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Survey of the Anti-Ascaridiose Activity of *Barringtonia Racemosa* (Lecythidaceae)

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Abstract

Ascariasis is one of the most common diseases affecting all age groups in third world countries. This study evaluates the anti-ascariasis activity of dichloromethane and methanol extracts from the leaves of the plant *Barringtonia racemosa*. Their effects on hypermobility, lysis, worm viability, and ability to inhibit egg development were studied. Egg development was monitored under microscopic observation at magnifications of X10 and X40 for 18 days. In the absence of extracts, the worms remained immobile and viable for 12 hours. Worms incubated in the presence of methanolic extract at 40 and 80 mg/mL exhibited hypermobility, but for a short duration of 16.80 ± 0.86 min and 10.20 ± 0.86 min. Those incubated in dichloromethane extract were less mobile, with a duration of 26.20 ± 3.46 min and 23.80 ± 1.40 min. However, after 8 hours, in the presence of the methanol extract, 60 ± 1.25% and 90 ± 1.60% of the worms were killed, compared to 30 ± 1.58% and 40 ± 1.6% at 12 hours of incubation in the presence of dichloromethane extract at concentrations of 40 and 80 mg/ml. In the presence of Albendazole at 20 mg/ml, the worms were hypermobile and 100% killed after 11 hours of incubation. On day 18, the *Ascaris lumbricoides* eggs had developed into stage 2 larvae. However, in the presence of extracts at 40 and 80 mg/mL and Albendazole at 20 mg/mL, the egg walls were altered and the processes of embryogenesis and larval development were inhibited.

Keywords: Anti-Ascariasis, Parasitosis, Intestinal Helminthiasis, *Ascaris Lumbricoides*, *Barringtonia Racemosa*, Eggs

1. Introduction

Traditional medicine is the art of healing. It encompasses all the knowledge and practices of traditional practitioners who diagnose and treat various diseases. It is also based on skills that rely on theories, beliefs, and experiences specific to a culture (Ba AS, 1994) [3]. Among the diseases treated traditionally, intestinal parasitic infections are conditions that affect the entire intestine and represent the pathological results of contact between a parasite and its host (Benouis A., *et al,* 2013) [4]. In humans, helminthiasis is caused by cestodes, nematodes, and trematodes (Adjanohoun, JE, 1996) [1]. In developing countries, parasitic diseases, including ascariasis, are among the most neglected diseases and constitute a major public health problem. Intestinal ascariasis is a disease of dirty hands, linked to fecal contamination and poor hygiene (Solofomalala A., 2016) [18]. It is also caused by contact between a parasite and its host, which frequently manifests as a digestive disorder. This digestive disorder begins with diarrhea, constipation, and may or may not be accompanied by abdominal pain (Benouis A., *et al.,* 2013) [4]. In Madagascar, due to the high cost of modern medicines, intestinal helminthiasis is often treated primarily with three endemic plants belonging to the Combretaceae family, namely: *Poivrea obscura* (Tul.), *Poivrea phaneropetala* (Baker) H. Perrier, and *Poivrea grandidieri* (Baill.) H. Perrier (Rakotoniriana E., 2010). This study was conducted to evaluate the anti-ascariasis activity of *Barringtonia racemosa* (Lecythidaceae), another plant also used in traditional Malagasy medicine to treat jaundice, malaria, and intestinal parasitosis.

2. Materials and Methods

2.1 Plant Material

Barringtonia racemosa leaves were collected in Amparihilava, Marovoay, Boeny Region, Madagascar, in February 2019. They were then identified by botanists from the "Biodiversity and Conservation" program, Life and Environmental Sciences program at the Faculty of Science, Technology, and Environment (FSTE) of the University of Mahajanga, Madagascar. These leaves were dried away from light and at room temperature in a well-ventilated room at the Biotechnology, Environment, and Health Research Laboratory (LRBES). After drying, they were ground using an electric grinder (BROOK CROMPTONSERIE 2000). The powder obtained was placed in a tightly closed plastic box and stored in a dry place, away from light and moisture, until use.

2.2 Phytochemical Studies

A phytochemical screening was performed using crude methanol extract (MeOH) to determine the main chemical families contained in *Barringtonia racemosa* leaf extract, following the method commonly used by various authors (Fong H., 1977).

2.3 Biological Studies

The antihelminthic activity of *Barringtonia racemosa* extracts was studied on adult *Ascaris lumbricoides* (ASCARIDEAE) intestinal worms and their eggs (Bussieras J, Chermette R., 1988) [6].

