JOURNAL OF PURE AND APPLIED MICROBIOLOGY, Sept. 2015. Vol. **9**(3), p. 2175-2184

In-vitro Antibacterial Activity Screening of Herb Extracts against Foodborne Pathogenic Bacteria from Thailand

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(Received: 10 February 2015; accepted: 20 April 2015)

Herbs have been used as traditional medicine since the ancient times as primary health care for local people. This experiment was aimed to study the individual antibacterial activity of six Thai local herbs (*Tradescantia spathacea*, *Andrographis paniculata*, *Eleocharis acicularis*, *Acacia concinna*, *Phyllanthus niruri*, and *Tinospora cordifolia*) against six foodborne pathogenic bacteria (*Escherichia coli* ATCC25822, *Samonella enterica* Typhimurium U302 (DT1046), *S. enterica* Enteritidis (human), *S. enterica* 4,5,12: i-(human) US clone, *Bacillus cereus*, and *Listeria monocytogenes* 10403S) under five extractions (95% ethanol, chloroform, hexane, sterile distilled water, and autoclaving at 121°C 15 PSI for 15 minutes). Agar disc diffusion method was used to evaluate antibacterial activity. The results showed that 95% ethanol and chloroform extraction gave the highest antibacterial activity of all herb extracts against all bacteria. The range of antibacterial activity is between 7.0 mm to 10.5 mm. The highest antibacterial activity was chloroform extract of *T. cordifolia* against *S. enterica* Typhimurium U302 (DT1046). The range of MIC and MBC is between 32 µl/ml to 256 µl/ml. These results showed the promising of antibacterial activity of six Thai local herbs which are stepping stone for further application like food industry, pharmaceutical industry, and cosmetic industry.

Key words: Antibacterial activity, Thai local herb, foodborne pathogenic bacteria.

In recent years, food safety concerns have been focused on pathogens, such as Salmonella which is recognized as a primary cause of food poisoning worldwide and massive outbreaks have been occurred in several parts of the world [1]. It was estimated that globally, around 86% of salmonellosis cases are food-borne [2]. The *S. enterica* Typhimurium DT104b has a remarkable ability to survive and becomes the major cause of salmonellosis food-borne illness. This is reflected by the fact that 6.6% of food-borne outbreaks were attributable to this serovar in an international study [3]. For *S. enterica* 4,5,12:i:- (human) US clone is a serotype that appears to be antigenically similar and genetically closely related to *S. enterica* Typhimurium (which has the antigenic formula 4,5,12:i:1,2) but lacks expression of the second-phase flagella antigen, which is 1,2 in *S. enterica* Typhimurium. The *S. enterica* 4,5,12:i:- (human) US clone was the sixth most common *Salmonella* serovar among cases of human disease in the United States in 2006 and the fourth most common serovar among human isolates in Spain in 1998. Overall, the prevalence of *S. enterica* 4,5,12:i:- (human) US clone among human cases has increased considerably in many countries in the world over the last 10 year [4,5,6]. While in the last 20 years or so, *S. enterica* Enteritidis has become the single most common cause of food poisoning. *L. monocytogenes* is a Gram-positive bacterium, motile by flagella. The 1-10% of population may be intestinal carriers of L. monocytogenes[7]. It is quite hardy and resists the deleterious effects of freezing, drying, and heat [7]. About 2500 cases of listeriosis

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occur each year. Most cases of listeriosis and most deaths occur in adults with weakened immune systems, the elderly, pregnant women, and newborns [7]. Outbreaks of listeriosis have been linked to a variety of foods especially processed meats and dairy products made from unpasteurized[7]. *Bacillus cereus* is a Gram-positive, may change to Gram-negative when get older, rod-shaped with endospore and aerobe[8]. *B. cereus* themselves not tolerate to physical condition, basic or acidic, but their spores are heat resistant and active in wide range of pH[8]. *B* Growth of Bacillus sp. will gives an enterotoxin. Even *B. cereus* outbreaks is only 2% of all foodborne illness[8]. *Escherichia coli* bacteria normally live in the intestines of people and animals[9]. Most *E. coli* are harmless and actually are an important part of a healthy human intestinal tract[9]. The types of *E. coli* that can cause diarrhea can be transmitted through contaminated water or food, or through contact with animals or persons[9].

