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RESEARCH ARTICLE

Efficacy of *Thunbergia laurifolia* crude extracts against carcinogenic liver fluke, *Opisthorchis viverrini*

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ABSTRACT

The human liver fluke, *Opisthorchis viverrini*, is a significant risk factor for cholangiocarcinoma (CCA) in Thailand. Praziquantel (PZQ) is the primary treatment for opisthorchiasis but is associated with adverse effects and the potential development of CCA amid chronic infection and reinfection, prompting the search for alternative treatments. *Thunbergia laurifolia* is widely used for detoxification from lead poisoning and other toxins. The bioactive compounds of *T. laurifolia* are categorized into sterols, phenolics, carotenoids, glycosides, and unclassified steroids. It is considered an herb that contains a variety of active compounds, which may demonstrate effectiveness in eliminating parasites. This study evaluated the anthelmintic efficacy of crude extracts of *Thunbergia laurifolia* on newly excysted juveniles (NEJs) and adult stages of *O. viverrini.*The experiments were involved treating *O. viverrini* NEJs and adult worms with various concentrations of the crude extracts (NEJs: 5, 10, 20, 40 mg/ml; adult worms: 20, 30, 40 mg/ml), with PZQ and RPMI-1640 medium serving as positive and negative controls, respectively. An assessment of relative motility (RM) and survival index (SI) was conducted, along with a morphological evaluation using scanning electron microscopy (SEM) and biochemical tests to evaluate the generation of reactive oxygen species (ROS) as a response to stress. Results showed that *T. laurifolia* crude extract reduced RM and SI of the *O. viverrini* NEJs and adult worms. SEM revealed minimal tegumental damage when compared to the positive control group. Elevated ROS levels were significantly higher, suggesting oxidative stress as a potential mechanism of action. These findings suggest that *T. laurifolia* possess promising anthelmintic properties against *O. viverrini*, meriting further research to isolate active compounds and elucidate their mechanisms. Such studies could lead to the development of novel herbal treatments for opisthorchiasis.

Keywords: *Thunbergia laurifolia*; crude extracts; against-parasite; *Opisthorchis viverrini*.

INTRODUCTION

Human liver fluke, *Opisthorchis viverrini*, it causes of opisthorchiasis, prevalent in northern and northeastern Thailand, escalating to 14.3% in high-risk areas (Wattanawong *et al*., 2021). Thailand incurs healthcare costs exceeding USD $120 million annually due to *O. viverrini* infection (Kaewpitoon *et al*., 2015). *O. viverrini* has been classified as a Group 1 biological carcinogen by the WHO International Agency for Research on Cancer (IARC, 2024). The persistent inflammatory response induced by *O. viverrini* in the bile ducts poses a substantial long-term risk for cholangiocarcinoma (CCA) (Sripa *et al*., 2007, 2011; Pinlaor *et al*., 2009; Hanpanich *et al*., 2017). Currently, the drug of choice for treating opisthorchiasis is praziquantel (PZQ) (CDC, 2024). PZQ was initially selected for its anti-helminthic properties in the mid-1970s and has since been utilized to treat various human trematode infections (Cioli & Pica-Mattoccia, 2003). However, repeated treatment with PZQ and subsequent reinfection with *O. viverrini* are associated with an increased risk of developing CCA (Lawson *et al*., 1991; Pinlaor *et al*., 2009; Kamsa-Ard *et al*., 2015). Inflammation and the resulting stress induced by *O. viverrini* antigens are statistically associated with papillary and intrahepatic CCA, along with the repeated use of PZQ treatment (Luvira *et al*., 2018). The emergence of drug resistance and the side effects associated with PZQ treatment raise significant concerns (Fallon & Doenhoff, 1994; Cioli & Pica-Mattoccia, 2003; Erko *et al*., 2012). The importance of investigating alternative herbal remedies is highlighted by the potential of bioactive compounds, such as tannins, flavonoids, and alkaloids, which are well-known

for their effectiveness against diverse parasites (Bauri *et al*., 2015; Fomum & Nsahlai, 2017; Mushtaq *et al*., 2018). These compounds could serve as guiding principles for identifying key bioactive agents for parasite elimination in future research endeavors.

Several herbals for *O. viverrini* liver fluke treatment have been investigated. The treated *O. viverrini* adult worms exhibited reduced movement and increased mortality when exposed to crude extract of *Allium sativum*. This treatment also revealed significantly elevated reactive oxygen species (ROS) level and distinctive morphological damage (Pechdee *et al*., 2024). Studies have shown that extracts of *Garcinia mangostana* and *Thunbergia laurifolia* possess anti-inflammatory and antioxidant properties against *O. viverrini* infection (Wonkchalee *et al*., 2012, 2013; Aukkanimart *et al*., 2015). Additionally, the anthocyanin complex derived from blue butterfly pea, turmeric, and purple waxy corn cobs exhibited anti-inflammatory and anti-periductal fibrosis effects against *O. viverrini* infection (Intuyod *et al*., 2014). Furthermore, the effects of *Areca catechu* on the motility of *O. viverrini* NEJs and adult worms (Wannachat, 2020). In contrast, *T. laurifolia* is widely used for detoxification from lead poisoning and other toxins, as well as for its antioxidant, anti-inflammatory, hepatoprotective, antitumor, and antihyperglycemic properties (Palipoch *et al*., 2011). The *T. laurifolia* exhibits a range of pharmacological activities, including antiproliferative, hepatoprotective, detoxifying, antimicrobial, antidiabetic, anti-inflammatory, and non-toxic effects (Palipoch *et al*., 2011; Rocejanasaroj *et al*., 2014). A previous report identified and categorized the bioactive compounds of *T. laurifolia* crude extract into five main groups, which are considered significant: sterols, phenolics, carotenoids, glycosides, and unclassified steroids (Chan *et al*., 2011; Junsi & Siripongvutikorn, 2016). These classifications underscore the plant's potential medicinal properties, linking its chemical diversity to its traditional uses and therapeutic benefits. Significant compounds such as rosmarinic acid, apigenin, caffeic acid, allic acid, protocatechuic acid, and various vitamins have been identified in *T. laurifolia*. The apigenin is main bioactive compound in *T. laurifolia*, possesses antioxidant and anti-cancer properties (Oonsivilai *et al*., 2007; Chan *et al*., 2010). A previous study demonstrated that both fresh and dried *T. laurifolia* solutions significantly reduced inflammatory cells in hamsters infected with *O. viverrini*. Furthermore, the combination of *T. laurifolia* extract with praziquantel reduced inflammatory cell aggregation and inhibited the development of CCA, which correlated with these effects correlating to the serum alanine transaminase (ALT) levels in the infected hamsters (Wonkchalee *et al*., 2012, 2013).

Therefore, the aim of this investigation is to assess the impact of *T. laurifolia* crude extract on the human liver fluke, *O. viverrini* NEJs and adult worms. The protective properties of the extracts against *O. viverrini* NEJs and adult worms were studied through relative motility values (RM) and % survival index (SI), alongside viability confirmation, and morphological tegumental damage. Additionally, *O. viverrini* adult worms were assessed and included evaluating oxidative stress generation.

MATERIALS AND METHODS

Ethics approval

The Animal Ethics and Bio Ethics have been approved by Committee of the institute of research and development, Suranaree University of Technology, Thailand (Animal Ethics: Ethical Clearance No. SUT-IACUC-0013/2023 and Bio Ethics: Ethical Clearance No. SUT-IBC-008-2023).

Parasite preparation

Opisthorchis viverrini metacercariae were obtained from naturally infected cyprinid fish in an endemic region spanning Nakhon Ratchasima and Chaiyaphum Provinces in northeastern Thailand. The whole fresh cyprinid fish were cut into pieces and then digested in a 0.25% pepsin-hydrochloric acid solution. and incubated at 37°C for 1-2 hours. The solution was then filtered and centrifuged using 0.85% normal saline solution in a sedimentation jar (Srisawangwong *et al*., 1997). Metacercariae were identified based on their morphology under a stereomicroscope (Vajrasthira *et al*., 1961; Scholz *et al*., 1991). *O. viverrini* metacercariae were excysted in 0.25% trypsin in 1x phosphate-buffered saline (PBS) supplemented with 200 u/ml penicillin and 200 µg/ml streptomycin for 5-15 minutes at 37°C to obtain *O. viverrini* NEJs for the experiment (Arunsan *et al*., 2019).

Five male Syrian golden hamsters (*Mesocricetus auratus*) were used in the infection experiment because female hamsters are more aggressive and to avoid pregnancy during infection. The hamsters, aged 6-8 weeks, were orally infected with 50 *O. viverrini* metacercariae via intragastric intubation, facilitating the development of *O. viverrini* adult worms in the liver bile ducts over a period of 2-3 months (Sripa & Kaewkes, 2002). The infected hamsters were kept in stainless steel cages and were provided ad libitum access to a standard diet and water. The room temperature was maintained at 23 ± 2°C, with a relative humidity of 45-50%, under a 12-hour light-dark cycle. Consequently, the *O. viverrini*-infected hamsters were sacrificed to collect adult worms from the liver bile ducts for experimental purposes. Hamsters were euthanized with 1-3% isoflurane. A surgical incision was prepared to open the abdominal cavity. The liver was removed and placed immediately in 0.85% normal saline solution (NSS). Adult worms were pressed from the liver bile duct and incubated in RPMI-1640 culture media. Successively, the actively adult worms were collected for experimentation.

