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ANTIBACTERIAL POTENTIAL OF SOME THAI MEDICINAL PLANTS

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ABSTRACT

Ethanol and hexane extracts of twenty Thai medicinal plants, used in Thai traditional medicine for the treatment of infectious and septic diseases, were screened *in vitro* for antibacterial activity against *Bacillus cereus, Escherichia coli* and *Staphylococcus aureus,* using disc diffusion method. The ethanol extracts of *Clerodendrum inerme* (L.) Gaertn., *Combretum qurdrangulare* L., *Psidium guajava* Kurz., and *Stephania venosa* (Blume) Spreng. showed antibacterial activity against all three tested pathogens. Based on minimum inhibitory concentration (MIC) of these four medicinal plant extracts, the ethanol extract of *C. inerme* demonstrated the highest antibacterial potency. The determination of MIC and minimum bactericidal concentration (MBC) of the extract using microdilution method showed that such extract had MICs for *B. cereus*, *E. coli*, and *S. aureus* of 0.039, 0.312 and 0.156 mg/ml, respectively; and MBCs for *B. cereus*, *E. coli*, and *S. aureus* of 0.039, 0.625 and 0.312 mg/ml, respectively. These results confirmed antibacterial potential of the plant.

KEYWORDS: **Antibacterial potential, Thai medicinal plants, *Clerodendrum inerme*, Antipathogenic activity**

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INTRODUCTION

Infectious diseases are considered one of the major health problems in Thailand as well as other developing countries[1-2]. The treatment of these infections is mainly based on the uses of antibiotics[3]. Antibiotic resistance has become a global concern[4]. Various microorganisms have developed multiple drug resistance due to the indiscriminate uses of commercial antimicrobial drugs[5]. This is the major cause of failure in the treatment of infectious diseases[6]. The increasing failure of antibiotics has led to the investigation of the medicinal plants for their potential antimicrobial activity[7]. According to the World Health Organization, medicinal plants would be the best source to obtain a variety of drugs[5]. The advent of science into the search for antibiotics largely depends on some of these plants as raw materials[8]. Thailand is endowed with a rich biodiversity of plant species and it has a long history of herbal medicine practices[9]. Plenty of Thai medicinal plants are still commonly used in a wide range of clinical settings for infectious diseases[1]. Traditionally, Thai pharmacists extract active components from medicinal plants using high-polar solvents as extraction media, e.g. either boiling the specimen in water or using alcoholic macerate[10]. Despite the long history of Thai herbal therapy, antibacterial profiles of some Thai medicinal plants are not yet fully characterized. The aim of this investigation was to screen 20 medicinal plants (belonging to 15 families), traditionally prescribed in Thailand for the treatment of infectious diseases, for their antibacterial activities against *Bacillus cereus*, *Escherichia coli*, and *Staphylococcus aureus*. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the extract indicating their high antibacterial activity were also determined.

MATERIALS AND METHODS

1. Plant materials

Twenty plant materials were collected from Udon Thani and Kanchanaburi provinces, Thailand (Table 1). The plants were air-dried under the shade at room temperature and cut into small pieces before grinding into fine powder with an electric grinder. The ground materials were stored in airtight containers protected from light. Herbarium specimens of all twenty plants were deposited at The Forest Herbarium (BKF) at the Nation Park, Wildlife and Plant Conservation Department, Bangkok, Thailand. The voucher specimen numbers of the plants are also shown in Table 1.

2. Plant extracts

The plant powders were extracted with 95% ethanol and hexane. Two hundred and fifty grams of the plant sample was mixed with 2 L of solvent. The mixtures were left for 21 days at room temperature and then filtered through Whatman No.1 filter paper. The extracts were evaporated to dryness by a rotary evaporator at 50^{o}C under reduced pressure to obtain crude extract. Dried extracts were collected and stored in a dark room at 4^{o}C for subsequent antibacterial tests.

