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Evaluation of Antibacterial Activities of Medicinal Plants Widely Used Among AIDS Patients in Thailand

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Abstract

Antibacterial activity of 12 selected Thai medicinal plants used as self-medication by HIV/AIDS patients in Thailand was studied. Thirty-nine chloroform, methanol, and aqueous extracts from these plants were investigated for their antibacterial activity against important pathogenic bacteria commonly associated with AIDS infection. These included *Staphylococcus aureus*, methicillin-resistant *Staphylococcus aureus* (MRSA), *Streptococcus mutans*, and *Salmonella typhi*. Inhibition of growth was tested using paper disk agar diffusion method. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined by agar microdilution method and agar dilution method in Petri dishes with millipore filters. The Gram-positive bacteria were proved to be susceptible to the chloroform extracts of *Alpinia galanga* (L.) Willd., *Boesenbergia rotunda* Mansf. (L.), *Piper betle* (L.), *Spilanthes acmella* (L.) Murray, and *Zingiber zerumbet* (L.) Roscoe ex Sm. and the methanol extract of *Boesenbergia rotunda*. Chloroform extract of *Alpinia galanga* demonstrated the greatest inhibition zones of 29.1 and 23.7 mm against *Staphylococcus aureus* and MRSA, respectively. The MIC values of this extract against *Staphylococcus aureus* and MRSA were 128 and 256 μg/ml and the MBC values were 256 and 256 μg/ml, respectively. An active compound, 1′-acetoxychavicol acetate, was identified with MIC values against MRSA and *Staphylococcus aureus* of 64 and 128 μg/ml, respectively.

Keywords: HIV/AIDS, medicinal plants, MRSA, *Staphylococcus aureus*, *Streptococcus mutans*, *Salmonella typhi*.

Introduction

In Thailand, medicinal plants are popularly employed among AIDS patients as primary health care for the treatment of many opportunistic infections (Farnsworth & Bunyapraphatsara, 1992). A number of studies reported antibacterial activity of Thai medicinal plants against certain pathogenic bacteria including enterohemorrhagic *Escherichia coli* O157: H7 (Voravuthikunchai et al., 2002, 2004a,b), *Helicobacter pylori* (Voravuthikunchai et al., 2004c), *Streptococcus mutans* (Chen et al., 1989; Kitamura et al., 1990; Järwinen et al., 1993; Jagtap & Karkera, 2000; Koo et al., 2000; Taweechaisupapong et al., 2000; Leclercq, 2002), and methicillin-resistant *Staphylococcus aureus* (MRSA) (Cardoso et al., 1988; Boyce, 1992; Berns, 2003; Machado et al., 2003; Voravuthikunchai & Kitpipit, 2003).

Treatment failures of pathogenic bacteria due to drug-resistance problems (Boyce, 1992; Berns, 2003), stimulation of toxin production (Yoh et al., 1999), and undesirable side effects (Farnsworth & Bunyapraphatsara, 1992) have frequently been reported. In Thailand, a number of people suffering from AIDS tend to seek help from local traditional medicine practitioners. Nevertheless, medicinal plants have been used without

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any scientific evidence. Therefore, the current communication aimed to evaluate the antibacterial activity of some plants against infections that can become very serious in AIDS patients.

Alpinia galanga, *Boesenbergia rotunda*, *Piper chaba*, *Spilanthes acmella*, and *Zingiber zerumbet* are medicinal plants commonly used to treat cases of diarrhea (Farnsworth & Bunyapraphatsara, 1992). *Acanthus ebracteatus*, *Barleria lupulina*, and *Murraya paniculata* are popular for their uses against wound infection (Farnsworth & Bunyapraphatsara, 1992). *Piple betle* leaf was earlier demonstrated to have inhibitory activity against obligate oral anaerobes that cause halitosis (Ramji et al., 2002).

Twelve medicinal plant species, belonging to seven families, used in Thailand to cure bacterial infections (Farnsworth & Bunyapraphatsara, 1992) were primarily screened for attributed antibiotic properties against selected groups of both Gram-positive and Gram-negative bacteria. Both the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of extracts from effective medicinal plant species were established.

Materials and Methods

Medicinal plant materials

Twelve medicinal plant species used in Thai traditional medicine were collected from Songkla Province, Thailand. The plant parts were chosen on the basis of traditional uses among AIDS patients in Thailand (Table 1). Botanical identification of the plant materials was obtained. A classified reference voucher specimen was kept at the Herbarium of Faculty of Pharmaceutical Sciences, Prince of Songkla University, Thailand.

