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Antibacterial Activity of Selected Thai Indigenous Plants Against Food-Borne Pathogenic Bacteria

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Abstract. Consumer demand for less use of synthetic preservatives has led to research and use of "naturally derived" antimicrobials. Certain plants and their extracts are known to possess antimicrobial activity offering a potential alternative to synthetic preservatives. In this study, antibacterial activity of the edible portion of five selected Thai indigenous plants, namely, vegetable fern tree, *Mun-Poo* tree, bago leaf, djenkol seed and stink beam seed, against food-borne pathogenic bacteria (*Bacillus cereus*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella* Typhimurium and *Vibrio cholera*) was determined by agar-well diffusion method. It was found that only *Mun-Poo* leaf extract could exhibit the antibacterial activity against 4 food-borne pathogenic bacteria such as *B. cereus*, *L. monocytogenes*, *S. aureus* and *V. cholera* with the MIC value of 40, 80, 100 and 100 mg/ml and the MBC value of 80, 160, 200 and 200 mg/ml, respectively. In addition, *Mun-Poo* leaf extract contained the highest amount of total phenolic content (55.33 $\pm$ 0.20 mg GAE/g DW) and tannin content (9.77 ± 0.01 mg TAE/g DW) among the other Thai indigenous plant extracts. The antibacterial activity of *Mun-Poo* leaf extract could relate to its high content of total phenolic and tannin contents. The results obtained in this study pointed out that *Mun-Poo* leaf extract has a potential for use as a natural antibacterial agent to ensure consumers safe food products.

Keywords: Thai indigenous plant, Antibacterial activity, Total phenolic content, Tannin content

1. Introduction

Nowadays, consumer demand for less use of synthetic preservatives has led to research and use of "naturally derived" antimicrobials. Certain plants and their extracts are known to possess antimicrobial activity offering a potential alternative to synthetic preservatives [1]. Many naturally occurring compounds found in plants have been shown to possess antimicrobial functions and serve as a source of antimicrobial agents against food-borne pathogens [2]. Their application on controlling pathogens could reduce the risk of food-borne outbreaks and assure consumers safe food products [3].

Many indigenous plants are rich in phenolic compounds and exerting the antimicrobial effect. Besides that, they may preserve the foods by reducing lipid oxidation as they are reported to have significant antioxidant activity [4]. A wide variety of phenolic substances derived from indigenous plants possess potent biological activities, which contribute to their preservative potential [5].

The aim of this study was to determine the efficacy of some Thai indigenous plant extracts as antibacterials against six common food-borne pathogenic bacteria and reveals the relationship between bacterial inhibition and total phenolic and tannin contents.

2. Materials and Methods

2.1. Plant Materials and Preparation of Extracts

Five selected Thai indigenous plants used in this study (Table 1) were collected during October 2011 at Songkhla, Thailand. Edible portion of the collected samples was ground in a blender and dried at 45°C until

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moisture content less than 10%. Ground samples were extracted with 75% ethanol with 1:10 sample-solvent ratio by shaking at 200 rpm in the dark at 25°C for 2 h [6]. Then the extracts were filtered through Whatman No.1 filter paper, kept in air-tight amber bottles and stored in freezer at -20°C until analyzed.

Table 1 Inventory of Thai indigenous plants used in this study.

Botanical names	Common names	Plant part
Diplazium esculentum (Retz.) *Sw.*	Vegetable fern	Leaf and Soft peak
Glochidion Perakense Hook.F.	*Mun-Poo* tree	Leaf
Gnetum gnemon Limm. var.	Bago tree	Leaf
Archidendron jiringa I. C. Nielsen.	Djenkol tree	Seed
Parkia speciosa Hassk	Stink beam tree	Seed

2.2. Test Bacteria and Their Culture

Six common food-borne pathogens were selected which included Gram-positive species and Gram-negative species as showed in Table 2. They were provided by Department of Medical Sciences, Thailand (DMST) and American Type Culture Collection (ATCC). Those bacteria were kept frozen at -20°C in nutrient broth (NB) supplement with 20% glycerol as stock cultures. Working cultures were sub-cultured twice in NB at 37°C for 18 h before use.

Table 2 Inventory of Food-borne pathogenic bacteria used in this study.