Plants extract preparation

The fresh leaves of *T. laurifolia*, weighing 1 kg, were washed with distilled water (DW), loosely labeled, and subsequently placed in a hot air oven at 60°C for 24 hours. Subsequently, the material was finely ground into a powder using a grinder. The aqueous solvent was used as the extraction solvent to minimize interference in this test. According to previous studies, apigenin, caffeic acid, gallic acid, protocatechuic acid, and rosmarinic acid are key bioactive compounds of *T. laurifolia* that were extracted using aqueous extraction. (Chan *et al*., 2011; Rojsanga *et al*., 2018; Ruangpayungsak *et al*., 2018). A mixture consisting of 10 g of *T. laurifolia* was dissolved in 40 ml of DW. This blend underwent agitation in a 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 DW 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 $133x10^{-3}$ mBar for 48 hours. The resulting powdered extract was stored at -20°C until used. The percentage yield of each crude extract was calculated using the formula described by Abbas *et al.* (2021).

***O. viverrini* experimental allocation**

PZQ is the standard drug, and in this preliminary study, the PZQ concentration was based on the standard opisthorchiasis treatment (25 mg/kg orally as a single dose) (Chai *et al*., 2020). PZQ concentrations of 2 mg/ml (10x) for *O. viverrini* NEJs and 20 mg/ml for adult worms were prepared from 600 mg tablets (HK Pharmaceutical, Bangkok, Thailand) to observe changes in pathophysiological and physiological conditions. Based on preliminary results, *T. laurifolia* crude extracts were selected at concentrations of 5, 10, 20, and 40 mg/ml for *O. viverrini* NEJs and 20, 30, and 40 mg/ml for adult worms, respectively. Previous studies

have not reported the *in vitro* antiparasitic effects of *T. laurifolia*. However, its extract has been tested on *O. viverrine* infected hamsters (Wonkchalee *et al*., 2012, 2013).

Sixty *O. viverrini* NEJs were divided into six distinct groups, each consisting of 5 NEJs (with duplicates in each group). Group 1 received RPMI-1640 as the negative control, while Group 2 was treated with 2 mg/ml of PZQ as the positive control. Groups 3, 4, 5, and 6 were treated with 5, 10, 20, and 40 mg/ml concentrations of *T. laurifolia* crude extract, respectively. Forty *O. viverrini* adult worms were divided into five distinct groups, each consisting of 4 adult worms (with duplicates in each group). Group 1 received RPMI-1640 as the negative control, while Group 2 was treated with 20 mg/ml of PZQ as the positive control. Groups 3,4 and 5 were subjected to treatment with 20, 30, and 40 mg/ml concentrations of *T. laurifolia* crude extract. The negative control, positive control, and all *T. laurifolia* crude extract concentration groups were prepared in RPMI-1640 culture medium. Additionally, each group was supplemented with 100 µg/ml of streptomycin antibiotic (Arunsan *et al*., 2019).

Motility and survival assay

All groups of *O. viverrini* NEJs and adult worms were exposed to different time intervals: 0 and 5 minutes, 1, 3, 6, 12, and 24 hours for *O. viverrini* NEJs, and 0 and 30 minutes, 1, 3, 6, 12, and 24 hours for *O. viverrini* adult worms under 37°C. The motility assessment included evaluating relative mobility (RM) and % survival index (SI) based on predefined scoring criteria at various exposure times. The motility was assessed by examining *O. viverrini* NEJs and adult worms under a stereomicroscope and scored based on the criteria: 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. The RM values were determined employing the formula detailed below (Kiuchi *et al*., 1987; Lorsuwannarat *et al*., 2013).

$$\text{Motility index (MI)} = \frac{\Sigma nN}{N} \quad (1)$$

$$\text{\% Relative motility (RM) value} = \frac{\text{MI test} \times 100}{\text{MI control}} \quad (2)$$

n = motility score,
N = number of parasites with the score of ''n''

The SI was calculated to determine the percentage of live *O. viverrini* NEJs and adult worms at 0, 5, and 30 minutes, as well as at 1, 3, 6, 12, and 24 hours after incubation. *O. viverrini* NEJs and adult worms that exhibited a motility score of 0 were classified as died. The SI was calculated using the formula provided below (Kiuchi *et al*., 1987; Lorsuwannarat *et al*., 2013).

$$\text{\% Survival index (SI)} = \frac{\text{Number of live parasite (each group)} \times 100}{\text{Total parasite (each group)}} \quad (3)$$

Morphological study by SEM

The evaluation of morphological damage in *O. viverrini* NEJs and adult worms following 12 hours of incubation was performed using scanning electron microscope (SEM). The *O. viverrini* NEJs and adult worms underwent multiple washes with 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 washes with DW. Subsequently, the samples were dehydrated through a series of graded acetone solutions (30%, 50%, 70%, 90%, 95%, and 100%) 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 of 3.00 kV.

Measurement of stress generation due to ROS

After 6 hours of incubation, the *O. viverrini* adult worms were thoroughly rinsed with DW. Following the rinsing procedure, the worms were exposed to a 30 µM fluorogenic dye, H2DCFDA (Med Chem Express®, New Jersey, USA), and incubated in darkness at 37°C for 30 minutes. After incubation, the samples were washed again with DW to eliminate any surplus fluorogenic dye. Slides were prepared for fluorescent imaging using a fluorescence microscope (Ex/Em = 488/525 nm). The fluorescence levels from ROS were quantified by analyzing fluorescence microscopy images with ImageJ software (https://imagej.net/ij/download.htm). The corrected total worm fluorescence (CTWF) was determined by subtracting the integrated density from the product of the selected worm's area and the mean fluorescence of the background readings (El-Sharkawey, 2016).

Worm viability assay

After 24 hours of incubation, the *O. viverrini* NEJs and adult worms were exposed to a 0.4% trypan blue stain at room temperature for 2-3 minutes. They were washed for 3 times with 1x PBS, and their viability was assessed under a light microscope.

Data analysis

The RM and SI values were analyzed using their respective formulas. 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). A one-way ANOVA was conducted among five groups (negative control, positive control, and treated groups) to compare the mean motility scores. Statistical significance was determined by a *P*-value < 0.05. Probit regression analysis was performed to calculate the half-maximal inhibitory concentration IC_{50} of *T. laurifolia* crude extract in inhibiting the motility of both *O. viverrini* NEJs and adult worms.

RESULTS

Motility and viability of *O. viverrini* NEJs

The motility rates were not constant. It showed that fluctuating depending on the different concentration, the *T. laurifolia* treated groups at concentrations of 5, 10, 20, and 40 mg/ml showed normal motility in *O. viverrini* NEJs within 0 to 5 minutes across all groups (RM = 100 and SI = 100 in all concentration group). After 1 hour, the motility rate remained constant until 12 hours, with the following results: 5 mg/ml (RM = 66.67, 74.07, 65.38, and 46.43; SI = 100, 100, 100, and 100 for 1, 3, 6, and 12 hours, respectively); 10 mg/ml (RM = 66.67, 74.07, 50.00, and 46.43; SI = 100, 100, 100, and 100, for 1, 3, 6, and 12 hours, respectively); 20 mg/ml (RM = 56.67, 59.26, 46.15, and 42.86; SI = 100, 100, 100, and 100, for 1, 3, 6, and 12 hours, respectively); and 40 mg/ml (RM = 36.67, 37.04, 38.46, and 35.71; SI = 100, 100, 100, and 100, for 1, 3, 6, and 12 hours, respectively). At 24 hours, motility ceased, and the worms died in all groups (RM = 0 and SI = 0 in all groups). The negative control group displayed normal motility from 0 minutes to 12 hours (RM = 100 and SI = 100), with a slight decrease in motility observed at the conclusion of the experiment at 24 hours (RM = 100 and SI = 60). The positive control group displayed normal motility within the first 0 to 5 minutes (RM = 100 and SI = 100). However, after 1 hour, the parasites exhibited a rapid decrease in movement (RM = 53.57 and SI = 100), remaining relatively stable from 3 to 6 hours (RM = 51.85 and 53.85, SI = 100 and 100, respectively), and further declining at

12 hours (RM = 39.29 and SI = 100). Motility ceased entirely by the 24 hours mark (RM = 0 and SI = 0) (Figure 1a and 1b). A comparative analysis was indicated significant differences between the negative control group and the groups treated with 20 and 40 mg/ml of *T. laurifolia* for NEJs. However, there were no significant differences in mean motility scores between the positive control group and the *T. laurifolia* treated groups ($P < 0.05$) (Figure 2). Viability confirmation via trypan blue staining showed no staining in the negative control group (Figure 3a). In contrast, extensive trypan blue staining was observed in the positive control group (Figure 3b), as well as in all groups treated with *T. laurifolia* (Figure 3c-3f). The inhibitory motility concentration of *T. laurifolia* crude extract on *O. viverrini* NEJs was recorded at LC_{50} = 22.723 mg/ml for average exposure times of 0, 5, and 30 minutes, as well as for 1, 3, 6, 12, and 24 hours.

