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RESEARCH ARTICLE

Effect of *Allium sativum*, *Thunbergia laurifolia*, and *Eurycoma longifolia* crude extracts on the minute intestinal fluke, *Haplorchis taichui*

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ABSTRACT

Haplorchis taichui is the minute intestinal fluke (MIF), presents a significant public health concern in Thailand. Despite praziquantel (PZQ) being the main treatment, concerns over recurrent use and drug resistance have surfaced. Thus, local herbal alternatives effective against gastrointestinal parasites could be crucial for reducing issues, necessitating exploration of herbal sources for alternative treatments. The objective of this study is to evaluate the efficacy of crude extracts from *Allium sativum*, *Thunbergia laurifolia*, and *Eurycoma longifolia* against *H. taichui* newly excysted juveniles (NEJ). *H. taichui* NEJs were exposed to varying concentrations of *A. sativum* (5, 10, and 20 mg/ml), *T. laurifolia*, and *E. longifolia* (100, 200, and 400 mg/ml), alongside PZQ (20 mg/ml) and RPMI culture medium as controls. Motility assessment at different exposure times and morphological surface changes were conducted by scanning electron microscopy (SEM). Treatment with *A. sativum*, *T. laurifolia*, and *E. longifolia* inhibited motility in *H. taichui* NEJs, resulting in reduced relative motility (RM) values and survival index (SI). Significant differences were noted in *A. sativum* and *E. longifolia* treated groups, with *T. laurifolia* showing no significant differences compared to the negative control. Morphological damage, especially tegumental swelling, was evident across all treatment groups, notably severe in the *A. sativum* group. These findings suggest the potential effectiveness of crude extracts of *A. sativum*, *T. laurifolia*, and *E. longifolia* against *H. taichui* NEJs. However, further investigations are necessary to understand their mechanisms and key bioactive compounds for developing effective anti-parasitic agents against helminthic infections.

Keywords: *Allium sativum*; *Thunbergia laurifolia*; *Eurycoma longifolia*; crude extracts against-parasite; *Haplorchis taichui*.

INTRODUCTION

Foodborne trematodes, specifically the minute intestinal fluke (MIF) *Haplorchis taichui*, are prevalent in Thailand, posing a significant public health challenge, particularly among low to middle-income populations (Chai & Jung, 2022). The most recent extrapolation estimates a global prevalence of foodborne trematode infections at 74.7 million individuals as of 2015-2016, with an annual increase of 0.2 million new cases (Fürst *et al*., 2019). In Thailand, the status of MIF indicates an overall infection rate of 1.31% (Wattanawong *et al*., 2021). A study conducted in the northern and northeastern regions of Thailand observed a substantial prevalence of MIF infection, reaching 44.80% (56/125) in freshwater fish (Myint *et al*., 2022). Human infection with *H. taichui* occurs through the consumption of raw or undercooked fish containing the infective stage metacercaria, notably in traditional dishes such as Koi pla, Pla som, and Pla ra in Northeastern Thailand. Despite praziquantel (PZQ) being the primary treatment choice for MIF, it has been the mainstay of therapy since its introduction as a broad-spectrum anthelmintic in 1975 for the treatment of human-infecting trematodes and cestodes (Cioli & Pica-Mattoccia, 2003). The first report of PZQ resistance in *Schistosoma mansoni* infected mice was published by Fallon and Doenhoff (1994). PZQ resistance is a complex issue, and studies investigating potential drug resistance mechanisms in *S. mansoni* may involve drug efflux mechanisms. (Wang *et al*., 2012; Summers *et al*., 2022) Additionally, the emergence of side effects associated with PZQ treatment continues to be a concern (Erko *et al*., 2012). Currently, research on drug resistance in intestinal trematode parasites has not included studies on PZQ resistance. Furthermore, there are no anti-intestinal trematode drugs undergoing clinical trials in humans. Therefore, investigating effective herbal remedies against intestinal trematode parasites presents an alternative approach to reducing

reliance on chemical treatments, which may lead to resistance and adverse effects in the future. Previously, studies have found that the bioactive compounds, including tannins, flavonoids, and alkaloids, are renowned for their effectiveness against various parasites (Mushtaq *et al*., 2018). Thus, locally available herbal alternatives that are effective against gastrointestinal parasites may be a crucial option for reducing issues. Therefore, there is a necessity to explore herbal sources for alternative gastrointestinal parasites infection.

Promising avenues for further research include the exploration of *Allium sativum* (Garlic), a traditional treatment with a rich history of use against various illnesses. *A. sativum*'s primary bioactive components, such as diallyl disulfide, s-allyl cysteine, methyl cysteine, and allicin, exhibit potent antibacterial, anticancer, and antiparasitic effects. Notably, allicin, extensively studied, demonstrates therapeutic potential against cardiovascular diseases, provides antioxidant effects, and offers protection to the liver from toxins and alcohol (Rabinkov *et al*., 1998; Corzo-Martínez *et al*., 2007). The efficacy of *A. sativum* is extensively documented in both *in vivo* and *in vitro* studies, particularly highlighting its inhibitory effects on helminths. *A. sativum* has proven effective against *Haemonchus contortus* nematodes in ruminant animals (Palacios-Landín *et al*., 2015), *Anisakis* sp. in marine fish (Morsy *et al*., 2021), and *Gyrodactylus turnbulli*, a monogenean parasite (Schelkle *et al*., 2013). Previous studies indicated the influence of *A. sativum* on *Schistosoma mansoni*, including adult worms, cercaria, schistosomula, and miracidia stages (Mantawy *et al*., 2012; Schelkle *et al*., 2013; Aly *et al*., 2017; Cortˈs *et al*., 2017). *A. sativum* has been found to enhance the immune response in mice infected with *S. mansoni* (El Shenawy *et al*., 2008; Mantawy *et al*., 2011, 2012; Metwally *et al*., 2018). Furthermore, *A. sativum* extract has demonstrated an impact on the morphological damage of *Fasciola gigantica* (Singh *et al*., 2009).

On the other hand, *Thunbergia laurifolia* (Rang Chuet) is widely used for detoxification from lead poisoning or other toxins (Palipoch *et al*., 2011). The significant compounds such as rosmarinic acid (RA), apigenin, caffeic acid, allic acid, protocatechuic acid, and various vitamins have been identified. The several reports have been reported that apigenin bioactive compound in *T. laurifolia* has antioxidant and anti-cancer properties (Oonsivilai *et al*., 2007; Chan *et al*., 2010). The previous report showed that *T. laurifolia* fresh and dried *T. laurifolia* solutions that clearly reduced the inflammatory cells in hamster infection with *O. viverrini* Moreover, *T. laurifolia* extract combined with PZQ to reduced inflammatory cell aggregation and inhibiting cholangiocarcinoma (CCA) development, which were correlated to the serum Alanine transaminase (ALT) level of *O. viverrini* infection in hamster (Wonkchalee *et al*., 2012, 2013).

Moreover, *Eurycoma longifolia* (Pla Lai Puek) is the one of tropical herbal medicinal plants in Southeast Asian countries, has potential in aphrodisiac, anti-malarial, anti-cancer, and anti-microbial (Ur Rehman *et al*., 2016). The quassinoids, cathin-6-one alkaloids, squalene type triterpenes, tirucallane-type triterpenes, and biphenyl neolignans are several major bioactive compounds that were reported in *E. longifolia* (Bhat & Karim, 2010). Previous studies have documented the effects of *E. longifolia* extract on various parasites. Specifically, its impact on *Blastocystis* sp. (Girish *et al*., 2015), effects on alterations in the cell wall of *Toxoplasma gondii* (Kavitha *et al*., 2012a, 2012b). The extract has also shown anti-plasmodial activity against *Plasmodium falciparum*, both *in vitro* and *in vivo* (Jiwajinda *et al*., 2002; Chan *et al*., 2004; Kuo *et al*., 2004; Mohd Ridzuan *et al*., 2005; Hout *et al*., 2006; Sriwilaijaroen *et al*., 2010). Furthermore, it has been reported that *E. longifolia* extract reduces the movement and egg-laying of *Schistosoma japonicum* (Jiwajinda *et al*., 2002).

