Wudani leaf extract (Quisqualis indica linn) as traditional medicine to control the incidence of cattle worm

Ida Bagus Komang Ardana,1 Made Suma Anthara,2 Anak Agung Gede Oka Dharmayudha,2 Anak Agung Ngerah Subawa,3 D. K. Harya Putra4

ABSTRACT

Background: The present research work was aimed to study whether application of 10% extract of wudani leaf (Quisqualis indica Linn) may decrease the potential of becoming embryos of eggs of Fasciola gigantica and Paramphistomum sp. worms under in-vitro evaluation. Methods: It was used three doses of the extract; those were 0.5 ml/40 ml of physiological NaCl, 1.0 ml/40 ml NaCl and 2.0 ml/40 ml of NaCl and with 5 repetitions for each dose. Assessments of the ovicidal effect of extract that is reducing the potential of eggs to become embryo were conducted at day-10 and day-30 at the Parasitology Laboratory of The Faculty of Veterinary Medicine, Udayana University, Bali. Results: Results showed that assessment at day-10, the ovicidal ability of the extract at 0.5 ml/40 ml NaCl for Fasciola gigantica eggs was 23.8% and was significantly lower (P < 0.05) than that of the dose of 1 ml/40 ml (33.1%) and of the dose of 2 ml/40 ml (35.9%). However, it was significantly higher than the control (10.4%). Moreover, the ovicidal ability of dose of 1 ml/40 ml and 2 ml/40 ml did not differ significantly (P > 0.05). At Day-30, the ovicidal ability of 1 ml/40 and 2 ml/40 ml was very high, that was 88.8% and 88.5%, respectively. Furthermore, for eggs of Paramphistomum sp., assessment at day-10 showed that the ovicidal ability of the dose of 2 ml/40 ml was the highest (23.3%) and significantly higher (P < 0.05) than that of the dose of 1 ml/40 ml (21.6%), 0.5 ml/40 ml (11.6%) and of the control (9.4%). On the other hand, there were no significant difference were noted between the dose of 1 ml/40 ml, 2 ml/40 ml and the control (P > 0.05). Likewise, at day-30 of assessment, the ovicidal ability of 1 ml/40 ml (51.9%) and 2 ml/40 ml (53.4%) was significantly higher (P < 0.01) than that of control. The high ovicidal ability of 10% extract of wudani leaf at the dose of 1 – 2 ml/40 ml NaCl may be related to damage of egg shells caused by active ingredient of the extract. Conclusion: Since infection of lungworm predominantly occurs in cattle, the present results may reveal the opportunity of applying the wudani leaf extract to control lungworm infection in cattle. Further study is definitely needed.

Keywords: eggs of Fasciola sp. and Paramphistomum sp., extract of wudani (Quisqualis indica Linn), ovicidal ability, in-vitro study.


INTRODUCTION

It has been known that in Indonesia, disease infestations are still major problems for cattle rearing and production. One of those is Fasciolosis due to infection of Fasciola hepatica, which may cause a total loss of 513 million rupiah per year. The disease causes loss of body weight, untreated liver damage and reproductive constraints.1 Moreover, it has been reported that worm infections have widely spread in Indonesia. Generally, the prevalence of worm infection in Indonesia is very high, particularly in agricultural landscape, near lakes and areal with high temperature and humidity. In Bali, the high prevalence of Fasciola infection was noted in the Region of Karangasem (18.29%) and it can be related with seasonal change, poor management and sanitation and low level of education of farmers. Meanwhile, the prevalence of Fasciola hepatica infection recorded in abattoir in Samarinda in 2009 was 55.56%.1 Furthermore, Agustina et al. reported that prevalence of Toxocara vitulorum infection in Bali cattle raised in eastern part of Bali is 39.4%, whereas there were no records reported for Indonesia.2 However, survey conducted in 2014 noted that Paramphistomum sp. were found in almost all cattle slaughtered at Pesanggrahan and Darmasaba abattoirs in Bali.

