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Anti-Bacterial Activity of *Cryptolepis buchanani* Aqueous Extract

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Abstract: The aqueous extract of *Cryptolepis buchanani* leaves was tested against food-borne pathogen bacteria (*S. aureus* ATCC 25923, *E. coli* ATCC 25922 and *S. typhimurium* ATCC 14028), nosocomial infection bacteria (*K. pneumoniae* ATCC 10031, *P. vulgaris* ATCC 13315 and *Ps. aeruginosa* ATCC 9721) and normal flora bacteria (*L. plantarum* ATCC 14917 and *S. epidermidis* ATCC 12228). The plant aqueous extract showed inhibitory effect against *S. aureus* ATCC 25923, *E. coli* ATCC 25922, *S. typhimurium* ATCC 14028, *K. pneumoniae* ATCC 10031, *P. vulgaris* ATCC 13315, *B. subtilis* ATCC 6633, *L. plantarum* ATCC 14917 and *S. epidermidis* ATCC 12228. The MICs (Minimal Inhibitory Concentrations) and MBCs (Minimal Bactericidal Concentrations) of this plant against all tested bacteria are in the range of 1-16 and 2-32 g L⁻¹, respectively. In conclusion, *C. buchanani* leaves aqueous extract showed broad-spectrum antimicrobial activity against food-borne pathogen bacteria, nosocomial infection bacteria and some normal flora bacteria at low concentration. This may supported the used of *C. buchanani* aqueous extract as food-borne pathogen bacterial growth control additive and nosocomial infections treatment remedy.

Key words: *C. buchanani*, food-borne contaminates, food-borne pathogen bacteria, nosocomial infection, anti-microbial, aqueous extract

INTRODUCTION

Food-borne infections are important public health concern worldwide (Busani *et al.*, 2006). In food production countries to control the growth of bacterial in the product is important. The most common bacteria causing food-borne illness are *Escherichia coli*, *Staphylococcus aureus*, *Samonella* sp., *Samonella typhimurium*, *Listeria monocytogenes*, *Clostridium botulinum*, *Vibrio vulnificus*, *Vibrio parahaemolyticus* and others (Busani *et al.*, 2006; Van *et al.*, 2007; Gerner-Smidt and Whichard, 2008).

The application of medicinal plants in health care are in great interesting of topical countries (Kolawole, 2007). The development of medicinal plant as medicine consider the good way for natural resource management especially in topical countries that enrich of plant species. However, the developments of medicinal plant as remedy need scientific results support. Especially, the development of medicinal plants against food-borne pathogen bacteria that may give useful results that may indicate the efficacy of the plant in food additive applications.

Cryptolepis buchanani belongs to the family Periplocoideae and subfamily Asclepiadaceae (Paulo and Houghton, 2003). The plant has been used in Indian folkloric medicine (Ayurveda) for anti-diarrhoeal, anticulcerative, anti-inflammatory, blood purifier, cough treatment, curing rickets in children and antibacterial (Kaul *et al.*, 2003). In Thailand, the alcoholic extract of stem of this plant has been

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used for treatment of inflammatory conditions such as arthritis, muscle and joint pain (Panthong *et al.*, 1986; Laupattarakasem *et al.*, 2003). Since, it has been used for antimicrobial in ayurveda folkloric medicine but still no scientific result to supported the used of *C. buchanani*. It would be interested to investigate the antimicrobial activity of *C. buchanani* aqueous extract whether the polar compound of it have antimicrobial activity or not. The objective of this study was to investigated antibacterial activity of *C. buchanani* leaves aqueous extract against food-borne pathogen, nosocomial infection and normal flora bacteria.

MATERIALS AND METHODS

Tested Bacteria

In this study gram positive (*Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* ATCC 12228, *Micrococcus luteus* ATCC 9341, *Bacillus subtilis* ATCC 6633, *Lactobacillus plantarum* ATCC 14917) and gram negative (*Escherichia coli* ATCC25922, *Salmonella typhimurium* ATCC 14028 *Klebsiella pneumoniae* ATCC 10031, *Proteus vulgaris* ATCC 13315, *Pseudomonas aeruginosa* ATCC 9721) bacteria were used. Among bacteria, there are food-borne pathogenic bacteria (*S. aureus* ATCC 25923, *E. coli* ATCC 25922 and *S. typhimurium* ATCC 14028), nosocomial infection bacteria (*K. pneumoniae* ATCC 10031, *P. vulgaris* ATCC 13315 and *Ps. aeruginosa* ATCC 9721) and normal flora bacteria (*L. plantarum* ATCC 14917 and *S. epidermidis* ATCC 12228) were included in the susceptibility test.

Plant Sample and Extraction

C. buchanani was collected in on May 2008 from Pattani Province, Thailand. The plant was identified by Department of Biology, Faculty of Science, Mahasarakham University, Thailand.

The leaves of plant were air dried and ground into powder. Ten gram of plant's leaves dried powder was boiled in 500 mL water and the residue of extraction where repeated the boiling process for 3 times. The water was filtrated, pooled and spray-dried. The yield of extraction was 1.2-2.5% of dried weight of dried grind plant powder.

