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Research

Mimosa pudica L. Leaves Ethanol Extract In Vitro Analysis of Anthelmintic Activity to *Ascaridia galli*

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Abstract: *Mimosa pudica* leaves contain of metabolites such as tannin, flavonoid and alkaloid which have the anthelmintic effects. This effect would be expected to minimalize the infection of parasitic worm *Ascaridia galli* in chicken. The research aim was to determine the effectivity of ethanol extract from mimosa leaves to *A. galli* worms in vitro. Mimosa leaves were extracted using maceration process. Worm samples were grouped randomly, consists of the positive control (Piperazine 1%), negative control (CMC Na 0,5%) and the treatment groups (10%, 20% and 50% of extract concentration). Mortality and paralysis data from the worm samples were collected and then analyzed using Kruskal Wallis and Mann Whitney Test. The phytochemical analysis showed that mimosa leaves extract contains all the secondary metabolite which can cause paralysis and mortality of *A. galli*. The 50% concentration of mimosa leaves extract can cause paralysis in 90 minutes equal with the positive control. The mortality time achieved by positive control, 50%, 20% and 10% of ethanol extract of mimosa leaves sequentially as follows 120, 270, 645 and 780 minutes. In conclusion, 50% mimosa leaves extract concentration have the best effect of anthelmintic, the paralysis effect equal with Piperazine but the mortality time is still slower than Piperazine.

Keywords: *Mimosa pudica*, Anthelmintic, *Ascaridia galli*

INTRODUCTION

Ascariasis in free ranging bird include chicken is most common caused by the infection of *Ascaridia galli*. This nematode infecting the small intestine of chicken and can cause financial loss in poultry [13,15]. Overall, the prevalence of ascariasis in laying hens with free-range eggs production due to *A. galli* can up to 84% [17]. In Kahsmir the prevalence was found around 35.5% and it can be higher in September [15]. The prevalence of *A. galli* infection in some region in Indonesia was vary, Jakarta is around 16% [12], 34.5% found in Jimbaran-Bali [13] and in Muara Badak-Kutai is about 53.3% [8].

The treatment widely used for ascariasis in chicken are carvacrol, piperazine citrate, levamisole, flubendazole, and fenbendazole [9,11,14,15,19]. All of these medicines are synthetic anthelmintic which have been found had several problems such as chemical residues, toxicity issue, and possibility lead to resistance [11,14]. Moreover, these chemical treatments are expensive, cannot afforded by most poultry farmer in Indonesia. To overcome this issue, many research done to find herbal medicine for ascariasis in chicken. Pineapple leaves extract also have the anthelmintic activity which can reduce the infection of *A. galli* in chicken, though it was not as good as piperazine citrate and levamisole [9]. Another research found

that *Ocimum sanctum* leaves [11], *Allium sativum*, *Acacia oxyphylla*, papaya latex [19], and *Mentha longifolia* [15] can also act as herbal anthelmintic for *A. galli*. Herbal anthelmintic will be expected as an easy resource to find and prepared, cheap, and reduced the helminth resistance to ascariasis treatment.

Mimosa pudica L. or prickly plant also called 'putri malu' in Indonesian is an abundant weed that can be found in deserted field, around the bush near the road, or in the backyard. This plant was found in 1753 by Carl Linnaeus [21]. Mimosa is a short prickly plant, their branches growing close to the ground and the plant height can reach up to half a meter. Their special character is their leaves which organized in bipinnate, fern like, the color is pale green, and has a tendency to closing when disturbed [16]. Mimosa plant have secondary metabolite consists of alkaloid, flavonoid, tannin and phenolic [10]. Almost every part of mimosa plant can be used, leaves, stem, root and seed. The extract of this plant traditionally used as the medicine for dysentery, inflammation, asthma, urinary infection, etc [1]. The use of mimosa plant extract now developed for pharmaceutical need such as antimicrobial, wound healing, antimalaria, antivenom, antidepressant, nefroprotector, etc [18, 22].

Among the uses of mimosa plant extract in pharmaceutical, one of them is as the anthelmintic. The research about anthelmintic activity from mimosa was already done in seed and leaves extract against *Pheretima posthuma* and several ruminants nematode. It was found that ethanol and water extract of mimosa have a good anthelmintic activity [4,6,16].

Mimosa was an abundant plant in Indonesia. The analysis of mimosa activity as anthelmintic still need to be developed. *Ascaridia galli* infection in chicken in Indonesia also quite high. Therefore, this research was need to be done and the aim was to determine the effectivity of ethanol extract from mimosa leaves to *Ascaridia galli* worms in vitro.

METHOD

Sample Collection

The mimosa plants were collected by hand picking in Marong, Middle Lombok, West Nusa Tenggara, Indonesia. The plants were washed and air-dry for several days. The dry leaves were collected, crushed with a blender and sifted to produced a very fine powder.

The *Ascaridia galli* worms were collected exactly before the examination in the laboratory from free-range chicken intestines which gathered from traditional market in Ampenan, Lombok, West Nusa Tenggara, Indonesia. These worms were placed in saline water and kept in 37ºC until ready to use.

Extraction and Phytochemical Analysis

The mimosa leaves powder was extracted using maceration process. Mimosa leaves powder macerated using 70% ethanol, left for 18 hours, and stirred at the interval of 6 hours. The maceration product was filtered and concentrated using rotary evaporator to produced mimosa leaves extract. The phytochemical analysis was done to the extract to test the alkaloid, flavonoid, tannin, saponin, terpenoid and steroid constituent.

