khrua khao (*Pueraria candollei*), sugar pea (*Pisum sativum*), soy bean (*Glycine max*), mung bean (*Vigna radiata*) and white popinac (*Acacia auriculiformis*); 5). Phenolics: kaffir lime (*Citrus hystrix*), ginger (*Zingiber officinale*), tree basil (*Ocimum gratissimum*) and coriander (*Coriandrum sativum*); 6). Saponin: kidney tea plant (*Orthosiphon aristatus*) and chinese bitter melon (*Momordica charantia*); 7). Phytisterol: white sesame (*Sesamum indicum*), black sesame (*Sesamum indicum*) and corn (*Zea mays*); and 8). Sulfide/Thiols: garlic (*Allium sativum*), onion (*Allium cepa*) and shallot (*Allium ascalonicum*).

2.2. Preparation of plant extract

Prior to extraction, fresh herbal plants were sun dried for 1-2 weeks then ground to a powder by using mortar and pestle. The powder plants were extracted with 150 ml of 95% ethanol. The plant extraction were placed at room temperature and shaken for 72 hours at 150 rpm. Then, the samples were filtered through filter paper and the supernatant collected. The supernatants were evaporated in a water bath at 40°C until the pellets appeared and were