Local herbs become potential natural source of antibiotics and medicinal properties. They have active compound that effective as antimicrobial. This research was aimed to study *in vitro* the individual antibacterial activity of six Thai local herbs; *Tradescantia spathacea* (Oyster plant), *Andrographis paniculata* (Kariyat), *Eleocharis acicularis* (Needle- Spike Rush), *Acacia concinna* (Soap pod), *Phyllanthus niruri* (Stonebreaker), and *Tinospora cordifolia* (Gulancha), against six foodborne pathogenic bacteria; *E. coli* ATCC25822, *S. enterica* Typhimurium U302 (DT1046), *S. enterica* Enteritidis (human), *S. enterica* 4,5,12: i-(human) US clone, *B.cereus*, and *L. monocytogenes* 10403S under five extraction conditions; 95% ethanol, chloroform, hexane, sterile distilled water, and autoclaving at 121°C 15 PSI for 15 minutes.

MATERIALS AND METHODS

Plant sample preparation

Herb samples; *T. spathacea* (Oyster plant), *A. paniculata* (Kariyat), *E. acicularis* (Needle- Spike Rush), *A. concinna* (Soap pod), *P. niruri* (Stonebreaker), and *T. cordifolia* (Gulancha) were collected locally from Pakthongchai, Nakhonratchasima provience, Thailand. The whole part of fresh herb wwas used. Each herb was clean using tab water and cut into small pieces. Then, herbs were stored in refrigerator at 6°C until use.

Extraction

Each herb was maculated into each solvents; 95% ethanol, chloroform, sterile distilled water, hexane, using ration 1:3 for 48 hours at room temperature and were stirred every 12 hours. For autoclaving, each herb was added into distilled water using ration 1:3 then was autoclaved at 121°C, 15 PSI for 15 minutes. And then, each herb was filtered and centrifuged at 5000 rpm for 5 minutes. All crude extracts were concentrated using water bath at 45°C until became slurry. All crude extracts were kept in freezer at -20°C until use.

Antibacterial Assay

BSAC disc diffusion method for antimicrobial Susceptibility Testing version 8[10] was used for antibacterial activity assay. The 100 µL of bacteria (approximately 1.5×10^8 CFU/ml) was swab on Mueller-Hinton agar (MHA) plate. The 15 µl of 15 mg/ml each crude extracts were used to test antibacterial activity against *E. coli* ATCC25822, *S. enterica* Typhimurium U302 (DT1046), *S. enterica* Enteritidis (human), *S. enterica* 4,5,12: i-(human) US clone, *B.cereus*, and *L. monocytogenes* 10403S.

The 15 µl of each solvents and 100 mg/ml Penicillin-G were used as negative and positive control, respectively. The inhibition zones were measured expressed as cm of inhibition zone to determine the effectiveness of the each crude extracts against each bacterium. The experiment was done in duplicate and three replications independently

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The MIC (Minimum Inhibitory Concentration) method was modified from BSAC disc diffusion method for Antimicrobial Susceptibility Testing version 8[10]. Only herb, which showed positive antibacterial activity, was tested for MIC. For MIC test, the herb extracted as following concentration; 0, 32, 64, 128, and 256 µl / ml, was added into 1 ml fresh NB. Then 100 µl/ml of culture with OD600 reach 0.1 (early log phase) was inoculated, and incubated at 37°C for 24 hours.

The negative MIC result tubes were chosen for MBC determination by taking 1 loop of negative MIC was streaked on NA then incubated

at 37°C 24 hours. The growth of culture in each plate was observed. The experiment was done in duplicate and three replications independently

RESULTS AND DISCUSSION

The results showed that the antibacterial activity was mostly in 95% ethanol and chloroform extraction conditions in all six crude extracts; *T. spathacea*, *A. paniculata*, *E. acicularis*, *A. concinna*, *P. niruri*, and *T. cordifolia* as in Table 1-6.

