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The Anthelmintic Activity of Ethanol 96% Extract of Papaya Stem (*Carica papaya* l.) from Indonesia

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Abstract

Introduction : Soil transmitted helminth (STH) infection incidence is still high in the world, become main problem in developing countries including Indonesia. Papaya's stem was traditionally used as anthelmintic in Indonesia.

Objective : This study aims to determine the anthelmintic effect of 96% ethanolic extract of papaya (*Carica papaya* L.) stem.

Material and Method : This study used a laboratory experimental research design with posttest only with control group design approach. The samples were 90 *Ascaris suum* worms, divided into 6 groups, 3 times repeated. The first group was treated by Aquadest as negative control, the second group was treated by Pyrantel Pamoate as positive control in 0.5 concentration. The third, fourth, fifth and sixth groups were treated by 96% ethanolic extract of papaya (*Carica papaya* L.) stem in 0.2 b/v, 0.4 b/v, 0.6 b/v and 0.8 b/v concentration respectively. Data were collected by observation of total mortality time of *Ascaris suum* worm every 3 hours. The anthelmintic effects was analysis by Post hoc Mann-Whitney with significancy level at $p<0.05$.

Result : There was significant difference between the 96% ethanolic extract of papaya (*Carica papaya* L.) stem (0.2 b/v, 0.4 b/v, 0.6 b/v and 0.8 b/v) with control negative with Pvalue < 0.05 by Mann Whitney Test.

Conclusion : The 96% ethanolic extract of papaya (*Carica papaya* L.) stem have potential as anthelmintic.

Keywords : *Carica papaya* L., *Ascaris suum,* Anthelmintic effect

Introduction

Infectious disease is one of diseases that become the main problems in developing countries, including Indonesia. A WHO report in 2016 stated that more than 1.5 billion or 24% people in the world are infected by *soil-transmitted helminths (STH)*. STH worms group is *Ascaris lumbricoides*, *Trichuris trichiura* and hookworms (*Anclyostama duodenale* and *Necator americanus*). Estimated there are 807-1221 million people of the world population are infected by *Ascaris lumbricoides* (Natadisastra and Agoes, 2009; CDC, 2016).

Approximately 270 million pre-school aged children and 600 million school children domisile in transmission area of *Ascaris sp* worms and are in need of medications and ascariasis mitigation. In South-East Asia, STH infections reached 354.5 million cases (WHO, 2016). In 2012, Indonesia was one of 11 countries with ascariasis endemic (Kemenkes RI, 2012). Estimated 55 million Indonesian people in 2016 were infected by STH out of 260.1 million population (Tiwow et al., 2013).

The terrible sanitation owned by poor communities enables the ascariasis prevalence in Indonesia remains high. Prevalence of ascariasis in Tegal was 7,5%, in Sumatra was 78%, while 79% in Kalimantan, 88% in Sulawesi, 92% in Nusa Tenggara Barat, 90% in Jawa Barat. The result of an investigation held by Sub Dit Diare on ascariasis and another digestive tract infection between 2002 – 2009 in 398 Sekolah Dasar (SD)/Madrasah Ibtidaiyah (MI) across 33 provinces reported that the average prevalence on ascariasis is 31.8% (Kemenkes RI, 2012; Sardjono, 2017; Novianty et al., 2018; Bestari et al., 2020).

Everyone, regardless their age, has the chance to be infected by ascariasis with the highest prevalence lies on children. Although rarely leading to death, worms infection causes patients undergoing malnutrition, physical, mental, cognitive, and intellectual developing disruption. On heavy infection of ascariasis, it can be intestinal obstruction and intususeption. Sometomes worms can migrate to outer intestine including appendix, ductus biliaris and diverticulum Meckel. Is worm migrate to ductus hepaticus and biliaris, become hepatic ascariasis. Beside complication, ascariasis can also become reinfection (Tiwow et al. 2013; Zaman, 2014; Zerdo et al., 2015). Ascariasis infection could be treated with anthelmintic medication. At the moment, the medicine options in the market to encounter ascariasis are pirantel pamoat, albendazol, and mebendazol (Soedarto, 2011; Gunawan, 2014; Sungkar et al., 2017). Pirantel pamoat is single-dose medicine and is drug of choice in ascariasis

infection therapy. However, this medicine offers a side effect; digestive tract disruption, fever and headache. Application on pregnant women and under 2 year old children were not recommended while they are high risk on ascariasis (Roy, et al., 2012; Wekesa et al., 2017; WHO, 2019). Moreover, the trend of back to nature lifestyle increases due to the number of reports on side effects caused by modern medications. Indonesian society has known traditional plants as anthelmintic. *Carica papaya* L. is one of them.

