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Antimicrobial Effects of *Chrysophyllum albidum* on Bacterial Isolates from Urinary Catheters

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Abstract

Aqueous and ethanolic extracts obtained from *Chrysophyllum albidum* plant parts (leaves, roots, and seeds) were used to evaluate the potential antibacterial activity against bacteria isolated from urinary catheter tips. The following isolates were recovered: *P. aeruginosa* (7), *S. aureus* (15), *E. coli* (11), *P. mirabilis* (11), and *K. aerogenes* (6). The agar well diffusion method was used. The average percentages of antimicrobial resistance to gentamicin among the isolates were 45.5% for P. aeruginosa, 42.1% for E. coli, 46.9% for K. aerogenes, and >90% for the other isolates. The ethanolic extract mixtures (leaf, root, and seed) had the greatest effect on all the isolates, with inhibition zones (IZs) ranging from 8 to 26 mm and MICs ranging from <16 to 32 mg/mL. The Potencies of the *C. albidum* extracts based on the IZ and MIC values were greater in the extract mixtures, followed by those in the roots. Phytochemical screening revealed that all extracts contained phenols, except the seeds, while tannins were present in all extracts except the leaves. The activity of the ethanolic extracts of each part at both high and low concentrations was greater than that of the aqueous extracts at the same concentrations ($p < 0.05$). The acute toxicity results showed that the LD_{50} of the extracts was greater than 5000 mg/kg body weight, indicating no toxicity. The antibacterial activities of the extract mixtures and roots against the isolates confirmed the use of *C. albidum* in folk medicine for the treatment of catheter-associated urinary tract infections (CAUTIs), indicating its potential for use in novel antibiotic production.

INTRODUCTION

Indwelling urinary catheters are standard medical devices used to prevent involuntary urine discharge, facilitate bladder evacuation, and retain urine in the bladder during hospitalization. They are also used in clinics, nursing homes, and medical care-giving centres for the same purposes [1]. The most common urinary catheter in use is known as the Foley indwelling urethral catheter. The Foley system is closed, sterile, and consists of a tube passed through the urethra and held in place by a balloon, which is inflated immediately after insertion and deflated before removal to allow drainage of the bladder. As a result of the frequent use of urinary catheters in hospitals, the placement skills of medical personnel, and the use of indwelling catheters during hospitalization, approximately 21-50% of patients are exposed to complications associated with the insertion and removal of these devices [2]. For patients with indwelling urinary catheters, their bladder could be contaminated with microorganisms; (i) at the time the catheter is being inserted by medical personnel, (ii) due to the ascent of microorganisms via the catheter lumen from a drainage system that is already contaminated, and (iii) due to the migration of microorganisms present in the urethra around the catheter [2]. There are various definitions of CAUTI (catheter-associated urinary tract infection) within published works, and the terms 'bacteriuria' and 'urinary tract infection' (UTI) are often used without a clear difference between them [3]. Bacteriuria or funguria levels >103 colony-forming units (CFUs) strongly indicate the presence of CAUTI, These values increase to 105 CFUs between 24 and 48 hours [4]. There are cases in which other researchers consider CAUTI to be present when the prevailing organism growth is ≥ 10^2 CFU, in which case pyuria is also found [4].

Fever, urgency, dysuria, leukocytosis, and flank pain, which are signs and symptoms of CAUTI, have been shown to have low positive predictive values for predicting CAUTI, as 90% of patients do not experience any symptoms. This likely results from a urinary tract catheter constantly inhibiting bladder contractions, which is associated with bladder distension and inflammation, such as urgency and pollakiuria. An indwelling

urethral catheter likewise prevents constant urethral exposure to a large number of microorganisms in infected urine and averts urethritis; therefore, urgency and disuria occur [1]. Urinary catheters are used worldwide solely for controlling, diagnosing, repairing, and treating UTIs. The infectious rate associated with the method employed during catheterization is approximately 1–2%. This rate increases to 3–7% per catheter per day, as almost every urinary catheter user develops bacteriuria after about 30 days of catheter insertion [5].

The average daily risk of CAUTI is 5%, while some researchers have observed that among the population whose catheters are inserted permanently, a greater number of these urinary catheter users will manifest bacteriuria after using them for 5 days and that the daily risk of infection is 2.7% for patients who are chronic compared with 0.14% for those who use the catheter intermittently [6]. In a hospital setting, approximately 15–20% of patients use urinary catheters during hospitalization, while approximately 4% use permanent urinary catheters at home [7]. Approximately 16.3% of patients have been reported to have extrahospital urinary infections connected to the use of vesical catheters [5]. Additionally, short- and long-term catheterizations have a conventional point of 30 days for distinguishing them [8].

The United Kingdom Health Security Agency reported substantial changes in both the bacterial spectrum and antimicrobial resistance following research conducted in the United Kingdom between 2022 and 2023. They also observed a higher incidence of polymicrobial infections, which have important clinical implications. Therefore, while the catheter remains in situ, CAUTIs could become more difficult to treat, as it is necessary to determine the antibiotic(s) to be used to combat the bacteria present in the urethra [9].

