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MOLLUSCICIDAL, CERCARIACIDAL, LARVICIDAL AND ANTIPLASMODIAL PROPERTIES OF BARRINGTONIA RACEMOSA FRUIT AND SEED EXTRACTS

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Abstract: Aqueous extracts of Barringtonia racemosa (Linn) Roxb. [Family: Lecythidaceae] fruit (pericarp) and seed have been evaluated for molluscidicidal, cercariacidal, mosquito larvicidal and antiplasmodial activities. The plant extracts produced approximately equipotent molluscidicidal, cercariacidal, mosquito larvicidal and antiplasmodial properties in the experimental models used. It is speculated that the observed biological effects of the plant aqueous extracts may largely be due to the triterpenoid saponins, especially barringtonenol and barringtonogenic acid, present in the fruit and seed of the plant.

Key Words: Barringtonia racemosa, fruit and seed extracts, triterpenoid saponins, molluscidicidal, cercariacidal, antiplasmodial, mosquito larvicidal activities.

INTRODUCTION

Malaria and schistosomiasis are the two leading parasitic diseases of tropical and subtropical countries of the world in terms of their overall prevalence, morbidity, socio-economic and public health burden, and human impact. Effective control and management of these infectious diseases require holistic approaches which not only include curative, chemotherapeutic and chemoprophylactic measures, but also preventive methods which include control measures that interfere with the life-cycles of the parasites. In developing, poor third-world countries, availability of cheap, effective, safe and environmentally-friendly indigenous alternatives to the existing expensive, toxic, synthetic, imported drugs used in the control and/or management of the parasitic diseases are certainly warranted. In our search for local, effective, safe alternatives for the control and/or management of malaria and schistosomiasis in South Africa, we have screened several species of higher plants from various families and diverse genera. One of the few such medicinal plants that have shown some promise as potential candidates for malaria and schistosomiasis control and/or management is Barringtonia racemosa.

Barringtonia racemosa (Linn) Roxb. [Family: Lecythidaceae] occurs as a small to medium-sized tree (4-10 metres) in KwaZulu-Natal Province of South Africa. Common names of this tree include 'brackwater mangrove', 'horse chestnut' and 'powder puff tree'. The Zulus of South Africa refer to this tree as ilboqo, iliboqo, umlukuluka or umuluku (Pool, 1993). The fruit of B. racemosa is said to make a good emetic solution against malaria (Hutchings et al, 1996; Palgrave, 1977). The stem-bark is reported to have insecticidal effects (Palgrave, 1977), and extracts of the fruit and stem-bark are claimed to be toxic to mosquitoes, snails and brine shrimp (Hutchings et al, 1996). In India, the root- and stem-barks of the tree are used for stomachache and skin diseases, while the fruit sap is applied to eczema (Watt and Breyer-Brandwijk, 1962). The stem-bark is often used in decoctions dabbed on bodies of sick people, and is also used in non-venerale stricture in Australia. The fruit is used to poison wild pigs in the...
MATERIALS AND METHODS

Plant Material: Mature fruits of *Barringtonia racemosa* were collected from the University of Durban-Westville's Campus in Durban, South Africa, between March and August, 2001. The fruits were identified by the Taxonomist/Curator of the Department of Botany, University of Durban-Westville, as those of *Barringtonia racemosa* (Linn) Roxb. [Family: Lecythidaceae].

Extraction Procedure: One kilogramme (1000 g) each of Barringtonia racemosa’s dry fruit (pericarp) and seeds were finely ground in an industrial mill. The ground materials were separately extracted twice, on each occasion with 5 litres of distilled water, at room temperature for 24 hours, with shaking. The aqueous solubles obtained were filtered and concentrated to dryness under reduced pressure at 30±1°C. The crude, aqueous extracts thus obtained were freeze-dried, finally yielding 1.5% and 6.1% powdery crude aqueous fruit and seed extract residues respectively. Aliquot portions of the crude fruit and seed extracts were weighed out and dissolved in distilled water on each day of our experiment.