T. spathacea or Hawaiian Dwarf, or *Rhoeo spathacea* is a succulent herb in the Family Commelinaceae *T. spathacea* have been recognized as a functional food particularly in South America [11]. In Thai folkmedicine, it is used to relieve fever, cough and bronchitis [12]. Only 95% ethanol and chloroform *T. spathacea* crude extract showed antibacterial activity against both positive; *B. cereus*, and *L. monocytogenes* 10403S, and negative bacterial; *S. enterica Typhimurium* U302 (DT1046), *S. enterica* Enteritidis (human),

Table 1. The antibacterial activity (clear zone in mm) of *T. spathacea* crude extracts under five different extractions condition against six foodborne pathogenic bacteria

Microorganism	95% Ethanol	Chloroform	Hexane	Sterile distilled water	Autoclave at 121°C 15 PSI for 15 minutes
E. coli ATCC25822	7.75±0.96	-	-	-	-
S. enterica Typhimurium U302 (DT1046)	9.25±0.50	10.50±1.29	-	-	-
S. enterica Enteritidis (human)	8.75±0.96	8.75±1.71	-	-	-
S. enterica 4,5,12: i-(human) US clone	9.25±1.50	9.25±0.96	-	-	-
B. cereus	8.00±0.82	8.00±0.82	-	-	-
L. monocytogenes 10403S	6.25±0.50	8.75±0.96	-	-	-

Table 2. The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of *T. spathacea* crude extracts under five different extractions condition against six foodborne pathogenic bacteria

Microorganism	MIC (µl/ml)		MBC (µl/ml)	
	95% Ethanol	Chloroform	95% Ethanol	Chloroform
E. coli ATCC25822	128	-	128	-
S. enterica Typhimurium U302 (DT1046)	128	64	128	64
S. enterica Enteritidis (human)	128	32	128	32
S. enterica 4,5,12: i-(human) US clone	128	64	128	64
B. cereus	128	32	256	32
L. monocytogenes 10403S	128	32	128	64

Table 3. The antibacterial activity (clear zone in mm) of *A. paniculata* crude extracts under five different extractions condition against six foodborne pathogenic bacteria

Microorganism	95% Ethanol	Chloroform	Hexane	Sterile distilled water	Autoclave at 121°C 15 PSI for 15 minutes
E. coli ATCC25822	7.25±0.50	-	-	-	-
S. enterica Typhimurium U302 (DT1046)	8.25±0.96	9.50±1.73	-	-	-
S. enterica Enteritidis (human)	7.25±0.50	8.50±1.29	-	-	-
S. enterica 4,5,12: i-(human) US clone	9.25±1.26	10.75±0.96	-	-	-
B. cereus	8.00±0.82	8.25±0.50	-	-	-
L. monocytogenes 10403S	6.50±0.58	8.25±2.06	-	-	-

S. enterica 4,5,12: i-(human) US clone, excepted for *E. coli* ATCC25822. The highest antibacterial activity was chloroform extract against *S. enterica* Typhimurium U302 (DT1046) which diameter of clear zone is 10.50±1.29 mm.

Only 95% ethanol and chloroform *T. spathacea* crude extract, which had the antibacterial activity, were assayed for minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) as shown in table 2. The MIC of *T. spathacea* crude extracts was between 32-128 µl/ml against six bacteria while the MBC was between 32-256 µl/ml. *T. spathacea* extract contained phenolic compounds, tannin and flavonoid [11]. It was reported to possess antimicrobial, insecticidal, anti-inflammatory, anticancer and anti-fertility activities [13]. *T. spathacea* have stimulating activity on human lymphocytes[12]. The *T. spathacea* aqueous leaf extracts in the forms of decoction and infusion, were found to have flavonoid and antioxidant activity [11]. The decoction and infusion also

Table 4. The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of *A. paniculata* crude extracts under five different extractions condition against six foodborne pathogenic bacteria

Microorganism	MIC (µl/ml)		MBC (µl/ml)	
	95% Ethanol	Chloroform	95% Ethanol	Chloroform
E. coli ATCC25822	128	-	128	-
S. enterica Typhimurium U302 (DT1046)	128	64	128	64
S. enterica Enteritidis (human)	128	64	128	64
S. enterica 4,5,12: i-(human) US clone	128	32	128	32
B. cereus	128	32	256	32
L. monocytogenes 10403S	128	32	128	32