In medical practice, the use of chronic vesicle catheters is common because of the diverse associated pathologies. Among permanent catheter users, infection risk varies, and this variability depends on the size of the population, the reason for hospital placement of the catheter, and the skill of medical personnel during catheter insertion. There are other potential problems associated with catheter use beyond urinary infection, including urethral stenosis in males, urethrorrhagia, vesicoureteral reflux, bacteremia, catheter obstruction, false pathways, and stone formation [10].

The indiscriminate use of antibiotics by either irregular or chronic urinary catheter users has made most of these bacteria resistant to one or multiple drugs in use, thus promoting the advancement of dangerous and hard-to-treat UTI,s. Patients could then face complications due to inadequate, insufficient, and inopportune medical treatment. CAUTIs, which represent more than 40% of the most common hospital-acquired infections, are a serious health concern due to their frequent recurrence and associated complications, and account for 80% of hospital-acquired UTIs [4]. These UTIs are often caused by *Pseudomonas aeruginosa, Escherichia coli, Proteus mirabilis,* and *Staphylococcus aureus* [4]. Gram-negative bacterial species, such as *E. coli*, that cause CAUTI exhibit a variety of virulence factors, including adhesins, motility, biofilm formation, immunoevasion, and nutrient acquisition, all of which are harmful to patients. Studies have shown that, despite the impending threat of infections caused by these potent, opportunistic, nosocomial, multidrug-resistant strains, the majority of bacteriuria cases linked to catheter use are asymptomatic. However, when CAUTI is associated with signs and symptoms, the consequences can include mild fever, cystitis and urethritis, life-threatening pyelonephritis, renal scarring, calculus formation, and bacteremia. These infections, if left without proper treatment, could be fatal [4].

A great number of hospital-acquired infections, particularly in patients who are critically ill, are caused by medical devices. Infections associated with medical devices can have significant medical and economic consequences. Bacterial colonization of indwelling medical devices can be a preliminary cause of infection in patients and device malfunction. The origin and development of infections associated with medical devices are centered on the multifaceted relationships among the bacteria invading, the patient, and the medical device in use. In the pathogenesis of infection, bacteria-associated factors are apparently the most important, whereas medical device factors can be modified solely to prevent infection. Some, but not all, of the bacterial receptors satisfy the proposed “adherence/infection” version of Koch’s postulate [11]. Antimicrobial coating of medical devices, a traditional surface-modifying approach for preventing medical device-associated infections, has yielded variable clinical success rates [12].

Plants referred to as ‘Medicinal’ are those that contain compounds applicable in medicine for therapeutic reasons or those that are precursors for useful drug production [13]. Many years ago, medicinal plants were employed in various disease treatments. In Africa alone, more than 5000 plants have been used as medicinal plants, but only a few have been described or studied [14]. Products extracted naturally from plants are valuable sources for biological research and could be excellent [15]. This study investigated the use of *C. albidum* in the southeastern part of Nigeria for the treatment of urinary tract infections. It is true that scientists are not relenting in their fight against these drug-resistant bacteria, but there are reasons why people, especially in rural areas, have resorted to traditional medicine as an alternative.

These reasons include: (i) the high cost of broad-spectrum antibiotics, which are not affordable for people in rural areas, (ii) the unavailability of such drugs in this locality,; (iii) the proximity of traditional doctors to them, and (iv) the low cost of these herbs used in traditional medicine. Additionally, people living in urban centres who can afford antibiotics often resort to traditional medicine because some bacteria have become resistant to these antimicrobial drugs. The incessant strike action by healthcare workers also forces urban dwellers to turn to traditional medicine. *Chrysophyllum albidum* is used in traditional medicine to treat urinary problems. Hence, the purpose of this research was to determine the efficacy of extracts from *Chrysophyllum albidum* plant parts on bacteria isolated from urinary catheters, particularly those resistant to broad-spectrum antibiotics.

MATERIALS AND METHODS

Research locations, Ethical considerations and Study design

This valuable experimental research was conducted at the Microbiology Department, University of Nigeria, Nsukka, and the Microbiology Laboratory of Enugu State University Teaching Hospital (ESUTH), Parklane, both located in Enugu State, Nigeria. This research work was carried out in compliance with the laid down ethical guidelines after receiving ethical clearance from Enugu State Ministry of Ethical Committee on Research and Ethical permission on Research Preparations from Enugu State University Teaching Hospital, then being headed by Dr. C.J.C Orjiakor; Chief Consultant Physician ESUTH, and Chairman Enugu State Ethical Committee on Research.

Sample collection

Collection of catheter tips

The catheter tips used for this work were collected from different hospitals around the Enugu state metropolis. These hospitals include the University of Nigeria Teaching Hospital (UNTH), Ituku-Ozalla, the Enugu State University Teaching Hospital (ESUTH) Parklane, Enugu, and the Annunciation Specialist Hospital, Emene, Enugu State. The lengths of stay of the catheters in the patients were ascertained orally from either the patient or the medical personnel who removed the catheter, and this information was authenticated from the patients' files. Thus, a total of 53 catheter tips were collected. Of the 53 catheter samples, 25 were from UNTH Ituku-Ozalla, 18 were from ESUTH Parklane, and 10 were from Annunciation Specialist Hospital, Emene.

