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Original Research Article

Anti-*Streptococcus pyogenes* Activity of Selected Medicinal Plant Extracts Used in Thai Traditional Medicine

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Abstract

Purpose: To evaluate the anti-*Streptococcus pyogenes* activity of selected medicinal plants used in Thai traditional medicine.

Methods: Sixty-nine extracts of 51 selected Thai medicinal plant species were tested for anti-*S. pyogenes* activity by paper disc agar diffusion and broth microdilution methods.

Results: Ten plants including *Boesenbergia pandurata* (Roxb.) Schltr., *Cinnamomum bejolghota* (Buch.-Ham.) Sweet, *Cinnamomum porrectum* (Roxb) Kosterm, *Eleutherine americana* Merr., *Gymnopetalum cochinchinensis* (Lour.) Kurz, *Piper betle* L., *Quercus infectoria* G. Olivier, *Quisqualis indica* L, *Rhodomyrtus tomentosa* (Aiton) Hassk., and *Walsura robusta* Roxb. demonstrated good antibacterial activity against *S. pyogenes* NPRC 101. These plants were selected and further evaluated for their anti-*S. pyogenes* activity against 11 isolates of *S. pyogenes* from patients with upper respiratory tract infections. Three plants including *Boesenbergia pandurata*, *Eleutherine americana*, and *Rhodomyrtus tomentosa* exhibited good antibacterial activity against all *S. pyogenes* isolates and produced similar activities against different tested isolates. *Boesenbergia pandurata* and *Rhodomyrtus tomentosa* demonstrated antibacterial activity with the same minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) range of 3.91 - 31.25 µg/ml whereas *Eleutherine americana* displayed MIC and MBC values of 250 and 250-500 µg/ml against all *S. pyogenes* isolates.

Conclusion: *Boesenbergia pandurata*, *Eleutherine americana*, and *Rhodomyrtus tomentosa* have great antibacterial potentials against *S. pyogenes*.

Keywords: Antibacterial activity, *Boesenbergia pandurata*, *Eleutherine americana*, *Rhodomyrtus tomentosa*, *Streptococcus pyogenes*, Thai medicinal plant, Upper respiratory tract infections

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INTRODUCTION

Upper respiratory tract infections (URTIs) are the most common human infection, mostly caused by viruses and bacteria. *Streptococcus pyogenes* is a major upper respiratory tract bacterial pathogen that causes a wide variety of diseases. It is the most common cause of bacterial pharyngitis and is linked to many serious complications [1]. Viral infections in upper respiratory tract are usually not an appropriate indication for the use of antibiotics. Bacteria that cause URTIs should be taken and cultured to determine the particular type of bacteria and antibiotics treatment are performed if necessary [2]. However, unnecessary and irrational self-medication with antibiotics seems to be common for URTIs [3,4] and these result in resistance to many bacteria including *S. pyogenes* [5,6]. Antibiotic misuse for URTIs is a serious problem that not only results in selection of resistant strains of bacteria but also waste of resources. Correlation of antibiotic resistance in *S.*

pyogenes with antibiotic consumption has been recorded. Increase in antibiotic use has resulted in increased prevalence of antibiotic-resistant *S. pyogenes* [7,8]. Hence, the search for alternative treatment dealing with URTIs caused by *S. pyogenes* is necessary. Medicinal plants are a great source of alternative treatment for many infections. The use of medicinal plants may substitute antibiotic consumption for URTIs or decrease antibiotic-resistant bacteria.

In traditional Thai medicine, many medicinal plants have been in use since ancient times. Herbal medicines are relatively safer than synthetic drugs and offer profound therapeutic benefits [9]. A number of Thai medicinal plants have been studied for their antibacterial activities. There are several reports on antibacterial activity of plants that inhibit various bacterial pathogens, but only limited number of studies on *S. pyogenes*, an important bacterial human pathogen, have been published. Therefore, this study was aimed to evaluate the antibacterial activity of selected medicinal plants commonly used in traditional Thai medicine for bacterial infections against *S. pyogenes* isolated from upper respiratory tract infections.

