



Investigation on Antibacterial and Antioxidant Activities, Phenolic and Flavonoid Contents of Some Thai Edible Plants as an Alternative for Antibiotics

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ABSTRACT: This study was aimed to examine the antibacterial and antioxidative properties of seven edible plants from Thailand to develop alternative antibiotics as feed additives. The plants include *Citrus aurantifolia* Swingle (Lime) fruits and its leaves, *Sesbania grandiflora* L. (Agati sesbania) leaves, *Piper sarmentosum* Roxb (Wild betel) leaves, *Curcuma domestica* Valeton (Turmeric) roots, *Morinda citrifolia* L. (Beach mulberry) leaves, *Cassia siamea* britt (Siamea cassia) leaves, and *Cocos nucifera* L. (Coconut) peels. The plants were extracted by methanol, *n*-hexane, chloroform, ethyl acetate, butanol and water. Antibacterial activities with minimum inhibitory concentration (MIC) were determined by agar diffusion assay against *Escherichia coli*, *Burkholderia* sp., *Haemophilus somnus*, *Haemophilus parasuis*, and *Clostridium perfringens* that were considered pathogenic strains in livestock infection. Methanol extracts of *C. aurantifolia* Swingle fruits and leaves showed the broadest spectrum of antibacterial activities except for *C. perfringens*. Butanol extract of *S. grandiflora* L. leaves showed the strongest activity against *Burkholderia* sp. with MIC, 135 µg/mL. *P. sarmentosum* Roxb leaves showed antibacterial activities against *E. coli*, *Burkholderia* sp. and *H. parasuis*. Ethyl acetate and water extracts from *C. domestica* Valeton roots showed MIC of 306 µg/mL and 183 µg/mL, respectively against only *C. perfringens*. Antioxidative activity was determined by 2-diphenyl-2-picryl hydrazyl photometric assay. The methanol extracts of *C. aurantifolia* Swingle fruits and *P. sarmentosum* Roxb leaves showed the highest antioxidant activity among all the extracts with 3.46 mg/mL and 2.70 mg/mL effective concentration 50% (EC₅₀) values, respectively. Total contents of phenolics and flavonoids were measured from the plant extracts. Methanol extracts of *S. grandiflora* L. and chloroform extracts of *C. domestica* Valeton were found to have the highest amount of total phenolics, 41.7 and 47.8 µg/mL, respectively. Flavonoid content of methanol extracts in *S. grandiflora* L. T was 22.5 µg/mL and the highest among plant extracts tested. These results indicated that *C. aurantifolia* Swingle, *S. grandiflora* L., *P. sarmentosum* Roxb, and *C. domestica* Valeton have antibacterial and antioxidant activities and can be used as alternative antibiotics or potential feed additives for the control of animal pathogenic bacteria. (**Key Words:** Plant Extract, Antibacterial Activity, Antioxidant Activity, Polyphenol, Flavonoid)

INTRODUCTION

Natural plant compounds that can substitute the use of

antibiotics have been emerged from health threat of residual antibiotics and outcome of multi-drug resistant pathogenic bacteria in food including animal products. Various plant products in different area were tested and reported as antimicrobial agents and bioactive natural products (Cowan, 1999; Gautam et al., 2007; Magadula and Erasto, 2009). The plants showing antioxidant activity were also known in many reports (Wolfe et al., 2003; Kaur et al., 2006; Ordon et al., 2006; Alviano et al., 2008; Doddola et al., 2008).

The regulation has been reinforced and use of antibiotics has no scope for promotion of livestock growth. From 2006, in-feed antibiotics used in animal production

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have been banned by the European Union. Recently, South Korea has also banned them from the second half of 2011. For rearing of livestock without chemical antibiotics, feeding of natural products with the function such as antimicrobial agents, antioxidants and immune stimulants should be helpful for animal health. Therefore, alternative antibiotics have been regarded as most important feed additives in animal farming. Specially, plant extracts has received much attention in the research for the development of alternative antibiotics (Esquenazi et al., 2002; Wannissorn et al., 2005). The production amounts of plants are much more abundant in subtropical and tropical countries including Thailand compared with northern hemisphere including South Korea with typical four seasons.