Table 5. The antibacterial activity (clear zone in mm) of *E. acicularis* crude extracts under five different extractions condition against six foodborne pathogenic bacteria

Microorganism	95% Ethanol	Chloroform	Hexane	Sterile distilled water	Autoclave at 121°C 15 PSI for 15 minutes
E. coli ATCC25822	8.00± 1.15	-	-	-	-
S. enterica Typhimurium U302 (DT1046)	9.75±2.22	10.50±1.91	-	-	-
S. enterica Enteritidis (human)	-	10.00±1.63	-	-	-
S. enterica 4,5,12: i-(human) US clone	7.75± 0.96	8.50±0.58	-	-	-
B. cereus	8.50± 0.58	8.00±0.82	-	-	-
L. monocytogenes 10403S	8.50± 1.00	9.25±0.96	-	-	-

Table 6. The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of *E. acicularis* crude extracts under five different extractions condition against six foodborne pathogenic bacteria

Microorganism	MIC (µl/ml)		MBC (µl/ml)	
	95% Ethanol	Chloroform	95% Ethanol	Chloroform
E. coli ATCC25822	64	-	64	-
S. enterica Typhimurium U302 (DT1046)	64	64	32	32
S. enterica Enteritidis (human)	-	-	32	32
S. enterica 4,5,12: i-(human) US clone	128	128	64	64
B. cereus	128	256	32	256
L. monocytogenes 10403S	128	128	32	256

exhibited antibacterial activity against six species of Gram positive and four species of Gram negative bacteria, notably methicillin-resistant *Staphylococcus aureus* and *Neisseria gonorrhoeae* [11]. The primary anthocyanin in *T. spathacea* leaves was isolated and identified by NMR to be rhoeonin which contribute as color [14]. For *T. spathacea*, only 95% ethanol and chloroform extracts showed the antibacterial activity against all bacteria, while other three extraction conditions had no antibacterial activity. These reports [11,14] confirm the results in this experiment that photochemical compounds in *T. spathacea* are the active compound which have antibacterial activity.

A. paniculata or Kariyat or Creat, or Chuanxinlian is a seasonal plant in the Family of Acanthaceae [15]. The whole plant has bitter taste. *A. paniculata* also contained many of flavanoids and polyphenol as their active compounds [15]. In this experiment, only 95% ethanol and chloroform *A. paniculata* crude extracts showed the

Table 7. The antibacterial activity (clear zone in mm) of *A. concinna* crude extracts under five different extractions condition against six foodborne pathogenic bacteria

Microorganism	95% Ethanol	Chloroform	Hexane	Sterile distilled water	Autoclave at 121°C 15 PSI for 15 minutes
E. coli ATCC25822	7.25±0.50	7.25±0.50	-	-	-
S. enterica Typhimurium U302 (DT1046)	8.75±2.06	10.00±0.82	-	-	-
S. enterica Enteritidis (human)	-	8.25±0.50	-	-	-
S. enterica 4,5,12: i-(human) US clone	10.50±1.29	9.25±0.96	-	-	-
B. cereus	7.25±0.50	7.50±0.58	-	-	-
L. monocytogenes 10403S	7.25±0.96	9.25±0.96	-	-	-

Table 8. The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of *A. concinna* crude extracts under five different extractions condition against six foodborne pathogenic bacteria

Microorganism	MIC (µl/ml)		MBC (µl/ml)	
	95% Ethanol	Chloroform	95% Ethanol	Chloroform
E. coli ATCC25822	64	32	64	32
S. enterica Typhimurium U302 (DT1046)	256	64	256	32
S. enterica Enteritidis (human)	-	-	32	32
S. enterica 4,5,12: i-(human) US clone	128	64	128	64
B. cereus	128	32	256	256
L. monocytogenes 10403S	128	32	128	256

Table 9. The antibacterial activity (clear zone in mm) of *P. niruri* crude extracts under five different extractions condition against six foodborne pathogenic bacteria

