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Evaluation of Phytochemical Constituents and Antimicrobial Activity of Calotropis procera Root Extract against some Pathogenic Microorganisms in Yola, Adamawa State, Nigeria

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Abstract

Calotropis procera has been traditionally used for medicinal purposes in Nigeria, with various parts of the plant utilized by traditional healers without scientific validation of their therapeutic properties. Therefore, this study sought to assess the phytochemical constituents and antimicrobial efficacy of root extracts from C. procera against pathogenic microorganisms. The extraction of C. procera roots was carried out using aqueous, chloroform, and n-hexane solvents, and the qualitative analysis of the phytochemical constituents was conducted following standard procedures. The antimicrobial activity of the extracts was evaluated against Staphylococcus aureus, Salmonella typhi, Klebsiella pneumoniae, Candida albicans, and Aspergillus flavus through the agar well diffusion method. Phytochemical screening identified the presence of tannin, saponin, alkaloids, phenolics, flavonoids, and reducing sugars in all extracts, with terpenoids exclusively found in the aqueous extract and cardiac glycosides absent in all extracts. The extracts demonstrated significant antimicrobial activity against all tested pathogens, with the chloroform extract exhibiting the largest inhibition zone (23.50±0.38 mm) against S. aureus, while the n-hexane extract displayed the smallest inhibition zone (6.38±0.52 mm) against A. flavus. Minimum inhibitory concentration (MIC) values were determined as 25 mg/ml for the chloroform extract against S. aureus and S. typhi, and 50 mg/ml for both aqueous and n-hexane extracts against K. pneumoniae and C. albicans. The minimum bactericidal concentration (MBC) was established at 100 mg/ml for S. aureus, S. typhi, and all extracts. The root extracts of C. procera exhibited promising antimicrobial effects against S. aureus and S. typhi, indicating its potential as a rich source of bioactive compounds and a viable alternative to antibiotics for treating infectious diseases.

Keywords: Antimicrobial, Calotropis procera, Microorganisms, Phytochemical compounds, Root extracts

INTRODUCTION

According to Zhu *et al.* (2022), bacterial infections rank among the world's major causes of illness and mortality, and antibiotic resistance is increasing globally. The declining efficacy of antibiotics poses a significant challenge in the treatment of infectious diseases, leading to a decrease in the quality of life, prolonged hospital stays, and increased financial burden. This issue is particularly pronounced in underdeveloped countries like Nigeria due to limited medical resources (Marasini *et al.*, 2015; Jamiu and Bello, 2018). The misuse of antibiotics to treat various medical conditions has further fueled the emergence of drug-resistant organisms. Without the discovery of alternative treatments, the World Health Organization (WHO) has projected that antibiotic resistance could result in approximately 10 million deaths annually by 2050.

Natural sources, such as medicinal plants, are being researched to provide alternative treatment solutions. These sources show promise as a cost-effective supply of antimicrobial compounds (Kitula, 2007; Bello and Jamiu, 2017). In rural parts of many developing nations, the utilization of medicinal plants as traditional and alternative medicine is widely recognized, accepted, and promoted globally (Ezekwesili-Ofili and Okaka, 2019; Obeta *et al.*, 2020). It has been demonstrated that plants can produce secondary metabolites, which are aromatic compounds that act as

defense mechanisms for plants against insects, microbes, and other herbivores (Makhuvele *et al.*, 2020). Nevertheless, humans appreciate the medicinal qualities that these protective chemicals offer to plants.