Therefore, the objective of this investigation is to evaluate the effectiveness of extracts obtained from *A. sativum*, *T. laurifolia*, and *E. longifolia* against newly excysted juvenile flukes (NEJ) of *H. taichui*. Evaluation criteria include relative motility (RM) and survival index (SI) at different time intervals, along with morphological surface changes assessed through scanning electron microscopy (SEM). Thus, the examination of crude extracts from *A. sativum*, *T. laurifolia*, and *E. longifolia* represents a crucial step in identifying promising herbal candidates for the potential development of alternative therapeutic agents for parasitic infections.

MATERIALS AND METHODS

Ethical approval

The present study obtained Bio Ethics clearance from the Ethics Committee of the Institute of Research and Development, Suranaree University of Technology (SUT), Thailand, with reference number, No. SUT-IBC-16/2021. The research was conducted between January 2022–January 2023.

Parasite preparation

H. taichui metacercariae were obtained from naturally infected cyprinid fish in an endemic area of Chaiyaphum Province, Northeastern Thailand. Fresh cyprinid fish were subjected to digestion in a 0.25% Pepsin-Hydrochloric acid solution, followed by incubation at 37°C for 1-2 hours. Subsequently, the resulting solution underwent filtration and centrifugation using a 0.85% normal saline solution (NSS) in a sedimentation jar. Identification of metacercariae was performed based on their morphological characteristics observed under a stereomicroscope. The *H. taichui* metacercariae were elliptical and had a baseball glove-shaped ventrogenital sac with rodlets and an O-shaped excretory bladder occupying large portion of posterior body (Phyo Myint *et al*., 2020). *H. taichui* metacercariae were excysted in 0.25% trypsin in 1x phosphate buffer saline (PBS) supplemented with 2×200 U/ml penicillin, 200 µg/ml streptomycin for 5-15 mins at 37°C atmosphere to obtained *H. taichui* NEJs for the experiment (Arunsan *et al*., 2019).

Crude extract preparation

The *A. sativum* was collected from a local market in Nakhon Ratchasima Province (Figure 1a). *T. laurifolia* leaves was harvested in the Nakhon Ratchasima Province (Figure 1b). The roots of *E. longifolia* crude was purchased from local herbal shop that adheres to standards (Figure 1c), with dried roots ground into powder. The aqueous solvent was used as the extraction solvent to minimize interference in this test. Pressing extraction of *A. sativum* effectively preserves allicin (Curtis *et al*., 2004; Bar *et al*., 2022), and dehydration during powdering does not destroy key compound of *A. sativum* (Khorshed & Obydul Hoq, 2016). The aqueous extract of *T. laurifolia* leaves yields key bioactive compounds such as apigenin, apigenin glucosides, and phenolic acids (Oonsivilai *et al*., 2007; Chan *et al*., 2011). Additionally, the aqueous extract from *E. longifolia* roots is a valuable source of phytochemicals, including eurycomanone, proteins, polysaccharides, and glycosaponins (Farag *et al*., 2022).

A. sativum cloves were meticulously peeled and washed with filtered water to remove any dust or particulate matter. One hundred fifty grams of cloves were ground, and *A. sativum* water was extracted by pressing it through three layers of gauze. *A. sativum* water underwent centrifugation at 5,000 RPM for 10 minutes to collect the supernatant, which was further filtered using Whatman filter No.1. The resulting liquid was then subjected to freeze-drying to yield a powdered extract (Labconco Free Zone Dry®, Kansas, USA) under conditions of $133x10^{-3}$ mBar for 48 hours. The powdered extract was stored at -20°C until used.

The fresh leaves of *T. laurifolia*, weighing 1 kg, were washed with distilled water, loosely labeled, and subsequently placed in a hot air oven at 60°C for approximately 24 hours. Subsequently, the material was finely ground into a powder using a grinder. The

Figure 1. (a) *A. sativum* cloves, (b) *T. laurifolia* leaves, (c) *E. longifolia* dried roots.

extraction was performed using the same method. The powders of *T. laurifolia* leaves and *E. longifolia* roots were each weighed at 10 g. and then dissolved in 40 ml of distilled water (DW) in separate 50 ml tubes. Each mixture was incubated in shaking water bath at 140 rpm and 25°C for 15 minutes (Memmert WTB50®, Schwabach, Germany). The resulting solution was then subjected to centrifugation at a speed of 3000 g for 3 minutes (Hermert Z446K®, Fujian, China). The supernatant was carefully collected, and an additional 40 ml of distilled water was introduced, repeating steps 3-5 twice. The combined supernatant was subsequently processed through filtration using Whatman filter paper No. 1. The resultant extract underwent evaporation to eliminate water content utilizing (Rotavapor R-300® Flawil, Switzerland) until a concentrated extract was achieved, followed by freeze-drying to produce a powdered extract (Labconco Free Zone Dry®, Kansas, USA) under conditions of 133×10^{-3} mBar for 48 hours. The resulting powdered extract was stored at -20°C until used. The percentage yield of each crude extract was determined using the formula below (Abbas *et al*., 2021).

$$\text{Yield (\%)} = \frac{\text{Weight of the extract (g) x100}}{\text{Dried extract weight}} \quad (1)$$

In preliminary tests, *A. sativum* crude extract was chosen at concentrations of 5-20 mg/ml, while *T. laurifolia* and *E. longifolia* extracts were selected at 100-400 mg/ml. *A. sativum* has been studied against various parasites, especially trematodes, with *in vitro* concentrations ranging from 0.1 to 100 mg/ml and *in vivo* from 5 mg/kg to 2 g/kg (El Shenawy *et al*., 2008; Singh *et al*., 2009; Mantawy *et al*., 2011, 2012; Jeyathilakan *et al*., 2012; Riad *et al*., 2013; Aly *et al*., 2017; Metwally *et al*., 2018). *T. laurifolia* reduced inflammation in hamsters infected with *O. viverrini* at 100 mg/kg/day for 30 days, though no *in vitro* studies exist (Wonkchalee *et al*., 2012, 2013). *E. longifolia* has been investigated for quassinoid compounds against *S. japonicum* at 2, 20, and 200 µM (Jiwajinda *et al*., 2002), but no *in vitro* antiparasitic studies on other trematodes have been reported.

Anthelmintic activity

H. taichui NEJs were allocated into five distinct groups of each herb (5 NEJs/group, duplicate in each group): Group 1 received RPMI-1640 culture medium, while group 2 was treated with the standard drug PZQ at a concentration of 2 mg/ml (a concentration approximately 80-100 from 25 mg/kg orally as a single dose standard treatment). Groups 3 to 5 received treatments with varying concentrations of *A. sativum* crude extract (5, 10, and 20 mg/ml) and *T. laurifolia* and *E. longifolia* crude extracts (100, 200, and 400 mg/ml). All treatments were diluted in RPMI culture medium and supplemented with 100 µg/ml of streptomycin antibiotic. All groups were exposed at different time intervals (0, 10, 20, 30 minutes, 1, 2, 3, 6, and 12, hours) under 37°C atmosphere. The motility was assessed by examining *H. taichui* NEJs under a stereomicroscope and scored based on the criteria established by Jiraungkoorskul *et al.* (2005) (3 = moving whole body, 2 = moving only parts of the body, 1 = immobile but alive, and 0 = died).

