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Research Article

In-Vivo anthelmintic Activities of Ethanol Extract of Croton (*Codiaeum variegatum* L. Blume) Against Tapeworm *Hymenolepis microstoma*

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Abstract: As per reported in a previous study, the extract of the croton twig possesses an anthelmintic activity against the *Hymenolepis spp* cestode in an in Vitro model. The aim of the present study was to determine the anthelmintic activity of the croton twig extract against the tapeworm *Hymenolepis microstoma* in Vivo. The study was started out by experimentally infecting mice with *H. microstoma.* Infected mice were divided into five groups consisting of one control negative group, one control positive group, and three test groups of three different doses; 125, 250, and 500 mg/kg BW. Evaluation was carried out by the fecal egg count reduction (%FECR) and the worm count reduction. Statistical analysis showed that all test group have lower egg per gram count and lower worm count than that of the control negative group. Result showed at dose 125 mg/kg BW, the croton twig extract has the highest ability to reduce the number of eggs with an %FECR of 63,25%, whilst the highest ability of reducing the number of worms was shown by the extract at dose 500 mg/kg with a %WCR of 63%.It is suggested that further works including safety, effective dose need to be carried out before using twigs *C.variegatum* as anthelmintic agent.
Keywords: Anthelmintic, *Codiaeum variegatum*, tapeworms, *Hymenolepis microstoma*

INTRODUCTION

Worm disease is a disease caused by parasitic worms . This disease is generally chronic and endemic . Intestinal worms can cause immunosuppressive effects on the host , allowing the occurrence of complications due to co-infection by other pathogens [1]. The most common intestinal worms attacking the world's population is ascariasis ,enterobiasis , cestodiasis , and ancylostomiasis . Cestodiasis in Southeast Asia reported a higher infection than other intestinal worms , which is about 40 million cases[2]. Cestodiasis a pathophysiological condition in which Cestoda (tapeworms) of adults living in the body of the definitive host. Adult worms live in the host gastrointestinal tract, while cysticercoids (larvae) worms can migrate to the subcutaneous tissue, brain, liver, and lungs so that the onset of the condition of cysticercosis, hidatidosis, andsenurosis which may cause central nervous system disruption if not treated immediately [3-5].

Control of intestinal worms are still dependent on the provision of anthelmintic. Anthelminticis a medication that works locally to remove worms from the gastrointestinal tract or other organs, canal so radicate the wormon stage larvae [6]. Anthelmintic of choice for treating cestodiasisis praziquantel(PZQ), which works against adult worms and metacestodanya forms (larvae and eggs). However, reported cases of acquired resistance to the synthetic drug in humans caused by worms life cycle of parasitic worms that are complex [6]. New development needs to be done to overcome the problem of resistance to anthelmintic, especially the development of anthelmintic derived from plant materials that are empirically known as an anthelmintic.

Twigs of the plant croton (*Codiaeum variegatum* (L.)Blume) is empirically known to kill tape worms in the patient's body cestodiasis [7]. Chemical compounds contained in C. variegatum are alkaloids, cardiacglycosides, saponins, sterols, tannins, and flavonoids [8-9] which is thought to have anthelmintic work. It was also reported that the extracts of twigs croton has anthelmintic activity against the genus. Hymen olepissp pin in-vitro models [10]. These data support this research, which aims to determine the anthelmintic activity of crotont wigs against Cestoda parasites in-vivo by using a model consisting of amurine intestinal worms, beetle *Tribolium castaneum*, and parasitic Cestoda *Hymenol epismicrostoma*.

MATERIALS AND METHODS

Plant material:

The plant material used in this study is the branch *Codiaeum variegatum* (L.) Blume obtained from

the experimental garden of medicinal plants Manoko - Lembang, Bandung - West Java.

Chemicals:

Chemicals used were amyl alcohol (Merck), ammonia (Merck), hydrochloric acid (Merck), sulfuric acid (Bratachem ®), ethanol 95% (Bratachem ®), chloroform (Merck), potassium hydroxide (Merck), ether (Bratachem ®), distilled water, physiological saline (CAS), PGA (Bratachem ®), sugar-saturated salt solution (500 grams of sugar and 400 g of salt in 100 mL of distilled water), tablets praziquantel (Cesol - Merck), and silica gel GF254 TLC plates (Merck). Reagents used were of reagent iron III chloride (FeCl3 - Merck), 1% gelatin solution, Dragendorff reagent (potassium iodide bisumt - Merck), Mayer's reagent (potassium mercury idodida - Merck), Liebermann-Burchard reagent (a mixture of acetic acid anhydride and concentrated sulfuric acid - Merck), magnesium powder (Merck), and vanillin powder (Merck). All materials unless otherwise indicated were pa grade.