The catheter tips were immediately collected after removal from the patients and kept in dry, sterile bottles. Immediately after sample collection, the samples were transported in coolers packed with ice blocks to the Microbiology Laboratory of the Research Unit, Enugu State University Teaching Hospital, Parklane, for subsequent analysis. All data collected was anonymised to protect participant privacy and confidentiality. In line with ethical best practices and data-sharing policies, anonymised study data will be made publicly available via the WHO ESPEN portal upon publication, thereby contributing to global health data repositories and facilitating further research.

The population studied consisted of adults who underwent catheterization during the course of this research. The basic skills and methods employed by the medical personnel involved in catheter insertion and removal were noted, and the hygiene measures practiced were equally assessed. The acceptable compliance rate was 60%. The preceding stage of the research was conducted in the Microbiology Laboratory of Enugu State University Teaching Hospital and the Microbiology Department Laboratory, University of Nigeria, Nsukka. Both medical and surgical departments of the hospital were included in the study. Additionally, patients who provided verbal consent or were in a coma and for whom caregivers provided consent were included. Immediately after sample collection, the samples were taken to the laboratory for culture for no more than one hour.

Collection of medicinal plant parts

The medicinal plant used for this study was *Chrysophyllum albidum* (roots, leaves, and seed cotyledons). This plant was chosen because it is widely used in ethnomedicine in the southeastern and western regions of Nigeria for the treatment of urinary tract infections. The plant parts were collected from Obiofu Nenwe in Aninri L.G.A. and Obollo-afor in Udenu L.G.A., both of which are located in Enugu State. The plant parts were identified and authenticated at the Botany Department, University of Nigeria, Nsukka.

Cultivation of microorganisms on the catheter tips

All samples used in this study were collected and cultured according to the CLSI guidelines for the collection and cultivation of microbiological samples [16]. In the laboratory, the cut part of the catheter was held, and a tiny smear was made with the tip on an already prepared Cystein Lactose Electrolyte Deficient (CLED) agar medium. A sterile wire loop was used to spread the smear evenly across the entire surface of the CLED agar using the streak-plating method. The cultured plates were then incubated for 24 hrs at 37 ºC.

Purification and preparation of stock cultures

Purification

The morphological characteristics of the cultivated organisms on each CLED agar plate were thoroughly checked, and distinct colonies were subcultured onto nutrient agar and blood agar plates to obtain pure cultures and determine whether the isolate was haemolytic. The samples were labeled and incubated for 24 hours at 37 °C. This process further helped in the morphological identification of the organisms.

Preparation of stock cultures

Nutrient agar slants were prepared using Bijou bottles. These bottles were washed and sterilized before use. The purified organisms were cultured on nutrient agar slants, labeled, and incubated for 24 hours at 37 °C. After incubation, the agar slants were stored in a refrigerator at 4 ºC. However, before use, each agar slant culture was subcultured to further purify the organism and to obtain a new, young, and active culture.

Extraction of plant parts

The quantities (1 kilogram each) of plant leaves, roots, and seed cotyledons were examined with the naked eye for the presence of undesirable materials or contaminants, such as molds and insects. After the examination, each plant part was washed in distilled water, air-dried at room temperature (28–32°C), and left for 14 days in a well-ventilated room. The direct effect of sunlight was completely avoided during the drying days to prevent the drying of the extractable substances in the plant parts. The extraction and maceration techniques described by Adebayo *et al* were used [15]. The air-dried plant parts —leaf, roots, and seed cotyledons — were crushed into a coarse powder. The plant parts used in this project (leaves, roots, and seed cotyledons) were prepared in the form of herbal extracts.

Aqueous extraction

A total of 250 g each of dried leaves, roots, and seed cotyledons (coarse powder) was cold-macerated by soaking in 2000 mL of distilled water in a conical flask for 12 hours, and each sample was labeled accordingly. The aqueous extracts of each plant part were first filtered through a clean muslin cloth before being further filtered with Whatman No. 2 (110 mm) paper. The extracts were concentrated using a forced air current (rotary table fan), and the paste formed (residual extracts) from each sample was placed in a clean airtight container. Additionally, the known weight was determined, and the samples were stored in a refrigerator at 4 ºC.

Ethanolic Extraction

A total of 250 g each of dried leaves, roots, and seed cotyledons (coarse powder) was cold-macerated by soaking in 2000 mL of 80% ethanol in a conical flask for 24 hours, and each sample was labeled accordingly. The ethanolic extracts of each plant part were first filtered with a clean muslin cloth before further filtering with Whatman No. 2 (110 mm). The extracts were concentrated using a forced-air current (rotary table fan), and the paste formed (residual extracts) from each sample was placed in a clean, airtight container with known and labelled weights before storage in a refrigerator at 4 ºC.

Phytochemical analysis

The procedures described by Sofowara [17] and Harborne [18] were used to investigate the phytochemical constituents of the plant parts (leaves, roots, and seed cotyledons). Alkaloids, saponins, tannins, terpenoids, flavonoids, and cardiac glycosides were determined. Briefly, for the saponins, 2 g of each dried powdered plant part was placed in 20 mL of distilled water in a

test tube, boiled in a water bath for 2 minutes, and filtered immediately. Cooled filtrates were subjected to both the Frothing and Emulsion tests. For the Frothing test, 10 mL of each filtrate was placed in a test tube and shaken vigorously to produce persistent froth, which confirmed the presence of saponins. For the emulsion test, three drops of olive oil were added to 1 mL of each plant part filtrate, and the mixture was shaken vigorously.