The purpose of this study was to examine antibacterial and antioxidant activities and total contents of phenolics and flavonoids of some edible plants of Thailand and to develop natural feed additive for animal health. The plants, viz. *Citrus aurantifolia* Swingle (Lime) fruit and leaves, *Morinda citrifolia* (Beach mulberry) leaves, *Sesbania grandiflora* L. (Agasti sesbania) leaves, *Piper sarmentosum* (Wild betel) leaves, *Curcuma domestica* Valetton (Turmeric) roots, *Cassia siamea* leaves, and *Cocos nucifera* L. (Coconut) fruits. Tested pathogens using the plant extracts are related to wasting diseases of livestock and causative to execute great economical loss in animal production. *Haemophilus parasuis*, *Haemophilus somnus*, and *Burkholderia* sp. are known to induce pneumonia (Palzer et al., 2008), respiratory disease (Norcia et al., 2004), and meliodiosis (Sprague and Neubauer, 2004; Millan et al., 2007), respectively. *Escherichia coli* and *Clostridium perfringens* are causative agents of diarrhoea in piglet (Hendriksen et al., 2006).

MATERIALS AND METHODS

Plants and preparation of crude extracts

The plants studied were *C. aurantifolia* Swingle (Lime) fruits and leaves, *M. citrifolia* (Beach mulberry) leaves, *S. grandiflora* L. (Agasti sesbania) leaves, *P. sarmentosum* (Wild betel) leaves, *C. domestica* Valetton (Turmeric) roots, *C. siamea* leaves, and *C. nucifera* L. (Coconut) peels. The plants were cut into smaller species and air-dried in the shade for a week and finely ground. The materials were supplied by K.-C Shin (Bangkok, Thailand) and authenticated by Chang-Won Choi (Department of Biology and Medicinal Science, Paichai University, South Korea). Voucher specimens were deposited at Animal Resources and Research Center, Konkuk University. A sample of 3 g was suspended in 27 mL of methanol and the mixture was agitated in 150 rpm for 6 h. The extract was filtered and evaporated. The remained extract was resuspended with 2

mL of methanol. Methanol extract (25 μ L) was used for antibacterial activity. One mL of methanol extract was mixed with 5 mL of distilled water and 5 mL of *n*-hexane. The mixture was vigorously mixed and then phase separation was developed by standing. Upper phase of *n*-hexane layer was evaporated and further solvents extractions were done as same manner of previous step. Used solvents were chloroform, ethyl acetate, and butanol, subsequently as described in Figure 1. All of prepared extracts was stored at 4°C until assay was performed. Dry matter content was analyzed by estimation of weight loss during heat dry.

Bacterial strains

Animal pathogenic bacteria used in this study, *H. somnus*, *H. parasuis*, *Burkholderia* sp., *E. coli*, and *C. perfringens*, are related to respiratory disease and diarrhea of piglet and other animals. All of them were collected from stock of National Veterinary Research Quarantine Service in South Korea.

Paper disc diffusion method

Antibacterial activities of each six fractions from 7 plants were determined using paper disc diffusion method, described elsewhere (Valsaraj et al., 1997). Each bacterial culture (0.1 mL) grown in Luria-Bertani broth (LB, Difco) at 37°C for 18 h was suspended in 10 mL of sterilized 0.8% agar solution. To prevent solidification of agar and heat damage of bacterial cells, 0.8% agar solution was maintained at 50°C until use. After suspension of test strain, agar solution was poured onto LB agar plate and then allowed to be solidified. Plant extracts (25 μ L) was put on 6 mm sterilized paper disk and it was dried for 30 min to evaporate solvent before bacterial cells placed on suspended

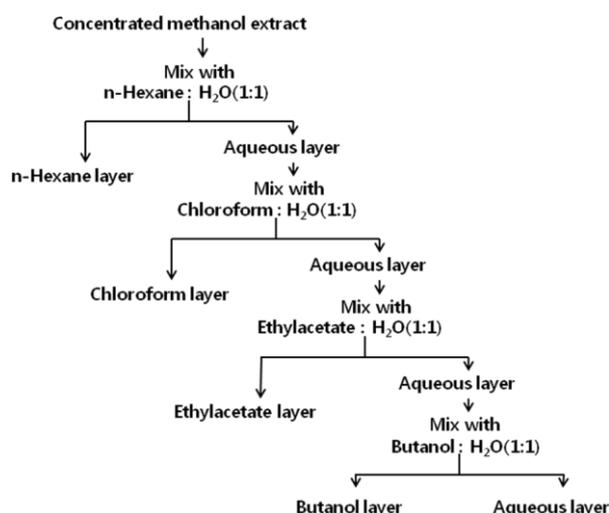


Figure 1. Schematic diagram of plant extract preparation with various solvents.

agar plate. Prepared test plates were incubated at 37°C for 18 h and then appeared diameters of clear zone were measured.

