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In Vitro Antihelmintic Activity of the Hydroethanolic Leaf Extracts of *Avicennia marina* (White Mangroves) on *Eudrilus eugeniae* (African Nightcrawlers)

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ABSTRACT

Research is needed to determine the *Avicennia marina* hydroethanolic leaf extract is effective against adult *E. eugeniae*, despite research showing that it can eliminate helminths such as *Pheretima posthuma*. The purpose of this study was to ascertain the hydroethanolic leaf extracts of *A. marina* antihelmintic effectiveness against adult *E. eugeniae in vitro*. Using *E. eugenie* adults (6–8 cm in length and 0.2–0.3 cm in breadth), the percentage vermicidal activity of three concentrations of dried crude hydroethanolic leaf extract of *A. marina*, 25, 50, and 75 mg/mL of distilled water, was assessed every three hours for 12 hours. Five replicates, three trials, distilled water as a negative control, and mebendazole as a positive control were all examined. According to the findings, the hydroethanolic leaf extracts of *A. marina* have shown antihelmintic efficacy against *E. eugenie* adults *in vitro* at doses of 50 mg/mL and 75 mg/mL utilizing Kruskal-Wallis and LSD on the 3, 6, 9, and 12 hours, with a p-value of less than .05. In both concentrations, 50 and 75mg/ml, until the 12-hour exposure to the extract, there was no discernible difference between them. *In vitro* antihelmintic activity was best demonstrated at 50 mg/mL for 3 hours (mean = 91.1; sd = 26.6), and the *in vitro* antihelminthic activity was significantly superior to that of the positive control for the same duration (mean = 2.22; sd = 8.61). The results scientifically demonstrated the antihelmintic efficacy of hydroethanolic leaf extract of *A. marina* against *E. eugeniae* and provided the foundation for testing against *Ascaris*, *Trichuris trichiura*, and hookworms.

INTRODUCTION

According to estimates, 1.5 billion people, or 24% of the world's population, are affected by helminth infections, making it one of the most common infections in the world (World Health Organization, 2023). It is especially common in third-world countries, where poor sanitation and hygiene create the perfect conditions for parasitic helminth infection and extensive transmission. Between 33.8% to 75.9% of Filipinos were afflicted with soil-transmitted helminth (STH) infections in the Philippines, with schoolchildren being the most susceptible (Mationg *et al.*, 2021). The roundworms *Ascaris*, *Trichuris trichiura*, and hookworms are the most frequent causes. Numerous health problems, such as stomach pain, diarrhea, loss of blood and protein, rectal prolapse, and physical and cognitive growth retardation, can be caused by significant helminthic infections in humans (CDC, 2022).

Over the past few decades, numerous anthelmintic studies have been developed as a result of the increased incidence and prevalence of helminthic illnesses. Using roundworm species that are morphologically similar to parasitic species is one strategy. Ogen *et al.* (2017) proposed using the common soil earthworm *Pheretima posthuma* as test subjects because of its morphological similarity to intestinal roundworms. In the Philippines, *E. eugeniae* (African nightcrawlers) are especially common and make excellent subjects for anthelmintic research. Additionally, they are helminths, which and intestinal roundworms have comparable anatomy (Pueblos *et al.*, 2015; Asiimwe *et al.*, 2023; Hogade *et al.*, 2014).

Commercial medications such as Albendazole, Mebendazole, Levamisole, and Pyrantel Pamoate were frequently used to treat helminth infections in humans (Moser *et al.*, 2017). Despite their effectiveness, these medications have a number of drawbacks when used over extended periods of time. In their research, Kotze *et al.* (2019) observed that helminth parasites have a propensity to become resistant to some drugs following prolonged therapy. Agricultural research, which focused on natural chemicals with anthelmintic action, was therefore very interested in novel natural alternatives.

Other plants with comparable phytochemical components, including Leucaena leucocephala (Lead Tree), Carica papaya (Pawpaw), and Cayriata auriculata (ear-leafed Cayratia), have been the subject of numerous studies and have been shown to be effective anthelmintic agents (Kancherla *et al.*, 2019; Widaad *et al.*, 2022). In particular, the synergistic anthelmintic effects of flavonoids and tannins mostly functioned by making the nematode cuticle stiffer, which inhibited growth and encouraged death (Greiffer *et al.*, 2022).

