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Research Article

Anthelmintic Activity of Ethanolic and Aqueous Extracts of *Allium fistulosum* L. Leaves on *Ascaris lumbricoides*

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ABSTRACT

The current study was to assess the anthelmintic activity of ethanolic and aqueous extracts of *Allium fistulosum* leaves using *Ascaris lumbricoides* as test worm. Three concentrations of the ethanolic and aqueous extracts of *A. fistulosum* leaves (50, 100, and 200 mg/ml) were separately tested against *A. lumbricoides* at room temperature for 7 hours. The results were expressed in terms of time to paralysis and time to death of the worms. Pyrantel pamoate was used as positive control, sodium chloride and carboxymethyl cellulose were served as negative and vehicle control, respectively. Phytochemical screening was also conducted to identify the chemical constituents of *A. fistulosum* leaves extracts. The results showed that the ethanol and aqueous extracts of *A. fistulosum* leaves to have anthelmintic activity at concentrations of 50, 100, and 200 mg/mL. Time to paralysis and time to death of the worms were doses dependent manner. Both ethanolic and aqueous extracts of *A. fistulosum* leaves have anthelmintic activity against roundworm *A. lumbricoides*. The leaf etanolic and aqueous extracts of the plant consisted of saponins, flavonoids, and steroids. Further studies are suggested to isolate of the active compounds from the extract of *A. fistulosum* leaves that responsible for the anthelmintic activity.

Keywords: anthelmintic activity, *Allium fistulosum*, *Ascaris lumbricoides*, ethanolic extract, aqueous extract

INTRODUCTION

Helminthiasis is still a health problem in the world. Tropical and subtropical regions, especially in sub-Saharan Africa, the Americas, China and East Asia are region that has high worm infection cases[1]. In Indonesia, it is currently estimated more than 4 million people suffer because of intestinal worm's infection. Soil-transmitted helminth disease also remains a major problem in Indonesia public health with the prevalence reaches 45-65%[2,3]. Helminthiasis is caused by infection of one or more parasitic intestinal worms such as roundworms (*A. lumbricoides*), whipworms (*Trichuris trichiura*), or hookworms (*Necator americanus* and *Ancylostoma duodenale*)[1]. *A. lumbricoides* is a human parasitic worm with the highest prevalence where it globally infects more than one million people[4]. The Ascaris is found in the human and pig intestines[4-6] which leads to ascariasis disease[5,7]. In human, ascariasis widespreads in all of ages with the highest prevalence in children. Ascariasis causes impaired growth and development of children[8,9]. In addition, migration of *A. lumbricoides* to the biliary[10,11], bronchus[12] or appendix[13] leads to an emergency situation in patient. *A. fistulosum* L. (Liliaceae) is one of horticultural commodities originating from Southeast Asia. In Indonesia, this plant is used as an ingredient for cooking because it provides a fragrant aroma and delicious flavor. The plant is also traditionally used as an anthelmintic[14]. Species of Liliaceae commonly contain tannins[15,16], steroids, triterpenoids and flavonoids[15,17] which are known to have an anthelmintic effect. This study was carried out to determine the anthelmintic activity of leaf ethanolic and aqueous extract of *A. fistulosum* against roundworm *A. lumbricoides*.

MATERIALS AND METHODS

Plant material

A. fistulosum leaves were collected from traditional market in Medan, Indonesia. The plant specimen was deposited and identified in Herbarium Medanense, Department of Biology, Faculty of Mathematic and Natural Sciences, University of Sumatra Utara.

Experimental worms

All experiments were conducted using roundworm *A. lumbricoides*. Roundworms isolated from intestinal pork were obtained from local slaughter house in Medan. The worm was washed with normal saline to remove all the faecal matter and then stored in normal saline solution. The roundworms with 10 – 30 cm in lenght were used for experiment. *A. lumbricoides* were identified at Animal Taxonomy Laboratory, Department of Biology, Faculty of Mathematic and Natural Science, University of Sumatera Utara.

Preparation of extracts

A. fistulosum leaves were cleaned, chopped and dried on

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Table 1: Time to Paralysis and Time to Death of *A. lumbricoides* Under the Ethanolic and Aqueous Extracts of *A. fistulosum* Leaves Treatments.