Microorganism	95% Ethanol	Chloroform	Hexane	Sterile distilled water	Autoclave at 121°C 15 PSI for 15 minutes
E. coli ATCC25822	8.25±0.50	-	-	-	-
S. enterica Typhimurium U302 (DT1046)	7.50±0.58	10.25±1.50	-	7.75±0.50	-
S. enterica Enteritidis (human)	-	9.25±1.71	-	-	-
S. enterica 4,5,12: i-(human) US clone	8.75±0.96	9.75±0.96	-	-	-
B. cereus	7.50±0.58	8.25±0.50	-	-	-
L. monocytogenes 10403S	8.25±1.50	10.00±1.41	-	-	-

antibacterial activity against both gram positive and negative bacteria, while other three extraction conditions had the antibacterial activity. The highest antibacterial activity was chloroform extract against *S. enterica* 4,5,12: i-(human) US clone which diameter of clear zone is 10.75±0.96 mm as showed in table 3.

Only 95% ethanol and chloroform *A. paniculata* crude extract, which had the antibacterial activity, were assayed for minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) as shown in table 4. The MIC of *A. paniculata* crude extracts was between 32-128 µl/ml against six bacteria while the MBC was between 32-256 µl/ml. The major active compound of *A. paniculata* are lactone group; andrographolide, deoxy-andrographolide, neoandrographolide, dehydroandrographolide [16].

Table 10. The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of *P. niruri* crude extracts under five different extractions condition against six foodborne pathogenic bacteria

Microorganism	MIC (µl/ml)			MBC (µl/ml)		
	95% Ethanol	Chloroform	Sterile distilled water	95% Ethanol	Chloroform	Sterile distilled water
E. coli ATCC25822	64	-		64	-	-
S. enterica Typhimurium U302 (DT1046)	64	64	256	64	64	>256
S. enterica Enteritidis (human)	-	32	-	-	32	-
S. enterica 4,5,12: i-(human) US clone	128	32	-	128	32	-
B. cereus	128	32	-	256	256	-
L. monocytogenes 10403S	128	32	-	128	256	-

Table 11. The antibacterial activity (clear zone in mm) of *T. cordifolia* crude extracts under five different extractions condition against six foodborne pathogenic bacteria

Microorganism	95% Ethanol	Chloroform	Hexane	Sterile distilled water	Autoclave at 121°C 15 PSI for 15 minutes
E. coli ATCC25822	7.75±0.96	7.25±0.50	-	-	-
S. enterica Typhimurium U302 (DT1046)	9.00±1.41	10.50±2.08	-	7.75±0.96	-
S. enterica Enteritidis (human)	-	9.25±2.06	-	-	-
S. enterica 4,5,12: i-(human) US clone	8.25±0.50	9.25±1.26	-	-	-
B. cereus	7.25±0.50	8.75±1.26	-	-	-
L. monocytogenes 10403S	7.00±0.82	8.75±0.96	-	-	-

Table 12. The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of *T. cordifolia* crude extracts under five different extractions condition against six foodborne pathogenic bacteria

Microorganism	MIC (µl/ml)			MBC (µl/ml)		
	95% Ethanol	Chloroform	Sterile distilled water	95% Ethanol	Chloroform	Sterile distilled water
E. coli ATCC25822	128	32	-	128	32	-
S. enterica Typhimurium U302 (DT1046)	128	64	256	128	64	>256
S. enterica Enteritidis (human)	-	64	-	-	64	-
S. enterica 4,5,12: i-(human) US clone	128	64	-	128	64	-
B. cereus	128	32	-	256	256	-
L. monocytogenes 10403S	128	32	-	128	128	-

