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Preliminary study on the anthelmintic activity of the leaf ethanolic extract of Indonesian *Curanga fel-terrae* (Lour.) Merr.

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Abstract: The present study was carried out to investigate the anthelmintic activity of the leaf ethanolic extract of *Curanga fel-terrae* against *Pheretima posthuma*. The leaf of *C. fel-terrae* was extracted in 96% ethanol by maceration. Anthelmintic activitiy was performed by exposing *P. posthuma* to the leaf ethanolic extract of *C.fel-terrae* solution at the concentration of 10, 20, 30, dan 40 mg/ml for 5 hours. Standard albendazol at dose of 10 mg/ml was used as positive control. While normal saline and 1% ethanol solution were served as negative and solvent control, respectively. The anthelmintic effect of the plant extract was determined by observing the paralysis and death time of the earthworm. The results showed that the leaf ethanolic extract of *C. fel-terrae* at all doses caused *P. posthuma* was paralysed at 3.43 - 41.28 minutes and then death at 7.92 – 47.26 minutes. In addition, the anthelmintic effects of the plant extract were produced in a dose dependent manner. While paralysis and death of the earthworm exposed to albendazol 10 mg/ml were 31.27 and 56.26 minutes, respectively. The results demonstrated that the anthelmintic activity of the ethanolic extract at the doses of 20, 30, and 40 mg/ml were significantly more potent compared with standard albendazole ($p<0.05$). This study indicates that the leaf ethanolic extract of *C. fel-terrae* has potential anthelmintic activity against *P. posthuma*.
Keywords: Curanga fel-terrae, puguntano, anthelmintic, earthworm, Pheretima posthuma..

Introduction

Helminthiasis is an infection disease caused by nematode worm such as *Ascaris lumbricoides*, *Trichuris trichiura*, *Necator americanus*, and *Ancylostoma duodenale*. Infection occured when ingesting food-contaminated eggs or larvae, hands or utensils or through penetration of the skin by infective hookworm larvae in contaminated soil[1]. World Health Organization estimated that more than 1.5 billion (24%) of world population are infected by parasite worm. The parasite worm infection cases are commonly occured in tropic and sub-tropic regions with the most cases in Africa, America, China, and the Southeast Asia[2]. In Indonesia, helminthiasis remains a serious health problem. It estimated more than 4 millions Indonesian people are infected by the parasite worm[3]. These conditions to be complicated when the parasite worm is resistant to anthelmintics[4-10]. In addition, the current drugs for human parasite worm infection are also limited and the development of new anthelminitic is rarely performed. It causes medication therapy for the worm infection become to be arduous. Therefore, development of new anthelmintic is very urgent[11].

Medicinal plants has important role to eliminate soil-transmitted helminthiasis. Diversity of the medicinal plants is potential resource for developing new drug, especially as lead compound that can be

synthesized to its more potent derivates[12]. Anthelmintic potential activity of plant metabolites such as alkaloids, flavonoids, glycosides, quinolones, lignans, saponins, and terpenoids has been reported. *In vitro* studies indicated that plant spesies from famili *Amaranthaceae*, *Arecaceae*, *Asteraceae*, *Crassulaceae*, *Dryopteridaceae*, *Fabaceae*, *Lamiaceae*, *Lythraceae*, *Moraceae*, *Myrsinaceae*, *Rutaceae*, and *Schropulariaceae* capable to kill human parasite worms[13].

Indonesian *C. fel-terrae* was known as *puguntano*, *kukurang*, *tamah raheut*, and *mempeduh tanah* belongs to famili Scrophulariaceae. The plant is distributed in Sumatra, Java, Kalimantan and Maluku regions[14]. In Indonesia, the leaf of this plant was commonly used as traditional medicine to cure helminthiasis[15]. However, very litle information is available for its anthelmintic activity. Therefore this study was conducted to investigate anthelmintic activity of the plant against *Pheretima posthuma*.

Materials and methods

Chemicals

The chemicals reagents of analytical grade used in this study were obtained from Merck, Germany, including ethanol 96%, chloride acid, potassium iodide, mercury chloride, ferri chloride, nitric acid, lead acetic, sodium hydroxide, acetic anhydride acid, sulfuric acid, ether, chloroform, methanol, petroleum ether, zinc powder, magnesium powder, isopropanol, benzene, sodium picric, *n*-hexane, dimethylsulfoxide, and sodium chloride. Standard albendazol was obtained from Sigma-Aldrich, USA.

Equipments

The equipments used in this work were balance (Sartorius, Germany), oven (Memmert, Germany), grinder (Panasonic, Japan), rotary evaporator (Eyela, USA), autoclave (Webeco, Germany), hot plate (Cimarec, USA), petridish (CMSI, Indonesia), and stopwatch (Saturn, UK).

Preparation of plant extract

Plant materials were collected from Dairi District, North Sumatra Province, Indonesia. The plant specimen was determined and stored by LIPI, Bogor, Indonesia. The leaf of *C. fel-terrae* was cleaned through tap water, dried, and then reduced to powder using electrical grounder. The dried powder of plant leaves (100 g) was extracted in 1 L of 96% ethanol by maceration. The extract was evaporated under reduced pressure to produce the crude ethanolic extract.

Phytochemical screening

Phytochemical screening of the dried material and the etanolic extract of *C. fel-terrae* leaves were carried out to identify alkaloids, flavonoids, glycosides, saponins, tannins, and terpenoids/steroids[16].

