



## Research Article

### THE ANTHELMINTICS EFFECT OF *MOMORDICA CHARANTIA* L. LEAVES AND *ANDROGRAPHIS PANICULATA* NESS. FROM INDONESIA

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#### ABSTRACT

*Momordica charantia* L. leaves and *Andrographis paniculata* Ness. leaves were traditionally used as anthelmintic in Indonesia.

**Objective:** This study aims at examining the anthelmintic effects of 70% ethanolic extract of *Momordica charantia* L. leaves and *Andrographis paniculata* Ness. leaves.

**Material and Method:** This research was an experimental research with only post test controlled group design. The samples were 72 *Ascaris suum* worms, divided into 8 groups. The first group was treated by NaCl 0.9% solution as negative control, the second group was treated by pyrantel pamoate as positive control in 0.236 % concentration. The third, fourth and fifth group were treated by 70% ethanolic extract of *Momordica charantia* L. in 20%, 40%, 80% concentrations respectively. The sixth, seventh and eighth groups were treated by 70% ethanolic extract of *Andrographis paniculata* Ness. in 20%, 40% 80% concentrations respectively. Data were collected by observation of total mortality time of *Ascaris suum* worm every 1 hour. The anthelmintic effects test analysis is performed by Kruskal Wallis test and followed by Mann Whitney test with significance level at  $p < 0.05$ .

**Result:** There are is significant difference between the 70% ethanolic extract of *Momordica charantia* L (20%, 40%, 80%) and *Andrographis paniculata* Ness. (20%,40%,80%) with control negative with P value  $< 0.05$  by Mann whitney test.

**Conclusion:** The 70% ethanolic extract of *Momordica charantia* L. and *Andrographis paniculata* Ness. leaves have potential as anthelmintic.

**KEYWORDS:** *Momordica charantia* L., *Andrographis paniculata* Ness., *Ascaris suum*, anthelmintic effect.

#### INTRODUCTION

Helminthic infection is one of health problems in the world. The most common helminthic infection is Ascariasis, which is caused by *Ascaris* worm. This is estimated to infect about 0.8-1.22 billion people, with an estimated prevalence of 25%<sup>1</sup>. The prevalence of ascariasis is highest in children aged 2-10 years, with the highest intensity of infection occurring in children aged 5-15 years who have simultaneous infections with other soil-transmitted helminths such as *Trichuris trichiura* and hookworm<sup>2</sup>. Usually present with no specific symptom, ascariasis mostly occurs in children of tropical and

developing countries, where they are perpetuated by contamination of soil by human feces or use of untreated feces as fertilizer<sup>3</sup>. Symptoms that show on ascariasis include growth retardation, pneumonitis, intestinal obstruction, or hepatobiliary and pancreatic injury. Ascariasis may exist as a zoonotic infection corelated with pigs and use of hog manure<sup>4</sup>. Children with a marginal diet may be susceptible to protein, caloric, or vitamin A deficiency, resulting in retarded growth and increased susceptibility to infectious diseases such as malaria<sup>5</sup>. Even though if estimates of disability-adjusted years of life due

to ascariasis have fallen because of development and management programs during the 1990s, especially in Asia, but still constitute a significant burden in some countries and might be equivalent to malaria or tuberculosis worldwide<sup>2</sup>. Large and tangled worms may cause intestinal (usually ileum), common duct, pancreatic, or appendiceal obstruction. Mean worm burden varies from more than 16 to 4 and appears related to host factors, particularly age, geophagy<sup>6</sup>, and immunity. Worms do not multiply their quantity in the host body. An infection case that persists more than the 2-year maximum lifespan of the worms, is predicted caused by re-exposure. Some children look very severely infested, it may be caused by multiple cumulative exposures over time and/or relative immunodeficiency<sup>7</sup>.

*Ascaris suum*, a swine nematode, has been suspected responsible for zoonotic infection. It is difficult to differentiate between *A. lumbricoides* and *A. suum* is difficult, because the difference between them are only 6 (1.3%) nucleotides in the first internal transcribed spacer (ITS-1) and by 3%-4% in the mitochondrial genome sequence<sup>7</sup>. *A. suum* appears to cause nearly in all ascariasis cases in well-developed countries with excellent sanitation (e.g., Denmark<sup>8</sup>, United States<sup>4</sup>, UK<sup>9</sup>). In the cases like this, those who are infected will only have a low worm burden and may present with not too severe manifestation, such as cough, acute eosinophilia, or eosinophilic liver lesions visible on CT scans. However, a molecular genetic study from China casts doubt that infections in pigs are the cause of most human infection<sup>10</sup>.