The high prevalence of worm infection in cattle may be related to farmer ignorance and their lack of knowledge in preventing the diseases, the unaffordable expensive medicines to control the diseases, and the improper use of medicines that lead to disease resistant. Due to particularly problems related to availability and the expensive price of medicines, alternative solution to control the diseases should be found for the benefit of farmers. It has been known that Indonesia has abundance of herbs that has medical properties and, therefore, research work should be encouraged to explore...
them. One of those herbs that has been known to have such medical properties is wudani (Quisqualis indica Linn) that available widely throughout Indonesia and has various names i.e dani, udani, bidai, kacekluk, kaceklik, wedani, tikao, rabedani, and saradengan. At the level of community, wudani belonging to the Combretaceae family has been used as traditional medicine to cure various illness such as diarrhea, headache, rheumatism, immune-modulator, anti-inflammation, antioxidant, and vermicide. Condensed tannins (CT) extracted from various forage can markedly decrease the viability of the larval stages of several nematodes in sheep and goats. Suma Antara et al reported that application of 10% wudani extract to piglets at a dose of 5 ml/8 kg of body weight each day for 3 days can diminish infection of Ascaris suum and Trichuris sp. However, they did not find out whether its effect is through its property as either ovicidal, larvaecidal, or vermicidal. Thus, the work reported in this paper dealt with such research questions.

METHODS

Extraction and Suspension of wudani leaf
The extract was made by macerating 50 grams of fresh wudani leaf, ground, added with 70% ethanol, and following which it was stored in capped container away from sunlight for 2 days. After 2 days of storage, it was then filtered and the residue obtained was macerated again with 70% ethanol by following the same procedure. Maceration was done until it was obtained clear macerate which then evaporated in rotating vacuum evaporator at 40°C and then dried in freeze drier. Suspension of extract wudani leaf was made at concentration of 10% w/v; 10 grams of the extract was added with aquabidest till the total volume was 100 ml.

Phytochemical screening of the wudani leaf extract
Phytochemical screening was used in order to detect the content of available chemical substances that have biological activities. Method to detect the availability of alkaloid, flavonoid, tannin, saponin and steroid are as follows.

1. Detection of alkaloid:
   a. With the use of Wagner reactant; one ml of wudani leaf extract is added with few drops of reactant; the reaction is considered positive when brown sediment is formed.
   b. With the use of Meyer reactant; one ml of extract is added with few drops of concentrated HCl and Mg powder. Positive reaction occurs when red-orange color is formed.

2. Detection of flavonoid was conducted as follows.
   a. With the use of 10% NaOH reactant; one ml of the extract was added with few drops of reactant and reaction is considered positive when specific color is formed.
   b. With the use of Wilstater reactant; one ml of the extract was added with few drops of concentrated HCl and Mg powder. Positive reaction occurs when red-orange color is formed.

3. Detection of saponin was conducted by adding hot water to the extract and then agitated. Reaction is considered positive when foam is formed.

4. Detection of poliphenol was conducted by adding few drops of 1% FeCl$_3$ reactant into one ml of the extract; Positive reaction occurs when black or dark blue color is formed.

5. Detection of poliphenol was conducted by adding few drops of unlhidrate acetate and concentrated H$_2$SO$_4$ into one ml of the extract. When green-blue color is formed, it indicates the positive reaction towards steroid and the color formation of red-violet, brown indicates the positive reaction towards tripernoid.

Determination of ovicidal ability
Collected eggs of Fasciola gigantica and Paramphistomum sp. obtained from the abattoir was initially put to hatch in media of 40 ml of physiological NaCl and with density of 7-9 eggs/µl. Then they were added with 10% wudani leaf extract at dose of 0.5 ml/40 ml NaCl (P1), 1 ml/40 ml (P2), and 2 ml/40 ml NaCl (P3). The control group (P0) was without wudani leaf extract. Six duplications were made for each group. After 24 hours, the wudani extract was taken away and replaced by physiological NaCl and then incubated for as long as 30 days. The doses used in the current work were based on previous results by Antara et al. They recorded that 10% wudani leaf extract, at a dose of 5 ml/8 kg of body weight or 0.625 ml/kg of body weight was effective in killing Ascaris suum in pigs. Moreover, in a preliminary study on cows, they found an effective dose of...
The incubated eggs were aerated every day in order to observe egg development and embryo ability of each egg was noted at day 10 and day 30 of incubation. Data for embryo ability was then collected and examined as follows. The number of fertile eggs was calculated from 100 eggs from each group and the embryo ability is the percentage of fertile eggs or eggs with embryo in it.