Diterpenoids , flavanoids and polyphenols are also major active compound in *A. Paniculata* [15]. The other study reported that the water extract of boiling roots have effective *against S. aureus* [17]. For methanol extract of stem have antibacterial activity against *Proteus vulgaris*, stem and leaves powder extract have antibacterial activity against *Shigella* bacteria but it is not effective against *Cholera* [17]. The ethanol extract of leaves have antibacterial activity against *S. aureus* and *E. coli* [17]. The study of ethanol extract of *A. paniculata* powder extracted is effective against *B. cereus* and *L. monocytogenes* under both normal and osmotic stress [18,19]. However, it may be that individual antibacterial flavonoids have multiple cellular targets, rather than one specific site of action; inhibition of nucleic acid synthesis, cytoplasmic membrane function, and energy metabolism [20]. There was a study indicated that 2´,4´- or 2´,6´ dihydroxylation of the B ring and 5,7-dihydroxylation of the A ring in the flavanone structure was important for anti-methicillin-resistant *S. aureus* activity [21]. The former study investigated that no antibacterial activity of andrographolide against any of *E. coli, Shigella sonnei, S. aureus, P. aeroginosa, S. pneumoniae, S. pyogenes, Legionella pneumophila*, and *Bordetella pertusis* [22]. These reports [15,16,17,18,19,20,21] confirm the results in this experiment that photochemical compounds in *A. paniculata* are the active compound which have antibacterial activity.

E. acicularis or needle spikerush or dwarf hairgrass is in family *Cyperaceae*. In this experiment, only 95% ethanol and chloroform *E. acicularis* crude extracts showed the antibacterial activity against both gram positive and negative bacteria, while other three extraction conditions had the antibacterial activity. The highest antibacterial activity was chloroform extract against *S. enterica* Typhimurium U302 (DT1046) which diameter of clear zone is 10.50±1.91mm as showed in table 5. *E. acicularis* crude extract showed antimicrobial activity against *S. aureus* with inhibition zone 10±0.7 mm. and also had antioxidant activity (DPPH-IC50) more than 20 ìg/mL[23]. *E. acicularis*'s seed crude extract also had antioxidant activity [24].

Only 95% ethanol and chloroform *E. acicularis*'crude extract, which had the antibacterial activity, were assayed for minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) as shown in table 6. The MIC of *E. acicularis*'crude extracts was between 32-256 µl/ml against six bacteria while the MBC was between 32-256 µl/ml. *Eleocharis* sp. contained "a high concentration of â-sitosterol and lupeol but the most dominant component was â-sitostanol (24-ethylcholestan-3â-ol) [25]. This plant have no report on antibacterial activity. It means that â-sitosterol and lupeol have antibacterial capacity to against most of bacteria that used in this experiment.

A. concinna or som-poi is important medicinal plant belonging to family Acaciaceae [26]. The bark contains high levels of saponins, which are foaming agents that are found in several other plant species [27]. In this experiment, *A. concinna*'s leaf was used. It was found out that only 95% ethanol and chloroform *A. concinna* crude extracts showed the antibacterial activity against both gram positive and negative bacteria, while other three extraction conditions had the antibacterial activity. The highest antibacterial activity was 95% ethanol extract against *S. enterica* 4,5,12: i-(human) US clone which diameter of clear zone is 10.50±1.29 mm as showed in table 7.

Only 95% ethanol and chloroform *A. concinna* crude extract, which had the antibacterial activity, were assayed for minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) as shown in table 8. The MIC of *E. acicularis*'crude extracts was between 32-256 µl/ml against six bacteria while the MBC was between 32-256 µl/ml. In previous study, the crude extracted of *A. concinna* pod can inhibit *B. subtilis, E.coli, S. aureus, P.aeruginosa,* and *K. pneumonia* [28]. The other study of bark extract is effective against *S.typhi, P.nirabilis, S.aureus, Yersinia, S.epidermis, K. pnemonia, P. aerogenosa, E.coli,* and *B.subtilis* under methanol, ethanol, and chloroform extraction condition. These extraction can extracted active compounds included Phenol, Tannin, fat and fixed oil, Flavanoids, Saponin, and quinine [29]. The benzene, methanol and aqueous extracts of fresh pods of *A. concinna* showed maximum activity against K. *pneumoniae, B. subtilis, E. coli*, followed by *P. aeruginosa* and *S. aureus* [30]. Phytochemical study showed that the crude extract of *A. concinna* pod consisted of

alkaloids, flavonoids, saponin and tannin but none of antraquinone and cyanotic glycosides [31]. It means that all these compounds have antibacterial activity against most bacteria used in this experiment.

