

# The effects of extracts from anti-diarrheic Thai medicinal plants on the in vitro growth of the intestinal protozoa parasite: *Blastocystis hominis*

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## Abstract

The activities of *n*-hexane, dichloromethane and methanol extracts from five anti-diarrheic Thai medicinal plants, *Acacia catechu* (Fabaceae) resin, *Amaranthus spinosus* (Amaranthaceae) whole plant, *Brucea javanica* (Simaroubaceae) seed, *Piper longum* (Piperaceae) fruit and *Quercus infectoria* (Fagaceae) nut gall were tested against the in vitro growth of fresh isolates of the intestinal protozoan parasite, *Blastocystis hominis*. The extracts at concentrations that ranged from 62.5 to 2000 µg/mL, were incubated with several isolates of *Blastocystis hominis* for 48 h. The activities were classified as killed, inhibited, moderately inhibited and not-inhibited. Dichloromethane and methanol extracts from the *Brucea javanica* seed and a methanol extract from *Quercus infectoria* nut gall showed the highest activity. At a concentration of 2000 µg/mL, the three extracts killed 82, 75 and 67% of the *Blastocystis hominis* samples tested and inhibited 94, 100 and 76% of them, respectively. Metronidazole, used as a reference antiprotozoan drug, at a concentration of 40 µg/mL, killed 97% of the *Blastocystis hominis* isolates and inhibited all samples tested at concentrations that ranged from 1.25 to 20 µg/mL.

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**Keywords:** *Blastocystis hominis*; Medicinal plant; *Acacia catechu*; *Amaranthus spinosus*; *Brucea javanica*; *Piper longum*; *Quercus infectoria*

## 1. Introduction

*Blastocystis hominis* is the most common protozoan parasite detected in the human intestine (Stenzel and Boreham, 1996). In some areas of the world and in particular Thailand, the prevalence can be as high as 40% or even higher, especially when several techniques of identification are used (Saksirisampant et al., 2003; Taamasri et al., 2002; Zaman and Khan, 1994).

The role of *Blastocystis hominis* as a human pathogen is still controversial. In general, this parasite is believed to be not harmful. However, several researchers have reported that *Blastocystis hominis* can cause severe intestinal complications (Ricci et al., 1984; Sheehan et al., 1986; Sinniah and Rajeswari, 1994). It was claimed to cause

diarrhea in immunocompromised hosts (Brites et al., 1997; Gassama et al., 2001). The most likely mode of transmission for this organism is drinking water via the faecal–oral route (Taamasri et al., 2000) and in some areas, it may be considered to be a zoonosis (Garavelli and Scaglione, 1989).

The most commonly recommended drug for treatment of *Blastocystis hominis* and other pathogenic intestinal protozoa is metronidazole. This drug can cause undesirable side effects and failures in treatment are frequently reported (Johnson, 1993; Lemee et al., 2000; Llibre et al., 1989; Tracy and Webster, 1996; Voolmann and Boreham, 1993). This is why there is a need to develop a safe and effective alternative antiprotozoal agent.

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. In the present study, extracts from *Acacia catechu* (L.f.) Willd. (Fabaceae) resin (Acr), *Amaranthus spinosus* L. (Amaranthaceae) whole

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plant (Asw), *Brucea javanica* (L.) Merr. (Simaroubaceae) seed (Bjs), *Piper longum* L. (Piperaceae) fruit (Plf) and *Quercus infectoria* Oliv. (Fagaceae) nut gall (Qin) were evaluated for their effect on the in vitro growth of *Blastocystis hominis* because these products are routinely prescribed as anti-diarrheal drugs in Thai traditional medicine. Although the in vitro assays may in some cases not be related to direct in vivo activities (Ghoshal et al., 1996), it is still an important approach to activity screening which may provide a firm basis for improving basic community health care to the population.

