Anthelmintic activity assay of *Stachytarpeta jamaicensis* L. Vhal tea against Fasciola sp

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**Abstract.** *Stachytarpeta jamaicensis* L. Vhal (SJ) tea has been empirically used as an anthelmintic, but scientific evidence on its use as an anthelmintic against Fasciola sp. is still limited. The aim of this study was to determine the effective concentration of SJ tea as an anti-Fasciola. *S. jamaicensis* leaves were cut, dried and then packed in the form of tea bags. The presence of secondary metabolites in the tea was also analyzed phytochemically. The anthelmintic activity assay of SJ tea was carried out using an *in vitro* experimental design with a post-test with control group design. The *in vitro* test consisted of negative control (0.9% NaCl), positive control (10% Albendazole) and SJ tea with a concentration of 10%, 5%, and 2.5%. The worm’s movement was observed and the time required for the death of Fasciola was recorded and analyzed. Phytochemical analysis shows that SJ tea contained alkaloid compounds, flavonoids, saponins, tannins and triterpenoids. Time required for death of Fasciola in the positive control, the negative control, the tea concentration of 10%, 5%, and 2.5% were 18.75, 168.75, 23.75, 42.5, and 66.25 min, respectively. *S. jamaicensis* tea with a concentration of 10% showed comparable effectiveness (p>0.05) to standard reference 10% Albendazole as an anti-Fasciola and resulted in the fastest death of Fasciola fluke compared to other concentrations of the tea. The results of this study indicate that SJ tea can be used as an alternative in overcoming Fasciola infestation. Further works are required to determine its safety when used *in vivo.*

1. **Introduction**

Fasciola sp. also known as liver flukes are commonly found in the liver tissue of ruminants [1] and cause a disease called Fasciolosis. The prevalence of ruminants Fasciolosis in Indonesia varies with the range from 40 to 95%, and is a major problem in the development of the livestock industry [2,3]. In cattle, prolonged mild liver fluke infection can cause inability to gain weight, weakened body condition, decreased appetite, swelling under the jaw, bloated stomach and can cause death. In a chronic condition, the liver fluke can damage liver tissue, resulting in cows experiencing weight loss, decreased milk production, anemia, slowed growth, bile inflammation and even death [4]. Fasciolosis in general causes huge economic losses of US$ 200 million per year for the livestock sector [1]. Therefore, special control of this disease is important to prevent economic losses and transmission of the disease to other livestock or to humans.

Fasciolosis control can be carried out pharmacologically or non-pharmacologically. Non-pharmacological controls involve regular livestock inspections, improving feeding procedures such as avoiding taking hay obtained from around the cage and terminating the life cycle of worms. Termination
of the life cycle can be done by avoiding grazing cattle in the morning, so that livestock do not consume grass tips that are still wet with morning dew that may contain metacercaria. Pharmacological controls involve the use of five groups of chemical substances including halogenated phenol groups (Bithionol, Hexachlorophene, Nitroxinyl), salicylanides (Klosantel, Rafoxanide), benzimidazoles (Triclabendazole, Albendazole, Mebendazole), sulfonamides (Chlorsulone), and phenoxyalkanes such as Diamfenetide [1].

The first-line treatment of Fasciolosis in cattle and other ruminants that is widely used is Albendazole 15 mg/kg BW orally with the ability to kill worms by 60-70% [5,6]. Albendazole is an anthelmintic with a very broad spectrum. Pharmacologically, benzimidazole works by inhibiting mitochondrial fumarate reductase, releasing phosphorylation and binding to beta-tubulin, thereby inhibiting polymerization. In adult worms, Albendazole and its metabolites act by inhibiting microtubule synthesis, thereby reducing glucose uptake irreversibly, resulting in paralysis of the worms [6].

Long-term use of high doses of Albendazole, however, may cause hepatic injury [7], congenital abnormalities and can even damage the fetus [8]. Inappropriate use of Albendazole can cause resistance [9,10] and has been reported to result in drug residues in meat as well as the environment [11,12]. To overcome the negative effects of Albendazole usage, it is necessary to explore alternative treatments using a safe and environmentally friendly agent such as anthelmintic derived from plant materials.

