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Evaluation of the Antimycobacterial Activity of Extracts from Plants Used as Self-Medication by AIDS Patients in Thailand

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Abstract

Chloroform, methanol, and water extracts from medicinal plants used as self-medication by AIDS patients in Thailand were evaluated for their antimycobacterial activity using the microplate Alamar blue assay. The crude extracts exhibited antimycobacterial activity with minimum inhibitory concentrations (MICs) of 0.12–1000 μg/ml. The chloroform extract of *Alpinia galanga* rhizomes and *Piper chaba* fruits had strong inhibitory effects with MIC values of 0.12 and 16 μg/ml, respectively. The active compounds, 1′-acetoxychavicol acetate from *Alpinia galanga* and piperine from *Piper chaba* had MIC values of 0.024 and 50 μg/ml, respectively.

Keywords: 1′-Acetoxychavicol acetate, AIDS patients, Alamar blue assay, antimycobacterial, piperine, Thai medicinal plants.

Introduction

Plants have been used worldwide in traditional medicine for the treatment of diseases. In Thailand, many medicinal plants are recommended for use as a primary health care system (Farnsworth & Bunyapraphatsara, 1992). Some of them are used by AIDS patients to treat their symptoms and opportunistic infections. Plants used in this study have been tested for their HIV-1 protease inhibitory activity (Tewtrakul et al., 2003). Only a few of them showed inhibitory activity. Tuberculosis is one of the most serious infections in AIDS patients. Antituberculosis drug resistance is a major public health problem that threatens global tuberculosis control. We, therefore, tested the activity of extracts of these plants against *Mycobacterium tuberculosis*. We report here on the antimycobacterial activity of 38 crude extracts of 12 medicinal plants used by AIDS patients in Thailand.

Materials and Methods

Plants

The plants were collected at the Botanical Garden of Prince of Songkla University and the area around Songkhla province, southern Thailand, in October 2001. The voucher specimens were identified and deposited in the Herbarium of the Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Prince of Songkla University. Each plant part was chosen on the basis of their known use by AIDS patients in southern Thailand. The names and parts of the plants used are shown in Table 1.

Preparation of samples

Plant materials were oven-dried at 50°C and powdered. Each sample (10 g) was extracted by maceration for 1 week with 3 consecutive 200-ml lots of either chloroform or methanol at room temperature. The solid material left from the methanol extraction was then extracted with 3 consecutive 200-ml lots of boiling water for 3 h. The 3 consecutive extracts from each solvent were combined and solvent was evaporated to dryness *in vacuo* and the residue dissolved in dimethyl sulfoxide (DMSO) to give chloroform-, methanol-, and water-extract test solutions, respectively. The yields of the extracts are given in Table 1.

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Table 1. Medicinal plants used in antimycobacterial assay.

Botanical name (Voucher specimen no.)	Family	Part used	% yield		
			$CHCl_3$ extract	MeOH extract	Water extract
Acanthus ebracteatus Vahl. (SN4501010)	Acanthaceae	Leaf, stem	2.2	5.4	9.0
Alpinia galanga (L.) Willd. (SN4412030)	Zingiberaceae	Rhizome	5.6	12.0	7.5
Baleria lupulina Lindl. (SN4501001)	Acanthaceae	Leaf	11.0	23.0	10.1
Baleria lupulina Lindl. (SN450105)	Acanthaceae	Stem	1.5	7.2	5.8
Boesenbergia pandurata (Roxb.) Schltr. (SN4412015)	Zingiberaceae	Rhizome	1.6	10.4	8.4
Coccinia grandis (L.) Voigt (SN4412050)	Cucurbitaceae	Leaf	6.3	9.6	13.1
Eclipta prostata (L.) L. (SN4412025)	Compositae	Whole plant	5.7	7.5	17.4
Gynura pseudochina (L.) D.C. var. *hispida* Thv. (SN4701001)	Compositae	Whole plant	ND	ND	ND
Murraya paniculata (L.) Jack (SN4412040)	Rutaceae	Leaf	11.3	17.3	8.4
Piper betle L. (SN4412035)	Piperaceae	Leaf	16.4	14.7	7.3
Piper chaba Hunter (SN4412020)	Piperaceae	Fruit	9.2	5.1	10.5
Spilanthes acmella (L.) Murray (SN4412045)	Compositae	Whole plant	2.3	12.3	15.9
Zingiber zerumbet (L.) Roscoe ex Sm. (SN4412010)	Zingiberaceae	Rhizome	1.9	4.3	14.1

ND, not determined.

