The Vermicidal Effectivity of Wudani Leaf Extract (*Quisqualis Indica* Linn) in Treating Infection Caused by Gastrointestinal Worms in Cattle

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**Abstract**

The present study was aimed to study whether application of 100% extract of the wudani leaf (*Quisqualis indica* Linn) was able to kill gastrointestinal worms *Strongylus* sp. (Vermicidal effect). In order to make a comparison with a commercial drug commonly used, one group was treated with Rheindazol (100 ml of Rheindazol containing 10 gram of albendazole) at a single dose of 10 mg/kg of body weight (BW). The other 3 groups were subjected to a single treatment with 100% wudani leaf extract at 10 mg, 15 mg, and 20 mg/kg of BW, respectively. Assessment of its vermicidal effect was carried out by conducting an assessment on fecal egg count reduction (FECR). The results showed that the vermicidal property of 100% wudani leaf extract given at 10mg/kg BW was 100% and this was significantly (P < 0.01). Higher than that of dose of 15 kg/kg BW (92.4%), 20 mg/kg BW (96%) and even compared to that of albendazole treatment (96%). Thus, from the present in vivo study, it can be concluded that 100% wudani leaf extract, due to its vermicidal property, can be effectively used to cure gastrointestinal worm infection and, therefore, can be subsequently applied to overcome worm infection in cattle.

**Keywords:** Gastrointestinal worm, Wudani leaf extract, Vermicidal property.

**Introduction**

Gastrointestinal worm parasite, with nematode, in particular, its prevalence is very high in cattle reared in developing countries [1]. In Bali cattle, trematode worms are also found, and the cattle are subjected to various worm infections, such as fascioliasis, pharamfistomiasis, and *Toxocara vitulorum* infection [2]. It has been reported that prevalence of *Fasciola* spp infection in Bali cattle reared in Karangasem was 18.29% and at Samarinda abattoir in 2009 was 55.56% [3]. Moreover, the prevalence of *Toxocara vilorum* in Bali cattle in East Bali was 39.4% [4] whereas that for *Pharamfistomenum* in cattle reared in Indonesia has not been reported. However, a survey conducted in 2004 at Darmasaba abattoir in Denpasar showed that almost all cattle slaughtered were infected by *Pharamfistomum* (unpublished data). It has been widely known that gastrointestinal worm infection hampers growth and reproductive performances [5,6]. Moreover, it has been reported that infection of *Strongylus* sp to cattle at a young age may seriously affect the growth rate of the animal [6].

In regard to Bali cattle, this breed of cattle has great potentials to survive and adapt to a poor environmental condition in relation to the availability of good quality feed, and it has high fertility rate [7]. When such potentials are also accompanied by minimum health problems particularly of worm infection and parasite in general, then Bali cattle will be able to show its optimum
growth rate and its reproductive performance. Therefore, attempts should be made to reduce worm infection in Bali cattle. Indeed various drugs to overcome worm infections have been available commercially. Since their prices are relatively high for farmers, alternative drugs with lower price but with high efficacy are needed.

It has been widely understood that plants have various beneficial properties for a human being. One of those is the utilization of plants as traditional drugs to overcome problems with worm infections, anti-inflammatory, diarrhea, headache, rheumatism, immunomodulator, and antioxidant. Wudani (*Quisqualis indica* Linn) is one example of plants that can potentially be used as a traditional drug to overcome worm problems. Methanolic extract of wudani leaf has been reported to reduce hyperlipidemia in rats that passively exposed to cigarette smoke [8] and it has the antibacterial effect to *Salmonella typhi* infection in vitro [9].

In order to get cheap alternative drugs, focuses have been made on plants or herbal properties. Antara *et al.* [10] reported that application of 10% wudani leaf extract at a dose of 5 mg/kg to pigs for 3 days may diminish *Ascaris suum* infection in heavily infected pigs; the similar results were also noted for *Trichuris Sp* infection. Its property as the drug may be related to its alkaloid, tannin, and glycoside contents. Some research works have shown that alkaloid, tannin, and glycoside were effectively used as drugs to overcome worm infections [11] and extracts of condense tannin from various plant sources may reduce development to larvae stadium of nematode worms in goat and sheep [12]. However, it has not been reported whether its drug properties are related to its ovicidal, larvicide, or vermicidal effect of the active chemical substances. Therefore, further studies should be made.