The formation of emulsions confirmed the presence of saponins. For alkaloids, an oven heated to 55°C was used to evaporate 15 mL of the extracts to dryness, and the residue was dissolved in 10 mL of hydrochloric acid (10% v/v). To precipitate the alkaloids, 10 mL of a 10% (v/v) ammonia solution was added, and the mixture was then extracted with 15 mL of ether. The extracted ether was evaporated to dryness, and hydrochloric acid (1.5 mL) was added. A total of 0.5 mL of the solution (ether and hydrochloric acid) was measured, and 2–3 drops of Mayer's reagent were added, which led to the formation of an opaque precipitate. This confirms the presence of alkaloids.

To detect flavonoids, 0.2 g of each dried plant part was placed in test tubes containing 10 mL of ethyl acetate, boiled in a water bath for 3 minutes, filtered, and cooled. The filtrates were subjected to the following steps: (a) Ammonium test – 4 mL of each filtrate was shaken with 1 mL of 1% dil. Ammonia. The formed layer was allowed to separate, and a red colouration in the ammonia layer was observed, indicating the presence of flavonoids. (b) Aluminum chloride test – 4 mL of each filtrate was shaken with 1 mL of Aluminum chloride solution and observed for a yellow color in the aluminum chloride layer, which confirmed the presence of flavonoids. For the analysis of cardiac glycosides, 10 mL of 50% H_2SO_4 (tetraoxosulphate(vi) acid) was added to 2 g of each plant extract (paste), which were then placed in a boiling water bath for 5 mins. Five millilitres each of Fehling solutions A and B were added to each solution until the pH was alkaline, as indicated by litmus paper. The alkaline solutions were boiled in a water bath for 2 minutes and observed for the formation of brick-red precipitate, indicating the presence of glycosides. For the identification of terpenoids, 2 g of each plant part extract (paste) was mixed with chloroform (2 mL), and conc H_2SO_4 (3 mL) was added cautiously until a layer formed. A reddish-brown colour was observed at the interface, indicating the presence of terpenoids.

Quantitative determination of the phytochemicals

Two grams of each plant part were defatted with diethyl ether (100 mL) for 2 hours using a Soxhlet apparatus before use. For total alkaloids, 5 g of each plant part (defatted) was placed in beakers containing 200 mL of 10% acetic acid in ethanol each. These mixtures were allowed to stay for 4 hours while it was covered. After this time, the mixtures were filtered, and the extracts were concentrated in a water bath to one-quarter of their original volume. To the extracts, Conc. NH_4OH (Ammonium hydroxide) was added drop by drop until the precipitation stopped. The precipitates were allowed to settle and were collected by filtration, washed with dilute NH4OH, and filtered again. The alkaloid residues were dried and then weighed. For total phenols, 5 g of each plant part (defatted) was boiled for 15 minutes in 50 mL of ether to extract the phenols. 5 mL of the extracts were pipetted into 50 mL flasks, and 10 mL of distilled water was added to each flask. NH_4OH solution (2 mL) and concentrated amyl alcohol (5 mL) were also added to the flasks. The flasks were brought up to the 50 mL mark and allowed to react for 30 minutes due to the formation of an orange color. After the formation of the orange colour, each sample was measured at 505 nm using a spectrophotometer.

For total tannins, 500 milligrams (mg) of each plant part were weighed into plastic bottles containing 50 mL of distilled water, and a mechanical shaker was used to shake them for 1 hour. Filtration of each sample was performed, and each was placed into a separate 50 mL volumetric flask, with the volume adjusted accordingly. Then, 5 mL of the filtrate was pipetted into test tubes containing 2 mL of 0.1 M FeCl3 in 0.1% HCl and 0.008 M potassium ferrocyanide. After 10 minutes, the absorbance was measured and recorded at 720 nm.

For total saponins, three conical flasks, each containing 100 cm^3 of 20% aqueous C_2H_5OH (ethanol), were measured, and 20 g of each of the plant parts was added. The samples were boiled in a water bath at 55 °C and the water was stirred continuously for 4 hours. The residues of the mixtures were obtained by filtration and further extracted with another 200 mL of 20% C_2H_5OH. The combined plant parts extracts were reduced to 40 mL in a water bath at 90 °C. The mixtures were concentrated and transferred into a 250 mL separating funnel containing 20 mL of diethyl ether and vigorously shaken. The ether layers were discarded, leaving behind the aqueous layer. Then, 60 mL of n-butanol was added to the aqueous layer, and the mixed extracts were washed twice with 10 mL of a 5% aqueous NaCl solution.

The solution was heated to dryness in a water bath. The samples were oven-dried to a constant weight, and the saponin content was recorded as a percentage. For flavonoids, 10 g of each plant part was repeatedly extracted with 100 mL of 80% aqueous CH_3OH (methanol) at room temperature. The solutions were filtered using Whatman filter paper No. 42 (125 mm). The filtrates were transferred to a crucible, evaporated to dryness over a water bath, weighed to constant weight, and then recorded.

