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(Study on Pharmacological Activity of Medicinal Plants Used as Self Medication by AIDS Patients)

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คณะเภสัชศาสตร์
มหาวิทยาลัยสงขลานครินทร์
HIV-protease inhibitory effects of medicinal plants used as self medication by AIDS patients.

HIV-1 protease inhibitory substances from the rhizomes of Boesenbergia pandurata Holtt.

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Antifungal activities of extracts from Thai medicinal plants against opportunistic fungal pathogens associated with AIDS patients.

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

The anti-amoebic activity of some medicinal plants used by AIDS patients in southern Thailand.

Inhibitory activity of Thai condiments on pandemic strain of Vibrio parahaemolyticus.


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จากคำจดศิพในผู้ป่วยโรคอัลชิเด
HIV-1 protease inhibitory effects of medicinal plants used as self medication by AIDS patients

Supinya Tewtrakul¹, Sanan Subhadhirasakul² and Sopa Kummee³

Abstract

Thirty-six chloroform-, methanol-, and water- extracts of some plants used as self medication by AIDS patients were investigated for their HIV-1 protease (HIV-1 PR) inhibitory activities. Of these extracts, Boesenbergia pandurata (rhizome, chloroform extract) showed the most potent inhibitory activity against HIV-1 PR, followed by Boesenbergia pandurata (rhizome, MeOH extract) and Alpinia galanga (rhizome, MeOH extract) with the inhibitions of 64.92, 51.92 and 48.70%, respectively, at concentration of 100 µg/ml.

Key words: HIV-1 protease, inhibitory effect, self medication, AIDS patients

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Thai medicinal plants were studied for their activities against HIV-1 PR; most of them have been used in the primary health care project of Thailand but the HIV-1 PR inhibitory activities of these plants have not been reported. They were Zingiber zerumbet (rhizome), Boesenburgia pandurata (rhizome), Piper chaba (fruit), Eclipta prostrata (whole plant), Barleria lupulina (leaf, stem), Acanthus ilicifolius (leaf and stem), Alpinia galanga (rhizome), Piper betel (leaf), Spilanthes acmella (whole plant), and Coccinia grandis (leaf). Therefore, the aim of the study was to investigate HIV-1 PR inhibitory effects of these Thai medicinal plants used as self medication for AIDS treatment.

Plant materials and preparation of extracts

The plants were collected at the botanical garden of Prince of Songklk University and some areas in Songkhla province, Thailand. The voucher specimens are deposited at the Herbarium of Faculty of Pharmaceutical Sciences, Prince of Songkla University, Thailand.

Ten grams of dried plant were extracted successively by maceration for 1 week (3 times) with 200 ml of chloroform and methanol. After that, the marc left from methanol extraction was then extracted with boiling water 200 ml for 3 hrs (3 times). The solvents were removed under reduced pressure to give chloroform-, methanol- and water extracts, respectively. The extracts were
HIV-1 protease inhibitory substances from the rhizomes of *Boesenbergia pandurata* Holtt.

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Abstract
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HIV-1 protease inhibitory substances from the rhizomes of *Boesenbergia pandurata* Holtt.

Four flavonoids (pinostrobin, pinocembrin, cardamonin and alpinetin) isolated from the ethan:
extract of *Boesenbergia pandurata* Holtt. (yellow rhizome) were tested for their activities against HIV-1 protease (HIV-PR). The result showed that cardamonin exhibited an appreciable anti-HIV-1 PR activity with an IC30 value of 31 μg/ml.

Key words: HIV-1 protease, inhibitory substance, *Boesenbergia pandurata*

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HIV-I protease inhibitory substances

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Boesenbergia pandurata Holtt., yellow variety (so called Kra-chai in Thai) is a perennial herb belonging to the Zingiberaceae family. The rhizome has been reported to contain essential oil (Ultee, 1957), boesenbergin, cardamonin, pinostrobin (Jaipetch et al., 1982), 5, 7-dimethoxyflavone, 1,8-cineole, panduratin (Pancharoen et al., 1987). In the primary health care project of Thailand, the rhizome of this plant is used for the treatment of dyspepsia. As regards its biological activities, B. pandurata exhibited antibacterial (Ungsurungsie et al., 1982), antifungal (Achararit et al., 1983), anti-inflammatory, analgesic, antipyretic (Pathong et al., 1989), antispasmodic (Apisaksiriyakul and Ananthasarn, 1984; Thamaree et al., 1985), antitumor (Murakami et al., 1993) and insecticidal activities (Areekul et al., 1987).