EXPERIMENTAL

Preparation of plant extract

Fifty-one medicinal plant species used in traditional Thai medicine for bacterial infections were selected. The plant materials were collected from various areas of the southern region of Thailand from 2006 - 2007. *Quercus infectoria* nut galls were purchased from an herb shop in Songkhla, Thailand. Botanical identification was performed by Dr. Oratai Neamsuvan, an ethnobotanist at the Faculty of Traditional Thai Medicine, Prince of Songkla University, where their voucher specimens are deposited. All plant materials were cut into small pieces and dried at 60 °C overnight. The dried plant materials were crushed in a mechanical mortar and soaked in extracted solvent for 7 days (3 times). The solvent was then filtered and dried using a rotary evaporator. All extracts were stored at -4 °C, and dissolved in dimethyl sulfoxide (DMSO, Merck, Germany) before use. The aliquots were checked for sterility by streaking with a sterile loop on brain heart infusion (BHI) agar and incubating at 37 °C overnight.

Bacterial strain and culture conditions

Eleven clinical isolates of *S. pyogenes* (NPRC 101-111) from patients with tonsillitis or pharyngitis were obtained from Department of Microbiology and Natural Products Research Center, Faculty of Science, Prince of Songkla University. All isolates were susceptible to erythromycin and penicillin G. These isolates were stored in BHI broth supplement with 20 % glycerol at -70 °C. All the isolates were routinely cultured in BHI broth or blood agar (BA) plates and incubated with 5 % CO_2 at 37 ^{o}C for 24 h.

Screening for anti-*S. pyogenes* activity

The preliminary screening of all plant extracts for their anti-*S. pyogenes* activity was carried out by disc agar diffusion method [10]. The extracts were dissolved in DMSO (250 mg/ml) and then 10 µl were applied to sterile filter paper discs (Whatman No. 1; 6 mm in diameter) so that each disc finally yielded 2.5 mg of the extract. Dry discs (dried at 37 °C overnight) were applied onto the surface of 5% blood Mueller Hinton agar (MHA) plates seeded with the culture of *S. pyogenes*. The plates were then incubated with 5% CO_2 at 37 °C for 20 h. Dimethyl sulphoxide, extraction solvents, and antibiotic discs including erythromycin (15 µg) and penicillin G (1 µg) were used as controls. The experiment was carried out in duplicate and the average diameter of inhibition zone was calculated.

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

A broth microdilution method according to Clinical and Laboratory Standards Institute Guidelines (CLSI) was used to determine the MIC and MBC of the plant extracts against *S. pyogenes* [10]. Erythromycin was used as reference antibiotic. MIC was recorded as the lowest concentration that produced a complete suppression of visible growth. MBC was taken as the concentration that gave significant MIC values using a sterile loop streaking on fresh media. All assays were carried out in triplicate.

RESULTS

Screening for anti-*S. pyogenes* activity

The antibacterial activities of 51 medicinal plant species used in Thai traditional medicine for bacterial infections against *S. pyogenes* NPRC 101 are presented in Table 1. Among the plants tested, nearly all extracts, except *Murdannia loriformis*, produced inhibition zones on *S. pyogenes* NPRC 101. The inhibition zones ranged from 7 - 26 mm. Ethanol extracts of *Piper betle*, *Coriandrum sativum*, *Quercus infectoria*, and *Eleutherine americana* demonstrated large

zones with diameters of 26, 24, 23, and 23 mm, respectively. Thirty four extracts (26 plant species) from a total of 69 extracts (51 plant species) possessed MIC values ≤ 1000 µg/ml on *S. pyogenes* NPRC 101. Only 22 extracts (17 plant species) exhibited bactericidal activity at MBC values ≤ 1000 µg/ml. Among the plant species tested, *Rhodomyrtus tomentosa* (flower, fruit, and leaf extracts) and *Boesenbergia pandurata* (rhizome extract) produced better activity against *S. pyogenes* NPRC 101, as indicated by lower MIC and MBC values.

Table 1: Antibacterial activity of selected Thai medicinal plants against *Streptococcus pyogenes* NPRC 101