## 2. Materials and methods

### 2.1. Isolation and cultivation of *Blastocystis hominis*

The cultures of *Blastocystis hominis* used in this experiment were isolated from patients' faecal specimens obtained at Songkhlanajarind hospital, Songkhla, Thailand. Faeces identified as *Blastocystis hominis* positive were cultured in Boeck & Drbohlav medium with some modification, as described elsewhere (Sawangjaroen et al., 1993). Calf bovine donor serum (10%) was used instead of horse serum. The cultures were incubated at 37 °C. *Blastocystis hominis* along with their associated bacteria were sub-cultured every 48 h.

### 2.2. Plant materials

Acr, Bjs, Plf and Qin were purchased from the medicinal plant store while Asw was collected from the area around Hat Yai, Songkhla, Thailand. Voucher specimens for Plf and Qin have been deposited at The Prince of Songkla University Herbarium, Department of Biology, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla, Thailand, under voucher specimen numbers K. SAWANGJAROEN 1 (PSU) and K. SAWANGJAROEN 2 (PSU), respectively. Voucher specimens for Asw, Acr, and Bjs were kept at the herbarium of the Faculty of Pharmaceutical Sciences, Prince of Songkla University, Thailand, under voucher specimen numbers N. SAWANGJAROEN 1, N. SAWANGJAROEN 2 and N. SAWANGJAROEN 3, respectively.

### 2.3. Preparation of crude plant extracts

The plants (or parts) were washed, cut into small pieces and dried in sunlight or in an oven at 50 °C maximum. The dried plant material was successively extracted by *n*-hexane, dichloromethane and methanol at the ratio of 1 kg dried plants per 3 L of solvent and supernatants were collected after 7 days. The procedure was repeated four times. The supernatants were pooled and filtered. The solvents were removed under reduced pressure, at 55 °C. Each residue was then dissolved in dimethyl sulfoxide (DMSO) and stored at 4 °C until used.

### 2.4. In vitro test for determining the effect of plant extracts and metronidazole against *Blastocystis hominis*

The plant extracts were used at concentrations of 62.5–2000 µg/mL throughout the experiments. Metronidazole, a standard drug, at concentrations between 1.25 and 40 µg/mL and PBS pH 7.4, with added DMSO were used as negative and positive control, respectively. Briefly, a test tube containing 3 mL egg slant and 5 mL of plant extract or metronidazole, at the required concentration, in PBS pH 7.4, supplement with 10% Calf bovine donor serum was mixed with *Blastocystis hominis* to give 10<sup>5</sup> cells/mL. Each sample was tested in duplicate. The numbers of *Blastocystis hominis* cells from each tube were counted twice, using a haemocytometer after 48 h incubation at 37 °C. It was compared against the control and the growth from each tube was reported as inhibited, moderately inhibited and not-inhibited, according to the system of Zierdt et al. (1983). A drop of sediment from any tube with no parasite found was transferred into fresh medium and reported as killed when no organisms were found after a further 48 h incubation.

### 2.5. Statistical methods used

The percentage of samples showing inhibition (cell count reduced by >50 fold) and the percentage of samples showing no growth (killed) obtained after treatment with plant extracts and metronidazole were transformed into probit values. The concentration (µg/mL) of active extracts and metronidazole required to kill all cells present in 50% of cases (KC<sub>50</sub>) and the effective concentration required to produce an "inhibition" in 50% of the *Blastocystis hominis* samples used (EC<sub>50</sub>) were calculated from the best straight line determined by regression analysis of the plot of the probit value against the log concentrations of the plant extract.

## 3. Results

### 3.1. Extraction of medicinal plants

The percent yield of each extract from each medicinal plant is shown in Table 1. A value of <0.5% produced

Table 1  
Percent yield (w/w) of extracts from of *Acacia catechu* resin, *Amaranthus spinosus* whole plant, *Brucea javanica* seed, *Piper longum* fruit and *Quercus infectoria* nut gall after successive extractions with *n*-hexane, dichloromethane and methanol

Medicinal plants	Percent yield (w/w) when extract with		
	<i>n</i> -Hexane	Dichloromethane	Methanol
<i>Acacia catechu</i> resin	0.1	3.4	77.3
<i>Amaranthus spinosus</i> whole plant	0.4	4.0	2.8
<i>Brucea javanica</i> seed	18.4	1.6	3.6
<i>Piper longum</i> fruit	4.6	4.4	2.3
<i>Quercus infectoria</i> nut gall	0.2	0.2	50.1

