# *In-vitro* Ovicidal and Larvicidal Activity of Two Local Plants (*Ficus septicum* and *Urena lobata*) Extracts on *Haemonchus contortus*

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## ABSTRACT

*Haemonchus contortus* is one of the health biggest constrains in a development of small ruminant production in all over the world. The annual cost for treatment is up to US $103 million a year, thus alternative method to control *Haemonchus contortus* created interest in research of the development of anthelmintic based on the local/medicinal plant. The purpose of this research is to examine the effect of crude aqueous extract of *Ficus septicum* and *Urena lobata* leaves on *Haemonchus contortus* eggs and larvae. Variables measure were ovicidal and larvicidal activity (egg hatch assay and larvae development assay) as well as adult motolity assay. The extracts were tested at 5 concentrations 3.125, 6.25, 12.5, 25, and 50 mg/ml. Oxfendazole or ivermectine super and distilled water were used as positive and negative control respectively. The crude aqueous extract of *Urena lobata* leaves showed highly significant ($P<0.05$) on ovicidal activity compared to the extract of *Ficus septicum* leaves. At concentration 50mg/ml extract inhibited larvae development by 65.40% (*Ficus septicum*) and 57.80% (*Urena lobata*). The two extracts showed reduced motility of worms about 40% two hours after incubation, while Oxbendazole inhibited motility of worm up to 80% two hours post exposure. In conclusion, *Urena lobata* leaves extract was more ovicidal than that of *Ficus septicum* leaves extracts. Further research on in-vivo experiment and toxicological evaluation are still needed.

**Key words:** ovicidal, larvicidal, *Ficus septicum, Urena lobata*, and *Haemonchus contortus*

## INTRODUCTION

Goat production is an attractive enterprise for smallholder farmer in Indonesia, due to low cost in initial investment, hight reproductive performance, and ability to use marginal land. One of the major obstacle in the development of goat production is gastro intestinal parasite especially *Haemonchus contortus*. This parasite causes weaknesses, anorexia, decreased feed efficiency, reduced weight gain and decreased productivity. In Bengkulu, because of this parasite, mortality rate could reach 66.7% (Suteky and Dwatmadji, 2011). It was reported that enormous of money has been spent for synthetic anthelmintics to control this disease every year.

The use of synthetic anthelmintics are less suitable for small holder farmer especially in developing country such as Indonesia, especially regarding to high costs, accesibility, as well as residue in meat, milk or in the environment. Exploring an alternative method to control *Haemonchus contortus* lead researchers to develop a natural anthelmintic substances based on local and available medicinal plant (Suteky and Dwatmadji, 2011; Suteky *et al*., 2014; Qi *et al*., 2015; Achmed *et al*., 2013; Mohammed *et al*., 2013; Acharya *et al.*, 2014; Nawaz *et al*., 2014; Akther *et al.*, 2015; Islam *et al*., 2015). Many researchers are also focusing on secondary plant metabolites that showed promising anthelmintic activitity (Alemu *et al.*, 2014).

Our previous findings showed that infected sheep which grazed a moderate quantity of *Ficus septica* Burm dan *Urena lobata* were more resistance with *Haemonchus contortus* infestation. *Ficus septica* Burm or loal name *Awar-awar* contain contains 8 a new alkaloid B-D (1-3), 19R, 13AR-Tylophorine N-oxide, 10R, 13aR-tylocrebrine N-oxide, 10 S, 13aR-isotylocebrine N-oxide, 10S. 13 aR-sotylocrebrine N-oxide, henanthroindolizidine, ten flavonoid compounds, viz. kaempferol (1), rutin (2), quercetin (3), afzelin (4), astragalin (5), tiliroside (6), kaempferol-3-O-β-D-glycopyranoside-7-O-α-L-rhamnoside (7), kaempferol-7-O-α-L-rhamnoside (8), kaempferol-7-O-α-L-rhamnoside-4'-O -β-D-glycopyranoside (9) and crenuloside (10) (Anonimous, 2015).

The used of *Ficus septica* Burm as medicinal plants have been documented as an antirheumatic, antipyretic, stomachic, vermifuge, and anti bacterial. It was found that the leaves shown inhibit the

growth of *Bacillus subtilis* and *Escherichia coli* in vitro. Nugroho et al (2013) found that *n*-hexane insoluble fraction (HIF) of *Ficus septica* leaves is potential to be developed as co-chemotherapeutic agent for breast cancer. In the Congo, part of a herbal concoction used for abdominal inflation associated with schistosomiasis, dysentery and diarrhea (Anonimous, 2015).