The anthelmintic activity of *A. marina* is one of the plants that can be investigated. This kind of mangrove can be found in rivers, streams, coastal regions, and the intertidal zones of seabeds. For coastal barangays, it was a natural defense, particularly in remote, impoverished

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areas. The effectiveness of *A. marina* tree extracts against helminths, including the earthworm *Pheretima posthuma*, has been the subject of a limited number of studies (Kondepudi, 2018). Furthermore, previous research had shown that phytochemicals like phenolic compounds, alkaloids, flavonoids, sugars, carotenoids, aliphatic alcohols, amino acids, hydrocarbons, fatty acids, tannins, saponins, and terpenes were present (Wilfred *et al*., 2023). In support of this, Mughal *et al.* (2016) also found that *A. marina* contains phytochemicals proteins, amino acids, flavonoids, phenols, carbohydrates, and alkaloids. Accordingly, the phytochemical substances saponins, flavonoids, and tannins were nematicidal chemicals according to research by Wilfred *et al.* (2023) and Mughal *et al.* (2016); D'Addabbo *et al.* (2022).

The use of *A. marina* leaf extract hydroethanolic against adult *E. eugeniae* has not been studied, despite the fact that it has been scientifically demonstrated to kill helminths like P. posthuma. The overall goal of this work was to ascertain the hydroethanolic leaf extracts of A.marina's antihelmintic effectiveness against *E. eugeniae in vitro*. The study's specific goal was to ascertain the hydroethanolic leaf extracts of A.marina's *in vitro* antihelmintic activity against adults of *E. eugeniae* using varying concentrations (25, 50, and 75 mg/mL) and time (3, 6, 9, and 12 hours) in terms of percent vermicidal activity (Va) in comparison to the negative control, distilled water and the positive control, Mebendazole (25 mg/mL). In terms of percent vermicidal, the study identified the optimal concentrations (25, 50, and 75 mg/mL) and times (3, 6, 9, and 12 hours) that demonstrated the hydroethanolic leaf extracts of *A. marina*'s *in vitro* anthelmintic activity against *E. eugeniae* adults. The study's findings gave *A. marina*'s vermicidal action against E.eugeniae a scientific foundation.

MATERIALS AND METHODS

A quantitative post-test, fully randomized approach was employed in the investigation. The % vermicidal (mortality) effects of A.marina at three different doses (25, 50, and 75 mg/mL) against *E. eugeniae* adults were evaluated. The study was reviewed by a private university's recognized Ethics Review Committee. In addition, the study received biosafety and ethics clearance.

E. eugeniae were gathered from a vermicomposting region in Antique Province, *A. marina* were gathered from the riverbanks in Dumangas, Iloilo. The test was carried out in Antique between February and April of 2024.

Identification of Samples

The pictures of trees, fruits, and plant leaves (Figure 1) were sent to the Department of Environment and Natural Resources for verification. This sample's taxonomic classification is as follows:

Kingdom: Plantae
Phylum: Tracheophyta
Class: Magnoliopsia
Order: Lamiales
Family: Acanthaceae
Genus: Avicennia
Species name: marina
Common name: api-api or white/gray mangrove

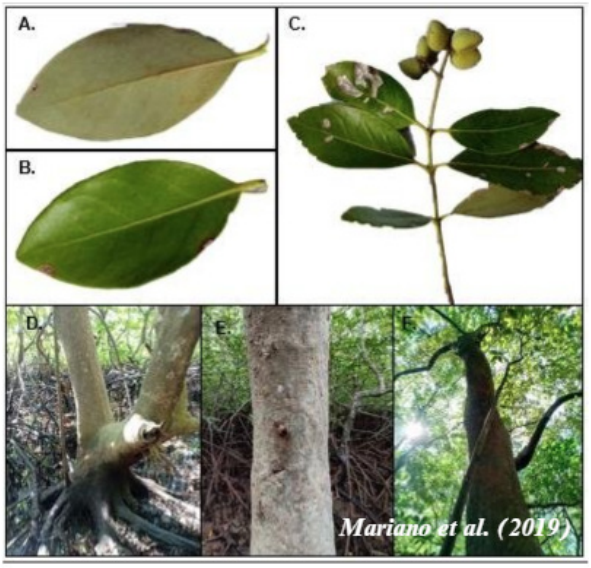

Figure 1: Images of the plant *A. marina*: A. yellowish leaf abaxial surface; B. edge of the leaf (adaxial surface); C. arrangement of leaves (opposite); D. roots (pneumatophores that resemble pencils); E. trunk of a tree (lenticels and smooth bark); F. The crown of a tree

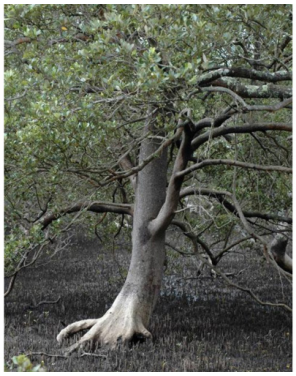

Figure 2: Photograph of *A. marina* whole plant

Collection of plant material

A. marina leaves were gathered in Dumangas, Iloilo. Samples of plants were gathered 100 meters from the road. The leaves were picked between 6:00 and 7:00 a.m. Leaves that were not budding or decaying were used. Leaf samples were free of insect bites, trauma, and irregularities. Collection was avoided within 3 days after rainfall in the area.

