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Phytochemical Analysis and Antimicrobial Efficacy of *Calotropis procera* Stem Bark Extracts and Isolated Compounds Against Clinical Pathogens

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ABSTRACT

Introduction: *Calotropis procera* is a medicinal plant traditionally used for treating various ailments, but the bioactive constituents of its stem bark require further investigation. This study aimed to evaluate the phytochemical composition and antimicrobial potential of *C. procera* stem bark extracts and to isolate and characterize active compounds against a panel of clinical isolates.

Methods: Methanol and ethyl acetate extracts of the stem bark were subjected to phytochemical screening. Their antimicrobial activity was assessed against clinical isolates of methicillin-resistant *Staphylococcus aureus* (MRSA), *Escherichia coli*, *Pseudomonas aeruginosa*, *Candida albicans*, and *Salmonella typhi* using agar well diffusion, broth dilution for minimum inhibitory concentration (MIC), and minimum bactericidal/fungicidal concentration (MBC/MFC) assays. Bioassay-guided fractionation of the active ethyl acetate extract led to the isolation of three compounds (SCP-A, SCP-B, SCP-C) using column chromatography.

Results: Phytochemical analysis confirmed the presence of tannins, saponins, flavonoids, and other secondary metabolites. The methanol extract demonstrated broad-spectrum activity, particularly against MRSA, *E. coli*, *P. aeruginosa*, and *C. albicans*, with MIC values ranging from 0.63 to 1.25 mg/mL. The isolated compounds SCP-A and SCP-B exhibited significant and superior antimicrobial effects compared to the crude extracts, showing potent activity against most tested pathogens. SCP-C showed more limited activity.

Conclusion: The stem bark of *C. procera* contains bioactive compounds with promising antimicrobial properties against key clinical pathogens, including multidrug-resistant MRSA. These findings justify further investigation into the development of these compounds as potential antimicrobial agents.

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Introduction

Calotropis procera, commonly known as swallowwort, Dead Sea apple, or Sodom apple, is a hardy shrub widely distributed in tropical regions across the world (Wadhwani et al., 2021; Kundu, 2021; Kumari & Chaudhary, 2021; Vaishnav et al., 2024). This species belongs to the family Apocynaceae (subfamily Asclepiadoideae) and is characterized by its abundant latex-producing sap (Yaniv & Koltai, 2018; Kundu, 2021). Native to the sunny regions of tropical Africa, it is known by various local names, such as *bomubomu* in Yoruba and *tumafafiya* in Hausa, reflecting its cultural significance. Traditionally, the aerial parts of this plant have been used for their antidiarrheal properties (Felix-Cuencas, 2022), contributing to the management of gastrointestinal disorders (Rabelo et al., 2023).

C. procera has also demonstrated anti-eczema activity (Aliyu et al., 2015) and antifungal effects against dermatophytes associated with skin infections such as dermatophytoses and dermatitis (Ali et al., 2023). Despite these therapeutic applications, the plant contains potent bioactive compounds that may also exert toxic effects (Waikar & Srivastava, 2015), highlighting its dual nature as both a medicinal resource and a potential toxic agent in pharmacological contexts (Rabelo et al., 2023; Alshahi, 2024).

Ongoing research on *C. procera* (as shown in Plate 1) and similar medicinal plants is essential, particularly in the context of increasing resistance of pathogens to conventional antimicrobial and antimalarial agents, including chloroquine and modern drugs such as artemisinin, artesunate, and artemether (Korsik & Todd, 2019). Harnessing the rich diversity of bioactive compounds in medicinal plants like *C. procera* is therefore crucial for addressing the challenges posed by evolving drug-resistant pathogens. Thus, the main goal of this study is to investigate the botanical classification, geographical origin, cultural significance, traditional uses, and pharmacological duality of *C. procera* as both a therapeutic agent and a potential toxin.

Materials and Methods

C. procera stem bark was collected at Lugbe, Abuja Municipal Area Council (AMAC). A voucher number NIPRD/H/7070 was assigned to the sample and stored in the Herbarium at the National Institute for Pharmaceutical Research and Development (NIPRD) in Abuja. The stem barks were thoroughly cleaned and air dried in the shade and weighed. The samples were then stored in airtight containers until they could be analyzed in the laboratory later. A fine powder was prepared from the dried samples using a TRP80 hammer mill. For thin layer chromatography (TLC), the spots were examined under UV light at wavelengths of 254 nm and 366 nm, and they were visualized using iodine vapor and a concentrated H_2SO_4-MeOH spray. The antimicrobial tests included clinical isolates of bacteria and fungi, specifically *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Candida albicans*, *Salmonella typhi*, and methicillin-resistant *Staphylococcus aureus* (MRSA). These were sourced from the Department of Medical Microbiology at Ahmadu Bello University Teaching Hospital in Zaria, Kaduna State, Nigeria.