Morphological surface study of *O. viverrini* NEJs

Surface morphological alterations of *O. viverrini* NEJs were examined via SEM after 12 hours of treatment. The tegumental surface of *O. viverrini* NEJs in the negative control group were showed normal of tegumental sureface, presence the normal of sensory papillae (Pa), under of the ventral sucker (Vs) region displays the small spines (Sp) (Figure 4a-4d). In the positive control group treated, the tegumental surface of *O. viverrini* NEJs showed a swollen appearance with blebs present around the ventral sucker (Vs). The entire body's tegument exhibited swelling and stretching (Figure 4e-4h). *T. laurifolia* treated groups, the tegumental surface of *O. viverrini* NEJs displaying morphological abnormalities exhibited slight swelling of the body tegument compared to the negative control group (Figure 4i-4l).

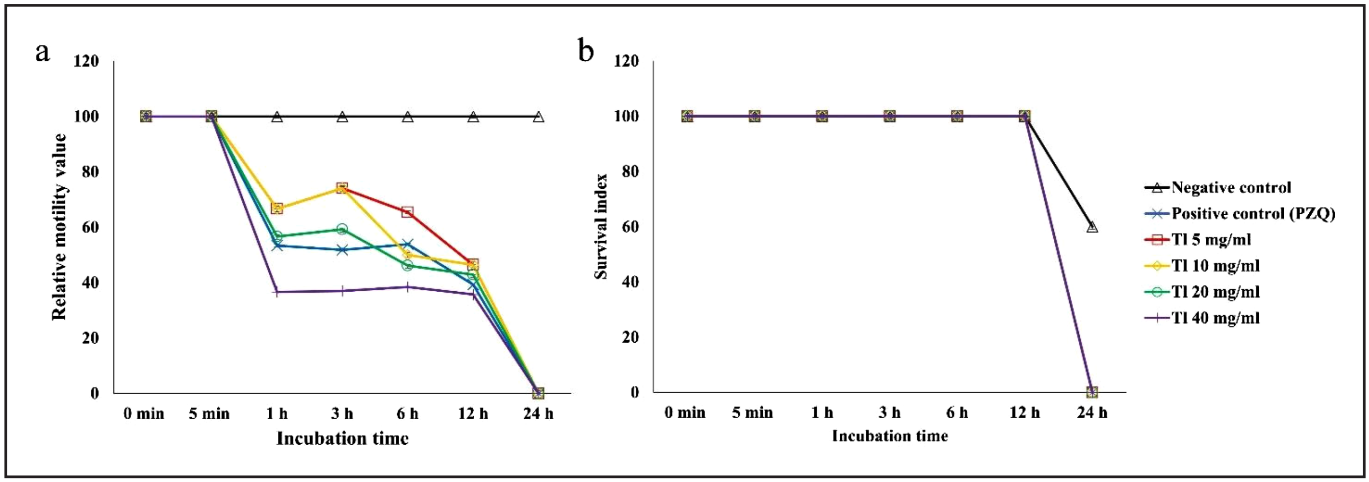

Figure 1. The (a) RM and (b) SI values of *T. laurifolia* treated groups against *O. viverrini* NEJs were evaluated following treatment with concentrations of 5, 10, 20, and 40 mg/ml.

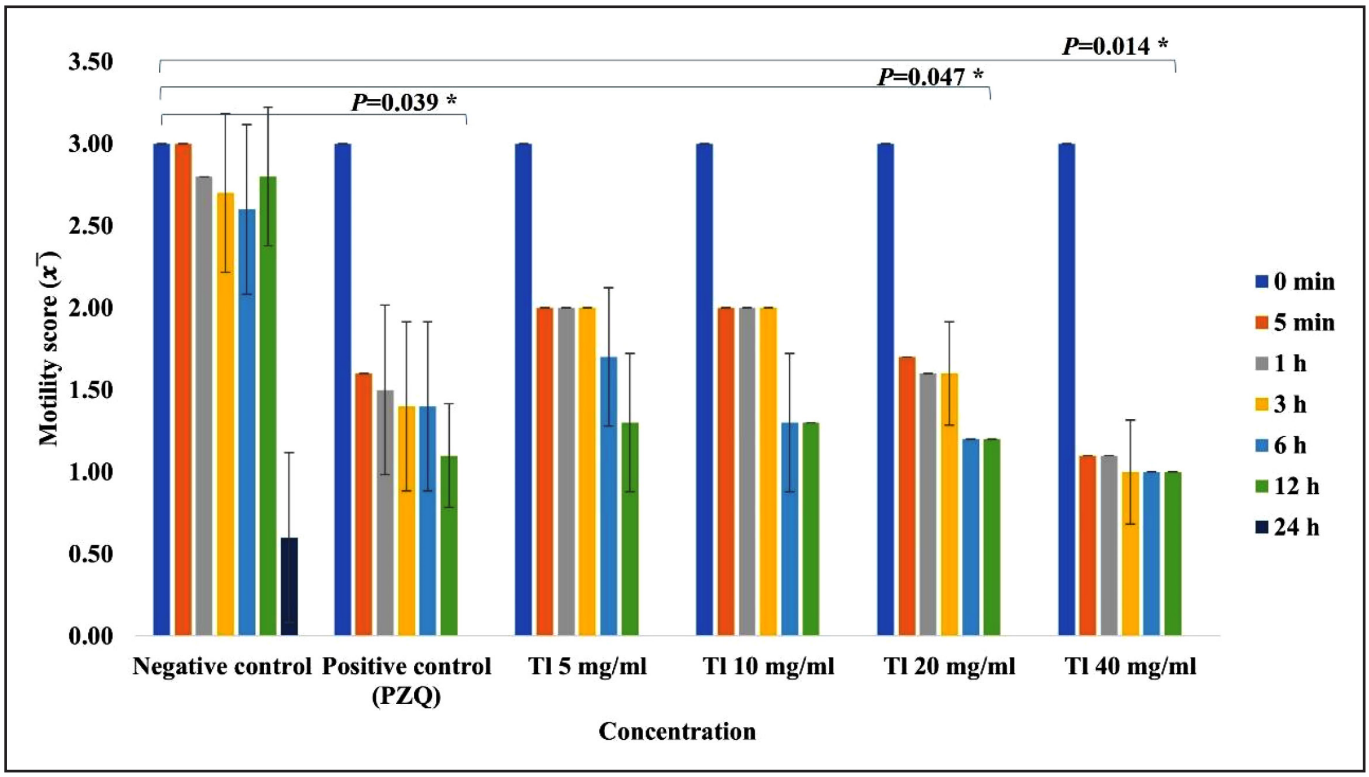

Figure 2. The motility scores of the *T. laurifolia* treated group showed significant differences compared to the negative control group, as well as between the groups treated with 20 and 40 mg/ml on NEJs ($P < 0.05$). However, no significant differences in mean motility scores were observed between the positive control group ($P > 0.05$). * = Significant difference.

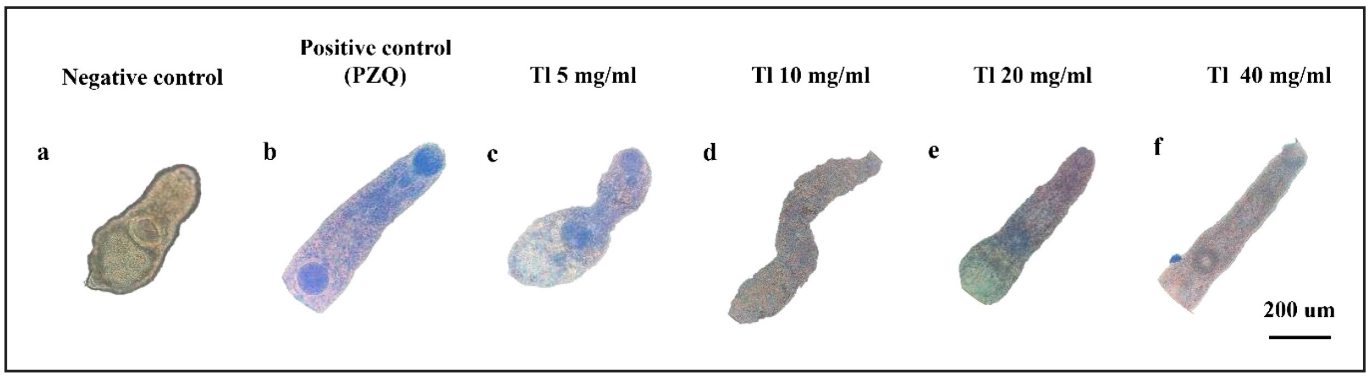

Figure 3. Viability of *O. viverrini* NEJs was assessed using trypan blue staining: (a) the negative control group, no staining was observed and evidenced, (b) the positive control group, (c), (d), (e), and (f) *T. laurifolia* treated groups. Scale bar = 200 µm.

