

SCHISTOSOMICIDAL ACTIVITY OF THE CRUDE EXTRACT OF *ARTOCARPUS LAKOOCHA*

Narin Preyavichyapugdee¹, Manaw Sangfuang¹, Saowapark Chaiyapum¹, Suwatcharaporn Sriburin¹, Yupa Pootaeng-on¹, Phiraphol Chusongsang², Wannee Jiraungkoorskul³, Mananya Preyavichyapugdee⁴ and Prasert Sobhon⁵

¹Faculty of Animal Sciences and Agricultural Technology, Silpakorn University, Petchaburi IT Campus, Petchaburi; ²Applied Malacology Unit, Department of Social and Environmental Medicine, Faculty of Tropical Medicine, Mahidol University, Bangkok; ³Department of Pathobiology, Faculty of Science, Mahidol University, Bangkok; ⁴Faculty of Agricultural Technology, Phetchaburi Rajabhat University, Phetchaburi; ⁵Department of Anatomy, Faculty of Science, Mahidol University, Bangkok, Thailand

Abstract. Puag-Haad is a traditional anthelmintic drug used to treat taeniasis in Thailand and Lao PDR. It is derived from the aqueous extract of the plant *Artocarpus lakoocha*. We investigated the *in vitro* anthelmintic properties of Puag-Haad against *Schistosoma mansoni*. Adult worms were incubated in M-199 medium containing 250, 500 and 750 µg/ml of Puag-Haad or praziquantel (PZQ) at a concentration of 175 µg/ml for 3, 6, 12 and 24 hours. The relative motility (RM value), survival index (SI) and tegument alterations seen under scanning electron microscope were assessed at each incubation time. The results showed the crude extract of *A. lakoocha* at a concentration of 250 µg/ml was more effective in causing damage than PZQ at a concentration of 175 µg/ml using RM and SI values. The major target organ affected by Puag-Haad was the tegument. The damage was greater at higher concentrations of the crude extract. It is likely tetrahydroxystilbene (THS), the main compound in Puag-Haad, caused the damage. THS could be a future candidate as a schistosomal drug. Further studies are needed to explore its mechanism, efficiency and safety *in vivo*.

Keywords: *Artocarpus lakoocha*, medicinal plant, anthelminthic drug, schistosomiasis, oxyresveratrol

INTRODUCTION

Schistosomiasis, caused by the blood flukes of *Schistosoma* spp, is an important public health problem in Africa, Asia and South America. Schistosomiasis ranks

second after malaria in terms of parasite-induced human morbidity and mortality, with more than 207 million people in 76 countries currently infected by the disease and 779 million people at risk (Colley *et al*, 2001; Gryseels *et al*, 2006; Steinmann *et al*, 2006). Among the five major species causing health problems in humans, *Schistosoma mansoni* is the most widespread, present in 54 countries, predominantly in Africa, parts of Arabia, in northern and eastern parts of South America and in some

Correspondence: Narin Preyavichyapugdee, Faculty of Animal Science, Silpakorn University, Phetchaburi Campus, Cha-am, Phetchaburi 76120, Thailand.

Tel: +66 (0) 32 594037; Fax: +66 (0) 32 594038

E-mail: jnnarin@hotmail.com

Caribbean islands (Chitsulo *et al*, 2000).

At present praziquantel (PZQ) is the drug of choice for treating schistosomiasis (Cioli and Pica-Mattoccia, 2003). However, reliance on a single drug is a problem since PZQ resistance has been reported among some strains of *S.mansoni* (Fallon *et al*, 1995; Stelma *et al*, 1995; Gryseels *et al*, 2001; Doenhoff *et al*, 2008). PZQ lacks efficacy against schistosomula and the young developing stages of *Schistosoma* spp (Sabah *et al*, 1986), possibly making it less effective in eradicating schistosomiasis where it is endemic (Cioli and Pica-Mattoccia, 2003). There is a need to search for a new treatments and preventions against schistosomiasis.

Artocarpus lakoocha Roxb. is an indigenous tree found widely in South and Southeast Asia (Gardner *et al*, 2000). In Thailand and in Lao PDR, a brown powder extract of *A. lakoocha* is known as Puag-Haad (PH) has been locally used to treat taeniasis (Charoenlarp *et al*, 1989; Pierce Salguero, 2003). The crude extract of *A. lakoocha* has been reported to be effective against the intestinal fluke *Haplorchis taichui* (Wongsawad *et al*, 2005) and the ruminant trematode *Fasciola gigantica* (Saowakon *et al*, 2009). A 70% crude aqueous extract of *A. lakoocha* as the phenolic compound a trans-2,3',4,5'-tetrahydroxystilbene (THS) (Mongkol-suk *et al*, 1957; Poopyruchpong *et al*, 1978; Likhitwitayawuid *et al*, 2006), is believed to be the active antihelminthic chemical.

The efficacy of PH against taeniasis and other intestinal and liver flukes makes it an appealing candidate for controlling other parasites. The extract of this plant has not been tested against blood flukes of *Schistosoma* spp. This compound could provide an alternative to PZQ as an anti-schistosomal drug.

In the present study we aimed to: 1) evaluate the anthelmintic effects of PH in killing *S. mansoni*, and 2) investigate its effects on the tegument of both male and female parasites using a scanning electron microscope (SEM).

MATERIALS AND METHODS

Mice

Six-week old Institute of Cancer Research (ICR) male mice with a mean weight of 25.59±1.13 g were obtained from the National Laboratory Animal Center, Mahidol University, Nakhon Pathom Province, Thailand. The animals were housed in shoe-box type cages (18×30×13 cm) containing sterile wood-shaving bedding in a strictly hygienic conventional animal room at the Faculty of Tropical Medicine, Mahidol University. A standard diet and tap water were available *ad libitum*. Room temperature was kept at 22-25°C with a relative humidity of 60-70% and a 12:12 hour light-dark cycle. All experimental animals used in this study were treated in accordance with the Guidelines for the Care and Use of Laboratory Animals, authorized by the Animal Care and Use Committee, Faculty of Tropical Medicine, Mahidol University (FTM-ACUC 006/2005).

Experimental design

ICR mice ($n=40$) were infected each with 150 *S. mansoni* cercariae shed from experimentally infected *Biomphalaria glabrata* snails, after exposure to artificial light for at least 4 hours, by the tail immersion method (Olivier and Stirewalt, 1952). Forty-nine days postinfection the mice were sacrificed and adult flukes were collected by perfusion using 0.1 M citrate in 0.15 M NaCl solution (Sornmani *et al*, 1973). After washing with saline several

times healthy flukes showing normal appearance and good motility were selected and kept in culture medium 199 (M-5017, Sigma, St Louis, MO, Lot No. 077K83001) containing antibiotics (penicillin 50 IU/ml; streptomycin 50 µg/ml) until used.

