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Antimicrobial Properties of *Cinnamomum verum* Aqueous Extract

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Abstract: The aim of this study is to determine the antimicrobial activity of *Cinnamomum verum* stem bark aqueous extract against food-borne pathogen bacteria, nosocomial infection bacteria and normal flora. Extraction with an aqueous system from the dried stem barks of *C. verum* yielded 2.5% of the dried plant. Among 10 test strains of bacteria, *C. verum* showed inhibitory effect on the growth of *Krebsilla pneumoniae* ATCC 10031, *Straphylococcus epidermidis* ATCC 12228 and *E.coli* ATCC 25922 in an agar diffusion test. The Minimal Inhibitory Concentrations (MICs) and the Minimal Bactericidal Concentrations (MBCs) were in the range of 4-16 and 16-32 g L^{-1}, respectively. In conclusion, *C. verum* stem bark aqueous extract showed interesting inhibitory effect on the growth of *S. epidermidis*, *K. pneumoniae* and *E. coli* at low minimum concentration. This may give additional information of antimicrobial activity of *C. verum* stem bark aqueous extract.

Key words: *Cinnamomun verum*, food-borne pathogen bacteria, nosocomial infection, anti-microbial, aqueous extract

INTRODUCTION

The food-borne pathogen is the major concern in food production factories especially for ecporting countries such as Thailand and Vietnam. There are reports on the food borne- pathogen bacteria in food products in Thailand (Padungtod *et al*., 2008) andan increase of antibiotic resistance in food-borne pathogen bacteria in Vietnam (Tillotson *et al*., 2008; Gerner-Smidt and Whichard, 2008; Dowzicky and Park, 2008; Van *et al*., 2007). This is the reason why food preservative compound is added to food products.

In nature, plants contain a variety of compounds called phytochemical and some of them have medicinal properties. Long time ago humanity learned to used plants for disease treatment or control. Today, scientific research reveals that not only the chemical from the plant has effect against a particular disease, but, that the antioxidant property of the plant's extract also gives beneficial effect to human health.

The use of food preservatives today is strictly regulated because of toxicity. In addition, chemicals as food preservative (indicated by labeling in food packages) is a major concern for consumer (Mau *et al*., 2001). Therefore, natural antimicrobial compounds from plant have to be explored. Since, cinnamon is a common food ingredient and hence probably non-toxic, it is very interesting to evaluate the biological activity of this plant.

Cinnamomum verum (Syn *Cinnamomum zeylanicum* Blume), or cinnamon belongs to the family Laureceae. Cinnamon is a wooden tree that grows in tropical Asia and Africa. The bark of the tree has a special smell andis usually used as a spice in food and dessert recipes. In western countries, the extract of cinnamon bark is used as an aroma powder. Cinnamon is rich in essential oils and tannins

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which inhibit microbial growth (Mau *et al*., 2001). Cinnamon essential oil has been reported to have antimicrobial activity against Escherichia *coli* o157:H7 (Senhaji *et al*., 2007), *Listeria monocytogenes*, *Salmonella choleraesuis*, *Aspegillus flavus*, *Candida albican* (Lopez *et al*., 2007). In addition, the mixture of clove and cinnamon essential oil showed antimicrobial activity against food born pathogen bacteria (*Aspergillus flavus*, *Pennicillium roqueforti*, *Mucor plumbeus*, *Eurotium* sp., *Debaryomyces hansenii*, *Pichia membranaefaciens*, *Zygosaccharomyces rouxii* and *Candida lipolytica*) in modidified condition (Matan *et al*., 2006). The essential oil part of cinnamon showed significant inhibitory effect on the growth of food-borne pathogen bacteria. However, the antibacterial activity of the aqueous extract of this plant has never been reported. The objective of the present study is to investigate the antimicrobial activity of *C. verum* aqueous extract against various bacteria.

MATERIALS AND METHODS

Tested Bacteria

The micro-organisms used in this study consist of five gram positive (*Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* ATCC 12228, *Micrococcus luteus* ATCC 9341, *Bacillus subtillis* ATCC 6633, *Lactobacillus plantarum* ATCC 14917) and five gram negative (*Escherichia coli* ATCC25922, *Salmonella typhimurium* ATCC 14028 *Klebsiella pneumonia*e ATCC 10031 *Proteus vulgaris* ATCC 13315, *Pseudomonas aeruginosa* ATCC 9721). All are reference strains obtained from the American Type Culture Collection.

Among the bacteria, there are 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) that were included in the susceptibility test.

Plant Material

The dried stem barks of *Cinnamomum verum* were bought in April 2008 from a local Chinese medicine shop in the Mahasarakham province, Thailand. Identification was confirmed by the Department of Biology, Faculty of Science, Mahasarakham University, Mahasarakham, Thailand.

Extraction

Ten grams of dried stem bark of *C. verum* were boiled in 1 L of water. For each dried stembark the extraction was repeated 3 times. The filtrate of the extraction was spray dried into powder form. The yield of extraction was 2.5% of the dried plant.