Molluscicidal Evaluation: The World Health Organization’s method (WHO, 1965) for screening molluscicides was used. The intermediate host of *Schistosoma mansoni* in Nigeria, *Biophalaria pfeifferi* snails, collected from a stream in Edun-Abon near Ille-Ife in Osun State of Nigeria, were separately exposed to graded concentrations of *B. racemosa* fruit and seed aqueous extracts. Niclosamide (Bayluscide®), a synthetic, chemical molluscicide, was used as the reference, control molluscicide for comparison. Ten snails were used per extract or niclosamide dose, with three replicates per dose of the extract or control. The snails were exposed to each extract dose for 24 hours, after which the snails were recovered and mortality recorded.

Cercariacidal Testing: Infected *Biophalaria pfeifferi* snails were placed in petri-dishes and then exposed to artificial light (100 Watt bulbs) for 30 minutes. The emerging cercariae were pooled and counted with the aid of a microscope. Laboratory bred, male albino mice (weighing 20-25 g) were inoculated intraperitoneally, with 0.1 ml of the suspension containing 200 cercariae (pre-determined infective dose) per mouse. The method used was adapted from that described by Ogboli and co-workers (2000).

Mosquito Larvicidal Screening: 0.01%, 0.1% and 1.0% (w/v) solutions of each extract were made-up in distilled water. Third (3rd) instars of *Anopheles arabiensis* mosquito larvae were obtained from the Medical Research Council (MRC) of South Africa, Durban Office.

The larvicidal assay method used in this study was adapted from the one described in detail earlier by Marston and co-workers (1993). The experimental room temperature was set at 28-30°C, with a relative humidity of 70-80%. 20-ml of each of the test solutions (0.01%, 0.1% and 1.0% w/v) were placed in 100-ml beakers. 20-ml distilled water (placed in 100-ml beakers) was used as controls for the aqueous extracts. ‘Test’ and ‘control’ beakers were set-up in triplicates. Into each of the ‘test’ and ‘control’ beakers were placed 20 third instar *Anopheles arabiensis* larvae. Mortality count was taken 1, 3, 6, 12 and 24 hours after exposing the larvae to the plant extract solutions. This was done by gently swirling and agitating the contents of the beakers, and then allowing them to settle for 5 minutes, before counting the number of dead and living larvae. Whereas dead larvae sank to, and remained motionless at the bottom of beakers, those that were alive were able to swim back to the surface of the solutions in the beakers.

Antiplasmodial examination: This assay part of the study was carried out in the Department of Pharmacology of the University of Cape Town, Cape Town, South Africa. Chloroquine-sensitive *Plasmodium falciparum* D10 strain was used for this assay. Stock solutions of the plant extracts (2 mg/ml) were made up in water. 100 μl of each of the above stock solutions was added to 900 μl of the assay medium to give a final concentration of 200 μg/ml. Red blood cells with 2% parasitaemia as well as a stock of unparasitized red blood cells were also used in this assay. The experiments were carried out in 96-well microtiter plates (see Figure 1). The assay procedure was carried out under sterile conditions, within a laminar flow hood. Firstly, 100 μl medium was placed into all the wells except those in column 3. Into each of the wells in column 3 were placed 200μl of the plant extracts (with concentration of 200μg/ml). This was done in duplicate with the same extract being tested in rows A and B. In this way, four plant extract concentrations were tested on each plate. The plant extract solutions were then serially diluted across the plate from column 3 to column 12, with the final concentration of plant extract in each row being 100 μg/ml, 50 μg/ml, 25 μg/ml, 12.5 μg/ml, 6.25 μg/ml, 3.13 μg/ml, 1.56 μg/ml, 0.78 μg/ml, 0.39 μg/ml, and 0.20 μg/ml. A volume of 100 μl of unparasitized red blood cells was placed in all the wells of column 1 and 100 μl of 2% parasitized red blood cells were placed into all the wells of columns 2 to 12. The plate was covered and placed into an incubation chamber. The chamber was then gassed...
for 5 minutes with filtered gas comprising 3% oxygen, 4% carbon dioxide, and 93% nitrogen. The chamber was thereafter placed into a 37°C incubator for 48 hours. After 48 hours incubation, the plates were removed from the incubator. The red blood cells in each well were carefully re-suspended. A volume of 100 μl Malstat and 25 μl NBT/PES was placed into all the wells of a new 96 well microtiter plate. Next, 15 μl of the re-suspended red blood cells from the original microtiter plates were placed into the corresponding wells of the plate containing the Malstat and NBT/PES, and the plate was immediately placed into a microplate reader (Cambridge Technology, Inc. – Microplate Reader Series 7500 VER-3.00), set at a wavelength of 620 nm. After 5 minutes (the time within which the tetrazolium would have been reduced to the blue formazan salt), readings were taken and recorded.