The ability of 10% wudani leaf extract in killing embryo or its ovicidal ability was calculated by dividing the number of unhatched eggs with the number of eggs examined times 100%.

### RESULTS

Prior to its application, screening of wudani leaf extract was made for its contents and the results were shown in Table 1.

**Table 1** Results on assessment of chemical substances found in wudani leaf extract

<table>
<thead>
<tr>
<th>No.</th>
<th>Phytochemical screening</th>
<th>Reactant</th>
<th>Change of color</th>
<th>Note</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alkaloid</td>
<td>HCl 2N + Wagner reactant + chloroform</td>
<td>Brown sediment occurred; Chloroform phase was red in color.</td>
<td>Alkaloid (+)</td>
</tr>
<tr>
<td>2</td>
<td>Flavonoid</td>
<td>Wilstater</td>
<td>Brownish green changes into dark brown. Brownish green changes into yellowish green</td>
<td>Flavonoid (+)</td>
</tr>
<tr>
<td>3</td>
<td>Saponin</td>
<td>aquadest, warmed, stirred K, + HCl 2M</td>
<td>Brownish green changes into blackish green</td>
<td>Saponin (+)</td>
</tr>
<tr>
<td>4</td>
<td>Fenol</td>
<td>FeCl₃</td>
<td>Brownish green changes into blackish green</td>
<td>Fenol (+)</td>
</tr>
<tr>
<td>5</td>
<td>Triterpenoid/Steroïd</td>
<td>Lieberman-Burchard H₂SO₄</td>
<td>Brownish green changes into blackish green</td>
<td>Steroid (+)</td>
</tr>
<tr>
<td>6</td>
<td>Tannin</td>
<td>FeCl₃</td>
<td>Brownish green changes into blackish green</td>
<td>Tannin (+)</td>
</tr>
</tbody>
</table>

Note: (+) indicate the availability of chemical substance

**Table 2** Ovicidal ability of 10% wudani leaf extract towards eggs of Fasciola gigantica following incubation for 30 days

<table>
<thead>
<tr>
<th>Group</th>
<th>10 days</th>
<th>30 days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fasciola gigantica</td>
<td>Fasciola gigantica</td>
</tr>
<tr>
<td>P0</td>
<td>10.346 a</td>
<td>13.098 a</td>
</tr>
<tr>
<td>P1</td>
<td>23.812 b</td>
<td>51.494 b</td>
</tr>
<tr>
<td>P2</td>
<td>33.104 c</td>
<td>86.810 c</td>
</tr>
<tr>
<td>P3</td>
<td>35.849 c</td>
<td>88.502 c</td>
</tr>
</tbody>
</table>

Note: P0: Control
P1: Dose of 0.5 ml extract/40 ml physiological NaCl
P2: Dose of 1 ml extract/40 ml physiological NaCl
P3: Dose of 2 ml extract/40 ml physiological NaCl

0.625/50 ml of rumen fluid or 0.0125 ml/ml for similar purposes.

The incubated eggs were aerated every day in order to observe egg development and embryo ability of each egg was noted at day 10 and day 30 of incubation. Data for embryo ability was then collected and examined as follows. The number of fertile eggs was calculated from 100 eggs from each group and the embryo ability is the percentage of fertile eggs or eggs with embryo in it. The ability of 10% wudani leaf extract in killing embryo or its ovicidal ability was calculated by dividing the number of unhatched eggs with the number of eggs examined times 100%.

**Ovicidal ability of wudani leaf extract towards Fasciola gigantica eggs**

Table 2 showed the ovicidal ability of 10% wudani leaf extract towards eggs of Fasciola gigantica. It has been noted that at day 10 of assessment, ovicidal ability of the extract towards the eggs was significant (P < 0.05) whereas on day 30 it was highly significant (P < 0.01).

