Aktivitas Anthelmintis Ekstrak Etanol Umbi Porang Terhadap *Fasciola gigantica* In Vitro

The Anthelmintic Activity Of Etanol Extract of Porang Tubers Against *Fasciola gigantica In Vitro*

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ABSTRACT

This study aims to determine the anthelmintic activity ethanol extract of porang tubers (*A. oncophillus*) against *F. gigantica* worms *in vitro*. There were five treatments and each treatment was done in five replications and used 10 *F. gigantica*. The treatments of this research were K- with CMC Na 1%, K+ with Albendazole 2.4 mg/ml, P1 with extract concentration 5%, P2 with extract concentration 10%, P3 with extract concentration 20%. The results showed that the extract of Porang tuber (*A. oncophillus*) had an anthelmintic effect against *F. gigantica* worms *in vitro*. In the extract with a concentration of 20%, there were anthelmintic properties that almost the same as Albendazole. The higher the extract concentration, the higher the anthelmintic properties. The longer the immersion time, the higher the number of dead worms. The morphological changes was evaluated by light microscopic examination and the results showed many histopathological changes on the morphology of *F. gigantica*. The results indicate that the possible use of the tubers as a potential anthelmintic against *F. gigantica*.

Keywords: *Amorphophallus oncophillus, Fasciola gigantica,* anthelmintic, *in vitro*, tegument

Received: 02-09-2021 **Revised:** 03-10-2021 **Accepted:** 16-11-2021

INTRODUCTION

Amorphophallus oncophillus is a nutraceutical plants that have the effect drugs with a preventive role as well as curative of a disease (Sutriningsih and Ariani, 2017). Diseases in farm animals can cause various kinds of losses for farmers. One disease which causes loss significant is fasciolosis. According to Alemneh et al. (2019), fasciolosis caused by worms F. hepatica and F. gigantica of the trematodes class. Prevalence the incidence of fasciolosis in some areas Indonesia can reach 90% (Manus and Dalton, 2006) and problems in fasciolosis is usually associated with zoonoses and interests economy (Putratama, 2009).

Prevention and treatment disease really needs to be done to controlling fasciolosis. Carmona and Tort (2016) mention that one of the control methods fasciolosis is the use of chemical drugs as anthelmintics. Moment these, albendazole, mebendazole, metronidazole and nitazoxanide are used as a treatment option for fasciolosis (Keiser et al., 2005).

Various types of herbal plants has anthelmintic activity in vitro by decreasing motility, damage the network structure, as well as speed up the time of paralysis and mortality of F. gigantica (Muslina, 2016), including porang tuber ethanol extract. F. gigantica has a tegumentary structure which is highly absorptive and becomes main target of anthelmintics (McKinstry et al., 2003)

METHODS

The sample used in This research is F. gigantica as much as 250 collected from beef liver after post-mortem examination at Home Slaughtering Animals (RPH) Pegirian Surabaya.

Research on activities anthelmintic tuber extract of A. oncophillus against F. gigantica in vitro will carried out in the Laboratory of the Division Veterinary Parasitology, Faculty Veterinary Medicine, University Airlangga. Production of tuber extract A. oncophillus carried out in the laboratory Division of Veterinary Basic Medicine, Faculty of Veterinary Medicine, University Airlangga. This research was conducted on December 2020 – February 2021.

Equipment used in this study is surgical scissors, scalpel, tweezers, storage case worm, petri dish, pipette, measuring cup 100 ml, 500 ml Beaker, Erlenmeyer flask 250 ml, gloves, mask, thermometer, rotary evaporator, glass stirring rod, cloth, water bath, incubator, scale digital, microscope, extraction tools, microtome and microscope.

Materials used in In this study, the tubers of A. oncophillus obtained from PT. Paidi Indo Porang, F. gigantica from beef liver obtained from the Slaughterhouse (RPH) Pegirian Surabaya City, aquades, physiological NaCl suspension, Albendazole® 16% (PT. Tekad Mandiri Citra) as drug control, CMC-Na as suspension, Technical Ethanol 96%, buffered formalin, graded alcohol (70%, 80%, 90%, absolute alcohol), xylol, liquid paraffin, and Hematoxylin and Eosin.