The RM value was computed based on the motility scores across all experimental groups. Notably, the negative control group, where all parasites were scored 3, demonstrated an RM value of 100. Accordingly, a diminished RM value observed in the all-treated group suggested a more robust inhibition of motility due to the *A. sativum*, *T. laurifolia*, and *E. longifolia* crude extract. The RM values were determined employing the formula detailed below (Kiuchi *et al.,* 1987; Lorsuwannarat *et al.,* 2013).

$$\text{Motility index (MI)} = \frac{\sum nN}{\text{N}} \quad (2)$$

$$\text{Relative motility (RM) value} = \frac{\text{MI test} \times 100}{\text{MI control}} \quad (3)$$

n = motility score,
N = number of worms with the score of "n"

The SI was calculated to determine the percentage of live worms at a specific time after incubation. Worms that exhibited a motility score of 0 were classified as died, whereas those with other scores (3, 2, and 1) were regarded as still alive. The SI was calculated using the formula provided below (Kiuchi *et al*., 1987; Lorsuwannarat *et al*., 2013).

$$\text{\% Survival index (SI)} = \frac{\text{Number of live worm (each group)}}{\text{Total worm (each group)}} \times 100 \quad (4)$$

Morphological study

The evaluation of morphological damage in *H. taichui* NEJs, following 12 hours of incubation, was performed using SEM. Subsequently, the *H. taichui* NEJs underwent multiple washes with distilled water (DW). The specimens were fixed overnight in a glutaraldehyde fixative solution at 4°C. Following fixation, the worms underwent three sequential 10-minute washes with DW. Post-fixation involved immersion in a 1% osmium tetroxide fixative solution in 0.1 M PBS with a pH of 7.2 for 1 hour, followed by three 10-minute DW washes. Subsequently, the samples were dehydrated through a series of graded acetone solutions (30%, 50%, 70%, 90%, 95%, and 100% alcohol) in two cycles. They were then desiccated using a

critical point dryer (Leica CPD 300® Vienna, Austria), coated with a layer of gold using an Au ion sputtering device on conductive tape, and examined under scanning electron microscopy (FESEM/Carl Zeiss Auriga® Dresden, Germany) at an electric high tension 3.00 Kilovolt (kV).

Data analysis

The RM values and SI were analyzed through the respective formulas, the RM values indicated stronger extract activity, while the survival indices reflected the percentages of live flukes at a given time post-treatment (Lorsuwannarat *et al*., 2013). Mean scores and standard deviations of motility were calculated for each group. Data analysis was performed using IBM SPSS Statistics 26 (SPSS Inc., Chicago, USA) with a One-Way ANOVA conducted among groups to compare the mean motility scores. Statistical significance was determined by a *P*-value < 0.05.

RESULTS

Motility test and viability assay

The specified time interval was reached, parasites were observed for motility scoring and calculated RM and SI value (Figure 2a and Figure 2b). This study will highlight the differences in the extracts of *A. sativum*, *T. laurifolia*, and *E. longifolia* at their maximum concentrations (20, 400, and 400 mg/ml, respectively). The results at other concentrations are presented in Table 1. The negative control group exhibited normal motility at 0 minutes and initiated slight movement at 10 minutes, continuing until the conclusion of the experiment at 12 hours (RM = 100 and SI = 100). The positive control group was observed that the *H. taichui* NEJs exhibited normal motility at 0 minutes (RM = 100 and SI = 100). However, at 10 minutes, the parasites began to rapidly decrease their movement (RM = 100 and SI = 66.67). There was a slight increase in the rate at 20 minutes, followed by a continuous decline at 30 minutes, 1 hour, 2 hours, 3 hours, and 6 hours (RM = 70.00, 62.50, 62.50, 50.00, 50.00, and 50.00, with SI = 100 at all concentrations, respectively). The movement was ceased entirely at the 12-hour mark (RM = 0 and SI = 0). *A. sativum* treated group at a concentration of 20 mg/ml, it was observed that when the parasites encountered the herbal extract, they began to exhibit a reduction in movement (RM = 66.67 and SI = 100) at 10 minutes. Subsequently, there was a slight increase in the rate of movement (RM = 90.00 and SI = 100) at 20 minutes. However, the parasites showed a rapid decrease in motility (RM = 62.50, 50.00, and 25.00, with SI = 100) at 30 minutes, 1 hour, and 2 hours. The movement was ceased entirely at 3 to 12 hours (RM = 0 and SI = 0). *T. laurifolia* treated group at a concentration of 400 mg/ml, it was observed that when the parasites were exposed to the extract at 0, 10, 20, and 30 minutes, the parasites initially exhibited normal motility, followed by a slight decrease (RM = 100 and SI = 100). However, the parasites consistently showed a steady decline in motility (RM = 50.00, 50.00, 50.00, and 25.00, with SI = 100) at 1 to 3 hours. There was another reduction in movement (RM = 25.00 and SI = 50.00) at 6 hours, leading to complete cessation of motility at 12 hours (RM = 0 and SI = 0). *E. longifolia* treated group at a concentration of 400 mg/ml, it was observed that when the parasites were exposed to the extract at 0 minutes, they exhibited normal motility (RM = 100 and SI = 100). However, at 10 and 20 minutes, the parasites displayed initially normal movement, followed by a rapid reduction (RM = 66.67 and SI = 100). Between 20 and 30 minutes, there was a slight resurgence in parasite activity (RM = 80.00 and 100.00, with SI = 100). Subsequently, at 1, 2, and 3 hours, the parasites exhibited a rapid decline in motility (RM = 50.00, 50.00, and 50.00, with SI = 100). At 6 hours, there was another decrease in movement (RM = 25.00 and SI = 50.00), leading to complete cessation of motility at 12 hours (RM = 0 and SI = 0).

A comparative analysis indicated significant differences between the average motility scores of the negative control group and those of both the *A. sativum* and *E. longifolia* treated groups. Conversely, no significant differences were observed between the negative control group and the *T. laurifolia* treated group. Furthermore, no significant differences were noted among the positive control group and the *A. sativum*, *E. longifolia*, and *T. laurifolia* treated groups ($p < 0.05$) (Figure 3). Additionally, no significant differences were observed among the average motility scores across the crude extracts of *A. sativum*, *E. longifolia*, and *T. laurifolia*; there were also no significant differences within the same crude extract ($p < 0.05$).

Surface morphological changes

The surface morphological changes of *H. taichui* NEJs were assessed using SEM post of treatment for 12-hour. The tegumental surface of *H. taichui* NEJs in the negative control group were showed normal of tegumental sureface, illustrates the surface around the oral sucker (Os) presence the normal of sensory papillae (Pa). The anterior region showcasing prominent large spines and posterior (Po) region displays the smaller size of the spines (Ss) (Figure 4a-d). The positive control group treated with a 2 mg/ml PZQ solution, the tegumental surface of *H. taichui* NEJs (Os). Furthermore, the overall tegument of the body showed mild swelling, particularly along the edges of both large spines (Ls) and small spines (Ss) (Figure 4e-h). The *A. sativum* treated group (20 mg/ml) was presented the tegumental surface of *H. taichui* NEJs displaying morphological abnormalities to show pronounced swelling. The observation extended to the

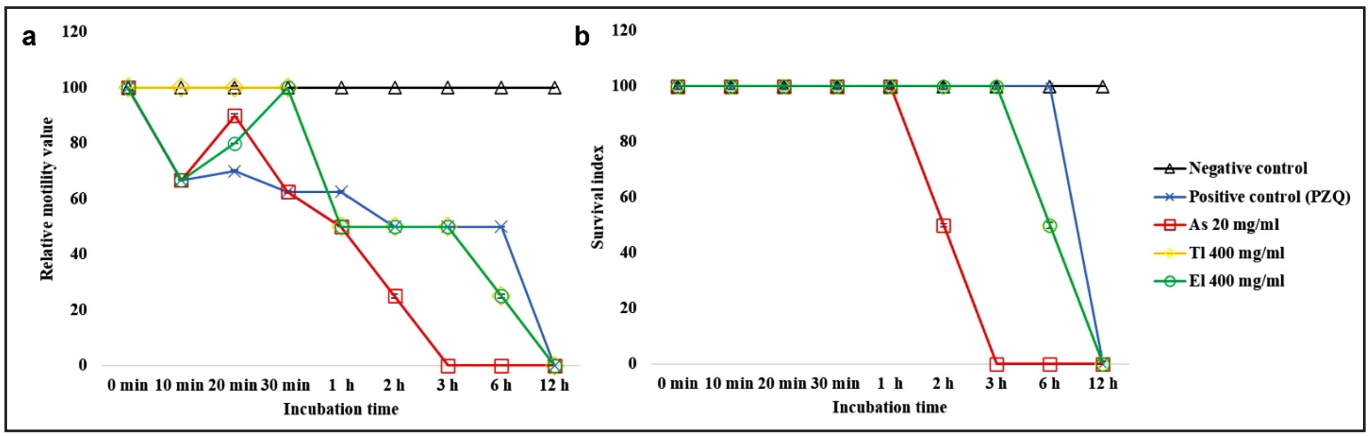

Figure 2. (a) The RM values and (b) SI of *H. taichui* NEJs were evaluated following treatment with 20 mg/ml *A. sativum* crude extract, 400 mg/ml *T. laurifolia* crude extract, and 400 mg/ml *E. longifolia* crude extract. The results demonstrated a decrease in both RM values and SI over time compared to both negative and positive controls, across various time intervals.