Pure compounds were isolated by chromatographic techniques and the spectroscopic data compared with those previously described (de Cleyn & Verzele, 1973; Itokawa et al., 1981; Tanaka et al., 1985; Burke & Nair, 1986). 1′-Acetoxychavicol acetate isolated from *Alpinia galanga* rhizomes and piperine from *Piper chaba* fruits were also tested for their antimycobacterial activity.

Antimycobacterial assay

The antimycobacterial activity was performed using the microplate Alamar blue assay (MABA) (Collins & Franzblau, 1997). Briefly, 100 µl of test solution in Middlebrook 7H9 medium was mixed with 100 µl of the same medium containing 10^5 cfu/ml of *Mycobacterium tuberculosis* H37Ra to give a final concentration of extract of 1000 µg/ml. After incubation at 37°C for 7 days, 20 µl of Alamar blue was added to the control well. If the dye turned pink, indicating bacterial growth, the dye was then added to all remaining wells. The results were read on the following day using fluorescence spectroscopy. Active extracts were retested at lower concentrations to determine the minimum inhibitory concentrations (MICs). Standard drugs rifampin, isoniazid, and kanamycin showed MICs of 0.0023, 0.1, and 2.5 µg/ml, respectively, which were in the acceptable ranges for these drugs.

Results and Discussion

Extracts of medicinal plants used as self-medication by AIDS patients were screened for their antimycobacterial activity. All the extracts tested at the initial concentration of 1000 µg/ml inhibited the growth of *Mycobacterium tuberculosis* H37Ra. The MIC values of each extract are shown in Table 2. Chloroform extracts of most plants were more active than methanol and water extracts except for *Boesenbergia pandurata*. Kirdmanee et al. also found that the antimycobacterial activity of hexane and dichloromethane extracts of plants were higher than were water extracts. Thus, the active antimycobacterial agents might be nonpolar compounds. In this study, the chloroform extract of *Alpinia galanga* was found to be the most active with an MIC of 0.12 µg/ml and compared favorably with the antituberculous drug isoniazid (0.1 µg/ml).

Table 2. Minimum inhibitory concentration (MIC) of crude medicinal plant extracts against *Mycobacterium tuberculosis* H37Ra.

Botanical name	MIC (μg/ml)		
	$CHCl_3$ extract	MeOH extract	Water extract
Acanthus ebracteatus	1000	1000	1000
Alpinia galanga	0.12	1000	1000
Baleria lupulina (leaf)	1000	1000	1000
Baleria lupulina (stem)	500	1000	1000
Boesenbergia pandurata	1000	62.5	62.5
Coccinia grandis	1000	1000	1000
Eclipta prostata	125	1000	62.5
Gynura pseudochina var. *hispida*	200	1000	1000
Murraya paniculata	250	1000	1000
Piper betle	62.5	1000	1000
Piper chaba	16	125	1000
Spilanthes acmella	500	1000	1000
Zingiber zerumbet	125	1000	NA
Rifampin		0.0023	
Isoniazid		0.1	
Kanamycin		2.5	

NA, not applicable.

The chloroform extract of *Piper chaba* showed strong antituberculous activity (MIC 16 μg/ml). Other extracts that exhibited moderate activity (MIC 62.5–125 μg/ml) were the methanol and water extracts of *Boesenbergia pandurata*, chloroform and water extracts of *Eclipta prostata*, and chloroform extracts of *Piper betle* and *Zingiber zerumbet*.