Plant extraction and isolation of active fractions

Plant materials were collected and washed with distilled water. Samples were cut into small pieces and dried at 60°C overnight. The extraction procedures have been earlier described (Tewtrakul et al., 2003). The plant parts were crushed in a mechanical mortar and successively extracted with solvents of increasing polarity beginning with chloroform, methanol, and boiling water. The solvents were removed under reduced pressure in a rotary evaporator until they became completely dry. The percentage yield for each extract was determined (Table 1). For antibacterial assays, extracts and pooled fractions residues were adequately diluted in the corresponding extractive solvents (agar diffusion method) and in dimethyl sulfoxide (DMSO) for broth dilution (dilution method).

Isolation of pure compounds

All pure compounds were further isolated by means of chromatographic techniques. The identification of the isolated compounds was performed by comparing the spectroscopic data with those previously described (Itokawa et al., 1981; Tanaka et al., 1985; Burke & Nair, 1986). The crude chloroform extract (10 g) of *Alpinia galanga* (rhizome) gave 1′-acetoxychavicol acetate (634.2 mg). Three flavanones, pinostrobin (2982.7 mg), pinocembrin (477.1 mg), and alpinetin (650.7 mg), were isolated from the chloroform extract (9.91 g) of *Boesenburgia pandurata* (rhizome).

Table 1. Medicinal plant names, plant part used, and their extract yield.

Botanical name (Voucher specimen number)	Family	Plant part	Extract yield (%)		
			Chloroform extract	Methanol extract	Aqueous extract
Acanthus ebracteatus Vahl (SN4501010)	Acanthaceae	Leaf	2.2	5.4	9.0
Alpinia galanga (L.) Willd. (SN4412030)	Zingiberaceae	Rhizome	5.63	12.05	7.48
Baleria lupulina Lindl. (SN4501001)	Acanthaceae	Leaf	10.95	23.01	10.09
		Stem	1.46	7.20	5.75
Boesenbergia rotunda (L.) Mansf. (SN4412015)	Zingiberaceae	Rhizome	1.58	10.44	8.38
Coccinia grandis (L.) Voigt (SN4412050)	Cucurbitaceae	Leaf	6.25	9.60	13.07
Eclipta prostrata (L.) (SN4412025)	Asteraceae	Whole plant	5.68	7.49	17.04
Gynura pseudochina (L.) D.C. var. *hispida* Thv. (SN4701001)	Compositae	Whole plant	ND[a]	ND	ND
Murraya paniculata (L.) Jack (SN4412040)	Rutaceae	Leaf	11.27	17.31	8.38
Piper betle (L.) (SN4412035)	Piperaceae	Leaf	16.36	14.68	7.26
Piper chaba Hunter (SN4412020)	Piperaceae	Fruit	9.19	5.07	10.49
Spilanthes acmella (L.) Murray (SN4412045)	Asteraceae	Whole plant	23.32	12.29	15.87
Zingiber zerumbet (L.) Roscoe ex Sm. (SN4412010)	Zingiberaceae	Rhizome	1.87	4.26	14.12

[a]ND, Not determined.

Tested bacterial strains

Clinical isolates of MRSA, *Streptococcus mutans*, *Salmonella typhi*, and *Staphylococcus aureus* ATCC 25923 were used. Each bacterial strain was suspended in Mueller-Hinton broth (MHB, Oxoid, Hampshire, England) and incubated at 37°C for 18 h. Mueller-Hinton agar (MHA, Oxoid) was used for testing antibacterial activity.

Determination of antibacterial activity

Paper disk agar diffusion method

Sterile filter paper disks (Whatman no. 1; 5 mm in diameter) were soaked with 10 µl of extract residue diluted in the corresponding extractive solvent, so that each disk was impregnated with 100 µg of residue and dried at 35°C overnight. Disks were applied onto the surface of MHA plates seeded with a 24-h culture of the tested bacteria in Trypticase Soy Broth (TSB, Oxoid) and incubated at 35°C for 18 h. Antibiotic disks including amikacin, ampicillin, aztreonam, chloramphenicol, erythromycin, gentamicin, kanamycin, oxacillin, penicillin, tetracycline, and vancomycin were used as control. Susceptibility patterns were obtained according to National Committee for Clinical Laboratory Standards (NCCLS) (National Committee for Clinical Laboratory Standards, 2000b). The antibacterial activity was evaluated by measuring the diameter of the inhibition zone formed around the disk. The experiment was performed in triplicate, and the mean of the diameter of the inhibition zones was calculated.

