Antibacterial Activity of Thai Medicinal Plant Extracts Against Microorganism Isolated from Post-Weaning Diarrhea in Piglets

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Background: *Post-weaning bacterial infections in piglets caused by resistant strains have increased dramatically. Thai medicinal plants such as Piper retrofractum, Piper leptostachyum, Piper sarmentosum, Zingiber officinale, Plumbago indica, Piper betle, Caesalpinia sappan, Garcinia mangostana etc have been widely used in Thai traditional medicine to treat diarrhea. Thus, these plants were investigated antimicrobial activity against gram negative strain isolated from piglets with diarrhea.*

Objective: *To investigate antimicrobial activity of Thai medicinal plants and their isolated compounds in order to develop an alternative treatment against bacteria causing post-weaning diarrhea in piglets.*

Material and Method: *Antimicrobial activity of some Thai medicinal plants and their compounds were tested using disc diffusion and broth dilution methods against bacteria associated with diarrheal disease including Escherichia coli and Salmonella spp. The extraction was performed by maceration in 95% ethanol.*

Results: *The results showed that all tested strains were sensitive to P. betle extract. As well as Plumbagin compound from Plumbago indica also showed antimicrobial activity against all microbes the same of P. betle extract with MIC between 5 to 10 mg/ml, which are different from the group of antibiotics with MIC values between 0.19-2.5 mg/ml, P. betle extracts inhibited Escherichia coli and Salmonella spp while some antibiotics can inhibit only some types.*

Conclusion: *The results support the use of Thai medicinal plants for treatment of diarrhea caused by these bacteria. This study also provides basic knowledge on antimicrobial activity against diarrheal microbe isolated from piglets. These results lead to further development of an effective formula of Thai medicinal plants for diarrheal disease in post-weaned piglets and other infectious diseases in the future.*

Keywords: *Antibacterial activity, Thai medicinal plants, Post-weaning diarrhea*

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Post-weaning diarrhea (PWD) is a multi-factorial condition characterized by frequent discharge of watery feces that can cause a growth check, morbidity and mortality[1,2]. This condition is typically associated with fecal shedding of β-hemolytic enterotoxigenic strains of *Escherichia coli* (ETEC). These strains proliferate in the small intestine after they attach to epithelial receptors and release toxins[2] although it's recognized that seemingly healthy pigs may also shed small numbers of these bacteria[3,4].

Virulence attributes of the *E. coli* associated with PWD are poorly defined and seem to be far more complex than those of neonatal colibacillosis. Some of these isolates produce K88 adhesin[5] and one or more of three enterotoxins, a heat-labile enterotoxin (LT) and two heat-stable enterotoxins (ST). Some produce cytotoxins (VT) detectable by Vero cell assay[6-8] but the role of VT in diarrhea and its relationship to the VT produced by edema disease strains[9] are unknown. Some enterotoxigenic strains associated with the PWD do not have known adhesion factors[10].

Thai traditional medicine used many plants to treat diarrhea. The Thadbunchop Scripture of Thai traditional medicine book is the lesson of the

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gastrointestinal tract system or diseases and feces characteristic[11]. This scripture composed many remedies for diarrheal treatment. Twelve plants which are in anti-diarrheal remedies for humans were chosen to investigate against bacterial causing PWD which isolated from post weaning piglets.

Hence, this study aims to investigate the antimicrobial activity of the extracts of Thai medicinal plants and their compounds against the bacteria in order to develop an effective formula for an alternative traditional plant-based treatment of bacterial infections. As a consequence, it would prevent and slow the emergence of resistance among the bacteria causing diarrhea in post-weaned piglets.

