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Anti-Microbial Properties of Clove (*Eugenia caryophyllum* Bullock and Harrison) Aqueous Extract Against Food-Borne Pathogen Bacteria

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Abstract: Anti-microbial activity of *Eugenia caryophyllum* Bullock and Harrison aqueous extract has been tested against food-borne pathogen bacteria (*S. aureus* ATCC 25923, *S. typhimurium* ATCC 14028 and *E. coli* ATCC 25922), normal flora (*S. epidermidis* ATCC 12228 and *L. plantarum* ATCC 14917) and other pathogen bacteria (*P. vulgaris* ATCC 13315). The agar diffusion susceptibility test revealed inhibition zone of *Eugenia caryophyllum* Bullock and Harrison aqueous extract against *S. aureus* ATCC 25923, *S. typhimurium* ATCC 14028, *E. coli* ATCC 25922, *S. epidermidis* ATCC 12228, *L. plantarum* ATCC 14917 and *P. vulgaris* ATCC 13315. The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) were determined by using agar dilution and broth macro-dilution methods. The MIC and MBC of clove against all tested bacteria were in the range of 1 to 4 g L⁻¹ and 2 to 8 g L⁻¹, respectively. In conclusion, the aqueous extract of *E. caryophyllum* showed good inhibitory effect on tested food-borne pathogen bacteria.

Key words: *E. caryophyllum*, food-borne, food-borne pathogen bacteria, anti-microbial, aqueous extract

INTRODUCTION

Food-borne illness is caused by eating food or drinking beverage contaminated with bacteria, parasites or viruses. Food-borne illness can cause symptoms that range from an upset stomach to more serious symptoms such as diarrhea, fever, vomiting, abdominal cramps and dehydration. Bacterial contamination is the most common cause of food-borne illnesses. The most common bacteria causing food-borne illness are *Escherichia coli*, *Staphylococcus aureus*, *Salmonella* sp., *Salmonella typhimurium*, *Listeria monocytogenes*, *Clostridium botulinum*, *Vibrio vulnificus*, *Vibrio parahaemolyticus* and others (Busani *et al.*, 2006; Van *et al.*, 2007; Hoque *et al.*, 2007; Gerner-Smidt and Whichard, 2008).

The pathogen bacterial contamination in food has recently led to the development of plant extracts as anti-microbial additives to control the growth of food borne pathogen bacteria. Spices are a common food ingredient in Indian, Thai, Chinese and other oriental foods (Arora and Kaur, 1999). Therefore, the investigation of the antimicrobial properties of spices used as food additives to control the growth of food-borne pathogen bacteria may give useful results.

Eugenia caryophyllum Bullock and Harrison or clove belongs to the plant family Myrtaceae. The use of clove as a food ingredient spice is common in oriental foods (Arora and Kaur, 1999). It has also been used in folklore treatment of toothaches, insect bites, gastroenteritis and intestinal parasites. It has been used as a sedative and as a cement material in dentistry (Markowitz *et al.*, 1992). Furthermore, antioxidant (Decker, 1997; Fugisawa *et al.*, 2002; Atsumi *et al.*, 2005) and anti-herpes simplex virus (Tragoopua and Jatisatienr, 2007) activities of eugenol have also been reported.

The essential oil from clove has been reported to have antimicrobial activity against food-borne pathogen bacteria (Yano *et al.*, 2006). The objective of this study was to assess the *in vitro* anti-microbial activity of clove aqueous extract against selected food-borne pathogen bacteria, to evaluate the potential use of clove aqueous extract as an anti-microbial additive to control the growth of food-borne pathogen bacteria.

MATERIALS AND METHODS

Plant sample and extraction: The clove was purchased from the local market on May 2008. The dried clove was ground using a grinder into a fine powder. Ten grams of

dried powder was boiled in 500 mL of water, the procedure was repeated 3 times. The pooled filtrates were spray-dried. The yield of extraction was 1-2% of dried weight of dried plant's powder. Several batches produced with the same extraction condition were used throughout the studies. The extraction and antimicrobial activity tested has been conducted on May, 2008-December, 2008 at Department of Chemistry, Faculty of Science, Mahasarakham University, Thailand.

Tested microorganisms: Laboratory isolates of the pure culture of gram positive (*Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* ATCC 12228, *Lactobacillus plantarum* ATCC 14917 and gram negative (*Escherichia coli* ATCC25922, *Salmonella typhimurium* ATCC 14028 *Proteus vulgaris* ATCC 13315) bacteria were obtained from the Department of Microbiology, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Thailand.

Antimicrobial susceptibility test:

Agar diffusion susceptibility test: Agar diffusion susceptibility determinations were made as described in the standard guideline technique (Lorian, 1996). 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 (10^8 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.

Spray dried powder of clove aqueous extract was dissolved in sterile water and put in sterile stainless steel cylinders (6 mm internal diameter and 10 mm height) that were placed on the inoculated agar surface. Different concentrations of plant extract solution were filled in the cylinders (300 µL cylinder⁻¹). 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:

The MICs of clove aqueous extract was determined by the agar dilution method (Merck) (Lorian, 1996) 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). 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. Values were means of three measurements.