Determination of Antimicrobial Activity of Extracts

In accordance with standard procedures, *C. procera* stem bark extract was examined for its antimicrobial activity (Reller et al., 2010). To test for growth of each test microbe, 0.1 ml of the sample was seeded on Mueller Hilton agar plates using a sterile cork borer, four uniform wells were bored into the agar surface. Wells were filled with different concentrations of extracts (5.0, 2.5, 1.25, 0.625 and 0.31 mg/ml). As controls, reference antibiotics were introduced into two holes bored on a plate at equal distances from each of the test organisms. After 40 minutes of pre-diffusion on the bench, the plate was incubated overnight at 37°C for 24 hours.

Determination of Minimum Inhibitory Concentration (MIC)

The MIC was defined as the lowest concentration that inhibited the growth of the organisms. The MIC of stem bark extracts and fractions was determined using a broth dilution method (Nayef, 2016). Extracts were tested at concentrations of (50, 25, 12.5, 6.25, 3.125 and 0.31) mg/ml in nutrient broth inoculated with the test organisms. After 24 hours of incubation at 37°C.

Determination of Minimum Bactericidal and Fungicidal Concentration (MBC/MFC)

1 ml of culture from the MIC tubes was collected and sub-cultured onto fresh agar plates free of drugs. These plates were also incubated at 37°C for 24 hours, and the MBC/MFC was recorded as

the lowest concentration at which no colony growth occurred.

Phytochemical Screening

Preliminary phytochemical screening was conducted following standard protocols (Iqbal, 2012), which confirmed the presence of various phytochemical classes in the extracts.

Isolation and Identification of Compounds

A glass column (40 cm length × 3 cm internal diameter) was packed with 150 g of silica gel to a bed height of 23 cm. The column was first washed with n-hexane to ensure compact and uniform packing. The ethyl acetate fraction (15.0 g) was loaded onto the column using the wet loading method. Elution was performed with a stepwise gradient of n-hexane–ethyl acetate (90:10 to 0:100, v/v) in increments of 10% polarity, yielding a total of 81 fractions, each of 75 mL.

Based on thin layer chromatography (TLC) profiles, fractions 11, 12, and 13 were pooled and individually washed with acetone to afford three pure compounds:

- SCP-A (white crystalline solid, 20 mg),
- SCP-B (yellowish crystalline solid, 25 mg), and
- SCP-C (white crystalline solid, 15 mg).

Statistical Analysis

Dunnett'spost hoc analysis was conducted in order to analyze the data after one-way analysis of variance (ANOVA). In the tables, the results are displayed as mean + standard error.

Results

Table 1 presents the results of the preliminary phytochemical screening of the methanolic stem extract, indicating the presence of major bioactive constituents. The extract tested positive for tannins, saponins, flavonoids, steroids, phenolic compounds, alkaloids, cardiac glycosides, terpenoids, and carbohydrates, suggesting a rich phytochemical profile and potential pharmacological relevance.

Table 2 reports the Zone of Inhibition (in mm) for various extracts against different microorganisms. The results indicate whether the extract/drug is susceptible (effective) or resistant (ineffective) against the pathogen.

Table 1: Preliminary phytochemical screening conducted on the methanol stem extract

Phyto-constituents	Stem extract
Tannins	+
Saponins	+
Flavonoids	+
Steroids	+
Phenolic acid	+
Alkaloids	+
Cardiac glycosides	+
Terpenoids	+
Carbohydrates	+

Table 2: Antimicrobial activity of *Calotropis procera* stem bark extracts [Zone of inhibition (mm)]

Test microorganisms	Treatments			
	Methanolic extract	**Ethyl acetate extract**	**Fluconazole (30 µg/ml)**	**Ciprofloxacin (10 µg/ml)**
MRS. Aureus	24.03 ± 0.05 (S)	20.43± 0.05 (S)	0.00 (R)	32.00 ± 0.45 (S)
S. aureus	0.00 (R)	0.00 (R)	0.00 (R)	32.00 ± 0.30 (S)
E. coli	27.08 ± 0.35 (S)	22.12 ± 0.25 (S)	0.00 (R)	30.00 ± 0.25 (S)
P.aeruginosa	23.07 ± 0.49 (S)	21.25 ± 0.43 (S)	0.00 (R)	32.00 ± 0.30 (S)
S.typhi	0.00 (R)	0.00 (R)	0.00 (R)	35.00 ± 0.10 (S)
C.albicans	25.00 ± 0.25 (S)	20.13± 0.15 (S)	35.00 ± 0.10 (S)	0.00 (R)

MRSA: Methicillin-resistant Staphylococcus aureus (written as MRS. aureus in some tables); S. aureus: Staphylococcus aureus; E. coli: Escherichia coli; P. aeruginosa – Pseudomonas aeruginosa; S. typhi – Salmonella typhi; C. albicans: Candida albicans; R= Resistant; S= Sensitive.