An acquired immunodeficiency syndrome (AIDS) has evolved rapidly into an epidemic and world-wide health crisis. Many researches have been carried out to discover compounds as anti-HIV-I agents and enzyme inhibitors of the HIV-I. However, the effective agents for treatment of this disease are still in demand since HIV-1 is resistant to some synthetic anti-HIV-1 PR inhibitors (Borman et al., 1996). HIV-I PR hydrolyzes viral polyproteins into functional enzymes and structural proteins that are essential for viral assembly (Kohl et al., 1988). Therefore, HIV-1 PR is considered to be an important target for development of anti-HIV-I drugs. The HIV-1 PR functions as a dimer which cleaves the amino acid sequence of Phe-Pro, Pro-Tyr or Leu-Phe in polyprotein (Orosalan, 1989).

In a previous study, the extract of Boesenbergia pandurata was screened for anti-HIV-1 PR activity (Tewtrakul et al., 2003). Herein, we report the isolation and the activity against HIV-1 PR of the isolated compounds from this plant.

Materials and Methods

The fresh rhizomes of B. pandurata Holtt. were bought from Hat Yai Market, Hat Yai, Thailand. The voucher specimen was identified and kept at the Herbarium of the Faculty of Pharmaceutical Sciences, Prince of Songkla University, Thailand (accession number: SKP 2060216).

Extraction and isolation

The fresh rhizomes (3 kg.) of B. pandurata Holtt. were homogenized in 95% ethanol (2 L.) and extracted by percolation for 3 days. After filtration, the residue was repeated twice by the same procedure. The solvent was evaporated from the combined extract, affording the crude extract and a pure compound, pinostrobin (compound 1, 3.4 g). The crude extract (15 g) was fractionated on a column of silica gel with n-hexane, dichloromethane, ethyl acetate and methanol as the mobile phase. Each fraction was evaporated to dryness under reduced pressure to give residues of 2.9, 1.8, 1.7 and 1.8 g of n-hexane, dichloromethane, ethyl acetate and methanol eluates, respectively. The hexane fraction was chromatographed over silica
Anti-HIV-1 integrase activity of medicinal plants used as self medication by AIDS patients

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Abstract

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Anti-HIV-1 integrase activity of medicinal plants used as self medication by AIDS patients

The extracts of selected medicinal plants used as self medication by AIDS patients were investigated for their inhibitory activities against HIV-1 integrase (HIV-1 IN) using the multiplate integration assay (MIA). Of these, the water extract of Eclipta prostrata (whole plant) exhibited the most potent inhibitory activity with an IC₅₀ value of 4.8 µg/ml, followed by the methanol extract of Eclipta prostrata (whole plant, IC₅₀ = 21.1 µg/ml), the water extract of Barleria lupulina (stem, IC₅₀ = 26.4 µg/ml), the chloroform extract of Barleria lupulina (stem, IC₅₀ = 33.0 µg/ml), the methanol extract of Barleria lupulina (stem, IC₅₀ = 38.2 µg/ml) and the chloroform extract of Piper betle (leaf, IC₅₀ = 39.3 µg/ml), respectively.

Key words: HIV-1 integrase, self medication, AIDS patients

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Anti-HIV-1 integrase activity of medicinal plants
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The effectiveness of combination therapy for HIV infection with drugs targeting protease and reverse transcriptase has recently been acknowledged. However, issues of patient compliance, drug toxicity, the emergence of multidrug-resistant phenotypes, and the presence of persistent reservoir of virus replication have launched the need to develop alternative therapeutic approaches utilizing other drug targets in the viral replication cycle. One of the essential enzymes in HIV-1 life cycle is the integrase. HIV-1 integrase (HIV-1 IN) integrates transcribed double strand DNA into the host chromosome (Kat and Skalka, 1994). Fusion of viral DNA with host chromosome subsequently triggers immunodeficiency that make patients susceptible to fatal opportunistic infections. Eleven Thai medicinal plants were studied for their inhibitory activities against HIV-1 IN. Most of them have been used in the primary health care project in Thailand and by AIDS patients. They are Zingiber zerumbet (rhizome), Boesenbergia pandurata (rhizome), Piper chaba (fruit), Eclipta prostrata (whole plant), Barleria lupulina (leaf, stem), Acanthus ilicifolius (leaf and stem), Alpinia galanga (rhizome), Piper betle (leaf), Spilanthus acmella (whole plant), and Coccinia glandis (leaf).