Table 1
List of twenty medicinal plants used in the experiment and their traditional uses

Species, Family, Voucher number	Thai name	Parts used	Traditional uses
Albizia chinensis (Osbeck.) Merr., Mimosaceae, BKF:154214	Kang luang	Roots	Treatment of infected wound and abscess[11].
Catunaregam tomentosa (Blume ex DC.) Tirveng., Rubiaceae, BKF:154226	Ma khet	Roots	Applied treatment of granular stomatitis, aphthous ulcer, cancerous or non-cancerous chronic ulcers[13].
Cleome viscosa L., Capparidaceae, BKF:154215	Phak sian phi	Whole plant	Applied treatment of spine tuberculosis, inflammation of Pattakaat line[14]; abscess[15-16].
Clerodendrum inerme (L.) Gaertn., Verbenaceae, BKF:154225	Samma nga	Roots	Treatment of skin diseases with pruritus, wound inflammation, rheumarthritis, hepatitis and cold[11].
Combretum qurdrangulare Kurz., Comberetaceae, BKF:154216	Sakae	Roots	Treatment of venereal diseases, anthelmintic[10]; and arthritis[11].
Dioecrescis erythroclada (Kurz.) Tiveng., Rubiaceae, BKF:154234	Ma khang daeng	Roots	Treatment of cancerous or non-cancerous chronic ulcers, yaws, leprosy and eczema[11].
Diospyros mollis Griff., Ebenaceae, BKF:154210	Ma kluea	Roots	Applied treatment of leukorrhea, abscess, yaws, leprosy, tertiary stage syphilis, tuberculosis, and infection[16].
Ixora javanica (Blume) DC., Rubiaceae, BKF:154213	Khem thong	Roots	Treatment of mucous bloody stool or sputum, eye diseases, anasarca and fire element tonic[11].
Lepisanthes senegalensis (Poir.) Leenh, Sapindaceae, BKF:154229	Chamma liang pa	Roots	Treatment of cerebral malaria, fever with vertigo, chest pain and nosebleed[18].
Luvunga scandens (Roxb.) Buch.-Ham., Rutaceae, BKF:154228	Chang nga diao	Roots	Treatment of internal and external abscess, disorders of urination and nephropathy[11].
Pavetta indica L., Rubiaceae, BKF:154219	Khem pa	Roots	Treatment of bronchial sputum occlusion, mucus in the gastrointestinal tract and blepharitis[11].
Phyllanthus emblica L., Euphorbiaceae, BKF:154222	Makham pom	Roots	Applied treatment of tong carcinoma[14]; splenomegaly caused by malaria[13]; urethral polyps, intestianal parasites and cancerous or non-cancerous chronic ulcers[15]; felon[19].
Polyalthia cerasoides (Roxb.) Benth, ex Bedd., Annonaceae, BKF:154232	Phaya rak dam	Roots	Treatment of muscular pain[18]; abscess, tuberculosis[11].
Pouzolzia hirta (Blume) Hassk., Urticaceae, BKF:154220	Khop cha nang	Whole plant	Treatment of venereal diseases and worm[11]. Apply locally for anti-inflammatory[10].
Psidium guajava L., Myrtaceae, BKF:154223	Farang	Leaves	Used as antidiarrheal and antidysenteric[10-11].
Scoparia dulcis L., Scrophulariaceae, BKF:154209	Krot nam	Whole plant	Applied treatment of infected wound, severe stage diarrhea[16].
Senna occidentalis (L.) Link, Fabaceae, BKF:154230	Khi lek thet	Whole plant	Treatment of fever, infected wound and pruritic rash[11].
Sida acuta Burm. f., BKF:154207	Ya khat mon	Whole plant	Treatment of manifestation of Malvaceae, infection[11].
Stephania venosa (Blume) Sreng., Menispermaceae, BKF:154235	Kra thom lueat	Bulb	Treatment of cancerous or non-cancerous chronic ulcers, leukorrhea[20], diarrhea[17], hypersputum and dysentery[11,21].
Suregada multiflorum (A.Juss.) Baill, Euphorbiaceae, BKF:154231	Khan thong	Roots	Treatment of skin diseases with pruritus, cancerous or non-cancerous chronic ulcers, worm, gingivitis[11,21].