Table 3 presents the minimum inhibitory concentrations (MICs) of the methanolic and ethyl acetate extracts against selected clinical isolates. The ethyl acetate extract generally exhibited stronger antimicrobial activity (MIC = 0.31 mg/mL) than the methanolic extract, particularly against MRSA, Escherichia coli, Pseudomonas aeruginosa, and Candida albicans. In contrast, both extracts showed no observable activity against Staphylococcus aureus and Salmonella typhi.

Table 3: Minimum Inhibitory Concentration of the methanolic and ethyl acetate extracts on some clinical isolates

Organisms	Treatments	
	Methanolic extract (mg/ml)	Ethyl acetate extract (mg/ml)
MRS. Aureus	1.25	0.31
S. aureus	NA	NA
E. coli	0.63	0.31
P.aeruginosa	0.63	0.31
S.typhi	NA	NA
C.albicans	0.63	0.31

MRSA: Methicillin-resistant Staphylococcus aureus (written as MRS. aureus in some tables); S. aureus: Staphylococcus aureus; E. coli: Escherichia coli; P. aeruginosa – Pseudomonas aeruginosa; S. typhi – Salmonella typhi; C. albicans: Candida albicans; NA: no growth.

Table 4 presents analyzing the Minimum Inhibitory Concentration (MIC) and the Minimum Bactericidal/Fungicidal Concentration (MBC/MFC) of the *C. procera* extract.

Table 4: Minimum Bactericidal/Fungicidal Concentration of *Calotropis procera* extract on some clinical isolates

Organisms	Minimum Bactericidal Concentration (mg/ml)	Minimum fungicidal concentration (mg/ml)
MRS. Aureus	2.5	2.5
S. aureus	NA	NA
E. coli	1.25	1.25
P.aeruginosa	2.5	2.5
S.typhi	NA	NA
C.albicans	2.5	2.5

MRSA: Methicillin-resistant Staphylococcus aureus (written as MRS. aureus in some tables); S. aureus: Staphylococcus aureus; E. coli: Escherichia coli; P. aeruginosa – Pseudomonas aeruginosa; S. typhi – Salmonella typhi; C. albicans: Candida albicans; NA: no growth.

Table 5 presents the zones of inhibition (in mm) for three isolated compounds—SCP-A, SCP-B, and SCP-C—derived from *C. procera* stem bark extracts, tested against a range of microorganisms.

Table 5: Antimicrobial activity of compounds isolated from stem bark extract of *Calotropis procera* against test clinical isolates [Zone inhibition (mm)]

Organisms	Compounds		
	SCP-A (µg/ml)	SCP-B (µg/ml)	SCP-C (µg/ml)
MRS. Aureus	12.5	12.5	25.0
S. aureus	25.0	25.0	NA
E. coli	12.5	25.0	25.0
P.aeruginosa	12.5	25.0	NA
S.typhi	NA	NA	NA
C.albicans	12.5	25.0	25.0

MRSA: Methicillin-resistant Staphylococcus aureus (written as MRS. aureus in some tables); S. aureus: Staphylococcus aureus; E. coli: Escherichia coli; P. aeruginosa – Pseudomonas aeruginosa; S. typhi – Salmonella typhi; C. albicans: Candida albicans; NA: no growth.

Table 6 summarizes the minimum concentrations of three isolated compounds (SCP-A, SCP-B, and SCP-C) required to kill—rather than merely inhibit—various test microorganisms, including both bacteria and fungi. Lower concentration values denote higher potency of the compound against the corresponding microorganism.

Table 6: Minimum Bactericidal/Fungicidal Concentrations of Isolated Compounds against the test Microorganisms

Organisms	Compounds		
	SCP-A (µg/ml)	SCP-B (µg/ml)	SCP-C (µg/ml)
MRS. Aureus	50.00	12.5	100.00
S. aureus	50.00	50.00	NA
E. coli	25.00	50.00	50.00
P.aeruginosa	25.00	50.00	NA
S.typhi	NA	50.00	NA
C.albicans	50.00	50.00	25.00

MRSA: Methicillin-resistant Staphylococcus aureus (written as MRS. aureus in some tables); S. aureus: Staphylococcus aureus; E. coli: Escherichia coli; P. aeruginosa – Pseudomonas aeruginosa; S. typhi – Salmonella typhi; C. albicans: Candida albicans; NA: no growth.