Six hundred adult flukes were randomly assigned to five groups (40 flukes per group, three replicates): group 1 (control group), the parasites were incubated in M199 medium containing 0.1% (v/v) DMSO and antibiotics (penicillin 50 IU/ml, streptomycin 50 µg/ml, gentamicin 30 IU/ml); group 2, the parasites were incubated in the same medium containing praziquantel (PZQ) (Medicpharma, Bangkok, Thailand, Lot No. MZ76807) at a concentration of 175 µg/ml (PZQ was diluted in 1% DMSO as a stock solution). Flukes in groups of 3-5 were incubated in the same base medium but also containing the crude extract of *A. lakoocha* at concentrations of 250, 500, and 750 µg/ml. The crude extract of *A. lakoocha* (PH) was bought from a traditional medicine drugstore (Chiang Mai, Thailand). According to traditional belief, PH crude extract is obtained by boiling the wood chips of *A. lakoocha* in water. The foam formed on the surface of the boiling-water containing *A. lakoocha* was continuously harvested and subsequently dried. The end product was a brown powder of PH used for further experiments. The PH was dissolved in 1% DMSO as a stock solution (the final concentration of solution was 0.1% DMSO). The PH which was diluted with the culture medium at a 250 µg/ml concentration contained 175 µg/ml of THS; THS constituted 70% of the PH as reported by Mongkolsuk *et al* (1957) and Poopyruchpong *et al*, (1978). The parasites in all the study groups were incubated in the culture medium in an incubator aerated with 5% CO₂ at 37°C. After 3, 6, 12, and

24 hours incubation, the motility, survival and tegument alterations were assessed by examination under an Olympus SZ-ST stereomicroscope (Tokyo, Japan).

Assays for the drug's activities

Motility criteria. Motility scores (n) were assigned using the following criteria: 3 = movement of the whole body, 2 = movement of only parts of the body, 1 = immobile but not dead and unstained with the vital dye methylene blue in 0.85% NaCl solution (1% (w/v), and 0 = immobile and stained with the vital dye. The efficacies of the tested drugs against adult *S. mansoni* were calculated as the relative motility (RM) value using the formula below (Kiuchi *et al*, 1987). A lower RM value indicated stronger drug activity, and when all the flukes died this value was 0.

$$\text{Motility index (MI)} = \sum Nn / N$$

$$\text{Relative motility (RM) value} = \text{MI test} \times 100 / \text{MI control}$$

n = motility score, N = number of flukes with the score of n.

Survival index. The survival index (SI) (the percentage of live flukes) was determined at each point of time during the incubation. Flukes with motility score of 0 (immobile and stained with the vital dye) were counted as dead, and those with other scores (3, 2, 1) were counted as still alive. The SI was calculated using the formula below, a SI = 0 indicated all the flukes were dead.

$$\text{Survival index (SI)} = (\text{number of live flukes} / \text{number of all flukes}) \times 100$$

Specimen preparation for scanning electron microscopy

Both male and female worms incubated in the M199 culture medium, PZQ 175 µg/ml, PH at 250, 500, and 750 µg/ml were collected and fixed in 2.5% glutaraldehyde-phosphate buffer (0.1 mol/l, pH 7.4) at 4°C for 24 hours and post-fixed in 1% osmium tetroxide for 1 hour. They were then dehydrated with a

series of increasing concentrations of ethanol and dried in a Hitachi HCP- 2 critical point dryer using liquid carbon dioxide as a transitional medium. After drying, they were mounted on aluminum stubs and coated with platinum and palladium in an ion-sputtering apparatus (Hitachi E-102) set at 10-15 mA for 6 minutes. They were then examined and photographed in a Hitachi scanning electron microscope S-2500 (Hitachi High Technologies, Hitachi-Naka City, Japan), operating at 15 kV.

Purification of standard oxyresveratrol

Oxyresveratrol was obtained from a dried aqueous extract (5 g) prepared from the heartwood of *A. lakoocha*. This was then dissolved in a small amount of ethyl acetate. Silica gel (15 g) was added and the mixture was dried under reduced pressure. The dried mixture was subject to silica gel column chromatography (4.5 cm i.d. x 15 cm) and eluted with a solvent system of *n*-hexane/ethyl acetate/acetone (47.5: 47.5: 5.0 v/v/v) to provide 2.46 g of crude light-yellow solid oxyresveratrol. Further purification by a silica gel CC (2.2 cm i.d. x 15 cm) and eluted with *n*-hexane/ethyl acetate/acetone (47.5: 47.5: 5.0 v/v/v) gave 102 mg, (68% yield from crude oxyresveratrol) of purified light-yellow crystalline oxyresveratrol. The purity of the oxyresveratrol was confirmed by thin-layer chromatography (TLC), melting point and high performance liquid chromatography (HPLC). The structure of the oxyresveratrol was confirmed by UV and NMR spectral data.

HPLC analysis of the oxyresveratrol in Puag-Haad

A standard solution of oxyresveratrol at a concentration of 0.16 mg/ml was prepared by dissolving 16.1 mg oxyresveratrol in 100 ml 95% methanol. Further dilutions were made to prepare

4 standard solutions at concentrations of 0.08, 0.04, 0.02 and 0.01 mg/ml using serial dilutions. Sample solutions were prepared by dissolving Puag-Haad (1.0 mg) in 10 ml 95% methanol. Chromatography was performed using a Thermo Scientific HYPERSIL ODS-2 column (250 x 4.6 mm, 5 μ m) at a flow rate of 1.0 ml/min, with a mobile phase composed of solvent A (water) and solvent B (0.78% acetic acid in 50% methanol) in a gradient system, initially with 30%B in a linear gradient to 30%B in 10 minutes, then a linear gradient to 100%B in 15 minutes, hold at 100%B for 3 minutes, then a linear gradient to 30%B in 5 minutes, and then held at 30%B for 5 minutes. Detection was conducted at 254 nm and quantification was based on the integrated peak areas with reference to external standards.

Statistical analysis

Comparisons of anthelmintic effects between groups was made using one-way analysis of variance with Duncan's test for multiple comparisons; significance was set at $p < 0.05$.

RESULTS

Relative motility and survival index values among parasites treated with praziquantel and crude extract of *A. lakoocha*

All worms in the negative control group lived and remained active during the 24 hour study period (RM = 100, SI = 100). The worms treated with PZQ or PH had reduced RM and SI values in direct proportion to their concentrations and incubation times (Fig 1A and B).