Material and Method

Plant materials and extraction

Twelve Thai medicinal plants were selected from Thai traditional remedies which used for diarrheal treatment in the present study. They are fruits of *Piper retrofractum, Phyllanthus emblica, Terminalia chebula* and *Terminalia bellirica*, root of *Piper sarmentosum* and *Plumbago indica*, stem of *Piper leptostachyum* and *Piper nigrum*, rhizome of *Zingiber officinale*, leaf of *Piper betle*, husk and peduncle of *Garcinia mangostana* and wood of *Caesalpinia sappan*. All plant materials were collected from many parts of Thailand. They were kept and identified voucher specimen (No. BKF) was deposited at the Bangkok Forest Herbarium, Herbarium Department of National Parks, Wildlife and Plant Conservation, Thailand and A voucher specimen (No. SKP) by Southern Center of Thai Medicinal plant at Faculty of Pharmaceutical Science, Prince of Songkhla University, Songkhla, Thailand. Some plant materials were deposited at the herbarium of Prince of Songkhla University the place of collection is shown in Table 1. Plant materials were cleaned with water, sliced to be small pieces and dried in a hot air oven at 50°C. Dried plant materials were grinded to be crude powder.

Extraction method

Maceration

The crude powder of each plant was macerated in 95% ethanol for 3 days and filtered through a Whatman No. 1 filter paper. The residues were continuously macerated 2 times. Crude extracts, from 3 cycles of maceration, were combined and concentrated by rotary evaporator followed with vacuum drying and the 95% EtOH crude extracts were stored at -20°C before used. This extract obtained the ethanolic extract and pure compounds from SIGMA® is a matter of substance compared to contain herbal extracts such as Plumbagin, Piperine, Eugenol, Myristicin, Gingerol, Shogaol and Brazilin.

All extracts were calculated the percentage of yield (Shown in Table 1).

Determination of antimicrobial activities

Microorganisms

Sources of infection were isolated from 32 cases of diarrheal piglets in Photharam, Ratchaburi. The bacterial strains were *Escherichia coli*, *Salmonella typhi* and *Salmonella* spp. These bacteria were identified by Betrago Science Center Co, Ltd. the *E. coli* ATCC25927 as standard microbe obtained from Department of Medical Science, Ministry of Public Health, Thailand.

Preparation of inoculums

Isolated colonies of bacteria were cultured into Mueller-Hinton broth (MHB) at 37°C for 2 hours. After that, suspension turbidity was adjusted to 0.5 McFarland standard by densitometer (GrantBio, England).

Preparation of test disc

The ethanolic extracts of all plants were dissolved in 1% dimethylsulphoxide (DMSO) to a final concentration 5 mg/ml.

Antimicrobial activities

The antimicrobial activities were determined by disc diffusion method according to NCCLS (2004)[12] for screening and microtiter plate-based antibacterial assay described previously[13] for determination of minimal inhibitory concentration (MIC) of the extracts against the bacteria. The concentration of Thai medicinal plants was 5 mg/ml per disc.

In briefly, sterilized filter paper discs (6 mm in diameter) were impregnated with 10 µl of the extracts. Turbidity to 0.5 McFarland standard were diluted with Mueller-Hinton Broth (MHB) and swabbed the entire the Mueller-Hinton Agar (MHA) surface evenly in three direction with sterile cotton swab, leave the plate for 3-5 minutes. After that, place the dried paper discs on to the lawn on the Mueller-Hinton Agar (MHA). Plates with microorganism and test samples were incubated at 37°C for 24 hours. Finally, measurement of inhibition zone (clear zone) around the disc was interpreted the susceptibility and resistance of the microorganism to test antimicrobial activity. The

Table 1. The ethnobotanical data and Thai traditional medicine used of Thai medicinal plants