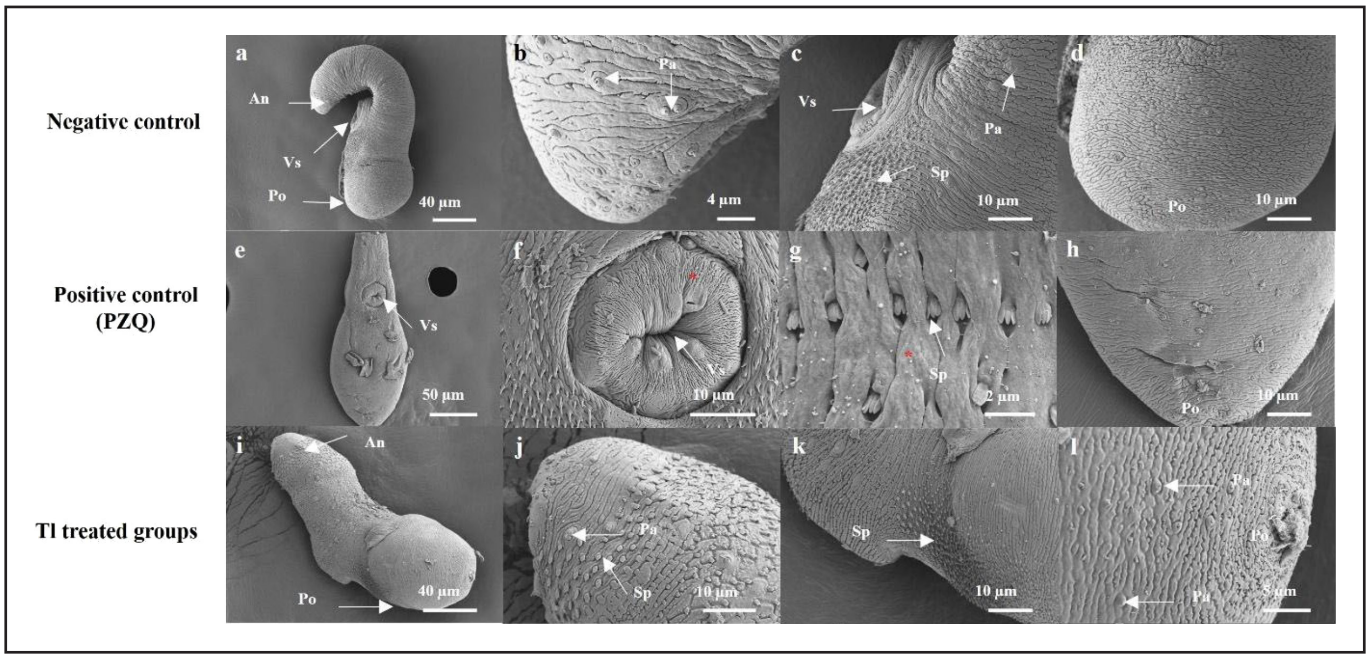

Figure 4. Tegumental surface of *O. viverrini* NEJs: (a), (b), (c), and (d) negative control group, scale bar = 40, 4, 10, and 10 µm, respectively. (e), (f), (g), and (h) Positive control group, scale bar = 50, 10, 2, and 10 µm, respectively. (i), (j), (k), and (l) *T. laurifolia* treated group, scale bar = 40, 10, 10, and 5 µm, respectively. * = Swelling of the tegumental surface. Abbreviations; An = Anterior region, Os = Oral sucker, Po = Posterior region, Pa = Papilla, Sp = spines, and Vs = Ventral sucker.

Motility and viability of *O. viverrini* adult worm

The *T. laurifolia* treated groups with concentrations of 20, 30, and 40 mg/ml exhibited normal motility in *O. viverrini* adult worms across all groups (RM = 100 and SI = 100 in all concentration groups). However, a decrease in motility was observed at 30 minutes in all groups, with the following results: 20 mg/ml (RM = 48.21, SI = 100), 30 mg/ml (RM = 51.79, SI = 100), and 40 mg/ml (RM = 50.00, SI = 100). This motility rate remained constant from 1 hour to 6 hours with the following results: 20 mg/ml (RM = 52.94, 50.97, and 60.98; SI = 100, 100, and 100 for 1, 3, and 6 hours, respectively); 30 mg/ml (RM = 64.71, 64.69, and 60.98; SI = 100, 100, and 100 for 1, 3, and 6 hours, respectively); and 40 mg/ml (RM = 50.98, 49.01, and 63.41; SI = 100, 100, and 100 for 1, 3, and 6 hours, respectively). Additionally, the motility rate in each group decreased again at the 12-hour mark: 20 mg/ml (RM = 40.00, SI = 50.00), 30 mg/ml (RM = 40.00, SI = 50.00), and 40 mg/ml (RM = 40.00, SI = 50.00). Motility ceased entirely at 24 hours (RM = 0 and SI = 0 in all concentration groups). The negative control group exhibited continuous active movement and sustained vitality (RM = 100 and SI = 100 in all groups). In the positive control, *O. viverrini* adult worms began to exhibit a continuous decrease in motility at 30 minutes, reaching the following values: 1 hour (RM = 50.98, SI = 100), 3 hours (RM = 43.13, SI = 100), 6 hours (RM = 39.02, SI = 75.00), and 12 hours (RM = 35.00, SI = 25.00). The motility rate reached complete cessation at the 24-hour mark (RM = 0 and SI = 0). (Figure 5a and 5b). A comparative analysis was indicated not significantly different between both the negative control and positive control groups compared to the *T. laurifolia* treated groups ($P < 0.05$) (Figure 6). The viability of *O. viverrini* adult worms was assessed using trypan blue staining. In the negative control group, no staining was observed. Conversely, extensive trypan blue staining was evident in the positive control group, as well as in all treated groups with *T. laurifolia* (Figure 7). The inhibitory motility concentration of *T. laurifolia* crude extract on *O. viverrini* adult worm was recorded at LC_{50} = 15.015 mg/ml for average exposure times of 0, 5, and 30 minutes, as well as for 1, 3, 6, 12, and 24 hours.

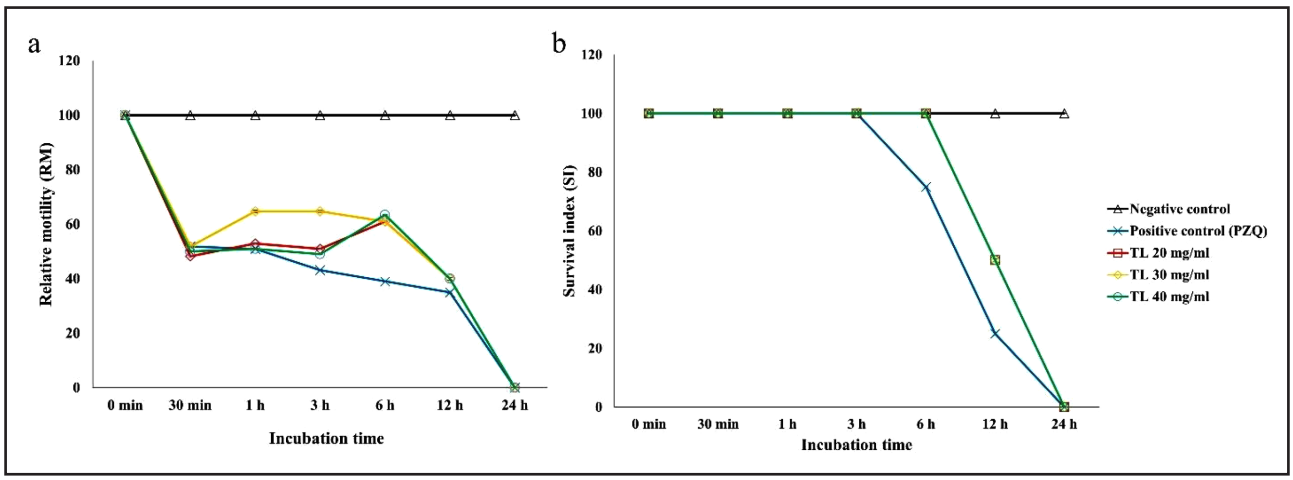

Figure 5. RM and SI values of *O. viverrini* adult worms: (a) RM values of groups treated with *T. laurifolia* and (b) SI of groups treated with *T. laurifolia* were evaluated after incubation with concentrations of 20, 30, and 40 mg/ml.