Apple of Sodom, or *Calotropis procera*, is a multipurpose plant that offers a wide range of ecological services. It has been widely employed in traditional medical systems in many parts of the world, particularly in Asia and Africa (Al Sulaibi *et al.*, 2020). *C. procera* has a long history of being recognized for its pharmacological actions against a variety of human illnesses, including fever, indigestion, diarrhea, elephantiasis, eczema, leprosy, asthma, rheumatism, and skin conditions (Al-Rowaily *et al.*, 2020). Mehmood *et al.* (2020) demonstrated that extracts derived from roots, bark, and leaves had antimicrobial properties against a variety of pathogens, such as *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Escherichia coli*, *Candida* spp., and *Aspergillus* spp. The antihyperglycemic potential of *C. procera* was demonstrated by the considerable decrease in blood sugar levels observed in its leaf and root extracts (Wadhwani *et al.*, 2021). Botanical chemicals constitute the plant's secondary metabolites and are responsible for *C. procera*'s therapeutic properties. Complex combinations of biologically active substances, including polyphenols, terpenoids, fatty acids, sterols, alkaloids, flavonoids, glycosides, saponins, tannins, and terpenes, are present in these phytochemicals (Makhuvele *et al.*, 2020).

Due to the substantial costs and potential side effects associated with administering antibiotics, research on therapeutic plants has increased in frequency. Plants with medicinal properties are frequently inexpensive to treat illnesses with and almost have no negative side effects. Therefore, it is critical to look for other therapeutic agents that are dependable and successful. *Calotropis procera* latex provides a multimodal strategy for counteracting microbiological problems. Its distinct antimicrobial potential is attributed to harboring a variety of bioactive compounds, destruction of cellular membranes, as well as inhibition of enzymes. To reconcile conventional wisdom with contemporary scientific understanding, it is crucial to investigate its bioactive components and their antimicrobial action. There is little information concerning *C. procera*'s antimicrobial ability against microbes in Northeastern Nigeria, especially around Adamawa State. Nevertheless, this study aimed to look into the phytochemical components and antimicrobial properties of *C. procera* against some clinical pathogens.

MATERIALS AND METHODS

Study Area

Adamawa State is bordered by Borno to the north, Taraba to the south, Gombe to the west, and Cameroon to the east. Covering approximately 39,742 km^2, which accounts for 4.4% of Nigeria's total land area, the state has a population density of 80 people per km^2, with a total population of 4,902,100 as of 2021 (National Bureau of Statistics, NBS, 2021).

Collection and Identification of Samples

From the Yola South region of Adamawa state, Nigeria, fresh *Calotropis procera* roots and leaves were collected. The plant materials were then sent to the Department of Plant Science at Modibbo Adama University in Yola for identification and authentication.

Four bacterial strains and two fungi, namely *Salmonella typhi*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Candida albicans*, and *Aspergillus flavus*, were obtained from the Microbiology Laboratory of the Modibbo Adama Teaching Hospital in Yola and used as test microorganisms for the study. These microbes were further confirmed using conventional biochemical techniques. The isolates were stored at 4°C on agar slants for future use.

After subculturing the bacterial strains in nutrient broth, a fresh inoculum was prepared and cultured for 18 hours at 37°C in a rotary shaker. Subsequently, the turbidity of each strain was adjusted to 10^8 cells/mL to adhere to the MacFarland standard.

A fungal isolate culture was utilized to generate a fungal inoculum, which was subsequently cultured for 48 hours in Saboraud dextrose broth. The spore density of the fungus was measured using a spectrophotometer at a wavelength of 595 nm, resulting in a final count of 10^6 spores/ml.

Preparation, Extraction, and Fractionation of Plant Extracts

After properly washing the leaves and roots with clean water to get rid of any dust, they were then spread out similarly in the shade to dry. They were first allowed to dry before being crushed into fine powder using a sterilized

mortar and pestle. For extraction, the plant material that had been powdered was kept at room temperature in an airtight polythene container.

The plant material was extracted using the procedure outlined by Zhang *et al*. (2018). In brief, 300 ml of aqueous, chloroform, and n-hexane solvent were used to soak 100 g of the plant powder individually for 48 hours at room temperature. The gauze was used to filter the mixture at first, and sterile filter paper was then employed to filter the filtrate. After the filtered extract was dried in a rotary evaporator, it was then weighed, recorded, and stored at 40^0C until needed.

