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Original Article

Evaluation of *In Vitro* Anthelminthic Activity of *Pseudocedrela kotschyi* Harms. (Meliaceae) a Dry Zone Ceder Stem Bark Aqueous Extract

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Helminths infections are chronic illness in human being and cattle. The use of alternate drugs has been a remedial measure against the resistant strains of helminth parasites, as well as means of reducing the cost of controlling diseases caused by helminthes. *Pseudocedrela kotschyi* is a medicinally valuable plant and possesses various pharmacological properties. *P. kotschyi* has been traditionally used as an anthelminthic agent. To justify the ethnomedicinal claims, the anthelminthic property of *P. kotschyi* was evaluated using *Lumbricus terrestris* as an experimental model because of similar anatomical features with intestinal helminthes. Piperazine citrate was used as the standard reference drug. Among the various concentrations tested (10-50 mg/mL), 50 mg/mL aqueous extract showed efficient paralysis effect (13.1 min), and also showed significant anthelminthic activity with 25.4 min death time . Standard drug at 10mg/mL concentration showed paralysis at 6.4 min and time of death was 19.1 min. This investigation revealed that aqueous extract of *Pseudocedrela kotschyi* showed significant anthelminthic activity against *Lumbricus terrestris*. It therefore validates the ethnomedicinal use of *Pseudocedrela kotschyi* stem bark as an anthelminthic agent.

Keywords:*Pseudocedrela kotschyi*, anthelminthic agent, *Lumbricus terrestris*, aqueous extract, paralysis

Infections with helminthes are among the most widespread infections in humans and their domestic animals.More than half of the population of the world suffers from various types of infections and also the majority of the cattles suffer from worm infections (1).

Pseudocedrela kotschyi Harms (Meliaceae) which is locally called "Tuunas" in Hausa language, is a savanna woodland plant, chiefly of the guineazone growing on moister heavy soil in valleys. The plant when fully grown resembles-*Khaya senegalensis* and is often found growing with it and with *Daniellii oleifera*. It is one of the largest trees of the savanna, but it suffers from forest and crooked stems. Trees are commonly 20-30 ft high but can grow up to 60 ft high and 6 ft girth on good sites. Large girths are more frequently seen than great girths. The crown is rounded with ascending branches. The bark is thick, silvery-grey and fairly regularly fissured into small square pieces. The trees

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are propagated from root suckers, the seed is often destroyed by bush fire (1-3).

Pseudocedrela kotschyi Harms (Meliaceae) is used for many purposes. Its average antimicrobial activity against microorganisms such as *Staphylococcus aureus*, *Streptococcus pyogenes*, *Bacillus subtilis*, and *Cladosporium herbarum*, was reported (4). Leaves, roots and stembarks extracts of Meliaceae family are utilized to treat inflammation, infections and skin diseases. *P. kotschyi* is one of the ten medicinal plants used in Nigeria and Africa as both traditional and alternative medicine. The plant also serves as an encouragement for the production of antibiotics locally, and is an example of the importance of natural endowed girth as nature.

*P. kotschyi*has been traditionally used as an anthelminthic agent. However, anthelminthic activity of *P. kotschyi* whole plant extract has not been scientifically proven or reported. To justify the traditional use of *P. kotschyi*, we made an attempt to assess *in vitro* the anthelminthic activity of *P. kotschyi* stem bark aqueous extract using earthworms.

Materials and methods

Plant collection, identification and extract preparation

Pseudocedrela kotschyi plant materials were collected from "Takalafiya" Ward, Bali Local Government Area, Taraba state, Nigeria. The plant was authenticated by Mr. Cletus A. Ukwubile of the Department of Science Laboratory Technology Federal Polytechnic Bali, voucher number MEL002. It was shade dried for three weeks. Furthermore, the bark of the plant was chopped finely and shade dried, powdered mechanically and subjected to cold extraction by soxhlet apparatus using water as the solvent system for 48 h. After every 24 h, fresh water was added and the aqueous phase containing the extract was separated. The aqueous extract was filtered and concentrated *in vacuo* under reduced pressure and allowed for complete evaporation of the solvent on rotary evaporator and finally vacuum dried. The crude extract was stored in a desiccator and the yield percentage was calculated. Aqueous extract and standard drug piperazine citrate (Benrock Chemicals, Nigeria) were dissolved in 0.5 % DMSO (Benrock Chemicals, Nigeria) in distilled water (v/v) and further used for evaluation of anthelminthic activity (5).

