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Antibacterial Activity of Selected Plant By-products Against Food-borne Pathogenic Bacteria

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Abstract. Food-borne diseases and diseases caused by the emergence of multi-drug resistant pathogens such as *Staphylococcus aureus* are globally recognized as environmental hazards to the food supply and human health. Natural inhibitors for food-borne pathogenic bacteria have been explored in many plants. In this study, the antibacterial activity against some food-borne pathogens by the extracts from selected plant by-products was evaluated using *in vitro* (agar-well diffusion) method. The 50% ethanolic extracts of cashew leave, resak tembaga leave and stink bean pod were potent inhibitor for *Bacillus cereus*, *Listeria monocytogenes*, *S. aureus*, *Escherichia coli*, *Vibrio cholerae* and *Salmonella* Typhimurium. Phytochemical analyses revealed the presence of active inhibitors including phenolics and tannins. The activity of resak tembaga leave extract was related to the highest content of total phenolics (158.14 mg GAE/g DW) while cashew leave extract was related to the highest content of tannins (124.57 mg TAE/g DW). Base on the results of this study, added-value items from plant by-products could provide health benefits to humans and may be employed in food preservation purpose.

Keywords: Plant by-product, Total phenolic content, Tannin, Antibacterial activity

1. Introduction

Food-borne illnesses are still a major concern for consumers, the food industry and food safety authorities. Meanwhile, consumers have been questioning the safety of synthetic preservatives of food. The worldwide spread of antibiotic-resistant pathogens such as methicillin-resistant *Staphylococcus aureus* has revived the search for antibacterial compounds from natural sources, including plants [1]. Among constituents of plants, polyphenols have received a great deal of attention due to their diverse biological functions. In addition, the antibacterial activity of the polyphenols, tannins and flavonoids, is well documented [2, 3]. In the previous study, they reported that plant by-products may be an abundant source of polyphenols and tannins [4]. Thus the use of the waste as a source of antibacterial compound has attracted much interest.

The study presents the antibacterial spectrum of extracts from plant by-products and reveals the relationship between bacterial inhibition and total phenolic and tannin contents of various plant by-product extracts.

2. Materials and Methods

2.1. Plant Materials and Preparation of Extracts

Fifteen selected plant by-products used in this study (Table 1) were collected during January to March in 2011 in Songkhla, Thailand. The collected samples were ground in a blender and dried at 45°C until moisture content less than 10%. Ground samples were extracted with 50% ethanol with 1:10 sample-solvent ratio by shaking at 200 rpm in the dark at 25°C for 2 h [5]. 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.

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Table 1: Inventory of plant by-products used in this study.

Botanical names	Common names	Plant part
Psidium guajava Linn.	Guava	Leaves
Nephelium lappaceum Linn.	Rambutan	Leaves
Hevea brasiliensis (A. Juss.) Muell. Arg.	Para rubber	Leaves
Manihot esculenta Crantz	Cassava	Leaves
Punica granatum Linn.	Pomegranate	Leaves
Anacardium occidentale	Cashew	Leaves
Garcinia mangostana Linn.	Mangosteen	Leaves
Cotylelobium melaoxylon Pierre	Resak tembaga	Leaves
Cotylelobium melaoxylon Pierre	Resak tembaga	Barks
Punica granatum Linn.	Pomegranate	Peels
Parkia speciosa Hassk.	Stink bean	Pods
Nephelium lappaccum Linn.	Rambutan	Peels
Garcinia mangostana Linn.	Mangosteen	Peels
Borassus flabellifer Linn.	Palmyra	Peels
Cocos nucifera Linn.	Coconut	Husks

2.2. Bacteria and Growth Conditions

Food-borne pathogenic bacteria used as indicator bacteria in this study were listed in Table 2. They were obtained from Department of Medical Sciences, Thailand (DMST) and American Type Culture Collection (ATCC). Bacteria indicators were kept frozen at -20°C in nutrient broth (NB) supplement with 20% glycerol. Working cultures were sub-cultured twice in NB at 37°C for 18 h.

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. Antibacterial Activity Assay

Antibacterial activity against bacteria indicators were performed by using agar-well diffusion assay as described by Mathabe *et al*. [6]. In brief, one milliliter of freshly prepared working cultures (10^7 CFU) 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. Total Phenolic Contents

The total phenolic content of ethanolic extract was quantified by using Folin–Ciocalteu's assay as described by Tan and Kissam [5] with some modifications. An amount of 1.0 mL of Folin reagent (10% v/v) was added to a test tube containing 100 µL 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 with deionised water 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 deionised water was used instead of the extract. The total phenolic content was expressed as gallic acid equivalents (mg of GAE/g dried weight) through the calibration curve of gallic acid. All samples were analyzed in three replications.

2.5. Tannin Contents

Tannin content was determined by Butanol/HCl method of Porter *et al*. [7]. 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 deionised water was used instead of sample.

The tannin content was expressed as tannic acid equivalents (mg of TAE/g dried weight) through the calibration curve of tannic acid. All samples were analyzed in three replications.

2.6. Statistical Analysis

Results were expressed as mean followed by standard deviation. Statistical significance was determined by one-way analysis of variance Fisher's test, with the level of significance at $P<0.05$.

3. Results and Discussion

3.1. Antibacterial Activity

Six food-borne pathogenic bacteria were tested for their sensitivity to ethanolic extracts from selected plant by-products. Table 3 presents radius of inhibition zones (clear zones around wells) exerted by the various extracts towards challenged bacteria. *B. cereus*, *S. aureus* and *V. cholera* were susceptible to almost all extracts (Table 3) while *E. coli*, *Sal.* Typhimurium and *L. monocytogenes* were resisted to most of the extracts,

While sterile distilled water (negative control) was inactive against tested indicator bacteria, the marked inhibition was associated with cashew leave, resak tembaga leave and stink bean pod extracts (Table 3). They have a broad activity since they were antagonistic for gram positives and gram negatives bacteria.

