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Antimicrobial activity and phytochemical components of ethanol stem bark extract of *Phellodendron amurense* on clinical isolates

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Abstract
The spread of drug-resistant pathogens is one of the most serious threats to successfully treating microbial diseases. Evaluating plant extracts and plant-derived products for antimicrobial potential has demonstrated that plants could serve as a promising source of new antibiotic prototypes. The current research was conducted to assess the antimicrobial efficacy of *Phellodendron amurense* stem bark extract on clinical isolates of *Escherichia coli*, *Pseudomonas aeruginosa*, *Candida albicans*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Bacillus* sp. Using a two-fold serial dilution, concentrations of 100, 50, 25, 12.5, and 6.25 mg/mL of the solution were generated and used for the antimicrobial assay. The antimicrobial properties of the crude extract of *Phellodendron amurense* stem bark were assessed by employing the agar well diffusion principles. From the result, the extracts effectively inhibited the growth of *S. aureus*, *E. coli*, and *P. aeruginosa*, but did not have any effect on the remaining organisms. At 100 mg/ml concentration, the extract of *P. amurense* exhibited the highest activity against *P. aeruginosa*, followed by *E. coli* and *S. aureus*, with an inhibitory zone diameter of 8.67 mm, 8 mm, and 5.67 mm, respectively with no activity observed on the other organisms at the various concentrations tested. Phytochemical analysis of the stem extract of *Phellodendron amurense* revealed the presence of alkaloids, tannins, saponins, and triterpenoids but no flavonoids. The result from this research has shown that different bacteria exhibited varying degrees of sensitivity and resistance to the extract of *Phellodendron amurense*.

Keywords: Antibacterial, *Phellodendron amurense,* drug-resistant, isolates, antimicrobial

Introduction
Background of study
Growing evidence highlights the effectiveness of medicinal plants for the cure of infectious diseases, emphasizing their potential as sources of innovative antimicrobial medicines [1]. Phytochemicals, in particular, demonstrate strong antimicrobial qualities against various human pathogens. These natural compounds offer advantages such as affordability, availability, and fewer side effects. Furthermore, certain plant-based compounds can boost the efficacy of conventional antimicrobial agents, presenting a promising approach to addressing antimicrobial resistance [2]. The spread of drug-resistant pathogens is widely recognized as a critical public health challenge, with increasing interest in plant-based antimicrobials due to the natural compounds they offer. Researchers focus on plant parts like flowers, seeds, leaves, bark, stems, fruits, and roots as promising sources of natural products that can combat resistant infections [3]. Interest in plant-based antimicrobials has grown significantly due to rising antibiotic resistance, mainly attributed to the misuse of conventional antibiotics and incomplete treatment courses. This trend has led researchers to explore the potential of plant-derived compounds as alternatives, as these natural sources often contain bioactive compounds capable of acting against multidrug-resistant pathogens. This approach has gained momentum in recent years, driven by the need for effective alternatives in the face of diminishing antibiotic efficacy. The therapeutic potential of medicinal plants is supported by their diverse active compounds, such as alkaloids, terpenoids, and phenolics, which can act on bacterial pathogens via various mechanisms like disrupting cell membranes, inhibiting biofilms, and interfering with bacterial signaling

pathways [4,5,6,3]. Selecting the right antibiotic based on the infecting organism is crucial to prevent the development of resistance, as infectious agents can evolve and become resistant to standard antimicrobial treatments [7]. The high cost of conventional medications, especially in resource-limited communities, has driven a greater reliance on plants as alternative treatments for infectious diseases. Plant extracts containing phytochemicals with strong antimicrobial properties hold significant value in combating these diseases. Studies screening plant extracts and products for antimicrobial effects have indicated that plants could serve as a promising source of new antibiotic prototypes [8]. The main advantage of plant-based medicines is their affordability compared to synthetic alternatives, providing significant therapeutic benefits at a lower cost. Many plant-based materials used in traditional medicine are easily accessible in rural areas, making traditional healthcare systems more economical than modern medical treatments.