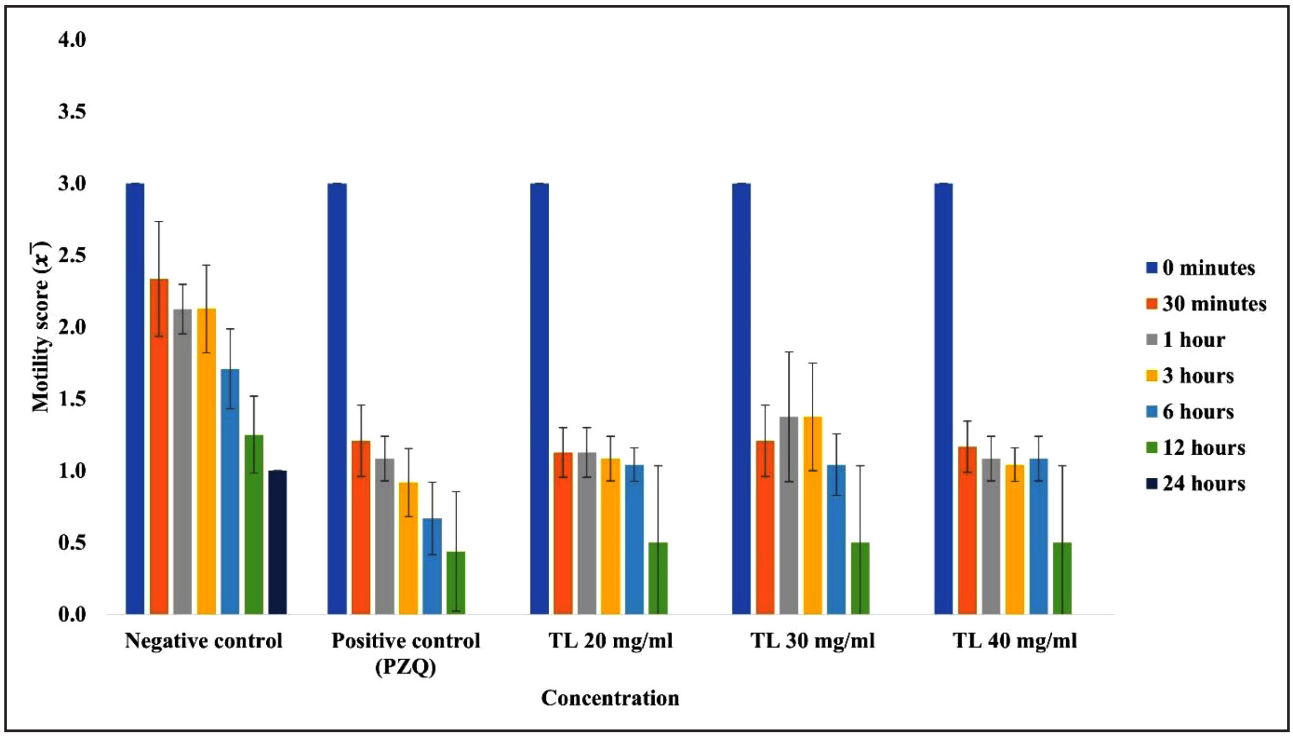

Figure 6. The mean motility scores of the *T. laurifolia*-treated group were not statistically significant when compared to those of the negative and positive control groups ($P > 0.05$).

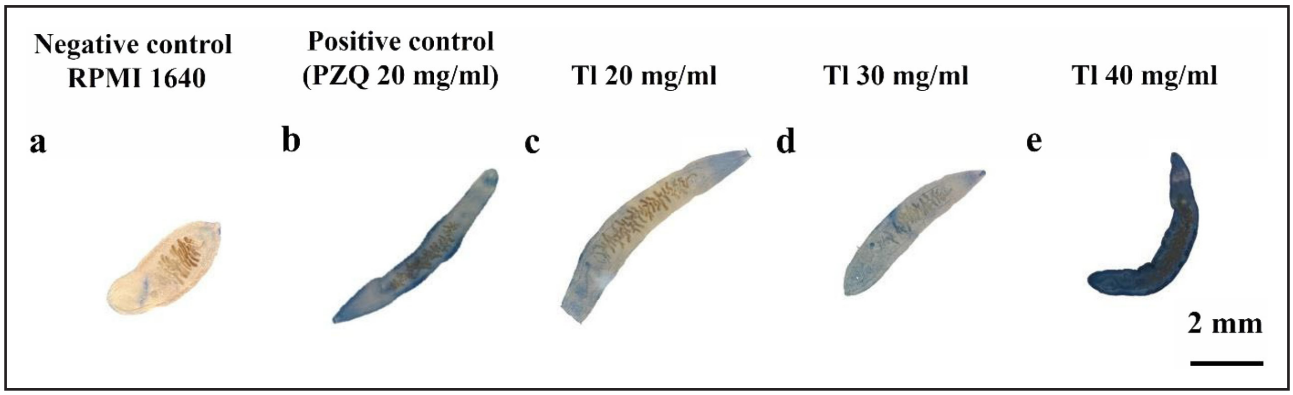

Figure 7. The viability of *O. viverrini* adult worms was assessed using trypan blue staining: (a) negative control group, (b) positive control group, and (c), (d), and (e) *T. laurifolia* treated groups at concentrations of 20, 30, and 40 mg/ml, respectively. Scale bar = 2 mm.

ROS Generation of *O. viverrini* adult worm

The *T. laurifolia* treated groups were showed elevated levels of ROS throughout the entire body of the *O. viverrini* adult worms, encompassing the anterior, middle, and posterior regions, in comparison to the negative and positive control groups. This effect was particularly pronounced in certain areas such as the reproductive organ (Ro) and area of genital pore (Gp), at concentrations of 20 mg/ml, 30 mg/ml, and 40 mg/ml respectively (Figure 8). Additionally, the CTWF were showed fluorescence intensity of *T. laurifolia* treated groups (20, 30, and 40 mg/ml) exhibited higher levels compared to the negative and positive control groups (Figure 9).

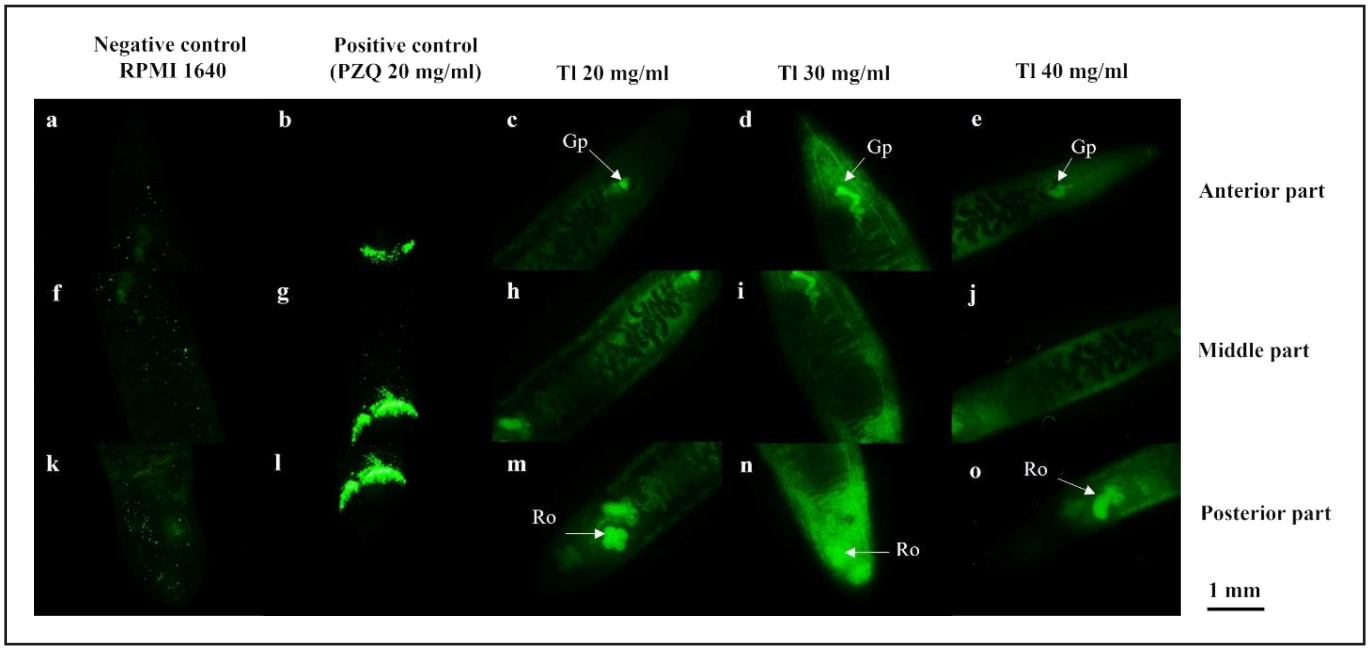

Figure 8. The ROS generation images of *T. laurifolia* treated groups at various parts of the adult worm were compared with those of the negative and positive control groups: (a), (f), and (k) represent the negative control group; (b), (g), and (l) represent the positive control group; (c), (h), and (m) represent the 20 mg/mL *T. laurifolia* treated group; (d), (i), and (n) represent the 30 mg/mL *T. laurifolia* treated group; and (e), (j), and (o) represent the 40 mg/mL *T. laurifolia* treated group. Scale bar = 1 mm. Abbreviations; Gp = genital pore and Ro = reproductive organ.