*Momordica charantia* L. (family: Cucurbitaceae) is a tropical and subtropical plant distributed in Asia, Africa, South America, Caribbean countries and commonly known as Pare in Indonesia. The plant is harvested for its bitter fruits, which usually used as a vegetable. Since ancient times, different parts of *M. charantia* are used in traditional medical management of diverse human diseases<sup>11</sup>. Fruit, leaf, seeds of the plant used as anthelmintic in different traditional medicinal systems<sup>12,13,14</sup>. Previous biological studies of *M. charantia* occurred that the plant parts possess anti-oxidant<sup>15,16</sup>, anti-diabetic<sup>17,18</sup>, anti-obesity<sup>19</sup>, anti-viral<sup>20</sup>, anti-bacterial<sup>21</sup>, insecticidal<sup>22</sup>, anti-cancer<sup>23</sup>, anti-tumor<sup>24</sup>, anti-depressant and anxiolytic<sup>25</sup>, anti-fertility<sup>26</sup>, wound healing<sup>27</sup>, analgesic and anti-pyretic<sup>28</sup> activity. *M. charantia* contains many potential pharmacologically active substances such as

charantine, goyaglycoside, mormodicoside, mormodicoside 3 $\beta$ , 25-dihydroxy-5 $\beta$ , 19-epoxycucurbita-6, (23E)-diene, momordicine-I, karavilagenin, karavilagenin C, karaviloside, karaviloside, kuguacin, kuguacin A, kuguacin B, kuguacin E<sup>13,29</sup>, 3,7,23-trihydroxy-cucurbita-5,24-diene-19-al<sup>29</sup>, 3,7,25-trihydroxy-cucurbita-5, 23-diene-19-al<sup>29</sup>, 3,7-dihydroxy-25-methoxycucurbita-5, 23-diene-19-al<sup>29</sup>. Ethanol extract of *Momordica charantia* L. has investigated for in vitro anthelmintic effect against *Ascaris suum*<sup>30</sup>. Another study reported that plant extract has better anthelmintic effect against *A. galli* than piperazine hexahydrate<sup>31</sup>.

*Andrographis paniculata* Nees, usually known as the "king of bitters," is an herbaceous plant belonging to the Acanthaceae and is found throughout tropical and subtropical Asia, Southeast Asia, and India. The predominant chemical substances exist in *A. paniculata* are andrographolide, andrographin, panicolin, andrographolide, diterpene glucoside-neoandrographolide, andrographidihnes, neoandrographolide, chlorogenic, myristic acide, homoandrographolide, andrographiside andropanoside, etc. *A. paniculata* are also available in the market to cure various diseases. This plant has antimalarial<sup>32</sup>, anti-inflammatory, antioxidant<sup>33</sup>, antihepatic<sup>34</sup>, antihyperglycemic<sup>35</sup>, anthelmintic<sup>36</sup>, antibacterial<sup>37</sup>, antipyretic<sup>38</sup>, and anticancer activity<sup>39</sup>. Anthelmintic effect of the extract may be attributed to the phytochemical substances such as sterols and terpenes, polyphenols, flavonoids, tannins saponins, and alkaloids. These compounds would be having strong activity due to the phytochemicals and could explain the anthelmintic activity of the plant. However, more detailed phytochemical analysis and experiments are required to isolate and characterize each active compound which is responsible for the anthelmintic activity and to know the exact mechanisms of action of this anthelmintic activity<sup>40</sup>.

In tropical developing countries helminthic infections causes a serious health problem for human. Even though gastrointestinal helminthic infections are not responsible for significant morbidity and mortality but they may cause a rather severe effects, for example malabsorption diarrhea, anemia and other states of poor health<sup>41,42</sup>. Although there are already a number of synthetic medicines for human, but it still do not distribute worldwide evenly, especially

in remote areas people still cannot afford it. Another key problem is drug resistance that often happens in case of managing parasite infections. In this context, use of medicinal plants tender a foremost and accessible source of health care to people<sup>41,42,43</sup>.

## MATERIAL AND METHOD

### Material

The plants used in this research were *Momordica charantia* L. and *Andrographis paniculata* Ness. were harvested from Karanganyar, Central Java, Indonesia in January 2015. The animal test were *Ascaris suum* obtained from butchery of hogs "Radjakaja" Surakarta.

### Preparation of extract

1.	Negative control	:	NaCl 0.9% 12.5 ml
2.	Positive control	:	Pyrantel Pamoate 0.236%
3.	Group III	:	The 70% ethanolic extract of <i>Momordica charantia</i> in 20% concentrations
4.	Group IV	:	The 70% ethanolic extract of <i>Momordica charantia</i> in 40% concentrations
5.	Group V	:	The 70% ethanolic extract of <i>Momordica charantia</i> in 80% concentrations
6.	Group VI	:	The 70% ethanolic extract of <i>Andrographis paniculata</i> Ness. in 20% concentrations.
7.	Group VII	:	The 70% ethanolic extract of <i>Andrographis paniculata</i> Ness. in 40% concentrations
8.	Group VIII	:	Ethanolic extract of <i>Andrographis paniculata</i> Ness. in 80% concentrations

### Anthelmintic test

Worms treated according to group giving all treatments carried out for 24 hours. To see if the worm has died after being put in the liquid extract, the worms are disturbed by stir bar every hour. The worm is said to still be alive if they move on and for the non-moving worm, the worm is immersed in distilled water 50° C, otherwise paralysis if after soaked and then stirred back worms will move and declared dead when once disturbed, remain there for the movement of the worms.

