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Research Article

ANTHELMINTIC ACTIVITY OF MELASTOMA MALABATRICUM EXTRACT ON HAEMONCHUS CONTORTUS ACTIVITY IN VITRO

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ABSTRACT

The in vitro efficacy of the extracts *Melastoma malabatricum* on egg hatch (AHA), larva development (LDA) and adult motility assay (AMA) was determined against *Haemonchus contortus* . The concentrations of 3.125, 6.25, 12.5, 25, and 50 mg/ml were incubated for 2 days (AHA) and 7 days (LDA) 12 hours (AMA), Oxfendazole or ivermectine super and distilled water were used as positive and negative control. The crude aqueous extract and hydro-ethanolic extract inhibited egg hatching and larva development, Chloroform extract showed less ovicidal and larvacidal activity compared to the other extracts. All extracts induced higher anthelmintic activity (AHA and LDA) significantly ($P<0.05$) compared to control negative. Non motile worms were observed 2 hours post exposure with aquaeus extract of *Melastoma malabatricum*. More over, Ivermectine super inhibited motility up to 100% two hours after incubation. In summary, the extracts of *Melastoma malabatricum* had anthelmintic activity in vitro against *Haemonchus contortus.*

Keywords: *Melastoma malabatricum*, Anthelmintic.

INTRODUCTION

Haemonchus contortus is a major limitation to the production of sheep and goats all over the world and are responsible for economic losses in grazing ruminant [1]. Some epidemiological studies revealed that the prevalence of this disease could reach 80 % in Indonesia. Anthelmintic application was commonly used to control *Haemonchus contortus* , however anthelmintic treatment are often unworkable and inapplicable in developing countries.

Moreover, the development of anthelmintic resistance causing increasing interest in the search for alternative solution in order to decrease the use of synthetic anthelmintic drugs[2] The use of medicinal plants as livestock dewormers is one of the methods readily adaptable to rural farming in developing countries. Therefore a lot of study have been done to examine the anthelmintic activity of medicinal plants [3,4,5]. However, the study on anthelmintic efficacy of plant commonly browse by goat or sheep remains insufficient.

Melastoma malabatricum or more known as senduduk or sengganen is belong to family Mastomaceae, it is familiar weed can be found easily throughout Indonesia, especially in cleared land , waste placed also under oil palm plantation.

Various parts of the plant are used in folklore remedies for the treatment of diarrhea and post-partum recovery[6]. Some phytochemical compound isolated from *Melastoma malabatricum* have also been reported as anti ulcerogenic [7], antiviral and cytotoxic activity [8], anti-helminthic and anti-spasmodic [9], antidiarrhoeal activity [10] .

In our previous research showed that parasite-infected sheep grazing/browsing moderate quantities of *Melastoma malabatricum* have shown more resistance to *Haemonchus contortus* infestation (Suteky and Dwatmadji, unpublished research). By the time, there is still limited scientific publication about in vitro study of *Melastoma malabathricum* as an anthelmintic. Therefore scientific validation of efficacy of this plant before their acceptance and use is needed.

MATERIALS AND METHODS

Preparation of extract

Crude Aqueous extract

The leaf of *Melastoma malabatricum* was collected from natural habitat in Bengkulu region during August-September 2010. The plant material were dried at room temperature (25-35 °C) and powdered using commercial electric blender [11] . The crude aqueous extract of *Melastoma malabatricum* was prepared according to the technique describe by Igbal *et al* [12] with minor modification. The powdered of *Melastoma malabatricum* (100 g) mix with 500 ml water in 2 L flask and boiled for 1.5 hours.

Following cooling to 40^0 C, the material filtered using muslin gauze and filter paper. The water in decocted material was removed by placing it on water bath. The weight of extract was recorded and the extract was stored at 4 ^{0}C for further used.

Hydro Ethanolic and Chloroform extract

Powdered material of *Melastoma malabatricum* leaf (about 100 g) exhaustively extracted with 90% ethanol in a Soxhlet`s apparatus until the solvent was clear. The extract was concentrated then dried at 60-70°C. The extract percentage yield of the semi solid extract was weight. The extract obtained were place in tubes and store in a refrigerator until used. The same method was used to prepare chloroform extract.

Egg Hatch Assay (AHA)

Egg hatch assay was conducted according the method describe by Al-Shaibani *et al.* [13] with minor modification. The stock solutions were prepared by disolving the crude extract in dimethylsulfoxide (DMSO) to improve it's solubility in water. Aliquots of start solution (50 mg/ml) were taken for preparation final concentrations of 3.125, 6.25, 12.5, 25.0 and 50.0 mg/ml.