Animals and Parasite Test

Animal testing in the form of white mice (*Musmusculus albinus*) and the beetle *Tribolium castaneum*. Parasites test were worms *Hymenolepis microstoma*. Mice used were as many as 50, are male, ddY strain, 1-2 months old weighing between 25-35 g were obtained from the Faculty of Veterinary Medicine, Bogor Agricultural University. Tapeworm *Hymenolepis microstoma* and beetle *Tribolium castaneum* maintained in Helminthologi Faculty of Veterinary Laboratory, Bogor Agricultural University with the goal of maintaining the life cycle of the worm specimen in a manner in continuous infection [11].

Methodology

Codiaeum variegatum Leaves were extracted and dried to obtain thick ethanol extract. Phytochemical screening of the extract conducted to determine the class of secondary metabolites present in the extracts of twigs croton . Screening was done to the class of alkaloid compounds ,polifenol at , tannins , flavonoids , monoterpenoid and seskuiterpenoid , terpenoids and steroids , and saponins .

Determination of the content of the extract compounds were analyzed by Thin Layer Chromatography method . The stationary phase used was silica gel GF254 , while the mobile phase were a solvent mixture of ethyl acetate - methanol - water (100 : 13.5 : 10) and toluene - ethyl acetate (93:7) for the determination of the profile of polar compounds and the profile of lipophilic compounds, respectively. Blots apparition used was vanillin - sulfuric (VS) [12].

Anthelmintic activity against *Hymenolepis microstoma,* Test

Testing was done by using the classical laboratory model of intestinal worms which consists of parasitic tapeworm *Hymenolepis microstoma,* , the beetle *Triboliumcastaneum*as an intermediate host , and white mice (*Musmusculus Albinus*) as the definitive host. Established models stems from the collection of gravid worms proglotid segments obtained from mice previously infected with a continuous specimen of *H. microstoma* who maintained their life cycle in the laboratory .Proglotid selected gravid already given to the beetle *T. castaneum* as feed , which previously had fasted for 5 days. Beetles dissected on day 16 post-administration of gravid *H. proglotid*microstoma to get cysticercoids infective .

A total of 50 mice were used as test models were divided randomly into 5 groups consisted of 10 mice each. All of the mice infected with each of as many as 15 tails cysticercoid infective *H. microstoma* which has been obtained from the abdominal beetle *T. castaneum* that had been dissected . Before being used for testing , all mice were adapted for 7 days to 15 days in the lab, observed health and activities, as well as removed the worm with a wide spectrum anthelmintics Albendazole 7.5 mg/kg BW to ensure free mice from infection natural worm .

Negative control group treated by administration of PGA 2 %, while for the positive control group treated by administration of anthelmintic praziquantel 25 mg/kg BW in a 2 % solution of PGA . Three other test group ie test group I, group II Test, and Test group III treated with the extract of twigs *C. variegatum* in suspension PGA 2 % respectively at a dose of 125 mg/kg BW, 250 mg/kg BW , and 500 mg/kg BW. Treatment test was conducted 21 days post-infection cysticercoids *H. microstoma* .

Anthelmintic activity of test materials known by checking the number of eggs per gram of feces (egg per gram , EPG count) and also the number of worms found (worm count recovery) in the intestine of mice .

To determine the effectiveness of the test material to the worm egg production, means data obtained EPG of feces into the formula calculated the percentage decline (fecal egg count reduction, % FECR) as by Presidente [13] and Dash *et al.*[14]

$$\text{FECR (\%)} = 100\% \left(1 - \frac{T_2 \times C_1}{T_1 \times C_2} \right)$$

Notes:

T_1 = Means EPG test group on H-1

T_2 = Means EPG test group on H+2, H+4 dan H+7

C_1 = Means EPG negative control group on H-1

C_2 = Means EPG negative control group on H+2, H+4 dan H+7

While the effectiveness of the test material to the number of worms, all mice at necropsy at the end of the study to calculate the number of worms *H. microstoma* surviving based on the number that can be found. The means of the number of worms that can be found from each treatment group can be calculated in reduction percentage as in the formula by Saeki *et al*. [15]

$$\%WCR = \frac{X_{negative\ \ control} - X_{test\ \ group}}{X_{negative\ \ control}} \times 100\%$$