Botanical name	Thai name	Plant Collected from	Voucher specimen number	Part of used	% yield of extracts	Thai traditional medicine used
1) *Caesalpinia sappan* Linn. LEGUMINOSAE	Fang	Kanchanaburi	BKF 192200/ SKP098031901	Stem	8.467	Blood tonic, menorrhagia, lecorrhoea and diabetes
2) *Garcinia mangostana* Linn. CLUSLACEAE	Mang-Khut	Yala	SKP083071301	Peel	27.087	Treatment of chronic diarrhea and intestinal diseases
3) *Phyllanthus emblica* Linn. PHYLLAMTHACEAE	Ma-Kham-Pom	Ratchaburi	SKP071160501	Fruit	28.260	Impregnate throat, cough expectorants, diarrhea and diuretic
4) *Piper betle* Linn. (Green leaf) PIPERACEAE	Phlu-Khiao	Ratchaburi	SKP1461602	Leaves	17.841	Treatment of dysentery, sluggish, bloated stomach and carminative
5) *Piper betle* Linn. (Yellow leaf) PIPERACEAE	Phlu-Luang	Ratchaburi	SKP1461602	Leaves	17.368	Treatment of dysentery, sluggish, bloated stomach and carminative
6) *Piper leptostachyum* Wall exMiq PIPERACEAE	Sa-Khan-Nu	Chiang Mai	BKF192199/	Stem	5.865	Treatment of carminatnive
7) *Piper retrofractum* Linn. PIPERACEAE	Dee-Pli	Ratchaburi	BKF192196/ SKP146160301	Fruit	7.182	Carminative, element tonic and antidiarrheal
8) *Piper nigrum* Linn. PIPERACEAE	Prig-Thri	Juntaburi	SKP146161401	Seed	6.454	Carminative, element, expectorate and diuretic
9) *Piper sarmentosum* Roxb. PIPERACEAE	Cha-Phlu	Ratchaburi	BKF192197/ SKP146161901	Root	6.329	Expectorant, carminative and flatulence
10) *Plumbago indica* Linn. PLUMBAGLNACEAE	Chettamun phloengdaeng	Bangkok	BKF192195/ SKP148160901	Root	14.782	Carminative, stomachache, antidiarrheic and hemorrhoids
11) *Terminalia bellirica* Roxb. COMBRETACEAE	Smo-Phi-Phek	Kanchanaburi	SKP206261501	Fruit	16.780	Treatment of laxative, Diarrhea diarrhea
12) *Terminalia chebula* Retz. COMBRETACEAE	Samo-Thai	Kanchanaburi	SKP049200301	Fruit	8.500	Laxative, Anti-diarrhea, Relieve cough
13) *Zingiber officinale* Roscoe ZINGIBERACEAE	Khing	Ratchaburi	BKF192198/ SKP206261501	Rhizome	8.528	Promote blood circulation, Carminative, Reduce phlegm, Antiemetic

BKF = A voucher specimen (No. BKF) was deposited at the Bangkok Forest Herbarium, Herbarium Department of National Parks, Wildlife and Plant Conservation, Thailand.
SKP = A voucher specimen (No. SKP) was deposited at the Faculty of Pharmaceutical Sciences, Prince of Songkla University, Thailand.

zone of inhibition (clear zone) were calculated by measuring the diameter. Ampicillin, gentamycin and norfloxacin as positive standard control were prepared in concentration 1 μg/ml.

The minimal inhibitory concentration (MIC) values were determined by microdilution assay. The ethanolic extracts for testing were dissolved in Dimethylsulfoxide (DMSO) (Merck Millipore, Tullagreen). After that, the samples were prepared to be concentration 10 mg/ml. The cultures were prepared for 24 hours. The inoculum was adjusted turbidily equal to 0.5 McFarland standard and diluted with sterile Mueller-Hinton Broth (MHB) at 1: 200 to give a final concentration of $5x10^5$ CFU/ml. Serial two-fold dilutions of each samples were diluted. Then, added 50 μl of the inoculums into sterile 96 wells microtiter plates. The plates were covered with a sterile plate sealer and plastic wrap. Then they were incubated at 37°C, 24 hours. After the specified time, added 10 μl of resazurin solution at concentration 1 mg/ml into each well. Then, they were continuously incubated at 37°C for 2 hours. The result was interpreted by the change of color of resazurin. The antimicrobial testing was performed in triplicate.

Statistical analysis

All data were carried out in triplicate. Values of different parameters were expressed as the mean ± standard deviation. Statistical analysis was performed using Prism Software.