Previously, we reported HIV-1 protease (HIV-1 PR) inhibitory effects and antifungal activities against opportunistic fungal pathogens of these Thai plants (Tewtrakul et al., 2003; Phongpaichit et al., 2005).

The aim of the present study was therefore to investigate HIV-1 IN inhibitory effects of these Thai medicinal plants used as self medication for AIDS treatment.

Materials and Methods

Plant materials and preparation of extracts
The plants were collected at the botanical garden of Prince of Songkla University and some areas in Songkhla province, Thailand, and were identified by Assoc. Prof. Dr. Sanan Subhadhirsakul. The voucher specimens are deposited at the Herbarium of Faculty of Pharmaceutical Sciences, Prince of Songkla University, Thailand.

Ten grams of each dried plant were extracted successively by maceration for 1 week (3 times) with 200 ml of chloroform and methanol. After that, the marc left from methanol extraction was extracted with boiling water 200 ml for 3 hrs (3 times). The solvents were removed under reduced pressure to give chloroform-, methanol- and water extracts, respectively. The extracts were dissolved in 50% DMSO for bioassay.

Enzymes and chemicals
HIV-1 IN protein was expressed in E. coli, purified according to the method described in the previous paper (Jenkins et al., 1996) and stored in -80°C before use.
Anti-HIV-1 protease activity of compounds from *Boesenbergia pandurata*

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Abstract—Searching for anti-HIV-1 protease (PR) inhibitors of Thai medicinal plants led to the isolation of a new cyclohexenyl chalcone named panduratin C (1) and chalcone derivatives (2-6) from the methanol extract of *Boesenbergia pandurata* rhizomes. The known compounds were identified to be panduratin A (2), hydroxypanduratin A (3), helichrysetin (4), 2',4',6'-trihydroxyhydrachalcone (5), and uvangoletin (6). The structures of all compounds were elucidated on the basis of chemical and spectroscopic methods. It was found that 3 possessed the most potent anti-HIV-1 PR activity with an IC_{50} value of 5.6 ± 1.1 μM, followed by 2 (IC_{50} = 18.7 ± 1.1 μM), whereas other compounds exhibited only mild activity. Structure-activity relationships of these compounds on anti-HIV-1 PR activity are summarized as follows: (1) hydroxyl moiety at position 4 conferred higher activity than methoxyl group; (2) prenylation of dihydrochalcone was essential for activity; (3) hydroxylation at position 4' reduced activity; and (4) introduction of double bond at C1' and C6' of chalcone gave higher activity. As regards active constituents contained in *B. pandurata* rhizomes, hydroxypanduratin A (3) and panduratin A (2) are active principles against HIV-1 PR.

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1. Introduction

*Boesenbergia pandurata* Hort., locally known in Thai as Kra-chai, is a perennial herb belonging to the Zingiberaceae family. The fresh rhizomes have a characteristic aroma and a slightly pungent taste. It is commonly used in Southeast Asia as a food ingredient, a folk medicine for the treatment of several diseases such as aphthous ulcer, dry mouth, stomach discomfort, leukorrhea, and dysentery.1 The rhizomes contain essential oil,2 pinostrobin, cardamomin, boesenbergin,3 5,7-dimethoxyflavone, 1,8-cineole, and panduratin.4 In the primary health care project of Thailand, the rhizomes of this plant are used for treatment of dyspepsia. Moreover, it has also been used as self-medication by AIDS patients in Thailand. As regards its biological activities, *B. pandurata* exhibits antibacterial,5 antifungal,6 anti-inflammatory, analgesic, antipyretic,7 antispasmodic,8 antitumor,9 and insecticidal activities.10

The human immunodeficiency virus type-1 (HIV-1), a member of retrovirus family, has been a causative organism in an acquired immunodeficiency syndrome (AIDS). One of the important enzymes necessary for the replication of this virus is HIV-1 protease (HIV-1 PR). HIV-1 PR belongs to the aspartyl protease class and functions as a dimer of 99 amino acids each. This enzyme plays a crucial role in the process of viral maturation and infectivity.12 Thus, searching for HIV-1 PR inhibitors from natural sources has become a promising approach.