Molluscicide & LD₅₀ values (in ppm) & & &

Barringtonia racemosa fruit (pericarp) aqueous extract & 521.48 (490.53-564.58) & & &

seed aqueous extract & 556.23 (540.36-581.76) & & &

iclosamide (Bayluscide®) & 0.112 (0.075-0.210) & & &

Table 1: Comparative toxicity of Barringtonia racemosa fruit (pericarp) and seed aqueous extracts on Biomphalaria pfeifferi snails.

RESULTS

Molluscidal Activity: The LD₅₀ values for Barringtonia racemosa extracts and niclosamide (Bayluscide®) on Biomphalaria pfeifferi snails are shown in Table 1. The fruit (pericarp) extract was found to possess slightly stronger molluscidal activity than the seed extract of the plant.

Cercariacidal Property: The non-infected, non-treated (negative control) mice had normal organs with no gross lesions, and with a mean liver weight of 1.16 g (Table 2). The infected but non-treated (positive control) mice had a mean liver weight of 2.37 g, and a mean worm load of 5.65/g of intestinal tissue. Pathologically, the mice in this positive control group had enlarged, fatty and friable liver, enlarged and congested spleen and kidney, and were anaemic. Infected mice treated with aqueous extracts of Barringtonia racemosa fruit (pericarp) and seed extracts showed a mean liver weight of 1.65 g and 1.71 g respectively, and a mean worm load of 1.61/g and 1.68/g of intestinal tissue respectively. Compared with the positive control mice, the latter values represent 71.50% and 70.27% reductions in the parasite load caused by the Barringtonia racemosa fruit- (pericarp) and seed-extract treatments respectively. Pathologically, all the Barringtonia reacemosa fruit- and seed-extract treated mice showed haemorrhagic and friable dark liver, enlarged spleen and kidney, and anaemic carcass. These observations are in tandem with the findings of Ogboli et al (2000).

<table>
<thead>
<tr>
<th>Mice group</th>
<th>Treatment</th>
<th>Mean liver weight (g)</th>
<th>Mean worm load (per g of intestinal tissue)</th>
<th>% Parasite reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>A(1)</td>
<td>B. racemosa fruit (pericarp) aqueous extract</td>
<td>1.65±0.018</td>
<td>1.61±0.75</td>
<td>71.50</td>
</tr>
<tr>
<td>B(2)</td>
<td>B. racemosa seed aqueous extract</td>
<td>1.71±0.25</td>
<td>1.68±0.81</td>
<td>70.27</td>
</tr>
<tr>
<td>C(3)</td>
<td>Positive control (Infected but not treated)</td>
<td>2.37±0.17</td>
<td>5.65±1.30</td>
<td>Nil</td>
</tr>
<tr>
<td>D(4)</td>
<td>Negative control (Non-infected and Non-treated)</td>
<td>1.16±0.05</td>
<td>Nil</td>
<td>Nil</td>
</tr>
</tbody>
</table>

Pathological signs

1. Enlarged, haemorrhagic, dark liver, spleen and kidney; anaemic carcass.
2. Enlarged liver, spleen and kidney with severe lesions; anaemic carcass.
3. Enlarged, fatty and friable liver, spleen and kidney; anaemic carcass.

Table 2: Effects of Barringtonia racemosa fruit (pericarp) and seed aqueous extracts on Schistosoma mansoni infected mice.