Table 1. The RM values and SI of *H. taichui* NEJs were evaluated following the treatment of all groups

Time	Negative control		Positive control (PZQ)		As 5 mg/ml		As 10 mg/ml		As 20 mg/ml		Tl 100 mg/ml		Tl 200 mg/ml		Tl 400 mg/ml		EL 100 mg/ml		EL 200 mg/ml		EL 400 mg/ml	
	RM	SI	RM	SI	RM	SI	RM	SI	RM	SI	RM	SI	RM	SI	RM	SI	RM	SI	RM	SI	RM	SI
0 min	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
10 min	100.00	100.00	66.67	100.00	66.67	100.00	66.67	100.00	66.67	100.00	100.00	100.00	100.00	100.00	100.00	100.00	66.67	100.00	66.67	100.00	66.67	100.00
20 min	100.00	100.00	70.00	100.00	80.00	100.00	80.00	100.00	90.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	80.00	100.00	80.00	100.00	80.00	100.00
30 min	100.00	100.00	62.50	100.00	75.00	100.00	87.50	100.00	62.50	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
1 h	100.00	100.00	62.50	100.00	50.00	100.00	50.00	100.00	50.00	100.00	50.00	100.00	50.00	100.00	50.00	100.00	50.00	100.00	50.00	100.00	50.00	100.00
2 h	100.00	100.00	50.00	100.00	25.00	50.00	25.00	50.00	25.00	50.00	75.00	100.00	62.50	100.00	50.00	100.00	50.00	100.00	50.00	100.00	50.00	100.00
3 h	100.00	100.00	50.00	100.00	25.00	50.00	25.00	50.00	0.00	0.00	50.00	100.00	50.00	100.00	50.00	100.00	50.00	100.00	50.00	100.00	50.00	100.00
6 h	100.00	100.00	50.00	100.00	25.00	50.00	25.00	50.00	0.00	0.00	50.00	100.00	50.00	100.00	25.00	50.00	50.00	100.00	50.00	100.00	25.00	50.00
12 h	100.00	100.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100.00	0.00	100.00	0.00	0.00	0.00	100.00	0.00	100.00	0.00	0.00

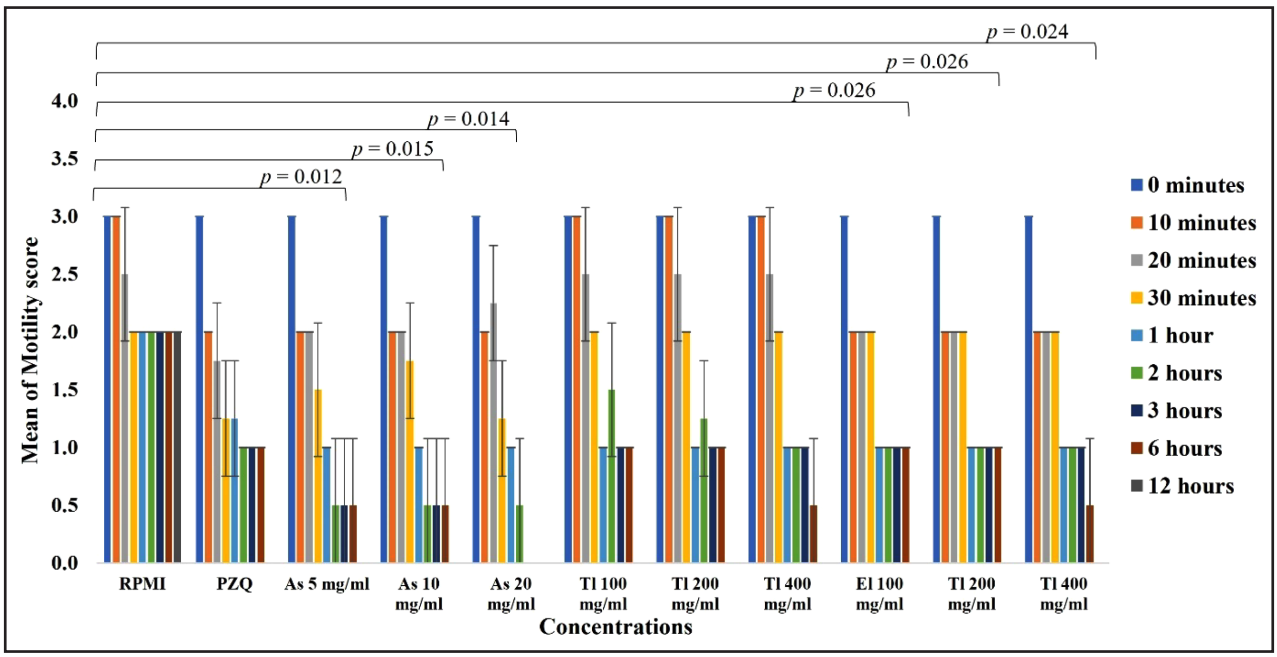

Figure 3. Motility scores exhibited statistically significant differences between the negative control group, treated with *A. sativum*, and crude *E. longifolia* extract, while no significant differences were observed between the negative control group and those treated with *T. laurifolia* extract. Conversely, no statistically significant differences in mean motility scores were observed among the positive control group and all treatment groups ($P < 0.05$).