A decrease in the RM occurred at 3 hours in the PZQ-treated group (RM = 38.73); the RM slowly declined until the end of incubation period at 24 hours (RM = 33.38). Some of the worms were dead at

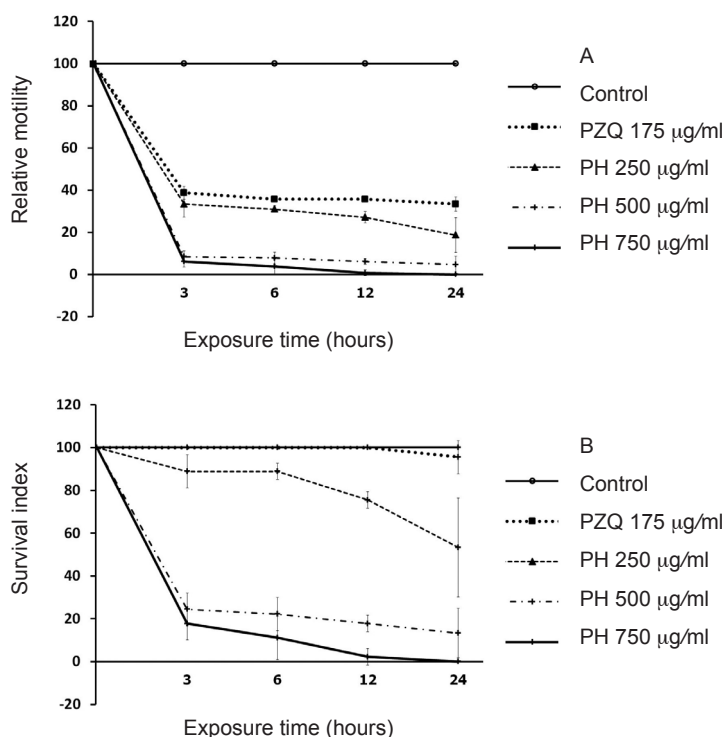


Fig 1–(A) Relative motility (RM) and (B) survival indices (SI) values of the control and the experimental worms treated with praziquantel (PZQ) and an aqueous extract of *A. lakoocha*-Puag-Haad (PH) at various concentrations and durations. Each point in the graph represents the response from 10 flukes (3 replicates). Data are expressed as mean \pm standard deviation (SD).

24 hours with a SI of 95.55. Worms incubated in PH at a concentration of 250 µg/ml exhibited reduced motility and died at a more rapid rate than in the PZQ-treated group, as the RM value dropped from the start of incubation, some of the worms took up vital dye, indicating they were dead by 3 hours (RM = 33.30, SI = 88.88). The RM and SI values gradually decreased until the end of the incubation period of 24 hours (RM = 18.71, SI = 53.33).

When comparing the anthelmintic effects of crude PH at 250 µg/ml and PZQ at 175 µg/ml (Fig 2), the PH had a signifi-

cantly lower ($p < 0.05$) RM and SI values than the PZQ (Fig 2). At 500 µg/ml of PH the RM and SI values dropped sharply at the start and then gradually decreased until the end of the experiment at 24 hours (RM = 4.70 and SI = 13.33). At 750 µg/ml of PH, most of the worms were immotile by 3-6 hours, and approximately 97% were dead by 12 hours; by 24 hours all the worms were dead (RM = 0, SI = 0).

Scanning electron microscopic observations

Control group. During the 24 hour incubation period in 0.1% DMSO (control group) the tegumental surface of the male worms still exhibited normal features comprised of numerous tubercles with small sharp spines distributed over the whole body of the worms. Both

oral and ventral suckers also appeared normal (Fig 3A and B). In females the surface features also appeared normal and comprised circumferential folds with smooth surface separated by furrows (Fig 3C and D).

Effects of PZQ. Among male worms 3 hours in the PZQ solution, their bodies become contracted and twisted (Fig 4A). By 6 hours, the tubercles on the surface became retracted and the spines became shortened or absent (Fig 4B) and numerous small blebs occurred in the intertubercular areas. By 24-hour incubation some parts

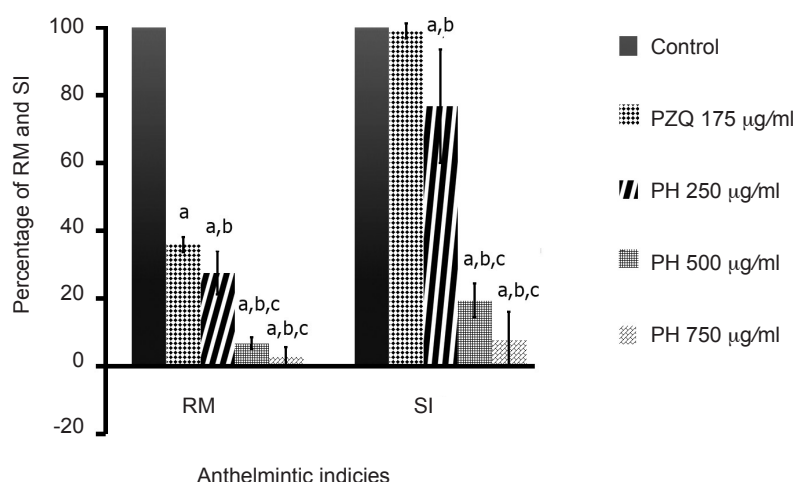


Fig 2—Comparative anthelmintic effects of PZQ at 175 µg/ml and PH at 250, 500 and 750 µg/ml on *S. mansoni* based on relative motility (RM) and survival index (SI). The RM and SI value from all time points were pooled and expressed as mean \pm SD. Significant difference between each group with negative control is indicated by “a”, between PZQ and PH at various concentrations is indicated by “b”, and between the consecutive doses of PH is indicated by “c” and “d” (compared with 250 and 500 µg/ml, respectively ($p < 0.05$)).

of the tegument sloughed off exposing subtegumental structures (Fig 4C and D).

Among female worms by 3 hours in the PZQ solution the tegument had swelling and focal erosion (Fig 4E). By 6 to 12 hours they had worse swelling and peeling of the tegument (Fig 4F and G). By 24 hours there was an extensive peeling of the tegument revealing the subtegumental layer (Fig 4H).

Effect of crude extract of *A. lakoocha*. Tegument damages in the parasites treated with PH were similar to those treated with PZQ, with the degree of severity depending on doses and times of incubation. In general the worms incubated with PH at 250 µg/ml showed a greater degree of body contraction and severity of tegumental alteration than the PZQ. Male worms

treated with PH had a disruption of tubercles and severe blebbing of the tegument by 3 hours (Fig 5A and B) and the suckers were damaged and had collapsed (Fig 5A inset). By 12-24 hours there were more ruptured tubercles and erosions that exposed the subtegumental structures (Fig 5C and D). Female worms treated with PH showed severely swollen teguments by 3 hours (Fig 5E). By 6-12 hours the tegument surface had extensive blebbing followed by erosion and peeling (Fig 5F and G). By 24 hours there was extensive peeling over the whole surface (Fig 5H).