Data were collected by recording the number of worms that die when do the treatment. Recording is done every hour by counting the number of worms that die every hour.

The *Momordica charantia* L. and *Andrographis paniculata* Ness leaves covered by black flannel cloth were dried under the sun. Once dried, the *Momordica charantia* L. and *Andrographis paniculata* Ness. leaves were blended to obtain the powder. The powder was macerated by 70% ethanolic extract for 4 days. The filtrates were aerated in the vacuum evaporator until thick extract was obtained. Extraction process was done in laboratory of Pharmacology of Faculty of Medicine of University Muhammadiyah Surakarta.

A total of 72 *Ascaris suum* were divided into 8 groups. Each group consists of 9 worms. All worms treated by extract / control appropriated test group, namely:

### Statistical analysis

The data of anthelmintic test were expressed by  $\pm$  SD and anthelmintic test was analyzed by Kruskal Wallis followed by Mann Whitney test.

## RESULTS AND DISCUSSION

### Result

In the process of maceration of *Momordica charantia* L with 70% ethanol in get 450 grams of liquid extract of 1.8 kg of dried leaves and to *Andrographis paniculata* Ness. with 70% ethanol in get 580 grams of liquid extract of 2 kg dry leaves.

The 70% ethanolic extract of *Momordica charantia* L. leaves and *Andrographis paniculata* Ness. leaves can be seen in figure 1.



**Figure 1: The 70% ethanolic extract of *Momordica charantia* L. leaves and *Andrographis paniculata* Ness. leaves**

Anthelmintics effect of *Momordica charantia* L. leaf and *Andrographis paniculata* Ness. leaf can be seen in figure 2 and Table 1.



**Figure 2: Ascaris suum worm**

**Table 1 : The time of death of worms after the treatment**

Groups	Mean ± SD of time of death worm (hours)	* P values (vs. negative control)
Negative control	24 ± 0	
Positive control (pyrantel pamoate)	3.67 ± 2.45	0.000
The 70 % ethanol extract of <i>Momordica charantia</i> L. 20 %	16.44 ± 9.57	0.012
The 70 % ethanol extract of <i>Momordica charantia</i> L. 40 %	12 ± 9.81	0.002
The 70 % ethanol extract of <i>Momordica charantia</i> L. 80 %	9.89 ± 7.85	0.000
The 70% ethanol extract of <i>Andrographis paniculata</i> Ness. 20 %	15.22 ± 9.32	0.001
The 70% ethanol extract of <i>Andrographis paniculata</i> Ness. 40 %	10.89 ± 8.88	0.000
The 70% ethanol extract of <i>Andrographis paniculata</i> Ness. 80 %	5.89 ± 5.84	0.000

\* P value of the test Man Whitney

From the Kruskal Wallis test, it is showed a significant with a P value of 0.000 ( $P < 0.05$ ). Based on Table 1 obtained significant difference between groups 70 % ethanol extract of *Momordica charantia* L. and 70% ethanol extract group *Andrographis paniculata* Ness. to the negative control ( $P < 0.05$ ).

It can be concluded that the 70% ethanol extract of *Momordica charantia* L group and the group *Andrographis paniculata* Ness. can be used as anthelmintics.

#### DISCUSSION

The result of anthelmintic effect test of 70% ethanol extract of *Momordica charantia* L. and *Andrographis paniculata* Ness. leaves of this

research is linear with previous study. The result shows that extract of *Momordica charantia* L. and *Andrographis paniculata* Ness leaves can influence the speed of the worm mortality compared with negative controls. So that extracts of *Momordica charantia* L. and *Andrographis paniculata* Ness. leaves have the anthelmintic effects.

The anthelmintic effect of this research is linear with previous research among others: *Andrographis paniculata* Ness. leaves known to contain saponins, tannins, and andrografolid. Saponins can be potentially act as anthelmintics for work by inhibiting the enzyme acetylcholinesterase, so the worm will become muscle paralysis and lead to death. Alkaloids tannin has the effect of anthelmintics by means of the worm body protein agglomerate. This activity can interfere with the metabolism and homeostasis in the body worm, so the worms will die. While andrografolid which is a bitter substance on bitter herbs can kill worms to cause alkaline conditions of the intestine, causing conditions uncomfortable for the life of worms. Besides, andrografolid also act as immunomodulators and antioxidants.

*Momordica charantia* L. leaves contain compounds that are anthelmintic such as saponins, tannins, flavonoids and triterpene glycoside<sup>45</sup>. Saponins may irritate the mucous membrane channel gastrointestinal worms that interfere with the absorption of food<sup>46</sup>. While the tannins can free protein binding in the intestinal tract that can lead to the death of worms. Flavonoid compounds may lead to the degeneration of neurons in the worm's body resulting in the death of worms anyway<sup>47</sup>. Triterpene glycosides in bitter melon leaves also can cause inhibition of spontaneous motility in the worms<sup>48</sup>. All this will eventually cause the worm into paralysis or death.

## CONCLUSION

70% ethanol extract of *Momordica charantia* L. and *Andrographis paniculata* Ness leaves has the effect of anthelmintics.

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