Results

From disc diffusion testing showed in Table 2, the ethanolic extracts of *P. betle* and *C. sappan* were highly effective in antimicrobial activities against all types of microbe; *Escherichia coli* and *Salmonella* spp. which are isolated in diarrheal piglets, as well as Plumbagin. Surprisingly, all antibiotic had no

Table 2. Antimicrobial activity of extracts of Antibiotics, pure compounds and single herbs by disc diffusion method

Substance	Extract	Gram-negative mean ± SD disc diffusion (mm)			
		E. coli ATCC25927	*E. coli* (Pathogen)	*Samonella typhi*	*Samonella* spp. (Pathogen)
Antibiotics	Ampicillin	20.75±0.50	NI	NI	NI
	Gentamicin	21.25±0.50	NI	20.00±0.50	7.18±0.40
	Norfloxacin	33.50±0.58	NI	29.5±0.58	20.09±0.54
Pure compounds	Plumbagin	14.75±0.50	10.90±0.70	10.00±0.68	14.09±0.30
	Piperine	NI	NI	NI	NI
	Eugenol	NI	NI	NI	NI
	Myristicin	NI	NI	NI	NI
	Gingerol	NI	NI	NI	NI
	Shogaol	NI	NI	NI	NI
	Brazilin	NI	NI	NI	NI
Single herbs	*Piper retrofractum*	NI	NI	NI	NI
	Piper sarmentosum	NI	NI	NI	NI
	Piper leptostachym	NI	NI	NI	NI
	Plumbago indica	NI	NI	NI	NI
	Zingiber officinale	NI	NI	NI	NI
	Phyllanthus emblica	NI	NI	NI	NI
	Terminalia chebula	NI	NI	NI	NI
	Terminalia bellirica	NI	NI	NI	NI
	Piper nigrum	NI	NI	NI	NI
	Piper betle (Green leaf)	12.00±0.82	11.33±0.66	13.67±0.58	12.18±0.40
	Piper betle (Yellow leaf)	13.50±0.58	12.10±0.62	13.69±0.58	11.82±0.40
	Caesalpinia sappan	7.50±0.58	NI	8.09±0.30	8.09±0.30
	Garcinia mangostana	NI	NI	NI	NI
	Pedicle of mangosteen	NI	NI	NI	NI

NI = no inhibition

zone against *E. coli* which isolated from diarrheal piglets or pathogen. *P. betle* and *C. sappan* exhibited higher antibacterial activity against resistant strain *E. coli* pathogen than ampicillin, gentamicin and norfloxacin. They also showed higher effective against *Salmonella* pathogen and *Salmonella typhi* than ampicillin. Ampicillin had no effect against two types of *Salmonella* spp. but *P. betle* showed clear zone 8.09-13.96 mm. However, they showed less antibacterial against two *Salmonella typhi* than gentamicin and norfloxacin which produced clear zone 20.0-29.5 mm except gantamicin which showed less antibacterial against *Salmonella* pathogen than *P. betle* (clear zone = 7.08 and 12.18 mm and MIC $\geq$10 and 10 mg/ml, respectively). Green leaves of *P. betle* showed no significant difference antibacterial activity against all type of gram negative bacteria from yellow leaves. Only one pure compound plumbagin, a main compound from *Plumbago indica*, showed high antibacterial activity against all microbes in range of 10.0-14.75 mm, but *Plumbago indica* had no antimicrobial activity. However, considering from MIC values and green leaves and yellow leaf of *P. betle* showed MIC less than 10 mg/ml (Table 3) and *P. betle* also showed the best antibacterial activity against all types of microbe, the other plants showed MIC more than 10 mg/ml. In addition, *P. betle* showed better activity against *E. coli* pathogen than ampicillin, gentamycin and norfloxacin. Thus *P. betle* should be developed as antibiotic against *E. coli* and *Salmonella* spp. in piglets with diarrhea.