Determination of minimum inhibitory concentration

According to the results of screening of antibacterial activities, the minimum inhibitory concentration (MIC) of selected ten plant extracts were determined by serial dilution method on 96 well plates with turbidity measurement (Alviano et al., 2008). Plant extract was diluted with sterilized LB broth, ranged from 0.01 to 10 mg/mL, and dispensed into 96 well plate. Test strains were inoculated in prepared each well with 1:100 ratio and incubated at 37°C for 18 h. Bacterial growth was determined by measuring of turbidity at 600 nm. The MIC was determined as the lowest concentration of extracts which showed no visible growth of the test strains.

Antioxidant activity assay

The free radical scavenging activities were determined *in vitro* using 2-diphenyl-2-picryl hydrazyl (DPPH) photometric assay (Tadic et al., 2008) with some modification. Each extract (1 to 10 µL) was mixed into its solvent to make a total volume of 100 µL. It was mixed with 900 µL of 0.04 mg/mL DPPH in methanol solution and the mixture was reacted for 30 min with gentle agitation. Reduction of violet color induced by free radical scavenging activity was estimated spectrophotometrically at 517 nm. Each solvent used in extraction was applied as negative control and *tert*-butyl hydroxytoluene was used as positive control. The inhibition percentage was calculated by comparing absorbance with negative control and it was plotted against the concentration of the samples. The extract concentration that represented 50% of activity was calculated by linear regression analysis and expressed as EC₅₀.

Determination of total polyphenols

Total polyphenol contents in the extracts were determined by the modified Folin-Ciocalteu method (Wolfe et al., 2003). Each sample (0.1 mL) was added into 2 mL of 2% Na₂CO₃. After the tubes were vortexed for 10 min at room temperature, 0.1 mL of 50% Folin-Ciocalteu reagent was added. The tubes were vortexed for 15 s and allowed to stand for 30 min at 40°C for color development. Absorbance was then measured at 700 nm using the Shimadzu UV-1601 spectrophotometer. Samples of extracts were evaluated at a final concentration of 1 mg/mL. Total polyphenol contents were calculated as quercetin (25 to 500 ppm) using the following equation based on the calibration curve: $y = 0.0033x - 0.0111$, $R^2 = 0.9979$, where x was the absorbance and y was the quercetin equivalent (µg/mL) (Kiselova et al., 2006).

Determination of total flavonoids

Total flavonoids were estimated using the method (Ordon et al., 2006). Each sample (0.5 mL) was added and mixed into 1.5 mL of 95% ethanol. To it, 0.1 mL of 10% aluminum chloride, 0.1 mL of 1 M potassium acetate and 2.8 mL of distilled water were added. After the tubes were mixed for 30 min at room temperature, the absorbance was measured at 415 nm. A yellow color indicated the presence of flavonoids. Extracts were evaluated at a final concentration of 1 mg/mL. Total flavonoid content was calculated as quercetin (5 to 100 ppm) using the following equation based on the calibration curve: $y = 0.0068x - 0.0165$, $R^2 = 0.9974$, where x was the absorbance and y was the quercetin equivalent (µg/mL).

RESULTS

Antibacterial activity assay

Methanol extracts of *C. aurantifolia* Swingle fruits showed the most broad spectrum antibacterial activity compared to other solvent extracts, except for *C. perfringens* (Table 1). Butanol and water fractions of it also showed very high antibacterial activities against *H. somnus*, related to porcine respiratory disease causative to wasting syndrome in young pigs. Antibacterial activities of *C. aurantifolia* Swingle leaves appeared different spectrum compared to its fruits. Methanol extract of leaves showed the antibacterial activities against both *Burkholderia* sp. and *H. somnus*, but not against *E. coli*, *H. parasuis*, and *C. perfringens*. However, the *n*-hexane and chloroform extracts from leaves showed antibacterial activities for *Burkholderia* sp. only whereas its fruits did not. Only butanol fraction of *S. grandiflora* leaves showed antibacterial activity against *Burkholderia* sp. related to melioidosis in pig, even in human. Only methanol extracts of *P. sarmentosum* leaves showed very mild antibacterial activities against both *E. coli* and *Burkholderia* sp. and moderate activity against *H. parasuis*. Antibacterial spectrum of ethyl acetate and water extracts from *C. domestica* Valetton (Turmeric) roots showed moderate to strong against *C. perfringens*, only. *M. citrifolia* L. leaves, *C. siamea* britt leaves, and *C. nucifera* L. fruits did not show any antibacterial activity against the tested pathogens in this study.