Bacterial Isolate Identification

The identification of the isolated bacteria was carried out based on their individual morphology, Gram staining results (positive or negative), and biochemical tests, as described by Ryan and Ray [19]. The isolated bacteria were characterized phenotypically based on their individual morphology, Gram staining results, and biochemical reactions. An elaborate characterization of the isolates is shown below:

1. *Proteus mirabilis*: These bacteria were gram-negative, motile (swarming), and urease-positive.
2. *Pseudomonas aeruginosa*: The bacterial isolates presented as gram-negative rods in single colonies, produced the green pigment - pyocyanin, and had positive oxidase test results.
3. *Staphylococcus aureus*: These isolates were gram-positive cocci in clusters and reacted positively to both the coagulase and catalase tests.
4. *Escherichia coli*: The bacterial isolates presented as Gram-negative rods in single colonies, were motile, had positive results in the indole test, and were lactose fermenting.
5. *K. aerogenes*: These isolates were gram-negative, rod-shaped, oxidase- and indole-negative, catalase and citrate-positive, and capable of motility.
6. Yeast cells: The cells were characterized on the premise of their structure through KOH wet mount microscopy.

Antibiotic susceptibility

The Kirby-Bauer method was used for bacterial colony identification after bacterial colony purification, and bacterial susceptibility patterns were ascertained for different antibiotics [20,21]. The antibiotics used were amoxicillin, ampicillin, ciprofloxacin, tetracycline, doxycycline, nalidixic acid, cefoxitin, gentamicin, ofloxacin, novobiocin, gramicidin, and dimethromycin. The above antibiotics were selected because they are commonly found on the Ogbete main market in Enugu, Nigeria. The following is a brief description of the antibiotic

susceptibility testing methods used. The test organism was emulsified in 3-4 mL of sterile normal saline until the turbidity reached a level similar to 0.5% McFarland's standard, which is equivalent to 1.5 × 10^8 colony-forming units (CFU), prepared according to the method described by Monica [22].

A sterile wire loop was used to transfer a loopful of the suspension onto nutrient agar plates, and a sterile glass spreader was then used to spread the entire plate surface evenly. The antibiotic discs were placed on the surfaces of the nutrient agar plates using sterile forceps and incubated at 37 ˚C for 18–24 hrs. The zones with no visible growth around the antibiotic discs, referred to as the 'inhibition zone diameter' (IZD), were measured to the nearest millimeter and classified as either resistant or sensitive according to the breakpoint system of the Clinical and Laboratory Standards Institute (CLSI) [16]. During this process, any isolate found to be resistant to three or more of the tested antibiotics was classified as multidrug-resistant (MDR). Therefore, this particular MDR bacterium was preserved for use, and plant extracts were tested to determine their efficacy.

Evaluation of the antimicrobial activity of the plant parts extracts by the agar well diffusion method

The screening for antibacterial activity of the plant part extracts (leaves, roots, seed cotyledons, and their mixture – equal weight of each plant part extract) on the MDR isolates from the catheter tips (*S. aureus, E. coli, K. aerogenes, P. mirabilis,* and *P.aeruginosa*) was based on the agar well diffusion method by CLSI [16]. A sterile wire loop was used to pick at least two to three isolated colonies of similar morphology from an agar plate of pure cultures incubated for 18– 24 hrs and then inoculated into 3-4 mL of normal saline. I adjusted the inoculum to 0.5 McFarland turbidity standards, which resulted in a suspension of 1.5×10^8 CFU to check for density or turbidity. Nutrient agar plates were inoculated with the MDR bacteria and left to dry for about 5 minutes. Then, five wells were dug to a depth of 4 mm each in the agar using a sterile 1 mm-diameter cork borer and labelled accordingly.

The microdilution method, as described by the National Committee for Clinical Laboratory Standards (NCCLS) [24], was used for standardizing each plant part extract and its mixture (aqueous and ethanolic). The serial dilutions were twofold, i.e 2^{-1}, 4^{-1}, 16^{-1}, and $256^{-1,}$ etc. Serial dilutions were performed to obtain different concentrations of each plant part extract. The concentrations obtained from each plant part extract (aqueous and ethanolic) in the above serial dilutions were approximately 250 mg/mL, 125 mg/mL, 62 mg/mL, 32 mg/mL, and 16 mg/mL, respectively. Finally, 0.5 mL from each plant part concentration was pipetted into each of the five wells, with the volumes tallied according to the labelling. This was done for each plant part extract (aqueous and ethanolic). To allow the extracts to be absorbed into the medium, the plates were placed in a refrigerator and allowed to stand for up to 1 hour. Then, the plates were incubated at 37°C for 24 hours, and the inhibition zones were measured in millimeters and recorded.

Acute toxicity studies (lethal dose LD_{50})

All nine male albino Wistar strain rats were purchased from the Nigerian Institute of Medical Research (NIMR) Yaba- Lagos for the study. Their weights ranged from 100 to 160 g, and the rats were preserved under laboratory conditions, with humidity, a temperature between 23 °C and 25 °C, and a light cycle of at least 12 hours (light/dark cycle). The animals were handled as specified by the National Institute of Health (NIH) as a guideline for the upkeep and use of laboratory animals for research purposes [23]. The animals were acclimatized for four weeks before use.