Notes: X = the means worm found

RESULTS AND DISCUSSION

Extraction Results

The extraction obtained extracts shaped thick, greenish-black, herbal characteristic odor, and obtained yield of 6.865%. While the test results show the water content of the extract containing 10.5% moisture. Tale 1.shows phytochemical screening results of twig Extract *C. Variegatum.*

Table 1: Phytochemical screening of branch *C. variegatum* ethanol extract

Compund groups	Results
Alkaloid	+
Flavonoid	-
Polyphenol	+
Tannin	+
Monoterpenoid & sesquiterpenoid	-/-
Steroid & triterpenoid	-/-
Quinon	-
Saponin	+

Notes: (+) = detected (-) = not detected

Most phytochemical compounds contained in an extract from the stalk in croton are alkaloids and tannins [9]. Both compounds are thought to be effective in lowering the number of tapeworms found in host pest. It strongly supported the existence of the in vitro experiments from extracts of the worm genus croton twigs *Hymenolepis spp* [10]. Similarly, other studies have reported the role of plant alkaloids of *Mimosa pudica* L. as anthelmintic against *Hymenolepis nana* worms on testing in Vivo [16]. Tapeworms generally absorb glucose, fat and protein (in the form of amino acids). While the process of absorption of the protein as a nitrogen source active transport is done through a special locus on tegument [17]. Alkaloids generally include alkaline compounds containing one or more nitrogen atoms, usually in combination, as part of a cyclic system [18] Nitrogen atom of the toxic alkaloids that inhibit the absorption will be undertaken by the amino acid microvilli tegumen tapeworms [19], resulting in a deficiency of nitrogen in the worm and in the end there is disruption of protein synthesis that is essential for worm development.

Besides alkaloids, tannins also affect the decrease in the number of worms in the host. Tannins can react with proteins to form solid copolymer is not soluble in water. This reaction is called the tanning reaction. Reaction that causes the protein is more difficult to be achieved by the digestive liquids [18]. Tannins are also able to destroy the lining of the intestinal mucosa [20]. Intestinal mucosa helps scolex worm continues strongly attached to the intestinal wall. This causes loss of mucosal lining in the scolex worm hook tool irrespective of the intestinal wall, and later the worm will come out along with the excreta increased peristaltic action due to a reaction to the destruction of the intestinal mucosa in the presence of tannins.

TLC results

Determination of the content of the extract compounds was conducted using thin layer chromatography (TLC) using a developer consisting of a mixture of ethyl acetate-methanol- water (100: 13.5: 10) for the determination of the profile of polar compounds, whereas a mixture of toluene-ethyl acetate (93: 7) was to determine the profile of lipophilic compounds. Due to the facts there were spots that were not visible under visible light, spotting apparition used. Vanillin-sulfuric apparition (VS) under UV light were then applied. Profiling results along with Rf can be seen in Figure 1.and Table 3.for system developers ethyl acetate-methanol-water (100:13,5:10), as well as in Figure 2.and Table 4.for system developers toluene - ethyl acetate (93:7).

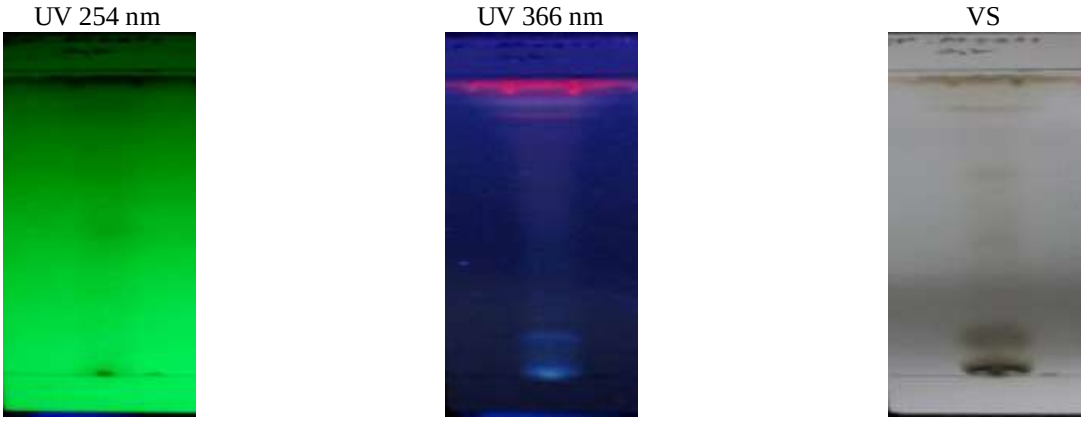

Figure 1: TLC results the ethanol croton extraton developer system ethyl acetate-methanol-water (100:13,5:10)