From the current results, it can be noted that ovicidal ability of the extract at 0.5 ml/40 ml NaCl was 23.8% which is significantly lower (P < 0.05) compared to that of dose of 1 ml/40 ml (33.1%) and of those of 2 ml/40 ml NaCl (35.9%). However, it was significantly higher than ovicidal ability of the control group (10.4%). On the other hand, the ovicidal ability of dose of 1 ml/40 ml and 2 ml/40 ml was not differ significantly (P > 0.05). Moreover, ovicidal ability assessed at day 30 showed a highly significant increase (P < 0.01). Ovicidal ability of the extract at dose of 1 ml/40 ml and 2 ml/40 ml was not differ significantly (P > 0.05). When compared to dose of 0.5 ml/40 ml (51.5%) and the control group (13.1%), however, a highly significant difference (P < 0.01) was noted.

**Ovicidal ability of 10% wudani leaf extract towards eggs of Paramphistomum sp.**

Table 3 showed the ovicidal ability of 10% wudani leaf extract towards eggs of Paramphistomum sp. during in-vitro assessment.

It has been recorded that the extract has a significant ovicidal ability towards the eggs that were assessed at day 10 and day 30 of incubation. At day 10 of incubation, the ovicidal ability of the extract at dose of 2 ml/40 ml was the highest (23.3%) and significantly higher (P < 0.05) than that of dose of 1 ml/
Table 3  Ovicidal ability of 10% wudani leaf extract towards eggs of *Paramphistomum* sp. incubated for 10 and 30 days

<table>
<thead>
<tr>
<th>Group</th>
<th>Paramphistomum 10 days</th>
<th>Paramphistomum 30 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>P0</td>
<td>9.364 a</td>
<td>8.686 a</td>
</tr>
<tr>
<td>P1</td>
<td>11.626 ab</td>
<td>40.246 b</td>
</tr>
<tr>
<td>P2</td>
<td>21.630 bc</td>
<td>51.972 c</td>
</tr>
<tr>
<td>P3</td>
<td>23.342 c</td>
<td>53.466 c</td>
</tr>
</tbody>
</table>

Note:
P0 : Control
P1 : Dose of 0.5 ml extract/40 ml physiological NaCl
P2 : Dose of 1 ml extract/40 ml physiological NaCl
P3 : Dose of 2 ml extract/40 ml physiological NaCl

Figure 1  Morphology of egg shell of *Fasciola* sp. following soaking with physiological NaCl for 24 hours; the shell looks normal. Magnification: 500 ×

Figure 2  Morphology of egg shell of *Fasciola* sp. after soaking with 10% wudani leaf extract at a dose of 0.5 ml/40 ml physiological NaCl for 24 hours. Changing in surface of egg shell occurred. Magnification: 500 ×

Figure 3  Morphology of egg shell of *Fasciola* sp. following soaking with 10% of wudani leaf extract at a dose of 1 ml/40 ml physiological NaCl for 24 hours. Changing in surface of egg shell occur and porosity can be seen. Magnification: 500 ×

Figure 4  Morphology of egg shell of *Fasciola* sp. following soaking with 10% wudani leaf extract at a dose of 2 ml/40 ml physiological NaCl for 24 hours. Changing in shell surface and porosity can be observed. Magnification: 500 ×

Figure 5  Morphology of egg shell of *Paramphistomum* sp. following soaking with physiological NaCl for 24 hours. The shell looks normal. Magnification: 500 ×
40 ml (21.6%), of 0.5 ml/40 ml (11.6%), and of control (9.4%). However, among the latest three doses, there were no significant different (P > 0.05) was noted.

At day 30 of incubation, there was no difference (P > 0.05) in ovicidal ability between dose of 1 ml/40 ml (52%) and of 2 ml/40 ml (53.5%). However, it was significantly higher (P < 0.05) compared to dose of 0.5 ml/40 ml (40.3%) and even a highly significantly different (P < 0.01) compared to that of control (8.7%) was recorded.

The ability of 10% wudani leaf extract in damaging egg shells

**FASCIOLA GIGANTICA EGGS**

Assessment using a Scanning Electron Microscopy (SEM) on the effect of soaking eggs with 10% wudani leaf extract at dose of 0 ml (control), 0.5 ml/40 ml NaCl, 1 ml/40 ml NaCl, and 2 ml/40 ml NaCl on morphology of egg shell are presented in Figure 1, Figure 2, Figure 3 and Figure 4, respectively.