Table 2

Activities of extracts from *Acacia catechu* resin, *Amaranthus spinosus* whole plant, *Brucea javanica* seed, *Piper longum* fruit, *Quercus infectoria* nut gall and metronidazole after incubation with cultures of *Blastocystis hominis* for 48 h

Plants	Extraction solvents	Concentrations ( $\mu\text{g/mL}$ )	Samples tested	Number of samples (%)			
				<i>K</i> <sup>a</sup>	<i>I</i>	<i>M</i>	<i>N</i>
<i>Acacia catechu</i> resin							
	<i>n</i> -Hexane			ND <sup>b</sup>			
	Dichloromethane	2000	10	0(0)	5(50)	4(40)	1(10)
		1000		0(0)	4(40)	5(50)	1(10)
		500		0(0)	1(10)	7(70)	2(20)
		250		0(0)	0(0)	6(60)	4(40)
		125		0(0)	0(0)	5(50)	5(50)
		62.5		0(0)	0(0)	4(40)	6(60)
	Methanol	2000	10	0(0)	1(10)	6(60)	3(30)
		1000		0(0)	0(0)	4(40)	6(60)
		500		0(0)	0(0)	1(10)	9(90)
		250		0(0)	0(0)	1(10)	9(90)
		125		0(0)	0(0)	0(0)	10(100)
		62.5		0(0)	0(0)	0(0)	10(100)
<i>Amaranthus spinosus</i> whole plant							
	<i>n</i> -Hexane			ND <sup>b</sup>			
	Dichloromethane	2000	10	0(0)	0(0)	10(100)	0(0)
		1000		0(0)	0(0)	9(90)	1(10)
		500		0(0)	0(0)	6(60)	4(40)
		250		0(0)	0(0)	3(30)	7(70)
		125		0(0)	0(0)	1(10)	9(90)
		62.5		0(0)	0(0)	0(0)	10(100)
	Methanol	2000	10	0(0)	0(0)	7(70)	3(30)
		1000		0(0)	0(0)	3(30)	7(70)
		500		0(0)	0(0)	2(20)	8(80)
		250		0(0)	0(0)	1(10)	9(90)
		125		0(0)	0(0)	1(10)	9(90)
		62.5		0(0)	0(0)	0(0)	10(100)
<i>Brucea javanica</i> seed							
	<i>n</i> -Hexane						
	<i>n</i> -Hexane	2000	10	0(0)	0(0)	3(30)	7(70)
		1000		0(0)	0(0)	0(0)	10(100)
		500		0(0)	0(0)	0(0)	10(100)
		250		0(0)	0(0)	0(0)	10(100)
		125		0(0)	0(0)	0(0)	10(100)
		62.5		0(0)	0(0)	0(0)	10(100)
	Dichloromethane	2000	17	14(82)	16(94)	1(6)	0(0)
		1000		10(59)	12(71)	5(29)	0(0)
		500		7(41)	10(59)	7(41)	0(0)
		250		6(35)	7(41)	8(47)	2(12)
		125		1(6)	6(35)	3(18)	8(47)
		62.5		0(0)	5(29)	2(12)	10(59)
	Methanol	2000	16	12(75)	16(100)	0(0)	0(0)
		1000		9(56)	15(94)	1(6)	0(0)
		500		3(19)	11(69)	5(31)	0(0)
		250		2(13)	6(38)	8(50)	2(13)
		125		0(0)	3(19)	7(44)	6(38)
		62.5		0(0)	1(6)	8(50)	7(44)
<i>Piper longum</i> fruit							
	<i>n</i> -Hexane						
	<i>n</i> -Hexane	2000	16	3(19)	7(44)	8(50)	1(6)
		1000		1(6)	4(25)	8(50)	4(25)
		500		0(0)	1(6)	6(38)	9(56)
		250		0(0)	1(6)	4(25)	11(69)
		125		0(0)	0(0)	3(19)	13(81)
		62.5		0(0)	0(0)	0(0)	16(100)