The worms treated with PH at concentrations of 500 and 750 µg/ml had similar changes but more severe than at the concentration of 250 µg/ml. The higher the concentration and longer the incubation time, the more severe the changes. Severe disruption of the tubercles and dislodging of the spines were seen at the two higher concentrations of PH by 3 hours (Figs 6B, 7A). By 12 hours, there was extensive destruction of the tegument (Figs 6C, 7C). By 24 hours the tegument had severe disruption with peeling and exposure of the subtegumental structures, especially at a concentration of 750 µg/ml (Figs 6D, 7D). The female worms had severe disruption of the tegument comprised of swelling, extensive blebbing, erosions and peeling by 3 hours (Figs 6E-H, 7E-H).

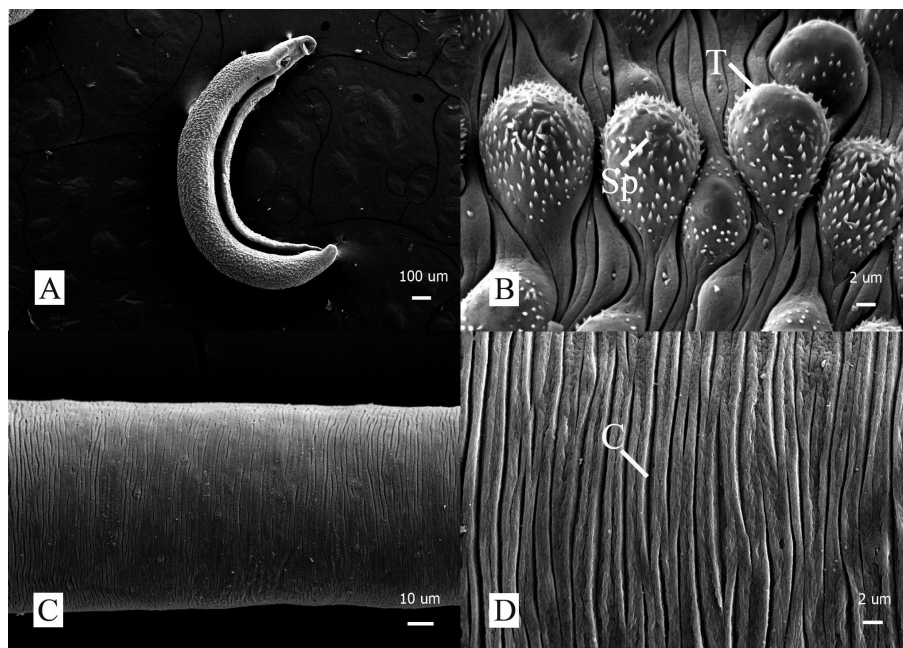


Fig 3—Scanning electron micrograph of a male (A-B) and a female (C-D) *S. mansoni* incubated in 0.1% DMSO. A) After 6 hours of exposure, the tegument of the male worm showed normal surface features. B) After 24 hours of exposure, at a higher magnification the tegument on the middle part still showed a normal appearance comprising of numerous spiny tubercles and smooth folds in between intertubercular areas. C) At 3 hours of exposure, the tegumental surface covering the middle part of the body of a female worm appeared as a flat sheet with circumferential streaks. D) At 24 hours of exposure, at a higher magnification of the tegument in the same area appears highly corrugated with circumferential folds intervened by narrow clefts (C).

Analysis of the oxyresveratrol in the Puak-Haad

The purity of the oxyresveratrol was confirmed with high performance liquid chromatography (HPLC) (Fig 8A). The structure of the oxyresveratrol was elucidated by nuclear magnetic resonance (NMR) as 2,3',4,5'-tetrahydroxystilbene which had a melting point (mp) of 199–201°C (mp_{Lit.} = 201°C) [1(UV λ_{max} (MeOH) nm (log ϵ): 325 (4.241), (log ϵ)_{Lit.}: 328 (4.329) (Mongkolsuk *et al*, 1975). The ¹H- and ¹³C-NMR spectral data with those of authentic sample (Kanchanapoom *et al*, 2002; Zhang *et al*, 2008). ¹H-NMR (CD₃COCD₃) δ : 6.25 (1H, *t*, J = 2.1 Hz), 6.34 (1H, *dd*, J = 8.4, 2.4

Hz), 6.42 (1H, *d*, J = 2.4 Hz), 6.53 (2H, *d*, J = 2.1 Hz), 6.88 (1H, *d*, J = 16.5 Hz), 7.32 (1H, *d*, J = 16.5 Hz), 7.38 (1H, *d*, J = 8.4 Hz), 8.33 (3H, *s*, D₂O exchangeable), 8.62 (1H, *s*, D₂O exchangeable), ¹³C-NMR (CD₃COCD₃) δ : 102.3, 103.6, 105.2, 108.5, 117.2, 124.3, 126.2, 128.3, 141.6, 156.7, 158.9, 159.3

The HPLC chromatogram of the oxyresveratrol in Puak-Haad is shown in Fig 8B. Quantification was based on the integrated peak areas with reference to an external standard (Fig 8A). The retention time of oxyresveratrol was 20.9 minutes and the naturally occurring percentage of oxyresveratrol in Puak-Haad was 72.6±1.8%.