**Eggs of Paramphistomum sp.**

Assessment using SEM on the effect of soaking with 10% wudani leaf extract at dose of 0 ml (control), 0.5 ml/40 ml physiological NaCl, 1 ml/40 ml physiological NaCl and 2 ml/40 ml physiological NaCl on morphology of egg shells are presented in Figure 5, Figure 6, Figure 7 and Figure 8, respectively.

**DISCUSSION**

The present research work has confirmed that extract of wudani leaf (*Quisqualis indica*) positively contains alkaloid, flavonoid, saponin, phenol, tripernoid/steroid and tannin. In addition to those contents, it has been found that it also contains vitamin, protein, amino acids, and glycoside. So far, as herbal plant, wudani has been used by people as traditional medicine to cure health problems related with worm, pain, diarrhea, head ache, rheumatism, as well as used as an imunomodulator, anti-inflammation, anti-staphilococcus, and anti-oxidant. The extract has property as anti-bacteria against *Salmonella typhi* in vitro as reported by Noorhamdani et al.

The current results showed the ovicidal ability of wudani leaf extract towards eggs of *Fasciola gigantica* (88.5%) and *Paramphistomum* sp. (53.5%).

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**Figure 6** Morphology of egg shell of *Paramphistomum* sp. following soaking with 10% wudani leaf extract at a dose of 0 ml/40 ml physiological NaCl for 24 hours. A significant damage in shell can be observed. Magnification: 500 ×

**Figure 7** Morphology of egg shell of *Paramphistomum* sp. following soaking with 10% wudani leaf extract at a dose of 1 ml/40 ml physiological NaCl for 24 hours. As in the previous picture, a significant damage can be observed. Magnification: 500 ×

**Figure 8** Morphology of egg shell of *Paramphistomum* sp. following soaking with 10% wudani leaf extract at a dose of 2 ml/40 ml physiological NaCl for 24 hours. Similar damage as shown in the previous pictures occurred. Magnification: 500 ×
The significant difference in ovicidal ability between the two species of worm indicated that its direct contact to egg shells has resulted in different effect to the egg shells. As mentioned above, wudani leaf extract contains alkaloid and glycoside and has ovicidal property. As reported earlier by Yongabi, alkaloid has the lowest ovicidal ability followed by glycoside and papain has the strongest ability. Moreover, as noted in the current SEM study, following soaking in the extract, structure of egg shells underwent changes as shown in Figures 2, 3, and 4 for eggs of *Fasciola gigantica* and in Figures 6, 7, and 8 for the *Paramphistomum* sp. eggs.

The eggs after being exposed to ethanol content of the extract may undergo pathological changes in albumin layer of egg shell in which some parts were thickened and others were thinner. Ardana reported that eggs of *Ascaris suum* soaked for 24 hours in extract of ripe banana seed also exhibit damage in their shells. The damage in egg shells of *Fasciola gigantica* and *Paramphistomum* sp., as noted in the current study, may be due to activity of alkaloid (carpain and carpasemin), tannin of ethanol extract that may have proteolytic property. It may cause coagulation of albumin. Changes in the egg shells may affect ability of eggs to form embryo. Bariah et al. reported that coagulation of albumin of egg shells prevent development of *Ascaris lumbricoides* eggs. Thus, the present results for *Ascaris lumbricoides* and *Paramphistomum* sp. have supported or in accordance with their finding.

In conclusion, coagulation of albumin layer of egg shells of *Fasciola gigantica* and *Paramphistomum* sp. following soaking in 10% extract of wudani leaf may lead to decrease in ability to form embryo or embryo development. In other word, the extract has an ovicidal property. This herbal plant has been used as traditional medicine to cure diseases caused by Ascaris and Taenia spp. have found that application of 10% extract of wudani leaf at dose of 5 ml per day for 3 days may overcome infection caused by *Ascaris suum* in pigs suffering from various degrees of infection; it also effective in combating *Trichuris* sp. infection.

**CONCLUSION**

*In vitro* application of 10% extract of wudani leaf is effectively used to overcome disease caused by worms (used as anthelmenthic). Thus, it may be further developed to be used in the future to overcome such problems in cattle.

**SUGGESTION**

Further study is needed to discovered the roles of extract of wudani leaf and the use of partition, fraction in order to know more about function of the substances or the chemical contents that have property as anthelmenthic, so that its practical application can be more effective.

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