Table 2 (Continued)

Plants	Extraction solvents	Concentrations ( $\mu\text{g/mL}$ )	Samples tested	Number of samples (%)			
				K <sup>a</sup>	I	M	N
	Dichloromethane			ND <sup>c</sup>			
	Methanol	2000	10	5(50)	6(60)	3(30)	1(10)
		1000		0(0)	4(40)	3(30)	3(30)
		500		0(0)	2(20)	1(10)	7(70)
		250		0(0)	0(0)	2(20)	8(80)
		125		0(0)	0(0)	2(20)	8(80)
		62.5		0(0)	0(0)	1(10)	9(90)
<i>Quercus infectoria</i> nut gall	<i>n</i> -Hexane			ND <sup>b</sup>			
	Dichloromethane			ND <sup>b</sup>			
	Methanol	2000	21	14(67)	16(76)	4(19)	1(5)
		1000		9(43)	15(71)	5(24)	1(5)
		500		4(19)	14(67)	6(29)	1(5)
		250		0(0)	13(62)	3(14)	5(24)
		125		0(0)	8(38)	7(33)	6(29)
		62.5		0(0)	8(38)	3(14)	10(48)
Metronidazole		40	32	31(97)	32(100)	0(0)	0(0)
		20		28(88)	32(100)	0(0)	0(0)
		10		17(53)	31(97)	1(3)	0(0)
		5		7(22)	17(53)	10(31)	5(16)
		2.5		0(0)	10(31)	15(47)	7(22)
		1.25		0(0)	2(6)	13(41)	17(53)

I: inhibited; cell count reduced by more than 50 fold; M: moderately inhibited; cell count reduced by 5–50-fold; N: not-inhibited; cell count reduced by less than five fold.

<sup>a</sup> K: killed; no cell was found after subculturing the sediment from tested samples into the fresh medium.

<sup>b</sup> ND: not done because the yield is not enough to test.

<sup>c</sup> ND: not done because this extract could not dissolve in any solvent.

insufficient material for testing. This was so for dried extracts obtained from Acr, Asw and Qin extracted with *n*-hexane and Qin extracted with dichloromethane.

### 3.2. Effects of plant extracts and metronidazole on *Blastocystis hominis* in vitro

The effects of the plant extracts and metronidazole on *Blastocystis hominis* are shown in Table 2. The KC<sub>50</sub> and EC<sub>50</sub> of active extracts and metronidazole are shown in Table 3. The inhibitory effects of plant extracts were clearly dose dependent. Extracts from Bjs appeared to be the most

effective inhibitors. At a concentration of 2000  $\mu\text{g/mL}$ , the dichloromethane and methanol extracts were able to kill *Blastocystis hominis* 82 and 75% and inhibited 94 and 100% of samples tested, respectively. The second most effective plant extract was Qin. At a concentration of 2000  $\mu\text{g/mL}$ , the methanol extract killed 67% and inhibited 76% of *Blastocystis hominis* samples. The remaining extracts at 2000  $\mu\text{g/mL}$ , all gave 50% or less killing and <60% inhibition. The EC<sub>50</sub> and KC<sub>50</sub> of the dichloromethane extract from Bjs was 249 and 643  $\mu\text{g/mL}$ ; and the EC<sub>50</sub> and KC<sub>50</sub> of the methanol extract from Bjs was 248 and 974  $\mu\text{g/mL}$ , respectively.

Metronidazole, at a concentration of 40  $\mu\text{g/mL}$ , killed 97% of *Blastocystis hominis* samples. All samples of *Blastocystis hominis* tested in this study were inhibited by metronidazole at concentrations from 1.25 to 20  $\mu\text{g/mL}$ . The EC<sub>50</sub> and KC<sub>50</sub> of metronidazole was 3 and 9  $\mu\text{g/mL}$ , respectively.