Respiratory tract infections (RTIs) remain the most common and significant cause of short-term diseases that prompt individuals to seek medical care, affecting populations in both developing and developed countries. Often, these infections are the first to occur after birth. RTIs result from the invasion of the respiratory system by infectious microorganisms, including bacteria and viruses. Bacterial pathogens, in particular, play a substantial role in causing illness and mortality associated with these infections. Common bacterial culprits include *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Klebsiella pneumoniae*, which often colonize the respiratory tracts of young children and the elderly as commensals. However, these bacteria can frequently cause disease under favorable conditions. Both the upper and lower respiratory tracts are impacted by respiratory tract infections, which can result in severe conditions such asthma, bronchitis, pneumonia, whooping cough, and tuberculosis, among others [9].

Phellodendron amurense commonly known as the Amur cork tree belongs to the family Rutaceae and is native to East Asia, including northern China, Japan, and Korea. Various parts of the plant like the root, fruits, leaves, and especially the bark have been found to possess many pharmacological properties as it is used in the treatment of various disorders [10]. It has also been observed to possess both antimicrobial and antibiotic properties due to the presence of alkaloids in the plant. The active constituents of this plant herb are Alkaloids (Berberine, candicin, palmatine, jatrorrhizine), saponins, coumarins and flavonoids. It has traditionally been used as a remedy for treating meningitis, pneumonia, tuberculosis, and dysentery. It also has a strong antibacterial effect and has been used internally to treat diarrhea, dysentery, vaginal infections, acute urinary tract infections, jaundice, and skin diseases [11].

Materials and Method

Collection and identification of plant

Fresh leaves along with the stem of *Phellodendron amurense* was collected from Akai Udo forest in Abak, Abak Local Government Area of Akwa Ibom State, Nigeria. The identification and verification of the plant was carried out by a taxonomist, Prof. Margaret Bassey, from the Department of Botany and Ecological Studies at the University of Uyo, Nigeria. A voucher specimen (UUPH46F) has been stored in the University of Uyo Faculty of Pharmacy Herbarium.

Preparation of extract

After being carefully washed with tap water to get rid of any contaminants, the freshly harvested stem bark of *Phellodendron amurense* was chopped into tiny pieces and allowed to air dry for ten days at room temperature in the lab. Once dried, the stem was crushed into smaller fragments using a mortar and pestle and later ground into a fine, uniform powder with a Kenwood electric blender. For seventy-two hours, the powdered material was macerated in 50% ethanol. The resulting mixture was filtered using a Buchner funnel and Whatman No. 1 filter paper while the liquid filtrate was evaporated until it was completely dry with the help of a water bath set at 40°C. The resulting crude-dried extract was then kept at -4°C in a refrigerator until further use.

Phytochemical screening of the extract

Phytochemical screening was conducted to determine the presence of various phytoconstituents such as tannins, and flavonoids following a standard method by Talukdar & Chaudhary [12], saponins described by Alamzed *et al* [13], alkaloids using Meyer's test [12], and Triterpenoids using a standard method by [14].

Antimicrobial Activity

Stock solution

Using an electronic balance, two grams (2g) of ethanol extract were weighed and then dissolved in 10 mL of dimethyl sulfoxide (DMSO) in beakers with adequate stirring to create a stock solution with a 200 mg/mL concentration. Subsequently, the following concentrations of solutions were generated: 100, 50, 25, 12.5, and 6.25 mg/mL, through a two-fold serial dilution. Five milliliters (5 mL) of DMSO solvent were added to each test tube, which was then labeled A through F and placed on a test tube rack. Using the two-fold serial dilution procedure, the extracts were diluted.

Test Microorganisms

Madonna Teaching Hospital in Elele, River State, Nigeria, supplied various clinical isolates, including *Escherichia coli*, *Staphylococcus aureus*, *Bacillus* sp., *Klebsiella* sp., *Pseudomonas aeruginosa*, and *Candida* sp. This clinical isolate's identity was verified at the Pharmaceutical Microbiology and Biotechnology Laboratory, Faculty of Pharmacy, Madonna University, following the method outlined by Bergey and Holt [15].