Botanical species	Family	Voucher no.	Plant part	Extract yield (%)	Inhibition zone[a] (mm)	MIC/MBC (µg/ml)
Acacia catechu (L.f.) Willd.	Fabaceae	NPRCP0001	core	5.60e	11	>1000/>1000
Aegle marmelos (L.) Correa	Rutaceae	NPRCP0002	fruit	5.37e	15	>1000/>1000
Alstonia scholaris (L.) R. Br.	Apocynaceae	NPRCP0003	wood	1.30a	8	>1000/>1000
Ardisia colorata Roxb.	Myrsinaceae	NPRCP0004	fruit	5.60a	11	>1000/>1000
				4.40e	9	>1000/>1000
Asclepias curassavica L.	Asclepiadaceae	NPRCP0005	wood	0.98e	14	>1000/>1000
Boesenbergia pandurata (Roxb.) Schltr.	Zingiberaceae	NPRCP0006	rhizome	1.58c	7	7.81/7.81
Cassia alata L.	Fabaceae	NPRCP0007	leaf	4.20a	9	>1000/>1000
Centella asiatica (L.) Urb.	Apiaceae	NPRCP0008	leaf	6.00e	15	>1000/>1000
Cinnamomum bejolghota (Buch.-Ham.) Sweet	Lauraceae	NPRCP0009	bark	14.68e	19	250/250
			wood	2.29e	16	125/125
Cinnamomum porrectum (Roxb.) Kosterm.	Lauraceae	NPRCP0010	bark	7.09e	19	500/1000
			wood	11.23e	16	250/250
Cleome gynandra L.	Capparaceae	NPRCP0011	whole plant	6.76e	11	1000/>1000
Coriandrum sativum L.	Apiaceae	NPRCP0012	fruit	2.00a	16	>1000/>1000
				4.00e	24	62.50/125
Curcuma zedoaria (Christm.) Roscoe	Zingiberaceae	NPRCP0013	rhizome	9.60e	11	500/500
Derris scandens Roxb. Benth.	Leguminosae	NPRCP0014	stem	11.40a	13	125/125
				3.20e	13	250/250
Dracaena loureoiri Gangnep.	Agavaceae	NPRCP0015	core	16.90e	15	1000/>1000
Dryopteris syrmatica O. Kze.	Polypodiaceae	NPRCP0016	wood	4.50a	8	>1000/>1000
				4.50e	13	125/125
Eleutherine americana Merr.	Iridaceae	NPRCP0017	bulb	4.80e	23	250/500
Euphorbia thymifolia L.	Euphorbiaceae	NPRCP0018	whole plant	1.30e	15	500/>1000
Gymnopetalum cochinchinensis (Lour.) Kurz	Cucurbitaceae	NPRCP0019	fruit	7.66e	15	250/500
Holarrhena antidysenterica (L.) Wall. ex A. DC.	Apocynaceae	NPRCP0020	bark	2.10e	15	500/500
Impatiens balsamina L.	Balsaminaceae	NPRCP0021	leaf	5.20e	15	>1000/>1000
Manilkara achras (Mill.) Fosberg	Sapotaceae	NPRCP0022	fruit	26.77e	9	>1000/>1000
Millingtonia hortensis L. f.	Bignoniaceae	NPRCP0023	flower	25.41e	8	>1000/>1000
Mimosa pudica L.	Fabaceae	NPRCP0024	whole plant	4.91e	10	>1000/>1000
Mitragyna speciosa (Korth.) Havil	Rubiaceae	NPRCP0025	leaf	5.96e	8	1000/>1000
Momordica charantia L.	Cucurbitaceae	NPRCP0026	vine	3.00e	15	1000/1000
Morinda citrifolia L.	Rubiaceae	NPRCP0027	fruit	7.36e	9	>1000/>1000
Murdannia loriformis (Hassk.) R.S. Rao & Kammathy	Commelinaceae	NPRCP0028	whole plant	7.67e	-[b]	NA[c]
Oroxylum indicum (L.) Kurz	Bignoniaceae	NPRCP0029	bark	3.71e	10	>1000/>1000
Peltophorum pterocarpum (DC.) Backer ex K. Heyne	Fabaceae	NPRCP0030	bark	0.03d	15	1000/>1000
				0.01h	12	>1000/>1000
				6.20m	15	1000/>1000
Phyllanthus amarus Schumach. & Thonn.	Euphorbiaceae	NPRCP0031	whole plant	7.82e	10	>1000/>1000
Piper betle L.	Piperaceae	NPRCP0032	leaf	9.19e	26	500/500
Piper chaba Hunter	Piperaceae	NPRCP0033	fruit	8.96e	9	>1000/>1000
Piper nigrum L.	Piperaceae	NPRCP0034	fruit	6.33e	10	>1000/>1000
Piper sarmentosum Roxb.	Piperaceae	NPRCP0035	leaf	4.72e	20	>1000/>1000
Pluchea indica (L.) Less.	Asteraceae	NPRCP0036	leaf	17.80e	14	500/>1000
Psidium guajava L.	Myrtaceae	NPRCP0037	leaf	2.80a	12	>1000/>1000
Quercus infectoria G. Olivier	Fagaceae	NPRCP0038	nut gall	57.15e	23	500/1000
Quisqualis indica L.	Combretaceae	NPRCP0039	flower	11.08e	17	250/500
Rhizophora mucronata Lam.	Rhizophoraceae	NPRCP0040	bark	11.67e	15	>1000/>1000
			fruit	10.75e	20	1000/>1000