The acute toxicity test was performed using the probit method [25]. The laboratory animals (rats) were divided into two groups (one for ethanolic extracts and the other for aqueous extracts), with the remaining group serving as a control. The crude extract of each *Chrysophyllum albidum* plant part (leaves, roots, and seed cotyledons and plant parts mixture) was placed in normal saline (0.9% (w/v) NaCl) to dissolve before oral administration to the test animals. In summary, the first clinical trial involved administering 500 mg/kg and 1000 mg/kg body weight ethanolic extracts of each plant part and the mixture to the labelled rats and to the control animals, with normal saline given. Locomotor activities, such as piloerection (goose bumps), movement suppression, spontaneous movement inhibition, emaciation, and anorexia, were observed from the time of oral administration of the extracts until three days after administration. They were also weighed daily for the three-day period. The second clinical trial was performed 2 weeks after the first, but 3000 mg/kg and 5000 mg/kg were administered. The above procedures and subsequent observations were also carried out.

RESULTS

A total of 58 bacterial isolates were recovered from 53 catheterized patients. The most frequently isolated organism was *Staphylococcus aureus* (25.7%), followed by *Escherichia coli* (19.0%) and *Proteus mirabilis* (19.0%). The results indicated that catheterization beyond one month yielded more mixed infections, which is particularly prominent in patients with old age, incontinence, and diabetes. Notably, yeast cells were isolated in 13.8% of cases, primarily from patients with diabetes and those with prolonged catheter use (**Table 1**).

The prevalence of multidrug-resistant (MDR) isolates recovered from urinary catheter tips is presented in **Table 2.** Of the 15 Staphylococcus aureus isolates, 6 (43%) were MDR, while the remaining 9 (57%) were non-MDR. *Escherichia coli* showed a lower MDR prevalence, with only 3 of 11 isolates (23%) resistant, compared to 8 (77%) susceptible. In contrast, *Pseudomonas aeruginosa* demonstrated a relatively high rate of resistance, with 4 out of 7 isolates (63%) classified as MDR, leaving 3 isolates (37%) as non-MDR. *Proteus mirabilis* displayed moderate resistance, where 4 of 11 isolates (39%) were MDR and 7 (61%) were non-MDR. The highest MDR prevalence was observed in *Klebsiella aerogenes*, with 4 of 6 isolates (67%) resistant, while only 2 (17%) remained non-MDR (**Table 2**).

Table 2. Prevalence of multidrug-resistant (MDR) bacterial isolates recovered from urinary catheter tips.

Isolate	Total number	Number of Multiple Drug Resistant Isolates (MDR) (%)	
		MDR (%)	Non-MDR (%)
S. aureus	15	6 (43)	9 (57)
E. coli	11	3 (23)	8 (77)
P. aeruginosa	7	4 (63)	3 (37)
P. mirabilis	11	4 (39)	7 (61)
K. aerogenes	6	4 (67)	2 (17)

MDR: Multiple Drug Resistant

Table 1. The distribution of bacterial isolates on catheter tips according to the length of catheterization.

Length of catheterization	Reason for catheterization & Number of patients involved	Bacterial Isolates (Number isolated)						
		S. aureus	*E. coli*	*P. aeruginosa*	*P. mirabilis*	*K. aerogenes*	Yeast cell	Total (n)
< one month	Gonorrhea (n=1)	0	0	0	0	0	0	0
	Surgery (n=5)	0	0	0	1	0	0	1
	Comatose (n=1)	0	1	0	0	0	0	1
One month	Unknown (n=7)	1	0	3	0	3	0	7
	Hepatitis (n=2)	0	0	1	0	1	0	2
	Accident (n=4)	3	0	0	1	0	0	4
	Paralysis (n=2)	2	1	0	0	0	0	3
	Old age (n=6)	1	3	0	0	2	0	6
	Incontinence (n=5)	1	0	2	1	0	2	6
	Urecterectomy (n=1)	0	0	0	1	0	0	1
	Diabetes (n=3)	2	2	0	0	0	1	5
	Dwarfism (n=2)	1	0	0	0	0	1	2
>One month	Unknown (n=1)	1	0	1	1	0	0	3
	Old age (n=6)	1	1	0	3	0	1	6
	Incontinence (n=2)	1	2	0	2	0	1	6
	Diabetes (n=3)	0	1	0	0	0	1	2
	Dwarfism (n=2)	1	0	0	1	0	1	3
Total (%)	53	15 (25.7%)	11 (19.0%)	7 (12.1%)	11 (19.0%)	6 (10.3%)	8 (13.8%)	58

Table 3. Antimicrobial susceptibility pattern.