Discussion

The isolated compounds SCP-A (Octadecan-2-yl acetate), SCP-B (propan-2-yl 2,2-dimethylpropanoate), and SCP-C (bis(2,2-dimethylpropyl)(cyclopentyl)silane) demonstrated notable antimicrobial activity, particularly at low concentrations, highlighting their potential as effective antimicrobial agents. The antimicrobial performance of these compounds, derived from *C. procera* stem bark extracts, was evaluated against selected clinical pathogens by measuring inhibition zones (mm), where larger values correspond to

stronger microbial suppression.

Among the tested compounds, SCP-A exhibited consistent and broad-spectrum antimicrobial activity against MRSA, Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, and Candida albicans, with the highest inhibition observed against E. coli (31.00 mm). SCP-B also showed substantial antimicrobial efficacy against most tested organisms, except Salmonella typhi. In contrast, SCP-C displayed a narrower activity spectrum, showing no inhibitory effect against S. aureus, E. coli, and S. typhi, but demonstrating measurable activity against MRSA, P. aeruginosa, and C. albicans. These results indicate clear variability in the antimicrobial potency and spectrum of action among the three compounds.

Minimum inhibitory concentration (MIC) analysis further supported these findings. SCP-A generally inhibited most microorganisms at 12.5 µg/mL, indicating high potency. SCP-B showed exceptional activity against MRSA at the same low concentration, suggesting a possible mechanism involving disruption of bacterial ribosomal function and inhibition of protein synthesis. SCP-C required higher concentrations for activity or showed no detectable effect against certain strains, indicating limited or selective antimicrobial action. The absence of activity (NA) reflects no observable inhibition at the tested concentrations.

Minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) analyses further confirmed the killing potential of these compounds, with lower values corresponding to higher antimicrobial strength. SCP-B in particular demonstrated strong bactericidal activity against MRSA at 12.5 µg/mL, reinforcing its high therapeutic potential.

Comparative literature supports these findings. Fatty acids such as oleic, palmitic, and linoleic acids derived from crocodile oil have been reported to exhibit strong antimicrobial effects against S. aureus, Klebsiella pneumoniae, and C. albicans. Optimal activity has been observed at 15% concentration, with anti-inflammatory effects peaking approximately three hours after administration. These fatty acids exert antimicrobial effects primarily through disruption of microbial cell membranes and inhibition of virulence factors, including biofilm formation and exotoxin production (Buthelezi et al., 2012; Desbois, 2012).

Phytochemicals such as tannins and flavonoids may further enhance antimicrobial efficacy by inhibiting bacterial efflux pumps, which are key mechanisms of antibiotic resistance, particularly in MRSA strains. This inhibition increases intracellular accumulation of antimicrobial compounds, potentially explaining the lower MIC values observed for isolated compounds compared with crude extracts. In addition, alkaloids and cardiac glycosides may interfere with essential microbial enzymes involved in ATP synthesis and DNA replication, thereby disrupting key metabolic pathways, especially in Candida albicans. The silicon-containing structure of SCP-C may contribute to its selective bioactivity through specific enzyme-target interactions. Furthermore, while tannins and flavonoids function as antioxidants in mammalian systems, they may induce oxidative stress in microbial cells by increasing reactive oxygen species (ROS) generation, ultimately destabilizing microbial homeostasis and enhancing antimicrobial effects (Rodríguez et al., 2023).

Conclusion

C. procera stem bark extracts, along with the isolated compounds SCP-A, SCP-B, and SCP-C, exhibited pronounced antimicrobial activity, thereby supporting their traditional medicinal use and demonstrating effectiveness against drug-resistant pathogens such as MRSA. The strong bioactivity of these compounds underscores their potential as lead candidates for targeted drug development, in agreement with reports on other medicinal plants such as Mirabilis jalapa L. (Mohammed, 2012). Future investigations should focus on in vivo efficacy and safety profiling, comprehensive toxicity assessments, and detailed elucidation of mechanisms of action using approaches such as molecular docking, proteomic analysis, and enzyme-target studies. These steps are critical for the development of safe, cost-effective, and novel antimicrobial and antimalarial agents capable of addressing the growing challenge of antimicrobial resistance, particularly in resource-limited settings. Overall, the broad-spectrum antimicrobial potential of *C. procera* and its diverse phytochemical constituents highlights its value as a promising source for future pharmacological exploration aimed at overcoming microbial resistance and expanding therapeutic options.

Declarations

Conflict of Interest

The authors declare no conflicts of interest. All authors have reviewed and approved the final version of the manuscript.

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Consent for Publications

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Ethical Considerations
In this paper, the authors strictly adhered to ethical standards, refraining from plagiarism, misconduct, data fabrication, falsification, duplication, and redundancy.

AI Use Disclosure
During the preparation of this work, the authors used a generative AI tool solely for language editing, grammar correction, and formatting assistance. The AI tool was not used for data analysis, interpretation, or generation of scientific conclusions. After utilizing the tool, the authors reviewed, revised, and verified all content and take full responsibility for the accuracy and integrity of the final manuscript.

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