ANTHELMINTIC ACTIVITY OF SUB-FRACTIONS FROM THE N-HEXANE FRACTION OF PICRIA FEL-TERRAE LEAVES ON PHERETIMA POSTHUMA

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INTRODUCTION

More than 1 billion of the world population is infected by parasitic worms. Helminthiasis cases commonly occur in tropic and subtropic regions, especially Africa, America, and South-East Asia. In Indonesia, helminthiasis also remains a health problem in which more than 4 million people infected are found [1]. In addition, the emergence of resistant worm strains lead to helminthiasis is difficult to eradicate [2,3].

To solve these problems, development of new anthelmintics from medicinal plants is very urgent [4]. Picria fel-terrae has been used to treat helminthiasis by Indonesian people [5]. The leaves ethanolic extract of this plant has anthelmintic activity on Pheretima posthuma Ascaridia galli. The plant extract contains flavonoids, saponins, tanmins, glycosides, and steroids/triterpenoids. However, the active compounds of plant extract that responsible for anthelmintic activities have not been identified, so it is need to be studied [6]. According to Yalavarthi and Thiruvengadarajan, triterpenoids compounds. The bands with reddish purple and bluish green may indicate the presence of steroids/triterpenoids compounds.

Keywords: Anthelmintic, Picria fel-terrae, Pheretima posthuma, Helminthiasis.

METHODS

Preparation of plant SF

P fel-terrae leaves were extracted with 96% ethanol. The plant extract solution was mixed with water in ratio of 7:3 and then fractionated with n-hexane by liquid-liquid extraction. The n-hexane fraction (19.4 g) was then separated on silica gel by vacuum liquid chromatography with n-hexane-ethyl acetate in ratios of (100:0), (90:10), (80:20), (70:30), (60:40), (50:50), (40:60), (30:70), (20:80), (10:90), and (0:100) and methanol as mobile phases. Each 250 mL of filtrate was collected and separated by thin layer chromatography with Lieberman-Burchard as detecting agent. The filtrates with same chromatogram pattern were combined to produce SF of the plant leaves.

Anthelmintic activity testing

The procedure from Agrahari et al. and Hounzangbe-Adote et al. were adopted in this study with slight modifications [8,9]. P. posthuma was acclimated in saline solution for 60 min. The worms were exposed to the plant SF and pyrantel at the concentration of 0.1%. Saline solution containing Na-CMC and tween-80 was included as negative control. Anthelmintic activity of these samples was determined by observing paralysis and death times of the worms during the experiment.

RESULTS

Fig. 1 described the chromatogram patterns of SF from n-hexane fraction of P. fel-terrae leaves on P. posthuma. The results showed that the plant fraction produced 4 SF, namely, SF1, SF2, SF3, and SF4. The paralysis time of worms treated by the plant SF was shorter than vehicle effects as well as the

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ABSTRACT

Objective: This study was to evaluate the anthelmintic activity of the subfractions (SF) from n-hexane fraction of Picria fel-terrae leaves on Pheretima posthuma.

Methods: The leaves ethanolic extract of P. fel-terrae solution was mixed with water in ratio of 7:3. The mixture was fractionated with n-hexane by liquid-liquid extraction. The n-hexane fraction was then separated on silica gel by vacuum liquid chromatography with n-hexane-ethyl acetate and methanol as mobile phases. The filtrates with same chromatogram pattern were combined to produce SF of the plant leaves. The SF at the concentration of 0.1% was tested on P. posthuma to evaluate its anthelmintic activity. The anthelmintic activity was determined by observing paralysis and death times of the worms. Pyrantel 0.1% and vehicle were included as positive and negative controls, respectively.

Results: The study showed that n-hexane fraction of P. fel-terrae leaves produced 4 SF, namely, SF1, SF2, SF3, and SF4. The paralysis time of worms treated with SF1, SF2, SF3, SF4, and pyrantel, respectively. The SF1, SF2, SF3, SF4, and pyrantel also caused the animal death at 123.00, 130.33, 78.67, 74.33, and 127.33 min when treated with SF1, SF2, SF3, SF4, and pyrantel, respectively. The animal paralysis and death times by those substances were shorter than negative control effects.

