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Research Article

Cytotoxic, Antibacterial, and Leishmanicidal Activities of *Paullinia pinnata* (Linn.) Leaves

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Abstract

Background: *Paullinia pinnata* leaves are employed traditionally for the treatment of various ailments which are of biological origin.

Objectives: The aim of this study was to explore cytotoxic, antibacterial, and antileishmanial properties of the leaves of *Paullinia pinnata* using in vitro models.

Methods: Brine shrimp lethality bioassay was used to determine the cytotoxic activity of the methanol leaf extract of *Paullinia pinnata*. The activity of the extract against the growth of cultured *Leishmania major* (DESTO) promastigotes was used to investigate the leishmanicidal activities. The agar well diffusion method was used to investigate the antibacterial activity against *Salmonella typhi*, *Pseudomonas aeruginosa*, *Shigella flexneri*, *Escherichia coli*, *Staphylococcus aureus*, and *Bacillus subtilis*.

Results: The methanol leaf extract of *P. pinnata* had no activity against *Artemia salina* (brine shrimp) and *L. major*. It showed a non-significant activity against *E. coli* and *B. subtilis* and no activity against *P. aeruginosa*, *S. flexneri*, *S. typhi*, and *S. aureus*.

Conclusion: The methanol leaf extract of *P. pinnata* did not exhibit any cytotoxic and anti-leishmanial properties. Moreover, the activity against various species of bacteria was not significant.

Keywords: *Paullinia pinnata* leaf extract, Antibacterial activity, Anti-leishmanial activity, Brine shrimp lethality

Background

Plants are now being explored for use in the treatment and management of clinical diseases (1-4). *Paullinia pinnata* is a woody or sub-woody climber of the family Sapindaceae. It originates from tropical America and is now common in the savanna zones of tropical Africa and Madagascar (5). The common names are “bread and cheese plant” and “sweet gum” (5,6).

Phytochemical screening of the leaves has shown the presence of cardiac glycosides, saponins, alkaloids, anthraquinones, flavonoids, and tannins (6-10). These secondary metabolites have been proposed and shown to be responsible for the observed effects in various investigations where the leaves of *P. pinnata* have shown antimalarial (11, 12), antioxidant (12-15), antidiarrhoeal (16), hematological (17), anti-typhoid (18), wound healing (19,20), phytotoxic (21), analgesic, and anti-inflammatory activities (8,22,23).

These investigations have served to support or validate some of the traditional applications of different preparations of the leaf of *P. pinnata* in the treatment and management of diverse diseases and ailments. However, its application in the amelioration of other health challenges still needs to be explored.

In the light of this, the aim of this study was to investigate the antileishmanial, antibacterial, and cytotoxic effects of *P. pinnata* leaves using various in-vitro bioassays, which may possibly lead to further investigations. The results of this study would further contribute to the existing knowledge on *P. pinnata* and provide observations which can be further explored.

Materials and Methods

Sample Collection and Extraction

Fresh leaves of *P. pinnata* were collected from the Forestry Research Institute of Nigeria (FRIN), Ibadan, Nigeria. They were authenticated and given the specimen voucher number FHI 106555 at the same institute. The leaves were shade-dried at room temperature and the dried leaves were milled and extracted using absolute methanol for 6 hours in a Soxhlet extractor. The extract was concentrated with a rotary evaporator (Heidolph HB, Germany) and a vacuum oven (Gallenhamp, England) at a temperature of 40°C. A 14% yield of the extract was realized that was stored refrigerated.

In Vitro Assays

Brine Shrimp Toxicity Assay

The modified method of Kivçak et al (24) was employed. First, 20 mg of *P. pinnata* was dissolved in 2 mL of

methanol. Various volumes of the solution (5, 50, and 500 µL) were prepared and transferred into three separate vials to make final concentrations of 10, 100 and 1000 µg/mL, respectively, and the solvent was evaporated overnight. This was done in triplicates. Then, 50 mg of the eggs of *Artemia salina* (brine shrimp) kept at 4°C was sprinkled into a hatching tray half-filled with filtered brine solution. For the eggs to hatch and develop into nauplii (larvae), the solution was incubated at 37°C for 2 days. With the help of a Pasteur pipette, 10 nauplii were deposited into each of the 9 vials and the volume was made up to 5 mL with sea water. To attract the nauplii, the vials were illuminated by a lamp and incubated at 22-27°C for 24 hours. For the negative and positive controls, 2 sets of 3 vials were prepared for the solvent and etoposide (7.4625 µg/mL, a cytotoxic drug), respectively. The lethal concentration 50 of the nauplii (LC_{50}) within 24 hours was determined.