Food-borne pathogenic bacteria	Code
Gram-positive bacteria	
Bacillus cereus	DMST 5040
Staphylococcus aureus	DMST 8840
Listeria monocytogenes	ATCC 19115
Gram-negative bacteria	
Escherichia coli	DMST 4212
Salmonella Typhimurium	DMST 562
Vibrio cholera non O1/non O139	DMST 2873

DMST: Department of Medical Sciences, Thailand; ATCC: American Type Culture Collection

2.3. Determination of Antibacterial Activity

The agar-well diffusion method was used to determine the radius of inhibition zone of the test bacteria as described by Mathabe *et al*. [7]. In brief, one milliliter of freshly prepared working cultures (10^7 cfu ml^{-1}) was aseptically added to a 9 ml sterilized Muller-Hinton soft medium (0.75% agar), immediately mixed, poured into Petri dish and left to soliolify at room temperature. After left to dry for 15 min in laminar air flow, 9 wells (6 mm in diameter) were made in media. Each well was filled with 20 µl of extracts. Sterile distilled water and tetracycline were used as a negative and positive control, respectively.

Plates were left at room temperature for 30 min to allow diffusion of material in media and were incubated at 37°C. After 24 h of incubation, the radius of inhibition zones in mm around wells was measured. The experiment was repeated three times.

2.4. Determination of Minimum Inhibition Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

MIC and MBC of the extracts were determined by broth dilution method as described by Jorgensen and Ferraro [8]. The minimum inhibitory concentration at which no bacteria grew in culture media was defined as MIC. After the MICs were determined, the samples showing no increases in turbidity were spread on nutrient agar plates to check bacterial survival. The MBC was determined as the lowest concentration at which the test samples killed the bacteria.

2.5. Total Phenolic Contents

The total phenolic content of ethanolic extract was determined by using Folin–Ciocalteu's assay as described by Tan and Kissam [6] with some modifications. An amount of 1.0 mL of Folin reagent (10% v/v) was added to a test tube containing 100 μL aliquot of appropriately diluted extracts or standard solutions of gallic acid. After 5 min, 800 μL of 1 M Na_2CO_3 was added and the solution was adjusted to 2.0 mL and mixed thoroughly. After incubation for 90 min in the dark at room temperature, the absorbance was measured at 750 nm. Blank was run in the same manner except the deionized water was used instead of the extract. The total phenolic content was expressed as gallic acid equivalents (mg of GAE/g dried sample) through the calibration curve of gallic acid. All samples were analyzed in three replications.

2.6. Tannin Contents

Tannin content was determined using Butanol/HCl method of Porter *et al*. [9]. Briefly, ten milligrams of sample was diluted with 0.5 mL of 70% acetone in screw cap tube. Then, 3 mL of acid butanol reagent (5% HCl in butanol) and 0.1 mL of the ferric reagent was added to the tubes. The tubes were capped and mixed thoroughly before placing in water bath at 95^{o}C for 60 min. After cooling the tubes, absorbance was recorded at 550 nm. The blank was run in the same manner except the deionized water was used instead of sample. The tannin content was expressed as tannic acid equivalents (mg of TAE/g dried sample) through the calibration curve of tannic acid. All samples were analyzed in three replications.

2.7. Statistical Analysis

Results were expressed as mean followed by standard deviation. Statistical significance was determined by one-way analysis of variance Fisher's test. Data indexed by the same letter are statistically not significantly different from each other ($p < 0.05$)

3. Results and Discussion

3.1. Antibacterial Activity

Six food-borne pathogenic bacteria were tested for their sensitivity to ethanolic extract from selected Thai indigenous plants. Table 3 presents radius of inhibition zones (clear zones around wells) exerted by the various extracts towards challenged bacteria. It was found that only *Mun-Poo* leaf extract could exhibited the antibacterial activity in which *B. cereus*, *L. monocytogenes*, *S. aureus* and *V. cholera* were susceptible to *Mun-Poo* leaf extracts (Table 3) while *E. coli* and *Sal.* Typhimurium were resisted to the extracts. Based on the agar-well diffusion studies, *Mun-Poo* leaf extract was selected for determination of MIC and MBC.