3. Bacterial strains

Bacillus cereus KB70, *Escherichia coli* ATCC 25922, and *Staphylococcus aureus* ATCC 25923 were used throughout in the determination of antibacterial activities of the plant extracts. All bacterial strains were obtained from Central Laboratory and Green House Complex, Kasetsart University (Bangkok, Thailand). Bacterial strains were maintained on nutrient agar[22] and subcultures were freshly prepared before use. Bacterial cultures were prepared by transferring two to three colonies into a tube containing 20 ml nutrient broth and incubated overnight at 37^{o}C. The turbidity of the culture was adjusted with sterile saline solution to match 0.5 McFarland standard.

4. *Disc diffusion assay*

(a) Antibacterial screening

The antibacterial activities of the 20 plant extracts were evaluated using disc diffusion method on Mueller-Hinton agar plates[23]. Sterile cotton swabs were dipped into standard bacterial strain suspensions (0.5 McFarland, containing 1.5 x 10^{8}cfu/ml) before inoculating evenly onto the entire surface of the agar plates. Plant extract solutions at different concentrations were prepared by dissolving the dried extracts obtained from the previous step in DMSO. All plant extract solutions were sterilized by Minisart® syringe filter (0.45 µm pore size; Sartorius, Germany) prior use. Sterile paper discs (6 mm diameter) were impregnated with 20 µl of the sterile plant extract solutions. The paper discs, containing 0, 0.625, 1.25, 2.5 and 5 mg of each extract/disc, were applied onto the inoculated agar plates. A paper disc containing only DMSO was used as a negative control. The plates were incubated at 37^{o}C for 24 hours. Clear zone around the paper discs, indicating the inhibition of bacterial growth, were measured and the average diameter of the clear zones were interpreted as the antibacterial activity of the extracts[24]. The experiment was performed in triplicate. The extracts which had high antibacterial activities were subsequently determined for their minimum inhibitory concentrations (MIC).

(b) Minimum Inhibitory Concentration (MIC) determination

The MIC endpoints were the lowest extract concentrations that showed absence of growth or complete growth inhibition (100% inhibition). MICs of the extracts with high antibacterial activities were carried out in the same manner as described previously[23]. Sterile paper discs (6 mm diameter) containing 0, 0.009, 0.019, 0.038, 0.078, 0.156, 0.312, 0.625, 1.25, 2.5 and 5 mg of each extract/disc were placed onto the inoculated agar plates before incubating at 37^{o}C for 24 hours. The extract exhibited the highest antibacterial activity was further investigated by broth dilution assay.

5. *Broth dilution assay*

Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) of the selected extract which exhibited the highest antibacterial activity were determined by broth dilution method[25].

(a) Determination of MIC

Each microdilution well containing 50 µl of serially two-fold diluted extract concentration was inoculated with 50 µl of diluted bacterial suspension (0.5x10^{6} CFU/ml) in 2x Mueller-Hinton broth. The microdilution trays were incubated at 37^{o}C for 24 hours. Turbidity of cell growth was measured by ELISA reader at 595 nm. The MIC endpoints were the lowest extract concentrations that showed absence of growth or complete growth inhibition (100% inhibition).

(b) Determination of MBC

MBCs of the antibacterial crude extract were determined by subculturing 5 µl of the 24-hour culture from each well of microdilution plates used for MIC determination onto Mueller-Hinton agar plates. The plates were incubated at 37^{o}C until growth was seen in the growth control subculture (usually before 48 h). The MBC was the lowest extract concentration that showed either no growth or fewer than three colonies to obtain approximately 99 to 99.5% killing activity.

6. Statistical analysis

For data on disc diffusion assays, independent sample t-test (SPSS program) was used to determine the significant difference ($P < 0.05$)

in anti-microbial activity between the ethanol and hexane extracts of each plant.