In the previous study, we reported the activity of some compounds isolated from *B. pandurata* on anti-HIV-1 PR activity.13 Herein, we report the isolation, structure elucidation of a new compound, and the activity against HIV-1 PR of chalcone derivatives from this plant.

2. Results and discussion

The MeOH extract of rhizomes of *B. pandurata* was fractionated by silica gel column chromatography and preparative TLC to obtain one new cyclohexenyl...
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 ocmella (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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The invitro anti-giardial activity of extracts from plants that are used for self-medication by AIDS patients in southern Thailand

Abstract This study evaluated the anti-giardial activity of chloroform, methanol and water extracts of 12 medicinal plants (39 extracts), commonly used as self medication by AIDS patients in southern Thailand. The plant extracts and a standard drug, metronidazole, were incubated with 2x10^3 trophozoites of Giardia intestinalis per millilitre of growth medium in 96-well tissue culture plates under anaerobic conditions for 24 h. The cultures were examined with an inverted microscope and the minimum inhibitory concentration and the IC50 value for each extract was determined. The chloroform extracts from Alpinia galanga, Boesenbergia pandurata, Eclipta prostrata, Piper betle, Piper chaba, Zingiber zerumbet, and the methanol extracts from B. pandurata and E. prostrata were classified as "active", i.e. with an IC50 of <100 μg/ml, whereas the chloroform extract from Murraya paniculata was classified as being "moderately active". This study shows that extracts from some medicinal plants have potential for use as therapeutic agents against G. intestinalis infections.

Introduction

Giardia intestinalis is one of the most common, universal pathogenic intestinal protozoan parasites of humans (Newman et al. 2001). It is becoming increasingly important among HIV/AIDS patients. There are reports that some cases of acute and chronic diarrhoea in AIDS patients may be associated with G. intestinalis infection (Merchant and Shroff 1996; Feitosa et al. 2001; Joshi et al. 2002; Mohandas et al. 2002). Although, Hailemariam et al. (2004) found no significant increase in infection rates with G. intestinalis in HIV/AIDS patients from Ethiopia; patients with some types of immunocompromised condition did have an increased probability of presenting with severe symptoms when infected with this organism (Janoff et al. 1988).

Metronidazole, the current drug of choice, can cause mutagenicity in bacteria (Legator et al. 1975) and is carcinogenic in rodents (Rustia and Shubik 1972; Shubik 1972). It also possesses undesirable side effects and treatment failures have been reported (Llibre et al. 1989; Johnson 1993; Voolmann and Boreham 1993; Tracy and Webster 1996; Lemee et al. 2000; Abboud et al. 2001). Furthermore, most Thai people with diarrhoea first tend to seek help from traditional healers dispensing traditional Thai medicines. For these reasons, our team is searching for an alternative drug suitable for use in preventing and treating cases of diarrhoea caused by the infection of HIV-positive patients with G. intestinalis. We therefore evaluated the ability of extracts from selected medicinal plants, used in a primary health care project by AIDS patients in southern Thailand, to inhibit the in vitro growth of G. intestinalis.

Materials and methods

Test organisms

A local Thai strain of G. intestinalis, originally described by Siripanth et al. (1995), was used throughout this
Antifungal activities of extracts from Thai medicinal plants against opportunistic fungal pathogens associated with AIDS patients

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Summary

In this study, 36 extracts derived from 10 plant species were selected to screen for their antifungal activity against clinical isolates of Candida albicans, Cryptococcus neoformans and Microsporum gypseum. Selection was based on their use by traditional Thai healers or their reported antimicrobial activities in an attempt to find bioactive medicines for use in the treatment of opportunistic fungal infections in AIDS patients. The disc diffusion and hyphal extension–inhibition assays were primarily used to test for inhibition of growth. Minimum inhibitory concentration was determined by dilution methods. The chloroform extracts of Alpinia galanga and Boesenbergia pandurata had pronounced antifungal activity against C. neoformans and M. gypseum, but exhibited weak activity against C. albicans. Alpinia galanga and B. pandurata are excellent candidates for the development of a remedy for opportunistic fungal infections in AIDS patients.

