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The in vitro anti-giardial activity of extracts from plants that are used for self-medication by AIDS patients in southern Thailand

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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 2×10^5 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 IC_{50} 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 IC_{50} 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.

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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

experiment. It was cultured axenically in screw-capped tubes at 37°C, under anaerobic conditions, on YI medium (Diamond et al. 1995) supplemented with 10% heat-inactivated horse serum. Subculture was performed every 48 h.

For assays, trophozoites were harvested by chilling the tube on ice for 15 min to detach the monolayer and centrifuged at 300 *g* for 5 min. The supernatant was decanted and cells were resuspended in fresh medium. The numbers of viable cells were calculated using a haemocytometer and 0.4% (w/v) trypan blue. The criteria used for viability were motility and dye exclusion.

Preparation of plant extracts

The names and parts of the plants used are shown in Table 1. They were collected in Songkhla Province, Thailand and voucher specimens are deposited at the herbarium of the Faculty of Pharmaceutical Sciences, Prince of Songkla University, Thailand. Each plant part was chosen on the basis of their known use by AIDS patients in southern Thailand. The preparation of plants, extraction procedures and methods for testing have been described by Tewtrakul et al. (2003). Briefly, dried plants were successively extracted with chloroform, methanol and boiling water. The solvents were removed under reduced pressure. Each dried extract was dissolved, at a concentration of 100 mg/ml, in dimethyl sulfoxide (DMSO) before assay. The extracts were further diluted with culture medium to a concentration of 2 mg/ml. The maximum concentration of DMSO in the test did not exceed 1%, at which level no inhibition of *G. intestinalis* growth occurred.

Anti-giardial activity of plant extracts

G. intestinalis, at a density of 2×10^5 trophozoites/ml of culture medium, were incubated in 96-well tissue culture plates (200 µl/well) in the presence of serial twofold dilutions of plant extracts that ranged from 31.25 to 1,000 µg/ml. Metronidazole, concentrations that ranged from 0.625 to 20 µg/ml, and complete medium with added DMSO were used as negative and positive controls, respectively. After 24 h of incubation at 37°C under anaerobic conditions, the trophozoites from each well were examined and counted using an inverted microscope. The appearance and numbers of trophozoites were scored from 1 to 4 with 1 showing the most inhibition of growth and 4 showing no inhibition according to Upcroft and Upcroft (2001), and the minimum inhibitory concentration (MIC) was recorded (the lowest concentration at which >90% of the trophozoites rounded up). The plates were chilled for 15 min to detach the trophozoites. The number of viable cells from every well was counted twice using trypan blue and a haemocytometer. The results were calculated as the percentage of growth inhibition when compared with the controls grown without plant extracts. A plot of the probit value against the log of the plant extract concentration was made. The best straight line was determined by regression analysis and the concentrations that caused 50% inhibition (IC_{50}) calculated. Each concentration was tested in duplicate and at least two experiments were performed on separate occasions.

As the criteria used for determining the degree of the anti-giardial effects seem to vary between different research groups, we used the slightly modified criteria from Tona et al. (1998) as follows: $IC_{50} < 20$ µg/ml = highly active, $20 < IC_{50} \leq 100$ µg/ml = active, $100 < IC_{50} \leq 250$ µg/ml = moderately active, $250 < IC_{50} \leq 500$ µg/ml = weekly active, $IC_{50} \geq 500$ µg/ml = inactive.

Results

The MIC values and the calculated IC_{50} values of the extracts are shown in Table 2. A chloroform extract of *Alpinia galanga* gave the highest activity with the MIC value of 125 µg/ml and an IC_{50} of 37.73 µg/ml. The chloroform extracts from *Boesenbergia pandurata*, *Eclipta prostrata*, *Piper betle*, *P. chaba*, *Zingiber zerumbet*, and the methanol extracts from *B. pandurata* and *E. prostrata* were classified as being active while the chloroform extract from *Murraya paniculata* was moderately

Table 1 Plant names and their parts used for the extracts tested for anti-giardial activity