RESULTS

1. Antibacterial screening of twenty medicinal plants

Table 2 illustrates the differences of antibacterial activities between the ethanol and hexane extracts of twenty medicinal plants against *B. cereus*, *E. coli* and *S. aureus*. Significant differences ($P < 0.05$) in antibacterial activity were observed between the ethanol and hexane extracts of *Psidium guajava* and *Stephania venosa*. However, it was found that both ethanol and hexane extracts obtained from *Dioecrescis erythroclada, Lepisanthes senegalensis*, *Pouzolzia hirta*, *Scroparia dulcis*, *Senna occidentalis*, *Sida acuta* and *Suregada multiflorum* did not exhibit antibacterial activity against the tested bacteria.

Table 2

Antibacterial activities of ethanol and hexane extracts of twenty medicinal plants at a concentration of 5 mg extract/disc using disc diffusion assay.

Medicinal Plants	Zone of inhibition (mm)								
	B. cereus			*E. coli*			*S. aureus*		
	Ethanol	Hexane	P<0.05	Ethanol	Hexane	P<0.05	Ethanol	Hexane	P<0.05
Albizia chinensis	< 6.0	< 6.0		10.0	9.0		< 6.0	8.5	*
Catunaregam tomentosa	8.0	< 6.0	*	< 6.0	< 6.0		< 6.0	< 6.0	
Cleome viscosa	6.5	6.5		< 6.0	< 6.0		8.0	7.0	
Clerodendrum inerme	11.0	8.0		11.5	< 6.0	*	10.5	8.0	
Combretum qurdrangulare	8.5	7.0		19.5	< 6.0	*	8.0	7.0	
Dioecrescis erythroclada	< 6.0	< 6.0		< 6.0	< 6.0		< 6.0	< 6.0	
Diospyros mollis	8.0	< 6.0	*	< 6.0	< 6.0		8.0	8.0	
Ixora javanica	7.0	8.0		< 6.0	< 6.0		8.0	18.0	*
Lepisanthes senegalensis	< 6.0	< 6.0		< 6.0	< 6.0		< 6.0	< 6.0	
Luvunga scandens	9.0	6.5	*	< 6.0	< 6.0		7.0	8.0	
Pavetta indica	7.0	6.5		< 6.0	< 6.0		7.0	7.0	
Phyllanthus emblica	7.0	< 6.0	*	10.0	9.0		< 6.0	< 6.0	
Polyalthia cerasoides	< 6.0	< 6.0		12.0	< 6.0	*	< 6.0	< 6.0	
Pouzolzia hirta	< 6.0	< 6.0		< 6.0	< 6.0		< 6.0	< 6.0	
Psidium guajava	9.5	< 6.0	*	13.5	< 6.0	*	12.0	< 6.0	*
Scroparia dulcis	< 6.0	< 6.0		< 6.0	< 6.0		< 6.0	< 6.0	
Senna occidentalis	< 6.0	< 6.0		< 6.0	< 6.0		< 6.0	< 6.0	
Sida acuta	< 6.0	< 6.0		< 6.0	< 6.0		< 6.0	< 6.0	
Stephania venosa	16.0	6.5	*	15.5	< 6.0	*	20.5	6.5	*
Suregada multiflorum	< 6.0	< 6.0		< 6.0	< 6.0		< 6.0	< 6.0	

**** indicates significant difference between the ethanol and hexane extracts of the same plant (P <0.05).***

Table 3
Susceptibility of the tested pathogens to the medicinal plant extracts at a concentration of 5 mg/disc using disc diffusion assay.

Medicinal Plants	Zone of inhibition[1] (mm)					
	B. cereus		*E. coli*		*S. aureus*	
	Ethanol	Hexane	Ethanol	Hexane	Ethanol	Hexane
Albizia chinensis	-	-	+	+	-	+
Catunaregam tomentosa	+	-	-	-	-	-
Cleome viscosa	+	+	-	-	+	+
Clerodendrum inerme	++	+	++	-	+	+
Combretum qurdrangulare	+	+	+++	-	+	+
Dioecrescis erythroclada	-	-	-	-	-	-
Diospyros mollis	+	-	-	-	+	+
Ixoraja vanica	+	+	-	-	+	+++
Lepisanthes senegalensis	-	-	-	-	-	-
Luvunga scandens	+	+	-	-	+	+
Pavetta indica	+	+	-	-	+	+
Phyllanthus emblica	+	-	+	+	-	-
Polyalthia cerasoides	-	-	++	-	-	-
Pouzolzia hirta	-	-	-	-	-	-
Psidium guajava	+	-	++	-	++	-
Scroparia dulcis	-	-	-	-	-	-
Senna occidentalis	-	-	-	-	-	-
Sida acuta	-	-	-	-	-	-
Stephania venosa	+++	+	++	-	+++	+
Suregada multiflorum	-	-	-	-	-	-