The results of the MIC were well agreed with the antibacterial patterns shown by paper disc diffusion method (Table 2). The strong activity (MIC 495 µg/mL) was shown by methanol extracts of *C. aurantifolia* Swingle fruits against *H. somnus*. Its leaves showed much lower MIC (198 µg/mL) by chloroform extracts than methanol (281 µg/mL) and *n*-hexane (810 µg/mL) extracts against *Burkholderia* sp. Butanol extracts of *S. grandiflora* L. showed antibacterial specificity to *Burkholderia* sp. with

Table 1. Antibacterial activities against animal pathogenic bacteria by various plant extracts

Plant tested (Common name) (Thailand local name)	Part used	Pathogenic bacteria	Solvent fraction ¹					
			M	H	C	E	B	W
<i>Citrus aurantifolia</i> Swingle (Lime) (Manaa)	Fruits	<i>Escherichia coli</i>	++	-	-	-	-	-
		<i>Burkholderia</i> sp.	++	-	-	-	-	+
		<i>Haemophilus somnus</i>	+++	-	-	-	+++	+++
		<i>Haemophilus parsuis</i>	++	-	-	-	-	-
		<i>Clostridium perfringens</i>	-	-	-	-	-	-
	Leaves	<i>Escherichia coli</i>	-	-	-	-	-	-
		<i>Burkholderia</i> sp.	+++	+	+++	-	-	-
		<i>Haemophilus somnus</i>	++	-	-	-	-	-
		<i>Haemophilus parsuis</i>	-	-	-	-	-	-
		<i>Clostridium perfringens</i>	-	-	-	-	-	-
<i>Sesbania grandiflora</i> L. (Agati sesbania) (Khaeban or Baikae)	Leaves	<i>Escherichia coli</i>	-	-	-	-	-	-
		<i>Burkholderia</i> sp.	-	-	-	-	+++	-
		<i>Haemophilus somnus</i>	-	-	-	-	-	-
		<i>Haemophilus parsuis</i>	-	-	-	-	-	-
		<i>Clostridium perfringens</i>	-	-	-	-	-	-
<i>Piper sarmentosum</i> Roxb (Wild betal) (Chaphulu or Baiphulu)	Leaves	<i>Escherichia coli</i>	+	-	-	-	-	-
		<i>Burkholderia</i> sp.	+	-	-	-	-	-
		<i>Haemophilus somnus</i>	-	-	-	-	-	-
		<i>Haemophilus parsuis</i>	++	-	-	-	-	-
		<i>Clostridium perfringens</i>	-	-	-	-	-	-
<i>Curcuma domestica</i> Valetton (Turmeric) (Khamin)	Roots	<i>Escherichia coli</i>	-	-	-	-	-	-
		<i>Burkholderia</i> sp.	-	-	-	-	-	-
		<i>Haemophilus somnus</i>	-	-	-	-	-	-
		<i>Haemophilus parsuis</i>	-	-	-	-	-	-
		<i>Clostridium perfringens</i>	-	-	-	++	-	+++
<i>Morinda citrifolia</i> L. (Beach mulberry) (Yaeyai or Baiyae)	Leaves	<i>Escherichia coli</i>	-	-	-	-	-	-
		<i>Burkholderia</i> sp.	-	-	-	-	-	-
		<i>Haemophilus somnus</i>	-	-	-	-	-	-
		<i>Haemophilus parsuis</i>	-	-	-	-	-	-
		<i>Clostridium perfringens</i>	-	-	-	-	-	-
<i>Cassia siamea</i> britt (Siamea cassia) (Kheelek)	Leaves	<i>Escherichia coli</i>	-	-	-	-	-	-
		<i>Burkholderia</i> sp.	-	-	-	-	-	-
		<i>Haemophilus somnus</i>	-	-	-	-	-	-
		<i>Haemophilus parsuis</i>	-	-	-	-	-	-
		<i>Clostridium perfringens</i>	-	-	-	-	-	-
<i>Cocos nucifera</i> L. (Coconut) (Maphraogere)	Peels	<i>Escherichia coli</i>	-	-	-	-	-	-
		<i>Burkholderia</i> sp.	-	-	-	-	-	-
		<i>Haemophilus somnus</i>	-	-	-	-	-	-
		<i>Haemophilus parsuis</i>	-	-	-	-	-	-
		<i>Clostridium perfringens</i>	-	-	-	-	-	-

M, methanol; H, *n*-hexane; C, chloroform; E, ethyl acetate; B, butanol; F, water.