Test were performed in 96 flat bottom wells micro plate (IWAKI, Asahi Glass co., Japan). Egg suspension of (100 uL: 100 eggs) was pipette into each well, and mixed with the same volume of different concentration (3.125, 6.250, 12.5, 25, 50 mg/ml) of crude aqueous, hydro ethanolic and chloroform extract of *Melastoma malabaricum*.

Moreover, oxfendazole (PT Sanbe Farma, Bandung, Indonesia) 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 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.* [14].

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

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

Table 1: Mean inhibition percentage of egg hatching ± SD of *Melastoma malabatricum* extracts at different concentrations against Haemonchus *contortus*

Concentrations (mg/ml)	Aqueous crude extract		Hydro-Ethanolonic extract		Chloroform extract	
	Mean	SD	Mean	SD	Mean	SD
50	84.44	3.17[b]	86.05	3.37 [b]	49.39	5.01[c]
25	73.21	1.00 [c]	77.42	6.07 [c]	32.43	0.20 [f]
12.5	64.39	2.13 [d]	67.83	6.96[d]	22.40	1.54[g]
6.25	63.52	3.97[d]	53.96	6.81[e]	18.25	1.77 [g]
3.125	55.98	6.05[e]	37.40	5.59 [f]	10.06	0.72[h]
Oxfendazole						
40	91.98	3.88 [a]	91.98	3.88 [a]	91.98	3.88 [a]
20	83.90	4.07[b]	83.90	4.07[b]	83.90	4.07[b]
10	75.00	1.58[c]	75.00	1.58[c]	75.00	1.58[c]
5	38.80	1.30 [f]	38.80	1.30 [f]	38.80	1.30 [f]
2.5	0.50	0.50[h]	10.50	0.50[h]	10.50	0.50[h]
Distilled water	10.50	0.82[h]	10.50	0.82[h]	10.50	0.82[h]

Table 2: Mean inhibition percentage of larval development ± SD of *Melastoma malabatricum* extracts at different concentrations on *Haemonchus contortus.*

Concentrations (mg/ml)	Aqueous crude extract		Hydro -Ethanolonic extract		Chloroform extract	
	Mean	SD	Mean	SD	Mean	SD
50	77.00	0.71[b]	79.48	1.66[a]	61.60	2.61[c]
25	65.84	1.31[c]	74.84	0.85[b]	55.52	1.03[d]
12.5	50.80	3.63[d]	52.76	2.30[d]	51.20	2.28[d]
6.25	42.00	3.74[e]	49.44	1.66[d]	31.00	2.24[f]
3.125	32.80	2.28[f]	39.92	1.43[f]	14.64	1.69[g]
Oxfendazole 40 mg/ml	80.80	2.69[a]	80.80	2.69	80.80	2.69
Distilled water	10.64	0.71[i]	10.64	0.71	10.64	0.71

Table 3: Mean motility of adult *Haemonchus contortus* after exposure with different concentration of aquaeus *Melastoma malabatricum* extract

Treatments	Mean of Motility adult *Haemonchus contortus* (hours after exoposure)													
	0		2		4		6		8		10		12	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Oxfendazole	10	0	2	0	0	0	0	0	0	0	0	0	0	0
Ivomec super	10	0	0	0	0	0	0	0	0	0	0	0	0	0
Air Destilasi	10	0	10	0	10	0	10	0	10	0	10	0	10	0
Melastoma malabatricum														
50	10.00	0.00	3.25	0.50	1.75	0.50	1.25	0.50	0.00	0.00	0.00	0.00	0.00	0.00
25	10.00	0.00	5.40	0.55	4.25	0.50	2.75	0.50	1.00	0.00	0.00	0.00	0.00	0.00
12.5	10.00	0.00	5.75	0.50	5.00	0.82	4.00	0.00	2.00	0.00	1.00	0.00	0.00	0.00
6.25	10.00	0.00	7.00	0.82	6.25	1.26	5.50	0.58	4.25	0.50	3.00	0.82	0.00	0.00
3.125	10.00	0.00	7.25	0.50	6.75	0.50	6.50	0.58	5.75	0.50	3.25	0.96	0.00	0.00

Larva Development Assay (LDA)

One hundred eggs in 100 µl were put into each well of 96-wellmicrotitre plate. A 20 µl of nutritive media (comprising of 1g yeast in 90 ml of normal saline and 10 ml Earle's balanced salt) was added into each well. The plates were then incubated under room temperature for 2 days. Then 100 µl of *Melastoma malabatricum* at the concentrations of 3.125, 6.250, 12.5, 25, 50 mg/ml were added to relevant plates. There were four replicates for each extract concentration and control. The plates were further incubated for 5 days (total of 7 days), further development was stopped by addition of one drop of Lugol`s iodine solution. All larvae in each well were counted under stereo microscope[13].

