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Evaluation of the anthelmintic property of the endophytic extract of *Senna alata* in mice model

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Abstract
Background: *Senna alata* belongs to the family, Fabaceae, which is widely distributed in the humid and tropical regions of the world. It has been ethnobotanically used in the management of diabetes, asthma, typhoid, malaria, worm infestation (ringworms, tinea infections), scabies, skin dermatitis such as eczema, blotch, and herpes. Hence, it is important to quantify the pharmacological properties of the plant, especially in the management of worm infestations.
Objectives: In this study, the endophytic extract of Senna alata was evaluated for its antihelmintic properties in mice.
Method: The leaves of *Senna alata* were obtained in Ufuma Orumba North Anambra state, were treated with 70% of ethanol for 3 minutes. Malt extract agar was prepared and transferred into several sterilized Petri dishes, allowed to solidify, before inoculation. Local rice used for the fermentation was prepared in several conical flasks by weighing 100g of the rice into each flask and mixing it with 200ml of water. At the end of the 3-day homogenization, the mixtures were decanted into sterile beakers. The filtrate was then concentrated by exposing them to room temperature for 5-7 days. The extracts were then stored at 25 ºC for further use. The anthelmintic activity was performed according to the method followed by Shelke *et al*., 2020, albendazole was used as the standard drug.
Result: It showed that the extract paralyzed worms at the same time Albendazole did and that it was more effective at higher concentrations of 50mg/ml.
Conclusion: This plant possesses potent anthelmintic activity in a dose-dependent manner, and may prove useful as a cost-effective and safe anthelmintic alternative.

Keywords: Homogenization, *Senna alata*, antihelmintic, ringworms, endophyte

Introduction
Parasitism, especially by helminth species, impairs health by causing lack of appetite, diarrhea, anemia, and, in severe cases, death (Athanasiadou and Kyriazakis 2004) [1]. Many countries throughout the world use synthetic anthelmintics to minimize losses caused by helminth infection. Furthermore, parasite resistance increases cost decreases production efficiency, and risks contamination of animal products with the environment (Saddiqi *et al*., 2010) [10]. These disadvantages have stimulated a search for alternative control methods such as the use of traditional medicinal plants. Screening and proper evaluation of medicinal plants could reveal bioactive compounds that may be sustainable and environmentally acceptable (Eguale *et al*., 2007; Nisa *et al*., 2010) [3, 7].
The cause of chronic illness and sluggishness among children is being recognized as helminthic infestations. In 2014, the World Health Organization estimated there were 2 billion helminth infections worldwide, and 100% of schoolchildren were at risk (Reznick, 2014) [9]. The major phyla of helminths are nematodes (roundworms) which are soil-transmitted helminths that mostly cause intestinal infection, filarial worms cause the onchocerciasis, and lymphatic filariasis, while platyhelminths (flatworms) also known as trematodes like schistosomes and cestodes causes cysticercosis (Liu C *et al*., 2015) [4]. Various studies estimate that more than half of the world's population carries intestinal helminth infections, including Ascaris, hookworms, Trichuris, Enterobius, Strongyloides, and tapeworms (Reznick, 2014) [9]. Animals with gastrointestinal parasites also suffer from infections resulting in lowered survival

rates, growth rates, and reproductive performance (Tripathi, 2003) [13]. Diabetic and lung cancer patients have a high incidence of nematode morbidity. Helminth parasites are found mainly in human tissues, although they may also migrate to intestinal tracts (Tu and Zhang 2015) [14]. Throughout the world, helminth control has been carried out through chemical means as well as improved management. There is some evidence that anthelmintic resistance is increasing year by year, resulting in intestinal digestive disturbances, nausea, and giddiness (Mejia-Carmona *et al*., 2015) [6]. As a consequence, alternative strategies against gastro-intestinal nematodes must be developed, which has led to the screening of medicinal plants for their antihelmintic properties.

It has been increasingly known that ethnomedicine and ethnoveterinary practices are used to treat various ailments throughout the world (Bizimenyera *et al*., 2006) [2] and that around 80% of the world population consumes plant-based medicine (Sarin 1996) [11]. A thorough evaluation and confirmation of the safety and effectiveness of medicinal plants are essential to their acceptance into scientific veterinary medicine (Rates, 2001) [8].

S. alata (L.), is a flowering shrub of the Fabaceae family. The inflorescences of this plant have the shape of candlesticks, thus gaining it the name "candle bush". Herbs of average height between one and four meters, growing in warm, humid climates, it is usually annual and occasionally biannual. A plant of this species has oblong leaves with 5 to 14 leaflets, slightly robust petioles (2 to 3 mm), caduceus bracts (2 × 3 by 1 × 2 cm), and dense flowers (20 × 50 by 3 × 4 cm). Flowers from this family are bright yellow in color with seven stamens and an ovary with pubescence. When the fruit is ripe, it has brown wings with many diamond-shaped brown seeds in a tetragonal pod that is 10 to 16 cm in size. It grows by seed and disperses up to 1500 m above sea level. A study was conducted on the antihelmintic properties of *Aspergillus striatus*, an endophyte.

Materials and Methods

Collection of plant materials

The leaves of *Senna alata* were obtained in Ufuma Orumba North Anambra state. The samples were identified and their botanical identities were authenticated and validated by Mr. Felix Ozioko of the Department of Botany University of Nigeria Nsukka, Nigeria.

Surface Sterilization of the Leaves of Senna Alata

The leaves were washed under running tap water to eliminate the soil particles before transferring them into a beaker containing 2% of hydrochloric acid for a duration of two minutes. The leaves were further submerged into a beaker containing 70% of ethanol for 3 minutes and then into a beaker containing 500ml water for 5minutes after which they were transferred to an aluminum foil placed aseptically on the laboratory table, from where the leaves were picked singly and cut into bits, exposing both the leaf blades and mid-ribs that were later inoculated onto a malt extract agar.