Bacterial Isolate			Antimicrobial Agents/Number of Isolates (%)													
Gram Negative	N	S/R	AMP	TET	SXT	GEN	CXM	CTX	AMK	CIP	LEV	CAZ	MEM	TZP	AMC	FOX
E. coli	11	S	9(82)	10(91)	11(100)	7(64)	10(91)	9(82)	4(36)	8(73)	10(91)	9(82)	10(91)	4(36)	8(73)	10(91)
		R	2(28)	1(9)	0(0)	4(36)	1(9)	2(28)	7(64)	3(27)	1(9)	2(28)	1(9)	7(64)	3(27)	1(9)
P. aeruginosa	7	S	-	-	-	3(37)	-	-	2(25)	4(50)	6(75)	1(12)	6(75)	1(12)	-	6(75)
		R	-	-	-	5(63)	-	-	6(75)	4(50)	2(25)	7(88)	2(25)	7(88)	-	2(25)
Klebsiella sp.	6	S	-	6(100)	3(50)	1(17)	0(0)	0(100)	4(67)	3(50)	_	1(17)	6(100)	1(17)	0(0)	3(50)
		R	-	0(0)	3(50)	5(83)	6(100)	6(0)	2(33)	3(50)	_	5(83)	0(0)	5(83)	6(100)	3(50)
P. mirabilis	11	S	-	0(0)	6(55)	6(55)	-	7(64)	5(45)	9(82)	10(91)	8(73)	11(100)	5(45)	8(73)	5(45)
		R	-	11(100)	5(45)	5(45)	-	4(36)	6(55)	2(28)	1(9)	3(27)	0(0)	6(55)	3(27)	6(55)
			AMP	AMC	CC	CXM	FEP	GEN	LEV	SXT	TEC	TET	TZP	VA		
S. aureus	15	S	14(93)	5(33)	7(47)	7(47)	6(40)	5(40)	10(67)	8(53)	6(40)	13(7)	4(27)	12(80)		
		R	1(7)	10(67)	8(53)	8(53)	9(60)	9(60)	5(33)	7(47)	9(60)	2(13)	11(73)	2(20)		

S = Sensitive; R = Resistant; - = antibiotic not tested; AMK = Amikacin; AMP = Ampicillin; AMC = Amoxicillin/Clavulanic acid; CXM = Cefuroxime; CTX = Cefotaxime; CIP = Ciprofloxacin; CAZ = Ceftazidime; CL = Colistin; CC = Clindamycin ; ERY = Erythromycin, FEP = Cefepime; FOX = Cefoxitin, Gen = Gentamycin; LEV = Levofloxacin; MEM = Meropenem; Tet = Tetracycline; SXT = Trimethroprim/Sulfamethoxazole; TZP = Piperacillin/Tazobactam; TEC = Teicoplanin and VA = Vancomycin.

Table 4. Antimicrobial activity of *Chrysophyllum albidum* (aqueous and ethanolic) plant parts on MDR test bacteria with average minimum inhibitory concentrations.

MDR Test Bacteria	Plant part Av. Zn. Inh. (mm)	Aqueous Extract (mg/mL)					Ethanolic extract (mg/mL)					Av. MIC Aq. Ext. (mg/mL)	Av. MIC Eth. Ext. (mg/mL)
		250	125	63	32	16	250	125	63	32	16		
P. aeruginosa	Leaf	14	10	7	-	-	16	12	9	5	-	63	32
	Root	19	15	11	5	-	21	16	11	7	-	32	32
	Cotyledon	11	8	-	-	-	11	8	4	-	-	125	63
	Extracts mixture	23	20	16	13	7	26	22	18	11	9	16	16
P. mirabilis	Leaf	11	8	4	-	-	13	9	7	4	-	63	32
	Root	17	14	7	-	-	17	14	10	6	-	63	32
	Cotyledon	11	7	-	-	-	12	10	7	-	-	125	125
	Extracts mixture	19	16	11	7	-	23	19	14	11	8	32	16
S. aureus	Leaf	17	13	8	-	-	18	14	9	6	-	63	32
	Root	21	18	14	8	-	22	18	11	7	-	32	32
	Cotyledon	10	7	-	-	-	11	8	-	-	-	125	125
	Extracts mixture	22	18	15	8	-	20	16	13	8	-	32	32
E. coli	Leaf	11	8	4	-	-	12	9	5	-	-	63	63
	Root	15	11	6	-	-	17	14	10	6	-	63	32
	Cotyledon	10	7	-	-	-	10	6	-	-	-	125	125
	Extracts mixture	20	17	15	10	-	22	19	16	9	-	32	32
K. aerogenes	Leaf	10	7	4	-	-	12	8	6	-	-	63	63
	Root	14	9	4	-	-	15	9	5	-	-	63	63
	Cotyledon	12	8	-	-	-	9	4	-	-	-	125	125
	Extracts mixture	15	10	7	-	-	19	16	14	11	9	63	16

Antimicrobial susceptibility

As shown in **Table 3**, among the antimicrobial susceptibility patterns of the bacteria isolated, *Staphylococcus aureus* – a gram-positive bacterium most isolate susceptible to standard antibiotics such as ampicillin (93% susceptibility), tetracycline (87% susceptibility), and vancomycin (80% susceptibility), but was highly resistant to amoxicillin/clavulanic acid (63% resistance), followed by cefepime and gentamycin (60% resistance). Among the Gram-negative isolates, E. coli had the highest resistance to Amikacin (64%), but was highly susceptible to trimethoprim/sulfamethoxazole (100%). It also showed susceptibility to ampicillin, tetracycline, cefuroxime, cefotaxime, levofloxacin, ceftazidime, meropenem, and cefoxitin, with 80% or greater susceptibility.

P. aeruginosa's resistance to the antibiotics tested was 88% to both ceftazidime and piperacillin/tazobactam. It was also susceptible to both levofloxacin and meropenem (75% susceptible to both). *Klebsiella* spp., on the other hand, was 100% susceptible to three antibiotics used in the study—tetracycline, cefotaxime, and meropenem. It also showed 100% resistance to both cefuroxime and amoxicillin/clavulanic acid. Finally, *P. mirabilis* was 100% resistant to tetracycline and 100% susceptible to meropenem.