Table 3: TLC Rf of Croton ethanol extract on ethyl acetate-Methanol-Water (100:13,5:10)

No.	R_f	UV 254nm	UV 365nm	VS
1	0.98	Dark green	Red	Green
2	0.96	Green	Dark red	Faded red
3	0.92	-	Bright purple	Dark orange
4	0.89	-	Purple	-
5	0.87	Green	Pink	-
6	0.84	-	Dark purple	-
7	0.80	-	Purple	-
8	0.76	-	Dark purple	-
9	0.66	-	-	Faded brown
10	0.45	Green	-	Faded brown
11	0.27	-	-	Faded gray
12	0.17	-	-	Brown
13	0.14	-	Blue	Gray
14	0.04	-	Dark Blue	Brown
15	0.02	Green	Blue	Black

Notes: VS = apparition spotting vanillin-sulfuric

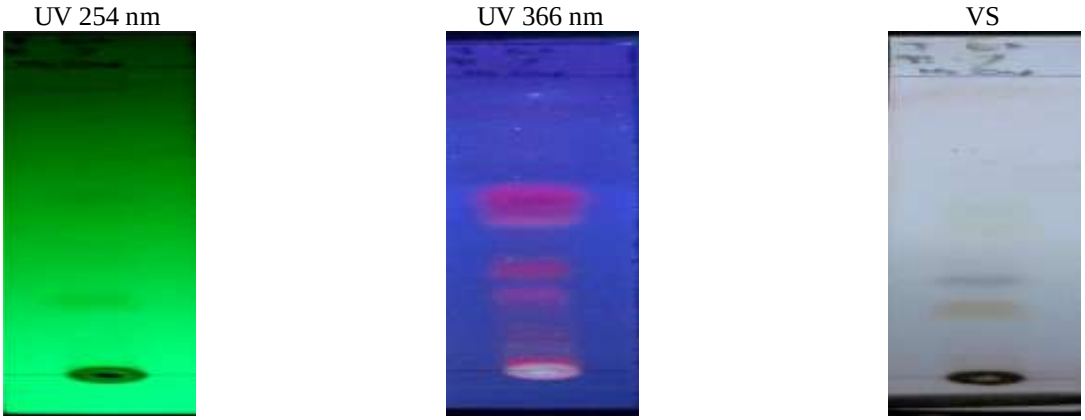

Figure 2: TLC results the ethanol croton extrat on developer system toluene – ethyl acetate (93:7)

Table 4: TLC Rf of Croton ethanol extract on toluene – ethyl acetate (93:7)

No.	R_f	UV 254nm	UV 365nm	VS
1	0.94	Green	Blue	Faded orange
2	0,60	-	Pink	-
3	0,58	-	Red	Faded yellow
4	0, 55	-	Purplish red	-
5	0,51	-	Pink	-
6	0,36	-	Dark red	-
7	0,33	-	Pink	Gray
8	0,25	Green	Pink	Faded orange
9	0,08	-	Faded red	-
10	0,06	-	Faded purple	Faded yellow
11	0,03	-	Red	Faded brown

Notes: VS = apparition spotting vanillin-sulfuric

Based on the figures and tables had been presented, obtained at least 15 compounds that were relatively polar, whereas the relative nonpolar compounds were detected at least 11. After spraying apparition VS spots on TLC with system developers ethyl acetate - methanol - water (100 : 13.5 : 10), if raised patches of red, brownish yellow , or bluish green principles indicated that bitter compounds including alkaloids and cardiac glycosides. The emergence of spots that are red, yellow, blue, or brown indicated the presence of essential oils [12]. . While spraying apparition VS spots on TLC with system developers toluene - ethyl acetate (93 : 7) indicated the presence of essential oil compounds if there were spots that were red, yellow, blue, or brown. If there were patches of light blue or brown under 365nm UV light without spraying apparition patches on the system, then there was a coumarin compound [12].