Very little is known about the antimycobacterial activity of the medicinal plants used in this study. Only the crude extract of *Alpinia galanga* has been demonstrated to have an activity similar to that of isoniazid. 1′acetoxychavicol acetate was isolated from *Alpinia galanga* and *Alpinia nigra* and this had antituberculous activity (Palittapongarnpim et al., 2002). In this study, a bioassay-guided isolation of the chloroform extract from *Alpinia galanga* yielded a known compound 1′-acetoxychavicol acetate as mycobacterial inhibitor. 1′-Acetoxychavicol acetate exhibited very potent antimycobacterial activity with an MIC value of 0.024 μg/ml (Table 3). *Alpinia galanga* is commonly used in traditional medicines. Previous work on antimicrobial activities of this plant has been reported (Janssen & Scheffer, 1985; Farnsworth & Bunyapraphatsara, 1992; Haraguchi et al., 1996; Sawangjaroen et al., 2005). This study confirmed the antimycobacterial activity of the chloroform extract and 1′-acetoxychavicol acetate from *Alpinia galanga*.

Piper chaba Hunter or *Piper retrofractum* Vahl. is used in traditional medicines by many people in Asia (Kirtikar & Basu, 1980; Farnsworth & Bunyapraphatsara, 1992). Various parts of this plant have been used in different traditional formulations. In our study, we found that piperine is one of the active principles in the chloroform extract of *Piper chaba* fruits. Its MIC value was 50 μg/ml (Table 3), which was 3-times higher than that of the chloroform extract. Piperine is one of the major ingredients of *Piper* species. It exhibits several pharmacological and biochemical effects including antimicrobial, antifungal, and hepatoprotective (Reddy & Lokesh, 1992); antimetastatic activity (Pradeep & Kuttan, 2002), as well as immunomodulatory and antitumor activity (Pradeep & Kuttan, 2004; Sunila & Kuttan, 2004) and antidepressant-like activity (Lee et al., 2005). Moreover, Balakrishnan et al. (2005) reported that piperine augmented transcription inhibitory activity of rifampicin several-fold in *Mycobacterium smegmatis*. Thus, the chloroform extract of *Piper chaba* fruits containing piperine may be useful in the treatment of tuberculosis in AIDS patients treated with antituberculous drugs.

Table 3. Minimum inhibitory concentration (MIC) of active compounds against *Mycobacterium tuberculosis* H37Ra.

Medicinal plants	Active compounds	Activity at 200 μg/ml	MIC (μg/ml)
Alpinia galanga	1′-Acetoxychavicol acetate	Active	0.024
Piper chaba	Piperine	Active	50.0

In Thailand, tuberculosis is the top opportunistic infection in AIDS patients. The majority of AIDS cases in Thailand have been laborers with low incomes (MOPH, 2005). Thus, Thai HIV/AIDS patients tend to seek remedies to relieve their symptoms using traditional and cheaper medicines. Among these active extracts, only the methanol extract of *Boesenbergia pandurata* and the water extract of *Eclipta prostata* had HIV-1 protease inhibitory activity (Tewtrakul et al., 2003). Although these plants have less activity against HIV, some of them, especially *Alpinia galanga* and *Piper chaba*, are useful for HIV patients requiring treatment for tuberculosis.

Plants in this study have also been investigated for their antibacterial (Voravuthikunchai et al., 2005), antifungal (Phongpaichit et al., 2005), and anti-giardial activities (Sawangjaroen et al., 2005). The chloroform extracts of *Alpinia galanga* and *Boesenbergia pandurata* had pronounced antifungal activity against *Cryptococcus neoformans* and *Microsporum gypseum* while the chloroform extracts of *Alpinia galanga*, *Boesenbergia pandurata*, *Eclipta prostrata*, *Piper betle*, *Piper chaba*, *Zingiber zerumbet*, and methanol extracts of *Boesenbergia pandurata* and *Eclipta prostrata* were active against *Giardia lamblia*.

Conclusions

Based on the strong antimycobacterial activity of the crude chloroform extracts of *Alpinia galanga* and *Piper chaba* and their bioactive compounds, it might be suggested that these two plants could be useful for the treatment of tuberculosis in AIDS patients.

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