2011 2nd International Conference on Biotechnology and Food Science
IPCBEE vol.7 (2011) © (2011) IACSIT Press, Singapore

Determination of Anthelmintic Potential in *Terminalia catappa* by Modified Selected *In Vitro* Bioassay

Azrul, L. M.
Department of Agrotechnology,
Faculty of Agrotechnology and Food Science;
Institute of Marine Biotechnology
Universiti Malaysia Terengganu
Kuala Terengganu, Terengganu, Malaysia
azrullokman@umt.edu.my

Effendy, A. W. M.
Institute of Marine Biotechnology;
Department of Biological Sciences,
Faculty of Science and Technology,
Universiti Malaysia Terengganu
Kuala Terengganu, Terengganu, Malaysia
effendy@umt.edu.my

Adzemi, M.A.
Department of Agrotechnology
Faculty of Agrotechnology and Food Science
Universiti Malaysia Terengganu
Kuala Terengganu, Terengganu, Malaysia
adzemi@umt.edu.my

Imelda, L. V.
Haematology and Parasitology Unit
Veterinary Research Institute
59, Jalan Sultan Azlan Shah
Ipoh, Perak, Malaysia
imelda@yahoo.com

Nurulaini, R.
Haematology and Parasitology Unit
Veterinary Research Institute
59, Jalan Sultan Azlan Shah
Ipoh, Perak, Malaysia
nur1709@yahoo.com

***Abstract*—In this study, anthelmintic potential from *Terminalia catappa* leaves were determined using selected *in vitro* bioassay that was modified based on other established assays. Larvae was distributed at a concentration of 50 L3 (n=±50) per well in a 96 multiwells plate, incubated with diluted crude extract of *T. catappa* at a ratio of 1:1 at 20°C for 3 hours and 5 hours. Control was conducted using PBS as positive control and distilled water as negative control. After incubation, larvae motility were observed and counted using inverted microscope. All the non-motile L3 were identified in order to ensure survivality and motility of the larvae. Results showed that after 3 hours, reduction percentage for *T. colubriformis, C. curticei* and *H. contortus* was 70%, 63% and 73% respectively while at 5 hours incubation, reduction percentage for each species was at 77%, 67% and 80% respectively. Reduction percentage is calculated by comparing the number of L3 before and after incubation period. Control showed no reduction in terms of motility with standard deviation at 5-10%. From the results, it could be suggested that *T. catappa* leaves are a potential alternative to be used as anthelmintic.**

Keywords-Terminalia catappa, larvae motility, reduction percentage, anthelmintic

I. INTRODUCTION

Goats are one of major important animals in livestock industry in worldwide [1]. Unfortunatley, nowadays, a range of diseases become a main factor that reduces number of goats' production. There are a lot of problems and diseases in goats [1]. According to Department of Veterinary Services of Malaysia, parasitic worm or helminths infection is one of major cause for reduced productivity in livestock industry.

To overcome this problem, the conventional method is by using the chemical known as anthelmintic or dewormer that based on drugs [1]. However, according to [2], these manufactured anthelmintics had some serious disadvantages, such as non availability in some developing countries including Malaysia, high cost, and risk of misuse leading to drug resistance, environmental pollution and food residues. According to this phenomenon especially the resistance, naturally produced plant anthelmintics offer an alternative that can overcome some of these problems. The studies about the alternative anthelmintic are becoming urgent because of the rapid escalation of anthelmintic resistance worldwide [3,4]. Besides that, the use of plant as anthelmintic is both sustainable and environmentally acceptable [2,5]. Traditional knowledge about the anthelmintic plants was widely explored nowadays.

Thus, in this study, potential plant that claimed to have anthelmintic potential based on ethnobotanical knowledge was used as an experimental plant namely *Terminalia catappa* or locally known as Ketapang [6]. *T. catappa* or

locally known as Ketapang tree in Malaysia is a plant widely distributed on tropical and subtropical beaches [7]. This plant has a vast natural distribution in near marine-coastal area of the Indian Ocean, through tropical Asia, and into the Pacific Ocean. It extends from the Seychelles through India, the Andamans and adjacent islands, and throughout Southeast Asia (Myanmar, Thailand, the Malay Peninsula, Vietnam, Philippines, and Indonesia) to Papua New Guinea and northern Australia as far south as the Tropic of Capricorn [8]. The leaves of this plant have been used as a folk remedies in India and Philippine to treat dermatitis, helminthiasis and hepatitits [7]. From previous study, we know that this plant had several bioactivities such as antioxidant, hepatoprotective, anthelmintic and antiinflammatory. There are also few phytochemical research studies of this plant, focused on volatile and tannin [7].