[1]***Zone of inhibition was interpreted as follows: resistant (-) = < 6 mm, intermediate susceptible (+) = 6-10 mm, moderate susceptible (++) = 11-15 mm, susceptible (+++) = 16-20 mm.***

Susceptibility of the bacteria to the treatments of the medicinal plant extracts were determined from inhibition zones obtained in screening assay (Table 3). The results showed that *B. cereus* was susceptible (+++) to the hexane extract of *S. venosa,* and moderate susceptible (++) to the ethanol extract of *C. inerme*. With regards to *E. coli*, the bacterial population was susceptible (+++) to the treatment of *C. qurdrangul are* ethanol extract, and moderate susceptible (++) to the ethanol extracts of *C. inerme*, *P. cerasoides, P. guajava* and *S. venosa*. The results also showed that *S. aureus* was susceptible (+++) to the treatment of *S. venosa* ethanol extract, and moderate susceptible (++) to the treatment of *P. guajava*. Both ethanol and hexane extracts obtained from seven plants (*D. erythroclada, L. senegalensis*, *P. hirta, S. dulcis*, *S. occidentalis*, *S. acuta* and *S. multiflorum*) seemed to have no inhibition effects on the growth of all three bacterial strains tested. The results from the screening steps revealed that, of the twenty medicinal plants studied, the ones with high potential to inhibit the growths of all three bacterial strains included the ethanol and hexane extracts obtained from *C. inerme*, *C. qurdrangul are*, *P. guajava* and *S. venosa*. Therefore, these four medicinal plants extracts were selected for further determination of their minimum inhibitory concentrations (MICs).

3.2 Determination of Minimum Inhibitory Concentration (MIC) of the selected medicinal plant extract.

The determination of MICs of the four selected plants extracts are shown in Table 4. It was found that all bacterial strains tested were sensitive to ethanol extracts at lower concentration than those of hexane extracts. Ethanol extract of *C. inerme* had the lowest MIC value of 0.019 mg/ml against *B. cereus* and 0.039 mg/ml against *S. aureus*. Ethanol extract of *C. qurdrangulare* had the lowest MIC value of 0.039 mg/ml against *E. coli* but it exhibited low antimicrobial activity against *S. aureus* (2.5 mg/ml MIC). Of the four plants studied, the ethanol extract of *C. inerme* exhibited the highest antibacterial potency.

Table 4
Minimum Inhibitory Concentration (MIC) values of the ethanol and hexane medicinal plant extracts against tested bacterial strains using disc diffusion assay.

Medicinal plants	MIC (mg/ml)					
	Ethanol extraction			Hexane extraction		
	B.cereus	*E.coli*	*S.aureus*	*B.cereus*	*E.coli*	*S.aureus*
Clerodendrum inerme	0.019	0.078	0.039	0.156	> 5.0	0.156
Combretum qurdrangulare	0.625	0.039	2.5	2.5	> 5.0	1.25
Psidium guajava	0.625	0.078	0.625	> 5.0	> 5.0	> 5.0
Stephania venosa	0.078	0.625	0.078	2.5	> 5.0	2.5

3.3 Determination of MIC and MBC of the ethanol extract of C. inerme root

The ethanol extract from the root of *C. inerme* was selected for subsequent analysis. As shown in Table 5, among the three pathogens tested, the extract was the most effective in inhibiting the growth of *B. cereus* with the lowest MIC and MBC values of 0.039 mg/ml.

Table 5
Minimum inhibition concentration (MIC) and minimum bacterial concentration (MBC) of the ethanol extract obtained from the root of Clerodendrum inerme using micro dilution assay.

