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Anthelmintic screening of the stem bark of *Berlina grandiflora*

N.M. Enwerem[1]*, C.O. Wambebe[2], J.I. Okogun[1], P.A. Akah[3], K.S. Gamaniel[2]

1. Department of Medicinal Plant Research and Traditional Medicine, National Institute for Pharmaceutical Research and Development [NIPRD], Abuja, Nigeria.
2. Department of Pharmacology and Toxicology, NIPRD, Abuja.
3. Department of Pharmacology and Toxicology, University of Nigeria, Nsukka, Nigeria.

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Abstract

Objective: To screen the anthelmintic properties of *Berlina grandiflora*. Materials and methods: The dried stem bark of *Berlina grandiflora* was extracted with 95% ethanol. Hexane, ethyl acetate and methanolic fractions were obtained successively from crude 95% ethanolic extract, leaving behind a crystalline residue. The crude extract and the resulting fractions were screened for anthelmintic activity and compared with piperazine using rats infected with *Nippostrongylus brasiliensis*. Qualitative phytochemical analysis was performed on the ethanolic extract and the residue. Results: At a dose of 100 mg/kg orally, the extracts have caused over 50% deparasitization in rats infected with *Nippostrongylus brasiliensis*. The highest anthelmintic activity was recorded in rats treated with the residue. The residue was found to be rich in tannins. Conclusion: *Berlina grandiflora* exhibits remarkable anthelmintic activity against *N. brasiliensis* and the results substantiate its traditional use as an anthelmintic.

Keywords: *Berlina grandiflora, Nippostrongylus brasiliensis,* anthelmintic, deparasitization.

1. Introduction

Helminthic infestations affect about 3000 million people worldwide [1]. Helminthiasis and its prevalence are intense especially in children in countries with tropical climate, poor sanitation, low standard of living and poor health education [2]. Unfortunately, available anthelmintics are either expensive or less effective because of resistance by the worms. Thus, the need for, inexpensive drugs, which will not induce resistance in the helminths becomes urgent and much effort is being made at the discovery of new anthelmintic from medicinal plants. Helminthic infestations are usually asymptomatic. Extent of debility and ill health caused by worms are related to the worm load, type and duration of infections. Treatment is directed primarily to reduction of worm load. This is achieved through different mechanisms. For example, mebendazole acts on the parasites tubulin, by inhibiting glucose uptake [3]. The action of many, if not all anthelmintics is due to their ability to reduce larval motility [4].

*Corresponding Author
Email: Nkyenwerem@aol.com

Berlina grandiflora, Hutch and Dalz (Caesalpinaceae) is a tropical shrub with leaves arranged in pinnate form. The leaf is pinkish when young with a swollen base. The flowers are large, white, conspicuous in ample panicles and as long as the leaves [5]. *Berlina grandiflora* has many Nigerian vernacular names, Hausa - "Dokarrafi'', Nupe - "Baborochi bata'', Yoruba - "Apado'', and Ibo - "buba". These names have been attributed to its may uses in ethnomedicine.

The bark infusion is used in Congo Brazzaville as a purgative and the leaves are used for treating intestinal problems [6]. In Ghana, the stem is used as a chewing stick and in the preparation of enema against constipation. The bark and fruits are used to stupefy fish in South Africa [6]. In the Eastern State of Nigeria, it is used in combination with *Pilostigma thonningii,* Schum, in the ratio of 3:2 for treating gastrointestinal worms [6]. However, as far as ascertained no report of the anthelmintic activity of *Berlina grandiflora* is found in the literature. In the present study, an attempt has been made to study the anthelmintic potential of the plant.

2. Materials and methods

2.1. Plant material

Stem bark of *Berlina grandiflora* was collected from Umuahia, Abia State, Nigeria in the month of May, 1994. It was identified by the late Mr.A.O.Ohaeri, Taxonomist of the Department of Medicinal Plant Research and Traditional Medicine, National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria and a herbarium specimen (N0 3370), is preserved in the Herbarium of the National Institute for Pharmaceutical Research and Development (NIPRD), Abuja.

2.2. Chemicals

Piperazine citrate used as anthelmintic control, was obtained from May and Baker, England. Pyrantel pamoate (a broad spectrum anthelmintic agent) used for deworming was obtained from Pfizer Co Plc., Nigeria. All solvents used were purified through distillation method.

2.3. Animals

Adult Wister albino rats (150-200 g) of either sex were used in the studies. The animals were obtained from the Animal Facility Center (AFC) of the National Institute for Pharmaceutical Research and Development, Abuja. The animals were kept in plastic cages at a room temperature of 30 ± 1°C and 55-60% relative humidity with a 12 h light/dark cycle. They had free access to drinking water and standard laboratory diet (Pfizer Feed PLC, Lagos Nigeria) *ad libitum*. The animals used for anthelmintic studies were dewormed using pyrantel pamoate in their drinking water (7 mg / ml) for three days, one month prior to the experiment.

2.4. Extract preparation

The bark of *Berlina grandiflora* was sun dried and reduced to coarse powder. 1.5 kg was extracted by maceration for 48 h at room temperature with 95% ethanol. The extract was filtered and then concentrated at 80°C using a rotary evaporator. The extract was brought to complete dryness over water bath. The yield was 30%. The crude extract (30 g) was then extracted to exhaustion successively with hexane, ethyl acetate and methanol leaving behind a crystalline residue. The different fractions obtained were subjected to anthelmintic screening.