Antimicrobial Susceptibility Assay

Agar well diffusion was used to measure antimicrobial sensitivity. For the bacterial isolates, Mueller Hinton agar-containing Petri dishes were prepared according to normal protocols. To promote fungal growth for *Candida* sp, another petri dish containing Mueller Hinton agar was prepared, but this time it was treated with 0.5 g/ml of methylene blue and 2% glucose. Mueller Hinton Agar (MHA) plates were aseptically coated with 0.1 mL (1×10^5 cells/mL) of the test bacterial isolates (*Bacillus* sp., *Escherichia coli*, *Klebsiella* sp., *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and the fungus Candida sp.), using specific agar supplements for Candida sp. After allowing the plates to dry for about 5 minutes, wells were created in the agar using a 7 mm diameter sterile cork borer. Then, 50 µL of each previously prepared diluted solution with different concentrations was added to each well using a micropipette and labeled with the corresponding extract concentration. For twenty-four hours, the plates were incubated at 37 °C. Using a divider and a meter ruler, the zone of inhibition was measured following incubation. The same protocol was used for the antibacterial test, where ciprofloxacin was used as the positive control for bacterial isolates, distilled water as the negative control, fluconazole as the positive antifungal control, and DMSO as the negative control. The results were presented as the mean of the two measurements and were recorded in duplicate [16, 17].

Minimum Inhibitory Concentration (MIC)

For this test, the broth dilution method was used to determine the Minimum Inhibitory Concentration, which is the lowest concentration of a substance that can prevent the growth of an organism throughout the course of a 24-hour incubation period. Molten Mueller Hinton agar supplemented with 2% glucose and 0.5 g/mL of methylene blue for the fungal plates were added to 19 mL of molten Mueller Hinton agar in bijou bottles. The bottles were filled with 1 mL of each concentration of the previously made extract. After that, the melted agar was cautiously transferred into empty Petri dishes and appropriately labeled. The test bacterial isolates including *Staphylococcus aureus*, *Bacillus* sp., *Escherichia coli*, *Klebsiella* sp., *Pseudomonas aeruginosa*, and the fungus *Candida* sp. were aseptically spread individually onto the agar surface. Mueller Hinton agar plates treated with the extracts were streaked with the fungal isolate. At 37 °C, all agar plates were incubated. The plates were examined for growth after 24 hours, and the MIC-the lowest concentration that prevented growth-was noted.

Results

Phytochemical component of stem bark extract of *Phellodendron amurense*

Phytochemical analysis of the stem bark of *Phellodendron amurense* revealed the absence of flavonoids but the presence of tannins, saponins, alkaloids, and triterpenoids, as shown in Table 1.

Table 1: Phytochemical components of stem bark of *Phellodendron amurense*

Phytoconstituents	Ethanol
Tannins	+
Saponins	+
Flavonoids	-
Alkaloids	+
Triterpenoids	+

KEYS: + = Positive; - = Negative

Antimicrobial activity of the stem bark extract of *P. amurense*

This study's antibacterial tests were conducted using the agar-well diffusion method, allowing quantification through the measurement of growth inhibition zone diameters and estimation of lowest inhibitory concentration values. The evaluation of antimicrobial activity was conducted in triplicate and the mean values were calculated. This was done to improve the reliability of the results. Diameters of the zone of inhibition are shown in Table 2. At a concentration of 100 mg/ml, the ethanol stem bark extract of *P. amurense* exhibited the highest activity against *P. aeruginosa*, with an inhibitory zone diameter of 8.67 mm, followed by *E. coli* at 8 mm and *S. aureus* at 5.67 mm. No activity was observed against the other clinical isolates at any concentration. In comparison, ciprofloxacin, used as a positive control, showed inhibition zone diameters of 22 mm for *E. coli*, 21 mm for *K. pneumoniae*, 25.33 mm for *S. aureus*, 22.33 mm for *P. aeruginosa*, and 17.33 mm for *B. subtilis* at the same concentration of 100 mg/ml. Fluconazole, used as a positive control for the fungus *C. albicans*, exhibited activity with a 20 mm inhibition zone at 100 mg/ml concentration. The standard antibiotics fluconazole and ciprofloxacin, which served as positive controls, showed the strongest activity.