3.2. Total phenolic and Tannin Contents

Total phenolic and tannin contents of cashew leave, resak tembaga leave and stink bean pod extracts are summarized in Table 4. According to the table, the highest concentration of total phenolic content was detected in Resak tembaga leaves (158.14 ± 1.22 mg GAE/g DW) while cashew leaves extract had the highest amount of tannin (241.41 ± 0.15 mg TAE/g DW). However, there was low correlation between total phenolic content and tannin content. Indeed extract with highest total phenolic content, Resak tembaga leaves, did not contain largest amount of tannin.

Plant phenolics are secondary metabolites, including simple phenols, phenolic acids, coumarins, flavonoids, stilbenes, steroids, tannins, saponins and cardiac glycosides, which are known to have antibacterial activity [8]. The site and number of hydroxyl groups on the phenol group are through to be related to their relative toxicity to microorganisms, with evidence that increased hydroxylation results increased toxicity [9].

Tannins are a complex and heterogeneous group of polyphenolic secondary metabolites of higher plants. They contain sufficient hydroxyls and other suitable groups to form effectively strong complexes with protein (gelatin and amino acid) and other macromolecules (polysaccharides and alkaloids) to insoluble or soluble tannin complexes [10]. The mode of antibacterial action may be related to their ability to inactive microbial adhesions, enzymes, cell envelope transport proteins, etc [11].

Table 3: Antibacterial activities of 50% ethanolic extract of selected plant by-products using agar well diffusion assay.

Sample*	Radius of inhibition zone (mm)					
	Gram positive bacteria			Gram negative bacteria		
	BC	LM	SA	EC	ST	VC
Guava leaves	1.2 ± 0.0	1.0 ± 0.0	1.1 ± 0.1	-	-	0.8 ± 0.1
Rambutan leaves	1.0 ± 0.1	-	0.8 ± 0.1	-	-	0.1 ± 0.1
Para rubber leaves	2.1 ± 0.2	0.3 ± 0.0	0.9 ± 0.1	-	-	0.8 ± 0.3
Cassava leaves	0.9 ± 0.2	-	1.1 ± 0.2	-	-	0.1 ± 0.1
Pomegranate leaves	2.8 ± 0.0	0.1 ± 0.0	0.1 ± 0.1	-	-	0.3 ± 0.2
Cashew leaves	2.0 ± 0.1	0.9 ± 0.1	1.3 ± 0.1	0.9 ± 0.2	1.0 ± 0.1	1.1 ± 0.2
Mangosteen leaves	2.8 ± 0.3	1.0 ± 0.1	1.7 ± 0.2	-	-	1.5 ± 0.4
Resak tembaga leaves	1.1 ± 0.1	2.8 ± 0.1	3.5 ± 0.3	0.2 ± 0.1	1.0 ± 0.2	4.3 ± 0.4
Resak tembaga barks	0.9 ± 0.0	2.3 ± 0.2	3.0 ± 0.1	-	0.6 ± 0.3	2.9 ± 0.1
Pomegranate peels	1.9 ± 0.4	-	-	-	-	-
Stink bean pods	1.8 ± 0.1	0.9 ± 0.1	0.9 ± 0.1	0.3 ± 0.1	0.7 ± 0.3	1.6 ± 0.4
Rambutan peels	1.8 ± 0.2	-	0.1 ± 0.1	-	-	0.3 ± 0.1
Mangosteen peels	4.6 ± 0.4	1.8 ± 0.0	4.3 ± 1.0	-	-	2.8 ± 0.3
Palmyra peels	1.1 ± 0.1	-	-	-	-	0.2 ± 0.0
Coconut husks	-	1.4 ± 0.0	1.9 ± 0.1	-	-	2.0 ± 0.0
Sterile distilled water	-	-	-	-	-	-
Tetracycline (10 µl)	5.4 ± 0.6	2.5 ± 0.4	5.6 ± 0.2	6.0 ± 0.1	4.4 ± 0.2	4.7 ± 0.7

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

Values are given as mean ± standard deviation (n=3). -: No inhibition; BC: *Bacillus cereus*; LM: *Listeria monocytogenes*; SA: *Staphylococcus aureus*; EC: *Escherichia coli*; ST: *Salmonella* Typhimurium and VC: *Vibrio cholerae*.

Table 4: Total phenolic and tannin contents of 50% ethanolic extract of selected plant by-products.

Sample	Total phenolic content (mg GAE/g DW)	Tannin content (mg TAE/g DW)
Cashew leaves	41.98 ± 1.03^{c}	124.57 ± 0.16^{a}
Resak tembaga leaves	158.14 ± 1.22^{a}	4.03 ± 0.13^{c}
Stink bean pods	81.54 ± 0.18^{b}	43.97 ± 0.25^{b}

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

4. Conclusion

While human pathogens such as *B. cereus*, *L. monocytogenes*, *S. aureus*, *E. coli*, *Sal.* Typhimurium and *V. cholera* which are ubiquitous in the environment showed resistance to most of the plant by-product

extracts *in vitro*. Cashew leave, resak tembaga leave and stink bean pod extracts could exhibited a board spectrum antibacterial activity against all selected food-borne pathogenic bacteria in this study. Moreover, the highest concentration of total phenolic content was found in Resak tembaga leaves while cashew leaves extract had the highest amount of tannin. Thus differences in the antibacterial activity of plant by-product extracts could be partially explained by strains sensitivity and variations in total phenolic and tannin contents of extracts. Furthermore, the use of the natural waste as a source of antibacterial compound could provide health benefits to humans and may be employed in food preservation purpose.

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