Table 1 (*contd.*): Antibacterial activity of selected Thai medicinal plants against *Streptococcus pyogenes* NPRC 101

Botanical species	Family	Voucher no.	Plant part	Extract yield (%)	Inhibition zonea (mm)	MIC/MBC (µg/ml)
Rhodomyrtus tomentosa (Aiton) Hassk.	Myrtaceae	NPRCP0041	flower	5.63e	17	15.6/62.50
			fruit	2.47e	16	15.6/62.50
			leaf	7.40e	18	7.81/62.50
			stem	7.17e	10	1000/>1000
Sandoricum indicum Cav.	Meliaceae	NPRCP0042	root	5.60a	11	500/500
				4.00e	15	>1000/>1000
Tamarindus indica L.	Fabaceae	NPRCP0043	leaf	4.80e	14	>1000/>1000
Terminalia bellirica (Gaertn.) Roxb.	Combretaceae	NPRCP0044	fruit	14.88e	15	>1000/>1000
Terminalia chebula Retz.	Combretaceae	NPRCP0045	fruit	17.20e	13	>1000/>1000
Terminalia sp.	Combretaceae	NPRCP0046	fruit	23.90e	17	>1000/>1000
Theobroma cacao L.	Sterculiaceae	NPRCP0047	pericarp	3.67e	9	>1000/>1000
Uncaria gambir	Rubiaceae	NPRCP0048	leaf,	65.40a	11	>1000/>1000
			branch	65.40e	11	500/>1000
Walsura robusta Roxb.	Meliaceae	NPRCP0049	leaf,	0.59a	17	>1000/>1000
			branch	1.00b	9	1000/>1000
				0.35et	16	125/500
				12.09m	17	>1000/>1000
Wrightia tomentosa (Roxb.) Roem. & Schult.	Apocynaceae	NPRCP0050	stem	3.90e	15	500/>1000
Xylocarpus granatum J. König	Meliaceae	NPRCP0051	pericarp	2.68e	10	>1000/>1000
			seed	6.77e	11	>1000/>1000
Erythromycin						0.125/0.125

[a]Concentration of the extract = 2.5 mg/disc; [b]No inhibition zone; [c]Not applicable.
a: Aqueous; b: n-Butanol; c: Chloroform; d: Dichloromethane: e: Ethanol; et: Ethyl acetate; m: Methanol; h: Hexane

Table 2: Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of 10 plant extracts against clinical *Streptococcus pyogenes* isolates (n = 11)

Botanical species	**Part tested**	**MIC range (µg/ml)**	**MBC range (µg/ml)**
Boesenbergia pandurata[a]	rhizome	3.91-31.25	7.81-62.50
Cinnamomum bejolghota[b]	bark	31.25->1000	31.25->1000
	wood	31.25-1000	31.25-1000
Cinnamomum porrectum[b]	bark	62.50->1000	62.50->1000
	wood	250->1000	250->1000
Eleutherine americana[b]	bulb	250	250-500
Gymnopetalum cochinchinensis[b]	fruit	31.25->1000	31.25->1000
Piper betle[b]	leaf	500-1000	500-1000
Quercus infectoria[b]	nut gall	125->1000	125->1000
Quisqualis indica[b]	flower	250->1000	250->1000
Rhodomyrtus tomentosa[b]	leaf	3.91-31.25	3.91-62.50
Walsura robusta[c]	leaf, branch	62.50->1000	62.50->1000
Erythromycin		<0.015-0.125	<0.015-0.125

[a]Chloroform extract; [b]Ethanol extract; [c]Ethyl acetate extract.

Anti-*S. pyogenes* activity of plant extracts

Ten effective plants, including *Boesenbergia pandurata*, *Cinnamomum bejolghota*, *Cinnamomum porrectum*, *Eleutherine americana*, *Gymnopetalum cochinchinensis*, *Piper betle*, *Quercus infectoria*, *Quisqualis indica*, *Rhodomyrtus tomentosa*, and *Walsura robusta* were selected based on their antibacterial activity against *S. pyogenes* NPRC 101 to further determine the variability of MICs and MBCs against 11 clinical isolates. The MIC and MBC values of these 10 effective plants against 11 clinical isolates of *S. pyogenes* are shown in Table 2. Variations in the MIC and MBC values were found among the bacterial isolates when tested with the extracts of *Cinnamomum bejolghota*, *Cinnamomum porrectum*, *Gymnopetalum cochinchinensis*, *Quercus infectoria*, *Quisqualis indica*, and *Walsura robusta*. These plants exhibited a wide range of MIC and MBC values, from 31.25 - >1000 and