Table 2. Antibacterial activity of the chloroform, methanol, and aqueous extracts of effective medicinal plant species (concentration 250 µg/disk).

Medicinal plant extracts and antibiotics	Mean values[a] of inhibition zone (mm)			
	MRSASK1	*Staphylococcus aureus* ATCC 25923	*Streptococcus mutans*	*Salmonella typhi*
Alpinia galanga				
Chloroform	23.7	29.1	9.4	—[b]
Methanol	—	—	—	—
Aqueous	—	—	—	—
Boesenbergia rotunda				
Chloroform	10.0	9.7	8.2	—
Methanol	10.5	11.4	8.1	8.0
Aqueous	—	—	—	—
Piper betle				
Chloroform	9.3	8.6	—	11.6
Methanol	—	—	—	—
Aqueous	—	—	—	—
Spilanthes acmella				
Chloroform	—	—	7.5	—
Methanol	—	—	—	—
Aqueous	—	—	—	—
Zingiber zerumbet				
Chloroform	9.5	9.8	8.6	—
Methanol	—	—	—	—
Aqueous	—	—	—	—
Amikacin (10 µg)	ND[c]	ND	ND	23.2
Ampicillin (10 µg)	ND	ND	ND	22.5
Aztreonam (30 µg)	ND	ND	ND	33.8
Chloramphenicol (30 µg)	24.5	23.5	22.3	25.2
Erythromycin (30 µg)	—	27.0	11.8	ND
Gentamicin (10 µg)	ND	ND	ND	21.4
Kanamycin (30 µg)	ND	ND	ND	21.3
Oxacillin (1 µg)	—	20.6	—	ND
Penicillin G (10 µg)	—	21.1	18.0	ND
Tetracycline (30 µg)	—	28.3	13.5	24.1
Vancomycin (30 µg)	16.7	16.6	21.5	ND

MRSA, methicillin-resistant *Staphylococcus aureus*.
[a]Mean values from triplicate.
[b]—, no inhibition zone.
[c]Not determined.

Determination of minimum inhibitory concentration and minimum bactericidal concentration

A modified agar microdilution method (National Committee for Clinical Laboratory Standards, 2000a) was used to determine the MIC of extracts of the medicinal plants that produced inhibition zones. Tetracycline and vancomycin were used as reference standards (Lorian, 1996). One microliter of an overnight culture of each bacterial strain containing approximately 10^4 CFU was applied onto MHA supplemented with the medicinal plant extracts. The microtiter plates were incubated at 35°C for 18 h. Observations were performed in triplicate and results expressed as the mean values of the lowest concentration of plant extracts that produced a complete suppression of colony growth, MIC. Minimum bactericidal concentration using the agar dilution method in Petri dishes with millipore filter was performed with the extracts that gave significant MIC values against each bacterial strain.

Results and Discussion

Opportunistic infections are quite inevitable among people suffering from AIDS. Serious infections caused by MRSA have increased significantly during the past decades in hospitals (Boyce, 1992; Santos et al., 1999) and the community (Cardoso et al., 1988). It is obvious that they become increasingly common among HIV/AIDS patients. Furthermore, viridians group streptococci, the most representative human carcinogenic agent, have been well-documented with AIDS. These bacteria are moderately resistant to antibiotics (Venditti et al., 1989) and other antimicrobial agents such as chlorhexidine (Jagtap & Karkera, 2000). Many other infections including diarrhea have often been reported (Farnsworth & Bunyapraphatsara, 1992). Scientific search for new ways to treat these infections stimulates the investigation of natural bioactive compounds as an alternative therapy.

We aimed to screen medicinal plants with attributed antibiotic properties. The ethnobotanical data of 12 medicinal plant species including botanical names, the plant parts employed, and the percentage extract yield of the selected plant species is summarized (Table 1). The plants were initially screened for their antibacterial activity against selected pathogenic bacteria. The results of the plants that produced inhibition zones are presented in Table 2. The analysis of the growth inhibition activity by the disk diffusion method showed that among 39 crude extracts tested, only six extracts (15.38%) of the plant species were demonstrated to have antibacterial activity. These were the chloroform and the methanol extracts of *Boesenbergia rotunda* and the chloroform extracts of *Alpinia galanga*, *Piper betle*, *Spilanthes acmella*, and *Zingiber zerumbet*. The inhibition zones ranged from 8.6 to 29.1 mm. The maximum zone of antibacterial effect against *Staphylococcus aureus* ATCC 25923 was produced from the chloroform extract of *Alpinia galanga*.