Mosquito Larvicidal Property: 1% (w/v) solutions of the fruit (pericarp) and seed extracts of Barringtonia racemosa produced far more pronounced larvicidal activities than the 0.1% and 0.01% (w/v) solutions of the extracts. Consequently, our discussion section of this paper will refer to the results obtained with the 1% (w/v) solutions.
The aqueous extracts of the fruit and seed of *B. racemosa* produced approximately equal larvicidal activity. A 50% mortality of the mosquito larvae were observed within 3 hours of exposing the larvae to 1% solutions of the extracts. 100% mortality of the larvae was obtained within 12 hours of incubating the larvae in 1% solutions of the extracts (see Figure 2). The distilled water control used in this set of experiments produced a maximum of 5% mortality (figure not shown). This observation strongly suggests that the plant extracts were actually responsible for the observed larvicidal activities.

![Figure 2](image_url)

**Figure 2**: Percentage mortality of *Anopheles arabiensis* larvae in 1% solutions of aqueous extracts of *B. racemosa* fruit (pericarp) and seed.

**Antiplasmodial Activity**: *Barringtonia racemosa* fruit and seed aqueous extracts displayed similar antiplasmodial activities (see Figure 3). They both gave IC50 values of approximately 30 μg/ml. Also worthy of note is the fact that the extracts caused 100% parasite death at 100 μg/ml. It is, therefore, speculated that the same chemical compound/s might be responsible for the anti-plasmodial activities of the two extracts. Although chemical samples with IC50 values of <10 μg/ml are usually considered active, it must be noted that the extracts tested in this study are very crude. Thus, in the present circumstances, the IC50 values of approximately 30 μg/ml may be considered significant in the sense that these crude extracts may contain antiplasmodial compounds but at low concentrations. The efficacy of the plant crude extracts may, therefore, only become obvious after their purification.

**DISCUSSION AND CONCLUSION:**

The results of this experimental study indicate that *Barringtonia racemosa* fruit and seed extracts possess molluscidal, cercaricidal, antiplasmodial and mosquito larvicalid properties. These observations are in consonance with some of our preliminary studies on the plant’s extracts (Nundkumar and Ojewole, 2002). The molluscicidal and cercaricidal properties of the plant extracts observed in this study, coupled with their antiplasmodial and mosquito larvicalid effects, make *B. racemosa* fruit and seed ideal candidates for scientific scrutiny in the search for new molluscidal and antimalarial agents among South African flowering plants. Indeed, *Barringtonia racemosa* fruits and seeds could assist mankind in the battle against schistosomiasis and malaria by providing chemical compounds to control the vectors, and also for the chemotherapy of the two debilitating and deadly parasitic diseases.

Our preliminary chemical analysis of *B. racemosa* fruit (pericarp) and seed aqueous extracts indicated the presence of flavonoids, terpenoids, fatty acids and saponins. Triterpenoids have been generally credited with mosquito larvicidal (Gbolade, 2000), molluscidical (Hostettmann *et al.*, 1982; Hostettmann, 1984) and cercaricidal (Ogoli, 2000) activities. Although it is premature at this stage, to attribute the biological activities of the plant extracts observed in this study to a specific group of chemical compounds, we speculate that the triterpenoid saponins, especially barringtonenol and barringtonenonic acid, present in the fruit and seed of *B. racemosa* (Hutchings *et al.*, 1996) might have contributed largely to the observed biological effects of the extracts. Further studies are in progress to determine the chemical constituent/s of the plant that is/are responsible for the observed biological activities obtained in this study, and to shed more light on the value of *B. racemosa* fruit and seed as potential plant molluscidides and larvicides.

![Figure 3](image_url)

**Figure 3**: Percentage parasite survival after treatment with aqueous extracts of *Barringtonia racemosa* fruit (pericarp) and seed. Chloroquine was used as the reference antiplasmodial drug for comparison.

**ACKNOWLEDGEMENTS**

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REFERENCES


The End

Figure 1: Diagrammatic representation of the ‘microtitre plate’ used for the antiplasmodial assay.