Production of tuber extract of A. oncophillus

The tubers of A. oncophillus were obtained from PT. Paidi Indo Porang. Process manufacture of porang plant tuber extract (A. oncophillus) refers to research Kurnijasanti (2019) and performed in Basic Medicine Division Laboratory Veterinary Faculty of Veterinary Medicine Airlangga University. Making process extraction using maceration method begins by peeling the skin of the tuber A. oncophillus, washing porang tuber flesh which has been peeled, then sliced thinly with a thickness of 0.5-1.0 cm, then soaked in salt water and dried by aerating. Tubers of A. oncophillus that have been dry made into flour with mashed using a blender. A. oncophillus tuber flour is added to the in a 250 ml Erlenmeyer flask, then macerated with 96% ethanol solvent, then stirred every day. The result of maceration in the form of filtrate is separated with the residue, then macerated again until the filtrate is clear. The filtrate is then evaporated using a vacuum rotary evaporator to obtain a thick extract of tuber A. oncophillus.

Preparation of various extract concentrations tubers of A. oncophillus

Determination of extract concentration tuber A. oncophillus refers to research that has been done by Dey and Ghosh (2010) with little modification. The concentration of tuber extract A. oncophillus is determined by the formula n, 2n and 4n, where the variable n is effective concentration of extract on reference test who have shown activity anthelmintic (Maulidya et al, 2017). On reference research found that Amorphophallus tuber extract can

exhibits anthelmintic activity significant against worms in concentration of 100 mg/ml (10%).

F. gigantica collection

250 F. gigantica worms is collected from beef cut in Pegirian Slaughterhouse (RPH) City of Surabaya. Sampling worms in this study were carried out by making an incision in the parenchyma heart. Worms are transferred to a petri dish and divided into five treatments. Each The treatment consisted of five repetitions and each cup consists of 10 F. gigantica. Preparation of albendazole suspension Albendazole used as a drug control is powder Albendazole® 16% (PT. Tekad Mandiri Citra) containing Albendazole 160 mg/g, while the standard dose for Albendazole usage is 15 mg/kg (Center for Veterinary Research, 2014).

Evaluation of extract's anthelmintic activity tuber A. oncophillus on mortality F. gigantica in vitro

Group 1 (K-): Soaking F. gigantica into 20 ml 1% CMC Na suspension.

Group 2 (K+): Soaking F. gigantica into 20 ml Albendazole suspension.

Group 3 (P1) : Immersion of F. gigantica into 20 ml tuber extract suspension A. oncophillus with concentration 50 mg/ml (5%).

Group 4 (P2) : Immersion of F. gigantica into 20 ml tuber extract suspension A. oncophillus with concentration 100 mg/ml (10%).

Group 5 (P3) : Immersion of F. gigantica into 20 ml tuber extract suspension A. oncophillus with concentration 200 mg/ml (20%).

Observation variables for worms is to observe the percentage worm mortality in terms of scores the motility of the worms given the treatment. Observation of the time of death of worms conducted every 1 hour and the deadline for observation is 5 hours.

Evaluation of anthelmintic activity of A. oncophillus tuber extract against microscopic changes in the tegument of F. gigantica in vitro

Hematoxylin and Eosin staining was carried out with reference to Prasetya's research (2019) which used standard procedures of the Pathology Laboratory of the Faculty of Veterinary Medicine, Airlangga University. The tissue processing steps consist of fixation using 10% buffered formalin, dehydration using graded alcohol (70%, 80%, 90%, absolute alcohol), clearing xylol, and paraffin infiltration using liquid paraffin. Then continue the process blocking, microtome cutting, incubation, and staining. The samples were then observed under a microscope (Nikon Eclipse E100LED MV R from Tokyo, Japan) which was calibrated by optilab camera at 100x and 400x magnification.

The observation variable for worms is to observe microscopic changes that occur in the tegumentary structureF. gigantica using porang tuber ethanol extractin vitro through light microscopy. Microscopic changes in the structure of the tegument F. gigantica interpreted based on organ abnormalities for further analysis descriptively.

Data analysis

The data obtained from the calculation results in the form of mortality F. gigantica analyzed using Analysis of Variance (ANOVA). If there is a significant difference, it will be continued with Duncan's Multiple Distance Test. Then, a probit analysis was carried out to determine the value of LC50, LC90, LT50, and LT90 extract for each hour of observation. Data processing is done using SPSS 25 for Windows.