¹ Antibacterial activities: -, no inhibition (8 mm); +, very mild inhibition (9 to 11 mm); ++, moderate inhibition (11 to 13 mm); +++, strong inhibition (13 to 17 mm).

MIC of 135 µg/mL. Methanol extracts of *Piper sarmentosum* Roxb had MICs, 502 µg/mL, 1,005 µg/mL, and 251 µg/mL against *E. coli*, *Burkholderia* sp., and *H. parasuis*, respectively. The ethyl acetate and water extracts from *C. domestica* Valetton roots have high specificities

against only *C. perfringens* with 306 µg/mL and 183 µg/mL MICs, respectively.

Antioxidant activity assay

C. aurantifolia Swingle fruit showed relatively high

Table 2. Minimum inhibitory concentration against animal pathogenic bacteria by various plant extracts

Plant tested	Part used	Fraction	Pathogenic bacteria				
			<i>Escherichia coli</i>	<i>Burkholderia</i> sp.	<i>Haemophilus somnus</i>	<i>Haemophilus parasuis</i>	<i>Clostridium perfringens</i>
----- Minimum inhibitory concentration (µg/mL) -----							
<i>Citrus aurantifolia</i> Swingle	Fruits	Methanol	1,980	990	495	990	ND ¹
		Butanol	ND	ND	660	ND	ND
		Water	ND	660	660	ND	ND
	Leaves	Methanol	ND	281	2,250	ND	ND
		<i>n</i> -Hexane	ND	810	ND	ND	ND
		Chloroform	ND	198	ND	ND	ND
<i>Sesbania grandiflora</i> L.	Leaves	Butanol	ND	135	ND	ND	ND
<i>Piper sarmentosum</i> Roxb	Leaves	Methanol	502	1,005	ND	251	ND
<i>Curcuma domestica</i> Valeton	Roots	Ethyl acetate	ND	ND	ND	ND	306
		Water	ND	ND	ND	ND	183

ND, not detected.

Table 3. Antioxidant activities of used plants extracts

Plant tested	Part used	Free radical scavenging activity	
		Fraction	EC ₅₀ value ¹ (mg/mL)
<i>Citrus aurantifolia</i> Swingle	Fruits	Methanol	3.46±0.2
		Butanol	8.89±1.2
		Water	12.16±0.6
	Leaves	Methanol	17.68±7.4
		<i>n</i> -Hexane	ND
		Chloroform	10.27±4.6
<i>Sesbania grandiflora</i> L.	Leaves	Butanol	ND
<i>Piper sarmentosum</i> Roxb	Leaves	Methanol	2.70±0.3
<i>Curcuma domestica</i> Valeton	Roots	Ethyl acetate	ND
		Water	18.18±0.8

ND, not detected.

¹ EC₅₀ value means the concentration at 50% of antioxidant activity. In the case of positive control determined by BHT, EC₅₀ was 0.3±0.04 mg/mL.

antioxidant activity (Table 3), specifically in methanol extract (EC₅₀, 3.46 mg/mL). Antioxidant activity of chloroform extract of *C. aurantifolia* Swingle leaves was higher than that (EC₅₀, 10.27 vs 17.68 mg/mL) of methanol extract. However, no activity was detected by *n*-hexane extract although it showed antibacterial activity (Tables 1 and 2). Butanol extract of *Sesbania grandiflora* L. leaves showed no antioxidant activity. On the other hand, *P. sarmentosum* Roxb showed the most strong antioxidant activity with EC₅₀, 2.70 mg/mL. Water extracts of *C. domestica* Valeton roots showed the moderate antioxidant activity (EC₅₀, 18.18±0.8 mg/mL) whereas ethyl acetate fraction did not.