2.3.1 Sample Preparation

Fecal matter from young pigs raised traditionally was collected at the Mahajanga slaughterhouse. It was stored in a clean container in an oven (JOUAN AFT054) maintained at 37°C. Fecal matter containing eggs was stored in a refrigerator at 4°C (Rousset J., 1993) [15]. A direct coprological examination was performed to isolate adult worms. This involved a direct macroscopic examination and an examination under an optical microscope of the intestinal contents and feces (Rousset J., 1993) [15].

2.3.2 Preparation of the Incubation Medium

During the direct coprological examination, the intestinal contents of pigs were collected to ensure the survival of adult Ascaris during the test (Rousset J., 1993) [15].

Five grams of fecal matter were mixed with 20 ml of distilled water. The mixture was filtered and the residue recovered. The total volume was then adjusted to 40 ml of distilled water and filtered again. The filtrate obtained was left to settle in a beaker for 3 hours. The supernatant was recovered in another container and used as a survival medium (Rousset J., 1993) [15].

2.3.3 Preparation of Eggs

To determine the presence of *Ascaris lumbricoides* eggs in the fecal matter, a direct microscopic examination was performed. To do this, a sample of feces was taken from the surface and bottom of the container using a thin rod. This sample was spread on a glass slide in a drop of warm 0.9% NaCl solution and Lugol's iodine, then observed under a microscope at 10x and 40 x magnification (Rousset J.,1993) [15]. The eggs were incubated at 28-32°C, under average humidity and in darkness, for 18 days in an incubator (Rousset J., 1993) [15].

2.3.4 Studies of the Effects of *Barringtonia Racemosa* Extracts on the Mobility and Viability of Adult *Ascaris Lumbricoides*

The adult worms were divided into six batches of five worms and incubated in the incubation medium. The worms in the control batch were exposed to a mixture of pig intestinal contents and distilled water. Those in the reference batch were incubated in the presence of albendazole dissolved in distilled water at 20 mg/mL. The other two batches of worms were incubated in the presence of Hex, DCM, and MeOH extracts of *Barringtonia racemosa* at 40 and 80 mg/mL, respectively. All samples were incubated in an oven at 37°C and average humidity for 12 hours (Aubry P., 2014) [2]. The effects of *Barringtonia racemosa* extracts on the motility of *Ascaris lumbricoides* intestinal worms were evaluated by determining the duration of motility and parasite lysis. Non-embryonated *Ascaris lumbricoides* eggs isolated from stool cultures were divided into six batches of five eggs. They were incubated in the absence (control batch) or in the presence of methanol (MeOH) and dichloromethane (DCM) extracts of *Barringtonia racemosa* at 40 and 80 mg/ml, respectively, or Albendazole at 20 mg/ml (reference batch). All samples were incubated in an oven at 28-32°C under medium humidity and in darkness for 18 days (Rousset J.,1993) [15]. Daily observations of the eggs were made under a 10x and 40x optical microscope (Bilong C., 2005) [5].

3. Results

3.1 Extraction Products

The 300 g of powdered leaves from the *Barringtonia racemosa* plant yielded three crude extracts: hexane (Hex), dichloromethane (DCM), and methanol (MeOH). Their respective characteristics and yields are presented in Table 1.

Table 1: Characteristics and extraction yields of *Barringtonia racemosa*

Extracts			
Code*	**Mass (g)**	Appearance and color	Yield (%)
Hex	2,71	Brown paste	0,90
DCM	4,22	Dark green paste	1,40
MeOH	29,73	Greenish paste	9,91

3.2 Chemical Composition of *Barringtonia Racemosa* Extract

Barringtonia racemosa leaf extract contains high levels (+++) of catechin tannins, moderate levels (++) of gallic tannins, phenolic compounds, and saponins, and low levels (+) of alkaloids. Polysaccharides could not be detected (-) using the method employed (Table 2).

Table 2: Chemical families present in *Barringtonia racemosa* extract

Familles chimiques	Tests	Résultats
Catechin tannins	Gélatin + Na Cl	+++
Gallic tannins	Gélatin + Fe Cl_3 10 %	++
Phenolic compounds	Gélatin 1 %	++
Saponin	Foam index	++
Alkaloids	Dragendorff	+
Terpenoids	Acetic anhydride + H_2SO_4	+
Polysacharids	3 Bände of ethanol	-
Anthocyanins	Concentrated HCl + water bath at 37°C	-

3.3 Effects of *Barringtonia Racemosa* Extracts on the Mobility and Viability of *Ascaris Lumbricoides*

Intestinal worms exposed to MeOH extract at 40 and 80 mg/mL were highly mobile, with short mobility times of 16,80 ± 0.86 min and 10,20 ± 0,86 min, respectively. Those exposed to DCM extract at the same concentrations were less mobile, with mobility durations of 26,20 ± 3,46 min and 23,80 ± 1.40 min, respectively. Worms treated with Albendazole exhibited hypermobility with an average duration of 6,20 ± 0,86 min (Fig 1).

