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Phytochemistry and antimicrobial properties of *Psydrax manensis* leaf

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Abstract

Pydrax is a densely populated genus in the Rubiaceae plant family, with about a hundred and thirty species. Some *Psydrax* species are used in traditional medicine as remedies for different diseases and have also been investigated for biological activities and phytochemicals. However, *Psydrax manensis* (Aubrév. & Pellegr.) Bridson, to the best of our knowledge, has not received any scientific evaluation of its chemical constituents nor bioactivities, and it is not used in traditional medicine. The antimicrobial properties of methanol extract and hexane, ethyl acetate, and butanol fractions of *P. manensis* leaves against two Gram-positive and Gram-negative bacteria and two fungal species were assessed using agar well diffusion and agar dilution methods for preliminary antimicrobial assay and MIC determination, respectively. In addition, the extract and fractions were screened for major phytochemical classes using standard methods. The antimicrobial result showed that *Pseudomonas aeruginosa* was the most susceptible to the test samples (MIC: 0.13 mg/mL) among the bacterial species tested, while fungal species were more resistant to the test samples than the test bacteria. *Candida albicans* was inhibited by ethyl acetate fraction, while *Aspergillus niger* was susceptible to the butanol fraction. The qualitative phytochemical analysis showed that the crude extract contained flavonoids, polyphenols, glycosides, steroids, tannins, and terpenes but not alkaloids. The fractions shared the phytochemicals in the extract in varying degrees according to their polarity. The outcome of this study presents the extract and fractions of *P. manensis* leaves as potential sources of antimicrobial molecules.

Keywords: Antimicrobial; Minimum inhibitory concentration; *Psydrax*; *Canthium*; *Psydrax manensis;* Phytochemicals

1. Introduction

Microbial infections are major health problems in the world, especially in developing countries, and they are caused by microbes such as viruses, fungi, bacteria, or protozoans that affect body parts or tissues. Various anti-infective drugs are adopted for treating these diseases. However, the indiscriminate use of commercial anti-infective agents has increased the multi-drug resistance of these microorganisms in humans. Thus, scientists continuously search for new and effective chemical compounds, especially antimicrobial molecules from various natural sources, including plants, that can tackle the health menace. Medicinal plants constitute the primary source of new pharmaceuticals and healthcare products (1–3). There are numerous plant natural products, including tannins, terpenoids, alkaloids, flavonoids, phenolic compounds, and others with antibacterial, antifungal, and antiprotozoal effects that could be used either systemically or locally (4).

An unpopular genus of the Rubiaceae family in traditional medicine, *Psydrax* (5), has few reports of its applications in the folkloric treatment of diabetes, malaria and fever, inflammations, bacterial infections, cardiovascular diseases,

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urinary tract infections and others (3,6–12). The name *Psydrax* was first mentioned by Joseph Gaertner in his book De Fructibus et Seminibus Plantarum in 1788 (13). However, *Psydrax* was abandoned for *Canthium* until Bridson reintroduced it in 1985 (14). Currently, over a hundred and thirty species are classified under *Psydrax*, according to the World Checklist of Selected Plant Families (WCSP) website (wcsp.science.kew.org), assessed on 16 November 2023. A recent review revealed that only 8% of these species have been reported for their ethnomedicinal uses, phytochemistry, and pharmacological activities with *P. subcordata* (DC.) Bridson being the most used and studied species (15). Some of the pharmacological properties of extracts and single compounds of *Psydrax* include antidiabetic, antimicrobial, antiplasmodial, anti-inflammatory, and anticonvulsant properties (10,16–19). The list of bioactive compounds of the few investigated species of *Psydrax* are summarized by (15).

One of the numerous unexplored species of *Psydrax is Psydrax manensis* (synonym: *Canthium manense* Aubrév. & Pellegr.), a medium-sized tree mostly seen in secondary forests in wet tropical regions. *P. manensis* is a native of Ivory Coast, Guinea, and Liberia but is scarcely found in Nigeria. To the best of our knowledge, *P. manensis* has not been evaluated for phytochemicals and biological properties, nor has it been reported to be used in ethnomedicinal treatment of any disease. This study, therefore, investigated the antimicrobial properties of *P. manensis* leaf extract and fractions and the phytochemicals they contain.

2. Materials and Methods

2.1. Collection Identification of Plant Materials

Fresh leaves of *P. manensis* were harvested from a secondary forest in Anaocha Local Government Area of Anambra State, Nigeria, in January 2021. The leaf sample was identified and authenticated by a taxonomist, Mr. Felix Nwafor, of the Department of Pharmacognosy and Environmental Medicine, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka, Nigeria. A sample of the leaf is deposited in the Herbarium of the same department and university with the voucher number PCG/UNN/0372.