Botanical name	Family	Part used
Acanthus ebracteatus Vahl	Acanthaceae	Leaf, stem
Alpinia galanga (L.) Willd.	Zingiberaceae	Rhizome
Barleria lupulina Lindl.	Acanthaceae	Leaf
B. lupulina Lindl.	Acanthaceae	Stem
Boesenbergia pandurata (Roxb.) Schltr.	Zingiberaceae	Rhizome
Coccinia grandis (L.) Voigt	Cucurbitaceae	Leaf
Eclipta prostrata (L.) L.	Asteraceae	Whole plant
Gynura pseudochina (L.) DC.	Asteraceae	Leaf
Murraya paniculata (L.) Jack	Rutaceae	Leaf
Piper betle L.	Piperaceae	Leaf
Piper chaba Hunter	Piperaceae	Fruit
Spilanthes acmella (L.) Murray	Asteraceae	Whole plant
Zingiber zerumbet (L.) Roscoe ex Sm.	Zingiberaceae	Rhizome

Table 2 The MIC and IC_{50} values of plant extracts incubated for 24 h with *Giardia intestinalis* growing in vitro

Plants	Extraction solvent	MIC (μg/ml)	IC_{50} (μg/ml)	
			Average[b]	SD
Acanthus ebracteatus	Chloroform	-[a]	-[f]	
	Methanol	-[a]	-[f]	
	Water	-[a]	-[f]	
Alpinia galanga	Chloroform	125	37.73[d]	1.88
	Methanol	-[a]	-[f]	
	Water	-[a]	-[f]	
Barleria lupulina leaf	Chloroform	-[a]	-[f]	
	Methanol	-[a]	-[f]	
	Water	-[a]	-[f]	
Barleria lupulina stem	Chloroform	-[a]	-[f]	
	Methanol	-[a]	-[f]	
	Water	-[a]	-[f]	
Boesenbergia pandulata	Chloroform	250	44.48[d]	5.85
	Methanol	250	78.30[d]	10.52
	Water	-[a]	-[f]	
Coccinia grandis	Chloroform	-[a]	-[f]	
	Methanol	-[a]	-[f]	
	Water	-[a]	-[f]	
Eclipta prostrata	Chloroform	250	45.63[d]	8.94
	Methanol	500	81.35[d]	7.83
	Water	-[a]	-[f]	
Gynura pseudochina	Chloroform	-[a]	-[f]	
	Methanol	-[a]	-[f]	
	Water	-[a]	-[f]	
Murraya paniculata	Chloroform	250	144.87[e]	19.45
	Methanol	-[a]	-[f]	
	Water	-[a]	-[f]	
Piper betle	Chloroform	250	51.57[d]	0.17
	Methanol	-[a]	-[f]	
	Water	-[a]	-[f]	
Piper chaba	Chloroform	250	45.47[d]	17.88
	Methanol	-[a]	-[f]	
	Water	-[a]	-[f]	
Spilanthes acmella	Chloroform	-[a]	-[f]	
	Methanol	-[a]	-[f]	
	Water	-[a]	-[f]	
Zingiber zerumbet	Chloroform	250	69.02[d]	0.92
	Methanol	-[a]	-[f]	
	Water	-[a]	-[f]	
Metronidazole		2.5[c]	0.48[c]	0.02

[a] MIC≥1,000
[b] Mean values obtained in at least two separate assays done in duplicate
[c] $IC_{50} < 20$ μg/ml = highly active
[d] $20 < IC_{50} \leq 100$ μg/ml = active
[e] $100 < IC_{50} \leq 250$ μg/ml = moderately active
[f] $IC_{50} \geq 500$ μg/ml = inactive

active. The other extracts showed no activity against this organism.

Discussion

In our research approach to detect new plant-derived compounds, aimed at treating *G. intestinalis* infections in HIV/AIDS patients, we tested 12 medicinal plants (39 extracts), commonly used by AIDS patients in southern Thailand. We found seven plant extracts that exhibited anti-giardial activity in vitro. These plant extracts, except for those from *E. prostrata*, were also active against another intestinal protozoan parasite pathogenic to humans, *Entamoeba histolytica* growing in vitro (N. Sawangjaroen et al., unpublished data).