Isolation and Purification of Endophytic Fungi

The prepared and autoclaved malt extract agar according to specifications was transferred into several sterilized Petri dishes, allowed to solidify, before inoculating the cut leaf blades and midribs onto the solidified agar using the sterile forceps. The Petri dishes were sealed with the aid of masking tape and incubated at room temperature for five days. After the fifth day, different and varying colonies of endophytic fungi were observed. These fungal colonies were continuously sub-cultured using the malt extract until pure cultures of particular fungal endophytes were finally obtained (Sa-LB2 & Sa-MR3). These pure endophytic fungi were further subjected to fermentation.

Fermentation of Endophytic fungi

These pure cultures were labeled accordingly to ease differentiation (Sa-LB2 & Sa-MR3). The local rice used for the fermentation was prepared in several conical flasks by weighing 100g of the rice into each flask and mixing it with 200ml of water. The flasks were plugged, autoclaved for 30 minutes, and allowed to cool maximally. The pure cultures together with the agar were cut into bits using a well-flamed spatula before introducing them accordingly into the conical flasks containing the cooled autoclaved rice. The flasks were plugged back and sealed very well with foil, thus allowing for 21 days' fermentation

Post-Fermentation

On the 21st day, the fermentation was halted by transferring 500ml of ethyl acetate into each of the conical flasks while turning the rice using a sterile glass rod. The mixture was homogenized by continuous shaking at intervals for three days, thus serving as a substitute for a one-day use of an electric shaker. At the end of the 3-day homogenization, the mixtures were decanted into sterile beakers. The filtrate was then concentrated by exposing them to room temperature for 5-7 days. The extracts were then stored at 25 ºC for further use.

Anthelmintic activity on Endophytic fungi

The anthelmintic activity was performed according to the method followed by Shelke *et al*. (2020) [12]. In all experiments, Nigerian adult earthworms were used due to their physiological and anatomical similarity with human intestinal roundworm parasites. They were collected from moist soil and washed with normal saline to remove all fecal matter. Five Petri-dishes were labeled group I-V. Group I contains 10 ml of 0.5% carboxymethyl cellulose (CMC), group II contains 10 ml of 20 mg/ml albendazole. Group III contains 10ml of 50mg/ml extract. While group IV contains 10 ml of 25 mg/ml extract, while group v contains 10 ml 12.5 mg/ml extract. Observations were made to determine whether a Petri dish was paralyzed or died when placed with 3 worms. After determining that worms neither moved when they were shaken nor exposed to external stimuli, we noted the meantime for paralysis (min). Also, the weights of the worms were taken before and after death. The test drug results were compared with the control group and reference compound (albendazole) treated group.

Statistical analysis

Data obtained from the study were analyzed using Statistical Package for Social Sciences (SPSS-20). Results were presented as mean ± Standard error of the mean (SEM) of sample replicates. Raw data were subjected to one-way analyses of variance (ANOVA) followed by a post hoc turkey's test. $p<0.05$ was considered to be statistically significant.

Results

Result of anthelmintic activity

The extracts produced a significant (p<0,01) anthelmintic

activity in a dose-dependent manner as shown in Table 1. The extract took less time to cause paralysis and death of the earthworms as compared to the group that received 10ml of 0.5% carboxymethyl cellulose. At 50mg/ml the time of paralysis was (10.32 ± 3.33) and the time of death was (14.88±2.55). At 25mg/ml the time of paralysis was (11.12±0.11) and the time of death was (16.64±4.76). At 12.5mg/ml the time of paralysis was (15.12±5.87) and the dead time was (23.92±3.54).

Table 1: Anti-Helminthic Result

Group	The initial weight of the worm	The final weight of the worm	Change in weight	Time of Paralysis	Time of death	Latent period before death
10ml of 0.5% CMC	0.46± 0.71	0.47±0.05	0.01	321.45±3.43	498.31±2.76**	176.86
Abendazole 20mg/ml	0.44±0.03	0.40±0.04	0.04**	12.23±5.12**	17.55±5.11**	5.32**
Crude extract 50mg/ml	0.39±0.09	0.34±0.04	0.05**	10.32±3.33**	14.88±2.55**	4.56**
Crude extract 25mg/ml	0.42±0.04	0.40±0.02	0.02*	11.12±0.11**	16.64±4.76**	5.52**
Crude extract 12.5mg/ml	0.44±0.05	0.36±0.03	0.08**	15.12±5.87**	23.92±3.54**	8.8**

Mean±SEM, n=3.
*= Significant (p<0.05)
**=extremely significant (p<0.01)

Discussion

Through appropriate experimental models, some of the herbs traditionally used have been proven to have powerful antihelmintic activity. Albendazole primarily causes flaccid paralysis in the worm, leading to its expulsion by peristalsis. Albendazole causes neural excitability to decline and muscle relaxation leading to flaccid paralysis and hyperpolarization of worm muscle membranes. It caused worm paralysis and death at the same time as Albendazole, especially at high concentrations of 50mg/ml.

Lastly, this study shows that the plant under research possesses significant anthelmintic activity based on its dose dependencies. Its potential for use as a cost-effective and safe alternative to insecticides has been recognized through studies so far, but further analysis is required to identify and isolate the specific chemicals responsible for the plant's anthelmintic activity to further improve its potency.

Conclusion

The plant showed marked anthelmintic activity, hence, an analysis of the safety and toxicity of the plant and other pharmacological parameters could reveal the potential as a safe and effective alternative to an anthelmintic and antimicrobial agent.

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