Stachytarpeta jamaicensis L. Vhal (SJ) leaves are empirically used as anti-parasitic drugs [13]. These leaves in some countries are used as tea, traditional medicinal tea, as a treatment for diseases caused by parasites [13]. S. jamaicensis plant contains alkaloids, flavonoid glycosides and tannins which are thought to function as anthelmintics [14,13]. An in vitro study had shown that 10% hydroalcoholic extract of SJ leaf can inactivate 90% of Strongyloides stercoralis, a worm from the nematode class, within 42 hours [15]. However, the anthelmintic effect of these leaves on worms of the Trematoda class, especially Fasciola sp. is very limited. This study aimed to determine the anthelmintic activity of SJ leaf tea against Fasciola sp.

2. Materials and methods

2.1. Preparation and manufacture of S. jamaicensis tea

S. jamaicensis leaves samples were obtained from Pagutan Village, Batukliang District, Central Lombok Regency. Prior to the preparation and manufacture of SJ leaf tea, the leaves samples were sent to the Bandungense Herbarium of School of Life Sciences, Bandung Institute of Technology for sample determination.

A total of 1 kg of SJ leaves were collected in a container. The leaves were then cleaned, sorted wet, cut, dried, sorted dry, and powdered into 10/40 coarse powder [16]. The resulting SJ powder was then packed in tea bags weighing 5 grams each. S. jamaicensis leaf tea was evaluated further for tea weight variation through weighing according to Agoes’s procedures [16], water content determination using the gravimetric method [17], and phytochemical screening based on procedure of Shanmugam et al. [18].

2.2. Collection and determination of Fasciola sp

Fasciola sp. (liver fluke) were obtained from the liver and bile ducts of cattle obtained at a slaughterhouse in Mataram City. The flukes were collected in a glass container containing a pH 7.8 physiological medium (0.9% NaCl, 0.1M NaOH). The fluke samples were then taken to the laboratory and identified morphologically to ensure that the samples used in the study were Fasciola sp.

2.3. Anthelmintic activity assay

This research was a laboratory experimental research using a post-test with control group design. The group consisted of three groups of test solutions made from the SJ leaf tea with concentrations (w/v) of 2.5% (SJ1), 5% (SJ2), and 10% (SJ3); negative control (N) was a physiological solution of pH 7.8
containing 0.9% NaCl and 0.1 M NaOH; positive control (P) was commercial anthelmintic Albendazole with 10% concentration. All treatments were replicated three times.

The negative control, positive control and all of the test solutions were each poured as much as 2 ml in 15 petri dishes, then incubated for 30 min at 37°C. After the incubation process, one sample of Fasciola fluke was immersed into each petri dish. All petri dishes were incubated and the fluke movement was observed using a digital microscope with a magnification of 40x every 5 min till its death (death was confirmed when the flukes lost their motility when pointing them with needle). The dead Fasciola flukes as a result of the treatment were marked by the staining with 1% methylene blue. Time required for the complete death of Fasciola flukes for each treatment was recorded for further analysis [19].

2.4. Data analysis

Data collected were analysed using Kruskal Wallis and Mann-Whitney tests using Statistical Package for Social Sciences (SPSS-16).

3. Results and discussion

3.1. Determination of plant sample and evaluation of S. jamaicensis tea

This research was a laboratory experimental research using a post-test with control group design. The group consisted of three groups of test solutions made from the SJ leaf tea with concentrations (w/v) of 2.5% (SJ1), 5% (SJ2), and 10% (SJ3); negative control (N) was a physiological solution of pH 7.8 containing 0.9% NaCl and 0.1 M NaOH; positive control (P) was commercial anthelmintic Albendazole with 10% concentration. All treatments were replicated three times.

Evaluation of SJ leaf tea includes tea weight variation, water content, and phytochemical screening.

The weight uniformity test aims to ensure that each tea bag contains simplicia powder with the same weight so that there is uniformity in the levels of active substances in it. The test results as depicted in Table 1 show that the weight uniformity test had met the requirements where none of the ten tea bags exceeded the maximum limit of 10% of 5 grams [16].