Identification and Collection of *E. eugeniae*

A veterinarian identified the worm. A veterinarian verified the authenticity of *E. eugeniae* worms that were gathered from vermicomposting facilities in the province of Antique. According to the research of Akande *et al.* (2018), the earthworms were measured to be 6–8 cm long and 0.2–0.3 cm wide. When the earthworms were collected, they were alive and moving. Following the

confirmation of the roundworm species, the *E. eugeniae* worms were cleaned of dirt and debris using a standard saline solution.

Preparation of *A. marina* Extract

Following collecting, the leaves were carefully cleaned with tap water and then rinsed with distilled water. The leaves were allowed to air dry at room temperature for an hour without exposure to sunlight in a screen bag. The leaves were allowed to air dry before being ground into a fine powder in a blender. One gram of powdered leaves was mixed with five milliliters of 95% ethanol for a duration of seventy-two hours in order to homogenize the leaves. After that, a muslin cloth was used to filter the homogenized leaves. The ethanol was then extracted from the filtrate using a rotary evaporator set to 45 °C. The filtrate was dried in an oven set to 45 °C in order to recover either the powdered or sticky form.To be sure there was no ethanol in the extract, a flame test was conducted. An anthelmintic test was performed on the dried, crude hydroethanolic extract against adult *E. eugeniae*. Ibrahim *et al.* (2022) modified this process and used it in their studies.

Antihelmintic Assay Preparation

The Kondepudi (2018) method was used to perform the anthelmintic assay. The ethanolic leaf extract was made into a stock solution at varying doses (25, 50, and 75 mg/mL). A positive control solution containing 25 mg/mL Mebendazole and a negative control solution consisting of distilled water were made. Each disposable plastic petri dish with a perforated lid and a 5.9-inch diameter was filled with three adult *E. eugeniae*. Each treatment's 5 mL solution is used to soak them. In accordance with the experiment carried out by Bestari *et al.* (2020), the percentage vermicidal of the helminth was measured at 3-hour intervals for 12 hours.

Validity and Reliability

A certified medical technologist verified the study's findings. To ensure the study's reliability, three concentrations—25 mg/mL, 50 mg/mL, and 75 mg/mL—were used in five replicates with three trials. To guarantee the precision of the tests conducted, the devices were also appropriately calibrated.

Waste Disposal

The adult *E. eugeniae* samples, both dead and alive, were disposed of appropriately by filling the petri plates with a 10% hypochlorite solution and securing the arrangement with tape. To guarantee the organisms' total demise, the sample was submerged for a whole day. After being put in the biohazard bag, the petri plates were disposed of in the proper garbage receptacle.

Statistical Analysis

The mean and SD of the results were displayed. Using varying quantities of A.marina hydroethanolic leaf extract, the study compared the mean results of vermicidal counts of *E. eugeniae* taken every three hours for 12 hours using the Kruskal-Wallis test. Statistical significance was defined as a p-value of 0.05. To determine whether there were any notable variations or differences between the treatments, a post-hoc Fisher's least significant difference (LSD) test was also employed.

RESULTS AND DISCUSSIONS

Table 1 demonstrates that *A. marina* hydroethanolic leaf extracts at 50 and 75 mg/mL demonstrated antihelmintic efficacy against *E. eugeniae*. Vermicidal activity (Va) showed $p < 0.05$ for *E. eugeniae* adults on the 3, 6, 9, and 12 hours using Kruskal-Wallis and LSD. The antihelmintic effects of the 50 mg/mL and 75 mg/5mL doses of *A. marina* extract did not significantly change from the 3 to the 12 hour. There was no antihelmintic effect from the 25 mg/ml. 50 mg/mL was the optimal dose and duration that demonstrated *in vitro* antihelmintic action for the three hours of exposure (mean = 91.1; sd = 26.6), and it was noticeably superior to the positive control during that period (mean = 2.22; sd = 8.61).