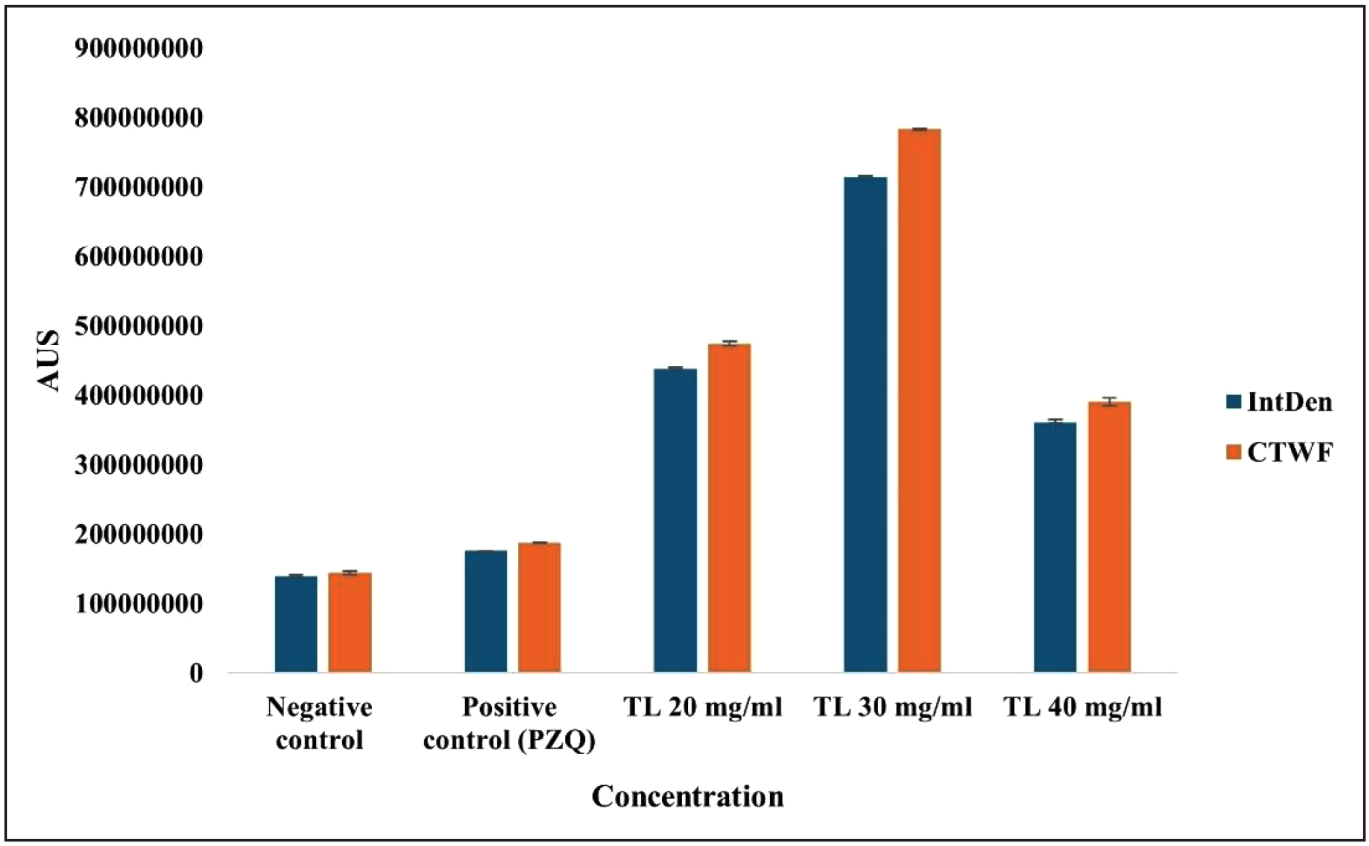

Figure 9. The CTWF of fluorescence intensity in the *T. laurifolia* treated groups was compared with the negative and positive control groups.

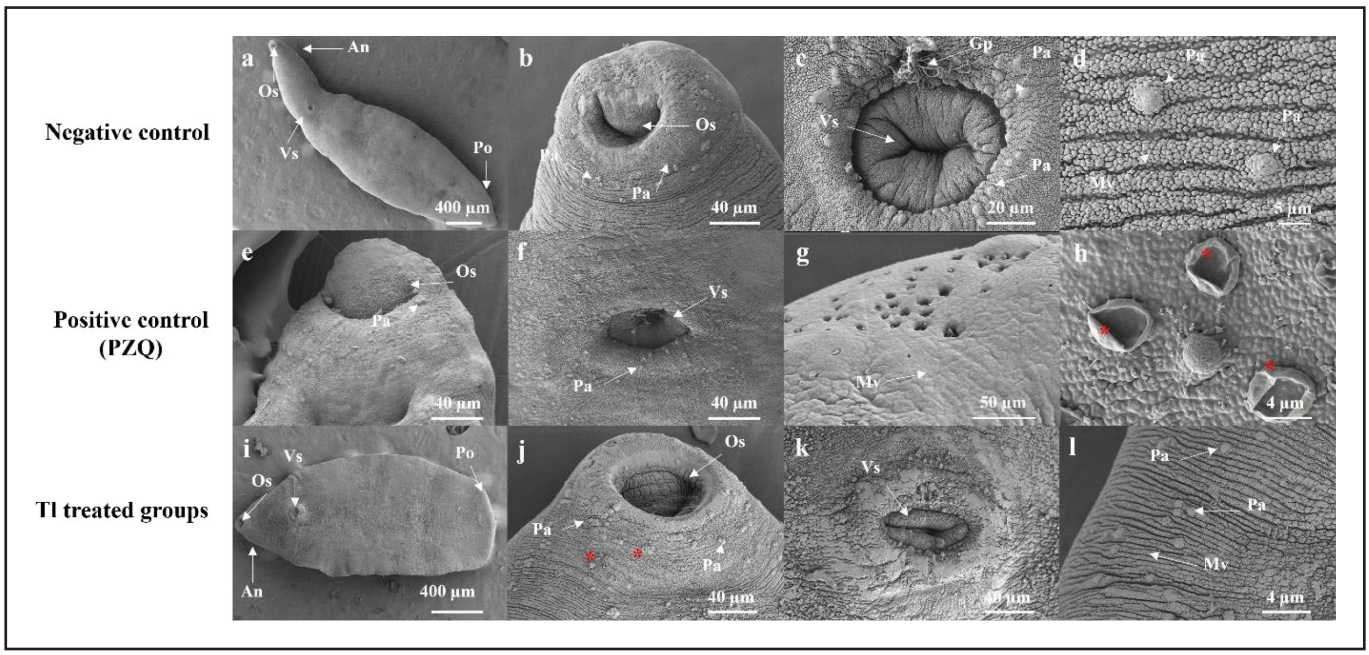

Figure 10. The tegumental surface of *O. viverrini* adult worms: (a), (b), (c), and (d) negative control group, scale bar = 400, 40, 20 and 5 µm, respectively, (e), (f), (g), and (h) positive control group, scale bar = 40, 40, 50 and 4 µm, respectively, (i), (j), (k), and (l) *T. laurifolia* treated group scale bar = 400, 40, 40, and 4 µm, respectively. * = Alterations to the tegumental surface (h) and (j) show swelling leading to rupture. Abbreviations; An = Anterior region, Mv = Microvilli, Os = Oral sucker, Po = Posterior region, and Vs = Ventral sucker.

Morphological surface study of *O. viverrini* adult worm

Surface morphological alterations of *O. viverrini* adult worm were examined via SEM after 12 hours of treatment. The negative control group (Figure 10a-10d), worms exhibited a regular, smooth surface, numerous short microvilli (Mv) and the typical distribution of papillae (Pa) around the oral suckers (Os) and ventral suckers (Vs). The arrangement of papillae (Pa) among the microvilli was also normal. In the positive control group, the microvilli (Mv) on the surface appeared swollen, and there was slight swelling of the papillae (Pa) around the ventral sucker (Vs) (Figure 10e-10h). The *O. viverrini* adult worm treated with *T. laurifolia* displayed a generally smooth surface across its body. There was noticeable swelling around the oral sucker (Ov) and ventral suckers (Vs), and upon closer inspection of the microvilli (Mv) region, slight surface swelling was observed. However, the papillae (Pa) remained clearly visible (Figure 10i-10l).