Ardana *et al.* [13] have studied the ovicidal effect of 10% wudani leaf extract on eggs of *Fasciola hepatica* and *Pharamfistomum in vitro* and found that it can be effectively used as anthelmintic due to its ovicidal effect in damaging the worm shell. The results are quite similar to those found when *Ascaris suum* eggs were rinsed with extract of ripe papaya seed [14]. The damage of worm egg shell following treatment with wudani leaf extract may be related to the activity of its alkaloid content (carpain and carpasemin) which has proteolytic property and of its tannin content [15]. Thus, it is expected that those previous results obtained by Ardana and co-workers and results presented in the current report may lead to future utilization of herbal drugs with low price but with high efficacy in overcoming problems with gastrointestinal worm infection in cattle.

**Materials and Methods**

**The Making of Wudani leaf Extract and Suspension**

Extract of wudani leaf was made by macerating 50 gram of fresh leaf, ground using mortar apparatus, added with 70% ethanol and finally kept for 2 days in sealed vessel away from sun light. The mixture was then filtered to obtain the macerate fraction. The residu was mixed with 70% ethanol following the same procedure, and it was repeated till clear macerate was obtained. The macerate was then evaporated in rotating vacuum evaporator at 40 °C and dried in a freeze dryer. Suspension of wudani leaf extract was made at a concentration of 100%.

**Phytochemical Screening of Wudani Leaf Extract**

Phytochemical screening was applied to detect the chemical substances available in order to get initial information about the biological activities of this particular plant. Methods of detection for alkaloid, flavonoid, tannin, saponin, and steroid are as follows.

**Determination of Alkaloid**

- Using Wagner reactant: One ml of extract was added with few drops of reactant; the reaction was considered positive when brown sediment was formed.
- Using Meyer reactant: One ml of extract was added with few drops of reactant; the reaction was considered positive when white sediment was formed.

**Determination of Flavonoid**

- Using 10% NaOH reactant: One ml of extract was added with few drops of reactant; formation of specific color indicates a positive reaction.
Using Wilstater reactant: On ml of extract was added with few drops of concentrated HCl plus a small quantity of Mg powder; the reaction was considered positive when red-orange colour was formed.

Using Smith-Metacalve reactant: One ml of extract was added with few drops of concentrated HCL and then was heated; formation of white colour indicates a positive reaction.

**Determination of Saponin (Foam Testing)**

One ml of extract was added with hot water and then shaken; the reaction was considered positive when long-lasting foam occurred.

**Determination of Polyphenol**

One ml of extract was added with 1% FeCl₃ reactant: formation of black or dark blue color indicates a positive reaction.

**Determination of Steroid and Triterpenoid**

One ml of extract was added with few drops of anhydrate acetate and concentrated H₂SO₄; changing color to greenish blue indicates a positive reaction to steroid whereas changing to reddish violet, brown indicates a positive reaction to triterpenoid.

**Assessment on Vermicidal Property**

Assessment *in vivo* on the vermicidal property of wudani leaf extract was performed using Bali cattle naturally suffered from worm infection, with a number of egg per gram (EPG) ranging from 250 to 2500 eggs (*International Harmonization & anthelmintic efficacy guideline*; Vercruysse). It was firstly done by checking worm eggs cooprostatically. Cattle suffered from helminthiasis were then treated as follows. One group considered as control (P0) was treated with Rheindazol (containing 10 g albendazol/100 ml Rheindazol) at a single dose of 10 mg/kg of body weight (BW). Treatment with 100% wudani leaf extract was performed at a single dose of 10 mg/kg BW (P1), 15 mg/kg BW (P2) and 20 mg/kg BW (P3). At Day 7 after the treatments, the number of worm eggs was counted in order to obtain data on EPG.