Total polyphenolic compounds and flavonoids

Among the plant extracts tested in the study, methanol extracts of *S. grandiflora* L. and chloroform extracts of *C. domestica* Valeton were found to have the highest amount of total polyphenol, 41.7 and 47.8 µg/mL, respectively

(Table 4). The highest amount of total flavonoid recorded among all plant extracts tested was 22.5 µg/mL, which was also from methanol extract of *S. grandiflora* L. Methanol extracts of *P. sarmentosum* Roxb showed high content (19.4±0.008 µg/mL) of total flavonoid. Most of the methanol extracts of tested plants showed high amounts of total polyphenol and total flavonoid compared to other solvent extracts except for *Curcuma domestica*, where chloroform extract showed highest (Table 4).

DISCUSSION

In this study, 6 kinds of solvent extraction were applied to 7 edible plants of Thailand and their antibacterial activities were determined by the spectrum against 5 animal pathogenic bacteria. Some bioactive components of these plants may differ in their solubility depending on the extractive solvents used. With these results, finally 10 extracts from 4 plants were selected and their MICs and

Table 4. Total polyphenolics and flavonoids in tested plants extracts

Plant tested	Part used	Solvent fraction	Contents ($\mu\text{g/mL}$) ¹	
			Total polyphenol	Total flavonoid
<i>Citrus aurantifolia</i> Swingle	Fruits	Methanol	28.5 \pm 0.004	15.8 \pm 0.007
		<i>n</i> -Hexane	18.2 \pm 0.009	9.4 \pm 0.005
		Chloroform	24.1 \pm 0.004	9.7 \pm 0.012
		Ethyl acetate	13.6 \pm 0.006	8.8 \pm 0.006
		Butanol	20.4 \pm 0.009	10.0 \pm 0.006
		Water	12.6 \pm 0.002	11.0 \pm 0.013
	Leaves	Methanol	31.8 \pm 0.003	17.5 \pm 0.003
		<i>n</i> -Hexane	18.6 \pm 0.004	5.9 \pm 0.007
		Chloroform	14.5 \pm 0.003	5.8 \pm 0.008
		Ethyl acetate	11.3 \pm 0.002	4.4 \pm 0.002
<i>Sesbania grandiflora</i> L.	Leaves	Methanol	41.7 \pm 0.006	22.5 \pm 0.006
		<i>n</i> -Hexane	20.7 \pm 0.008	7.7 \pm 0.006
		Chloroform	26.0 \pm 0.004	7.0 \pm 0.013
		Ethyl acetate	11.0 \pm 0.005	10.0 \pm 0.014
		Butanol	11.3 \pm 0.004	9.0 \pm 0.020
		Water	17.4 \pm 0.006	7.0 \pm 0.013
	Leaves	Methanol	38.8 \pm 0.006	19.4 \pm 0.008
		<i>n</i> -Hexane	17.6 \pm 0.009	10.2 \pm 0.012
		Chloroform	23.3 \pm 0.010	9.2 \pm 0.018
		Ethyl acetate	13.5 \pm 0.013	5.2 \pm 0.010
<i>Piper sarmentosum</i> Roxb	Leaves	Butanol	23.9 \pm 0.007	11.3 \pm 0.011
		Water	17.7 \pm 0.006	4.4 \pm 0.006
		Methanol	25.9 \pm 0.004	14.1 \pm 0.002
		<i>n</i> -Hexane	24.5 \pm 0.009	7.5 \pm 0.010
		Chloroform	47.8 \pm 0.008	14.1 \pm 0.012
		Ethyl acetate	19.7 \pm 0.003	5.8 \pm 0.008
	Roots	Butanol	18.5 \pm 0.004	7.0 \pm 0.015
		Water	27.1 \pm 0.011	12.7 \pm 0.006

¹ Total polyphenol and flavonoid contents were calculated as quercetin equivalent.

antioxidant activities were determined. As a result, *C. aurantifolia* Swingle fruits known as lime showed the most broad antibacterial spectrum with the strong antioxidant activity specially in methanol extracts. It has been traditionally regarded as an effective carminative fruit in Thailand and the antibacterial activity of its essential oil has been reported against *Salmonella typhimurium*, *S. enteritidis*, *E. coli*, *C. perfringens*, and *Campylobacter jejuni* (Wannissorn et al., 2005). In this study, we newly found that the fruit extracts of *C. aurantifolia* Swingle also had the antibacterial activities against *Burkholderia* sp., *H. somnus*, and *H. parasuis* including *E. coli*. However, we did not find the antibacterial activity from the fruit extracts of *C. aurantifolia* Swingle against *C. perfringens*. There may be a difference in concentration of effective compounds between essential oil and our solvent extraction. *C. aurantifolia* Swingle leaves contained more strong antimicrobial activity