Table 2: Zone of inhibition of *P. amurense* stem bark extract against the clinical isolates

	Concentration of extract					Ciprofloxacin 100 mg/ml	Fluconazole 100 mg/ml	Ethanol (Negative control)
	100 mg/ml	50 mg/ml	25 mg/ml	12.5 mg/ml	6.25 mg/ml			
Test Organisms	Zone of inhibition (mm)							
E. coli	8	-	-	-	-	22	-	-
P. aeruginosa	8.67	-	-	-	-	22.33	-	-
S. aureus	5.67	-	-	-	-	25.33	-	-
Klebsiella sp	-	-	-	-	-	21	-	-
B. subtilis	-	-	-	-	-	17.33		
C. albicans	-	-	-	-	-	-	20	-

Minimum inhibitory concentration of stem bark extract of *P. amurense*: The ethanol stem extract of *P. amurense* exhibited a minimum inhibitory concentration (MIC) of 100 mg/ml against *E. coli*, *S. aureus*, and *P. aeruginosa*, with no activity detected against the other organisms at any tested concentration, and this is presented in Table 3 below.

Table 3: Minimum inhibitory concentration of *P. amurense* extract on clinical isolates

	100 mg/ml	50 mg/ml	25 mg/ml	12.5 mg/ml	6.25 mg/ml
E. coli	+	-	-	-	-
P. aeruginosa	+	-	-	-	-
S. aureus	+	-	-	-	-
Klebsiella sp	-	-	-	-	-
B. subtilis	-	-	-	-	-
C. albicans	-	-	-	-	-

KEY WORDS: += Susceptible; - = Not susceptible

Discussion and Conclusion

Discussion

The clinical effectiveness of plant-based medicinal extracts has renewed interest in exploring medicinal plants as potential sources of new drugs. Medicinal plant usage worldwide dates back to times long before antibiotics and other modern medicines were introduced to Africa. Herbal medicine remains extensively utilized and serves as a key component of primary healthcare in countries such as China, Ethiopia, Argentina, and Nigeria. Additionally, a substantial number of pharmaceutical products currently in use are derived from plants [18]. Phytochemical analysis of the stem bark of *Phellodendron amurense* revealed the presence of alkaloids, tannins, saponins, and triterpenoids among the compounds tested. This is suggestive of the fact that the antimicrobial activity of *Phellodendron amurense* stem bark may be largely attributed to its phytochemical components and this is in line with an earlier report by [19].

The ethanol extract of *Phellodendron amurense* demonstrated greater antibacterial efficacy against Gram-negative bacteria, including *Escherichia coli*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae* compared to Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*). Following the antibacterial screening, the extract's minimum inhibitory concentration (MIC), the lowest concentration preventing observable bacterial growth, was ascertained. The fivefold serial dilution revealed that the MIC for *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli* was 100 mg/ml. The susceptibility of *Staphylococcus aureus*, *Escherichia coli,* and *Pseudomonas aeruginosa*, to the plant extract suggests its potential as a drug for combating these organisms, with the expectation that higher extract concentrations could yield greater antimicrobial effects. This aligns with previous research indicating that antimicrobial activity is directly proportional to increased extract concentration (%) [20].

It is important to note that the extract did not cause the inhibition of the growth of *Klebsiella pneumoniae*, *Bacillus subtilis*, or *Candida albicans* at any of the tested concentrations, indicating that these organisms are not susceptible to the extract at the studied concentrations. Therefore, further studies should be carried out with higher concentrations of the extract.

Conclusion

This research has shown that different bacteria exhibited varying degrees of sensitivity and resistance to the extract of *Phellodendron amurense.* In recent years, the majority of pathogenic organisms have developed antibiotic resistance; however, there has been considerable evidence that plant extracts contain active compounds with great potential as antimicrobial agents. Therefore, the discovery of novel active compounds against these pathogenic organisms is imperative, to overcome this problem.

Conflicts of interest

There are no conflicts of interest disclosed by the authors.

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