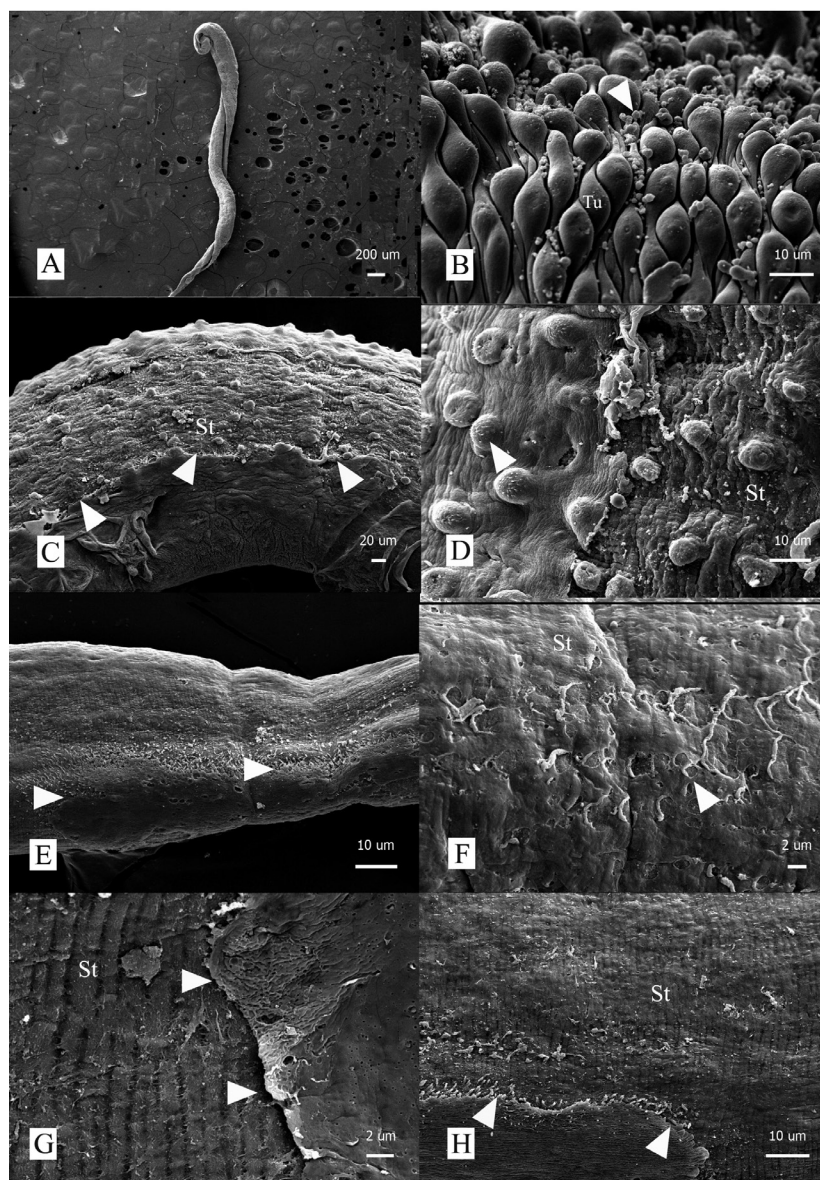


Fig 4—Scanning electron micrographs of male (A-D) and female (E-H) *S. mansoni* treated with 175 µg/ml PZQ. A) At 3 hours of exposure, a male worm showed contracted body. B) At 6 hours of exposure, the middle part of male worm showed swollen tubercle (Tu), some of which lost spines. Intertubercular areas were covered with clusters of blebs (arrowheads). C) At 24 hours of exposure, the tegument in the middle part showed peeling (arrowheads) and disrupted subtegumental tissues (St). D) At 24 hours of exposure, the tegument exhibited damaged tubercles (arrow) and some areas were peeled off exposing the subtegumental tissues (St). E) At 3 hours of exposure, a female worm began to show disruption of the tegumental surface (arrowheads). F) At 6 hours of exposure, disruption of the tegument (arrowhead) lead to the exposure of subtegumental tissues (St). G) At 12 hours of exposure, erosion of tegument (arrowheads) and exposed subtegumental layers (St) were evident. H) At 24 hours of exposure, a female worm exhibited peeling (arrowheads) of tegument which exposed the subtegumental layers (St).

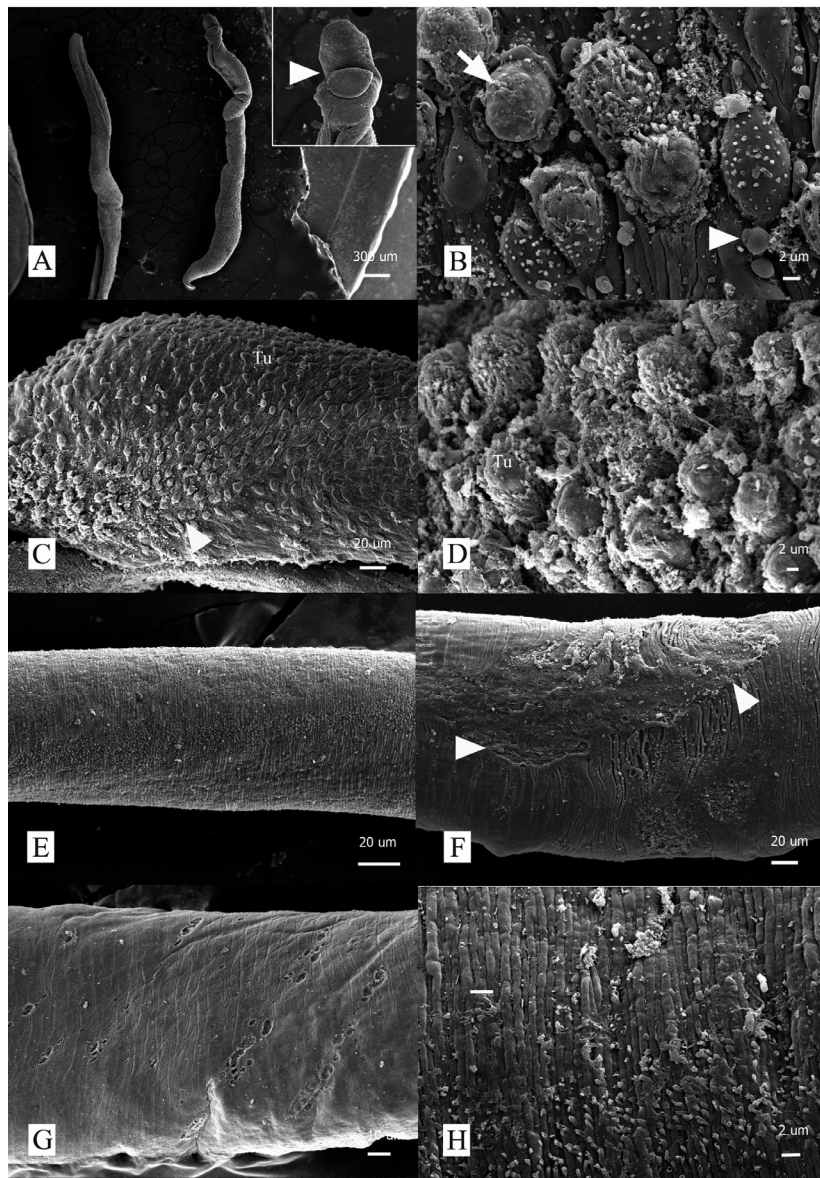


Fig 5—Scanning electron micrographs of male (A-D) and female (E-H) *S. mansoni* treated with 250 µg/ml of *A. lakoocha* crude extract at 3-24 hours of exposures. A) At 3 hours, a male worm showed swollen body surface. Inset shows destruction of the oral sucker (arrowhead). B) At 3 hours, the tubercles (arrow) were damaged and numerous blebs emerged from the tegumental surface around the tubercles (arrowhead). C) At 24 hours, tegumental surface showed severe erosion and disruption of tubercles. D) At 24 hours, the surface showed collapsed tubercles (Tu) and extensive peeling. E) At 3 hours, a female worm showed extensive swelling of the tegument covering the middle part of the body. F) At 6 hours, the surface showed extensive erosion and peeling (arrows). G) At 12 hours, the tegument showed peeling of tegument. H) At 24 hours the tegument was disrupted and some areas were peeled off.

