ภาคผนวก 6 : ผลงานวิจัยตีพิมพ์ เรื่อง The in vitro anti-giardial activity of extracts from plants that are used for self-medication by AIDS patients in southern Thailand.

Parasitol Res (2005) 95: 17–21
DOI 10.1007/s00436-004-1264-8

ORIGINAL PAPER

N. Sawangjaroen · S. Subhadhirasakul · S. Phongpaichit
C. Siripanth · K. Jamjaroen · K. Sawangjaroen

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

Received: 15 July 2004 / Accepted: 6 October 2004 / Published online: 18 November 2004
© Springer-Verlag 2004

**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 $2\times10^5$ 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.

N. Sawangjaroen (✉) · S. Phongpaichit · K. Jamjaroen
Natural Products Research Unit and Department of Microbiology, Faculty of Science, Prince of Songkla University, Hat-Yai, 90112 Songkhla, Thailand
E-mail: nongyao.s@psu.ac.th
Tel.: +66-74-446661
Fax: +66-74-446661

S. Subhadhirasakul
Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Prince of Songkla University, Hat Yai, 90112 Songkhla, Thailand

C. Siripanth
Department of Protozoology, Faculty of Tropical Medicine, Mahidol University, 10400 Bangkok, Thailand

K. Sawangjaroen
Natural Products Research Unit and Department of Pharmacology, Faculty of Science, Prince of Songkla University, Hat Yai, 90112 Songkhla, Thailand

## 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 $2\times10^5$ 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, $20<IC_{50}\leq100$ µg/ml = active, $100<IC_{50}\leq250$ µg/ml = moderately active, $250<IC_{50}\leq500$ µg/ml = weekly active, $IC_{50}\geq500$ 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.

**Acknowledgements** This research was partly funded by a grant from the Thai Government. The authors would like to thank Prof. Brian Hodgson for useful advice.

## References


Abboud P, Lemee V, Gargala G, Brasseur P, Ballet JJ, Borsa-Lebas F, Caron F, Favennec L (2001) Successful treatment of metronidazole- and albendazole-resistant giardiasis with nitazoxanide in a patient with acquired immunodeficiency syndrome. Clin Infect Dis 32:1792–1794

Agarwal AK, Tripathi DM, Sahai R, Gupta N, Saxena RP, Puri A, Singh M, Misra RN, Dubey CB, Saxena KC (1997) Management of giardiasis by a herbal drug 'Pippali Rasayana': a clinical study. J Ethnopharmacol 56:233–236

Diamond LS, Clark CG, Cunnick CC (1995) YI-S, a casein-free medium for axenic cultivation of *Entamoeba histolytica*, related *Entamoeba*, *Giardia intestinalis* and *Trichomonas vaginalis*. J Eukaryot Microbiol 42:277–278

Farnsworth NR, Bunyapraphatsara N (1992) Thai medicinal plants recommended for primary health care systems. Prachachon, Bangkok

Feitosa G, Bandeira AC, Sampaio DP, Badaro R, Brites C (2001) High prevalence of giardiasis and strongyloidiasis among HIV-infected patients in Bahia, Brazil. Braz J Infect Dis 5:339–344

Ghoshal S, Krishna Prasad BN, Laksmi V (1996) Antiamoebic activity of *Piper longum* fruits against *Entamoeba histolytica* in vitro and in vivo. J Ethnopharmacol 50:166–170

Hailemariam G, Kassu A, Abebe G, Abate E, Damte D, Mekonnen E, Ota F (2004) Intestinal parasitic infections in HIV/AIDS and HIV seronegative individuals in a teaching hospital, Ethiopia. Jpn J Infect Dis 57:41–43

Janoff EN, Smith PD, Blaser MJ (1988) Acute antibody responses to *Giardia lamblia* are depressed in patients with AIDS. J Infect Dis 157:798–804

Jeng JH, Wang YJ, Chang WH, Wu HL, Li CH, Uang BJ, Kang JJ, Lee JJ, Hahn LJ, Lin BR, Chang MC (2004) Reactive oxygen species are crucial for hydroxychavicol toxicity toward KB epithelial cells. Cell Mol Life Sci 61:83–96

Johnson PJ (1993) Metronidazole and drug resistance. Parasitol Today 9:183–186

Joshi M, Chowdhary AS, Dalal PJ, Maniar JK (2002) Parasitic diarrhoea in patients with AIDS. Natl Med J India 15:72–74

Larsen K (1996) A Preliminary checklist of the Zingiberaceae of Thailand. Thai Forest Bull, Bot 24:35–49

Legator MS, Connor TH, Stoeckel M (1975) Detection of mutagenic activity of metronidazole and nitridazole in body fluids of humans and mice. Science 188:1118–1119

Lemee V, Zaharia I, Nevez G, Rabodonirina M, Brasseur P, Ballet JJ, Favennec L (2000) Metronidazole and albendazole susceptibility of 11 clinical isolates of *Giardia duodenalis* from France. J Antimicrob Chemother 46:819–821