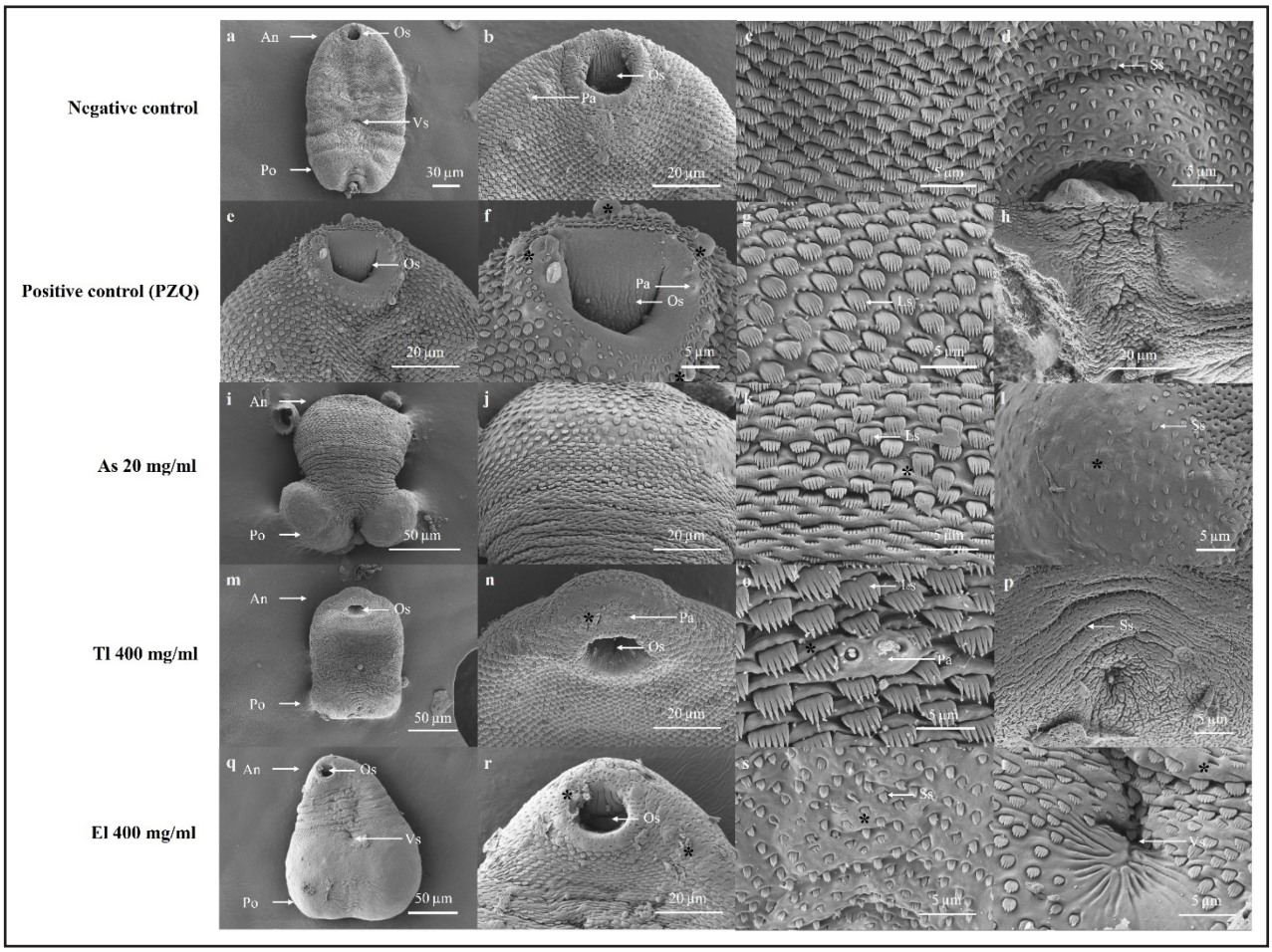

Figure 4. Tegumental surface of *H. taichui* NEJs was examined 12 hours post-incubation across all experimental groups. (a), (b), (c), and (d) Negative control group, scale bar= 30, 20, 5 and 5 µm, respectively. (e), (f), (g), and (h) Positive control group, scale bar=20, 5, 5, 20 µm, respectively. (i), (j), (k), and (l) *A. sativum* treated group at a concentration of 20 mg/ml, scale bar=50, 20, 5, and 5 µm, respectively. (m), (n), (o), and (p) *T. laurifolia* treated group at a concentration of 400 mg/ml, scale bar=50, 20, 5, and 5 µm, respectively. (q), (r), (s), and (t) *E. longifolia* treated group at a concentration of 400 mg/ml, scale bar=50, 20, 5, and 5 µm, respectively. Abbreviations; * = Swelling area, Os = Oral sucker, Pa = Papillae, Po = Posterior region, An = Anterior region, Ls = Large spines, and Ss = Small spines.

entire tegument of the body, where noticeable swelling was evident, particularly along the edges of both large spines (Ls) and small spines (Ss) (Figure 4i-l). In the *T. laurifolia* treated group (400 mg/ml), the tegumental surface of *H. taichui* NEJs displaying morphological abnormalities exhibited a swollen appearance covered with blebs around the oral sucker exhibited a pervasive swelling of the body tegument. The swelling was evident across the entire tegument of the body, with notable prominence along the edges of both large spines (Ls) and small spines (Ss), as well as around the oral sucker (Os) (Figure 4m-p). The *E. longifolia* treated group (400 mg/ml), the tegumental surface of *H. taichui* NEJs displaying morphological aberrations showed a comprehensive swelling and damage of the body tegument. The overall tegument of the body exhibited swelling along the edges of large spines (Ls) in the posterior region and small spines (Ss) in the ventral sucker (Vs) region (Figure 4q-t).

DISCUSSION

This study represents the effect of *A. sativum*, *T. laurifolia*, and *E. longifolia* crude extract on a gastrointestinal fluke, *H. taichui* NEJs. Exposure to *A. sativum*, *T. laurifolia*, and *E. longifolia* resulted in a substantial reduction in motility among *H. taichui* NEJs, leading to notable decreases in both relative RM values and SI. Noteworthy distinctions were observed between the *A. sativum* and *E. longifolia* treatment groups, where in *T. laurifolia* demonstrated no significant variations compared to the negative control. When compared to the positive control group, it was found that the groups treated with extracts of *A. sativum*, *T. laurifolia*, and *E. longifolia* did not exhibit statistically significant differences, indicating comparable efficacy to the PZQ standard drug. Additionally, the current findings align with the research conducted by Lorsuwannarat *et al*. (2013), which examined the *in vitro* anthelmintic effects of plumbagin on *S. mansoni*. In their study, the group treated with plumbagin demonstrated a more rapid reduction in RM values compared to the group treated with PZQ. Furthermore, they are consistent with the investigation by Songsri *et al.* (2019) into the impact of Chia oil (*Salvia hispanica* L.) on adult worms of *H. taichui*. In their research, adult worms were subjected to incubation with either PZQ (10 µg/ml) or Chia oil (1 or 10 mg/ml) for durations of 1, 6, 12, and 24 hours. The results indicated that all worms exhibited complete inactivity and contraction after just 1 hour of exposure to 10 mg/ml Chia oil, in contrast to 10 µg/ml PZQ. This observation underscores the efficacy of Chia oil in affecting the motility of *H. taichui* adult worms.

The SEM analysis, representative samples from all concentrations of the crude extracts of *A. sativum*, *T. laurifolia*, and *E. longifolia* to assess their effects on morphological damage, revealed morphological damage characterized by tegumental swelling evident across all treatment crude extract in this study, notably severe in the *A. sativum* treated group. Conversely, the negative control group exhibited a normal tegumental surface, while the positive control group showed swelling and blebbing around the oral sucker. The study concerning the examination of alternative extracts on the *H. taichui* in previous research. Previous study investigated the effects of aqueous extract from *Artocarpus takoocha* Roxb on the tegumental surface of *H. taichui*. They found that the aqueous extracts of *A. takoocha* exhibited anti-helminthic activity against *H. taichui*, inducing numerous small blebs and disruption of the tegument surface (Wongsawad *et al*., 2005). Previous investigation into the tegument of the parasite *H. taichui* was documented by Kumchoo *et al.* (2007), their study specifically examined the effects of niclosamide on the tegumental of adult *H. taichui* using SEM. Extended incubation with the drug led to notable alterations in the tegument, marked by substantial swelling and blebbing.

The analysis of morphological changes in the surface of *H. taichui* NEJs through SEM highlighted significant alterations upon exposure to crude extracts of *A. sativum*, *T. laurifolia*, and *E. longifolia*. Particularly, the group exposed to *A. sativum* exhibited distinct changes consistent with those reported (Riad *et al*., 2013; Aly *et al*., 2017). Their investigations suggested that the administration of *A. sativum* extracts to mice infected with the *S. mansoni* blood fluke had discernible effects on the parasite's integument. SEM analysis of the adult stage of the parasite revealed noteworthy alterations, including swelling, and blistering around the body, compared to the control group. Furthermore, *A. sativum* extract has been shown to have an impact on the morphological damage of *F. gigantica* (Singh *et al*., 2009). To date, there has been no direct investigation into the effects of *T. laurifolia* and *E. longifolia* extracts on the *H. taichui* gastrointestinal fluke. However, a previous study conducted in hamsters infected with *O. viverrini* effectively mitigated inflammation and hindered the aggregation of cells that could potentially evolve into cholangiocarcinoma cells (Wonkchalee *et al*., 2012, 2013). The reports on the efficacy of *E. longifolia* against parasites have been documented. It has been reported to influence *T. gondii*, as evidenced by SEM examination revealing cell wall alterations with the formation of invaginations followed by completely collapsed cells compared to normal *T. gondii* cells in response to the fractions (Kavitha *et al*., 2012a). *E. longifolia* significant inhibitory effect on adult schistosome movement (IM) and egg-laying of *S. japonicum* (Jiwajinda *et al*., 2002). Additionally, *E. longifolia* has demonstrated anti-plasmodial activity against *P. falciparum* both *in vitro* and *in vivo* (Mohd Ridzuan *et al*., 2005; Hout *et al*., 2006; Sriwilaijaroen *et al*., 2010). The morphological changes observed in the *H. taichui* NEJs can be explained by the following process: Cellular death resulting from disrupted respiration leads to hypoxia and metabolic alterations. Reduced ATP production causes a shift in metabolism to anaerobic pathways, which results in lactate accumulation and a decrease in pH. ATP depletion leads to lysosome swelling and enzyme release. The increased calcium influx due to tissue hypoxia damages cellular membranes and organelles, ultimately causing cellular self-digestion (autolysis) and tissue degradation (Cobb *et al*., 1996; Madea *et al*., 2014).