Test organisms

Earthworm was selected for anthelminthic activity due to its anatomical and physiological resemblance with the intestinal round worm parasites of human being (6-8). Adult earthworms *(Lumbricus terrestris)* were collected from the pond near by Bali river. The earth worms were maintained under normal vermicomposting medium with adequate supply of nourishment and water for about two weeks. Before starting the experiment, the earthworms were washed with normal saline. Adult earthworms of approximately 4 cm long and 0.2 – 0.3 cm width were used.

Anthelminthic activity

The anthelminthic activity of stem bark extract of *P. kotschyi* was assessed according to the method reported by Coles and Rush (9). Briefly, seven groups of earthworm (*Lumbricus terrestris)* with four worms in each group were included in the study. Each worm was separated into 10 mL of desired formulation in normal saline. In group 1, earthworms were released into 10 mL normal saline in a clean petri dish and were maintained as negative control. Group 2 earthworms were released in normal saline containing standard drug piperazine citrate (B.P.C Elixir) 10 mg/mL as positive control. Group 3, 4, 5, 6 and 7 earthworms were released in 10, 20, 30, 40 and 50 mg/mL of extract in 10 mL normal saline, respectively. Earthworms were observed for time of paralysis and death. Paralysis was observed based on the behavior of the worm with no revival of body state. Death was concluded based on the total loss of motility with faded body color (7). The experiment was repeated four times for each group and mean values were recorded.

Statistical analysis

The data obtained were expressed as mean ± SEM of four earthworms in each group. Statistical analysis was carried out using one way ANOVA followed by Duncan multiple range test. The difference in values at $P< 0.05$ was considered as statistically significant. Analysis of variance (ANOVA) was performed using graph pad prism to determine the mean and standard error of paralysis and death time of earth worms.

Results

Earthworms belonging to control group showed time of paralysis at 17. 1 min and death time at 56.1min,whereas the aqueous extract at the concentration of 10 mg/mL showed the time of paralysis and death at 15.3 and 28.1 min, respectively. The paralysis and death timesdecreased in a concentration dependent manner (Table 1).

Among various concentrations tested, the aqueous extract at 50 mg/mL showed more efficient paralysis and death effect (13.1 and 25.4 min, respectively) compared with other tested groups (Table 1). Standard drug at 10mg/mL showed time of paralysis at 6.4 min and death time at 19.1 min (Table 1).

Table 1. *In vitro* anthelminthic activity of aqueous extract of *P. kotschyi* against *Lumbricus terrestris*

Animal group	TOP (min)	TOD (min)
Group 1 (10 mL NS)	17.1±0.07	56.1±0.07
Group 2 (10 mg/mL PC)	6.4±0.09^{c}	19.1±0.05^{a}
Group 3 (10 mg/mL PKE)	15.3±0.03	28.1±0.07
Group 4 (20 mg/mL PKE)	14.3±0.05^{a}	27.3±0.05^{a}
Group 5 (30 mg/mL PKE)	14.0±0.04^{b}	27.0±0.04^{b}
Group 6 (40 mg/mL PKE)	13.4±0.04^{b}	26.1±0.09^{c}
Group 7 (50 mg/mL PKE)	13.1±0.12	25.4±0.04^{b}

Earthworms were released into: 10 mL normal saline (group 1), normal saline containing10 mg/mL piperazine citrate (group 2), 10, 20, 30, 40 and 50 mg/mL, respectively of *Pseudocedrela kotschyi* extract in 10 mL normal saline (groups 3-7, respectively). Results are presented as means ± SEM. TOP= time of paralysis; TOD= time of death. Numbers followed by letters are statistical significant at $P< 0.05$.

Discussion

The present study assessed the effectiveness of dry zone cedar *(Pseudocedrela kotschyi)* plant which is used locally against helminth disease and in the treatment of stomach pain, using *Lumbricus terrestris* as an experimental model. Anthelminthic assay was performed on the adult earthworms (*Lumbricus terrestris*) due its physiological resemblance with the intestinal helminth parasites of human beings (10-12). The shrinkage of the worm's cell content via plasmolysis at the clitelum region possibly depicts the same way the extract would act on internal worms. This investigation revealed that aqueous extract of *P. kotschyi* showed significant anthelminthic activity against *L. terrestris* ($P < 0.05$), which justifies the use of *P. kotschyi* as an anthelminthic prescription in traditional medicine. The results of the present study showed that the plant has anthelminthic property in a dose dependent fashion. The plant thus represents a potential source of anthelminthic drug development and an immediate remedy for many helminth infections.

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Conflict of interest

The authors declared no conflict of interest.

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