2.2. Sample Preparation and Extraction

The leaf sample was washed to remove dust and other particles, air-dried and ground into powder. Cold maceration of the powered leaves was done using methanol, followed by drying the extract with a rotatory evaporator. The extract was reconstituted in distilled water and subsequently fractionated sequentially with n-hexane, ethyl acetate and butanol (three times with each solvent). These fractions were also dried, adequately packaged, and stored for further studies. The solvents used were of analytical grades.

2.3. Qualitative Phytochemical Analysis

The crude extract and the three fractions (n-hexane, ethyl acetate and butanol) of *P. manensis* leaves were subjected to qualitative phytochemical analyses using the methods described by (20,21). They were tested for various phytochemical classes, such as flavonoids, tannins, steroids, glycosides, polyphenols, terpenes, and alkaloids.

2.3.1. Test for Terpenes

A 3mL of crude/fractions was mixed with 1mL of chloroform in a test tube. This was followed by gradually introducing concentrated sulphuric acid (1.5 mL) into the test tube. A reddish-brown colour on the interface indicates the presence of terpenes.

2.3.2. Test for Glycosides

The Fehling's solution test was done for the crude/fractions. Distilled water (5 mL) was added to the crude/fractions and boiled in a water bath for 5 min. The mixtures were filtered, and equal volumes of Fehling's solutions A and B (5 mL) were added to the filtrate and boiled for a few minutes. A brick-red precipitate indicates a positive result.

2.3.3. Test for Flavonoids

1 mL of lead acetate solution (10%) was added to an aqueous solution of the crude/fractions. The formation of yellow precipitates identifies the presence of flavonoids.

2.3.4. Test for Tannins

A neutral ferric chloride solution (5%) was added to an aqueous solution (5 mL) of crude/fractions. The formation of a dark green colouration indicates tannins' presence.

2.3.5. Test for Alkaloids

10 mg of the crude/fractions was dissolved in dilute hydrochloric acid and filtered. The filtrates were used for the following experiments:

Mayer's test: To a few mL of filtrate, two drops of Mayer's reagent were added, and an off-white precipitate indicates the presence of alkaloids.

Wagner's test: A few drops of Wagner's reagent were added to a small filtrate volume. The formation of a reddish-brown precipitate implies the presence of alkaloids.

2.3.6. Test for Polyphenols

Lead acetate test: Lead acetate solution (3 mL, 10%) was added to a 1.5 mL aqueous solution of the crude/fractions. The production of yellow precipitates shows the presence of polyphenols.

2.3.7. Test for Steroids

The crude/fractions (0.5 g) were mixed with acetic anhydride (2 mL) in a test tube, followed by a careful introduction of concentrated sulphuric acid. The mixture in the tube was cooled in an ice bath, and change in colour from purple to blue indicates the presence of steroids.

2.4. Microorganism and Culture Media

The test organisms used in this work were two Gram-positive bacteria, *Bacillus subtilis* and *Staphylococcus aureus*, two Gram-negative species, *Escherichia coli* and *Pseudomonas aeruginosa,* and two fungi, *Aspergillus niger* and *Candida albicans*. They were provided by the Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmaceutical Sciences Nnamdi Azikiwe University, Agulu Campus, Nigeria. The culture media used were Nutrient Broth (NB) and Mueller-Hinton agar (MHA), used for bacteria culture and Sabouraud dextrose agar (SDA), used for fungi culture. The growth media were prepared according to manufacturers' instructions.

2.5. Antimicrobial Assay

2.5.1. Agar Well Diffusion Method

The antibacterial assay for the crude extract, n-hexane, ethyl acetate and butanol fractions of the *P. manensis* leaves was carried out using the agar well diffusion method as described by (22), with slight modifications; the use of 8 mm cork borer instead of 6 mm and the volume of the extract and fractions used was 80 µL instead of 20 µL. The extract and fractions were reconstituted in 100% dimethyl sulfoxide (DMSO). The antimicrobial potentials of the extract and fractions were tested against laboratory bacteria (*S. aureus, E. coli, P. aeruginosa* and *B. subtilis*) and fungi species (*C. albicans and A. niger*). The concentration of the bacteria liquid cultures was adjusted to 0.5 McFarland turbidity standard and inoculated on sterile MHA plates. In contrast, standardized fungi cultures were inoculated on pure SDA plates. 8 mm (in diameter) wells were made with a sterile cork borer on each MHA and SDA plate. An aliquot (80 µL) of each extract/fraction dilution (1, 0.5, 0.25, 0.13 and 0.06 mg/mL) was introduced into each well in the inoculated plates. Ciprofloxacin (8 µg/mL) served as the positive control for bacteria, and miconazole (50 µg/mL) was the positive control against the fungal species, while DMSO (100%) was the negative control. The seeded plates were incubated at 37 °C for 24 h for bacteria and 28 °C for 48 h (fungi). The antimicrobial potentials of the extract and fractions were determined by measuring the diameter of the inhibition zone (DIZ) around each well (excluding the diameter of the well).

