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RESEARCH ARTICLE
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Liquid Chitosan from *Corbula faba* Hinds on Soil Transmitted Helminth (STH) in Pakcoy (*Brassica chinensis L.)*

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ABSTRACT

Worms are a health problem that is still commonly found in developing countries. One of the causes of helminthiasis is Soil-Transmitted Helminth (STH). The worms that cause worm problems are Ascaris lumbricoides, Trichuris trichiura, Hookworm (Necator americanus and Ancylostoma duodenale). Chitosan has been discovered to have a quality positively charged polycationic, leading to changes in cell permeability. Chitosan can be found in the framework of Crustacea sp., such as white mussel shell. The purpose of this research was to determine the effectiveness of liquid chitosan from white mussel shells (*Corbula faba* Hinds) against Soil-Transmitted Helminth on Pakcoy (*Brassica chinensis* L.). The research results at a concentration of 2% on average worm larvae death time of 5.25 minutes, 1.5% concentration on average worm death time of 5.15 minutes, the concentration of 1% and 0.5% did not occur death of worm larvae. There is a significant difference in the exposure of liquid chitosan from white shell to death time of worm larvae. These results show that the liquid chitosan from white mussel shell to Soil-Transmitted Helminth on mustard meat is effective at a concentration of 1.5% with an average death time of 5.15 minutes.

Keywords: Soil-Transmitted Helminth; chitosan; *Corbula faba* hinds

INTRODUCTION

Worms are a health problem that is still commonly found in developing countries. One of the causes of helminthiasis is Soil-Transmitted Helminth (STH). Soil-Transmitted Helminth (STH) is a Nematode worm that requires soil to establish its infective form. The worms that cause worm problems are Ascaris lumbricoides, Trichuris trichiura, Hookworm (Necator americanus and Ancylostoma duodenale) [(1)]. Based on the World Health Organization (WHO) data, more than 1.5 billion people, or 24% of the world's population, are infected with Soil-Transmitted Helminths (STH). In Asia, helminthiasis due to Soil-Transmitted Helminths (STH) reaches 67% [(2)]. In Indonesia, the prevalence of helminthiasis in several districts and cities in 2012 was above 20% [(3)]. Transmission of worm eggs to humans can occur from soil containing worm eggs. Soil-Transmitted Helminths (STH) eggs are excreted in the feces of an infected person. In areas that do not have adequate sanitation, these eggs will contaminate the soil. Eggs can stick to vegetables and are swallowed if the vegetables are not washed or cooked properly, in addition, eggs can also be ingested through contaminated drinking water and in children who play on the ground without washing their hands before eating [(4)]. There are still many people who wash vegetables poorly by just dipping them in water, so there is still some soil left in the vegetables. Poor washing and improper cooking of vegetables can lead to the ingestion of worm eggs and other parasites into the body. The existence of Kupang in East Java is found and spread along the coast of Sidoarjo, Surabaya, Bangil, Gresik, Pasuruan, and its surroundings. Kupang production in East Java, especially Sidoarjo, ranges from 8,540,400 kg to 8,675,300 kg per year. Fishers' fishing effort of mussels is carried out every day throughout the year because mussels do not have a fishing season. Kupang widely available in Sidoarjo is the white mussel (*Corbula faba* Hinds) [(5)], white mussel shell powder effectively kills and damages the morphology of Soil-Transmitted Helminth (STH).

METHODS

Type of research used was experimental with a pretest-posttest control group design. Population and sample are white mussel was obtained in Balungdowo village, Candi subdistrict, Sidoarjo, Indonesia. The sample used for research was all parts of pakcoy. The Federer formula above replicated this study four times, and the number of pieces required was 24 samples.

Chitosan Sample Preparation

The raw material comes from the shell waste of white mussel (*Corbula faba* Hinds), cleaned using water and rinsed with distilled water—then dried in the sun. After that, the dried white mussel shells were mashed using a mortar and stamper until they were finely powdered and sieved with a 200 mesh sieve.