31.25 - >1000 µg/ml, respectively. In contrast, three plant species including *Boesenbergia pandurata*, *Eleutherine americana*, and *Rhodomyrtus tomentosa* showed similar antibacterial activity among different 11 clinical isolates. *Boesenbergia pandurata* and *Rhodomyrtus tomentosa* demonstrated very good antibacterial activity with MIC and MBC values ranged from 3.91 - 31.25, 3.91 - 31.25 and 7.81 - 62.50, 3.91 - 62.50 µg/ml, respectively. Meanwhile the extract of *Eleutherine americana* demonstrated moderate activity against all isolates with the MIC and MBC values of 250 and 250 - 500 µg/ml, respectively. All isolates tested were sensitive to erythromycin (MIC ≤ 0.25 µg/ml).

DISCUSSION

A number of plant extracts can be screened for their anti-*S. pyogenes* properties quickly using paper disc assay. However, this assay is not classically quantitative and using the size of inhibition zone to indicate relative antibacterial activity is not sufficient. The zone of inhibition may be affected by many factors such as the evaporation, solubility, and diffusion rate of the active components through test medium. Zone of inhibition testing is particularly appropriate for determining the ability of water-soluble antimicrobial compounds to inhibit the growth of microorganisms. Therefore, the MIC/MBC values and zone of inhibition of some of the plant extracts in this study did not correlate. For example, *Boesenbergia pandurata* extract generated a very small zone of inhibition (7 mm) but possessed very good MIC and MBC values.

In this study, we found that the extracts of *Boesenbergia pandurata*, *Eleutherine americana*, and *Rhodomyrtus tomentosa* not only demonstrated good antibacterial activity against *S. pyogenes* isolated from upper respiratory tract infections but also produced similar activities against different *S. pyogenes* clinical isolates.

In Thai traditional medicine, the rhizomes of *Boesenbergia pandurata* are used to treat colic disorders, wound infections and inflammation [11]. The antibacterial activities of this plant have been reported [12]. Some antibacterial active components from this plant were isolated and studied for their activities. Pinostrobin isolated from this plant demonstrated anti-*Helicobacter pylori* activity [13]. Panduratin A from this plant displayed significant antibacterial activity against a number of staphylococci and enterococci clinical isolates. Notably, the antibacterial activity of panduratin A was more potent than many reference antibiotics [14,15]. Isopanduratin A from this plant demonstrated antibacterial activity against many streptococci including *Streptococcus mutans*, *Streptococcus sobrinus*, *Streptococcus sanguinis*, and *Streptococcus salivarius* [16,17].

Eleutherine americana is a herbal plant whose red bulb was used as a folk medicine and flavouring agent. In the screening test we found that the bulb extract of this plant demonstrated moderately strong activity against *S. pyogenes* when compared with other plant extracts. However, when we increased the number of bacterial isolates it could produce similar antibacterial activity against difference *S. pyogenes* isolates. The antibacterial activities of this plant have been previously reported against gram-negative [18] and gram-positive bacteria [19]. Several compounds from this plant such as eleuthinone A, eleuthraquinone A and B, and eleucanarol have been isolated and studied for their antibacterial activities against *Staphylococcus aureus* [20].

Rhodomyrtus tomentosa is a Thai medicinal plant used to treat skin, oral, gastrointestinal, and urinary tract infections. In this study, we found that the extracts of flower, fruit, and leaf demonstrated good antibacterial activity against *S. pyogenes*. The leaf extract of this plant has been reported for its antibacterial activity against many bacterial pathogens [19]. An isolated compound, named rhodomyrtone, from the leaf of this plant exhibited strong antibacterial activity against many pathogenic Gram-positive bacteria [21-23] including *S. pyogenes* [24]. Moreover, rhodomyrtone possessed noteworthy activity against methicillin-resistant *S. aureus,* displaying a stronger activity than vancomycin, a reference antibiotic [24].

CONCLUSION

Our study demonstrated that the plants species including *Boesenbergia pandurata*, *Eleutherine americana*, and *Rhodomyrtus tomentosa* have great potentials as antibacterial agents against *S. pyogenes*. Thus, these plants may yield biologically active compounds that might be valuable in the treatment of the diseases caused by *S. pyogenes*. Their active components, however, need to be isolated, and their toxicity and therapeutic activity *in vivo* evaluated.

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