Anthelmintic activity testing

Adult *P. posthuma* with approximately equal size was used in this study. The eartworms were collected from moist soil and cleaned with distilled water. Prior to expriment, the earthworm was determined by Animal Taxonomy Laboratory, Faculty of Mathematic and Natural Sciences, University of Sumatra Utara. The earthworm was acclimated in saline solution for 60 minutes[17]. The animal was divided into seven groups where each group consisted of 3 earthworms. The earthworm was placed into petridish containing 20 ml of solution test. Group I as negative control was treated with normal saline. Group II served as solvent control was treated by 1% ethanol. Group III treated with standard albendazol (10 mg/ml) was used as positive control. Group IV - VII were treated by the plant extract at the concentration of 10, 20, 30, and 40 mg/ml, respectively. Paralysis and death of individual earthworm were observed for 5 hours. Paralysis was defined if there is no movement of the worms except when shaken vigorously. Death was considered when the worms lost their mortality followed by fading away of their body color.

Statistical analysis

The results were presented as mean ± standard deviation. All data were analyzed by using SPSS software 17.0. Data were analyzed using one-way analysis of variance (anova) and followed with Tukey/Duncan (all mean) post hoc test.

Results

Phytochemical screening

The results of preliminary phytochemical screening of the leaf ethanolic extract of *C. fel-terrae* showed the presence of flavonoids, glycosides, saponins, tannins, steroids, and terpenoids

Anthelmintic activity

The ethanolic extract of *C. fel-terrae* leaves revealed anthelmintic activity against *P. phostuma* (Table 1). All doses exhibited the anthelmintic effect to the worms with the paralysis time ranging from 3.43 to 41.28 minutes and the death time ranging from 7.92 to 47.26 minutes. In addition, the anthelmintic effects of the plant extract were produced in a dose dependent manner. Statistical analysis indicated that the anthelmintic activity of the ethanolic extract of *C. fel-terrae* leaves at doses of 20, 30, and 40 mg/ml significantly difference with standard albendazole ($p < 0.05$). The anthelmintic activity of the plant extract at such doses was more potent than standard albendazol.

Table 1. Anthelmintic activity of the leaf ethanolic extract of *C. fel-terrae* against *P. posthuma*. Data presented as mean ± standard error of the mean (n = 3); *p<0.05 indicated significantly difference between the plant extract with standard albendazole.

Samples	Concentration (mg/ml)	Paralysis time (minute)	Death time (minute)
Normal saline solution	-	no effect	no effect
Ethanol 1%	-	no effect	no effect
Albendazol	10	31,27 ± 0,20	56,26 ± 0,41
Ethanolic extract	10	41,28 ± 0,41*	47,09 ± 0,80*
	20	23,27 ± 0,26*	27,41 ± 0,65*
	30	12,18 ± 0,07*	16.66 ± 0,13*
	40	3,43 ± 0,04*	7,92 ± 0,10*

Discussion

The present study only identified general phytochemical compounds the ethanolic extract of *C. fel-terrae* leave. However, other researchers successed to isolate flavonoid glucoronides, dehydrobryogenin glycoside, hexanorcucurbitacin F, phenylethanoid glycosides, cucurbitacin glycosides[18], triterpenoid saponins[19], and β-sitosterol[20] from *C. fel-terrae*. Plant species of family Schropulariaceae commonly contain flavonoids, saponins, tannins, glycosides, and terpenoids. In addition, typical secondary metabolites especially cucurbitacins and iridoids are widely distributed in *Bacopa moniera*, *Picria fel-terrae*, *Eremophila sp*., *Picrorhiza sp*., and *Scoparia dulcis*[21-26]. Cucurbitacins and iridoids were reported to have anthelmintic activity against *Leishmania donovani*[27].

Anthelmintic activity of *Schropulariaceae* spesies was also reported. Methanolic extracts from the aerial parts of 13 *Verbascum* species exhibited to have potential *in vivo* anthelmintic activity against *Aspiculuris tetraptera* at doses of 100 mg/kg body weight[28]. The essential oil of *Limnophila conferta* showed anthelmintic effect toward earthworm, roundworm, and tapeworm at doses ranging from 1.7 mg/ml to 2.0 mg/ml[29]. A good anthelmintic activity was also demonstrated by the methanolic extract of *Picrorrhiza kurroa* rhizome against *P. posthuma* in a dose dependent manner from 20 mg/ml to 120 mg/ml[30].

An anthelmintic is toxic substance that kills earthworm by paralyzing or energy depleting, so the worm can not fullfill their metabolic needs [31]. The leaf ethanolic extract of *C. fel-terrae* was also toxic on the *P. posthuma*, so it is very potential as anthelmintic source. The anthelmintic activity of plant extract might be due to the presence of its phytochemical compounds such as polyphenols, tannins, saponins, and glycosides[32-34]. The plant extracts contained tannins in high concentration indicated to have potential anthelmintic activity[35]. Tannins is a polyphenol which is able to disturb the earthworm metabolism through oxidative phosphorilation reaction[36]. The anthelmintic effects of tannins may also due to its capacity to bind free protein nutrition resulting in larval starvation or interact with glycoproteins of the worm cuticula causing the worm death[37]. Glycosides and steroids have antioxidant properties are able to decrease production of nitrate causing the

inhibition of worm development[38]. The anthelmintic mechanism also involves inhibition of glucose uptake system that leads to the lost energy of worm[39]. However, the anthelmintic mechanism of the leaf ethanolic extract of *C. fel-terrae* has not clear yet, therefore, the further studies are needed.

The leaf ethanolic extract of *C. fel-terrae* has potential anthelmintic activity. The results reported in this study also support the folklore claims of the usage of this plant for helminthiasis treatment.

Conflict of interest statement

We declare that we have no conflict of interest

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