Table 3

Concentration ( $\mu\text{g/mL}$ ) of active extracts and metronidazole which killed 50% (KC<sub>50</sub>) and effected 50% (EC<sub>50</sub>) of *Blastocystis hominis* samples tested

Plants	Extraction solvents	Concentrations ( $\mu\text{g/mL}$ )	
		KC <sub>50</sub>	EC <sub>50</sub>
<i>Brucea javanica</i> seed	Dichloromethane	643	249
	Methanol	974	248
<i>Piper longum</i> fruit	Methanol	2000	1426
<i>Quercus infectoria</i> nut gall	Methanol	1248	171
Metronidazole		9	3

## 4. Discussion

To date, the limited data available on the effects of drugs against *Blastocystis hominis* in vitro was obtained using various strains of axenic cultures (Dunn and Boreham, 1991; Yang et al., 1996; Zierdt et al., 1983). The *Blastocystis hominis* strains used in this study were all isolated from patients

and cultured with their associated bacteria. The organisms were tested after not more than 1 week of cultivation. The condition of these isolates should resemble more closely their condition in their normal habitat of the intestine.

This study focused on the effects of *n*-hexane, dichloromethane and methanol extracts obtained from certain medicinal plant against *Blastocystis hominis* in vitro. Acr, Bjs, Qin and Asw are routinely used as a traditional medicine in tropical countries for the treatment of diarrhea while Plf is widely used for preparing spicy Thai dishes as well as for treating intestinal disorders.

Results from the present study demonstrate that dichloromethane and methanol extracts from Bjs are the most effective against most strains of *Blastocystis hominis* used in this study. Bjs is well known for its antiameobic activities (Wright et al., 1988) and antiplasmodial activities (O'Neill et al., 1987). Yang et al. (1996) also reported that a water extract of Bjs was very effective against one axenic strain of *Blastocystis hominis*. We tested a wider range of *Blastocystis hominis* isolates and found that different isolates produced different responses to plant extracts. This may be due to the distinct karyotypic or isoenzyme patterns of different isolates as reported by several authors (Boreham et al., 1992; Carbajal et al., 1997; Upcroft et al., 1989).

The use of Plf against protozoal infections has been evaluated by several researchers. Ghoshal et al. (1996) reported the potential role of an ethanol extract of Plf on amoebiasis in rats. A similar activity in mice was also reported by Sawangjaroen et al. (2004). In addition, aqueous and ethanol extracts from Plf inhibited the growth of *Giardia lamblia*, 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). This is the first time that the effects of extracts from Plf against *Blastocystis hominis* in vitro have been described.

Although Qin is routinely prescribed for the treatment of diarrhea in Thai herbal medicine, the scientific data supporting the use of this plant as a herbal drug is scarce. Dar et al. (1976) reported that a methanol extract of Qin significantly reduced blood sugar levels in rabbits. Hwang et al. (2000) suggested the use of hexagalloylglucose isolated from methanol extracts of Qin as a safe hypoglycemic agent. However, at present there are only a few studies that show an effect of Qin against microorganisms. Sawangjaroen et al. (2004) showed some antiameobic activity of a methanol extract of Qin in mice. Hussein et al. (2000) demonstrated its activity against hepatitis C virus protease. The present study provides evidence of an inhibitory activity, ranging from moderately inhibited to killing by the methanol extract from Qin against most isolates of *Blastocystis hominis*. It is probably worthwhile to purify these active extracts to identify specific compounds responsible for the activities.

In conclusion, this study shows that some extracts from Thai medicinal plants have some potential use as therapeutic agents against *Blastocystis hominis*. Although, several extracts produced only moderately inhibited growth of

the organisms, they might be of use in reducing infection because patients always show no symptoms when only small numbers of *Blastocystis hominis* are present (Zierdt, 1983; Zaki et al., 1991). Furthermore, the mode of actions of these plants used in herbal medicine as anti-diarrheic medicinal plants could result from other pharmacological effects such as a reduction in intestinal motility or from antibacterial activity. Based on this study, extracts of effective plants will be further investigated to identify their active compounds.

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