Toxicity of Laboratory Animals to *Chrysophyllum albidum* Plants

No deaths occurred during the period of the administration of Wistar rats with any extract or the mixture of extracts of *Chrysophyllum albidum* parts. There was little change in locomotor activity, but this effect was observed only at the high concentration of 3000 mg/kg body weight. No visible or measurable changes in body weight were observed. The acute toxicity results showed that the LD_{50}values of the plant extracts and their mixture were greater than 5000 mg/kg body weight. The antimicrobial efficacy of *Chrysophyllum albidum* plant extracts, both aqueous and ethanolic, was assessed against five multidrug-resistant bacterial isolates: *Pseudomonas aeruginosa, Proteus mirabilis, Staphylococcus aureus, Escherichia coli,* and *Klebsiella aerogenes* (**Table 4**).

The ethanolic extracts exhibited greater antibacterial potency, as evidenced by larger average zones of inhibition and lower minimum inhibitory concentrations (MICs) in comparison to the aqueous extracts. Among all plant parts tested, the mixture of extracts (equal portions of leaf, root, and seed cotyledon) exhibited the strongest activity across all organisms. The results show that the maximum average inhibition zone of 26 mm was observed against the pathogen *P. aeruginosa* when the ethanolic extract mixture was applied. This corresponds to a minimum inhibitory concentration of 16 mg/mL. In addition, *S. aureus* and *P. mirabilis* exhibited significant susceptibility to the ethanolic mixture with inhibition zones of 20 mm and 23 mm, respectively. This corresponds with minimum inhibitory concentration (MICs) values as low as 32 mg/mL. Of all the extracts tested, the cotyledon extract showed the lowest efficacy in both solvents, particularly against the bacteria E. coli and K. aerogenes, which showed reduced inhibition and elevated MIC values to 125 mg/mL. The root extracts demonstrated greater efficacy than the leaf or cotyledon extracts alone.

DISCUSSIONS

Nigeria has forests and uncultivated farmlands that host various plant species with medicinal properties, as do many other countries in West Africa [14]. However, few studies have examined the use of these plants to prevent CAUTI. The antimicrobial activity of extracts of *Chrysophyllum albidum* parts (leaves, roots, and seed cotyledons) potently inhibited bacterium-induced CAUTI. The root extracts successfully inhibited the MDR bacterial isolates, and the leaves also inhibited the organisms at high concentrations. Seed cotyledons were inhibited only by high concentrations of the extracts. Moreover, extracts of mixed *Chrysophyllum albidum* plant parts (leaves, roots, cotyledons) also showed the greatest inhibitory effects on these MDR bacterial isolates.

The MICs showed that the plant parts extract mixture had the greatest activity against all the MDR urinary catheter bacterial isolates, followed by the root extract, while the seed cotyledon extract had the least activity. The high quantity of phenolic compounds in the *Chrysophyllum albidum* root extract (6.0 mg/100 g) may be responsible for its superior antibacterial activity compared to other plant parts. The acute toxicity results showed that the LD_{50} values of the extracts were greater than 5000 mg/kg body weight, indicating no toxicity. The inhibitory effects of plant extracts are mainly attributed to the phytochemicals present in the plants [26]. The phytochemical analysis of the extracts indicated the presence of typical plant constituents, including alkanoids, saponins, tannins, and phenolic compounds.

The presence of phenols in *C. albidum* could be the reason why it has antibacterial, antiseptic, and therapeutic properties. These findings align with those of Adewusi [27], who reported that *Chrysophyllum albidum* exudates or latex exhibit fungal properties against *Candida albicans* and antibacterial properties against *Streptococcus pyogenes*, *Staphylococcus aureus, Klebsiella pneumoniae,* and *Escherichia coli.* The inhibitory effect of the plant parts on these bacteria further clarifies why folk or traditional medicine practitioners use leaf, root, and cotyledon extracts of this plant as anticancer agents, urinary tract infection agents, and wound-healing agents. The phytochemical mechanism of action for inhibiting microorganisms could involve impairing a variety of enzyme systems, inhibiting those that produce energy, and interfering with the integrity of the cell membrane and the synthesis of structural components [28].

The phenolic content ranged from 2.03 mg/100 g in the plant leaves to 6.40 mg/100 g in the roots and 2.78 mg/100 g in the cotyledons. Phenolic compounds are considered to be bacteriostatic and fungistatic [29]. These compounds cause swelling of hyphal tips, plasma seeping around hyphae, leakage of plasma, cell wall distortion, abnormal branching or fusion of hyphae, and, consequently, wrinkling of hyphal surfaces [26]. The plant *Chrysophyllum albidum* is widely used in herbal medicine in southeastern and western Nigeria to treat injuries such as bruises, sprains, and wounds, as well as urinary tract infections.

CONCLUSION

The results of this study clearly demonstrate that extracts from the leaves, roots, and seeds of *Chrysophyllum albidum* possess qualitative properties suitable for use as antibacterial compounds, and further provide a rationale for the use of these plant parts in traditional or folk medicine practices in Nigeria for the treatment of UTIs, especially CAUTI. The plant parts studied in this research have also been proven to be a potential source of useful drugs.

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