DISCUSSION

O. viverrini NEJs and adult worms were demonstrated the potential of crude extracts of *T. laurifolia* in reducing the RM and SI to depend on concentrations and exposure times. *O. viverrini* NEJs, *T. laurifolia* treated group, significant differences were observed between the negative control group for 20 mg/ml and 40 mg/ml. The *O. viverrini* adult worms, *T. laurifolia* no significant difference compared to both the negative and positive control groups. This result is consistent with the anthelmintic effects of plumbagin on *Schistosoma mansoni*, as it showed a more rapid reduction in RM values compared to the group treated with PZQ (Lorsuwannarat *et al*., 2013). Similarly, the comparable outcomes using a crude extract from *Areca catechu* on *O. viverrini* NEJs and adult worms, indicating a swift decline in motility with escalating concentrations of *A. catechu* extract (Wannachat, 2020). Additionally, Ahmed *et al.* (2020) discussed that the observed anthelmintic effects of plant extracts may be due to the presence of secondary metabolites. Specifically, tannins, alkaloids, and phenolic compounds (acting as enzyme inhibitors) have been demonstrated to disrupt coupled oxidative phosphorylation and inhibit ATP synthesis in parasites. This disruption can result in the release of enzymes that degrade the worm membrane (Martin, 1997; Joshi *et al*., 2011; Wang *et al*., 2012). This study considered the IC50 value of *T. laurifolia* crude extract, it showed a lower value when compared to the highest concentration used in the test. This suggests the potential ability of the extract to inhibit the motility of *O. viverrini* NEJs and adult worms.

This study observed morphological surface alterations of *O. viverrini* NEJs and adult worms. The SEM analysis revealed morphological damage characterized by tegumental swelling evident across all treatment groups of *O. viverrini* NEJs and adult worm. *T. laurifolia* treated groups, exhibited slight swelling of the tegumental surface. This process involves cellular death due to halted respiration, leading to hypoxia and metabolic changes. Reduced ATP production shifts metabolism to anaerobic pathways, resulting in lactate accumulation and decreased pH. Lysosome swelling and enzyme release follow ATP depletion. Increased calcium influx from tissue hypoxia damages membranes and organelles, ultimately causing cellular self-digestion (autolysis) and tissue degradation (De Groot & Littauer, 1989; Cobb *et al*., 1996; Madea *et al*., 2014).

In contrast, *T. laurifolia* treated groups was elevated ROS levels throughout the entire body, particularly prominent in specific regions such as the reproductive organ (testes and ovary) and around of genital pore. Notably, minimal ROS generation was observed in the regions of the negative control group. This observation is consistent with the study conducted by Goel *et al*. (2020) on oxidative stress in *H. contortus* parasites treated with *Lansium parasiticum* aqueous extract-protected silver nanoparticles, indicating a metabolic shift in response to ROS-induced oxidative stress (Goel *et al*., 2020). Generally, the generation of stress-induced ROS entails the production of highly reactive molecules, such as superoxide anions, hydrogen peroxide, and hydroxyl radicals, within cells or organisms in response to various stressors. These stressors encompass physical, chemical, environmental, or biological factors capable of disrupting the normal balance in cells. Under stressful conditions, the overproduction of ROS can lead to oxidative stress, which is associated with impairment of proteins, lipids, and DNA, thus affecting cellular functionality (Finkel, 2011). The generation

of stress-induced ROS involves a complex process with numerous cellular pathways and mechanisms, playing critical roles in cellular processes such as signaling and immune responses (Schieber & Chandel, 2014). Therefore, the expression of ROS in *O. viverrini* adult worms in this study depends on the cellular characteristics of each parasite.

Currently, the drug used for the treatment of Opisthorchiasis is PZQ. Its mechanism of action involves increasing calcium influx, which causes the muscles of parasites to contract, affecting their movement and inducing paralysis (Pax *et al*., 1978; Becker *et al*., 1980; Greenberg, 2005; Angelucci *et al*., 2007). Rosmarinic acid, a major compound in *T. laurifolia* extract, has garnered significant interest due to its potential broad pharmacological effects (Guan *et al*., 2022; Woottisin *et al*., 2022). The mechanism through which *T. laurifolia* affects parasites has not been extensively studied. However, insights into this mechanism can be inferred from the general action of phenolic compounds on bacterial cells, as outlined by Resende *et al.* (2015). Phenolic compounds are known to effectively disrupt the membranes of gram-positive bacteria at the membrane-interface. This disruption can significantly compromise membrane plasticity, leading to weakened membrane integrity, destabilization, and subsequent disruption of the cell membrane and transport systems (Miceli *et al*., 2011; Resende *et al*., 2015). Despite some successful strategies, there is still considerable progress needed in implementing novel therapies for schistosomiasis or opisthorchiasis (Vale *et al*., 2020). Studies on parasite eradication in *T. laurifolia* have shown promising results demonstrated the effectiveness of *T. laurifolia* in reducing inflammation caused by pathological alterations in *O. viverrini*-infected hamsters (Wonkchalee *et al*., 2012, 2013).

CONCLUSION

The knowledge derived from the *T. laurifolia* crude extract, which exhibits antiparasitic properties against the *O. viverrini* NEJs and adult worms. This is evident from the reduction in parasite motility and the increased expression of ROS following exposure to *T. laurifolia* crude extract at concentrations of 20 mg/ml and 40 mg/ml. At the same time, no significant effects were observed in the adult worm experiment. To enhance our comprehension and application of *T. laurifolia* crude extract, future research should include extensive *in vivo* and *in vitro* studies. Despite the promising outcomes observed with the use of *T. laurifolia* crude extract against *O. viverrini* infections, it is crucial to emphasize the need for further studies to elucidate the specific mechanisms of action, identify the key bioactive components, assess cytotoxicity, and determine parasite specificity. This crucial research step is important for the future development of effective antiparasitic agents and herbal alternatives to control *O. viverrini* liver fluke infection.

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

Ahmed, A.H., Ejo, M., Feyera, T., Regassa, D., Mummed, B. & Huluka, S.A. (2020). *In vitro* anthelmintic activity of crude extracts of Artemisia herba-alba and Punica granatum against *Haemonchus contortus*. *Journal of Parasitology Research* **2020**: 4950196. https://doi.org/10.1155/2020/4950196

Angelucci, F., Basso, A., Bellelli, A., Brunori, M., Mattoccia, L.P. & Valle, C. (2007). The anti-schistosomal drug praziquantel is an adenosine antagonist. *Parasitology* **134**: 1215-1221. https://doi.org/10.1017/s0031182007002600

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

Aukkanimart, R., Boonmars, T., Sriraj, P., Songsri, J., Laummaunwai, P., Waraasawapati, S., Boonyarat, C., Rattanasuwan, P. & Boonjaraspinyo, S. (2015). Anthelmintic, anti-inflammatory and antioxidant effects of *Garcinia mangostana* extract in hamster opisthorchiasis. *Experimental Parasitology* **154**: 5-13. https://doi.org/10.1016/j.exppara.2015.03.007

Bauri, R., Tigga, M.N. & Kullu, S.S. (2015). A review on use of medicinal plants to control parasites. *Indian Journal of Natural Products and Resources* **6**: 268-277.

Becker, B., Mehlhorn, H., Andrews, P., Thomas, H. & Eckert, J. (1980). Light and electron microscopic studies on the effect of praziquantel on *Schistosoma mansoni*, *Dicrocoelium dendriticum*, and *Fasciola hepatica* (Trematoda) *in vitro*. *Zeitschrift für Parasitenkunde* **63**: 113-128. https://doi.org/10.1007/bf00927527

CDC (Centers for Disease Control and Prevention). (2024). Clinical Overview of Opisthorchis. Centers for Disease Control and Prevention. https://www.cdc.gov/liver-flukes/hcp/clinical-overview-opisthorchis/index.html. Accessed 30 June 2024.