Discussion

Two Thai medicinal plant extracts showed antibacterial activity against pathogen *E. coli* in piglets

Table 3. Antimicrobial activity of extracts of antibiotics, pure compounds and single herbs by minimum inhibitory concentration method

Substance	Extract	Gram-negative mean $\pm$ SD minimum inhibitory concentration (mg/ml)			
		E. coli ATCC25927	*E. coli* (Pathogen)	*Samonella typhi*	*Samonella* spp. (Pathogen)
Antibiotics	Ampicillin	2.5	NI	NI	NI
	Gentamicin	>10	NI	0.31	NI
	Norfloxacin	>10	NI	0.19	1.20
Pure compounds	Plumbagin	>10	>10	10	5
	Piperine	NI	NI	NI	NI
	Eugenol	NI	NI	NI	NI
	Myristicin	NI	NI	NI	NI
	Gingerol	NI	NI	NI	NI
	Shogaol	NI	NI	NI	NI
	Brazilin	NI	NI	NI	NI
Single herbs	*Piper retrofractum*	NI	NI	NI	NI
	Piper sarmentosum	NI	NI	NI	NI
	Piper leptostachym	NI	NI	NI	NI
	Plumbago indica	NI	NI	NI	NI
	Zingiber officinale	NI	NI	NI	NI
	Phyllanthus emblica	NI	NI	NI	NI
	Terminalia chebula	NI	NI	NI	NI
	Terminalia bellirica	NI	NI	NI	NI
	Piper nigrum	NI	NI	NI	NI
	Piper betle (Green leaf)	10	10	10	5
	Piper betle (Yellow leaf)	10	10	10	5
	Caesalpinia sappan	>10	>10	10	>10
	Garcinia mangostana	NI	NI	NI	NI
	Pedicle of mangosteen	NI	NI	NI	NI

NI = no inhibition

P. betle (Green leaf) and *P. betle* (Yellow leaf) while three antibiotics ampicillin, gentamicin and norfloxacin used as positive control had no activity. These results related with the previous report which found that the hydroxychavicol isolated from the chloroform extract of *P. betle* showed antibacterial activity against *E. coli* human pathogen with MIC level as 0.4-1 mg/ml[14]. However, this present study of *P. betle* is the first report showing the activity of *P. betle* against isolated diarrheal microbe from piglets. One of report of *C. sappan* barks extracted by maceration with methanol showed antibacterial activity against human *E. Coli* pathogen with inhibition zone value of 15 mm[15]. In contrast, its ethanolic extract of this plant had no activity against pathogen *E. coli* in piglets. However *C. sappan* extract activity showed against *E. coli* from ATCC. By the reason, the method of extract from solvent such as ethanol and methanol can affect antimicrobial activity. In addition, *P. betle* (Green leaf) and *P. betle* (Yellow leaf) also showed antibacterial activity against pathogen *Samonella* spp in piglets. Ethanolic extract of *P. betle* has ever been reported on against *Salmonella typhi* in human pathogen with inhibition zone value of 23 mm[16] which is higher than the results in this study that investigated microbe from piglets. It is possible that some bacterial are evolring resistant to antibiotics. In addition, the plant extract or antibiotic showed better sensitivity of antimicrobial activity in human than in piglet. Hydroxychavicol, which is the phenolic compound[17] and isolated from *P. betle* was reported that it has antibacterial property[18]. However, the results of these twelve plants and their ingredients against gram negative bacteria, the cause of diarrhea in piglets, are the first reported. *P. betle* should be developed to be antimicrobial drug against *E. coli* and *Salmonella* pathogen instead of antibiotics for reducing antibiotic use which causes resistant strain bacteria.

Conclusion

The ethanolic extracts of *P. betle* and *C. sappan* exhibited the highest antibacterial activity against the microorganisms, which are the cause of diarrhea in piglets such as *E. coli* and *Samonella* spp. Hydroxychavicol, an ingredient of *P. betle* should be continuously tested against *E. coli* and *Salmonella* pathogens in piglets in using it as a marker for developing product from these plants against these gram negative microbes that cause diarrhea in piglets. However, these results support the use of Thai medicinal plants for treatment of diarrhea caused by these bacteria. This study also provides basic knowledge on antibacterial activity which can lead to formula development for diarrheal disease in post-weaned piglets and other infectious diseases in future.

What is already known on this topic?

Some species of medicinal plants have been studied with some diarrhea pathogens isolated from cases in Thailand by susceptibility tests such as agar and broth dilution methods but reports are limited especially the Minimal Inhibitory Concentration value (MIC) and the relationship between the micro-organisms as the most common cause of diarrhea in piglets from *E. coli* and *Salmonella* spp. Most previous studies did not include medicinal plant activity against *E. coli* and *Salmonella* spp pathogens isolated from piglets; thus, the aim of this study was to determine susceptibility of the most common diarrhea casing pathogens to Thai medicinal plant extracts.