Dilution assay	*B. cereus*	*E. coli*	*S. aureus*
MIC (mg/ml)	0.039	0.312	0.156
MBC (mg/ml)	0.039	0.625	0.312

DISCUSSION

Twenty medicinal plants were screened for their antibacterial activities against *B. cereus*, *E. coli*, and *S. aureus*. These three pathogenic strains were selected because they were the major causes of various detrimental illness and clinical conditions[26-29]. Additionally, there were many studies reported that they have developed overall resistance to several antibiotics[30-34]. In this experiment, active substances in the plant samples were extracted using ethanol (high-polar) and hexane (low-polar) as extraction solvents. It was quite obvious from the results obtained that generally the hexane extracts had lower antibacterial activities than the ethanol extracts (Tables 2, 3 and 4). This could be because the antibacterial components in the plant materials investigated in this study are of high-polar families. The preliminary screening showed that extracts of four medicinal plants, *Clerodendrum inerme*, *Combretum qurdrangulare*, *Psidium guajava* and *Stephania venosa*, exhibited the highest antibacterial activities against all three bacterial strains. It was also observed that all bacterial strains tested were resistant to both ethanol and hexane extracts obtained from *Dioecrescis erythroclada, Lepisanthes senegalensis*, *Pouzolzia hirta, Scroparia dulcis, Senna occidentalis*, *Sida acuta* and *Suregada multiflorum*. Although these plants have long been prescribed to the treatment of infectious diseases as Thai traditional drugs (Table 1), it should be noted that most of Thai traditional medicines are compound drug containing more than one ingredient. Therefore, these plants may not be effective on their own but need to be combined with other ingredients for synergistic effects. At present, little is known about these plants, especially their correlation with other ingredients and their specific activities since there were only small numbers of scientific reports have been published[1]. In this study, it was clearly shown that both the ethanol and hexane extracts of these plants had no effect on growth inhibition of the pathogens tested.

Susceptibility results indicated that the extracts from four medicinal plants, *Clerodendrum inerme*, *Combretum qurdrangulare*, *Psidium guajava* and *Stephania venosa*, were promisingly effective in inhibiting the growth of all three bacterial strains. Of the four plant extracts selected, the

ethanol extract obtained from *C. inerme* root showed the highest antibacterial potency (the lowest MIC value). The MICs of such extract against *B. cereus*, *S. aureus*, and *E.coli* were 0.019, 0.039, and 0.078 mg/ml, respectively (Table 4). A previous study reported the MICs of methanol extract of *C. inerme* against *S. aureus* and *E. coli* of 0.078 and 0.312 mg/ml respectively[35]. It was found that the MIC values of the ethanol extract of *C. inerme* root observed in this study were lower than those of the methanol extract reported earlier, suggesting the higher antimicrobial activity of ethanol extract. It is therefore noteworthy to further explore its antibacterial activity against other microorganisms.

CONCLUSION

In this study, twenty Thai medicinal plant species were screened for antibacterial activity. The plants of interest that could be used in antibacterial product development included *Clerodendrum inerme*, *Combretum qurdrangulare*, *Psidium guajava,* and *Stephania venosa*. Among the extracts of these plants, the ethanol extract of *Clerodendrum inerme* root exhibited the highest antibacterial potency.