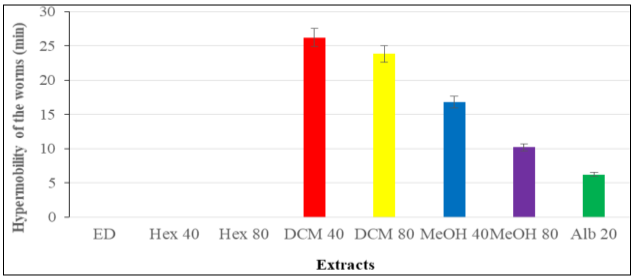

Fig 1: Variation in the mobility of *Ascaris lumbricoides* incubated in the absence (ED) or presence of hexane extracts (Hex) at 40 mg/mL and 80 mg/mL, dichloromethane (DCM) at 40 mg/mL, 80 mg/mL and methanol (MeOH) extracts at 40 mg/ml, 80 mg/ml from *Barringtonia racemosa* leaves or albendazole at 20 mg/ml (m ± s.e.m; n = 5; $p < 0.05$)

The mobility of intestinal worms exposed to MeOH extract is followed by rapid lysis and death of *Ascaris lumbricoids* after 8 hours, with mortality rates of approximately 60 ± 1.25% and 90 ± 1,60%, respectively. At the same time, intestinal worms incubated in the presence of Albendazole at 20 mg/ml are completely killed at 100 ± 0%. In contrast, worms exposed to the DCM extract showed mortality rates of 30 ± 1,58% and 40 ± 1,6% at 40 mg/mL and 80 mg/L, respectively, after 12 hours (Fig 2).

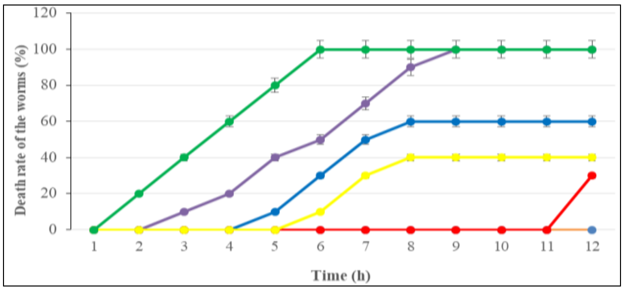

Fig 2: Variation in mortality rates of *Ascaris lumbricoides* intestinal worms incubated in the absence or presence of methanolic extracts at 40 mg/mL, 80 mg/mL and dichloromethane extracts at 40 mg/mL, 80 mg/mL from *Barringtonia racemosa* leaves or the reference product Albendazole at 20 mg/ml (m ± s.e.; n = 5; $p < 0.05$).

3.4 Effects of *Barringtonia Racemosa* Extracts on the Development of *Ascaris Lumbricoides* Eggs

After 18 days of treatment, the *Ascaris lumbricoides* eggs from the control batch treated with distilled water embryonated into stage 2 (L2) larvae and became infective (Figure 3A). In contrast, the eggs from the batches treated with the methanol extract (Figure 3B) and dichloromethane extract (Figure 3C) of *Barringtonia racemosa* at 80 mg/mL on the one hand, and with Albendazole at 20 mg/mL (Figure 3D) on the other hand, are altered. The embryonic masses become voluminous and occupy the central part of the eggs. The eggs lose their initial state as they gradually lose their walls. The embryonic masses lose their central position and tend to move outward as the internal and external walls are altered. There is inhibition of the process of embryogenesis and larval development in *Ascaris lumbricoides* eggs.