Research Method

This research utilized laboratory experimental research design with a method called *posttest only with controlled group design approach.* This research was conducted in Pharmacology Laboratory of Medicine Faculty of Universitas Muhammadiyah Surakarta on November 2017.

The independent variable of this research was 96% ethanol extract concentration of papaya stem. The dependent variable was death to every worm on every observation. While the controlled variable on this research was the length of the *Ascaris suum* and room temperature and the uncontrolled variable lies on psychological state and the age of the worms.

The samples of this research was *Ascaris suum* worms that fulfill the inclusive criteria. The test material of the antihelmintic activity was 96% ethanol extract of papaya (*Carica papaya* L.) stem, aquadest and pirantel pamoat. The process of manufacturing the extract of papaya (*Carica papaya* L.) stem utilized maseration method in Pharmacology Laboratory of Medicine Faculty of Universitas Muhammadiyah Surakarta. Furthermore, the process to evaporate ethanol solvent utilizes rotavapor tool and a water bath in Pharmacy Faculty of Universitas Muhammadiyah Surakarta until become ethanol extract of *Carica papaya* L. with concentrations of 0.2 b/v, 0.4 b/v, 0.6 b/v, and 0.8 b/v was obtained.

The positive control utilized 125 g pirantel pamoat tablet. While the negative control utililized aquadest. The manufacturing of the papaya stem extract was conducted in Pharmacology Laboratory of Medicine Faculty of Universitas Muhammadiyah Surakarta. The extract of papaya stem was applied for 3 days with details followed; 1.) concentration 0.2 b/v: 5 gram of papaya stem extract in 25 mL of aquadest, 2.) concentration 0.4 b/v: 10 gram of papaya stem extract in 25 mL of aquadest, 3.) concentration 0.6 b/v: 15 gram of papaya stem extract in 25 mL of aquadest, and 4.) concentration 0.8 b/v: 20 gram of papaya stem extract in 25 mL of aquadest.

The samples received treatments accordingly. A recuring treatment was given 3 times to those worms with an inclusion of 5 *Ascaris suum* worms by soaking them into solutions mentioned above. Beakers are prepared by filling them with 25 mL of papaya stem extract according its concentration, 25 mL of pirantel pamoat solution according its concentration and 25 mL of aquadest solution, respectively. Then 5 *Ascaris suum* worms are included into each beaker. An observation must be conducted to conclude whether the worms die, paralyze or live normally after incubation. The observation could be conducted by disturbing those worms' movements with a stirring bar. If the worms are stoned, then they must be transferred into a glass of warm water with a temperature of 50°C. After this, if the worms are still stoned, then they must have been collapsed. But if these worms are still able to move, then they only got paralyzed. The result was recorded every three hours.

Results

From this research, the average time of death of the worms is seen as Table 1. Then the summary of Mann-Whitney Post Hoc Test on the time of death of the worms is seen as Table 2.

Table 1. The average time of death of the worms

Group	Average time of death of the worms (hour) +- SD
G (-)	168.6 +- 7.84
G (+)	13.87 +- 8.95
TG 1	9.27 +- 3.84
TG 2	7 +- 1.46

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TG 3	7.8 +- 7.33
TG 4	18.8 +- 11.19

Graphic 2. Summary of *Mann-Whiteney Post hoc Test* on The Time of Death of The Worms

Group	G (+)	TG 1	TG 2	TG 3	TG 4
G (-)	0.009*	0.009*	0.008*	0.009*	0.009*
G (+)		0.207	0.113	0.246	0.295
TG 1			0.264	0.665	0.116
TG 2				0.991	0.113
TG 3					0.116