| No | Mean ± SD     | No | Mean ± SD     |
|----|---------------|----|---------------|
| 1  | 5.0565 ± 0.0002 | 6  | 5.0455 ± 0.0001 |
| 2  | 5.0277 ± 0.0001 | 7  | 5.0252 ± 0.0002 |
| 3  | 5.0435 ± 0.0004 | 8  | 5.0381 ± 0.0000 |
| 4  | 5.0241 ± 0.0001 | 9  | 5.0440 ± 0.0000 |
| 5  | 5.0043 ± 0.0001 | 10 | 5.0273 ± 0.0001 |

| Code | Water content percentage (%) | Code | Water content percentage (%) |
|------|------------------------------|------|------------------------------|
| 1    | 10.88                        | 6    | 11.28                        |
| 2    | 11.30                        | 7    | 11.39                        |
| 3    | 11.23                        | 8    | 11.28                        |
| 4    | 11.28                        | 9    | 11.43                        |
| 5    | 11.30                        | Mean ± SD | 11.26±0.156                |

Measuring the water content of a material is important to avoid the growth of microorganisms such as molds or fungus. High water content can also lead to enzymatic processes and hydrolysis processes that can damage simplicia [20]. Water content of the SJ tea as measured by gravimetric test (Table 2) shows an average water content of 11.26±0.156%. This result exceeds the water content standard set
by the Indonesian Food and Drug Administration which is 10% [21]. This high-water content may be due to the lack of drying of the material under the sun, and other factors such as storage of the teabags that do not use silica gel so that the humidity is not controlled.

Table 3 depicts the results of qualitative phytochemical analysis of the SJ leaf tea. The data show the presence of major secondary plant metabolites such as alkaloids, flavonoids, saponins, tannins, triterpenoids, but no steroids were found. The results of this study, especially the presence of secondary metabolites of steroids, differ from the results of a study conducted by Udodeme et al. [22], which reported the presence of steroids in the extract of *S. jamaicensis Linn. (Vahl) (Verbenaceae)*. Differences in environments where plants grow may have contributed to these results. A study conducted by Liu [23] (2000) showed that the biosynthesis of an indole alkaloid was affected by drought conditions. In line with the results of Liu's study, King et al. [24] (2004) also stated that environmental conditions such as water stress, nutrient availability and high CO$_2$ also affect the concentration of secondary metabolites in plants.

**Table 3. Phytochemical analysis of *S. jamaicensis* tea**

| Compound      | Results | Indicator                                                                 |
|---------------|---------|----------------------------------------------------------------------------|
| Alkaloid      | +       | Formation of a white precipitate with Mayer's reagent and a brown pre precitate on Dragendorff's reagent |
| Flavonoid     | +       | There was a change in colour of the solution to yellow with the addition of Mg powder and HCl concentrated. |
| Saponin       | +       | The formation of foam (1-3 cm) after shaking                               |
| Tannins       | +       | The appearance of blackish green colour after the addition of 1% FeCl$_3$   |
| Steroid       | -       | The colour changes to brownish red when reacted with H$_2$SO$_4$           |
| Triterpenoids | +       |                                                                           |

(+) = detected; (-) = not detected

3.2. Determination of Fasciola sp

The morphometric identification shows that the flukes collected in this study were Fasciola sp. namely *Fasciola gigantica* species (Figure 1).

**Figure 1.** Morphometric identification of worms. (a) Fluke sample from this study; (b) Fluke from the study of Valino et al. [25].

BW = Body width; BL = Body length; CW = Cone width; CL = Cone length

3.3. Evaluation of anthelmintic activity

Anthelmintic assay was conducted to determine the most effective concentration of SJ leaf tea used as an anthelmintic. In this assay, the negative control used was a physiological media to mimic the living
conditions of the worms in the host's body, as well as to determine the length of the test time. Whilst the positive control (10% Albendazole) was used to compare the effectiveness of the SJ tea with commercial drugs that have been used in the treatment of Fasciola sp. infestation. Kristiyani et al. [26] in their study reported that Albendazole has an effectiveness of 75% in killing adult worms and reducing helminth eggs. Albendazole acts by binding to β-tubulin on the colchicine-sensitive site of the cell wall thereby inhibiting polymerization and formation of worm tubules. Albendazole can also reduce glucose uptake so that the worms are paralyzed and even die. At a high concentration, Albendazole can interfere with metabolism and inhibit worm enzymes [27].