Anti-Microbial Susceptibility Test

The anti-microbial susceptibility test of *C. buchanani* was screened using agar diffusion susceptibility test as described in the standard guideline (Lorian, 1996). The plant extract spray-dried powder was dissolved in sterile water at concentration of 125, 250 and 500 g L⁻¹.

All test bacteria were cultured overnight on a Tryptic Soy Agar (TSA) slant at 37°C. Bacteria were washed from the surface of the agar slant with sterile Normal Saline Solution (NSS) (0.9% NaCl), which was then adjusted to match the turbidity of a standard Mcfarland No. 0.5 (10⁸ colony-forming units (cfu) mL⁻¹) before used as the starter solution. Twenty milliliter of Mueller Hinton Agar (MHA) was put in cultivation plates and swabbed with starter solution on the agar surface, by using swab cotton.

The plant solution were filled in sterile stainless steel cylinders (6 mm internal diameter and 10 mm height) that were placed on the inoculated agar surface. After pre-diffusion at room temperature for 1 h, the plates were incubated at 37°C for 19 h. The NSS filled in the cylinder was used as control and a 10 mg L⁻¹ gentamicin sulphate (Sigma Chemical Co., St. Louis, USA) solution was used as standard in same cultivation plate.

MICs and MBCs Determination Using Agar Dilution and Broth Macro Dilution Methods

MICs of *C. buchanani* aqueous extract was determined by the agar dilution method (Merck) while MBCs were determined by the broth macro-dilution method (Lorian, 1996) and

using gentamicin sulphate as reference antibiotics (Sigma Chemical Co., St. Louis, USA). Briefly, inoculates were prepared in the same medium at density adjusted to 0.5 McFarland turbidity standard (10^8 colony-forming units (cfu) mL^{-1}) and two fold dilution for the broth macro-dilution procedure. The inoculated tubes were incubated at 37°C and the MICs were recorded after 24 h of incubation. The MIC was defined as the lowest concentration of plant extract or gentamicin sulphate at which the microorganism tested did not showed visible growth, while MBC was defined as the minimum bactericidal concentration with negative subcultures on the agar medium.

RESULTS

In this study, the plant's leaves were extracted using aqueous system with 1.2-2.5% yield of dried grind plant's leaves powder. *Cryptolepis buchanani* aqueous extract was tested against 10 bacterial strains. There are selected food-borne pathogen bacteria (*S. aureus* ATCC 25923, *E. coli* ATCC 25922 and *S. typhimurium* ATCC 14028), nosocomial infection bacteria (*K. pneumoniae* ATCC 10031, *P. vulgaris* ATCC 13315 and *Ps. aeruginosa* ATCC 9721) and normal flora bacteria (*L. plantarum* ATCC 14917 and *S. epidermidis* ATCC 12228) were include in the susceptibility test.

The agar diffusion susceptibility test showed the inhibition zone of *C. buchanani* aqueous extract against 8 out of 10 tested strains. There are *S. aureus* ATCC 25923, *E. coli* ATCC 25922, *S. typhimurium* ATCC 14028, *K. pneumoniae* ATCC 10031, *P. vulgaris* ATCC 13315, *B. subtilis* ATCC 6633, *L. plantarum* ATCC 14917 and *S. epidermidis* ATCC 12228 were inhibited by *C. buchanani* aqueous extract (Table 1). The MICs and MBCs of this plant against all tested bacteria are in the range of 1-16 and 2-32 g L^{-1} , respectively (Table 2).

It was found that *C. buchanani* showed broad-spectrum antibacterial activity against 8 out of 10 tested strains. It showed very good inhibitory effect on food-borne pathogen bacteria (*S. aureus*, *E. coli* and *S. typhimurium*) and nosocomial infection bacteria (*K. pneumoniae*, *P. vulgaris* and *E. coli*) at low concentration. However, it also showed inhibitory effect on normal flora bacteria such as *L. plantarum* and *S. epidermidis*.

Table 1: Inhibition zone diameters of *C. buchanani* aqueous extract against various bacteria

Bacterias	Gram	Inhibition zone diameter of <i>C. buchanani</i> aqueous extract (mm)			Gentamicin sulphate (10 mg L^{-1})
		500	250	125	
<i>S. aureus</i> ATCC 25923	+	12.6±0.3	nz	nz	19.6±2.65
<i>S. epidermidis</i> ATCC 12228	+	14.8±0.7	10.9±0.8	nz	19.7±0.93
<i>M. luteus</i> ATCC 9341	+	nz	nz	nz	176.0±1.93
<i>B. subtilis</i> ATCC 6633	+	16.5±1.7	12.2±0.4	11.1±1.2	17.3±0.92
<i>L. plantarum</i> ATCC 14917	+	23.0±1.3	17.0±1.2	nz	21.3±1.23
<i>E. coli</i> ATCC 25922	-	21.0±0.6	15.1±0.5	12.4±0.9	21.6±0.57
<i>K. pneumoniae</i> ATCC 10031	-	19.6±0.7	16.7±0.5	14.4±1.2	19.3±0.84
<i>S. typhimurium</i> ATCC 14028	-	12.4±0.6	nz	nz	19.0±1.20
<i>Ps. aeruginosa</i> ATCC 9721	-	nz	nz	nz	20.2±1.26
<i>P. vulgaris</i> ATCC13315	-	14.2±0.3	12.8±1.2	nz	20.0±0.46