Previous studies had described a range of *in vitro* tests that can be conducted to evaluate the anthelmintic efficacy of any potential plants. In this study, the preliminary modification was done to the larval motility assay [9]. The principles of the egg hatch assay [10] and larval migration test [11] were referred to study the efficacy of *Terminalia catappa* leaves as natural anthelmintic against three targeted species of gastrointestinal nematode obtained from naturally infected Boer goats.

The objective of this study, is to determine the anthlemintic potential of this plant against three most important species of gastrointestinal nematode in ruminants which are *Haemonchus contortus, Trichostrongylus colubriformis* and *Cooperia* curticei. Modification was done to the selected anthelmintic bioassays that established before to give more choices of *in vitro* anthelmintic bioassays in the future.

II. MATERIALS AND METHODS

T. catappa leaves were freshly collected at the marine-coastal area of Kuala Terengganu beach, eastcoast of Peninsular Malaysia. The sampling process was conducted according to the correct agronomy practices. Drying process and followed by cutting process were done according to the FAO Protocol [10] and proceed to the next level until the crude powder of *T. catappa* was prepared. Crude powder was used in the anthelmintic bioassay with crude aqueous extract (CAE) form. The CAE was freshly prepared right before the bioassay was started.

For the targeted species of worms, the sample was obtained from the research centre was in mixture of species. Thus, the species differentiation must be done to differentiate the species and the percentage of each species was recorded. Then, each species was distributed (n=50) in each well in the 96-multiwells plate respectively, together with positive and negative control. The vehicle solution which is distilled water was used as a negative control while for the positive control, PBS solution was used. After the larvae (L3) were inserted into the well, it will be mixed up with the test and control solution. At every time of trial, two sets of well were prepared for each species as it have to incubated for two different incubation period, which is 3 hrs and 5 hrs. At the end of the incubation period, the well was taking out and being gently shaked to make sure the worms is not in dorman mode. Observation of motile and survived larvae was conducted using inverted microscope.

III. RESULTS

US-letter paper size. If you are using A4-sized paper, please close this template and download the file for A4 paper format called “CPS_A4_format”.

A. Maintaining the Integrity of the Specifications

The template is used to format your paper and style the text. All margins, column widths, line spaces, and text fonts are prescribed; please do not alter them. You may note peculiarities. For example, the head margin in this template

Figure 1. The percentage of larvae differentiation based on species from the whole samples

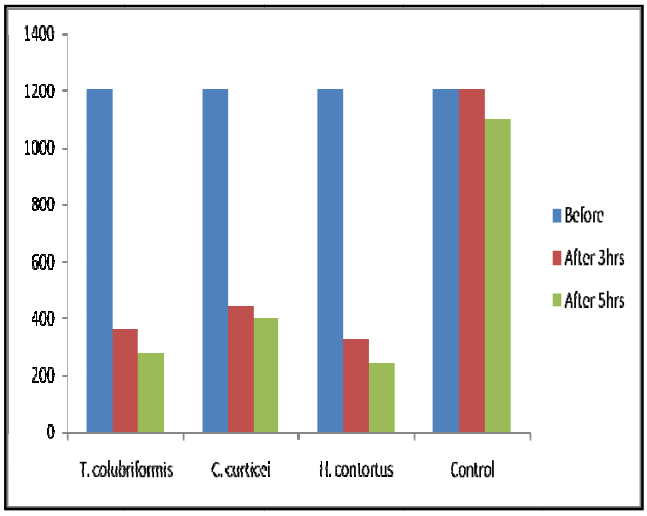

Figure 2. Percentage of L3 reduction after incubation with CAE of *T. catappa*

IV. DISCUSSION

After larvae culture was done, L3 were collected and observed under the microscope to identify their species. *T. colubriformis* is the most dominant species at 50% followed

by *C. curticei* and *H. contortus* at 30% and 20% of total population of this sample (Fig. 1).