Groups	Concentration	Time to Paralysis (minute)	Time to Death (minute)
NaCl	0.9%	…	…
CMC-Na	0.5%	…	…
Pyrantel	3 mg/mL	69.99±4.10*	93.16±5.03*
	6 mg/mL	60.71±4.34*	75.59±6.34*
Ethanolic extract	50 mg/mL	131.81±2.02*+	242.92±9.06*+
	100 mg/mL	105.13±5.12*	185.27±2.90*+
	200 mg/mL	88.54±3.81*	151.54±20.88*
Aqueous extract	50 mg/mL	140.90±14.43*+	255.90±24.62*+
	100 mg/mL	104.54±21.81*	232.00±24.60*+
	200 mg/mL	30.28±8.91*	181.45±38.23*

Data was presented as mean±SEM, n=3, *=p<0.05 with CMC-Na, + = p<0.05 with pyrantel pamoate

Table 2: Chemical compounds of ethanolic and aqueous extracts of *A. fistulosum* leaves

Compounds	Ethanolic Extract	Aqueous Extract
Alkaloids	-	-
Saponins	+	+
Flavonoids	+	+
Steroids	+	+
Triterpenoids	-	-

drying cabinet at 60^0C. Dried leaves of the plant were powdered with electrical grounder. Ethanolic extract was prepared by percolating the dried leaves powder of plant (800g) in ethanol (96%). Solvent was evaporated with rotary evaporator to obtain the crude ethanolic extract of *A.fistulosum* leaves. To prepare the aqueous extract, 900 g of *A. fistulosum* dried leaves were boiled in distilled water on water bath at 90^0C for 30 minutes. After filtration, liquid was evaporated and freeze dried to obtain the aqueous extract of the plant leaves.

Anthelmintic activity

Anthelmintic activity test was performed according to the method of Das et al.[18] and Pillai et al.[19] with slight modifications. Each *A. lumbricoides* adult worm was placed in a petridish containing 25 ml of test solution. Ethanolic and aqueous extracts of *A. fistulosum* leaves at the doses of 50, 100, and 200 mg/ml were used for experiment. Pyrantel pamoate (3 and 6 mg/ml), sodium chloride 0,9%, w/v, and carboxymethyl cellulose 0.5% w/v were served as positive control, negative control, and vehicle control, respectively. All test solution was prepared freshly prior to the experiments. The anthelmintic activity was determined by observing time to paralysis and time to death of the worms for 7 hours. Time to paralysis of worms was noted when no movement of any sort could be observed, except when the worm was shaken vigorously or immersion in 50^0C hot water. Time to death of worm was recorded after ascertaining that worm neither moved when shaken nor when immersed in water 50^0C.

Statistical analysis

Data were analyzed using one-way ANOVA tests followed by Tukey's tests. The *p* values under 0.05 were considered as significantly different.

RESULTS

As shown in Table 1, the ethanolic and aqueous extracts of *A. fistulosum* leaves revealed anthelmintic activity at the concentration of 50, 100, and 200 mg/ml. Both extracts led to paralysis and followed death of *A. lumbricoides*. At a dose of 200 mg/ml, the leaf ethanolic and aqueous extracts of plant were more potent to paralysis and kill the worms compared with pyrantel pamoate. The results also clearly indicated that the time to paralysis and time to death of the worms decreased as increasing doses of the plant extracts (Fig. 1). Therefore, the ethanolic and aqueous extracts of *A. fistulosum* leaves possessed potential wormicidal activity, so it's may be used as an anthelmintic agent. Table 2 described chemical compounds of the ethanolic and aqueous extracts of *A. fistulosum* leaves that indicated the presence of saponins, flavonoids, and steroids.