Data are expressed as Mean±SD (n = 3); nz: No inhibition zone

Table 2: The MICs and MBCs of *C. buchanani* aqueous extract against various bacteria

Bacterias	<i>C. buchanani</i> aqueous extract (g L^{-1})		Gentamicin sulphate (mg L^{-1})	
	MIC	MBC	MIC	MBC
<i>S. aureus</i> ATCC 25923	4	8	<0.5	nd
<i>S. epidermidis</i> ATCC 12228	2	4	<0.5	nd
<i>B. subtilis</i> ATCC 6633	4	16	<0.5	nd
<i>L. plantarum</i> ATCC 14917	1	2	<0.5	nd
<i>E. coli</i> ATCC 25922	2	4	<0.5	nd
<i>K. pneumoniae</i> ATCC 10031	4	16	<0.5	nd
<i>S. typhimurium</i> ATCC 14028	16	32	<0.5	nd
<i>P. vulgaris</i> ATCC 13315	4	16	<0.5	nd

nd: Not determine

In conclusion, the aqueous extract of *C. buchanani* leaves showed anti-microbial against food-borne pathogenic bacteria and nosocomial infection bacteria at low concentration. This suggests the possibility to use *C. buchanani* aqueous extract as the food-borne pathogen bacteria growth control additive in food.

DISCUSSION

The food-borne pathogen bacteria contaminations are the big problem in livestock production (pigs, chickens, cattle and aquatic animals) countries. There is a report about the food-borne pathogen bacteria contaminated in Thailand (Padungtod *et al.*, 2008) and Vietnam especially in Vietnam also found the antibiotic resistant strains (Van *et al.*, 2007). Therefore, the growth of food-borne pathogen bacteria should be control. However, using of antibiotic may cause adverse effect and in most countries the amount of antibiotic applies in food is under control.

It is well known that some plant have anti-microbial activity and has been apply as the remedy for local people even before the chemical medicine was existed. As the fact that in topical area have a variety of plants species, development the plant extract as the anti-food-borne pathogen bacteria is one useful possibility.

Cryptolepis buchanani was used in ayurvedic Indian folkloric medicine for the treatment of diarrhea, ulcer, inflammation, cough, infection and others (Kaul *et al.*, 2003). It has been reported anti-inflammatory activity (Laupattarakasem *et al.*, 2006) and immunopotentiating properties (Kaul *et al.*, 2003). It was also reported chemical component such as nicotinoyl glucoside (Dutta *et al.*, 1980), cryptosin (Venkateswara *et al.*, 1987, 1989) and buchananine (pyridine alkaloid) (Dutta *et al.*, 1978). This study was aimed to evaluate the efficacy of *C. buchanani* aqueous extract anti-bacterial activity.

C. buchanani has been used in Indian folkloric antimicrobial remedy (Kaul *et al.*, 2003) but its antimicrobial activity never been reported. This study was evaluated the possibility to used this plant as antifeed-borne pathogen bacteria. The plant's leaves aqueous extract was tested against 10 selected bacteria with 3 strains of food-borne pathogen bacteria (*E. coli* ATCC25922, *S. typhimurium* ATCC 14028 and *Ps. aeruginosa* ATCC 9721), 4 strains of nosocomial infection bacteria (*S. aureus* ATCC 25923, *M. luteus* ATCC 9341, *K. pneumoniae* ATCC 10031 and *P. vulgaris* ATCC 13315), 2 strains of normal flora bacteria (*S. epidermidis* ATCC 12228 and *L. plantarum* ATCC 14917) and *B. subtilis* ATCC 6633. The results showed inhibitory effect of *C. buchanani* leaves aqueous extract against *S. aureus* ATCC 25923, *E. coli* ATCC 25922, *S. typhimurium* ATCC 14028, *K. pneumoniae* ATCC 10031, *P. vulgaris* ATCC 13315, *B. subtilis* ATCC 6633, *L. plantarum* ATCC 14917 and *S. epidermidis* ATCC 12228 with low concentrations. The evidence supports the used of *C. buchanani* aqueous extract as antifeed-borne pathogen bacteria. It also showed the good antimicrobial activity against nosocomial infection bacteria (*K. pneumoniae* ATCC 10031 and *P. vulgaris* ATCC 13315) even though the plant aqueous extract cannot inhibit growth of *Ps. aeruginosa* ATCC 9721.

In conclusion, *C. buchanani* aqueous extract showed broad spectrum inhibitory effect on growth of all food-borne pathogen bacteria and some of nosocomial bacteria. This may support use of *C. buchanani* as antimicrobial remedy. However, it's active compound should also been studied to evaluated the mechanism of action and those toxicity of the plant as well as to supported the safety of used this plant extract as the food additive.

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