*Urena lobata* (Malvaceae) can be found growing in disturbed areas, waste grounds, roadsides, open woodlands, forest margins, coastal dunes, riparian areas, swamps, salt marshes, as well as in pastures and active and abandoned croplands in tropical and sub-tropical regions According to Fagbohun et al. (2012). the leaves contained moisture (7.21%), crude fibre (6.31%), carbohydrate (47.53%), crude protein (19.79%), fat (10.21%) and ash (8.95%). The mineral analysis in mg/100 g indicated that the leaves contained calcium (42.09), copper (0.31), iron (9.35), magnesium (35.38), manganese (0.81), phosphorus (24.91), potassium (35.38), sodium (29.48) and zinc (51.55). The phytochemicals detected in the leaves of U. Lobata L. were alkaloids, cardiac glycoside, tannins, terpenoid, and saponin. Yadav and Tangpu (2007) found that methanol extract Urena lobata have therapeutic implications as anti diarrhea. Adeloye et al. (2007) in their study found that leaf extracts of Urena lobata had varied antimicrobial activity against selected bacterial isolates such as Escherichia coli, Clostridium sporogenes, Bacillus subtilis, Bacillus cereus, and Bacillus stearothermophilus. Hocking (1997) said that the leaves used as emmolient, demulcent, hemostat, contraseptive,and for liver diseases.

## MATERIALS AND METHODS

**Preparation of extract Crude Aqueous extract**

The leaves of *Ficus septicum and Urena lobata* were collected from natural habitat in Bengkulu region.The extract was prepare based on method by Suteky and Dwatmadji (2011).

**Ovicidal activity /Egg Hatch Assay (AHA)**

Egg hatch assay was conducted according the method describe by Al-Shaibani *et al.* (2009). with minor modification. The crude extract was disolved in dimethylsulfoxide (DMSO) and used as stock solutions. Egg suspension of 100µl containing approximately 100 fresh eggs was pipette into each well in a 96-well flat-bottomed microtitre plate and mixed with the same volume of plant extracts at different concentrations ranging from 3.125, 6.25, 12.5, 25.0 and 50.0 mg/ml. Albendazole dissolved in 0.3% dimethyl sulfoxide (DMSO) and diluted at concentration of 2.5, 5, 10, 20, and 40 mg /ml was utilized as positive control, while distilled water was used as negative control. Five replicates of each extract and control were carried out. The number of eggs which had not hatched and number of hatched larvae were counted and percentage hatching calculated.

The plate then incubated in room temperature (26-27$^0$C) for 48 hour. One drop of Lugol's iodine solution was added in each well to stop further hatching, unhached egg and first larvae were calculated under dissecting microscope. The percentage of inhibition of egg hatching was calculated for each concentration using the following formula of Coles *et al.* (1992).

$$\% \text{ inhibition} = 100(1-X1/X2)$$

Where X1 is the number of eggs hatched in test extracts, X2 is the respective number in distilled water.

## RESULT AND DISCUSSION

In our past examination demonstrated that parasite-infected sheep grazing/brozing moderate amounts of *Ficus septicum* and *Urena lobata* have indicated more imperviousness to *Haemonchus contortus* infestation (Suteky and Dwatmadji, unpublished research).

Table 1 shows the mean efficacy of Ficus septicum and Urena lobata extracts on Haemonchus contortus eggs compared with Oxfendazole as a positive control.

Table 1. Mean inhibition percentage of egg hatching $\pm$ SD of *Ficus septicum* and *Urena lobata* extracts at different concentrations against *Haemonchus contortus.*

| Aquaous Extract | *Ficus septicum* | | | *Urena lobata* | | | *Oxfendazole (control)* | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Dose (mg/ml) | Mean | ± | Sd | Mean | ± | Sd | Dose (mg/ml) | Mean | ± | Sd |
| 50 | 53,91 | ± | 2.65 $^{e}$ | 70.08 | ± | 1.69$^{c}$ | 40 | 91.98 | ± | 3.88 $^{a}$ |
| 25 | 37,38 | ± | 1.27 $^{f}$ | 63.22 | ± | 2.70$^{d}$ | 20 | 83.90 | ± | 4.07$^{b}$ |
| 12.5 | 32,98 | ± | 3.99 $^{fg}$ | 48.18 | ± | 2.49$^{e}$ | 10 | 75.00 | ± | 1.58$^{c}$ |
| 6.25 | 28,2 | ± | 6.08$^{g}$ | 45.97 | ± | 1.72$^{e}$ | 5 | 38.80 | ± | 1.30$^{f}$ |
| 3.125 | 11.65 | ± | 1.36$^{h}$ | 31.42 | ± | 1.49$^{f}$ | Destilled water | 10,5 | ± | 0.82 $^{h}$ |

a,b,c means with different superscripts in same the colum and rows and are significantly different (P<0.01).