RESULTS AND DISCUSSION

Based on the results of observations, differences in the concentration of ethanol extract of porang tubers affect the length of time for worm mortality. The higher the concentration of the porang tuber ethanol extract used, the faster it is worm mortality time. The results of observations in the first hour showed that the K+, P2, and P3 treatments caused a decrease in motility and resulted in mortality in *F. gigantica*, while K- and P1 have not shown mortality *F. gigantica*, but causes a decrease in motility. This indicates that onset of action on K+, P2 and P3 starting in the first hour. The results of observations in the second hour showed that K+, P1, P2, and P3 caused an additional decrease in motility and mortality. *F. gigantica*, whereas in K- has not shown mortality *F. gigantica*, but showed an increase in the decrease in motility. Based on the decrease in motility F. gigantica, the results showed the order of anthelmintic activity from the highest to the lowest in a row by K+, P3, P2, P1, and finally K-.

F. gigantica's death in ethanol extract of porang tubers is thought to be caused by the chemical content in it, namely flavonoids, alkaloids, polyphenols, and tannins (Tue, 2019). Flavonoids have a pharmacological effect by causing capillary vasoconstriction and reducing the permeability of blood vessels which causes blood vessel disorders so that the absorption of nutrients and oxygen is disrupted and can accelerate the death of worms (Mahatriny et al., 2014). The tannin content in the ethanol extract of porang tubers will enter the worm's body by binding to the worm's tegument which consists of glycoproteins and mucopolysaccharides, then precipitation of these proteins, thereby preventing the worms from absorbing nutrients and eventually the worms will die (Maryam, 2017). Polyphenols have the potential to inhibit the formation of energy for worms through binding of glycoproteins to the tegument, causing the death of worms. Alkaloids in the ethanolic extract of porang tubers make the worms experience a decrease in motility intensity, muscle weakness, to paralysis and in a long time will causes the muscles in the digestive system of worms to not function so that the worms cannot digest food (Maryam, 2017).

In this study, the ethanol extract of porang tubers with a concentration of 20% showed the best mortality compared to other concentrations. This indicates that the higher the concentration of the porang tuber ethanol extract used, the higher the effectiveness of the anthelmintic which can kill F. gigantica. Based on the results of probit analysis, LC50 porang tuber ethanol extract against F. gigantica at 1 hour to 5 hours respectively by 3.160%, 1.918%, 1.432%, 1.027%, and 0.635%, while the LC90 the ethanol extract of porang tubers at 1 hour to 5 hours was 9.490%, 5.761%, 4.301%, 3.086%, and 1.908%, this indicates that the longer the soaking time, the smaller the LC50 value obtained, while the value of LT50 on Albendazole that is at 1,258 hours, the value of LT50 at 1% CMC- Na at 94,259 hours, and various concentrations of ethanol extract of porang tubers, namely 5% concentration (P1) at 3,986 hours, 10% concentration (P2) at 1,629 hours, and 20% concentration (P3) at 1,302 hours. Value of LT90 on Albendazole that is at 4.059 hours, the value of LT50 at 1% CMC-Na at 304.232 hours, and various concentrations of ethanol extract of porang tubers, namely 5% concentration (P1) at 12.866 hours, 10% concentration (P2) at 5.257 hours, and 20% concentration (P3) at 4.204 hours, so that concluded that the higher the concentration given to the treatment, the lower the time needed to kill F. gigantica. The results showed

that F. gigantica given the ethanol extract of porang tubers with various concentrations significantly reduced motility and caused death in F. gigantica. The results of microscopic examination using light microscopy with HE staining showed that the morphology of F. gigantica soaked in 1% CMC-Na suspension showed no microscopic changes in the tegumentary tissue and the tegumentary structure appeared normal without any significant damage. The spine, muscles, and basement membrane also show normal structure. The tegument is the outermost layer of the worm that maintains homeostasis, plays a role in the synthesis and secretion of antigens, protection against the host immune Figure 1. Histopathological changes in the tegument of F. gigantica after being given various treatments

Notes: Yellow arrows indicate erosion, blue arrows indicate vacuolization, green arrows indicate detachment of the tegument, red arrows indicate detachment of spine, T = tegument, S = spine, M = muscle, B = basal lamina, HE staining, 100x objective magnification. a) F. gigantica in CMC-Na suspension, b) F. gigantica in Albendazole suspension, c) F. gigantica in 5% porang tuber ethanol extract, d) F. gigantica in 10% porang tuber ethanol extract, e) F. gigantica in 20% ethanol extract of porang tubers.