Deproteination

White mussel powder was added with 3% NaOH in a 1: 6 (w/v) ratio and stirred at 85°C for 30 minutes. Then the results obtained were filtered, the residue obtained was washed with distilled water to a neutral pH. The filtrate obtained was tested with a biuret reagent; if the filtrate turns blue, the protein content has been lost. Then it was dried in an oven at 80°C for 24 hours. (6)

Demineralization

White mussel powder that has gone through the deproteination process was then added with 1 N HCl in a ratio of 1: 10 and stirred with a magnetic stirrer at a temperature of 75°C for 1 hour. After filtered the result, washed the residue obtained with distilled water to a neutral pH. The last filtrate obtained was tested with $AgNO_3$ solution; if there was no white residue, lost the remaining Cl. If there was still a white residue, repeated the demineralization process two times. It was drying in an oven at a temperature of 80°C for 24 hours. The residue from this process was called chitin powder. (7)

Deacetylation

Added chitin powder obtained from the demineralization process with 50% NaOH with a ratio of 1:10 (w/v) heated at a temperature of 90-100°C for 1 hour. The residue obtained was washed with distilled water and then added with a dilute HCl solution so that the pH was neutral and then dried. Then chitosan was formed. (8)

Chitosan Characterization Test

Chitosan characterization included water content, ash content, degree of deacetylation, pH, solubility, and yield. Testing as follows:

Solubility

The 0.5% (w/v) chitosan was dissolved in 1% (v/v) acetic acid, then filtered. The remaining chitosan indicated the percentage of chitosan solubility compared to the initial chitin. (9)

Liquid Chitosan Making

Chitosan samples concentration 2% dissolved in 3% acetic acid. From the 2% concentration, it was diluted to concentrations of 0.5%, 1%, and 1.5%. 2% liquid chitosan is made by dissolving 0.3 grams of powdered chitosan in 15 mL of 3% acetic acid, then stirring until the powder dissolves.

Liquid Chitosan Application Stage

Pakcoy samples were thoroughly washed in each part of the pakcoy with distilled water and soaked for 10 minutes, then centrifuged at 2500 rpm for 10 minutes. It was decanted, and the residue was taken using a dropper, placed on a glass object, and observed under a microscope with an objective lens magnification of 10x. Then drip liquid chitosan onto each glass object that is positive for the live Soil-Transmitted Helminth (STH) parasite and observe the parasite's survival at 10x magnification for 60 minutes.

RESULTS

After the chitosan powder made from white mussel shells was ready, then a characterization examination was carried out to determine whether the quality of the chitosan powder made was good or not. Based on table 1, the results of the chitosan characterization test made were the appearance of the form of chitosan in the form of a white powder with a particle size of 200 mesh. The moisture content of chitosan powder was 0.25%, the ash content produced was quite high, namely 97.02%. The degree of deacetylation of chitosan reached 72.19%, the solubility of chitosan powder was quite good, reaching 85.24%. While the yield was 9.83%, and the pH of the chitosan powder was very alkaline, getting pH 12. Based on table 2, the results showed that the worm larvae were exposed to various concentrations of liquid chitosan, ranging from 0.5%, 1%, 1.5%, 2%, and were also exposed to liquid worm medicine as a positive control. The exposure was carried out for 60 minutes and was replicated four times. At a concentration of 2%, the average time of death of larvae from Soil-Transmitted Helminth was 5 minutes 25 seconds; at a concentration of 1.5%, the average death time was 5 minutes 15 seconds. At concentrations of 1% and 0.5%, the average time was 60 minutes because until the observation limit does not experience.

Death of the Soil-Transmitted Helminth larvae was considered ineffective at this concentration. In positive control, the average time of death was 6 minutes, while the moderate negative control was 60 minutes because there was no death in Soil-Transmitted Helminth larvae. Based on table 3, the observation results found that many Soil-Transmitted Helminth larvae from Hookworm with rhabditiform and filariform stages were found, and some larvae were found from Strongyloides stercoralis. Meanwhile, Soil-Transmitted Helminth larvae other than Hookworm and Strongyloides stercoralis were not found during the observations.

Table 1. The results of the characterization test of chitosan powder from white mussel shell

Parameter	Results
Sightings	white powder
Particle size	200 mesh
Water content	0.25%
Ash content	97.02%
Degree of deacetylation (DD)	72.19%
Solubility	85.24%
Yield	9.83%
Ph	12

Soil-Transmitted Helminth Larva Death Time Data

After researching the effectiveness of liquid chitosan from the shells of white mussels (*Corbula faba* Hinds) against Soil-Transmitted Helminth (STH) on pakcoy (*Brassica chinensis* L.)

Table 2. The results of the mortality time of Soil Transmitted Helminth larvae after being given liquid chitosan and exposed for 60 minutes

Concentration	Time of Death (minutes)				Average
	I	II	III	IV	
2% concentration	7	3	3	8	5.25
1.5% concentration	3	6	7	3	5.15
1% concentration	60	60	60	60	60
0.5% concentration	60	60	60	60	60
Positive Control	10	3	6	5	6
Negative Control	60	60	60	60	60

Types of Larvae Soil-Transmitted Helminth

Observation of the preparation of washing water from pakcoy that has been centrifuged and observed using a microscope with a magnification of 10x found larvae from Soil-Transmitted Helminth.