Adult motility Assay

Female mature live *Haemonchus contortus* worms were collected directly from the freshly abomasums of slaughtered goats naturally infected with *Haemonchus contortus* in the local abattoir. The worms were washed and suspended in PBS, 10 worms in 400 □L of distilled water were placed into multi well plate. 400 □L of aquaeus crude extract of *Melastoma malabatricum* at different concentration were added into each well and the multi well plate was incubated at 25°C for 24 hours. The motility of each worm was observed at 0 hour and then after 2, 4,6, 8,10, and 12 hour intervals, then the treated worms were kept for 30 minutes in the lukewarm fresh PBS to observe the recovery of motility, a worm was considered to be motile if it moved in a sinusoidal motion when stimulated by lukewarm fresh PBS. Oxfendazole and ivermectin super were used as positive control and distilled water are used as negative control

Statistical analysis

The percentage of inhibition of egg hatching and larva development was calculated, data from egg hatch assay/test and larval development and adult motility as assay/test were analyzed using one-way analysis of variance. The means were compared by the Duncan test with 5% significant level using the SPSS 15.0 programmed.

RESULTS AND DISCUSSION

Mean inhibition percentage of egg hatching and larva development was presented in Table 1 and 2. The crude aqueous and ethanolic extracts of *Melastoma malabatricum* demonstrated 84.44% and 86.05% of egg hatch inhibition respectively. In vitro anthelmintic activity against *Haemonchus contortus* of aqueous and hydro-alcoholic extracts of *Hedera helix* were studied, it was found that

there was no statistically significant difference in the activity of the two extract types[14]..

While, mean inhibition percentage of chloroform extract on egg hatching significantly (P<0.05) lower compared to the others extract used. Our finding is almost similar with previous studied by Al-Shaibani et al [13]both ethanolic and aqueous extracts exhibited ovicidal on egg hatching of gastro intestinal nematodes and the highest effectiveness (P<0.05) was at the concentration of 50 mg/ml. In general mean ovicidal activity of crude aqueous, hydro-ethanolic extracts and chloroform extract was significant higher (P<0.05) compared to distilled water.

The isolation of botanical compounds from plant material largely depends on the solvent and method of extraction[15] . *Furth*ermore, they found that the organic solvent extracted more bioactive compounds from plants compared to distilled water.

The effectiveness of *Melastoma malabricum* leave extracts on larva development is presented on Table 2. The present study revealed that hydro ethanolic extract showed inhibitory effect (79.08%) at concentration 50mg/ml, which is not significantly (P<0.05) different with positive control (oxfendazole 40 mg/ml). Moreover, it appear that all extracts exhibited larvacidal activity and the inhibitory seems to be dose-dependent.

There are two mechanism of uptake of anthelmintic drugs in nematodes ,the first is the diffusion of the anthelmintic drugs through eggshells or the cuticles of larvae and the second, the diffusion into the intestinal cells[16].

The results of adult motility assays performed after a period of 0,2,4,6,8,10 and 12 hours incubation are shown in Table 3. Non-motile worms was clearly express as a twitching motion or parts of body appear stiff, different with the smooth sinusoidal motion of control worms[17]. It is evident from Table 3 that crude aqueous extract of *Melastoma malabatricum* cause immotility about 66.25 % within 2 hours after exposure, this extract inhibited motility of adult worms completely at 8 hours after exposure. It look like that higher concentration of extract inhibit motility much earlier. Adults female *Haemonchus contortus* exposed to Ivermectin super were found 100 % non-motile 2 hours post exposure. While oxfendazole showed inhibited motility started 2 hours after incubation, in contrast distilled water showed no effect on motility test until end the end of experiment. The current study revealed that extracts of *Melastoma malabatricum* has a potential anthelmintic activity in vitro, further in vivo evaluation of the different parts and fractions is needed to make use of this plant for beneficial purposes.