This current research revealed that *Ficus septicum* and *Urena lobata* extracts have capability to inhibit egg hatch. But its efficacy was significanly (P<0.05) lower than that of the control positive used (Oxfendazole). Oxfendazole is a synthetic anthelmintic which contain pure active substances, whereas the extract contained several compounds and the active compound could be present only in small amounts. Table 1 also showed that mean inhibition percentage of egg hatching of *Urena lobata* extract in the same dose was always significantly (P<0.05) higher than those of *Ficus septicum* extracts.

Phytochemical analysis of *Urena lobata* has shown it to contain alkaloids, flavanoid, tannin, saponin, coumarin, steroid/triterperioid, furocoumarin, mangiferin and quercetin, Imperatorin and triglycerides/polyunsaturated fatty acids (Keshab, 2004). Purnomo *et al*. (2015) said that five active compounds, namely stigmasterol, gossypetin and b-sitosterol, mangiferin, and chrysoeriol, were identified in both *Urena lobata* water and ethanol extract. Azando *et al*. (2011) and Alemu *et al*. (2014) found that tannin extracts from various tannin rich plants had ability to inhibit egg hatchability therefore *Urena lobata* extract could be used as ovicidal for *Haemonchus contortus*. It was also found that ovicidal activity of *Urena lobata* (50mg/ml) extract similar or do not differ significantly (P>0.05) with Oxfendazole (10mg/ml) as a positif control. Table 1 also showed that mean ovicidal activity of aquaus extract *Ficus septicum* was 53,91% (50mg/ml), this activity is dose dependent. Alemu *et al*. (2014) reported that extract *Ficus sicomorus* inhibit 57,33% egg hatch. It seem that extract *Ficus sp.* show moderate anthelmintic activity.

Table 2. Mean larvicidal activity ± SD of *Ficus septicum* and *Urena lobata* extracts at different concentrations against *Haemonchus contortus.*

| Dose (mg/ml) | *Ficus Septicum* | | *Urena lobata* | |
|---|---|---|---|---|
| | Mean | Sd | Mean | Sd |
| 50 | 65.40 | 1.95 $^{c}$ | 57.80 | 1.48$^{cd}$ |
| 25 | 51.80 | 2.28$^{d}$ | 49.80 | 3.19$^{d}$ |
| 12.5 | 40.00 | 5.29 $^{e}$ | 36.88 | 3.13$^{f}$ |
| 6.25 | 29.60 | 2.61$^{f}$ | 20.40 | 1.67$^{g}$ |
| 3.125 | 18.00 | 2.00$^{g}$ | 12.40 | 0.89$^{h}$ |
| | | | | |
| Oxfendazole 40 mg/ml | 80.80 | 2.69$^{a}$ | 80.80 | 2.69$^{a}$ |
| Distilled water | 10.64 | 0.71 $^{i}$ | 10.64 | 0.71$^{i}$ |

a,b,c means with different superscripts in same the colum and rows and are significantly different (P<0.01).

The two extracts) than that of Oxfendazole 80.80% as a positif contol, and are significanty higher (P<0.05) than that showed inhibitory effect on larva development. The inhibitory effect are significanty lower (P<0.05of distilled water as negatif control. Al-Rofaai *et al* (2012) found that the larvicidal activity of *M esculleta* leaves extract depend on the solvent used. The larvicidal activity with the concentration 25mg/ml range from 4.33% (chloroform), 10.33% (hexan) and 59.33% (mehanol 80%). In the concentration 25mg/ml our finding showed that larvicidal activity of *Ficus*

*septicum* (51.80%) and *Urena lobata* (49.80%) is higher than that of *M. esculenta*. According to Martínez *et al*. (2010), the mode of action of tannins as anthelmintic is attributed to their capacity to bind to some proteins of the metabolism or larva's organs and muscles causing a change in their functions and resulting in the paralysis or death.

## CONCLUSION

The two aquaeos extract of *Ficus septicum* and *Urena lobata* have capability to inhibit the development of egg and larva of *Haemonchus contortus.*

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