The reduction percentages of L3 are shown in Fig. 2. After 3hrs of incubation, the reduction were 70%, 63%, and 73% respectively while for 5hrs of incubation, the reduction were 77%, 67% and 80% respectively. The reduction percentages were slightly different between both incubation periods. Decreasing trend can be seen in both periods; with the number of motile L3 at 5hrs incubation is less than motile L3 after 3hrs incubation. Significant difference ($P>0.05$) was determined for all three targeted species compared to the control when analyzed with Simple T-Test. Longer incubation period will reduce more number of motile L3.

These results proved that there is anthelmintic activity in *T. catappa*; by inhibiting the motility and survivality of larvae as mentioned previously in ethnoveterinary reports.

V. CONCLUSSION

As a conclusion, this preliminary modification can be use to evaluate the in vitro anthelmintic efficacy of this potential plant. By observing the motility and survival of the larvae following incubation process, the anthelmintic efficacy of *T. catappa* can be evaluated. For future study, the parameters of this assay should be optimized to obtain better results so that it can be widely used and promoted.

ACKNOWLEDGMENT

This work is supported by Fundamental Research Grant Scheme (Vot no. 59164), under Ministry of Science, Technology and Innovation of Malaysia. The authors wish to thank Dr. P. Chandrawathani, Mdm. Nurulaini and Ms. Imelda from Veterinary Research Institute of Ipoh for their help and valuable advices during this study. A million thanks to these particular person namely Ms. Rawaidah Anas, Ms. Khadijah Saad and Mr. Muhammad Embong for helping me during the plant sampling and data analysis.

REFERENCES

[1] Wahab A. R. Cacing dalam kambing; masalah dan penyelesaian. Siri Syarahan Umum Pelantikan Profesor . 2003. Univesiti Sains Malaysia.

[2] Hammond, J. A., Fielding, D., and Bishop, S. C. Prospects for plant anthelmintics in tropical veterinary medicine. Review Article, Veterinary Research Communications, 21. 1997. 213-228.

[3] Waller, P. J. Sustainable helminth control of ruminants in developing countries. Vet Parasitol. 71. 1997. 195-207.

[4] Jackson, F. and Coop, R. L. The development of anthelmintic resistance in sheep nematodes. Parasitology 120 (Suppl.), 2000. S95-S107.

[5] Githiori, J. B. (2004). Evaluation of anthelmintic properties of ethnoveterinary plant preparations used as livestock dewormers by pastoralists and small holder farmers in Kenya, Ph.D Thesis, Swedish University of Agricultural Sciences, Uppsala, Sweden.

[6] Tan, R. Sea Almond Tree. Available on http://www.naturia.per.sg/buloh/plants/sea_almond.htm; 2001. Accessed on 18th of January 2011

[7] Fan, Y. M., Xu, L. Z., Gao, J., Wang, Y., Tang, X. H., Zhao, X. N. and Zhangm Z. X. (2004). Phytochemical and antiinflammantory studies on Terminalia catappa. Fitoterapia 75. Pp 253-260.

[8] Thompson, L. A. J. and Evans, B. Terminalia catappa (tropical almond). Species Profile for Pacific Island Agroforestry. 2006. Ver. 2.2. Available on www.traditionaltree.org. Accessed on 18th January 2011.

[9] Kotze, A. C., Clifford, S., O'grady, J., Behnke, J. M. and McCarthy, J. S. An In Vitro Larval Motility Assay to Determine Anthelmintic Sensitivity for Human Hookworm and Strongyloides Species. Am. J. Trop. Med. Hyg., 71(5). 2004.Pp. 608-616

[10] Coles, G. C., Bauer, C. and Borgsteede, F. H. World Association for Advancement of Veterinary Parasitology (WAAVP) Methods for Detection of Anthelmintic Resistance in Nematodes of Veterinary Importance. Vet Parasitol. 1992 pp. 44: 35-43.

[11] Rabel, B., McGregor, R. and Douch, P. G. C. (1994). Improved Bioassay for Estimation of Inhibitory Effects of Ovine Gastrointestinal Mucus and Anthelmintics on Nematode Larval Migration. International Journal of Parasitology 24. 1994. 671- 676.

[12] Makkar, H. P. S., Bluemmel, M., Borowy, N. K. and Becker, K. Gravimetric determination of tannins and their correlations with chemical and protein precipitation methods. J. Sci. Food Agric. 61, 1993. 161-165.