Table 3. Types of larvae and stages found in the washing water preparation of pakcoy

Concentration	Type of larva			
	I	II	III	IV
2%	HR	HR	SSR	HR
1.5%	HR	HR	SSF	HR
1%	HF	HF	HR	HF
0.5%	HF	SSR	HR	HF
Positive control	HF	HR	HF	HR
Negative control	HF	HR	HR	HR

HR: Hookworm std. rhabditiform; HF: Hookworm std. filariform; SSR: Strongyloides stercoralis std. rhabditiform; SSF: Strongyloides stercoralis std. filariform

Based on the normality test using Kolmogorov-Smirnov, p-value was 0.009 (<0.05), so the data was not normally distributed. Based on the homogeneity test, p-value was 0.000 (<0.05), so data was not homogeneous. Because the data did not meet the parametric test requirements, the data analysis continued with the non-parametric test, namely Kruskal-Wallis test. Based on the results of the Kruskal-Wallis test, the p-value was 0.001 (<0.05), so there was the effectiveness of liquid chitosan in the shell of white mussel (*Corbula faba* Hinds) against Soil-Transmitted Helminth (STH) on pakcoy (*Brassica chinensis* L.). Based on the Post Hoc test results, the p-value was 0.000 (<0.05), so there was a significant difference for the concentration of 2% with 1%, concentration of 2% with 0.5%, concentration of 1.5% with 1%, concentration of 1.5% with 0.5%.

DISCUSSION

This study aimed to analyze the effectiveness of liquid chitosan from white mussel shells against Soil-Transmitted Helminth (STH) in Pakcoy (*Brassica chinensis* L.). This study uses liquid chitosan test material from white mussel shells from Balungdowo village, Candi sub-district, Sidoarjo. The powder from white mussel shells is processed in several stages. Among others was The deproteination process to remove protein and the demineralization process to remove minerals in white mussel shell powder. The last is the deacetylation process to release the acetyl group into a free amino group called chitosan. Liquid chitosan was made in 2%, 1.5%, 1%, and 0.5%, respectively, then dripped one drop of liquid chitosan on the preparation made, and Soil-Transmitted Helminth (STH) was found in practice from each – each concentration. Observations were made for 60 minutes. This research is said to be effective if the larvae of the Soil-Transmitted Helminth (STH) do not move due to muscle weakness of the larvae. Muscle weakness can be shown from a decrease in the level of movement [10]. Based on table 5.2, the results showed differences in the time of death of larvae from various concentrations. From the results obtained, the average death time of larvae is that the fastest time is 5 minutes 15 seconds at a concentration of 1.5% and the average death time at a concentration of 2% is 5 minutes 25 seconds. So based on the research results that liquid chitosan is effective at a concentration of 1.5%. In the positive control test, observations were made for 60 minutes with the addition of anthelmintic containing pyrantel pamoate, and the average time of death was 6 minutes. The time of death of positive control was longer than that of liquid chitosan. In contrast, there was no death of Soil-Transmitted Helminth (STH) larvae in the negative control test after 60 minutes of observation, and the larvae were still very active. The death of Soil-Transmitted Helminth (STH) larvae was due to chitosan's polycationic content, which caused a change in cell permeability due to the interaction between the positive charge of chitosan and the negative charge on the cell surface. The exchange of positively charged and negatively charged microbial cell membranes results in leakage of intracellular constituents [11]. Chitosan is a depressant on the larval central nervous system, causing larval death. In addition, chitosan is a positively charged polyamine compound that has linear chemical properties of polyamines, reactive amino groups, and hydroxyl groups. These properties allow chitosan to react with proteins in larvae and form complex compounds, resulting in enzyme inhibition and membrane damage [12]. Based on table 3, the study results found more larvae in Hookworm than Strongyloides stercoralis and other Soil-Transmitted Helminth (STH). The most common stage found was the rhabditiform stage rather than the filariform stage. The time of death between these stages is different because the immune system of the larvae is different. Differences in larval death time can occur due to the influence of varying Soil-Transmitted Helminth larval stages [13]

CONCLUSION

Based on the results, there is the effectiveness of liquid chitosan in the shell of white mussel (*Corbula faba* Hinds) against Soil-Transmitted Helminth (STH) on pakcoy (*Brassica chinensis* L.).

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