Key words: AIDS, Alpinia galanga, antifungal, Boesenbergia pandurata, Candida albicans, Cryptococcus neoformans, Microsporum gypseum.

Introduction

Opportunistic fungal infections are increasing as a consequence of the unprecedented increase in numbers of immunocompromised patients from various areas of the health care system. The situation has become even more alarming with the current pandemic of AIDS. The commonly encountered fungal infections in HIV patients are candidiasis, cryptococcosis and histoplasmosis. In Thailand, cryptococcosis and candidiasis are among the top five opportunistic infections in AIDS patients. Microsporum gypseum is a geophilic fungus with worldwide distribution. It generally causes infections of the skin and scalp. Although it is not common, it can cause disseminated infections in AIDS patients.

Approximately 46.6% of AIDS cases in Thailand have been labourers with low income. Thus, Thai HIV/AIDS patients tend to seek remedies to relieve their AIDS symptoms and opportunistic infections using traditional and cheaper medicines. Therefore, we investigated the antifungal activities of plant extracts used for self-medication by AIDS patients in southern Thailand. Selection of plants was based on their use by traditional healers and/or on previously reported antimicrobial activities.

Materials and methods

Plant materials and extract preparation

Plants used in this study with their reported biological activities are shown in Table 1. Plant materials were collected in Songkhla Province, Thailand and deposited at the Herbarium of the Faculty of Pharmaceutical Sciences, Prince of Songkla University. The extraction procedures had been described previously by Tewtrakul et al. [17]. Briefly, dried plants were successively extracted with chloroform, methanol and boiling water.
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 & Bunyaphaphatsara, 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 I.

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.
The anti-amoebic activity of some medicinal plants used by AIDS patients in southern Thailand

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Abstract The anti-amoebic activities of chloroform, methanol and water extracts from 12 Thai medicinal plants (39 extracts) commonly used by AIDS patients in southern Thailand were screened, at a concentration of 1,000 μg/ml against Entamoeba histolytica strain HUT-56:MUTM and strain HM1:IMSS growing in vitro. The extracts were incubated with 2×10⁵ E. histolytica trophozoites/ml of medium at 37°C under anaerobic conditions for 24 h. The cultures were examined with an inverted microscope and scored (1–4) according to the appearance and numbers of the trophozoites. The extracts that caused inhibition were selected and retested using the same conditions but with concentrations that ranged from 31.25 to 1,000 μg/ml using E. histolytica strain HM1:IMSS, and the IC₅₀ values for each extract were calculated. The chloroform extracts from Alpinia galanga (IC₅₀ 55.2 μg/ml), Barleria lupulina (IC₅₀ 78.5 μg/ml), Boesenbergia pandurata (IC₅₀ 45.8 μg/ml), Piper betle (IC₅₀ 91.1 μg/ml) and Piper chaba (IC₅₀ 71.4 μg/ml) and the methanol extract from B. pandurata (IC₅₀ 57.6 μg/ml) were all classified as “active”, i.e. with an IC₅₀ of less than 100 μg/ml, whereas those from Murraya paniculata (IC₅₀ 116.5 μg/ml) and Zingiber zerumbet (IC₅₀ 196.9 μg/ml) were classified as being “moderately active”. The IC₅₀ of a standard drug, metronidazole, was 1.1 μg/ml.

Introduction

Amoebiasis is an increasingly important parasitic disease among patients with HIV infection regardless of whether they have AIDS. Although HIV/AIDS patients are not especially prone to infection with Entamoeba histolytica, it has been suggested that they are more susceptible to an invasive form of the disease than are normal patients (Fäkenheuer et al. 1997; Hung et al. 1999; Liu et al. 2001). Infection with E. histolytica has also been reported to be an important cause of acute and chronic diarrhoea in HIV patients (Waywa et al. 2001; Joshi et al. 2002; Arenas-Pinto et al. 2003).