P. niruri or dukong anak or stonebreaker's leaf crude extract only 95% ethanol, chloroform, and sterile distilled water crude extracts showed the antibacterial activity against both gram positive and negative bacteria, while other three extraction conditions had the antibacterial activity. The highest antibacterial activity was chloroform extract against *S. enterica* Typhimurium U302 (DT1046) which diameter of clear zone is 10.25±1.50 mm as showed in table 9. The antimicrobial mechanism showed that the bacterial cells, after exposure to the *P. niruri* extract, showed complete alteration in their morphology, followed by collapse of the cells beyond repair [32]. The study revealed that the *P. niruri* methanolic extract may be an effective antibacterial agent to treat bacterial infections since the extract exhibited significant antimicrobial potency, comparable with that of the standard antibiotic chloramphenicol [32].

95% ethanol, chloroform and sterile distilled water *P. niruri* crude extract, which had the antibacterial activity, were assayed for minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) as shown in table 8. The MIC of *P. niruri* crude extracts was between 32-128 µl/ml against six bacteria while the MBC was between 32-256 µl/ml. *P. niruri* contained flavonoids, alkaloids, terpenoids, lignans, polyphenols, tannins, coumarins and saponins, have been identified from various parts have effect to against the Hepatitis B and other viral infections [33]. The other study report that this plant extract is effective against *Staphylococcus, Micrococcus*, and *Pasteurella* bacteria under methanol, DCM with methanol (1:1), and aqueous extraction [34]. These are some active compounds that can extract from *P. niruri*, Alkaloids, astragalin, brevifolin, carboxylic acids, corilagin, cymene, ellagic acid, ellagitannins, gallocatechins, geraniin, hypophyllanthin, lignans, lintetralins, and lupeols [34]. The *P. niruni* extracte had antibacterial activity against *Candida albicans, B. pumilus, Micrococcus luteus, K. pneumonia, S. aureus, B. subtilis*, and *E. col*i under mathanol extraction condition. These are the active compounds that can extract from this plant flavonoids, alkaloids, terpenoids, lignans, polyphenols, tannins, coumarins and saponins [35]. These active compounds had been report as for their antimicrobial activity.

T. cordifolia or guduchi crude extract only 95% ethanol, chloroform, and sterile distilled water crude extracts showed the antibacterial activity against both gram positive and negative bacteria, while other three extraction conditions had the antibacterial activity. The highest antibacterial activity was chloroform extract against *S. enterica* Typhimurium U302 (DT1046) which diameter of clear zone is 10.50±2.08mm as showed in table 11. *T. cordifolia* composed of difference active compounds, for example, alkaloids, diterpenoid lactones, glycosides, steroids, sesquiterpenoid, aliphatic [36,37]. These active compounds had been report as for their antimicrobial activity.

95% ethanol, chloroform and sterile distilled water *T. cordifolia* crude extract, which had the antibacterial activity, were assayed for minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) as shown in table 8. The MIC of *T. cordifolia* crude extracts was between 32-128 µl/ml against six bacteria while the MBC was between 32-256 µl/ml. *T. cordifolia* ether extract of stem (aerial part) is effective against Mycobacterium tuberculosis and aqueous extract is effective against *E. coli* and *S. aureus* with active compounds included alkaloids, diterpenoid lactones, glycosides, steroids, sesquiterpenoids, phenolics, aliphatic compounds, and polysaccharides [38].

aqueous, ethanol, methanol, and acetone *T. cordifolia* crude extracts had antibacterial activity *against E. coli, S. aureus, K. pneumoniae, P. vulgaris, S. typhi, S. flexneri, S. paratyphi, S. typhimurium, P. aeruginosa, E. aerogene,* and *Serratia marcesensesi* with active compounds included â-sitosterol, hydroxy ecdysone, ecdysterone, and giloinsterol [39,40].

CONCLUSION

The six Thai local herbs; *T. spathacea, A. paniculata, E. acicularis, A. concinna, P. niruri,* and *T. cordifolia*) showed the antibacterial activity against foodborne pathogenic bacteria. This is the stepping stone for further application in food

safety, cosmetic industry, and pharmaceutical industry.

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