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

Chai, J.Y., Sohn, W.M., Hong, S.J., Jung, B.K., Hong, S., Cho, S., Park, J.B., Kim, I.S., Kim, S., Lee, K.H. *et al.* (2020). Effect of mass drug administration with a single dose of albendazole on *Ascaris lumbricoides* and *Trichuris trichiura* infection among schoolchildren in Yangon region, Myanmar. *The Korean Journal of Parasitology* **58**: 195-200. https://doi.org/10.3347/kjp.2020.58.2.195

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

De Groot, H. & Littauer, A. (1989). Hypoxia, reactive oxygen, and cell injury. *Free Radical Biology & Medicine* **6**: 541-551. https://doi.org/10.1016/0891-5849(89)90047-6

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

Finkel, T. (2011). Signal transduction by reactive oxygen species. *Journal of Cell Biology* **194**: 7-15. https://doi.org/10.1083/jcb.201102095

Fomum, S.W. & Nsahlai, I.V. (2017). *In vitro* nematicidal activity of plant species possessing alkaloids and tannins. *Cogent Food & Agriculture* **3**: 1334295. https://doi.org/10.1080/23311932.2017.1334295

Goel, V., Singla, L.D. & Choudhury, D. (2020). Cuminaldehyde induces oxidative stress-mediated physical damage and death of *Haemonchus contortus*. *Biomedicine & Pharmacotherapy = Biomedecine & Pharmacotherapie* **130**: 110411. https://doi.org/10.1016/j.biopha.2020.110411

Greenberg, R.M. (2005). Are Ca2+ channels targets of praziquantel action? *International Journal for Parasitology* **35**: 1-9. https://doi.org/10.1016/j.ijpara.2004.09.004

Guan, H., Luo, W., Bao, B., Cao, Y., Cheng, F., Yu, S., Fan, Q., Zhang, L., Wu, Q. & Shan, M. (2022). A comprehensive review of rosmarinic acid: from phytochemistry to pharmacology and its new insight. *Molecules* **27**: 3292. https://doi.org/10.3390/molecules27103292

Hanpanich, P., Laha, T., Sripa, B., Mairiang, E., Sereerak, P., Upontain, S., Tangkawattana, P., Brindley, P.J. & Tangkawattana, S. (2017). Decreased risk of cholangiocarcinogenesis following repeated cycles of *Opisthorchis viverrini* infection-praziquantel treatment: magnetic resonance imaging (MRI) and histopathological study in a hamster model. *Parasitology International* **66**: 464-470. https://doi.org/10.1016/j.parint.2016.04.012

IARC (International Agency for Research on Cancer). (2024). List of classifications, agents classified by the IARC monographs, monographs on the evaluation of risk to humans. International Agency for Research on Cancer Web. https://monographs.iarc.who.int/list-of-classifications. Accessed 5 June 2024

Intuyod, K., Priprem, A., Limphirat, W., Charoensuk, L., Pinlaor, P., Pairojkul, C., Lertrat, K. & Pinlaor, S. (2014). Anti-inflammatory and anti-periductal fibrosis effects of an anthocyanin complex in *Opisthorchis viverrini*-infected hamsters. *Food and chemical toxicology* **74**: 206-215. https://doi.org/10.1016/j.fct.2014.09.021

Joshi, B., Kommuru, D., Terrill, T., Mosjidis, J., Burke, J., Shakya, K. & Miller, J. (2011). Effect of feeding *Sericea lespedeza* leaf meal in goats experimentally infected with *Haemonchus contortus*. *Veterinary parasitology* **178**: 192-197. https://doi.org/10.1016/j.vetpar.2010.12.017

Junsi, M. & Siripongvutikorn, S. (2016). *Thunbergia laurifolia*, a traditional herbal tea of Thailand: botanical, chemical composition, biological properties and processing influence. *International Food Research Journal* **23**: 923-927.

Kaewpitoon, N., Kootanavanichpong, N., Kompor, P., Chavenkun, W., Kujapun, J., Norkaew, J., Ponphimai, S., Matrakool, L., Tongtawee, T. & Panpimanmas, S. (2015). Review and current status of *Opisthorchis viverrini* infection at the community level in Thailand. *Asian Pacific Journal of Cancer Prevention* **16**: 6825-6830. https://doi.org/10.7314/apjcp.2015.16.16.6825

Kamsa-Ard, S., Luvira, V., Pugkhem, A., Luvira, V., Thinkhamrop, B., Suwanrungruang, K. & Bhudhisawasdi, V. (2015). Association between praziquantel treatment and cholangiocarcinoma: a hospital-based matched case-control study. *BMC Cancer* **15**: 776. https://doi.org/10.1186/s12885-015-1788-6

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

Lawson, L.D., Wang, Z.Y.J. & Hughes, B.G. (1991). Identification and HPLC quantitation of the sulfides and dialk (en) yl thiosulfinates in commercial garlic products. *Planta Medica* **57**: 363-370. https://doi.org/10.1055/s-2006-960119

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

Luvira, V., Kamsa-Ard, S., Kamsa-Ard, S., Luvira, V., Srisuk, T., Pugkhem, A., Pairojkul, C. & Bhudhisawasdi, V. (2018). Association between repeated praziquantel treatment and papillary, and intrahepatic cholangiocarcinoma. *Annals of Hepatology* **17**: 802-809. https://doi.org/10.5604/01.3001.0012.3140

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

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

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

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

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

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.

Pax, R., Bennett, J. & Fetterer, R. (1978). A benzodiazepine derivative and praziquantel: effects on musculature of *Schistosoma mansoni* and *Schistosoma japonicum*. *Naunyn-Schmiedeberg's Archives of Pharmacology* **304**: 309-315. https://doi.org/10.1007/bf00507974

Pechdee, P., Boonsuya, A., Arunsan, P., Thanchonnang, C., La, N., Rattanapitoon, N.K., Laha, T., Nuansing, W. & Rattanapitoon, S.K. (2024). Anthelmintic activity and pathophysiological effect of *Allium sativum* crude extract against carcinogenic liver fluke, *Opisthorchis viverrini*. *Tropical Biomedicine* **41**: 1-11. https://doi.org/10.47665/tb.41.4.002

Pinlaor, S., Prakobwong, S., Boonmars, T., Wongkham, C., Pinlaor, P. & Hiraku, Y. (2009). Effect of praziquantel treatment on the expression of matrix metalloproteinases in relation to tissue resorption during fibrosis in hamsters with acute and chronic *Opisthorchis viverrini infection*. *Acta Tropica* **111**: 181-191. https://doi.org/10.1016/j.actatropica.2009.04.011

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

Rocejanasaroj, A., Tencomnao, T. & Sangkitikomol, W. (2014). *Thunbergia laurifolia* extract minimizes the adverse effects of toxicants by regulating P-glycoprotein activity, CYP450, and lipid metabolism gene expression in HepG2 cells. *Genetics and Molecular Research* **13**: 205-219. https://doi.org/10.4238/2014.january.10.12

Rojsanga, P., Sithisarn, P. & Sanguansataya, T. (2018). Simultaneous determination of caffeic acid and vitexin contents in *Thunbergia laurifolia* leaf extracts collected from different provinces in Thailand by HPLC. *Science Asia* **45**: 205-212.

Ruangpayungsak, N., Sithisarn, P. & Rojsanga, P. (2018). High performance liquid chromatography fingerprinting and chemometric analysis of antioxidant quality of Thunbergia laurifolia leaves. *Journal of Liquid Chromatography & Related Technologies* **41**: 713-721. https://doi.org/10.1080/10826076.2018.1485038

Schieber, M. & Chandel, N.S. (2014). ROS function in redox signaling and oxidative stress. *Current Biology* **24**: R453-R462. https://doi.org/10.1016/j.cub.2014.03.034

Scholz, T., Ditrich, O. & Giboda, M. (1991). Differential diagnosis of opisthorchiid and heterophyid metacercariae (Trematoda) infecting flesh of cyprinid fish from Nam Ngum Dam Lake in Laos. *The Southeast Asian Journal of Tropical Medicine and Public Health* **22**: 171-173.

Sripa, B., Bethony, J.M., Sithithaworn, P., Kaewkes, S., Mairiang, E., Loukas, A., Mulvenna, J., Laha, T., Hotez, P.J. & Brindley, P.J. (2011). Opisthorchiasis and Opisthorchis-associated cholangiocarcinoma in Thailand and Laos. *Acta Tropica* **120**: S158-S168. https://doi.org/10.1016/j.actatropica.2010.07.006

Sripa, B. & Kaewkes, S. (2002). Gall bladder and extrahepatic bile duct changes in *Opisthorchis viverrini*-infected hamsters. *Acta Tropica* **83**: 29-36. https://doi.org/10.1016/s0001-706x(02)00052-9

Sripa, B., Kaewkes, S., Sithithaworn, P., Mairiang, E., Laha, T., Smout, M., Pairojkul, C., Bhudhisawasdi, V., Tesana, S. & Thinkamrop, B. (2007). Liver fluke induces cholangiocarcinoma. *PLoS Medicine* **4**: e201. https://doi.org/10.1371/journal.pmed.0040201

Srisawangwong, T., Sithithaworn, P. & Tesana, S. (1997). Metacercariae isolated from cyprinoid fishes in Khon Kaen District by digestion technic. *Southeast Asian Journal of Tropical Medicine and Public Health* **28**: 224-226.

Vajrasthira, S., Harinasuta, C. & Komiya, Y. (1961). The morphology of the metacercaria of *Opisthorchis viverrini*, with special reference to the excretory system. *Annals of Tropical Medicine & Parasitology* **55**: 413-418. https://doi.org/10.1080/00034983.1961.11686068

Vale, N., Gouveia, M.J. & Gärtner, F. (2020). Current and novel therapies against helminthic infections: The potential of antioxidants combined with drugs. *Biomolecules* **10**: 350. https://doi.org/10.3390/biom10030350

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

Wannachat, C., Kumpangnil, K., Krasuaythong, N., Kanharat, N. & Leksomboon, R. (2020). Effects of *Areca catechu* Linn. extract on motility of *Opisthorchis viverrini*. Rangsit University International Research Conference. Pathum Thani, Thailand.

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

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