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REFERENCES

1. Perry BD, Randolph RF, McDermott JJ, Sones KR, Thornton PK. Investing in animal health research to alleviate poverty. International Livestock Research Institute (ILRI), Nairobi, Kenya 2002.
2. Waller PJ. Anthelmintic resistance. Vet. Parasitol. 1997; 72:391-412.
3. Adama K, Gaston BAM, Hamidou T, Amadou T, Laya. S. In vitro anthelmintic effect of two medicinal plants (*Anogeissus leiocarpus* and *Daniella olivera*) on *Haemonchus contortus* , an abomasal nematode of sheep in Burkiso Faso. Afr J Biotechnol. 2009; 8 (18): 4690-4695.
4. Kamaraj C, Rahuman AA, Mohamed MJ, Elango G, Rajakumar G, Zahir AA, Santhoshkumar T, Marimuthu S. Ovicidal and larvacidal of crude extracts of *Melia azedarach* against *Haemonchus contortus* (Strongylida). Parasitol. Res.2010; 106: 1071-1077
5. Ademola IO, Eloff JN. Athelmentic activity of acetone extract and fractions of *Vernonia amygdalina* against *Haemonchus contortus* eggs and larvae.Trop Anim Health Prod. 2010; 42(2):521-527
6. Burkill IH. A dictionary of the economic products of the Malaya Peninsula. 1st edition. Kualalumpur, 1996.
7. Hussain F, Abdulla MA, Noor SM, Ismail, Sand Hapipah MA. Gastroprotective effects of *Melastoma malabathricum* aqueous leaf extract against ethanol-induced gastric ulcer in rats. Am J Biochem Biotechnol. 2008; 4 (4): 438-441
8. Lohezic-Le,F A, Devehat C,.Bakhtiar M,. Bezivin, Amoros, Boustie J. Antiviral and cytotoxic activities of some Indonesian plants. Fitoterapia.2002; 73: 400-405.
9. Osman K, Faridah HI, Ibrahim ZA, Latif A. A Guide book to medicinal plant of Perlis. 1 st edition, Jabatan Perhutanan Negeri Perlis, Kangar Perlis. 2002
10. Sunilson JAJ, Anandarajagopal K, Kumari A V A G, Mohan S. Antidiarrhoeal activity of leaves of *Melastoma malabathricum* Linn. Indian J Pharm Sci. 2009; 71(6): 691–695.
11. Bhinge SD, Hogade MG,Chavan C, Kumbhar M, Chature V. *In vitro* anthelmintic activity of herb extract of *Eclipta prostrates* L. against *Pheretima posthuma.* AJPCR 2010; 3(3):229-230
12. Iqbal T, Lateef ZM, Jabbar A, Akhter MS, Khan MN. Anthelmintic activity of *Vernonia anthelmintica* seeds against Trichostrongylid nematodes of sheep. 2006; *Pharm. Biol.*, 44: 563–567
13. .Al-Shaibani IRM, Phulan MS and AM Shiekh. Anthelmintic activity of *Fumaria parviflora* against gastrointestinal nematodes of sheep. Int J Agr Biol. 2009; 11:431-436.
14. Coles C, Bauer GC, Borgsteede C, Geerts FHM, Klei S, Taylor TR and Waller. J. World Association for the advancement of veterinary parasitology method for detection of anthilmintic resistance in nematodes of veterinary importance. Vet Parasitol. 1992; 44: 35-44
15. Eguale T, Tilahun G, Debella A, Feleke A, Makonnen E. *Haemonchus contortus*: *in vitro* and *in vivo* anthelmintic activity of aqueous and hydro-alcoholic extracts of *Hedera helix* Exp. Parasitol. 2007;116(4):340-345.
16. Alawa CBI, Adamu AM, Gefu OJ, Ajanusi P A, Abdu N P,. Chiezey J.N. Alawa, Bowman, DD. *In vitro* screening of two Nigerian medicinal plants (*Vernonia amygdalina* and *Annona senegalensis*) for anthelmintic activity. Vet Parasitol. 2003; 113: 173-181.
17. Alvarez LI, Mottier ML, Sanchez SF, Lanusse CE. *Ex vivo* diffusion of albendazole and its sulfoxide metabolite into *Ascaris suum* and *Fasciola hepatica*. Parasitol. Res. 2001;87: 929-934
18. Kotze C, Clifford S, O'Grady J,. Behnke JM, McCarthy JS. An *In Vitro* larval motility assay to determine anthelmintic sensitivity for human hookworm and *strongyloides* species. 2004; *Am. J. Trop. Med. Hyg.,* 71(5):. 608–616.