against *Burkholderia* sp. (Tables 1 and 2) compared to its fruits. Antibacterial properties of different parts in *C. aurantifolia* Swingle were reported against Gram-positive bacteria such as *Staphylococcus aureus*, Gram-negative bacteria including *E. coli*, *S. paratyphi*, *Pseudomonas aeruginosa*, and *Candida albicans* (Aibinu et al., 2007). Patil et al. (2009) reported that the chloroform extracts of *C. aurantifolia* Swingle fruit had much higher free radical-scavenging activity than acetone and methanol extracts. Immunomodulatory effect of concentrated lime juice extract was also observed on activated human mononuclear cells (Gharagozloo et al., 2001).

Although a few suggestions about antibacterial activity against skin and gastrointestinal microbes have been reported (Denyer, 1990; Doddola et al., 2008), there is little information about antimicrobial activities of *S. grandiflora* L. leaves. Antibacterial activity against *Burkholderia* sp.

was found (Tables 1 and 2) in butanol fraction of *S. grandiflora* L. leaves. Zaidan et al. (2005) reported the antibacterial activities of methanol extracts of *P. sarmentosum* leaves against *S. aureus* and *P. aeruginosa*. In this study, *P. sarmentosum* Roxb also showed the antibacterial activities and the highest antioxidant activity, especially, its methanol extracts showed the antibacterial activities against *E. coli*, *Burkholderia* sp. and *H. parasuis*. *C. domestica* Valetton roots have been widely cultivated in Southeast Asia for the use of an additive in cooking and a traditional medicine to treat diarrhea (Ahmad-Raus et al., 2001). Interestingly, antibacterial spectrum of ethyl acetate and water extracts from *C. domestica* Valetton roots showed specificities against *C. perfringens* (Tables 1 and 2), in contrary to the report of Wannissorn et al. (2005).

M. citrifolia L. is widely grown in the Hawaii and Asia and it has been commonly called as “Noni”. It has been used as an herbal plant with effectiveness on diabetes, hypertension and immunomodulation (Takashima et al., 2007). Schäfer et al. (2008) showed the bactericidal effect against *Escherichia coli* in calves fed *M. citrifolia* (Noni) puree. Blood samples from Noni puree-fed calves displayed significantly more bacterial killing against *E. coli* than did controls on day 14. *C. siamea* britt is commonly found tree in Southeast Asia and its bark, root and flowers have been known to have medicinal values in diabetes, hypertension and insomnia (Kaur et al., 2006). Methanol extracts of *C. siamea* exhibited antibacterial activities against both *Bacillus cereus* and *Listeria monocytogenes* whereas *M. citrifolia* L. had no antibacterial activities for all tested microorganisms (Nanasombat and Teckchuen, 2009). In this study, the antibacterial activities of *M. citrifolia* L., *C. siamea* britt and *C. nuricifera* L. were not detected against tested pathogens. Husk of *C. nuricifera* L. fruits have various phenolic compounds that have the physiological effects including antileishmanial, antibacterial, antiviral and anti-neoplastic (Alviano et al., 2008). In addition, Esquenazi et al. (2002) reported that extracts of *C. nuricifera* showed the antibacterial activity against *S. aureus*. In an earlier study (Wannissorn et al., 2005), the antibacterial activity against *C. perfringens* was reported as very high, however, no activity was found at all of solvent fractions in this study.

Biological activities related to antibacterial and antioxidant activities may be correlated with total polyphenol and flavonoid (Rice-Evans et al., 1997; Cushnie and Lamb, 2005; Daglia, 2011). Even though the correlation was not perfectly matched in this study, methanol extracts of both *Citrus aurantifolia* Swingle and *Piper sarmentosum* Roxb possessed high antibacterial and antioxidant activities in proportion to high amounts of total polyphenol and flavonoid. In conclusion, the extracts from *Citrus aurantifolia* Swingle (Lime), *Sesbania grandiflora* L. (Agati sesbania), *Piper sarmentosum* Roxb (Wild betal) and

Curcuma domestica Valetton (Turmeric) showed high antibacterial and antioxidant activities. They should be further tested *in vivo* animal experiments as alternative antibiotics in potential feed additives for the inhibition of respiratory disease and meliodiosis with diarrhea in livestock.

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