The percentage yield of the fractions was calculated using the formula:

Percentage yield (%) = (dry weight of extract x 100) / dry weight of plant material

Screening of Phytochemical Components

The procedure outlined by Aly *et al*. (2019) was followed to conduct the qualitative phytochemical evaluation. The aqueous, chloroform, and n-hexane root extracts of *C. procera* were tested for tannin, saponin, alkaloids, flavonoids, steroids, cardiac glycosides, reducing sugar, phenolics, and terpenoids.

Determining The Antimicrobial Activity of the Root Extracts

The agar well diffusion method was employed to test for antimicrobial susceptibility following Clinical Laboratory Standard Institutes (CLSI) specifications (CLSI, 2021). In brief, 1-millilitre aliquots of the overnight broth cultures of every bacterial isolate were transferred into sterile Petri dishes after being adjusted to achieve the turbidity equivalent of 0.5 McFarland standards. Molten Muller-Hinton agar was added to the plates using aseptic methods, and the plates were gently swirled to ensure that the bacterial isolates were evenly dispersed throughout the medium. After allowing the agar plates to harden, homogeneous wells were made using a sterile, 9 mm-diameter cork borer. Next, the wells designated for each extract were filled with 0.5 ml of various extract concentrations. After that, the plates were incubated for 24 hours at 37°C. SDA was used for the test on the fungal organisms, and the plates were incubated for 72 hours at 30°C. Sterile water was used as the negative control in the experiment, and amphotericin B and ciprofloxacin were used as the positive controls for bacteria and fungi, respectively. There were three duplicates of the experiment run. The clearer zone of inhibition surrounding each well was measured in millimetres using a transparent ruler, and the outcome was noted.

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The tube dilution method, as outlined by Makhuvele *et al*. (2020), was used to determine the extracts' minimum inhibitory concentration (MIC). The MIC was identified by evaluating the extracts that exhibited a positive zone of inhibition in the agar well diffusion test. Different concentrations of the root extracts (400, 200, 100, 50, 25.12.5, 6.25 3.13, 1.56, 0.78 mg/ml) were prepared in separate tubes, each containing one milliliter. To ensure uniformity, five (5) milliliters of Mueller Hinton broth was added to each tube and thoroughly mixed. For both the chloroform and n-hexane extracts, tubes containing only DMSO were used as controls, and for the aqueous extract, distilled water was used. After adding a standard suspension (0.1 ml) of each bacterial isolate culture to the tubes, they were incubated for 24 hours at 37°C, and the presence or absence of turbidity was noted. The minimum inhibitory concentration (MIC) was defined as the lowest extract concentration that inhibited the test strains from growing.

Mueller Hinton agar was inoculated with 10 µL of each aliquot from the MIC that did not exhibit any turbidity, and the plate was incubated for 24 hours at 37°C to determine the MBC. The extract's lowest concentration at which no growth is visible is called the MBC. Every test was conducted in triplicates.

Data Analysis

The data from the study were inputted into the Statistical Package for Social Science (SPSS) software version 25.0 worksheet. Descriptive findings were analyzed using percentage and frequency analysis. Group comparisons were conducted using ANOVA.

RESULTS

Percentage Yield of Root Extracts from *C. procera*

Table 1 displays the percentage yield of the various solvent fractions containing *Calotropis*

procera roots. Based on the acquired data, the maximum yield (17.00 ± 0.50 %) occurred in the aqueous extract, while the lowest yield (8.50 ± 1.00 %) was obtained in the n-hexane extract indicating a significant difference in the percentage yield of the extracts (P<0.05).