**Determination of EPG** was conducted following modified MC Master Method.

- Four (4) gram of cattle feces was weighed.
- The feces were then put into a plastic dish with a number on it.
- The saturated salt solution was added, and the mixture was stirred until it became homogeny.
- The mixture was then filtered using cloth and the solution obtained was placed in 100 ml cylinder tube.
- The saturated salt solution was added to the mixture until the total volume became 60 ml and stirred homogenously.
- By using 10 ml pipette, the mixture was placed in the MC Master Counting Chamber and kept there for 5 – 10 minutes.
- Finally, the number of eggs was counted from each counting chamber under a light microscope at a magnification of 10 x (See Appendix 1 and 2).
- Egg per gram (EPG) was counted following the formula below.

\[
\text{EPG} = \frac{\text{solution volume}}{\text{Feces weight}} \times \frac{\text{X \ average egg number}}{\text{counting chamber volume}}
\]

Note: Solution volume = 60, feces weight = 2 gram, counting chamber volume = 0.15. The average number of eggs was that of note during the counting.

After data on EPG has been collected, determination of vermicidal property was made by counting the fecal egg count reduction (FECR); that is by counting the percentage of reduction in EPG after the experimental animals have been subjected to treatments as mentioned above. Thus, EPG before the treatment and EPG after the treatment till Day 7 were assessed, and the FECR was calculated by the following formula.
Data Analysis

Data on fecal egg count reduction (FECR) and the embryonic ability of worm eggs in feces of cattle suffering from helminthiasis was analyzed following Analysis of Variance [16] and then with Duncan’s multiple range test [17].

Results and Discussion

Table 1: Results on phytochemical screening of wudani leaf extract (Quisquallis indica Linn)

<table>
<thead>
<tr>
<th>No.</th>
<th>Phytochemical test</th>
<th>Reactant</th>
<th>Colour changing</th>
<th>Availability</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alkaloid</td>
<td>HCl 2N + Wagner reactant + chloroform</td>
<td>Formation of brown sediment</td>
<td>Alkaloid (+)</td>
</tr>
<tr>
<td>2</td>
<td>Flavonoid</td>
<td>Wilsttater reactant + Bate Smith-Metcalfe reactant</td>
<td>Chloroform phase red in color</td>
<td>Flavonoid (+)</td>
</tr>
<tr>
<td>3</td>
<td>Saponin</td>
<td>NaOH 10% + Aquadest, heated, stirred + HCl 2M</td>
<td>Brownish green to yellowish green</td>
<td>Saponin (+)</td>
</tr>
<tr>
<td>4</td>
<td>Phenol</td>
<td>FeCl₃</td>
<td>Brownish green to blackish green</td>
<td>Phenol (+)</td>
</tr>
<tr>
<td>5</td>
<td>Triterpenoid/</td>
<td>Lieberman-Burchard reactant</td>
<td>Brownish green to green</td>
<td>Steroids (+)</td>
</tr>
<tr>
<td></td>
<td>Steroid</td>
<td>H₂SO₄ reactant</td>
<td>Brownish green to green</td>
<td>Steroids (+)</td>
</tr>
<tr>
<td>6</td>
<td>Tannin</td>
<td>FeCl₃</td>
<td>Brownish green to blackish green</td>
<td>Tannin (+)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Gelatin</td>
<td>Formation of white sediment</td>
<td></td>
</tr>
</tbody>
</table>

Note: (+) indicates chemical substances being checked were available

It has been found that tannin is quite effective in controlling infection caused by gastrointestinal parasites in ruminants reared in the tropical countries [12]. Moreover, tannin extracted from the plant has been reported as an alternative drug to kill Haemonchus worm [20].

Our previous in vitro study [13] revealed that 10% wudani leaf extract given at a dose of 1-2 ml/40 ml has resulted in the highest ovicidal effect on Fasciola gigantica eggs (88.5%) and Paramphistomum sp. eggs (53.5%). The significant difference in ovicidal effect on those two different species of worms indicated that direct contact of 10% wudani leaf extract to egg shells caused the different rate of shell damage. Yongabi [11] reported that the chemical substances available in herbal extract have a different rate of ovicidal effect, with alkaloid at the lowest followed by glycoside and papain has the strongest property.

The effectiveness of vermicidal effect of certain chemical substances or in other words the anthelmintic property of drugs to cure gastrointestinal worm infection can be evaluated from a reduction in a number of worms as indicated by increasing fecal egg count reduction (FECR) and by anthelmintic efficacy (%) after drug application. Thus, the increase in FECR indicates that the number of gastrointestinal worms in the infected animals is decreasing. It has been widely understood that chemical substances that are considered to have anthelmintic property are those who have efficacy more than 70%.