RESULTS

In this study, the antimicrobial activity of clove aqueous extract was determined against standard strains of food-borne pathogen bacteria *S. aureus* ATCC 25923, *S. typhimurium* ATCC 14028 and *E. coli* ATCC 25922 and normal flora *S. epidermidis* ATCC 12228 and *L. plantarum* ATCC 14917. Furthermore, bacteria also found in fecally contaminated food *P. vulgaris* ATCC 13315 were also included in this study.

Antimicrobial activity of clove aqueous extract was screened using the agar diffusion susceptibility test. The aqueous extract of clove showed an inhibition zone against *S. aureus* ATCC 25923, *S. typhimurium* ATCC 14028, *E. coli* ATCC 25922, *S. epidermidis* ATCC 12228, *L. plantarum* ATCC 14917 and *P. vulgaris* ATCC 13315 (Table 1). The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

Table 1: Inhibition zone diameters of clove aqueous extract against some food-borne pathogen and normal flora bacteria

Bacteria	Gram	Inhibition zone diameter (mm)			
		Clove aqueous extract (500 g L ⁻¹)	Clove aqueous extract (250 g L ⁻¹)	Clove aqueous extract (125 g L ⁻¹)	Gentamicin sulphate (10 mg L ⁻¹) (mean±SD)
<i>S. aureus</i> ATCC 25923	+	23.7	20.0	12.5	21.6±0.9
<i>S. epidermidis</i> ATCC 12228	+	16.2	13.2	nz	20.9±0.9
<i>L. plantarum</i> ATCC 14917	+	19.7	13.4	nz	19.3±0.9
<i>E. coli</i> ATCC 25922	-	23.5	14.6	nz	21.2±1.0
<i>S. typhimurium</i> ATCC 14028	-	17.3	11.9	nz	17.9±1.8
<i>P. vulgaris</i> ATCC 13315	-	17.4	13.1	nz	19.9±1.3

nz = No inhibition zone

Table 2: The MICs and MBCs of clove aqueous extract against some food-borne pathogen and normal flora bacteria

Bacteria	Clove aqueous extract		Gentamicin sulphate	
	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>L. plantarum</i> ATCC 14917	1	2	<0.5	nd
<i>E. coli</i> ATCC 25922	4	8	<0.5	nd
<i>S. typhimurium</i> ATCC 14028	2	4	<0.5	nd
<i>P. vulgaris</i> ATCC 13315	2	4	<0.5	nd

nd = Not determine

were determined by using agar dilution and broth macro-dilution methods. The MIC and MBC of clove against all tested bacteria were in the range of 1 to 4 g L⁻¹ and 2 to 8 g L⁻¹, respectively (Table 2).

In conclusion, aqueous extract from clove exhibited antibacterial activity against food-borne pathogen bacteria *in vitro* (*S. aureus* ATCC 25923, *S. typhimurium* ATCC 14028 and *E. coli* ATCC 25922). Therefore, aqueous extract from clove can be used to control food-borne pathogen bacteria in food. It also showed inhibitory effect on in fecally contaminated bacteria *P. vulgaris* ATCC 13315. However, it also inhibited the normal flora strain bacteria such as *S. epidermidis* ATCC 12228 and *L. plantarum* ATCC 14917. This may support the use of aqueous extract from clove as an antibacterial additive against food-borne pathogen bacteria.

DISCUSSION

Food-borne infections are an important public health concern worldwide (Busani *et al.*, 2006). The food-borne pathogen bacteria are the big problem in livestock production countries. In Thailand, there has been reported the contamination of livestock production with food-borne pathogen bacteria such as *Salmonella* sp. and *Campylobacter* sp. (Padungtod *et al.*, 2008). Furthermore, there are reports of antibiotic resistance in food-borne pathogen bacteria contaminants in Vietnam (Van *et al.*, 2007).

Essential oil from clove has been studied for antimicrobial activity against food-borne pathogen bacteria such as *Vibrio parahaemolyticus* (Yano *et al.*, 2006), *Bacillus cereus*, *Campylobacter jejuni*, *Salmonella enteritidis*, *Escherichia coli*, *Staphylococcus aureus* and *Listeria monocytogenes* with the range of MICs 0.5-8 g L⁻¹ while in this study the MICs of clove aqueous extract was in the range of 1-4 g L⁻¹ which is in good range compared with essential oil. However, there are some limitations in using essential oil from spice in food (1) essential oil usually has a strong smell and taste, (2) it

may cause some unsatisfactory taste and (3) the essential oil may react with oxygen and generate undesirable smell or taste. Thus, the aqueous extract has less toxicity compared with solvent extraction and usually gives less smell compared with essential oil.

This study was to evaluate the antimicrobial activity of clove aqueous extract against food-borne pathogen bacteria and other pathogen bacteria. The results showed good inhibitory effect of clove aqueous extract against food-borne pathogen bacteria (*S. aureus* ATCC 25923, *S. typhimurium* ATCC 14028 and *E. coli* ATCC 25922). The results from this study may give additional data for antimicrobial activity of *E. caryophyllum* aqueous extract. This may supported the used of clove as food additive to control growth of food-borne pathogen bacteria in food products.

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