Antibacterial Bioassay

Bacillus subtilis, Escherichia coli, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Shigella flexneri*, and *Staphylococcus aureus* were the bacteria strains used for the bioassay. Overnight cultures containing 10^8 colony forming unit/mL of microorganisms were used and diluted with sterile distilled water to obtain turbidity equivalent to a 0.5 McFarland turbidity standard. The agar well diffusion method was employed (24,25). On day 1, the nutrient broth was inoculated with single colony of bacterial culture and incubated for 24 hours at 37°C. On day 2, 100 µL of fresh bacteria was inoculated into soft agar that was melted and cooled to 40°C before shaking the mixture, which was then added to the plate containing the nutrient agar. For even distribution, the plate was rotated and allowed to solidify. A 6 mm diameter sterile cork borer was employed to make wells in the plate and appropriate codes were assigned to them. Subsequently, 100 µL of the stock solution of the methanol leaf extract of *P. pinnata* (3 mg of extract dissolved in 1 mL of dimethyl sulfoxide (DMSO) (i.e. the concentration of 3 mg/mL) was placed in respective agar well plate according to bacterial culture. Wells supplemented with DMSO and imipenem (standard drug) served as negative and positive controls, respectively. The zone of inhibition was documented after incubating the plates at 37°C for 24 hours.

Leishmanicidal Assay

The method of Saeed et al (26) was applied in this study. *Leishmania major* (DESTO) promastigotes were cultured at 22-25°C in RPMI- 1640 (Sigma) supplemented with 10% fetal bovine serum (FBS) which had been inactivated by heat at 56°C for 30 minutes. Under this condition, the promastigote culture in the logarithmic phase of growth was washed three times with physiological saline after centrifugation at 2000 rpm for 10 minutes. Subsequently, to have a final concentration of 10^6 cells/mL, fresh culture medium was used to dilute the parasites. In a 96-well microtiter plate, 180 µL of the medium was placed in the first row and 100 µL of the same medium was placed in the other well. Serial dilution of the extract was carried out after adding 20 µL of the extract to the medium and 100 µL of parasite culture was added into all the wells. One row contained DMSO and served as control. Another row contained Amphotericin B and Pentamidine which were the standard drugs. The numbers of the parasites which survived were counted microscopically with the aid of the Neubauer chamber (Marienfeld, Germany) after incubating the plate at 21-22°C for 72 hours. The mean of the three different experiments was used as the final result. A Windows-based EZ-Fit Enzyme Kinetics Software version 5.03 (Perrella Scientific Inc.) was used to calculate the 50% inhibitory concentrations (IC_{50}).

Results

The in vitro brine shrimp lethality bioassay of the methanol leaf extract of *P. pinnata* showed that the standard drug (etoposide) had 50% lethality at 7.4625 µg/mL while the extract was not toxic at any of the three concentrations (Table 1).

In Table 2, the antibacterial bioassay shows that the zone of inhibition of the methanol leaf extract of *P. pinnata* for *E. coli* and *B. subtilis* were 9 mm and 12 mm, respectively, while it was zero for *S. flexneri*, *S. aureus*, *P. aeruginosa*, and *S. typhi*. The zone of inhibition of the standard drug, imipenem, ranged from 24 to 33 mm for various bacteria species.

Table 3 reveals that the methanol leaf extract of *P. pinnata* showed no inhibitory activity on *L. major* at the concentration of 100 µg/mL while the standard drugs (amphotericin B and pentamidine) had 50% inhibition

Table 1. Activity of the Methanol Leaf Extract of *Paullinia pinnata* Against *Artemia Salina* Nauplii

	Concentration (µg/mL)	Number of Shrimps	Number of Survivors	LC_{50} (µg/mL)
Etoposide	7.4625	30	15	7.4625
	10	30	30	-
P. pinnata	100	30	30	-
	1000	30	28	-

LC_{50}: concentration at which 50% of the larvae are killed within 24 hours; -: no activity

Table 2. Effect of the Methanol Leaf Extract of *Paullinia pinnata* Against Some Bacterial Pathogens

Name of Bacteria	Zone of Inhibition of Imipenem (mm)	Zone of Inhibition of *P. pinnata* (mm)
Escherichia coli	30	9
Bacillus subtilis	33	12
Shigella flexneri	27	-
Staphylococcus aureus	33	-
Salmonella typhi	25	-
Pseudomonas aeruginosa	24	-

-: no activity

Table 3. Activity of Methanol Leaf Extract of *Paullinia pinnata* against *Leishmania Major* (DESTO)

	Inhibition (%)	IC_{50} (µg/mL)
Amphotericin B	50	0.48 ± 0.02
Pentamidine	50	2.53 ± 0.01
P. pinnata	0	>100

IC_{50}: 50% inhibitory concentration

of *L. major* at 0.48 ± 0.02 µg/mL and 2.53 ± 0.01 µg/mL, respectively.