Antimicrobial Assay

Agar Diffusion Susceptibility Test

The antimicrobial activity of *C. verum* was evaluated using the agar diffusion method as described in the standard guideline (Lorian, 1996). The spray dried powder of plant extract was prepared into solutions by dissolving in sterile water at concentrations of 125, 250 and 500 mg mL^{-1}. Three hundred microliter of plant solution were injected into sterile stainless steel cylinders (6 mm internal diameter and 10 mm height) that were placed on the inoculated Mueller Hinton agar (MHA) surface. After pre-diffusion at room temperature for 1 h, the plates were incubated at 37°C for 19 h. The Normal Saline Solution (0.9% NaCl) (NSS) injected into the cylinder was used as control and a 10 μg mL^{-1} gentamicin sulphate (Sigma Chemical Co., St.Louis, USA) solution was used as standard in the same cultivation plate. The inhibition zones were measured and data are mean of three measurements.

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

MICs for *C. verum* aqueous extract was determined by the agar dilution method (Lorian, 1996) while MBCs were determined by the broth macro-dilution method (Lorian, 1996) andusing 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 AND DISCUSSION

The result in Table 1 shows that the plant extract inhibited the growth of *Krebsilla pneumoniae* ATCC 10031, *Straphylococcus epidermidis* ATCC 12228 and *E. coli* ATCC 25922. The Minimal Inhibitory Concentrations (MICs) were in the range of 4-16 mg mL^{-1} while Minimal Bactericidal Concentrations (MBCs) were in the range of 16-32 mg mL^{-1}, respectively (Table 2).

Food-borne pathogen bacteria cause problems for food industry and consumers. Food-borne disease can be the cause of serious health problems and even death in humans (Busani, 2006). The food borne pathogen bacteria was found to contaminate live stock production countries such as Thailand (Padungtod *et al*., 2008) and Vietnam (Van *et al*., 2007). Bacteria that often contaminate food are *S. aureus*, *K. pneumoniae*, *S. typhymurium*, *Campylobacter* sp. and *Listeria* sp. (Padungtod *et al*., 2008).

In earlier study, the antimicrobial activity of *C. verum* essential oil against food-borne pathogen bacteria was reported and was found that the essential oil of *C. verum* showed inhibitory effect against *Staphylococcus aureus*, *Enterococcus faecalis*, *Listeria monocytogenes*, *Bacillus cereus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Yersinia enterocolitica*, *Salmonella choleraesuis*, *Aspergillus flavus*,

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

		Inhibition zone diameter of *C. verum* aqueous extract (mm)			
		500	250	125	Gentamicin sulphate
Bacteria	Gram	(mg mL^{-1})			(10 µg mL^{-1})
S. aureus ATCC 25923	+	nz	nz	nz	20.3±1.23
S. epidermidis ATCC 12228	+	12.4±0.9	nz	nz	21.1±0.67
M. luteus ATCC 9341	+	nz	nz	nz	18.2±0.52
B. subtilis ATCC 6633	+	nz	nz	nz	17.3±0.92
L. plantarum ATCC 14917	+	nz	nz	nz	20.2±0.73
E. coli ATCC 25922	-	14.3±0.6	nz	nz	22.1±0.44
K. pneumoniae ATCC 10031	-	14.6±0.7	21.1+0.5	nz	19.8±0.84
S. typhimurium ATCC 14028	-	nz	nz	nz	19.8±0.78
Ps. aeruginosa ATCC 9721	-	nz	nz	nz	21.2±0.78
P. vulgaris ATCC13315	-	nz	nz	nz	20.0±0.46

Data are mean±SD (n = 3); nz: No inhibition zone

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

	C. verum aqueous extract		Gentamicin sulphate	
	MIC	MBC	MIC	MBC
Bacteria	(mg mL^{-1})		(µg mL^{-1})	
S. epidermidis ATCC 12228	16	32	<0.5	nd
E. coli ATCC 25922	4	16	<0.5	nd
K. pneumoniae ATCC 10031	4	16	<0.5	nd

nd: Not determined

Penicillium islandicum and *Candida albicans* (Lopez *et al*., 2007; Senhaji *et al*., 2007; Becerril *et al*., 2007; Matan *et al*., 2006; Mau *et al*., 2001; Singh *et al*., 2007). Additionally, *in vivo* antitumorigenic activity of *C. verum* has been reported (Amara *et al*., 2008). The major chemical constituents in *C. verum* reported are thymol, carvacol, carvone, cinnamaldehyde, eugenol and linalool as components in its essential oil (Friedman *et al*., 2000). However, the antimicrobial activity of *C. verum* aqueous extract has never been reported. The results of the present study indicate that *C. verum* showed inhibitory effect against *Krebsilla pneumoniae* ATCC 10031, *Straphylococcus epidermidis* ATCC 12228 and *E. coli* ATCC 25922. This finding also indicates that the *C. verum* aqueous extract showed better inhibitory effect on the growth of gram negative bacteria than gram positive bacteria. Due to the difference of cell wall composition between gram positive and gram negative bacteria, this may indicate that the *C. verum* aqueous antimicrobial activity should involve the cell wall of the bacterium because it showed inhibitory effect in gram negative bacteria more than gram positive bacteria. The result gives additional data for the antimicrobial activity of *C.verum* aqueous extract. As mention before, this is the first reported of antimicrobial activity of *C. verum* aqueous extract. In conclusion, *C. verum* aqueous extract showed antimicrobial activity against *Krebsilla pneumoniae* ATCC 10031, *Straphylococcus epidermidis* ATCC 12228 and *E. coli* ATCC 25922 at low concentration. This might give additional data to support the use of *C. verum* against food-borne pathogen bacteria.

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