Four of the seven plant extracts that were active against *G. intestinalis* in this study (*A. galanga*, *B. pandulata*, *P. chaba* and *Z. zerumbet*) are from plants frequently prescribed by practitioners of traditional Thai medicine for treating cases of diarrhoea or dysentery, (Farnsworth and Bunyapraphatsara 1992). However, these plants have been used for these purposes without any scientific evidence that they work. Our findings confirm the traditional therapeutic claims for the use of these herbs to treat diarrhoea that may be caused by infection from *G. intestinalis*.

A. galanga or *Languas galanga* or "Khaa" in Thai and *B. pandurata* are from the same Zingiberaceae family as *Z. zerumbet*, and all are effective against both *E. histolytica* (N. Sawangjaroen et al., unpublished data) and *G. intestinalis*. Zingiberaceae rhizomes are commonly used in Thai traditional medicine. The active compounds present in these extracts that are responsible for inhibiting *G. intestinalis* growing in vitro have yet to be characterized.

The fresh rhizome of *B. pandurata*, currently known as *B. rotunda* (Larsen 1996) or "Kra-chai" in Thai, is commonly used to treat colic disorders as well as inflammation. Panduratin A, sakuranetin, pinostrobin, pinocembrin and dihydro-5,6-dehydrokawain, isolated from chloroform extracts of this rhizome, are, according to Tuchinda et al. (2002), responsible for the anti-inflammatory effect. The antimutagenic effect (Trakoontivakorn et al. 2001), as well as the hepatocarcinogenic effect (Tiwawech et al. 2000), of several agents extracted from this rhizome were also reported. In addition, its chloroform extract was shown to have potent HIV-1 protease inhibitory activity (Tewtrakul et al. 2003). Whether or not the anti-giardial activities stem from these compounds still needs to be determined.

The hepatoprotective potential of extracts from *E. prostrata* in rats and mice against hepatotoxicity induced by carbon tetrachloride was reported by Singh et al. (2001). We report here an additional effect of extracts from *E. prostrata* against the in vitro growth of *G. intestinalis*.

P. betle is a tropical plant the leaves of which are often chewed in Thailand and many other Southeast Asian countries to prevent malodor. It was found that hydroxychavicol, a major phenolic compound in *P. betle* leaves, is related to the incidence of oral submucous fibrosis (Jeng et al. 2004). The anti-adherence effect on early plaque settlers (Razak and Rahim 2003), its vasodilatory activity (Runnie et al. 2004), and the hepatoprotective and antioxidant effects (Saravanan et al. 2002, 2003) of the aqueous extract of *P. betle* have been reported. Furthermore, allylpyrocatechol from *P. betle* leaves shows inhibitory activity against obligate oral anaerobes that cause halitosis (Ramji et al. 2002). However, an effect of this plant and its active compounds on intestinal microbes has not been previously

reported. This is the first report of an extract from the leaves of *P. betle* inhibiting the human pathogen, *G. intestinalis*, growing in vitro.

An aqueous and ethanol extract of *Piper longum*, a closely related species to *P. chaba*, was reported to inhibit the growth of *G. intestinalis* both in vitro and in vivo (Tripathi et al. 1999), and it has been successfully used as part of a drug formulation to treat giardiasis in patients in India (Agarwal et al. 1997). In addition, several researchers (Ghoshal et al. 1996; Sawangjaroen et al. 2004) have also reported its ability to inhibit *E. histolytica* both in vitro and in vivo. However, Ghoshal et al. (1996) revealed that piperine, a major plant alkaloid present in this group, was not active against *E. histolytica* in vitro. Therefore, the compounds responsible for the anti-giardial activities in the chloroform leaf extracts of *P. betle* and *P. chaba* need to be further identified.

In conclusion, several plants that are being used by AIDS patients of southern Thailand for their reputed medicinal properties are good candidates for further studies on their potential use in the systemic therapy and/or prophylaxis of *G. intestinalis* infections. These findings should assist in initiating therapy for such patients to reduce the morbidity and mortality caused by this pathogen.

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