Discussion

In this study, it was discovered that the methanol extract of *P. pinnata* leaves does not possess cytotoxic and leishmanicidal activities. Its effect against bacterial pathogens was insignificant. This is a preliminary study from which further studies can be developed.

Brine shrimp lethality bioassay is a simple, rapid, low-cost but highly efficient and sensitive laboratory assay which is considered a useful tool for the detection of antitumor compounds (27), nano-structures (28), pesticidal compounds (29), fungal toxins (30), plant extract toxicity (31-33), heavy metals (34), cyanobacteria toxins (29), anti-*Trypanosoma cruzi* (35), and cytotoxicity of dental materials (36). The assay is based on the ability to kill laboratory cultured *A. salina* nauplii. Moreover, Hamidi et al (37) have shown that crude plant extracts give more accurate results than pure compounds. Additionally, isolated pure compounds seem to lose specific bioactivity, especially with brine shrimp. The LD_{50} values below 249 µg/mL are regarded as highly toxic, 250-499 µg/mL as median toxicity, and 500-1000 µg/mL as light toxicity, and values above 1000 µg/mL are regarded as non-toxic (38). Since the methanol leaf extract of *Paullinia pinnata* did not show any sign of toxicity against *A. salina* at 10, 100, and 1000 µg/mL, it implies that the extract may have no anti-tumor, pesticidal, anti-*Trypanosoma cruzi* or anti-fungal properties. This observation is inconsistent with the findings of the study conducted by de Dieu Tamokou et al (15), which showed that *P. pinnata* leaves had anti-cancer properties. This may be because these investigators employed various specific cancer cell lines, suggesting that cancer cell lines are more sensitive in assessing cytotoxicity.

Bacteria are prokaryotes that are usually single-celled organisms, most of which have cell walls that contain peptidoglycan. They are abundant in air, water, and soil, and are major inhabitants of the skin, mouth, and intestines (39). Some of them are pathogenic and are therefore responsible for various bacterial diseases. The agar well diffusion method has been shown to be more sensitive than the disc diffusion method (40). Moreover, it is used to test the antimicrobial activity of plants or microbial extracts (41). The methanol leaf extract of *P. pinnata* showed no significant activity against *E. coli* and *B. subtilis*, and had no activity against *S. flexneri*, *S. aureus*, *P. aeruginosa*, and *S. typhi*. This supports the findings of de Dieu Tamokou et al who investigated the leaf extract of *P. pinnata* for activity against *Salmonella typhimurium*, *Listeria monocytogens*, *E. coli*, and *S. aureus* but reported no activity (15). Our findings also corroborate the observations of Ajayi et al (42) who investigated essential oil extracted from *P. pinnata* but did not report any activity against *Klebsiella pneumonia*, *Bacillus megaterium*, *B. subtilis*, *Proteus mirabilis*, *P. aeruginosa*, and *E. coli*. However, Ikhane et al (6) demonstrated that the methanol leaf extract had activity against *P. aeruginosa* and *S. aureus* at high concentrations. It can be suggested from the results of this study that fractionation of the methanol extract of the leaves may improve its activity against *B. subtilis* which had an inhibition zone of 12 mm.

Leishmaniasis, a tropical and sub-tropical disease, is endemic in 98 countries and the most common in Europe, Northern Africa, the Middle East, Asia, and parts of South America (43,44). It is mainly caused by a genus of parasitic protozoa known as *Leishmania*, which is transmitted by the bite of infected female phlebotomine sand flies. There are three forms of leishmaniasis: visceral (which is the most serious form of the disease), cutaneous (the most common form), and mucocutaneous (45). leishmaniasis affects either the skin or the internal organs. *Leishmania major* was used in this study because it is a species of protozoa in the genus which is responsible for the disease condition known as zoonotic cutaneous leishmaniasis (46). The methanol leaf extract of *P. pinnata* showed no activity against *L. major*; therefore, it does not possess antileishmanial activity and may not be recommended for the management of the disease.

Conclusion

Based on the findings of this study, it can be concluded that the leaves of *Paullinia pinnata* (Linn.) possess insignificant activity against bacterial pathogens; however, the activity against *Bacillus subtilis* may be explored. Additionally, the leaves had no activity against *Leishmania* and may not be cytotoxic; therefore, they may not be of considerable benefit when used for these purposes.

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Conflict of Interest Disclosures

The authors declare no conflict of interests.

Ethical Issues

This is not applicable because they are all in-vitro assays.

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