Results of the current study indicated that treatment with 100% wudani leaf extract at various doses to cattle suffering from gastrointestinal worm infection led to an increase in FECR (Table 2). The average FCR was the highest for treatment with 100% wudani leaf extract at a dose of 10 mg/kg BW (100%) which was even higher than that of commercial drug albendazole (96%). Treatments with the extract at a dose of 15 mg/kg BW and 20 mg/kg BW resulted in FECR of 92.2% and 96%, respectively.

<table>
<thead>
<tr>
<th>No.</th>
<th>Reactant</th>
<th>Colour changing</th>
<th>Availability</th>
</tr>
</thead>
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<tr>
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<td>Formation of brown sediment</td>
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<td>NaOH 10% + Aquadest, heated, stirred + HCl 2M</td>
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<td>FeCl₃</td>
<td>Brownish green to blackish green</td>
<td>Phenol (+)</td>
</tr>
<tr>
<td>5</td>
<td>Lieberman-Burchard reactant</td>
<td>Brownish green to green</td>
<td>Steroids (+)</td>
</tr>
<tr>
<td></td>
<td>H₂SO₄ reactant</td>
<td>Brownish green to green</td>
<td>Steroids (+)</td>
</tr>
<tr>
<td>6</td>
<td>FeCl₃</td>
<td>Brownish green to blackish green</td>
<td>Tannin (+)</td>
</tr>
</tbody>
</table>

Note: (+) indicates chemical substances being checked were available

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Table 2: FECR in feces of cattle suffering from helminthiasis (*Strongyloides* ssp) at Day 3 after the treatment with various doses of 100% wudani leaf extract

<table>
<thead>
<tr>
<th>Treatment</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
<th>V</th>
<th>VI</th>
<th>Total</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>P0</td>
<td>96,0</td>
<td>98,0</td>
<td>94,6</td>
<td>95,4</td>
<td>94,0</td>
<td>98,0</td>
<td>576</td>
<td>96,0</td>
</tr>
<tr>
<td>P1</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>600</td>
<td>100</td>
</tr>
<tr>
<td>P2</td>
<td>92,2</td>
<td>94,0</td>
<td>90,0</td>
<td>91,4</td>
<td>93,6</td>
<td>92,0</td>
<td>553,2</td>
<td>92,2</td>
</tr>
<tr>
<td>P3</td>
<td>96,0</td>
<td>95,4</td>
<td>96,6</td>
<td>98,0</td>
<td>94,0</td>
<td>96,0</td>
<td>576</td>
<td>96,0</td>
</tr>
</tbody>
</table>

P0: albendazole at 10 mg/kg BW  
P1: 100% wudani leaf extract at 10 mg/kg BW  
P2: 100% wudani leaf extract at 15 mg/kg BW  
P3: 100% wudani leaf extract at 20 mg/kg BW

The summary of statistical analysis following Analysis of Variance of FECR data presented in Table 2 was as follows (Table 3).

Table 3: Summary of statistical analysis of FECR of cattle suffering from helminthiasis after treated with 100% wudani leaf extract at various doses

<table>
<thead>
<tr>
<th>Source</th>
<th>Type III Sum of Squares</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>182.580</td>
<td>3</td>
<td>60.860</td>
<td>36.055</td>
<td>.000</td>
</tr>
<tr>
<td>Error</td>
<td>33.760</td>
<td>20</td>
<td>1.688</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corrected</td>
<td>216.340</td>
<td>23</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>23</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

As shown in the above table (Table 3), statistical analysis of FECR revealed that treatment with 100% wudani leaf extract significantly (P < 0.01) affected the reduction in fecal egg count that is ranging from 92% to 100%. In order to find out the differences between treatments, further statistical analysis employing Duncan’s multiple range tests was performed. The FECR of cattle receiving wudani leaf extract at 10 mg/kb BW (P1 group) was 100% and significantly (P < 0.01) differed from those of treatment with the extract at 20 mg/kg BW (P2) and 15 mg/kg BW (P3) and even with those receiving the commercial anthelmintic drug albendazole (P0). Statistically, no difference in FECR between albendazole treatment (P0) and treatment with the extract at 20 mg/kg BW (P3) was noted, though it was slightly higher than P2 group (Table 4).