G (-) : Negative control group; *Ascaris suum* in aquadest
G (+) : Positive control group; *Ascaris suum* in a solution of 0.5% pirantel pamoat
TG 1 : Treatment group 1; *Ascaris suum* in an extract of papaya stem 0.2 b/v
TG 2 : Treatment group 2; *Ascaris suum* in an extract of papaya stem 0.4 b/v
TG 3 : Treatment group 3; *Ascaris suum* in an extract of papaya stem 0.6 b/v
TG 4 : Treatment group 4; *Ascaris suum* in an extract of papaya stem 0.8 b/v
* : Meanifully different (p<0,05) on *Mann-Whitney Post hoc Test*

Discussion

Based on the result with the help of *Kruskal-Wallis Test* -the test to compare 6 groups data all at once which are related or unrelated- we obtained value p (sig) = 0.006 (p<0.05) that leads to an existance of, at least, 2 groups with different meanings. In order to find out the analysis result on which data is different at meaning between each treatment group, we conduct *Mann-Whitney non-parametric test*.

A research on antihelmintic activity of 96% ethanol extract of papaya (*Carica papaya* L.) stem. Table 1 shows the data on the everage time of death of the worms. The comparing result of *post hoc test* between negative control group and positive control group states that p<0.5, which means the positive control is appropriate. When negative control group is compared with the other treatment groups, it resulted a data of p<0.05. At this state, a conclusion can be drawn; there is a significant difference between negative control group and the other treatment groups. It means that treatment groups with concentration 0.2 b/v, 0.4 b/v 0.6 b/v, and 0.8 b/v experience an antihelmintic activity. At the other hand, the result of *post hoc test* between positive control group and treatment groups indicates p>0.05, which means the antihelmintic acitivity of 96% ethanol extract of papaya stem, which involving a 0.5% of pirantel pamoat, have no significant difference.

As a comparison, we are going to present a slightly similar research result regarding the same topic. Long before this research comes to an existence, a slightly similar research has been conducted by Roy et al. (2012). His research indicates that there is an antihelmintic activity on an extract of papaya leaves and stem. It is estimated that bioactive compounds like glikosida saponin and alkaloid in the extract of papaya leaves and stem play a role as antihelminth (Roy et al., 2012). A similar research conducted by Agarti et al. (2017) indicates an antihelmintic activity by infuse of papaya seed (*Carica papaya* L.) *studi in vitro.*

The death of *Ascaris suum* worms in 96% ethanol extract, approximately, is caused by bioactive compunds such as saponin, alkaloid, tannin, and papain (Pal and Mazumder, 2017). Alkaloid inside papaya stems is a karpain that posseses an antihelmintic effect. Karpain presses worms' central nervous system causing them to lose nervous coordination that makes them experiencing a paralysis. Based on the researcher's observation, the worms' posture looked benign and paralyzed, unlike the worms' on negative control group whose postures are firm like rubber bands (Faradila et al., 2013).

Saponin solution could block asetilkolinesterase enzyme, so that worms would undergo a muscle paralysis. It is assumed that tanin compund causes the enzymes produced by large roundworm of pig (*Ascaris suum*) to absorb nutrition, being bundled. This causes absorption process being disrupted and leads to nutrition deficiency (Faradila et al., 2013; Kuntari, 2008).

On a research conducted by Roy et al. (2012), extraction result of papaya leaves and stem with soxhlet extractor, which then being tested utiilizing thin layer chromatography, indicates that there is an existence of glikosida, saponin, and alkaloid compound. In this case, the absence of tannin, possibly, is caused by a denaturation at the soxhletation process. In this research, tannin was not extracted, also, due to a denaturation while undergoing evaporation process utilizing rotavapor which has been conducted in laboratory of Pharmacy Faculty of Universitas Muhammadiyah Surakarta.

Study on some research regarding active compounds inside the 96% ethanol extract of papaya stem stated before was only a literature review. The uncertainty of the worms availability on this research became the problem of the research uniformity. This could possibly be the cause of the overly high time span of death (+- 11.19 hour) on the treatment group with concentration 0.8 b/v. However, statistically, the comparing result of treatment group with concentration 0.8 b/v with the other treatment groups was $p>0.05$, which means there was no significant difference on antihelmintic effect.

Conclusion

Based on the research result and discussion above, a conclusion could be drawn; that there was an antihelmintic activity demonstrated by 96% ethanol extract of papaya stem (*Carica papaya* L.).

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