The administration of Albendazole suspension at a dose of 2.4 mg/g causes integument F. gigantica looks swollen, ruptured, and found some vacuole formation. By in vitro Albendazole has a tissue-damaging effect on worms, causing microscopic changes in the form of swelling and rupture (Guimaraes et al., 2018). Swelling of the tegument is an early sign of osmotic changes and disturbances due to damage to the structure of the tegument. On the administration of Albendazole, tegumental changes showed the same pathological damage as the ethanol extract of porang tubers but with less severity.

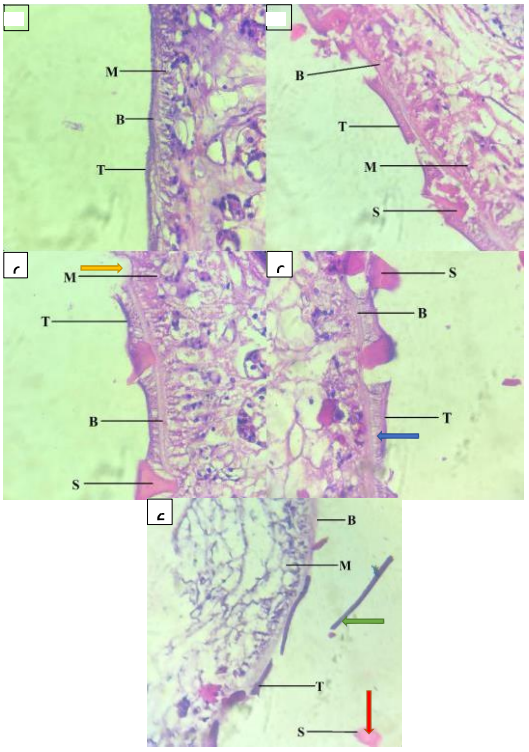

Figure 1. Histopathological changes in the tegument of F. gigantica after being given various treatments

Tegument F. gigantica is the main target of anthelmintics because this organ can make direct contact with anthelmintic compounds. Tegument changes test results F. gigantica using ethanol extract of porang tubers with concentrations of 5%, 10% and 20% illustrates that there are various damages to the entire tegument F. gigantica, depending on the concentration given to each treatment. Tegument damage was more clearly found in samples with higher concentrations of porang tuber ethanol extract. The results showed that the ethanol extract of porang tubers worked effectively in vitro In inhibiting motility, it causes partial disturbances in the form of structural changes in almost the entire tegument surface such as shrinkage or erosion of the tegument that results in thinning of the tegument surface, the spine looks submerged by the swollen tegument and some of the spines appear

detached, vacuolization and disintegration of the tegument, erosion of the tegumentary cells that destroys the basement membrane, until the complete loss of the structure of the tegument leads to complete destruction of the tegument structure. due to the activity of the active compound isolated from the ethanol extract of porang tubers. After damage to the tegument, F. gigantica become paralyzed and then die.

CONCLUSION

From the research results, it can be concluded that Tuber extract A. oncophillus has anthelmintic effectiveness against F. gigantica by in vitro with an effective concentration of 20% with a time of 5 hours. LC50 porang tuber ethanol extract against F. gigantica at 1, 2, 3, 4, and 5 hours respectively were 3.160%, 1.918%, 1.432%, 1.027%, and 0.635% while the LC90 ethanol extract of porang tubers 1, 2, 3, 4, and 5 hours respectively were 9.490%, 5.761%, 4.301%, 3.086%, and 1.908%. LT50 Albendazole at 1.258 hours and ethanol extract of porang tubers with a concentration of 5% (P1) at 3.986 hours, 10% (P2) at 1.629 hours, and 20% (P3) at 1,302 hours. LT90 Albendazole at 4,059 hours and ethanol extract of porang tubers with a concentration of 5% (P1) at 12,866 hours, 10% (P2) at 5,257 hours, 20% (P3) at 4,204 hours. The administration of porang tuber ethanol extract causes microscopic changes in the tegument structure F. gigantica. The higher the concentration of porang tuber ethanol extract given, the more obvious the microscopic changes found.

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