Extract anthelmintic activity test against *Hymenolepis microstoma*

This test was intended to determine the anthelmintic activity of extracts of twigs *Codiaeum variegatum* against *Hymenolepis microstoma* which were infected artificially in mice. Tests carried out by using a model consisting of mice as the definitive host, the beetle *Tribolium castaneum* flour as an intermediate host, and the tapeworm *Hymenolepis microstoma*.

EPG was an examination of the initial parameters for the observation of anthelmintic activity of a test substance to the worms. Generally, the number of eggs found in the faeces of mice was positively correlated with the number of surviving worms in the host body. EPG examination conducted by collecting faeces from each treatment group to count the number of eggs that could be found. Faeces were collected on the day before treatment (H - 1), two days after treatment (H +2), four days after treatment (H +4), and seven days after treatment (H +7). Data on the means EPG before treatment and after treatment can be seen in table 4. and chart patterns based on the data in the table decline can be seen in Figure 3.

Table 5: Means EPG on each observation

Groups	K-	K+	Test I	Test II	Test III
H-1	9800 ± 2255,42	13250 ± 638,36	11500 ± 3421,62	12766,67 ± 957,00	12683,33± 2483,11
H+2	10650 ± 3931,60	0 ± 0	5550 ± 2901,29	10550 ± 2851,92	9916,67 ± 2636,44
H+4	10083,33 ± 625,17	0 ± 0	3233,33 ± 1155,78	11700 ± 3118,09	5533,33 ± 2413,68
H+7	10150 ± 1905,91	0 ± 0	4533,33 ± 678,85	5166,67 ± 896,29	6383,33 ± 1284,85

Notes:

K- = Negative control; PGA 2%
K+ = Positive control; PZQ 25 mg/kg BW
Test I = *C. variegatum* extract 125 mg/kg BW
Test II = *C. variegatum* extract 250 mg/kg BW
Test III = *C. variegatum* extract 500 mg/kg BW
H-1 = observations on EPG number the day before treatment
H+2 = observations on EPG number two days after treatment
H+4 = observations EPG number four days after treatment
H+7 = observations EPG number seven days after treatment

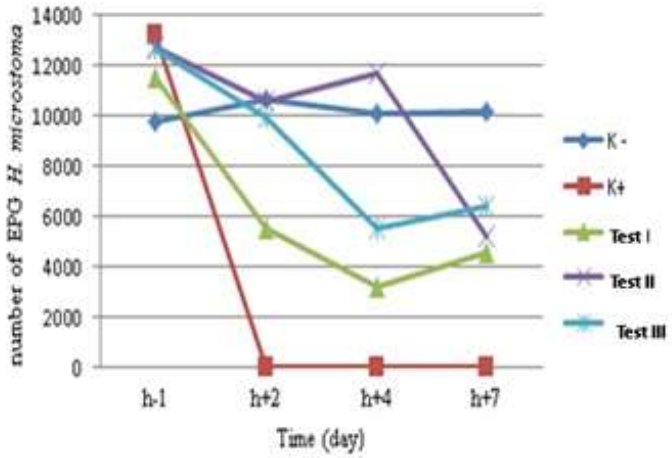

Figure 3: Chart patterns means reduction of EPG in faeces on the H-1, H +2, H +4 and H +7

Notes:
K- = Negative control; PGA 2%
K+ = Positive control; PZQ 25 mg/kg BW
Test I = *C. variegatum* extract 125 mg/kg BW
Test II = *C. variegatum* extract 250 mg/kg BW
Test III = *C. variegatum* extract 500 mg/kg BW
H-1 = observations on EPG number the day before treatment
H+2 = observations on EPG number two days after treatment
H+4 = observations EPG number four days after treatment
H+7 = observations EPG number seven days after treatment

Means EPG in the negative control group was relatively stable in number, while the means EPG in the positive control group decreased dramatically; eggs were not found at all in the H +2, H +4, and H +7. Means EPG in the treatment groups decreased after administration of croton twig extract. On means EPG of both Test I and Test group III decreased relative to each observation, except at H +7; both test groups experienced small fluctuations. Means EPG test group II relative fall in H +2, then fluctuated on H +4, and experienced a significant drop in H +7.At the end of the study, all mice was necropsied to count the number of worms that can be found in the gastrointestinal tract. The means of the number of worms that can be found per treatment group are presented in Table 6.