Currently, the drug used to eradicate intestinal parasite is PZQ. Its mechanism of action involves increasing calcium influx, which causes the muscles of parasites to contract thereby affecting their movement and causing paralysis (Martin *et al*., 1997). Allicin is the major bioactive compound of *A. sativum,* in a study investigating the mechanism of allicin, a bioactive compound of *A. sativum*, on parasites, research has been conducted on protozoa *Leishmania* sp. by Corral *et al*. (2016). Their findings illustrated that allicin induces dysregulation of calcium homeostasis and oxidative stress, which cannot be controlled by the antioxidant defense of the cell. This leads to mitochondrial dysfunction and a bioenergetic catastrophe resulting in cell necrosis and cell cycle arrest in the premitotic phase. Rosmarinic acid is a major compound in *T. laurifolia* extract and has garnered significant interest due to its potential broad pharmacological effects (Woottisin *et al*., 2022). The mechanism by which *T. laurifolia* affects parasites is not well understood. However, insights can be drawn from the known effects of phenolic compounds on bacterial cells describe how these compounds disrupt gram-positive bacterial membranes at the interface, compromising membrane plasticity and integrity, which destabilizes the cell membrane and transport systems (Miceli *et al*., 2011; Resende *et al*., 2015). Quassinoids are one of the major bioactive groups in *E. longifolia* (Bhat & Karim, 2010). The mechanism of action of *E. longifolia* was investigated, revealing that extract fractions target *T. gondii* within its cytoplasmic region. It was suggested that

these fractions may induce intracellular oxidative stress through an indirect pathway involving mitochondria. This process could lead to uncontrolled superoxide bursts within mitochondria, thereby increasing oxidative stress, which plays a critical role in hypoxia/reoxygenation injury in parasite cells (Kavitha *et al*., 2012a).

CONCLUSION

The knowledge acquired from herbal crude extracts, specifically those derived from *A. sativum*, *T. laurifolia*, and *E. longifolia*, which demonstrate anti-parasitic properties against the *H. taichui* gastrointestinal fluke, holds considerable potential for the development of herbal extracts designed to control and treat infections caused by gastrointestinal flukes. This avenue offers a viable alternative to existing pharmaceutical interventions. To further advance our understanding and application of these herbal extracts, forthcoming research endeavors should encompass comprehensive investigations involving both *in vivo* and *in vitro* studies. These studies are crucial for elucidating the underlying mechanisms and assessing the overall impacts of *A. sativum*, *T. laurifolia*, and *E. longifolia* crude extracts on *H. taichui* NEJs. Despite the promising effects of these herbal extracts in combating *H. taichui* gastrointestinal fluke, it is essential to emphasize the need for future investigations into their specific mechanisms and the identification of key bioactive compounds. Additionally, assessing cytotoxicity against human cells is crucial. Comprehensive in vivo studies and clinical trials are necessary to develop effective antiparasitic agents, which may offer promising alternatives for the treatment of MIF infection. This crucial research step is essential for the development of effective anti-parasitic agents, positioning herbal extracts as viable alternatives for the treatment of gastrointestinal fluke infections. As we move forward, a more comprehensive understanding of the underlying biological processes and active components will pave the way for the formulation of targeted and efficacious treatments in the ongoing pursuit of addressing parasitic infections.

Conflict of interest

The authors declare that there are no conflicts of interest.

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REFERENCES

Abbas, A., Naqvi, S.A.R., Rasool, M.H., Noureen, A., Mubarik, M.S. & Tareen, R.B. (2021). Phytochemical analysis, antioxidant and antimicrobial acreening of *Seriphidium oliverianum* plant extracts. *Dose Response* **19**: 15593258211004739. https://doi.org/10.1177/15593258211004739

Aly, I., Taher, E.E., Hoda, E.S., Mohammed, F.A., ELnain, G., Hamad, R.S. & Bayoumy, E.M. (2017). Efficacy of soluble glycoprotein fraction from *Allium sativum* purified by size exclusion chromatography on murine *Schistosomiasis mansoni*. *Microbial Pathogenesis* **107**: 243-248. https://doi.org/10.1016/j.micpath.2017.03.039

Arunsan, P., Ittiprasert, W., Smout, M.J., Cochran, C.J., Mann, V.H., Chaiyadet, S., Karinshak, S.E., Sripa, B., Young, N.D. & Sotillo, J. (2019). Programmed knockout mutation of liver fluke granulin attenuates virulence of infection-induced hepatobiliary morbidity. *Elife* **8**: e41463. https://doi.org/10.7554/eLife.41463

Bar, M., Binduga, U.E. & Szychowski, K.A. (2022). Methods of isolation of active substances from garlic (*Allium sativum* L.) and its impact on the composition and biological properties of garlic extracts. Antioxidants **11**: 1345. https://doi.org/10.3390/antiox11071345

Bhat, R. & Karim, A. (2010). Tongkat Ali (*Eurycoma longifolia* Jack): a review on its ethnobotany and pharmacological importance. *Fitoterapia* **81**: 669-679. https://doi.org/10.1016/j.fitote.2010.04.006

Chai, J.Y. & Jung, B.K. (2022). General overview of the current status of human foodborne trematodiasis. *Parasitology* **149**: 1262-1285. https://doi.org/10.1017/S0031182022000725

Chan, E.W., Eng, S.Y., Tan, Y.P. & Wong, Z.C. (2011). Phytochemistry and pharmacological properties of *Thunbergia laurifolia*: a review. *Pharmacognosy Journal* **3**: 1-6. https://doi.org/10.5530/pj.2011.24.1

Chan, E.W.C., Lim, Y.Y., Chong, K.L., Tan, J.B.L. & Wong, S.K. (2010). Antioxidant properties of tropical and temperate herbal teas. *Journal of Food Composition and Analysis* **23**: 185-189. https://doi.org/10.1016/j.jfca.2009.10.002

Chan, K.L., Choo, C.Y., Abdullah, N.R. & Ismail, Z. (2004). Antiplasmodial studies of *Eurycoma longifolia* Jack using the lactate dehydrogenase assay of *Plasmodium falciparum*. *Journal of Ethnopharmacology* **92**: 223-227. https://doi.org/10.1016/j.jep.2004.02.025

Cioli, D. & Pica-Mattoccia, L. (2003). Praziquantel. *Parasitology research* **90**: S3-S9. https://doi.org/10.1007/s00436-002-0751-z

Cobb, J., Hotchkiss, R., Karl, I. & Buchman, T. (1996). Mechanisms of cell injury and death. *British Journal of Anaesthesia* **77**: 3-10. https://doi.org/10.1093/bja/77.1.3

Corral, M.J., Benito-Peña, E., Jiménez-Antón, M.D., Cuevas, L., Moreno-Bondi, M.C. & Alunda, J.M. (2016). Allicin induces calcium and mitochondrial dysregulation causing necrotic death in *Leishmania*. *PLOS Neglected Tropical Diseases* **10**: e0004525. https://doi.org/10.1371/journal.pntd.0004525

Cortés, A., García-Ferrús, M., Sotillo, J., Guillermo Esteban, J., Toledo, R. & Muñoz-Antolí, C. (2017). Effects of dietary intake of garlic on intestinal trematodes. *Parasitology Research* **116**: 2119-2129. https://doi.org/10.1007/s00436-017-5511-1

Corzo-Martínez, M., Corzo, N. & Villamiel, M. (2007). Biological properties of onions and garlic. *Trends in Food Science and Technology* **18**: 609-625. https://doi.org/10.1016/j.tifs.2007.07.011

Curtis, H., Noll, U., Störmann, J. & Slusarenko, A.J. (2004). Broad-spectrum activity of the volatile phytoanticipin allicin in extracts of garlic (*Allium sativum* L.) against plant pathogenic bacteria, fungi and Oomycetes. *Physiological and molecular plant pathology* **65**: 79-89. https://doi.org/10.1016/j.pmpp.2004.11.006