REFERENCES

1. Trakulsomboon, S, Kummalue, T, and Jiratchariyakul, W, Antibacterial Activities of Four Thai Medicinal Plants. J Med Assoc Thai, 89(9):1466-71, (2006).
2. Ahmad, I. And Arina, Z.B. Antimicrobial and phytochemical studies on 45 Indian medicinal plants against multi-drug resistant human pathogens. Journal of Ethnopharmacology, 74 (2001): 113–123, (2000).
3. Kowti, R, Harsha, R, Mohammed, GA, Hareesh, AR, Thammanna, GSS, Dinesha, R, Satish, KBP and Irfan, AM. Antimicrobial activity of ethanol extract of leaf and flower of *Spathodeacampanulata P*. Beauv. RJPBCS, 1: 692-698, (2010).
4. Parekh, J, Jadeja, D, and Chandah, S, Efficacy of Aqueous and Methanol Extracts of Some Medicinal Plants for Potential Antibacterial Activity. Turk J Biol, 29: 203-210, (2005).
5. Gislene, GFN, Locatelli, J, Paulo, C, Giuliana, F, and Silva, L. Antibacterial activity of plant extracts and phytochemicals on antibiotic resistant bacteria. Brazilian Journal of Microbiology, 31: 247-256, (2000).
6. Davis, J. Inactivation of the antibiotics and the dissemination of resistance genes. Science, 264: 375-382, (1994).
7. Parekh, J, and Chanda, SV, In vitro Antimicrobial Activity and Phytochemical Analysis of Some Indian Medicinal Plants. Turk J Biol, 31: 53-58, (2007).
8. Jayalakshmi, B, Raveesha, KA, and Amruthesh, KN, Phytochemical investigations and antibacterial activity of some medicinal plants against pathogenic bacteria. Journal of Applied Pharmaceutical Science, 1(5): 124-128, (2011).
9. Wiyakrutta, S, Sriubolmas, N, Panphut, W, Thongon, N, Danwisetkanjana, K, Ruangrungsi, N. and Meevootisom, V, Endophytic fungi with anti-microbial, anti-cancer and anti-malarial activities isolated from Thai medicinal plants. World Journal of Microbiology &Biotechnology, 20: 265–272, (2004).
10. Saralamp, P, Chuaku, W, Temsiririrkkul, R and Clayton, T, Medicinal plants in Thailand Volume I. Amarin Printing and Publishing, Bangkok, (1996).
11. Pongboonrod, S, Plants from abroad and Thailand, properties of the drug from abroad and Thai drug, 1st Edn. Bangkok, (1950).
12. Veeraphajuk, K, Meeteam, T, Paiboonwangcharoun, P, Mapamo, K, Sirisompan, J, Chowsoun, A, Kunsri, S and Punnawanichsiri, S, WatRatchaorasaramRatchaworawihan inscription pharmacopoeia. Fine Arts Department. Artit Products Group, Bangkok, (2002).

