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Acute Toxicity Profile and Protection Assay of Alchornea Cordifolia Leaves Extract Against Selected Multi-Drug-Resistant Bacterial Pathogens from Surgical Site Infections

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Abstract: *The increasing challenge of multidrug-resistant (MDR) pathogens presents a significant threat in managing surgical site infections (SSIs), necessitating the exploration of alternative therapeutic agents. Alchornea cordifolia, a medicinal plant used in ethnomedicine, is renowned for its wound healing potentials based on its antimicrobial and anti-inflammatory properties. This study investigated the acute toxicity profile and in vivo protective efficacy of the ethanol extract of A. cordifolia leaves, against selected MDR bacterial isolates obtained from patients clinically diagnosed of SSIs, in five major hospitals within Calabar-Nigeria. Acute toxicity evaluation was conducted in mice following standard protocol, to determine the extract's safety. The in vivo efficacy of the extract was also assessed on its ability to mitigate the establishment of infection in murine models infected with the MDR-SSI test isolates. Results revealed a favourable safety profile of the extract, with an LD_{50} of 1,732.0mg/kg. Further in vivo assessments demonstrated notable protective efficacy/antibacterial activity of the A. cordifolia extract, as it exerted 100% protection against mortality in mice due to induced infection with E. cloacae, K. pneumoniae, P. mirabilis, A. baumannii, S. aureus, and P. aeruginosa test isolates, while also exerting 50% protection against mortality in mice due to induced infection with E. coli and S. epidermidis test isolates. These findings suggests that the ethanol extract of A. cordifolia leaves holds promise as a safe and effective therapeutic option against surgical site infection caused by MDR bacterial pathogens, and underscores the need for integrating modern pharmacological approaches in the validation of ethnomedicinal plants.*

Keywords: *Alchornea cordifolia*, surgical site infections, antibacterial activity, acute toxicity, pathogens.

INTRODUCTION

Surgical site infections (SSIs) represent a leading cause of hospital-acquired infections globally, significantly contributing to patient morbidity, extended hospital stays, and increased healthcare costs (Allegranzi *et al*., 2016; Olowo-Okere *et al*., 2019; Upula *et al*., 2022). The global burden of SSIs has been exacerbated by the emergence of multidrug-resistant (MDR) bacterial pathogens, including *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* (Magill *et al*., 2014; Owusu *et al*., 2021). These pathogens exhibit resistance to multiple antibiotics, complicating treatment protocols and necessitating the development of novel therapeutic strategies (Magill *et al*., 2014; Upula *et al*., 2022; WHO, 2017).

Medicinal plants have historically been a vital cornerstone of drug discovery, offering a diverse reservoir of bioactive compounds with therapeutic potential (Owusu *et al*., 2021; Adeonipekun *et al*., 2018; Akinrinlola *et al*., 2018). Among these, *Alchornea cordifolia*, a medicinal plant native to tropical Africa, has gained prominence for its traditional use in treating wounds, infections, and inflammatory conditions underscoring its therapeutic properties (Ogungbe *et al*., 2013; Akinmoladun *et al*., 2020). Phytochemical investigations reveal that *A. cordifolia* is rich in alkaloids, flavonoids, tannins, and saponins, which possess antimicrobial and anti-inflammatory properties (Adeonipekun *et al*., 2018; Akinrinlola *et al*., 2018).

Acute toxicity studies are critical in evaluating the safety profile of plant-based therapeutics. These studies assess the potential adverse effects of extracts in biological systems and establish safe dosage limits for further pharmacological evaluations (John-Africa *et al*., 2019; Tseha *et al*., 2022). Additionally, *in vivo* protection studies are indispensable in determining the efficacy of medicinal plants against infections in real-world biological contexts (Yadav and Rohane, 2021; Olorunniyi *et al*., 2023). Such studies not only provide insights into the antimicrobial activity of plant extracts but also evaluate their potential to promote wound healing and reduce bacterial burdens in infected tissues (Olayemi *et al*., 2019; Nigussie *et al*., 2021). Therefore, this study evaluated the acute toxicity profile and protection potentials of the ethanol extract of *A. cordifolia* leaves in order to provide a robust foundation for the potential clinical application of *A. cordifolia* in the treatment of SSIs caused by MDR bacterial pathogens.