El Shenawy, N.S., Soliman, M.F. & Reyad, S.I. (2008). The effect of antioxidant properties of aqueous garlic extract and *Nigella sativa* as anti-schistosomiasis agents in mice. *Revista do Instituto de Medicina Tropical de São Paulo* **50**: 29-36. https://doi.org/10.1590/s0036-46652008000100007

Erko, B., Degarege, A., Tadesse, K., Mathiwos, A. & Legesse, M. (2012). Efficacy and side effects of praziquantel in the treatment of *Schistosomiasis mansoni* in schoolchildren in Shesha Kekele Elementary School, Wondo Genet, Southern Ethiopia. *Asian Pacific Journal of Tropical Biomedicine* **2**: 235-239. https://doi.org/10.1016%2FS2221-1691(12)60049-5

Fallon, P.G. & Doenhoff, M.J. (1994). Drug-resistant schistosomiasis: resistance to praziquantel and oxamniquine induced in *Schistosoma mansoni* in mice is drug specific. *The American Journal of Tropical Medicine and Hygiene* **51**: 83-88. https://doi.org/10.4269/ajtmh.1994.51.83

Farag, M.A., Ajayi, A.O., Taleb, M., Wang, K. & Ayoub, I.M. (2022). A Multifaceted review of *Eurycoma longifolia* nutraceutical bioactives: Production, extraction, and analysis in drugs and biofluids. *ACS omega* **8**: 1838-1850. https://doi.org/10.1021/acsomega.2c06340

Fürst, T., Yongvanit, P., Khuntikeo, N., Lun, Z.R., Haagsma, J.A., Torgerson, P.R., Odermatt, P., Bürli, C., Chitnis, N. & Sithithaworn, P. (2019). Food-borne trematodiases in East Asia: epidemiology and burden. In: Neglected Tropical Diseases-East Asia. Dordrecht: Springer, pp. 13-38.

Girish, S., Kumar, S. & Aminudin, N. (2015). Tongkat Ali (*Eurycoma longifolia*): a possible therapeutic candidate against *Blastocystis* sp. *Parasites and Vectors* **8**: 1-7. https://doi.org/10.1186/s13071-015-0942-y

Hout, S., Chea, A., Bun, S.S., Elias, R., Gasquet, M., Timon-David, P., Balansard, G. & Azas, N. (2006). Screening of selected indigenous plants of Cambodia for antiplasmodial activity. *Journal of Ethnopharmacology* **107**: 12-18. https://doi.org/10.1016/j.jep.2006.01.028

Jeyathilakan, N., Murali, K., Anandaraj, A. & Abdul Basith, S. (2012). *In vitro* evaluation of anthelmintic property of ethno-veterinary plant extracts against the liver fluke *Fasciola gigantica*. *Journal of Parasitic Diseases* **36**: 26-30. https://doi.org/10.1007/s12639-011-0064-1

Jiraungkoorskul, W., Sahaphong, S., Tansatit, T., Kangwanrangsan, N. & Pipatshukiat, S. (2005). *Eurytrema pancreaticum*: the *in vitro* effect of praziquantel and triclabendazole on the adult fluke. *Experimental Parasitology* **111**: 172-177. https://doi.org/10.1016/j.exppara.2005.07.004

Jiwajinda, S., Santisopasri, V., Murakami, A., Kawanaka, M., Kawanaka, H., Gasquet, M., Eilas, R., Balansard, G. & Ohigashi, H. (2002). *In vitro* anti-tumor promoting and anti-parasitic activities of the quassinoids from *Eurycoma longifolia*, a medicinal plant in Southeast Asia. *Journal of Ethnopharmacology* **82**: 55-58. https://doi.org/10.1016/s0378-8741(02)00160-5

Kavitha, N., Noordin, R., Chan, K.L. & Sasidharan, S. (2012b). *In vitro* anti-*Toxoplasma gondii* activity of root extract/fractions of *Eurycoma longifolia* Jack. BMC Complement. *Alternative Medicine Studies* **12**: 1-8. https://doi.org/10.1186/1472-6882-12-91

Kavitha, N., Noordin, R., Kit-Lam, C. & Sasidharan, S. (2012a). Real time anti-*Toxoplasma gondii* activity of an active fraction of *Eurycoma longifolia* root studied by *in situ* scanning and transmission electron microscopy. *Molecules* **17**: 9207-9219. https://doi.org/10.3390/molecules17089207

Khorshed, A. & Obydul Hoq, M. (2016). Medicinal plant Allium sativum= A Review. *Journal of Medicinal Plants Studies* **72**: 72-79.

Kiuchi, F., Miyashita, N., Tsuda, Y., Kondo, K. & Yoshimura, H. (1987). Studies on crude drugs effective on visceral larva migrans. I. Identification of larvicidal principles in betel nuts. *Chemical and Pharmaceutical Bulletin* **35**: 2880-2886. https://doi.org/10.1248/cpb.35.2880

Kumchoo, K., Wongsawad, C., Vanittanakom, P., Chai, J. & Rojanapaibul, A. (2007). Effect of niclosamide on the tegumental surface of *Haplorchis taichui* using scanning electron microscopy. *Journal of Helminthology* **81**: 329-337. https://doi.org/10.1017/s0022149x07381108

Kuo, P.C., Damu, A.G., Lee, K.H. & Wu, T.S. (2004). Cytotoxic and antimalarial constituents from the roots of *Eurycoma longifolia*. *Bioorganic and Medicinal Chemistry* **12**: 537-544. https://doi.org/10.1016/j.bmc.2003.11.017

Lorsuwannarat, N., Saowakon, N., Ramasoota, P., Wanichanon, C. & Sobhon, P. (2013). The anthelmintic effect of plumbagin on *Schistosoma mansoni*. *Experimental Parasitology* **133**: 18-27. https://doi.org/10.1016/j.exppara.2012.10.003

Madea, B., Henssge, C., Reibe, S., Tsokos, M. & Kernbach Wighton, G. (2014) Postmortem changes and time since death. In: Handbook of forensic medicine. 2nd edition. New York: Wiley, pp 75-133. https://doi.org/10.1002/9781119648628.ch7

Mantawy, M.M., Ali, H.F. & Rizk, M.Z. (2011). Therapeutic effects of *Allium sativum* and *Allium cepa* in *Schistosoma mansoni* experimental infection. *Revista do Instituto de Medicina Tropical de São Paulo* **53**: 155-163. https://doi.org/10.1590/s0036-46652011000300007

Mantawy, M.M., Aly, H.F., Zayed, N. & Fahmy, Z.H. (2012). Antioxidant and schistosomicidal effect of *Allium sativum* and *Allium cepa* against *Schistosoma mansoni* different stages. *European Review for Medical and Pharmacological Sciences* **16**: 69-80.

Martin, R., Robertson, A. & Bjorn, H. (1997). Target sites of anthelmintics. *Parasitology* **114**: 111-124. https://doi.org/10.1017/S0031182097001029

Metwally, D.M., Al-Olayan, E.M., Alanazi, M., Alzahrany, S.B. & Semlali, A. (2018). Antischistosomal and anti-inflammatory activity of garlic and allicin compared with that of praziquantel *in vivo*. *BMC Complement. Alternative Medicine Studies* **18**: 1-11. https://doi.org/10.1186/s12906-018-2191-z

Miceli, N., Trovato, A., Marino, A., Bellinghieri, V., Melchini, A., Dugo, P., Cacciola, F., Donato, P., Mondello, L. & Güvenç, A. (2011). Phenolic composition and biological activities of *Juniperus drupacea* Labill. berries from Turkey. *Food and Chemical Toxicology* **49**: 2600-2608. https://doi.org/10.1016/j.fct.2011.07.004

Mohd Ridzuan, M.A.R., Noor Rain, A., Zhari, I. & Zakiah, I. (2005). Effect of *Eurycoma longifolia* extract on the Glutathione level in *Plasmodium falciparum* infected erythrocytes *in vitro*. *Tropical Biomedicine* **22**: 155-163.