13. Veesapen, V, Wutisan, S, Wanaudon, S, Nunchuntee, A, Rawarin, N. and Chanawech, A, Ancient pharmacopoeia from Kantarawichai, Mahasarakam province, Technical Paper No. 1. Northeast Bailan inscribed conservation project, Mahasarakam University. Siritam Offset Printing, UbonRatchathani, (2005).
14. S, Schematic representation of abscess from Pijitmahawong and Tipmala scripture, Tell the symptoms and cure, and illustration. Augsoncharountusana Printing, Bangkok, (1940).
15. Veesapen, V, Wutisan, S, Wanaudon, S, Nunchuntee, A, Rawarin, N. and Chanawech, A, Ancient pharmacopoeia from WatSrisompon, aumporChaingyoun, Mahasarakam province, Technical Paper No. 2. Northeast Bailan inscribed conservation project, Mahasarakam University. Siritam Offset Printing, UbonRatchathani, (2005).
16. Traditional medical school faculty, Pharmacopoeia stone inscriptions in WatPhachetuponwimolmungkararam (Wat Po) Bangkok, His Majesty King Rama Rama graciously the inscription on the year 1832, Complete Edn. WatPhachetuponwimolmungkararamratchaworamahawihan(Wat Po). Mahamakut College Printing, Bangkok, (1962).
17. Boonyaphapas, N. and Chokchaipon, A. Folk medicinal plants 4thEdn, Faculty of Pharmacy, Mahidol University; National Center for Genetic Engineering and Biotechnology, (2000).
18. Chuakul, W, Saralamp,P, Paonil, W, Temsiririrkkul, R and Clayton, T. Medicinal plants in Thailand Volume II. Amarin Printing and Publishing, (1997).
19. Veesapen, V, Wutisan, S, Wanaudon, S, Nunchuntee, A, Rawarin, N. and Chanawech, A, Ancient pharmacopoeia from Kosumpisai and Nacheak, Mahasarakam province, Technical Paper No. 3. Northeast Bailan inscribed conservation project, Mahasarakam University. Siritam Offset Printing, UbonRatchathani, (2005).
20. Chotianun et al. Family of Thai sacred and magical plants 2ndEdn. Doungkamol Publishing, Bangkok, (2010).
21. Rujjanawate, C, Kanjanapothi, D, Amornlerdpison, D, Somwong, R. and Hargreave, O, 20th Annitersary HRH Princess MahaChakriSirindhorn Herbs Garden. PTT Public, Bangkok, (2005).
22. Collee, JG, and Marr, W. Cultivation of bacteria. In: Collee JG, Fraser AG, Marmion BP, Simmons A. (eds). *Mackie and McCartney Practical Medical Microbiology*, 3rdEdn. Churchill Livingstone, London, 1989, pp. 432-454.
23. NCCLS. Performance standards for antimicrobial disc susceptibility tests, Approved standard. M2-A8. National Committee for Clinical Laboratory Standards, Pennsylvania, USA (2003).
24. Ruengsakul, S, Antibacterial Properties of Some Medicinal Plants. Department of Biology, Faculty of Science, Srinakarinvirot (Prasanmitr) University, Abstract of the Research, (1987).
25. NCCLS. Methods for Dilution Antimicrobial Susceptibility Test for Bacteria that Grow Aerobically; Approved standard. M7-A6. National Committee for Clinical Laboratory Standards, Pennsylvania, (2003).
26. Lattuada, PC. and McClain, D. Examinaion of meat and poultry products for *Bacillus cereus*. Microbiology Laboratory Guidebook, 3rdEdn. Food Safety and Inspection Service, United States Department of Agriculture, (1998).
27. Olawale, AK and Akintobi, AO, Antibiotic Resistant *Bacillus cereus* in Some Selected Foods in Osun State, Nigeria. Report and Opinion 3(1): 21-25 (2011).
28. Schlegelova, J, Brychta, J, Klimova, E, Napravnikova, E and Babak, V, The prevalence of and resistance to antimicrobial agents of *Bacillus cereus* isolates from foodstuffs. Vet. Med.–Czech, 48(11): 331–338, (2003).
29. Spicer, JW, Clinical Microbiology and Infectious Diseases, 2ndEdn. Churchill Livingstone, London, (2008).
30. Cosgrove, SE, Sakoulas, G, Perencevich, EN, Schwaber, M J, Karchmer, AW, and Carmeli, Y.

Comparison of Mortality Associated with Methicillin-Resistant and Methicillin-Susceptible *Staphylococcus aureus* Bacteremia: A Meta-analysis. Clinical Infectious Diseases, 36: 53–9.(2003).

31. Whong, CMZ, and Kwaga, JKP, Antibiograms of *Bacillus cereus* isolates from some Nigerian Foods. Nigerian food Journal, 25(1): 178-183, (2007).
32. Manges, AR, Johnson, JR, Foxman, B, O'Bryan, TT, Fullerton, KE and Riley, LW. Widespread distribution of urinary tract infection caused by a multidrug-resistsnt *Escherichia coli* clonal group. N Engl J Med, 345(14): 1007-1013, (2001).
33. Tadesse, DA, Zhao, S, Tong, E, Ayers, S, Singh, A, Bartholomew, MJ, and McDermott, PF, Antimicrobial Drug Resistance in *Escherichia coli* from Humans and Food Animals, United States, 1950–2002. Emerging Infectious Diseases, 18(5): 741-749, (2012).
34. Waters, AE, Contente-Cuomo, T, Buchhagen, J, Liu, CM, Watson, L, Pearce, K, Foster, JT, Bowers, J, Driebe, EM, Engelthaler, DM, Keim, PS, and Price, LB, Multidrug-Resistant *Staphylococcus aureus* in US Meat and Poultry. Clinical Infectious Diseases, 52(10): 1-4,(2011).
35. Chahal, J.K., Sarin, R. and Malwal, M. Efficacy of *Clerodendrum inerme* L. (Garden Quinine) against some human pathogenic strains. International Journal of Pharma and Bio Sciences 1(4): 219-223, (2010).