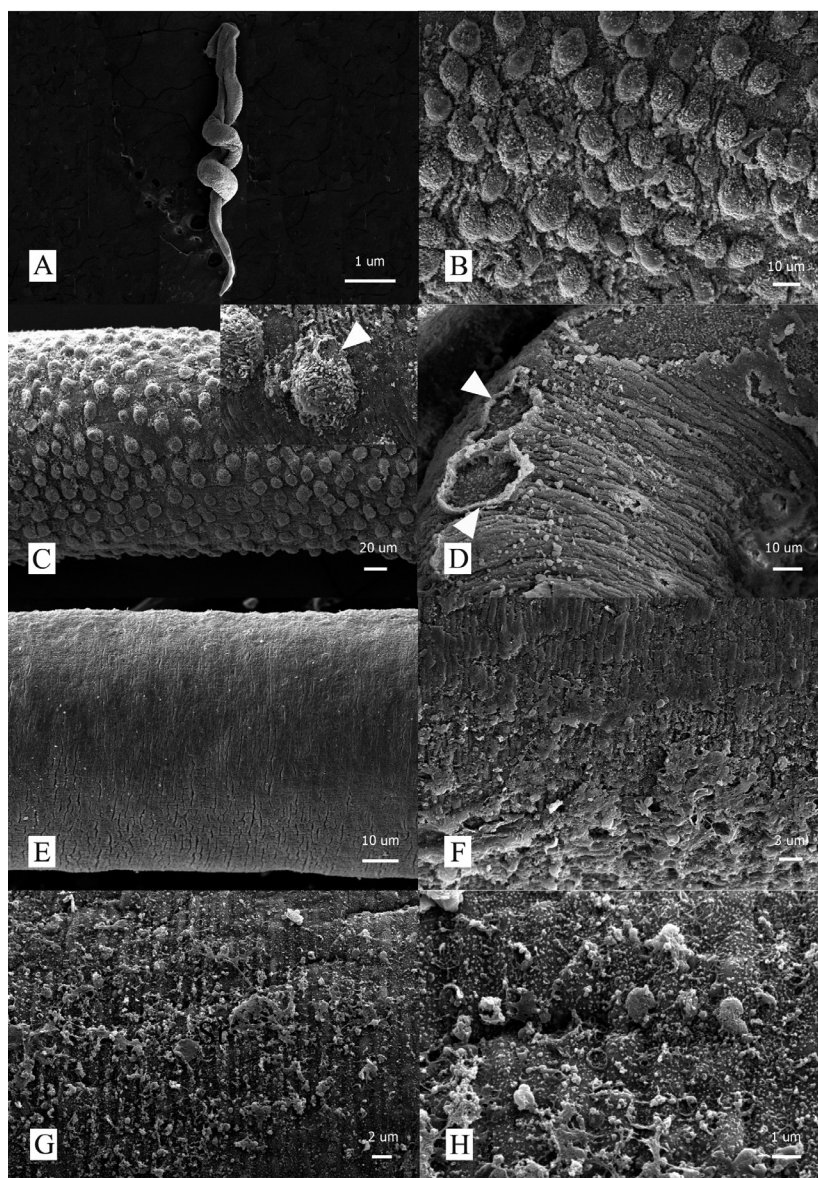


Fig 6—Scanning electron micrographs of male (A-D) and female (E-H) *S. mansoni* treated with 500 µg/ml of *A. lakoocha* crude extract at 3-24 hours of exposure. A) At 3 hours, a male worm showed tightly coiled and contracted body. B) At 3 hours, there were extensive erosion and peeling of the tubercles. C) At 12 hours, the tegument exhibited extensive disruption of the tubercles. Inset showing severe disruption of the tubercle (arrow). D) At 24 hours, the tegumental surface showed severe erosion and peeling (arrows). E) At 3 hours, a female worm showed extensive swelling of the tegument covering the middle part of the body. F) At 6 hours of exposure, the tegument showed erosion and peeling. At 12 hours (G), and 24 hours (H), the tegument exhibited severe disruption and erosion.

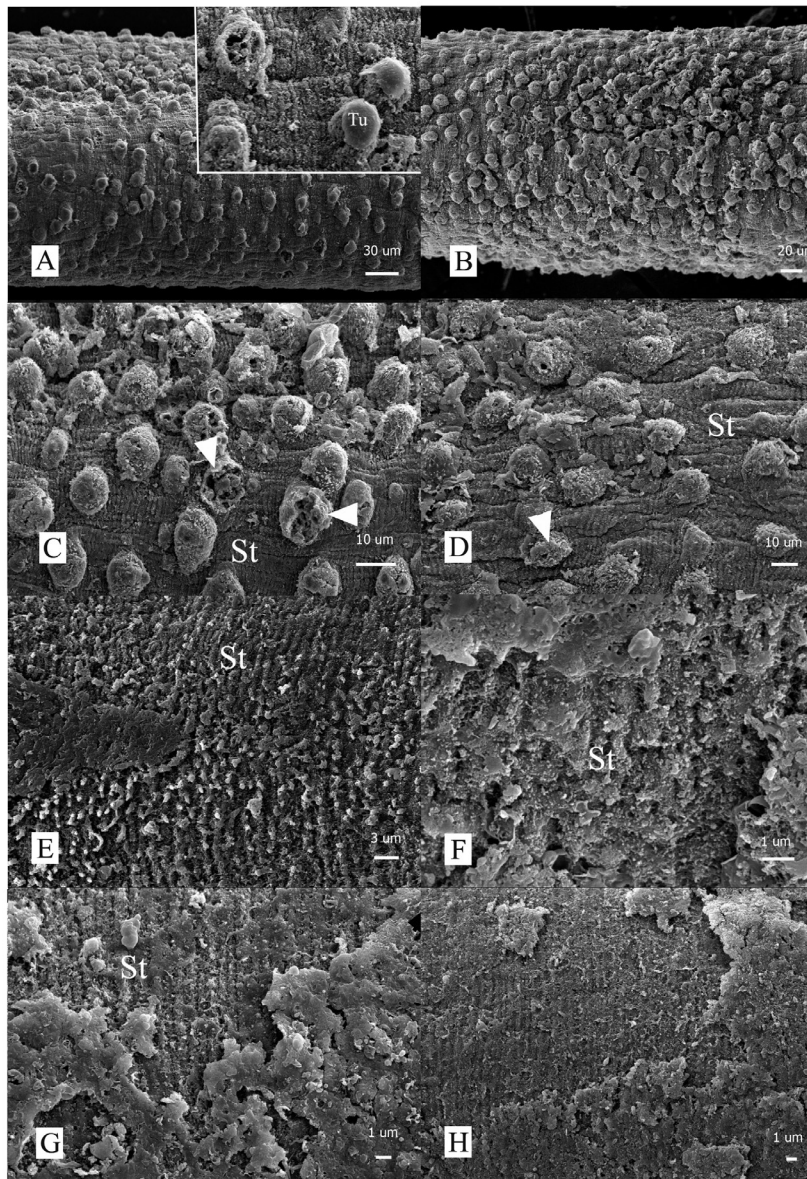


Fig 7–Scanning electron micrographs of male (A-D) and female (E-H) *S. mansoni* treated with 750 µg/ml *A. lakoocha* at 3-24 hours of exposure. A) At 3 hours, the micrograph of the worm showed erosion of tegumental surface in the middle part of the body. Inset shows destruction of the tubercles (Tu). B) At 6 hours, tubercles in the middle part of the body were collapsed. C) At 12 hours, there were severe disruption of the subtegumental layer (St) and collapse of damaged tubercles (arrows). D) At 24 hours, there were severe destruction of the tubercles (arrows) that lead to the exposure of the subtegumental tissues (St). E) At 3 hours, a female worm showed extensive swelling of tegumental ridges in the middle part of the body. F) At 12 hours, there was focal erosion of the tegument covering the middle part of the body with exposed subtegumental layer (St) in some areas. G, H) At 24 hours, there were extensive erosions and peelings in many areas revealing the subtegumental layers (St).