What this study adds?

The study will gather information about extracting some Thai medicinal plants. Data will be obtained about treatment of diarrhea in piglets by pathogenic *E. coli* and *Salmonella* spp which are resistant to antibiotics. The data will help to develop effective treatment for the disease, so that poor farmers' costs do not escalate further, and will help keep the pork industry profitable.

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Potential conflicts of interest

None.

References

1. Hampson DJ. Post-weaning *Escherichia coli* diarrhoea in pigs. In: Gyles CL, editor. *Escherichia coli* in domestic animals and humans. Wallingford,

UK: CAB International: 1994: 171-91.
2. Madec F, Bridoux N, Bounaix S, Cariolet R, Duval-Iflah Y, Hampson DJ. Experimental models of porcine post-weaning colibacillosis and their relationship to post-weaning diarrhoea and digestive disorders as encountered in the field. Vet Microbiol 2000; 72: 295-310.
3. Osek J. Prevalence of virulence factors of *Escherichia coli* strains isolated from diarrheic and healthy piglets after weaning. Vet Microbiol 1999; 68: 209-17.
4. Schierack P, Steinruck H, Kleta S, Vahjen W. Virulence factor gene profiles of *Escherichia coli* isolates from clinically healthy pigs. Appl Environ Microbiol 2006; 72: 6680-6.
5. Smith HW, Linggood MA. Observations on the pathogenic properties of the K88, Hly and Ent plasmids of *Escherichia coli* with particular reference to porcine diarrhoea. J Med Microbiol 1971; 4: 467-85.
6. Gannon VP, Gyles CL, Friendship RW. Characteristics of verotoxigenic *Escherichia coli* from pigs. Can J Vet Res 1988; 52: 331-7.
7. Konowalchuk J, Dickie N, Stavric S, Speirs JI. Comparative studies of five heat-labile toxic products of *Escherichia coli*. Infect Immun 1978; 22: 644-8.
8. Smith HR, Scotland SM. Vero cytotoxin-producing strains of *Escherichia coli*. J Med Microbiol 1988; 26: 77-85.
9. Dobrescu L. New biological effect of edema disease principle (*Escherichia coli*-neurotoxin) and its use as an in vitro for this toxin. Am J Vet Res 1983; 44: 31-4.
10. Svendsen J, Larsen JL, Bille N. Outbreaks of post weaning *Escherichia coli* diarrhoea in pigs. Nord Vet Med 1974; 26: 314-22.
11. Li Siam K. Water aerobics [Internet]. 2016 [cited 2016 Jan 1]. Available from: http://www.stou.ac.th/Schools/Shs/booklet/book543/thai.html.
12. National Committee for Clinical Laboratory Standard (NCCLS). Performance standards for antimicrobial susceptibility testing: fourteenth informational supplement. NCCLS document M100-S14. Wayne, PA: NCCLS; 2004.
13. Sarker SD, Nahar L, Kumarasamy Y. Microtitre plate-based antibacterial assay incorporating resazurin as an indicator of cell growth, and its application in the in vitro antibacterial screening of phytochemicals. Methods 2007; 42: 321-4.
14. Jesonbabu J, Spandana N, Aruna Lakshmi K. In vitro antimicrobial potentialities of chloroform extracts of Ethanomedicinal plant against clinically isolated human pathogens. Int J Pharm Pharm Sci 2012; 4 (Suppl 3): 624-6.
15. Mohan G, Anand SP, Doss A. Efficacy of aqueous and methanol extracts of *Caesalpinia sappan* L. and *Mimosa pudica* L. for their potential antimicrobial activity. South As J Biol Sci 2011; 1: 48-57.
16. Bangash FA, Hashmi AN, Mahboob A, Zahid M, Hamid B, Muhammad SA, et al. In vitro antibacterial activity of *Piper betel* leaf extracts. J App Pharm 2012; 3: 639-46.
17. Bhalerao SA, Verma DR, Gavankar RV, Teli NC, Rane YY, Didwana VS. et al. Phytochemistry, pharmacological profile and therapeutic uses of *Piper betle* Linn.–An Overview. RRJPP 2013; 1: 10-9.
18. Nair R, Chanda S. Antimicrobial Activity of *Terminalia catappa*, Manilkarazapota and Piper betel Leaf Extract. Indian J Pharm Sci 2008; 70: 390-3.