Anthelmintic Effectivity Examination

The *A. galli* worms were divided into five groups for anthelmintic effectivity assessment which were positive control (Piperazine 1%), negative control (NaCl 0.9%), and 3 groups of treatment (10%, 20% and 50% of mimosa leaves extract). There were three *A. galli* worms in each testing groups and all groups were incubated in 37ºC. The time of paralysis and worm mortality would be observed

every 15 minutes and the observation time was limited according to the worm life span in the NaCl 0.9%.

Data Analysis

The data of paralysis and mortality time were analyzed statistically using Kruskal-Wallis and Mann Whitney Test (significant at $p>0.05$).

RESULTS

Phytochemical analysis results showed that mimosa leaves extract contain all of the alkaloid, flavonoid, tannin, saponin, terpenoid and steroid metabolites (Table 1.). Alkaloid, flavonoid and tannin are the metabolites which have anthelmintic activity.

Paralysis time of *A. galli* after exposed to the mimosa leaves extract is shown in Figure 1. The 50% concentration of mimosa leaves extract begin to paralyze the worm in 90 minutes and cause 100% worm paralysis in 150 minutes. Piperazine 1% (positive control) also begin the worm to paralyze in the minutes of 90, but causing 100% of paralysis in the minutes of 105. The starting point of paralysis between mimosa leaves extract and Piperazine was equal in 90 minutes. The other concentration of mimosa leaves extract, 10% and 20% caused paralysis much slower, 720 and 525 minutes respectively. These results indicated that the 50% mimosa leaves extract is as good as Piperazine to cause of *A. galli* paralysis.

Each concentration of mimosa leaves extract and 1% of Piperazine gives a mortality to *A. galli* in different time. Piperazine cause 100% of worm death after 120 minutes, this is the fastest mortality time compared to mimosa leaves extract. The mortality time in mimosa leaves extract from the fastest to the slowest are as followed, 50% in 270 minutes, 20% in 645 minutes and 10% in 780 minutes (Figure 2.). These results showed that Piperazine is still the best anthelmintic that cause 100% of *A. galli* mortality.

The statistical analysis from the paralysis and mortality time data showed that the increasing of mimosa leaves extract concentration will give the fastest time of *A. galli* paralysis and death. The mortality time of 50% mimosa leaves extract (270 minutes) is not as good as 1% of Piperazine (120 minutes). Nevertheless, the paralysis time of the 50% mimosa leaves extract is comparable with Piperazine (90 minutes). Therefore, the 50% of mimosa leaves ethanol extract is a good concentration so far for anthelmintic against *A. galli* (Table 2).

DISCUSSION

The paralysis of *A. galli* in mimosa leaves extract is caused by the alkaloid compound in the extract. The alkaloid, just like Piperazine is a chemical compound that can disrupt the worm's nervous system and cause paralysis. In this research, flaccid paralysis due to hyperpolarization and muscle relaxation is the type of paralysis cause by both mimosa leaves extract and Piperazine in *A. galli*. Piperazine usually work at ion chloride conductance in the worm muscle membrane, produces hyperpolarization and decrease the muscle excitability which then leads to flaccid paralysis [5]. Alkaloid has a neurotoxic activity and perform as agonist or antagonist to neuroreceptor or ion channels. In the worm's nervous system, alkaloid will inhibit the acetylcholine receptor which then stop the acetylcholine to enter the muscle and cause paralysis [20].

Flavonoid and tannin compound in mimosa leaves extract are assumed to be the cause of *A. galli* mortality. Flavonoid will disturb the energy consumption, makes the worm body weaker until death. Tannin has the effect on the tegument, it can bind to the tegument cuticle glycoprotein and damaging the cuticle. The damaging on the cuticle will then leads to the penetration of flavonoid and alkaloid and cause the death of the worm. Tannin binds to worm gastrointestinal and

tegument which can cause the death of the worm [3]. Moreover, tannin also can binds to collagen protein and protein rich in proline and hydroxyproline on the worm's cuticle. Tannin which binds to these proteins will cause the substantial structural damage to the cuticle and tegument [2]. Mimosa is a legumes plants, therefore the tannin from mimosa leaves extract is a condensed tannin. This type of tannin is effective against gastrointestinal nematode parasite. This tannin will bind to proteins and rotating the tegument of nematode to become inactive and cause mortality [7].

According to the statistical data analysis, the *A. galli* mortality time will increase significantly to the escalation of mimosa leaves extract concentration. The highest concentration of mimosa leaves extract (50%) can affect 100% mortality of worm in 270 minutes. This result showed that mimosa leaves extract has the anthelmintic ability but it is not as good as Piperazine which cause worm mortality in just 120 minutes. The work of flavonoid and tannin in mimosa leaves extract to alter mortality in *A. galli* is not comparable with Piperazine.

The statistical analysis of paralysis time showed significant differences between mimosa leaves extract treatment groups. The increasing of mimosa leaves extract concentration will faster the paralysis time. The difference of paralysis time which is not significant was found between 50% of mimosa leaves extract and Piperazine. Either Piperazine nor 50% of mimosa leaves extract can cause paralysis of *A. galli* in 90 minutes. This result showed that the activity of alkaloid in mimosa leaves extract is comparable with Piperazine to cause flaccid paralysis.

CONCLUSION

In conclusion, 50% of mimosa leaves extract concentration have the best effect of anthelmintic compared to 10% and 20%. The paralysis effect of this concentration equal with Piperazine but the mortality time is still slower than Piperazine.

DECLARATION

The authors declare that there is no conflict of interest.

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