Table 1: Anthelmintic activity of *A. marina* hydroethanolic leaf extract against *E. eugeniae* taken at different hours using different concentrations in terms of % vermicidal activity(Va)

Treatments	Time (hours)							
	3		6		9		12	
	%Va		%Va		%Va		%Va	
	m	sd	M	sd	m	sd	m	Sd
25 mg/mL	$^{a}8.89$	15.4	$^{a}20^{a}$	24.6	$^{a}33.3^{a}$	30.9	$^{a}46.7^{a}$	35.2
50 mg/mL	$^{b}91.1^{a}$	26.6	$^{b}93.3^{a}$	25.8	$^{c}95.6^{a}$	17.2	$^{c}97.8^{a}$	8.61
75 mg/mL	$^{b}91.1^{a}$	26.6	$^{b}93.3^{a}$	25.8	$^{c}93.3^{a}$	25.8	$^{c}100^{a}$	0.0
Negative-Control (Distilled Water)	$^{a}0.0^{a}$	0.0	$^{a}0.0^{a}$	0.0	$^{a}0.0^{a}$	0.0	$^{a}15.6^{b}$	30.5
Positive-Control Mebendazole) 25 mg/mL	$^{a}2.22^{a}$	8.61	$^{a}6.67^{a}$	13.8	$^{b}51.1^{ab}$	39.6	$^{b}84.4^{ab}$	27.8

Note: letters before the numbers mean comparison with the treatments; while letter after number means comparison among the hours

Discussions

Significant anthelmintic activity was demonstrated by the hydroethanolic leaf extract of *A. marina* at concentrations of 50 and 75 mg/mL in terms of percent vermicidal. Significant anthelmintic action was demonstrated by concentrations of 50 mg/mL and 75 mg/mL in terms of percent vermicidal, with the peak activity occurring after 12 hours. Our results suggest that the phytochemical compounds present in *A. marina*, including alkaloids, flavonoids, sugars, carotenoids, aliphatic alcohols, amino acids, hydrocarbons, fatty acids, phenolic compounds, tannins, saponins, and terpenes, may have contributed to the anthelmintic activity (Wilfred *et al*., 2023; Mughal *et al*., 2016). Greiffer *et al.* (2022) found that flavonoids and tannins particularly inhibit helminth proliferation and cause helminth mortality.

In addition to *A. marina*, several plants were reported to possess these chemicals, including L. leucocephala (Widaad *et al*., 2022). Because of its physical similarity to diseased helminths, *E. eugeniae* was used in the study as a reference for creating synthetic anthelmintic treatments.

Notably, the strongest anthelmintic activity against *E. eugeniae* was obtained at concentrations of 50 mg/mL and 75 mg/mL. The results of Kondepudi's (2018) investigation are consistent with the observed trend, which shows that higher concentrations exhibit stronger anthelmintic activity. The highest level of anthelmintic activity of *A. marina* was similarly seen at a concentration of 75 mg/mL to P. posthuma.

As demonstrated by the notable impacts on mortality employing varying doses of hydroethanolic leaf extracts of *A. marina*, the results gave a scientific foundation for the vermicidal actions of A.marina against *E. eugeniae*. For future research on helminths or organisms that share a biological morphology with African nightcrawler worms, such as hookworms, *Trichuris trichiura*, and roundworms (*Ascaris* suum), a concentration of 50 mg/mL may be utilized as a reference.

Given the persistently high incidence of helminth infections in the Philippines, the experimental findings are positive about the best use of readily available plants with anthelmintic qualities. The results show that natural products are reliable and strong enough to serve as a pointer to other possible sources of helminth infection treatment. This is especially true for higher concentration therapies. Furthermore, by placing more significance and focus on the product's source, this may also have positive economic effects. Last but not least, our research may help address the ongoing concern about the spread of drug-resistant parasites (Fissiha & Kinde, 2021) by introducing a novel antidote that significantly slows the development of undesired resistance.

Only the hydroethanolic leaf extract of *A. marina* at concentrations of 25 mg/mL, 50 mg/mL, and 75 mg/mL and the anthelmintic assay method produced these results. Different results can be obtained by using a different extractant, concentration, technique, or part of the plant.

CONCLUSION

Comparing the extract concentrations at 50 mg/mL and 75 mg/mL to the negative control (distilled water), there was notable antihelmintic activity based on the percentage of vermicidal activity, from 3 to 12 hours. On the 3rd hour, the optimal concentration and duration of *in vitro* anthelmintic action were 50 mg/mL. On the 3rd hour, the extract doses of 50 mg/mL and 75 mg/mL exhibited similar vermicidal effectiveness to the positive control, Mebendazole. The results scientifically demonstrated the antihelmintic efficacy of hydroethanolic leaf extract of *A. marina* against *E. eugeniae* and provided the foundation for testing against *Ascaris*, *Trichuris trichiura*, and hookworms. Since the study demonstrated the plant's effectiveness against E. eugenia *in vitro*, it is advised that a study on *in vitro* human helminths be carried out utilizing the crude ethanolic extract of *A. marina* leaves. The phytochemical of *A. marina* leaves should be used to identify or isolate the particular components that give it its anthelmintic effects.

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