2.5.2. Determination of Minimum Inhibitory Concentration (MIC)

The MIC of the active extract/fractions was determined for each test organism using the agar dilution method described by (23) with a modification. In brief, stock solutions of 10 mg/mL of crude extract and fractions were prepared in 100% DMSO. Then, two-fold serial dilutions were made to get 5, 2.5, 1.25 and 0.625 mg/mL concentrations. After that, a 10-fold dilution of each concentration was made using 9 mL of sterile molten agar, which was allowed to solidify. The microbial broth cultures standardized to 0.5 McFarland turbidity were streaked on the inoculated agar plates. The plates were incubated for bacteria at 37 °C for 24 h and 28 °C for 48 h for fungi species.

3. Results and Discussions

3.1. Phytochemicals

The powdered leaves of *P. manensis* (500 g) produced 122.94 g of methanol extract, and 100 g of the crude yielded 21.9, 10.9 and 1.42 g of butanol, ethyl acetate and n-hexane fractions, respectively. The crude extract and fractions showed varying quantities of phytochemicals when analysed. The crude indicated the presence of all the tested classes of compounds in varying degrees, except alkaloids. This finding coincides with previous studies on other species of *Psydrax* where alkaloids were missing among other phytochemicals identified in leaf, root bark, and stem extracts of *P. acutiflora*, *P. peruviana*, and *P. subcordata* (10,24–28). On the other hand, flavonoids, glycosides, polyphenols, and tannins were the most abundant, followed by steroids, and the least were terpenes in this study. This phytochemical result corroborates, to some extent, the result obtained in a survey by (8) on methanol leaf extract of *Psydrax horizontalis*, except for the presence of alkaloid in *P. horizontalis* leaf extract. The discrepancy in the two results could be linked to the genetic makeups of the two plant species, different geographical locations and harvesting season variations. This study also showed that the ethyl acetate and butanol fractions contained phytochemicals similar to the crude, except terpenes and saponins, which were absent in the fractions. In contrast, the n-hexane fraction showed the absence of many phytochemicals, except steroids and terpenes. Table 1 summarises the outcome of the qualitative analysis of the phytochemical classes of *P. manensis* leaf extract and fractions.

Table 1 Phytochemicals of the extract/fractions of leaves *P. manensis*

Phytochemicals	Crude extract	n-hexane fraction	Ethyl acetate fraction	Butanol fraction
Flavonoids	+++	-	++	+++
Glycosides	+++	-	+	+++
Polyphenols	+++	-	++	+++
Terpenes	+	+	-	-
Tannins	+++	-	++	+++
Steroids	++	+	-	-
Alkaloids	_	-	-	-

Keywords: (-): absent; (+): slightly present; (++): moderately present; (+++): abundantly present

3.2. Antimicrobial assay

The active extract/fractions showed a broad spectrum of activities against the test organisms in a concentration-dependent manner, as shown in Table 2. Among the test bacteria, *P. aeruginosa* was the most susceptible to the plant extract and all the fractions at higher concentrations (1 – 0.25 mg/mL) down to the smallest concentration for the butanol fraction, with DIZ ranging from 11 – 3 mm, and MIC of 0.13 – 0.25 mg/mL. This result contradicts the usual observation of Gram-positive bacteria being more susceptible to antibacterial agents than their Gram-negative counterparts (30). The butanol and ethyl acetate fractions with this unusual activity contain many of the phytochemicals investigated in this study, which could be linked to their positive antibacterial effects. *E. coli* was the least susceptible to the crude and fractions; it was inhibited by the crude and two fractions (n-hexane and ethyl acetate) at high concentrations with DIZ between 7 and 2 mm and MIC values of 0.25 – 0.5 mg/mL but unaffected by these samples at their lower concentrations (0.13 – 0.06 mg/mL). The extract and the three fractions were moderately effective against *S. aureus* with DIZ of 6.5 – 2 mm for the highest concentration and MIC of 0.5 mg/mL. Contrary to the preceding observations, *B. subtilis* was utterly resistant to the extract and fractions at all concentrations used in this study. Considering that Gram-positive bacteria are more susceptible to antibacterial agents (31), the complete resistance of *B. subtilis* to test samples was quite unusual.