The most commonly prescribed drug for treating intestinal protozoan infections, including the invasive form of E. histolytica, is metronidazole. However, metronidazole can have undesirable side-effects, and failures in treatment have been reported (Libre et al. 1989; Johnson 1993; Voolmann and Boreham 1993; Tracy and Webster 1996; Lemee et al. 2000). These problems lead our team to search for an alternative drug that could be suitable for use in preventing and treating E. histolytica infections in HIV-positive patients.

The use of medicinal plants by people in developing countries is popular because these products are safe, widely available at low cost and easy to access. We therefore evaluated the in vitro activity of selected medicinal plants, used in a primary health care project by AIDS patients in southern Thailand, against E. histolytica. Furthermore, in previous studies, we found that some of these plants are active against growth of Giardia intestinalis in vitro (Sawangjaroen et al. 2005). Although the in vitro assays may not be related to direct in vivo activities in some studies (Ghoshal et al. 1996), it still an important approach to activity screening, which may provide a firm basis for improving basic community health care to the population.
Inhibitory activity of Thai condiments on pandemic strain of Vibrio parahaemolyticus

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Abstract

Antibacterial activity of 13 condiments used in Thai cooking was investigated with a pandemic strain of Vibrio parahaemolyticus. Using a disk diffusion technique, freshly squeezed extracts from galangal, garlic and lemon, at a concentration of 10\textmu l/disk produced a clear zone of 13.6±0.5, 11.6±0.5 and 8.6±1.2 mm, respectively. The inhibitory activity of these 3 condiments on pandemic strains was not significantly different from that on non-pandemic strains of V. parahaemolyticus. Because of its popularity in seafood cooking, galangal was subjected to further investigation. Only a chloroform extract of galangal inhibited growth of V. parahaemolyticus producing a clear zone of 9.5±0.5, 12.0±0 and 13.5±0.5 mm diameter at concentrations of 25, 50 and 100\textmu g/disk, respectively. One active component is identified as 1'-acetoxychavicol acetate. The activity of galangal was not reduced at pH 3 or in the presence of 0.15\% bile salt but was reduced by freeze and spray drying. Hesitation for a fresh preparation of galangal to 100°C but not 50°C for 30 min also reduced growth inhibition. Therefore, using fresh galangal in cooking was recommended. The MIC and MBC of a freshly squeezed preparation of galangal were 1:16 and 1:16, respectively. This is the first report of an inhibitory activity of a Thai medicinal plant, galangal, that is used in Thai cooking, on the pandemic strain of V. parahaemolyticus.

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Keywords: V. parahaemolyticus; Thai condiments; Galangal; Antibacterial activity

1. Introduction

Vibrio parahaemolyticus is a halophilic Gram-negative bacterium that causes acute gastroenteritis in humans. Food-poisoning caused by this pathogen is generally associated with the consumption of raw or undercooked seafood. Clinical manifestations include diarrhea, abdominal cramps, nausea, vomiting, headache, fever and chills, with incubation periods of from 4 to 96 h. Most clinical strains of V. parahaemolyticus possess a major virulence factor, the thermostable direct hemolysin (TDH) which exhibit β-hemolysis on Wagatsuma agar (Miyamoto et al., 1969; Sakurai et al., 1973; Nishibuchi and Kaper, 1995). Another virulence factor, TDH-related hemolysin (TRH) has also been involved in some food-poisoning outbreaks (Honda et al., 1988). TDH and TRH are encoded by the \textit{tdh} and \textit{trh} genes. Serotypes of \textit{V. parahaemolyticus} can be differentiated into 13 O groups and 71 K types (Iguchi et al., 1995). A predominant new strain belonging to serovar O3:K6 appeared for the first time in February 1996 in Calcutta, India and later accounted for 50-80\% of the strains isolated from clinical specimens taken in India between February to August 1996 (Okuda et al., 1997). Since then, the new O3:K6 strains have been considered to be the first pandemic strains of \textit{V. parahaemolyticus}, and are involved in a high proportion of foodborne poisoning outbreaks in the United States (Center for Disease Control and Prevention (CDC), 1998, 1999) and several Asian countries including Thailand (Aragawa et al., 1999; Chiou et al., 2000; Matsumoto et al., 2000; Vuddhakul et al., 2000). In addition, O4:K68, O1:KUT and O1:K25 have been reported as pandemic strains and originate from the same clone as O3:K6 (Matsumoto et al., 2000;
HIV-1 protease- and HIV-1 integrase inhibitory substances from *Eclipta prostrata*