2.5. Phytochemical screening

The crude ethanolic extract and the residue were subjected to qualitative phytochemical analysis using standard methods [7].

2.6. Parasite culture and infection

Helminthic infestation and anthelmintic screening were performed as described by Cavier [2] and Standen [8]. The helminthes used for this study was *Nippostrongylus brasiliensis*. It is a natural helminthes parasite of Norwegian rat.

This helminthes can easily be adapted in albino mice, rats, hamsters and rabbits. It is a good helminthic model for experimental *helmintiasis* because of the ease with which it can be maintained through all its stages in the laboratory [9].

Table 1
Percentage deparasitization of rats infected with *N.brasiliensis* and treated with *B.grandiflora* extracts and piperazine citrate

Extract / Drug	Drug Dose (mg / kg)	Average number of worms	% Deparasitization
95% ethanol	100	51± 19**	78.0
Ethyl acetate	100	60 ± 13**	74.0
Methanol	100	90 ± 38**	61.0
Residue	100	28 ± 2**	88.0
Piperazine	100	11 ± 8**	95.0
Control [‘tween 80]	0.2 ml/animal	223 ± 9	00.0

Number of rats/group = 5; ** P < 0.01

Faecal pellets from infected rats were collected for culturing. The pellets were soaked in water for 4 hr after which,they were crushed in a porcelain mortar, and transferred into a 250 ml beaker. The material was mixed with water, strained through several layers of cotton gauze and allowed to stand for about 4 h in a glass jar. The supernatant fluid was discarded and the sediment mixed with vermiculate in labeled plastic petri dishes. The dishes were incubated at 25 ± 2°C for 7 days using a cool incubator. In 4 - 5 days, the larvae (L3) emerged from the egg, developed and migrated toward the margin of the vermiculate [8].

The larvae (L3) were harvested by transferring the cultures from the petri dishes into glass bottles. The glass bottles were half filled with warm water and inverted onto clean petri dishes. A little water was then placed around the bottles in the dishes. The apparatus was allowed to stand for at least 6 hr, after which the water was pipetted off from the dishes and transferred into the glass tubes. The L3 obtained was concentrated by washing several times with distilled water, and their number determined by dilution counts. The volume was then adjusted to contain 300 L3 in 0.2 ml for treatment and 2000 L3 in 0.2 ml for maintenance of the parasites [2, 8].

2.7. Infection of rats

Rats were dewormed 2 - 4 weeks prior to experiment using pyrantel pamoate (7 mg/ml of drinking water for 3 days) in order to maintain a helminthes free colony. Helminth-free rats were infected subcutaneously in the cervical region with 300 L3 of *N. brasiliensis*, in 0.2 ml of distilled water.

Fresh faecal pellets were obtained from each rat by squeezing them out of the rectum into labeled glass tubes on the seventh day after infection. Helminthes ova were recovered and examined qualitatively by floatation using 51% magnesium sulphate solution with distilled water. Rats shedding the ova of *N.brasiliensis* seven days post - infection were used for the chemotherapeutic trials[8].

2.8. Anthelmintic screening

Animals were treated orally on the 10th, 11th and 12th days post-infection with the extracts (100 mg / kg). Infected control rats received 0.2 ml of distilled water containing 3% Tween 80. The drug responsiveness of *N. brasiliensis* was tested by administering piperazine citrate 100 mg / kg to a group of rats. On the 14th day post-infection, the rats were starved overnight to clean the small intestine so as to enhance worm counting.

The rats were killed on the 15th day in a chloroform chamber. The rats were autopsied and the first 15 cm of the small intestine removed, sectioned longitudinally, enclosed between two glass plates and examined under a dissection microscope. The worms (dead and alive) which were easily visible were counted.

The percentage deparasitization was calculated using the formula: [2]

$$\frac{N - n}{N} \times 100$$

Where N = average number of worms in control animals; n = average number of worms in treated animals.

2.9. Statistical analysis

The results were analysed statistically using student's *t* - test. The minimum level of significance was fixed at $P<0.05$

3. Results and discussion

The anthelmintic effect of *B.grandiflora* extracts is shown in table 1.

The extracts caused more than 50% deparasitization in rats infected with *N. brasiliensis*. The residue was the most potent fraction, although less active than piperazine. The results clearly indicate that *B.grandiflora* exhibits remarkable anthelmintic activity and provide scientific basis for its popular traditional use as worm expeller.

During the preliminary experiments (data not shown) the extracts potently reduced fecal egg counts in the treated rats. Also the extracts neither influenced spontaneous contraction of the isolated rabbit jejunum nor acetylcholine evoked contractions of the isolated guinea pig intestine. Taken together, these results, the indirect effect of the extracts on the worm is excluded. Thus direct action is being suggested. The exact mechanism has not been determined.

The result of the phytochemical analysis revealed the presence of tannins, flavonoids, triterpenes and glycosides in the ethanolic extract while the residue contains mainly tannins and flavonoids. Tannins are associated with the ability to react with proteins and thus starve micro-organisms by rendering proteinaceous substrates non assimilable [10]. This may in part contribute to the mechanism of the anthelmintic activity of the extracts.

Further studies to isolate the active and other chemical constituents of *B.grandiflora* are in progress.

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