The extract and the fractions had less effect on the fungi than the bacteria used in this study. The crude showed no inhibition against the two fungi at all concentrations. However, the ethyl acetate fraction was active against *C. albicans* at the highest concentration (DIZ: 4.5 mm), while the butanol fraction, at 1 mg/mL, inhibited *A. niger* with a DIZ of 5 mm. Comparing the antimicrobial properties of the crude and the fractions across board, ethyl acetate and butanol fractions could be adjudged the most active, the crude at the middle and the n-hexane fraction the least effective. The antibacterial positive control, ciprofloxacin (8 µg/mL), showed zero activity against the test bacteria, while miconazole

was active only against *C. albicans* at 50 µg/mL. The negative control (100% DMSO) also did not inhibit any of the test organisms.

There are reports about the favourable antimicrobial properties of extracts and fractions of different parts of other species of *Psydrax* (17,19,30,32). However, it is quite difficult to directly compare their results with ours because of the following reasons: differences in plant species and test microorganisms investigated, the use of different extraction methods and solvents, and the use of other antimicrobial assay methods and concentrations of extracts/fractions. One thing that cuts across these variations is the positive antimicrobial effect observed in the studies.

Table 2 Antimicrobial activities of extract/fractions of the leaves of *Psydrax manensis*

Microorganisms	**DIZ (mm)**							**MIC (mg/mL)**
	Concentration of extract/fractions (mg/mL)							
	1	**0.5**	**0.25**	**0.13**	**0.06**	**Positive Control**	**DMSO (100 %)**	
Staphylococcus aureus								
Crude	6.5	4	4	0	0	0	0	0.5
Hexane	6.5	5	5	4	0	0	0	0.5
Ethyl acetate	2	0	0	0	0	0	0	>1
Butanol	5	5	4	0	0	0	0	0.5
Bacillus subtilis								
Crude	0	0	0	0	0	0	0	>1
Hexane	0	0	0	0	0	0	0	>1
Ethyl acetate	0	0	0	0	0	0	0	>1
Butanol	0	0	0	0	0	0	0	>1
Escherichia coli								
Crude	4	4	3	0	0	0	0	0.25
Hexane	7	5	4	0	0	0	0	0.5
Ethyl acetate	3.5	2.5	2	0	0	0	0	0.5
Butanol	0	0	0	0	0	0	0	>1
Pseudomonas aeruginosa								
Crude	7	6	5	0	0	0	0	0.25
Hexane	6	5	4	0	0	0	0	0.25
Ethyl acetate	9	8	7	3	0	0	0	0.25
Butanol	11	8	8	6	3	0	0	0.13
Candida albicans								
Crude	0	0	0	0	0	15	0	>1
Hexane	0	0	0	0	0	15	0	>1
Ethyl acetate	4.5	0	0	0	0	15	0	>1
Butanol	0	0	0	0	0	15	0	>1
Aspergillus niger								
Crude	0	0	0	0	0	0	0	>1

Hexane	0	0	0	0	0	0	0	>1
Ethyl acetate	0	0	0	0	0	0	0	>1
Butanol	5	0	0	0	0	0	0	1

Key: Positive controls: ciprofloxacin (8 µg/mL) for bacteria; miconazole (50 µg/mL) for fungi

4. Conclusion

From this study, it can be concluded that *P. manensis* leaf extract/fractions possess a broad spectrum of antimicrobial activities against *E. coli, S. aureus, P. aeruginosa*, *A. niger* and *C. albicans* at concentrations investigated, which could be linked to a wide range of phytochemicals they contain. Considering the similarities in phytochemical and antimicrobial properties of *P. manensis* leaf extract/fractions and other species of *Psydrax*, further tests are ongoing to ascertain the safety of the extract in animal models to introduce *P. manensis* leaf extract in ethnomedicine, just as other species of *Psydrax* are already in use in traditional medicine practice. In addition, the butanol and ethyl acetate fractions with antimicrobial activities against the microorganisms used in this study will be purified down to single active compounds.

Compliance with ethical standards

Disclosure of conflict of interest

There is no conflict of interest among the authors.

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