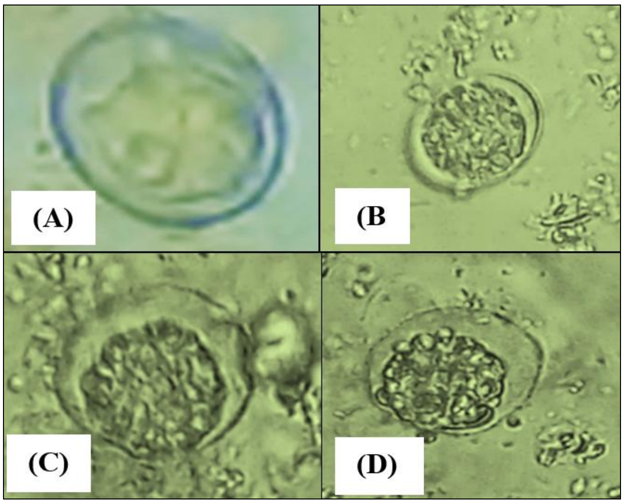

Fig 3: Effects of *Barringtonia racemosa* leaf extracts on the development of *Ascaris lumbricoides* eggs. The eggs were incubated in the absence (Control, A) or presence of methanolic (B) or dichloromethane (C) extract of *Barringtonia racemosa* at 80 mg/mL, or Albendazole at 20 mg/mL (Reference, D), respectively. The condition of the eggs was observed daily for 18 days under a 10x and 40x optical microscope.

4. Discussion

The effect of *Barringtonia racemosa* methanolic extract tested at concentrations of 40 and 80 mg/mL on adult *Ascaris lumbricoides* worms is considered high because the duration of worm hypermobility corresponding to 16.8 ± 0. 86 min and 10.2 ± 0.86 min, respectively, are short. Disrupting food intake for 24 hours is sufficient to kill most adult parasitic worms. These worms die if they become paralyzed and temporarily lose their ability to maintain their position in their environment (Harekrishna R., 2010). These findings are confirmed by studies conducted by Danquah C. and colleagues in 2012, which demonstrated that saponins can prevent the production of energy used by parasites, especially gastrointestinal nematodes (Danquah C., 2012).

Our results showed that 80 mg/mL of *Barringtonia racemosa* methanolic extract caused denaturation of the integument of Ascaris lumbricoides worms. Recent similar studies have shown that the main biological activity attributed to saponins is the permeability of the parasite membrane (Menin L., 2001). These compounds cause a change in membrane permeability by forming pores (Melzig M., 2001). In this case, the vermicidal effect of *Barringtonia racemosa* on Ascaris lumbricoides intestinal worms could be due to the saponins present in average concentrations in this extract. Alkaloids reduce glucose in worms by suppressing the distribution of sucrose to the small intestine. They are capable of suppressing sucrose transfer throughout the digestive system and also reducing glucose supply to the helminth (Shaibani I., 2009). These alkaloids are well known for their role in inducing paralysis by acting on the central nervous system (Shaibani I., 2009). Therefore, the vermicidal activity of the methanol and dichloromethane extracts of *Barringtonia racemosa* against the intestinal parasite Ascaris lumbricoides could be attributed to the alkaloids they contain. Our results also showed that the methanol and dichloromethane extracts of *Barringtonia racemosa* inhibited the development of *Ascaris lumbricoides* intestinal worm eggs. In this context, it has been shown that saponins can also penetrate the eggs of gastrointestinal worms, altering their biological functions and subsequently blocking and definitively inhibiting their normal development. These compounds are also capable of disrupting the enzymes involved in larval development, leading to the death of the larvae of these parasites (Cavalcanti G., 2016). In addition, saponins are generally involved in interacting with cell membranes, promoting destabilization and increasing cell membrane permeability (Francis G., 2002) and decreasing the absorption and transport of sugar by the helminth (Rashmi M., 2019) They are capable of stopping larval formation through three mechanisms, including (1) affecting the permeability of the egg wall; (2) inhibiting the effect of enzymes to block egg hatching; and (3) affecting the hatching receptors found in egg coats (Vargas M., 2014). These chemical compounds are thought to have inhibited the development of Ascaris eggs into embryonated eggs with larvae at stage 2 (L2) of their development (Sreelatha S., 2011). Consequently, the saponins present in *Barringtonia racemosa* extract could be responsible for the inhibition of *Ascaris lumbricoides* larval development observed in this study.

5. Conclusion

The *Barringtonia racemosa* plant is used in traditional Malagasy medicine to treat parasitic diseases. This study conducted on *Ascaris lumbricoides* worms and eggs demonstrated that the methanol and dichloromethane extracts of this plant exerted antihelminthic activity against these parasites. They inhibited the viability of adult worms and the larval development of *Ascaris lumbricoides* intestinal parasites during 18 days of incubation. However, the methanolic extract proved to be more active than the dichloromethane extract. The saponins and alkaloids present in this extract may be responsible for this vermicidal activity. Further studies will be undertaken to isolate and identify the active ingredient(s) responsible for this antihelminthic activity.

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