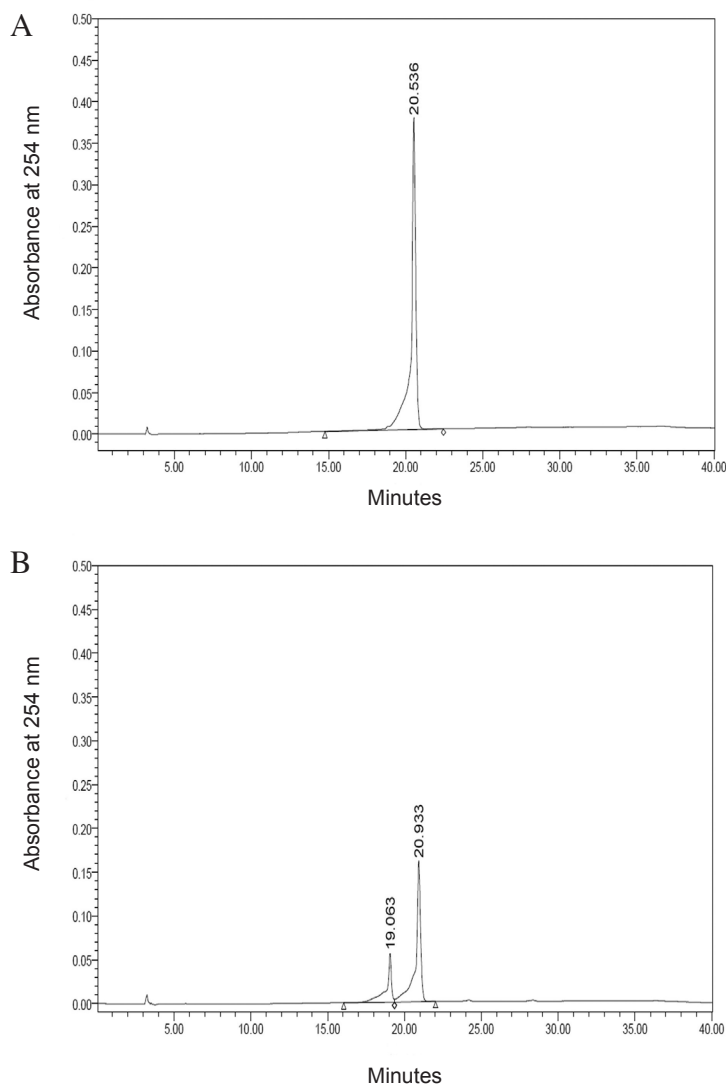


Fig 8—HPLC chromatogram of sample: (A) purified oxyresveratrol for external standard (0.161 mg/ml), (B) Puag-Haad (0.101 mg/ml). The detection was conducted at 254 nm and quantification was based on the integrated peak areas with reference to external standard. The retention time of oxyresveratrol was 20.9 minutes.

DISCUSSION

This is the first report of the anthelmintic effects of Puag-Haad, the crude extract of *A. lakoocha*, against adult *S. mansoni* parasites. HPLC analysis revealed the

major component of this extract is oxyresveratrol (PubChem CID: 5281717), similar to previous reports (Mongkolsuk *et al*, 1957; Poopyruchpong *et al*, 1978). Based on the RM and SI values, the crude extract containing an equivalent concentration of 2,3',4,5'-tetrahydroxystilbene (THS) to PZQ at 175 µg/ml, was more effective in causing reduction of the parasite motility and survival than PZQ. Observations with a SEM found the studied PH crude extract caused more damage to the tegument of adult *S. mansoni* parasites than PZQ, while the sequence of pathological change were similar. The severity and rapidity of the damage was directly related to its concentration and time of exposure.

Our findings support the potential use of *A. lakoocha* as anthelmintic, which has been used in traditional medicine by indigenous people in Thailand and Lao PDR (Pierce Salguero, 2003). Besides, the results are in agreement with previous studies of the susceptibility of the fish trematode, *Haplorchis taichui* and

the ruminant trematode, *F. gigantica*, to the crude extract of *A. lakoocha* (Wongsawad *et al*, 2005; Saowakon *et al*, 2009). Both those studies found the crude extract inhibited parasite motility, caused severe damage to the tegument and killed the worms.

The tegument of trematodes is important for osmoregulation, protection, synthesis and secretion and represents a primary drug target (Hallton, 2004). The disruption of the tegument can impair the above mentioned functions and allow better drug penetration, resulting in reduced motility and susceptibility to the host's immune system (Xiao *et al*, 2000). The damage to the suckers may reduce the ability of the worm to adhere to blood vessels, reducing nutrient intake (Xiao *et al*, 2000). Alteration in the female tegument may lead to reduction in egg production and prevalence of the parasite.

The crude extract of *A. lakoocha* contains a high concentration of THS, which is believed to damage the tegument (Saowakon *et al*, 2009). Corbett and Goose (1971), reported *Fasciola* spp could be killed by exposure to phenolic compounds such as nitroxylnil, hexachlorophane and oxyclozanide, causing uncoupling of oxidative phosphorylation followed by Na⁺ influx and alteration of the tegument morphology. The fact that *A. lakoocha* had a similar affect as the above chemicals suggests the active ingredient in *A. lakoocha* could be TSH, a phenolic compound.

The tegument of adult *S. mansoni* contains numerous mitochondria, which are commonly distributed throughout the tegumental syncytium (Morris and Threadgold, 1968; Hockley and McLaren, 1973), and could be a target of THS. Decreased production of ATP from these mitochondria could affect energy-dependent Na⁺/K⁺ ATPase ion pumps, resulting in instability of the osmoregulatory system (Pax *et al*, 1987). The influx of Na⁺ and water into the syncytium could lead to swelling, blebbing, disruption, erosions, and finally peeling of the tegument. Once the surface layer is damaged, the drug can penetrate deeper into the muscular layer

and cause motility reduction leading to parasite's death. In contrast, PZQ affects the permeability of the tegument surface membrane by increasing calcium influx (Pax *et al*, 1978). PZQ induces contraction of the parasites resulting in paralysis and death.