DISCUSSION

The purposes of this study were to evaluate the anthelmintic activity of the ethanolic and aqueous extracts of *A. fistulosum* leaves against *A. lumbricoides* and to identify its chemical constituents. Members of family Liliaceae has been widely used as medicinal plant for helminthiasis treatment. Anthelmintic activity of *Allium sativum*[20] and *Gloriosa superba*[21] against earthworms *Pheretima posthuma* has been reported. Their reports have relevance with our study, the ethanolic and aqueous extracts of *A. fistulosum* leaves led to A. lumbricoides paralysed and finally death. The anthelmintic effects of *A. fistulosum* leaves extracts also produced in doses dependent manner. The ethanolic and aqueous of *A. fistulosum* leaves extracts contained saponins, flavonoids, steroids and triterpenoids. The compounds may responsible for the anthelmintic activity of the plant extracts[15-17]. Other phytochemical compounds that produce the anthelmintic activity are alkaloids[22]. Patilaya and Husori[23] reported the anthelmintic activity of ethanolic extract of *Curanga fel-terrae* leaves against *P. phostuma*, the extract contains flavonoids, glycosides, saponins, tannins and terpenoids. Anthelmintic activity of alkaloids is related to the hydrophobicity[22,24]. Guimaraes et al.[25] reported that the alkaloid (epiisopiloturine) possess in vivo anthelmintic activity. Waghmare et al.[26] reported the anthelmintic activity of ethanolic and petroleum ether extract of *Murraya koenigii* fruits.

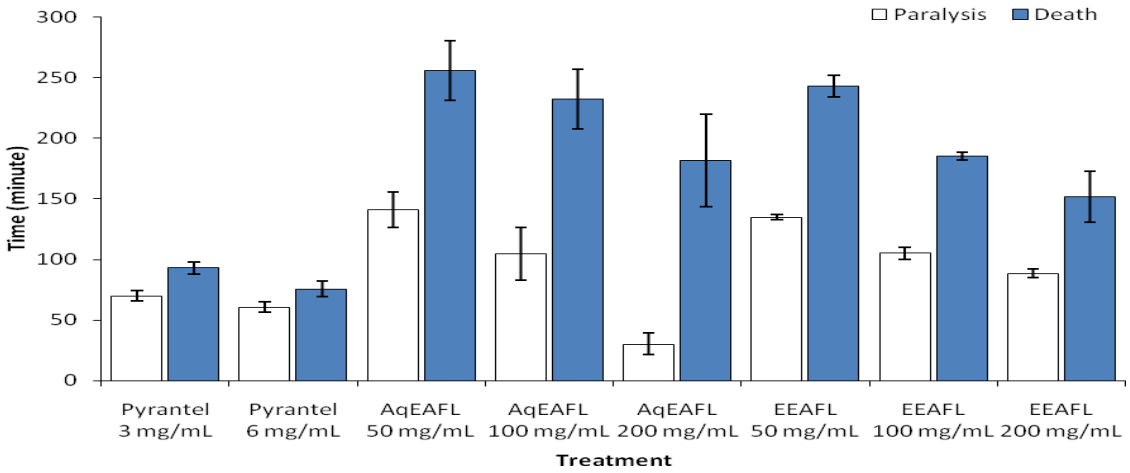

Figure 1: Paralysis and death time of *A. lumbricoides* vs Ethanolic (EEAFL) and Aqueous (AqEAFL) Extracts of *A. fistulosum* Leaves. Data were presented as mean±SEM, n=3.

Anthelmintic activity of steroid compounds was reported by Wang et al.[27], steroid saponin (dioscin and polyphyllin D) has potential activity against *Dactylogyrus intermedius*. While Hernández-Villegas et al.[28] found that saponins from *Phytolacca icosandra* exhibit ovicidal and larvicidal activity against *Haemonchus contortus*. Saponin isolated from *P. hydropiper* was also reported to have the in vitro cytotoxic activity with broad-spectrum[29]. The anthelmintic activity of triterpenoids was also studied by Patel et al.[30]. Unfortunately, the anthelmintics mechanism of action of phytocehmical compounds has not been reported clearly. However, Kamal et al.[31] claimed that flavonoids and saponins acted as antichlolinesterase to kill the infectious worms.

CONCLUSION

Based on this study we concluded that the ethanol and aqueous extracts of *A. fistulosum* have potential anthelmintic effects against roundworms *A. lumbricoides*. Further studies are suggested to isolate of the active compounds of the plant extracts which are responsible for the anthelmintic activity as well as their mechanism of actions.

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