Phytochemical Components of the Root Extracts

The results of the phytochemical screening revealed the presence of reducing sugar, tannin, steroids, phenolics, flavonoids, saponins, alkaloids, and terpenoids. However, cardiac glycoside was not detected in any of the extracts, and terpenoids were absent in the n-hexane and chloroform extracts (Table 2**).**

Antimicrobial Effect of *C. procera's* Root Extracts on the Isolates

Table 3 displays the antibacterial activity of *C. procera*'s aqueous, chloroform, and n-hexane fractions against the isolates. The outcomes demonstrated that every extract had antibacterial activity with a distinct zone of inhibition. The results indicate that the chloroform extract exhibited the maximum zone of inhibition (23.50±0.38 mm) on *Staphylococcus aureus*, whilst the n-hexane fraction showed the lowest inhibition zone (11.24±0.55 mm) on *K. pneumoniae*. The fungal isolates produced by chloroform against *C. albicans* have the maximum inhibition zone (17.24±0.95 mm), whereas the lowest inhibition zone (6.38±0.52 mm) was seen in n-hexane against *A. flavus*.

Table 1: Percentage yield of *C. procera* root extract from different solvents

Solvents	Yield from dry powder (g)	Percentage yield (%)
Aqueous	34± 0.25	17.00 ± 0.50
Chloroform	24.60± 0.67	12.30 ±1.34
n- Hexane	17.50± 0.50	8.50±1.00

Data from triplicate extractions are presented as means ± standard error.

Table 2: Phytochemical components of *C. procera* root extracts from different solvents

Constituents	Aqueous	Chloroform	n-Hexane
Tannin	+	+	+
Saponin	+	+	+
Flavonoids	+	+	+
Steroids	+	+	+
Terpenoid	+	-	-
Cardiac glycosides	-	-	-
Reducing sugar	+	+	+
Phenolics	+	+	+

Key: + = Presence – = Absence

Table 3: Antimicrobial Pattern of *C. procera* Root Extract against the Isolates

Test organism	Zones of inhibition (mm)			
	Aqueous	Chloroform	n-Hexane	Ciprofloxin/ Ketaconazole
S. typhi	10.24±0.95	21.48±0.52	10.25±0.35	32.05±0.90
S. aureus	11.44±0.55	23.50±0.38	12.33±0.28	33.00±0.50
K.pneumoniae	8.24±0.50	18.40±0.55	11.50±0.20	32.00±0.95
C. albicans	9.25±0.43	17.24±0.95	10.44±0.65	28.50±0.30
A. flavus	7.05±0.35	11.44±0.65	6.38±0.52	26.00±0.10

Data from triplicate extractions are presented as means ± standard error.

Minimum inhibitory concentrations (MIC) of the extracts on the isolates

Table 4 shows MIC and MBC values for the *C. procera* root extract. *S. aureus* and *S. typhi* exhibited a minimum inhibitory concentration (MIC) of 25 mg/ml, *K. pneumoniae* and *C. albicans* at 50 mg/ml, and *A. flavus* at 100 mg/ml for the chloroform and aqueous extract fraction. However, the minimum inhibitory concentration (MIC) of the n-hexane extract for *S. aureus* and *S. typhi* was 50 mg/ml, *K.*

pneumoniae and *C. albicans* were at 100 mg/ml, and *A. flavus* was at 200 mg/ml. *S. aureus* and *S. typhi* showed a minimum bactericidal activity of 100 mg/ml, while the remaining isolates had an MBC of 200 mg/ml.

Table 4: Minimum Inhibitory Concentration of Root Extract of *C. procera* on the Isolates

Test isolates	MIC Concentration of plant extract (mg/ml)															MBC
	Aqueous					Chloroform					n-Hexane					
	12.5	25	50	100	200	12.5	25	50	100	200	12.5	25	50	100	200	
S. typhi	+	+	-	-	-	+	-	-	-	-	+	+	-	-	-	100
S. aureus	+	+	-	-	-	+	-	-	-	-	+	+	-	-	-	100
K. pneumoniae	+	+	+	-	-	+	+	-	-	-	+	+	+	-	-	200
C. albicans	+	+	+	-	-	+	+	-	-	-	+	+	+	-	-	200
A.flavus	+	+	+	-	-	+	+	+	-	-	+	+	+	+	-	200