Llibre JM, Tor J, Manterola JM, Carbonell C, Foz M (1989) *Blastocystis hominis* chronic diarrhoea in AIDS patients. Lancet 1:221

Merchant RH, Shroff RC (1996) The prevalence of HIV infection in children with extensive tuberculosis and chronic diarrhoea. AIDS Asia 3:21–23

Mohandas, Sehgal R, Sud A, Malla N (2002) Prevalence of intestinal parasitic pathogens in HIV-seropositive individuals in northern India. Jpn J Infect Dis 55:83–84

Newman RD, Moore SR, Lima AA, Nataro JP, Guerrant RL, Sears CL (2001) A longitudinal study of *Giardia lamblia* infection in north-east Brazilian children. Trop Med Int Health 6:624–634

Ramji N, Ramji N, Iyer R, Chandrasekaran S (2002) Phenolic antibacterials from *Piper betle* in the prevention of halitosis. J Ethnopharmacol 83:149–152

Razak FA, Rahim ZH (2003) The anti-adherence effect of *Piper betle* and *Psidium guajava* extracts on the adhesion of early settlers in dental plaque to saliva-coated glass surfaces. J Oral Sci 45:201–206

Runnie I, Salleh MN, Mohamed S, Head RJ, Abeywardena MY (2004) Vasorelaxation induced by common edible tropical plant extracts in isolated rat aorta and mesenteric vascular bed. J Ethnopharmacol 92:311–316

Rustia M, Shubik P (1972) Induction of lung tumors and malignant lymphomas in mice by metronidazole. J Natl Cancer Inst 48:721–729

Saravanan R, Prakasam A, Ramesh B, Pugalendi KV (2002) Influence of *Piper betle* on hepatic marker enzymes and tissue antioxidant status in ethanol-treated Wistar rats. J Med Food 5:197–204

Saravanan R, Rajendra Prasad N, Pugalendi KV (2003) Effect of *Piper betle* leaf extract on alcoholic toxicity in the rat brain. J Med Food 6:261–265

Sawangjaroen N, Sawangjaroen K, Poonpanang P (2004) Effects of *Piper longum* fruit, *Piper sarmentosum* root and *Quercus infectoria* nut gall on caecal amoebiasis in mice. J Ethnopharmacol 91:357–360

Shubik P (1972) Current status of chemical carcinogens. Proc Natl Acad Sci U S A 69:1052–1055

Singh B, Saxena AK, Chandan BK, Agarwal SG, Anand KK (2001) In vivo hepatoprotective activity of active fraction from ethanolic extract of *Eclipta alba* leaves. Indian J Physiol Pharmacol 45:435–441

Siripanth C, Chintana T, Tharaphan Y, Lekkra A (1995) Cloning of Thai strain *Giardia intestinalis*. Asian Pac J Allergy Immunol 13:71–73

Tewtrakul S, Subhadhirasakul S, Kummee S (2003) HIV-1 protease inhibitory effects of medicinal plants used as self-medication by AIDS patients. Songklanakarin J Sci Technol 25:239–243

Tiwawech D, Hirose M, Futakuchi M, Lin C, Thamavit W, Ito N, Shirai T (2000) Enhancing effects of Thai edible plants on 2-amino-3, 8-dimethylimidazo(4,5-f)quinoxaline-hepatocarcinogenesis in a rat medium-term bioassay. Cancer Lett 158: 195–201

Tona L, Kambu K, Ngimbi N, Cimanga K, Vlietinck AJ (1998) Antiamoebic and phytochemical screening of some Congolese medicinal plants. J Ethnopharmacol 61:57–65

Tracy JW, Webster LTJr (1996) Drugs used in the chemotherapy of protozoal infection. In: Hardman JG, Limbird LE (eds) Goodman and Gilman's: The pharmacological basis of therapeutics. McGraw-Hill, New York, pp 987–1008

Trakoontivakorn G, Nakahara K, Shinmoto H, Takenaka M, Onishi-Kameyama M, Ono H, Yoshida M, Nagata T, Tsushida T (2001) Structural analysis of a novel antimutagenic compound, 4-hydroxypanduratin A, and the antimutagenic activity of flavonoids in a Thai spice, finger root (*Boesenbergia pandurata* Schult.) against mutagenic heterocyclic amines. J Agric Food Chem 49:3046–3050

Tripathi DM, Gupta N, Lakshmi V, Saxena KC, Agarwal AK (1999) Antigiardial and immunostimulatory effect of *Piper longum* on giardiasis due to *Giardia lamblia*. Phytother Res 13:561–565

Tuchinda P, Reutrakul V, Claeson P, Pongprayoon U, Sematong T, Santisuk T, Taylor WC (2002) Anti-inflammatory cyclohexenyl chalcone derivatives in *Boesenbergia pandurata*. Phytochemistry 59:169–173

Upcroft JA, Upcroft P (2001) Drug susceptibility testing of anaerobic protozoa. Antimicrob Agents Chemother 45:1810–1814

Voolmann Y, Boreham PFL (1993) Metronidazole resistant *Trichomonas vaginalis* in Brisbane. Med J Aust 159:490