Table 3 Antibacterial activities of 75% ethanolic extract of selected Thai indigenous plants using agar well diffusion assay.

Sample*	Radius of inhibition zone (mm)					
	Gram positive bacteria			Gram negative bacteria		
	BC	LM	SA	EC	ST	VC
Vegetable fern leaf	-	-	-	-	-	-
Mun-Poo leaf	3.43	2.60	2.53	-	-	2.46
Bago leaf	-	-	-	-	-	-
Djenkol seed	-	-	-	-	-	-
Stink beam seed	-	-	-	-	-	-
Sterile distilled water	-	-	-	-	-	-
Tetracycline (10 ug)	8.20	7.80	9.60	7.90	8.50	9.30

*Twenty microliters of filter sterile samples and distilled water were applied into each well.

Values are given as mean from triplicate determination. -: No inhibition; BC: *Bacillus cereus*; LM: *Listeria monocytogenes*; SA: Staphylococcus *aureus*; EC: *Escherichia coli*; ST: *Salmonella* Typhimurium and VC: *Vibrio cholera*e.

Table 4 shows the MICs and MBCs of *Mun-Poo* leaves extract. The MICs and MBCs of *Mun-Poo* leaves extract affecting all selected test bacteria range from 40 mg DW/ml to 200 mg DW/ml. *B. cereus* is most susceptible to the extract while *S. aureus* and *V. cholera* are more resistible.

Table 4 Minimum inhibitory concentration (MIC) values of *Mun-Poo* leaf extract against pathogenic bacteria using broth dilution method

Pathogenic bacteria	MIC (mg DW/ml)	MBC (mg DW/ml)
Bacillus cereus	40	80
Listeria monocytogenes	80	160
Staphylococcus aureus	100	200
Vibrio cholerae	100	200

Values are given as mean from triplicate determination. DW: Dry weight.

3.2. Total phenolic and Tannin Contents

Total phenolic and tannin contents of selected Thai indigenous plant extracts are summarized in Table 5. According to the table, the highest concentration of total phenolic and tannin content was detected in *Mun-Poo* leaves extract (55.33 ± 0.20 mg GAE/g DW and 9.77 ± 0.01 mg TAE/g DW). There were high correlation between total phenolic and tannin content with the antibacterial activity.

Table 5 Total phenolic and tannin contents of 75% ethanolic extract of selected Thai indigenous plants.

Sample	Total phenolic content (mg GAE/g DW)	Tannin content (mg TAE/g DW)
Vegetable fern leaf	2.23 ± 0.02^{d}	3.20 ± 0.02^{c}
Mun-Poo leaf	55.33 ± 0.20^{a}	9.77 ± 0.01^{a}
Bago leaf	4.60 ± 0.03^{b}	5.73 ± 0.01^{b}
Djenkol seed	2.15 ± 0.02^{d}	0.40 ± 0.01^{c}
Stink beam seed	4.24 ± 0.09^{c}	1.37 ± 0.02^{d}

Values are given as mean ± standard deviation (n=3). Different letters in the same column indicate significant differences ($P < 0.05$). GAE: Gallic acid equivalent; TAE: Tannic acid equivalent and DW: Dry weight.

4. Conclusion

In this study, food-borne pathogens such as *B. cereus*, *S. aureus*, *L. monocytogenes* and *V. cholera* which are cause of serious food-borne diseases showed sensitivity to *Mun-Poo* leaf extract *in vitro*. In addition, it was high content of total phenolic and tannins. Thus they might be the correlation between total phenolic and tannin content with the antibacterial activity of this extract.

Plant extracts have shown a considerable promise in a range of applications in the food industry and several plant extracts enjoy GRAS status. The antimicrobial activities of plant extracts may reside in a variety of different components, and several extracts owing to their phytochemical constituents have been shown to have antimicrobial activity. The antibacterial activity is most likely due to the combined effects of adsorption of polyphenols to bacterial membranes with membrane disruption and subsequent leakage of cellular contents. The use of plant extracts in consumer goods is expected to increase in the future due to the rise of “green consumerism”, which stimulates the use and development of products derived from plants, as both consumers and regulatory agencies are more comfortable with the use of natural antimicrobials.

5. Acknowledgements

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