Table 4: Summary of statistical analysis following Duncan’s multiple range test of the’ FECR of worm-infected cattle after treated with various doses of 100% wudani leaf extract and commercial anthelmintic drug

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Mean</th>
<th>0.05</th>
<th>0.01</th>
</tr>
</thead>
<tbody>
<tr>
<td>P0</td>
<td>96.000</td>
<td>A</td>
<td>a</td>
</tr>
<tr>
<td>P1</td>
<td>100.000</td>
<td>C</td>
<td>c</td>
</tr>
<tr>
<td>P2</td>
<td>92.200</td>
<td>B</td>
<td>b</td>
</tr>
<tr>
<td>P3</td>
<td>96.000</td>
<td>A</td>
<td>a</td>
</tr>
</tbody>
</table>

Note: the same letter at the same line indicates non-significant different (P >0.05)  
P0 = treatment with albendazole  
P1 = treatment with the extract at 10 mg/kg BW  
P2 = treatment with the extract at 15 mg/kg BW  
P3 = treatment with the extract at 20 mg/kg BW
Thus, the present results demonstrated that treatment with 100% ethanol extract of wudani leaf at various doses might significantly reduce the number of worm eggs in cattle suffering from gastrointestinal worm infection as indicated by the high FECR (fecal egg count reduction). Moreover, the FECR of cattle subjected to treatment with the herbal extract was as high as that of treatment with gold-standard of commercial drug albendazol as shown in the following histogram (see Graphic 1).

From the above histogram, it is quite obvious that the FECR of all treatments are more or less similar in their height that may indicate the similar reduction in the number of worm eggs in the infected experimental animals. Therefore, it may be concluded that the herbal extract tested in the present study is as effective as the widely used commercial anthelmintic drug albendazol in controlling gastrointestinal worm infection in cattle.

Assessment of FECR at Day 7 after the treatments showed results as presented in Table 5. The FECR for all treatments were 100%, respectively. Thus, the results may further confirm the efficacy of treatments with 100% wudani leaf extract as an alternative herbal drug for controlling gastrointestinal worm infection in cattle and with vermizidal property as good as that of commercial drug albendazol.

The high vermizidal property of ethanol extract of wudani leaf may due to its active chemical substances that may inhibit glucose uptake and reduce level of glycogen, phosphomonoesterase acid, and alkaline phosphomonoesterase that, in turn, may cause the gastrointestinal worms to undergo cholinesterase and increased lactic acid concentration (Sing and Nagaiah, 1999). It has been reported that treatment of goat suffering from nematode infection with tannin extracted from certain plants may reduce the number of worm eggs in the gastrointestinal tract of the goat.

Regarding traditional utilization of wudani (Quisqualis indica Iinn), this herbal plant has been known and used by the local community to control infection of various species of gastrointestinal worms. Antara et
al. [10] reported that application of 10% wudani leaf extract at a dose of 5 ml/day for 3 days to pigs might cure Ascaris suum infection as well as infection caused by Trikuris Sp.

As a conclusion, the ethanol extract of wudani leaf may be effectively used to control gastrointestinal worm infection in cattle due to its high vermicidal property that is comparable to that of commercial anthelmintic drug albendazole. Thus, this herbal extract may provide an alternative solution for controlling worm infections faced by farmers in Bali who reared Bali cattle that is susceptible to various worm infections. In addition to its efficacy that almost similar to that of the commercial drug, the ethanol extract of wudani leaf is much cheaper and thus should become the alternative problem solver chosen by farmers [21-27].

Conclusion and Suggestion

Conclusion

Results of the present in vivo study conclude that ethanol extract of 100% wudani leaf (Quisqualis indica L.) is affective for curing worm infection (as an anthelmintic drug) due to its vermicidal property. Therefore, this herbal extract can be used to control worm infection in cattle; such problem is quite common in Bali cattle reared in Bali. The current finding can be considered as an alternative way in overcoming worm problems in cattle in addition to commercial anthelmintic that can be easily found by farmers, but with relative expensive.

Suggestion

Further experiments on the beneficial roles of wudani leaf extract should be done; active chemical substances contained in this herbal should be thoroughly studied so that it can be more effectively used as alternative traditional drugs to combat worm infections that up to now become one of the common problems faced by local farmers.

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LIST OF APPENDIX

Appendix 1: Nematode eggs (gastrointestinal worm) at magnification of 45 x
Appendix 2: Gastrointestinal worm eggs at magnification of 100 x