ฤทธิ์ต้านเชื้อแบคทีเรียของสารสกัดสมุนไพรไทยต่อจุลินทรีย์ก่อโรคท้องร่วงของลูกสุกรหลังหย่านม

วิศิษย์ เกตุปัญญาพงศ์, อรุณพร อิฐรัตน์

ภูมิหลัง: ลูกสุกรหลังหย่านมมีการติดเชื้อแบคทีเรียก่อโรคท้องเสียที่เกิดจากเชื้อดื้อยาเพิ่มจำนวนมากขึ้นอย่างรวดเร็วและพืชสมุนไพรไทยหลายชนิด เช่น ผลดีปลี (Piper retrofractum), เถาสะค้าน (Piper leptostachyum), รากช้าพลู (Piper sarmentosum), เหง้าขิง (Zingiber officinale), รากเจตมูลเพลิงแดง (Plumbago indica), ใบพลู (Piper betle), แก่นฝาง (Caesalpinia sappan) และเปลือกมังคุด (Garcinia mangostana) ซึ่งใช้กันอย่างแพร่หลายในการแพทย์แผนไทยในการรักษาอาการท้องเสีย ดังนั้นพืชเหล่านี้ถูกนำมาทดสอบฤทธิ์ต้านเชื้อแบคทีเรียที่แยกจากลูกสุกรหย่านมเพื่อรักษาท้องเสีย

วัตถุประสงค์: ศึกษาฤทธิ์ของสมุนไพรไทยและส่วนประกอบของสมุนไพรแต่ละชนิดเพื่อพัฒนาสมุนไพรไทยให้เป็นทางเลือก สำหรับการรักษาโรคท้องร่วงในลูกสุกรหลังหย่านมที่เกิดจากการติดเชื้อ

วัสดุและวิธีการ: ศึกษาฤทธิ์ต้านเชื้อแบคทีเรียของพืชสมุนไพรไทยและสารประกอบของสมุนไพรแต่ละชนิดด้วยวิธี disc diffusion และ broth dilution เชื้อแบคทีเรียก่อโรคท้องร่วงในลูกสุกรหลังหย่านมได้แก่ Escherichia coli และ Salmonella spp. การสกัดสมุนไพรทำโดยใช้วิธีสกัดด้วย 95% เอทานอลได้ทำการศึกษาเปรียบเทียบกับยาต้านแบคทีเรียมาตรฐาน ได้แก่ Ampicillin, Gentamicin และ Norfloxacin

ผลการศึกษา: พบว่าเชื้อที่ทดสอบทุกชนิดมีความไวต่อสารสกัดพลูเช่นเดียวกับสารบริสุทธิ์คือ plumbagin โดยมีค่า MIC ระหว่าง 5 ถึง 10 mg/ml ซึ่งต่างจากกลุ่มของยาปฏิชีวนะที่มีค่า MIC ระหว่าง 0.19-2.5 mg/ml แต่สารสกัดจากพลูสามารถยับยั้งเชื้อ Escherichia coli และ Salmonella spp. ที่เป็นเชื้อก่อโรคได้ ส่วนกลุ่มของยาปฏิชีวนะสามารถยับยั้งเชื้อได้บางชนิดเท่านั้น

สรุป: ผลการศึกษาสนับสนุนการใช้พืชสมุนไพรไทย เช่น ฝางและพลูในการรักษาโรคท้องร่วงที่เกิดจากแบคทีเรีย ผลการศึกษายังเป็นความรู้ในการพัฒนาผลิตภัณฑ์สำหรับรักษาอาการท้องเสียหรือโรคอื่นๆ ในสุกรหลังหย่านม