Morsy, K., Ghamdi, A.A., Dajem, S.B., Bin-Meferij, M., Alshehri, A., El-Kott, A., Ibrahim, E., Ali, A., Hamdi, H. & Al-Doaiss, A. (2021). The oil of garlic, *Allium sativum* L. (Amaryllidaceae), as a potential protectant against *Anisakis* spp. Type II (L3) (Nematoda) infection in Wistar rats. *Revista Brasileira de Parasitologia Veterinária* **30**: e015920. https://doi.org/10.1590/s1984-296120201086

Mushtaq, S., Abbasi, B.H., Uzair, B. & Abbasi, R. (2018). Natural products as reservoirs of novel therapeutic agents. *EXCLI Journal* **17**: 420-451. https://doi.org/10.17179%2Fexcli2018-1174

Myint, E.P., Sereemaspun, A., Sjödin, H., Rocklöv, J., Lai, Y., Ribas, A., Pakdeenarong, N. & Nithikathkul, C. (2022). Surveillance model of parasitic zoonosis in cyprinoid fishes in northern zone and northeastern zone of Thailand and Myanmar using GIS. *International Journal of Geoinformatics* **18**: 129-139. https://doi.org/10.52939/ijg.v18i2.2165

Oonsivilai, R., Cheng, C., Bomser, J., Ferruzzi, M.G. & Ningsanond, S. (2007). Phytochemical profiling and phase II enzyme-inducing properties of *Thunbergia laurifolia* Lindl. (RC) extracts. *Journal of Ethnopharmacology* **114**: 300-306. https://doi.org/10.1016/j.jep.2007.08.015

Palacios-Landín, J., Mendoza-de Gives, P., Salinas-Sánchez, D.O., López-Arellano, M.E., Liébano-Hernández, E., Hernández-Velázquez, V.M. & Valladares-Cisneros, M.G. (2015). *In vitro* and *in vivo* nematocidal activity of *Allium sativum* and *Tagetes erecta* extracts against *Haemonchus contortus*. *Turkiye Parazitoloji Dernegi* **39**: 260-264. https://doi.org/10.5152/tpd.2015.4523

Palipoch, S., Jiraungkoorskul, W., Jaikua, W., Kosai, P., Tansatit, T. & Preyavichyapugdee, N. (2011). Protective efficiency of *Thunbergia laurifolia* leaf extract against lead (II) nitrate-induced toxicity in *Oreochromis niloticus*. *Journal of Medicinal Plant Research* **5**: 719-728.

Phyo Myint, E.E., Sereemaspun, A., Rocklöv, J. & Nithikathkul, C. (2020). Discovery of carcinogenic liver fluke metacercariae in second intermediate hosts and surveillance on fish-borne trematode metacercariae infections in Mekong region of Myanmar. *International Journal of Environmental Research and Public Health* **17**: 4108. https://doi.org/10.3390/ijerph17114108

Rabinkov, A., Miron, T., Konstantinovski, L., Wilchek, M., Mirelman, D. & Weiner, L. (1998). The mode of action of allicin: trapping of radicals and interaction with thiol containing proteins. *Biochimica et Biophysica Acta* **1379**: 233-244. https://doi.org/10.1016/s0304-4165(97)00104-9

Resende, F.A., Nogueira, L.G., Bauab, T.M., Vilegas, W. & Varanda, E.A. (2015). Antibacterial potential of flavonoids with different hydroxylation patterns. *Eclética Química* **40**: 173-179. https://doi.org/10.26850/1678-4618EQJ.V40.1.2015.P173-179

Riad, N.H., Taha, H.A. & Mahmoud, Y.I. (2013). Effects of garlic on *Schistosoma mansoni* harbored in albino mice: Molecular characterization of the host and parasite. *Gene* **518**: 287-291. https://doi.org/10.1016/j.gene.2013.01.023

Schelkle, B., Snellgrove, D. & Cable, J. (2013). *In vitro* and *in vivo* efficacy of garlic compounds against *Gyrodactylus turnbulli* infecting the guppy (*Poecilia reticulata*). *Veterinary Parasitology* **198**: 96-101. https://doi.org/10.1016/j.vetpar.2013.08.027

Singh, T.U., Kumar, D., Tandan, S.K. & Mishra, S.K. (2009). Inhibitory effect of essential oils of *Allium sativum* and *Piper longum* on spontaneous muscular activity of liver fluke, *Fasciola gigantica*. *Experimental Parasitology* **123**: 302-308. https://doi.org/10.1016/j.exppara.2009.08.002

Songsri, J., Ketthaisong, D., Boonmars, T., Boueroy, P., Laummaunwai, P., Vaeteewoottacharn, K., Rattanasuwan, P., Boonyarat, C., Lomthaisong, K. & Tangwongchai, R. (2019). *In vitro* and *in vivo* effect of Chia oil (*Salvia hispanica* L.) on *Haplorchis taichui*. *The Thai Journal of Veterinary Medicine* **49**: 393-400.

Sriwilaijaroen, N., Kondo, S., Nanthasri, P., Auparakkitanon, S., Suzuki, Y. & Wilairat, P. (2010). Antiplasmodial effects of *Brucea javanica* (L.) Merr. and *Eurycoma longifolia* Jack extracts and their combination with chloroquine and quinine on *Plasmodium falciparum* in culture. *Tropical Medicine and Health* **38**: 61-68. https://doi.org/10.2149/tmh.2009-11

Summers, S., Bhattacharyya, T., Allan, F., Stothard, J.R., Edielu, A., Webster, B.L., Miles, M.A. & Bustinduy, A.L. (2022). A review of the genetic determinants of praziquantel resistance in *Schistosoma mansoni*: Is praziquantel and intestinal schistosomiasis a perfect match? *Frontiers in Tropical Diseases* **3**: 933097. https://doi.org/10.3389/fitd.2022.933097

Ur Rehman, S., Kevin, C. & Hye Hyun, Y. (2016). Review on a traditional herbal medicine, *Eurycoma longifolia* Jack (Tongkat Ali): its traditional uses, chemistry, evidence-based pharmacology and toxicology. *Molecules* **21**: 331-361. https://doi.org/10.3390/molecules21030331

Wang, W., Wang, L. & Liang, Y.-S. (2012). Susceptibility or resistance of praziquantel in human schistosomiasis: a review. *Parasitology Research* **111**: 1871-1877. https://doi.org/10.1007/s00436-012-3151-z

Wattanawong, O., Iamsirithaworn, S., Kophachon, T., Nak-Ai, W., Wisetmora, A., Wongsaroj, T., Dekumyoy, P., Nithikathkul, C., Suwannatrai, A.T. & Sripa, B. (2021). Current status of helminthiases in Thailand: a cross-sectional, nationwide survey, 2019. *Acta Tropica* **223**: 106082. https://doi.org/10.1016/j.actatropica.2021.106082

Wongsawad, C., Wongsawad, P., Luangphai, P. & Kumchoo, K. (2005). *In vitro* effects of aqueous extract from *Artocarpus takoocha* Roxb on tegumental surface of *Haplorchis taichui*. *The Southeast Asian Journal of Tropical Medicine and Public Health* **36**: 112.

Wonkchalee, N., Boonmars, T., Laummaunwai, P., Aromdee, C., Hahnvajanawong, C., Wu, Z., Sriraj, P., Aukkanimart, R., Chamgramol, Y., Pairojkul, C. *et al.* (2013). A combination of Praziquantel and the traditional medicinal plant *Thunbergia laurifolia* on *Opisthorchis viverrini* infection and cholangiocarcinoma in a hamster model. *Parasitology Research* **112**: 4211-4219. https://doi.org/10.1007/s00436-013-3613-y

Wonkchalee, O., Boonmars, T., Aromdee, C., Laummaunwai, P., Khunkitti, W., Vaeteewoottacharn, K., Sriraj, P., Aukkanimart, R., Loilome, W., Chamgramol, Y. *et al.* (2012). Anti-inflammatory, antioxidant and hepatoprotective effects of *Thunbergia laurifolia* Linn. on experimental opisthorchiasis. *Parasitology Research* **111**: 353-359. https://doi.org/10.1007/s00436-012-2846-5

Woottisin, N., Sukprasert, S., Kulsirirat, T., Tharavanij, T. & Sathirakul, K. (2022). Evaluation of the intestinal permeability of Rosmarinic acid from *Thunbergia laurifolia* leaf water extract in a Caco-2 cell model. *Molecules* **27**: 3884. https://doi.org/10.3390/molecules27123884