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**Abstract** : The bioassay-guided fractionation led to the isolation of six compounds from the whole plants of *Eclipta prostrata* extract. They were indentified as 5-hydroxymethyl-(2, 2′: 5′, 2′′)-terthienyl tiglate (1), 5-hydroxymethyl-(2, 2′: 5′, 2′′)-terthienyl agelate (2), 5-hydroxymethyl-(2, 2′: 5′, 2′′)-terthienyl acetate (3), eclipctal (4), orobol (5) and wedelolactone (6). Among these, compound 6 showed the highest activity against HIV-1 integrase (IN) with IC₅₀ value of 4.0 ± 0.2 μM, followed by compound 5 (IC₅₀ = 8.1 ± 0.5 μM); whereas four terthiophene compounds were inactive (IC₅₀ > 100 μM). Regarding HIV-1 protease (PR) inhibitory activity, compound 1 exhibited appreciable activity against HIV-1 PR with IC₅₀ of 58.3 ± 0.8 μM, followed by compound 4 (IC₅₀ = 83.3 ± 1.6 μM) and compound 3 (IC₅₀ = 93.7 ± 0.8 μM), while compounds 2, 5 and 6 were inactive against HIV-1 PR.

**Keywords** : HIV-1 protease, HIV-1 integrase, Inhibitory activities, *Eclipta prostrata*
In vitro immunomodulatory activities of extracts from some Thai medicinal plants

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Abstract

Eleven medicinal plants commonly used in Thai tradition were extracted with either chloroform, methanol or water. Thirty six crude extracts were screened for activating human lymphocytes and phagocytes. Using flow cytometry, the marker used for detecting lymphocyte activation and proliferation were CD69 antigen expression. Effects on phagocytes were detected by measuring the migration of polymorphonuclear cells towards a chemoattractant under agarose. Four chloroform extracts from Alpinia galanga, Boesenbergia pandurata, Piper chaba and Zingiber zerumbet stimulated T lymphocytes to express CD69 antigen (19.6%, 21.0%, 29.0% and 31.6% respectively) compared to the control (6.2%). Boesenbergia pandurata extract was further purified and 1 of 3 fractions induced CD69 expression at the concentration of 50 and 100 µg/ml. None of the crude extracts stimulated the migration of polymorphonuclear cells but one chloroform extract from Zingiber zerumbet and 3 methanol extracts from Eclipta prostrata, Piper betle and Zingiber zerumbet suppressed polymorphonuclear cells migration.

Keywords: Immunomodulatory activity; Medicinal plant; Lymphocyte activation; Polymorphonuclear cells migration
บัฑชิตย์

สมุนไพรที่มีฤทธิ์ด้านเชื้อจุลินทรีย์ ที่มีฤทธิ์บดบู่ ได้แก่ ยาสิ่งที่มีฤทธิ์ที่อยู่ในหลาย

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ยาสิ่งที่มีฤทธิ์ที่อยู่ใน多元化
Antibacterial and antifungal activities against the infections in HIV infected patients of some Thai medicinal plants have been widely studied. However, their toxicity studies are quite rare. The objectives of this study are to study both acute and subchronic toxicity of the lead traditional medicines that have been shown to have antibacterial or antifungal activities such as “Galanga” in rats. In acute toxicity study, the LD50 of the chloroform extract of Galanga given orally in male and female rats were 3,200 and 6,300 mg/kg respectively. For the subchronic toxicity study, 40 rats per each sex were divided into 3 groups. The control group received 5% gum arabic (as vehicle of the extract) by orally intubation and the two treatment groups received chloroform extract of Galanga at the dose of 24 and 240 mg/kg/day (equivalent to about 10 and 100 times of routinely used) for 3 months. The result of the study has shown that the body weight gain and food consumption were significantly reduced in the rats (of both sexes) that received the extract at the dose of 240 mg/kg/day. Anyway, there was no abnormal behavior detected. Histopathological finding has shown some histological changes which were not related to the concentration of the extract and should be just incidental finding. In conclusion, there was no correlation between the dose and toxicity of the Galangal extract. Therefore, Galanga should be considered safe if it is used as in daily life.