Key: **+ - Inhibited growth**
- :- Growth not inhibited

DISCUSSIONS

The discovery of abundant bioactive compounds in medicinal plants makes them a viable, cost-effective, and readily available source of antibacterial agents. *C. procera* root extracts were used to extract secondary metabolites, which were then evaluated against various pathogenic bacteria using solvents with different polarities. The highest percentage extract yield (17.00 ± 0.50%) in this study was observed in the *C. procera* chloroform root extract. In contrast, Falana and Nuruddeen (2020) found the highest yield (14.6%) in an acetone extract, while Akindele *et al.* (2017) reported that the aqueous extract had the highest percentage yield (12.37%). Differences in extraction techniques and solvent compositions may account for these variations.

Saponins, tannins, flavonoids, alkaloids, phenols, steroids, reducing sugars, and terpenoids were among the secondary metabolites identified in the qualitative phytochemical screening of *C. procera* root extracts in this study. Previous research by Yasmeen *et al.*, 2008), Yissa *et al.*, 2022), and Behbani *et al.*, 2023) also reported the presence of flavonoids, tannins, and saponins in C. procera extracts. The antimicrobial properties of these secondary metabolites, as highlighted by Bello *et al.* (2020), align with the antibacterial activity observed in the plant extracts. This finding is consistent with studies using aqueous, ethanol, and n-hexane solvents conducted by Kawo *et al.*, (2009) and Yissa *et al.*, (2022). Tannins, due to their ability to interact with proteins and form stable compounds that disrupt bacterial cell membranes, have the potential to act as antimicrobial agents (El-Marie and Jonah, 2001). Similarly, saponins exhibit detergent-like properties that enhance bacterial cell membrane permeability, facilitating the entry of antimicrobial substances. Flavonoids, according to Neenah (2013), inhibit nucleic acid synthesis, cytoplasmic membrane function, and energy metabolism, thereby exerting antibacterial effects. Additionally, the antibacterial properties of terpenoids and alkaloids have been documented by Quazi *et al.*, 2013) and Wadhwani *et al.*, 2021).

The root extracts evaluated exhibited varying levels of antimicrobial activity against the test pathogens. This study revealed that *S. aureus* was the most susceptible strain, with *S. typhi*, *C. albicans*, *K. pneumoniae*, and *A. flavus* following in susceptibility. Specifically, the chloroform extract showed the highest antimicrobial activity (23.50±0.38) against S. aureus, while the n-hexane extract demonstrated the lowest activity (6.38±0.52) against *A. flavus*. This finding is consistent with previous research indicating that the chloroform extract of C. procera has stronger antibacterial activity compared to its aqueous and n-hexane counterparts (Naranyi *et al.*, 2012; Saadabi *et al.*, 2012). In contrast, Kareem *et al.* (2008) and Leonard *et al.* (2013) reported that the ethanolic extract of *C. procera* exhibited the highest efficacy against bacterial and fungal isolates. The antimicrobial activity of a plant extract is often attributed to the presence of multiple

secondary metabolites and the interactions among these constituents, rather than a single primary active ingredient. The variations in antibacterial activity can be attributed to the different levels of secondary metabolites present in the plant, which may be influenced by extraction methods and diffusion rates (Behbani *et al.*, 2023).

However, the research does have some drawbacks. Although *C. procera* is known to be poisonous, its potential toxicity was not evaluated in this study. Additionally, the focus was solely on in vitro studies; thus, well-designed clinical trials are needed to assess the safety and efficacy of this plant extract for human use.

CONCLUSION

The study's overall findings indicate that *C. procera* root extracts exhibit significant antimicrobial activity against the selected microorganisms. Furthermore, the presence of phytochemical compounds in the plant suggests that *C. procera* extracts may provide a feasible alternative to chemically synthesized drugs beyond the financial reach of the average individual. Further research is needed to explore the mode of action of *C. procera*, combination therapy, toxicity profile, and human-subject clinical trials.

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