The methanol extracts of *Boesenbergia rotunda* exhibited high activity against all bacterial species tested (Table 3). The chloroform extracts of *Alpinia galanga*, *Boesenbergia rotunda*, *Piper betle*, and *Zingiber zerumbet* exhibited inhibition zones against *Staphylococcus aureus*. The inhibition zones of *Streptococcus mutans* were demonstrated with the chloroform and the methanol extracts of *Boesenbergia rotunda* and the chloroform

Table 3. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of crude medicinal plant extract against pathogenic bacteria.

Medicinal plant extracts and antibiotics	Mean values[a] of MIC and MBC (μg/ml)							
	MRSA		*Staphylococcus aureus*		*Streptococcus mutans*		*Salmonella typhi*	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Alpinia galanga								
Chloroform extract	256	256	128	256	ND[b]	ND	ND	ND
Boesenbergia rotunda								
Chloroform extract	>512	ND	ND	ND	512	>512	ND	ND
Methanolic extract	>512	ND	>512	ND	512	>512	512	>512
Piper betle								
Chloroform extract	512	>512	ND	ND	ND	ND	512	>512
Tetracycline	ND	ND	ND	ND	ND	ND	1	32
Vancomycin	2	2	2	2	1	1	ND	ND

MRSA, methicillin-resistant *Staphylococcus aureus*.
[a]Mean values from triplicate.
[b]Not determined.

Table 4. Minimum inhibitory concentration (MIC) of active compounds against pathogenic bacteria.

Medicinal plants	Active compounds	MIC (μg/ml)	
		Staphylococcus aureus	MRSA
Alpinia galanga	Fraction 04 (1′-acetoxychavicol acetate)[a]	256	256
	Fraction 05 (1′-acetoxychavicol acetate)	256	256
	1′-Acetoxychavicol acetate	128	64
Boesenbergia rotunda	Fraction J (pinocembrin and alpinitin)	>512	>512
	Pinostrobin	>512	>512
	Pinocembrin	512	512
	Alpinitin	>512	>512

MRSA, methicillin-resistant *Staphylococcus aureus*.
[a]Main compound.

extracts of *Alpinia galanga*, *Spilanthes acmella*, and *Zingiber zerumbet*. The methanol extracts of *Boesenbergia rotunda* and the chloroform extract of *Piper betle* produced an inhibition zone against *Salmonella typhi*.

Significant antibacterial effects, expressed as MIC, of crude extracts against the pathogenic bacteria were presented at the concentrations of 128 to 512 μg/ml. The chloroform extract of *Alpinia galanga* was among the most active medicinal plant with the MIC values against *Staphylococcus aureus* and MRSA at 128 and 256 μg/ml, respectively.

As the chloroform extract of *Alpinia galanga* and both the chloroform and the methanol extracts of *Boesenbergia rotunda* were effective against the pathogenic bacteria tested, they were further studied for the presence of active compounds (Table 4). An active compound, 1′-acetoxychavicol acetate, was identified with the MIC values against *Staphylococcus aureus* and MRSA at 128 and 64 μg/ml, respectively.

Alpinia galanga, *Boesenbergia rotunda*, and *Zingiber zerumbet* (Zingiberaceae) are rhizomes popularly used in Thai traditional medicine. The anti-inflammatory effect (Tuchinda et al., 2002) as well as the hepatocarcinogenic effect (Tiwawech et al., 2000) of many compounds extracted from the rhizomes of *Boesenbergia rotunda* were reported. This is the detailed study on the compounds of the two plant species. A series of studies on these compounds are underway in our laboratory including the investigation of their effects on other important groups of pathogens such as *Mycobacterium tuberculosis*, *Candida albicans*, and *Giardia intestinalis* that are common among AIDS patients.

Conclusions

The chloroform extract of *Alpinia galanga* showed the most significant antibacterial properties. 1′-Acetoxychavicol acetate, isolated from the extract, is one of the active principles. The results obtained suggest this could be used in the treatment of opportunistic infection in AIDS patients such as infection caused by MRSA.

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