Table 6. The means of the number of worms found per test group

Groups	Worm Recovery Count
K-	8,4 ± 1,58
K+	0 ± 0
Test I	3,7 ± 1,83
Test II	5,8 ± 1,32
Test III	3,13 ± 0,43

Notes:
K- = Negative control; PGA 2%
K+ = Positive control; PZQ 25 mg/kg BW
Test I = *C. variegatum* extract 125 mg/kg BW
Test II = *C. variegatum* extract 250 mg/kg BW
Test III = *C. variegatum* extract 500mg/kg BW

Table 6. showed the number of worms were most commonly found in the negative control group, followed by the number of worms in the second test group, test group I and group III trials, whereas the positive control group, there were no worms at all.

The ability of anthelmintic activity of the extract could be determined by calculating the test percentage decrease in EPG in faeces (% FeCr) and also the percentage decrease in the number of worms found (% WCR). Data reduction in the means EPG data and the decrease of the number of worms and the percentage decrease of each data can be seen in Table 7.

Table 7: Means eggs per gram stool (EPG), Percent Reduction in Fecal EPG (% FeCr), Worm Recovery Count and Percentage Decrease in Number of worms in Mice (% WCR)

Groups	EPG	% FECR	Worm Recovery Count	%WCR
K-	-494,444 ± 342,51	0	8,4 ± 1,58	0
K+	13250 ± 638,36	100	0 ± 0	100
Test I	7061,1 ± 2115,18	63,25	3,7 ± 1,83	56
Test II	3627,77 ± 2704,38	31,85	5,8 ± 1,32	31
Test III	5405,55 ± 1335,87	45,37	3,13 ± 0,43	63

Description:
K-= negative control; PGA 2% suspension
K + = positive control; PZQ 25 mg / kg BW
Test I = *C. variegatum* twig extract 125 mg / kg BW
Test II = *C. variegatum* twig extract 250 mg / kg BW
Test III = *C. variegatum* twig extract 500 mg / kg BW

It was found (Table 7), the greatest ability to decrease the number of worm eggs is the first test extract (125 mg / kg body weight) while that has the greatest ability to decrease the number of Test III was a worm extract (500 mg / kg). The results of the calculation % FeCr different with the calculation results % WCR shows the influence of worm density on egg production . This phenomenon was known as " the crowding effect" by Smyth and McManus [21]. The crowding effect describes the phenomenon that the fewer the number of worms present in the host , increasing the value of the EPG which is caused by the reduced ability of the worm to form a new segment or lengthen new segments.

Differences effect of treatment on means EPG in faeces and also the means of the number of worms found were analyzed with statistical methods of analysis of variance (ANOVA). ANOVA test results for a decrease in the means EPG obtained p-value = 0.000 is less than $\alpha = 5$ % , which means that there was a marked influence of the five treatment groups. While the results of ANOVA test for the number of worms found obtained p-value = 0.000 ($\alpha < 5$ %) which means that there was a marked influence of the five treatment groups .

Further tests performed using Duncan's test to determine which treatments have different effects on the decrease in the average EPG in faeces and also the number of worms found. Duncan test results for the effect of treatment on the reduction in the means EPG in the treatment group showed fecal test I extract (125 mg / kg BW) had the effect of decreasing the amount equal to the extract EPG III trials (500 mg/kg BW) , and the extract treated group III trials had the effect of decreasing the amount equal to the extract EPG II trials (250 mg/kg BW) . However, treatment of test extracts I had a different effect with extract II trials. Duncan test results for the effect of treatment on the number of worms found there indicated that the effect did not differ between the groups of the first Test with the Test group III, although the means of the number of worms in the Test group III was relatively smaller than in Test I, while other treatment groups contained a different effect on decreasing the number of worms .

The conclusion that can be drawn based on % % FeCr and WCR and statistical analysis of the results for the second test of the data showed that the extract at a dose of 125 mg/kg BW have the best ability to lower the percentage of worm egg production decreased by 63.25 % , while the best ability to decrease the number of worms by test extract at a dose is 500 mg/kg BW, with the percentage decrease of 63 % .The results of this study concluded that the ethanol extract of *C. variegatum* twigs that can be used as anthelmintic agent. Further study, however is needed to find the effective dose of the extract against *Hymenolepis microstoma,*

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