Further studies are needed to elucidate the mechanism(s) of THS toxicity against schistosomes and to evaluate its suitability as an anthelmintic agent.

ACKNOWLEDGEMENTS

This study was supported by a New Researcher Grant funded by the Thailand Research Fund (TRF) and the Commission on Higher Education to Narin Preyavichyapugdee (MRG5180360) and a grant from Faculty of Science, Mahidol University (to Prasert Sobhon). The authors would like to thank Mr Jean Marcel Meyer, for assistance with English editing and Assoc Prof Mana Kanjanamaneesathian, Faculty of Animal Sciences and Agricultural Technology, Silpakorn University, for valuable comments.

REFERENCES

- Charoenlarp P, Radomyos P, Bunnag D. The optimum dose of Puag-Haad in the treatment of taeniasis. *J Med Assoc Thai* 1989; 72: 71-3.
- Chitsulo L, Engels D, Montresor A, Savioli L. The global status of schistosomiasis and its control. *Acta Trop* 2000; 77: 41-51.
- Cioli D, Pica-Mattoccia L. Praziquantel. *Parasitol Res* 2003; 90: S3-9.
- Colley DG, LoVerde PT, Savioli L. Medical helminthology in the 21st Century. *Science* 2001; 293: 1437-8.
- Corbett JR, Goose J. A possible biochemical mode of action of the fasciolicides nitroxylnil, hexachlorophene and oxyclozanide. *Pestic Sci* 1971; 2: 119-21.
- Doenhoff MJ, Cioli D, Utzinger J. Praziquantel:

- mechanisms of action, resistance and new derivatives for schistosomiasis. *Curr Opin Infect Dis* 2008; 21: 659-67.
- Fallon PG, Sturrock RF, Niang AC, Doenhoff MJ. Short report: diminished susceptibility to praziquantel in a Senegal isolate of *Schistosoma mansoni*. *Am J Trop Med Hyg* 1995; 53: 61-2.
- Gardner S, Sidisunthorn P, Anusarnsunthorn V. A field guide to forest trees of northern Thailand. Bangkok: Kobfai Publishing Project, 2000: 451.
- Gryseels B, Mbaye A, De Vlas SJ, *et al*. Are poor responses to praziquantel for the treatment of *Schistosoma mansoni* infections in Senegal due to resistance? An overview of the evidence. *Trop Med Int Health* 2001; 6: 864-73.
- Gryseels B, Polman K, Clerinx J, Kestens L. Human schistosomiasis. *Lancet* 2006; 368: 1106-18.
- Hallton DW. Microscopy and the helminth parasite. *Micron* 2004; 35: 361-90.
- Hockley DJ, McLaren DJ. *Schistosoma mansoni*: changes in the outer membrane of the tegument during development from cercaria to adult worm. *Int J Parasitol* 1973; 3: 13-25.
- Kanchanapoom T, Suga K, Kasai R, Yamasaki K, Kamel MS, Mohamed MH. Stilbene and 2-arylbenzofuran glucosides from the rhizomes of *Schoenocaulon officinale*. *Chem Pharm Bull* 2002; 50: 863-5.
- Kiuchi F, Miyashita N, Tsuda Y, Kondo K, Yoshimura H. Studies on crude drugs effective on visceral larva migrans. I. Identification of larvicidal principles in betel nuts. *Chem Pharm Bull* 1987; 35: 2880-6.
- Likhitwitayawuid K, Sornsute A, Sritularak B, Ploypradith P. Chemical transformations of oxyresveratrol (trans-2,4,3',5'-tetrahydroxystilbene) into apotent tyrosinase inhibitor and a strong cytotoxic agent. *Bio Med Chem Lett* 2006; 16: 5650-3.
- Mongkolsuk S, Robertson A, Towers R. 2,4,3',5'-Tetrahydroxystilbene from *Artocarpus lakoocha*. *J Chem Soc* 1957: 2231-3.
- Morris GP, Threadgold LT. Ultrastructure of the tegument of adult *Schistosoma mansoni*. *J Parasitol* 1968; 54: 15-27.
- Olivier L, Stirewalt MA. An efficient method for exposure of mice to cercariae of *Schistosoma mansoni*. *J Parasitol* 1952; 38: 19-23.
- Pax R, Bennett JL, Fetterer R. A benzodiazepine derivative and praziquantel: effects on musculature of *Schistosoma mansoni* and *Schistosoma japonicum*. *Naunyn Schmiedeberts Arch Pharmacol* 1978; 304: 309-15.
- Pierce Salguero C. A Thai herbal: tretraditional recipes for health and harmony. Findhorn Forres: Findhorn Press, 2003: 168 pp.
- Poopyruchpong N, Rungruangsak K, Nimmanpisut S, Panijpan B, Ratanbanangkoon K. Some physico-chemical properties of 2,4,3',5'-tetrahydroxystilbene. *J Sci Soc Thai* 1978; 4: 163-7.
- Sabah AA, Fletcher C, Webbe G, Doenhoff MJ. *Schistosoma mansoni*: chemotherapy of infections of different ages. *Exp Parasitol* 1986; 61: 294-303.
- Saowakon N, Tansatit T, Wanichanon C, Chanakul W, Reutrakul V. *Fasciola gigantica*: anthelmintic effect of the aqueous extract of *Artocarpus lakoocha*. *Exp Parasitol* 2009; 122: 289-98.
- Sornmani S, Kitikoon V, Schneider CR, Harinasuta C, Pathammavong O. Mekong schistosomiasis. 1. Life cycle of *Schistosoma japonicum*, Mekong strain in the laboratory. *Southeast Asian J Trop Med Public Health* 1973; 4: 218-25.
- Steinmann P, Keiser J, Bos R, Tanner M, Utzinger J. Schistosomiasis and water resources development: systemic review, meta-analysis, and estimates of people at risk. *Lancet Infect Dis* 2006; 6: 411-25.
- Stelma FF, Talla I, Sow S, Kongs A, *et al*. Efficacy and side effects of praziquantel in an epidemic focus of *Schistosoma mansoni*. *Am J Trop Med Hyg* 1995; 53: 167-70.
- Wongsawad C, Wongsawad P, Luangphai P, Kumchoo K. *In vitro* effects of aqueous extract from *Artocarpus takoocha* Roxb on

- tegumental surface of *Haplorchis taichui*. *Southeast Asian J Trop Med Public Health* 2005; 36: 112-6.
- Xiao SH, Shen BG, Chollet J, Tanner M. Tegumental changes in 21- day-old *Schistosoma mansoni* harboured in mice treated with artemether. *Acta Trop* 2000; 75: 341-8.
- Zhang Z, Jin J, Shi L. Protective function of cis-mulberroside A and oxyresveratrol from *Ramulus mori* against ethanol-induced hepatic damage. *Environ Toxicol Pharmacol* 2008; 26: 325-30.