Conclusion: This study suggests that the SF from n-hexane fraction of P. fel-terrae leaves has anthelmintic activity on P. posthuma. The effects of SF4 are stronger when compared with SF1, SF2, SF3, and pyrantel.

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Table 1 describes the effects of SF from n-hexane fraction of P. fel-terrae leaves on P. posthuma. The results showed that the SF of the plant caused paralysis and death of the worms. The paralysis time of worms treated by the plant SF was shorter than vehicle effects as well as the
due to prolonged activation of the excitatory nicotinic acetylcholine receptors on the body wall muscle [14]. Therefore, further study is needed to isolate the active compounds of *P. fel-terrae* leaves as well as its action mechanisms.

**CONCLUSION**

This study suggests that the SF from n-hexane fraction of *P. fel-terrae* leaves has anthelmintic activity on *P. posthuma*. The effects of SF4 are strongest when compared with SF1, SF2, SF3, and pyrantel.

**ACKNOWLEDGMENT**

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**REFERENCES**


**Table 1: The effects of SF from n-hexane fraction of *P. fel-terrae* leaves on the paralysis and death of *P. posthuma***

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Paralysis time* (min)</th>
<th>Death time* (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SF1</td>
<td>123.00±5.29</td>
<td>156.00±7.55</td>
</tr>
<tr>
<td>SF2</td>
<td>130.33±1.45</td>
<td>166.67±0.88</td>
</tr>
<tr>
<td>SF3</td>
<td>78.67±0.88</td>
<td>107.00±1.15</td>
</tr>
<tr>
<td>SF4</td>
<td>74.33±0.67</td>
<td>101.67±0.33</td>
</tr>
<tr>
<td>Pyrantel 0.1%</td>
<td>127.33±1.45</td>
<td>140.00±2.65</td>
</tr>
<tr>
<td>Vehicle</td>
<td>310.33±2.60</td>
<td>369.33±2.91</td>
</tr>
</tbody>
</table>

*The data are presented as mean±SE. SF: Subfractions, *P. posthuma*: Pheretima posthuma, *P. fel-terrae*: Picria fel-terrae.death time. Statistical analysis also indicated that the anthelmintic effects between samples tested were significantly different (p<0.05). It means that the effects of SF4 are strongest when compared with SF1, SF2, SF3, and pyrantel.

**DISCUSSIONS**

Different species of worms such as earthworms, *Ascaris, Nippostrongylus*, and *Heterakis* are used to assess the anthelmintic activity of natural products. However, the earthworms have been widely used for the initial study of *in vitro* anthelmintic activity [10]. According to Sen et al., substances that have anthelmintic activity on the earthworms is also effective against parasitic worms of human [11]. The present study exhibited the anthelmintic activity of SF4 is most potent to *P. posthuma* among the others even than the standard pyrantel. This result indicates that probable active compounds of *P. fel-terrae* leaves may concentrated in this SF. According to Kumanasringha et al., fraction derived from the dichloromethane: methanol extract of the whole plant exhibits anthelmintic activity on *Caenorhabditis elegans* [12].

This study also revealed that the active plant SF consists of steroids/triterpenoids compounds. Plant steroids have the ability to afford specific action mainly on muscle, while triterpenoids may inhibit glucose uptake and depletes the glycogen content in worms [13]. Interestingly, the plant SF SF4 is more potent than standard drugs, pyrantel. Pyrantel is nicotinic receptor agonist that produces muscle paralysis of worms due to prolonged activation of the excitatory nicotinic acetylcholine receptors on the body wall muscle [14]. Therefore, further study is needed to isolate the active compounds of *P. fel-terrae* leaves as well as its action mechanisms.

This study suggests that the SF from n-hexane fraction of *P. fel-terrae* leaves has anthelmintic activity on *P. posthuma*. The effects of SF4 are strongest when compared with SF1, SF2, SF3, and pyrantel.

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