The time required for death of Fasciola in all treatments is presented in Table 4. The positive control (P) showed the shortest worm death time (18.750±13.768 min). The worm death time in all concentrations of SJ tea (SJ1, SJ2 and SJ3) was faster than the negative control (N) indicating that the tea has an anti-Fasciola effect. The increase in the concentration of the test solution was positively correlated with the anthelmintic activity of the test solution. This is similar to the results of the study by Saowakon [28] which showed that the higher the concentration of water extract of *Artocarpus lakoocha* bark, the higher the anti-Fasciola activity. The anthelmintic effect of SJ tea is in accordance with the results of phytochemical screening which shows that the tea contained alkaloids, flavonoids, tannins, saponins and triterpenoids that can damage or kill worms. Many medicinal plants have been reported for use as deworming agents, especially those with a high content of condensed tannin known to possess a good anthelmintic effect [29,30,31].

| Group                                      | Worm death time (min) ± SD |
|--------------------------------------------|---------------------------|
| P (positive control)                       | 18.750±13.768             |
| N (negative control)                       | 168.750±22.867            |
| SJ1 (2.5% *S. jamaicensis* tea concentration) | 66.250±8.530              |
| SJ2 (5% *S. jamaicensis* tea concentration) | 42.500±11.900             |
| SJ3 (10% *S. jamaicensis* tea concentration) | 38.750±26.880             |

Each secondary metabolite has a different mechanism of action as an anthelmintic agent. The mechanism of action of alkaloids is to break down cell walls, proteins and lipid content of the worms, resulting in death. Flavonoids work by inhibiting the NAD+ catabolizing enzyme that regulates calcium content so that it interferes with worm motility [32] (Neves et al., 2015). Saponins can increase calcium flow so that there is an increase in worm muscle activity which causes paralysis [33] while tannins cause lysis of worm cell membranes due to the destruction of worm membranes and proteins [32]. Triterpenoid compounds work by inhibiting the enzyme thioredoxin glutathione reductase which plays a role in cellular processes such as DNA synthesis so that worm reproduction stops [32].

Further data analysis using Mann-Whitney test (Table 5) shows that all SJ tea concentrations caused significantly more Fasciola fluke death when compared to the negative control (p<0.05). The 10% SJ tea concentration had an anti-Fasciola activity comparable to 10% Albendazole. This was indicated by the non-significant difference between the 10% concentration of the SJ tea group and the positive control group (p>0.05). The results of statistical analysis also show that the tea with a concentration of 5% had an activity that was comparable to the concentration of 10% (p>0.05) but not comparable to the positive control of Albendazole. Thus, amongst the concentrations of the test solution used, the 10% concentration was the most effective against Fasciola in the *in vitro* test.
Table 5. Mann-Whitney test amongst test groups

| Groups          | Asymptotic Significance | Groups          | Asymptotic Significance |
|-----------------|-------------------------|-----------------|-------------------------|
| P vs N          | 0.000*                  | N vs SJ2        | 0.000*                  |
| P vs SJ3        | 0.064                   | N vs SJ1        | 0.000*                  |
| P vs SJ2        | 0.003*                  | SJ3 vs SJ2      | 0.332                   |
| P vs SJ1        | 0.000*                  | SJ3 vs SJ1      | 0.032*                  |
| N vs SJ3        | 0.000*                  | SJ2 vs SJ1      | 0.009*                  |

* = significantly different (p < 0.05)

P = 10% Albendazole (positive control); N = 0.9% NaCl (negative control); SJ1 = 2.5% *S. jamaicensis* tea; SJ2 = 5% *S. jamaicensis* tea; SJ3 = 10% *S. jamaicensis* tea

In vitro screening of plants to determine their potential anthelmintic activity may not correlate with the in vivo activity and toxicity profile of plants. The importance of conducting in vitro testing may lie in the ease and speed of finding the right plants as candidates for in vivo tests. Further studies are certainly needed to determine the anthelmintic activity of SJ leaf tea in vivo and to test its toxicity so that it can be widely used as an alternative in the treatment and control of Fasciolosis.

4. Conclusion

The study concludes that all concentrations of SJ tea tested in vitro had an anthelmintic activity against Fasciola sp. The anthelmintic activity corresponds to the presence of secondary metabolites such as alkaloids, flavonoids, saponins, tannins and triterpenoids. The SJ tea at a concentration of 10% showed anthelmintic activity comparable to the commercial anthelmintic Albendazole 10%. Plants-sourced medicine is considered as promising alternative treatments in the development of effective deworming agents.

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