MATERIALS AND METHODS

Identification of Bacterial Isolates

The bacterial isolates used in the present study were fully characterized SSI isolates from SSI patients admitted in five major specialist hospitals within Calabar-Nigeria namely: University of Calabar Teaching Hospital (UCTH), Nigerian Navy Reference Hospital Calabar (NNRH), General Hospital Calabar (GH), Bakor Medical Centre Calabar (BMC) and Nigerian Airforce Clinic Calabar (NAC). SSI samples were obtained from the study subjects, within 12 months and characterized as reported by the author in a previous article (Upula *et al*., 2022).

Acute Toxicity Assay

The acute toxicity study (LD_{50}) was conducted in accordance with Lorke's method as previously described (Ekong *et al*., 2004; Bulus *et al*., 2011). Albino mice (20 – 28 g) were obtained from the animal house facility of University of Uyo, Nigeria. All animals were housed under ambient conditions and fed on standard rodent diet with clean drinking water *ad libitum*, and the mice were handled according to the animal guidelines for care and use of animals as previously documented (John-Africa *et al*., 2019; Tseha *et al*., 2022). The bioactive extract (ethanol crude extract of *A. cordifolia* leaves) was freshly standardized to a stock concentration of 100.0 µg/mL and thereafter, seven (7) different concentrations (500.0 mg/kg, 1000.0 mg/kg, 1500.0 mg/kg, 2000.0 mg/kg, 3000.0 mg/kg, 4000.0 mg/kg, and 5000.0 mg/kg) were prepared. The seven (7) different concentrations constituted seven (7) experimental groups of six (6) albino mice which were randomly allotted into each group.

Using the intraperitoneal route, albino mice in each group were respectively dosed with graded concentrations of the bioactive extract based on their body weight, while the control group mice were dosed only with distilled water. The animals were observed within 24 h for physical and clinical signs of toxicity including locomotion, reaction to noise, reaction to pinch, aggressiveness, state of excrement, and mortality, and the LD_{50} was calculated thus:

$LD_{50} = \sqrt{ab}$; where a = maximum dose that produced 0 % mortality; and b = minimum dose that produced 100 % mortality. The LD_{50} of the extract was then interpreted based on Hodge and Sterner toxicity scale (Eco-Goldex, 2019; Yadav and Rohane, 2021).

Mouse Infection Model and Protection Assay

The bioactive extract's ability to protect experimental animals dosed separately with standardized broth cultures of test organisms was tested on albino mice (weighing 16-21 g) as previously described (Ekong *et al*., 2004; John-Africa *et al*., 2019). Firstly, albino mice in both the test and positive control groups were challenged intraperitoneally with 0.5 mL broth cultures of the SSI-MDR test isolates previously incubated for 18-24 h. Four (4) mice each were placed in eight (8) groups corresponding to each test-isolate in the experimental set-up while another four (4) mice each were placed in 8 groups corresponding to each test-isolate in the control set-up.

After 1 h post-inoculation of the test isolates, different volume of 30 % of the LD_{50} concentration were administered intraperitoneally to the albino mice in the experimental groups based on their body weight, while the albino mice in the positive control groups were administered normal saline water. Another set of mice were dosed orally with distilled water but no test organism (negative control). Within five (5) hours post-inoculation of the test isolates, a second dose of the same bioactive extract was administered to the albino mice in the experimental groups. All the mice were allowed access to water and food unrestricted (*ad libitum*) for five (5) days, during which period the animals were monitored for clinical presentations of infection, survival, or death as previously described (Ekong *et al*., 2004; John-Africa *et al*., 2019).

Ethical Consideration

This study was conducted in accordance with existing ethical guidelines. Ethical approval was obtained from the Ethical Committee of the Cross River State Ministry of Health, with REC No.: CRSMOH/RP/REC/2021/181.

RESULTS AND DISCUSSION

Surgical site infections (SSIs) remain a significant cause of postoperative complications, with multidrug-resistant (MDR) bacterial pathogens posing major treatment challenges (Allegranzi *et al*., 2016; Owusu *et al*., 2021; Upula *et al*., 2022). Eight bacterial pathogens frequently implicated in SSI causation was characterized and included in this study. The microscopic, biochemical and phenotypic characterization/identification of the eight (8) MDR-SSI test isolates included in this study are as shown (Table 1). Also, the antibiotic resistance profile and multiple antibiotic resistance (MAR) index of the test isolates are as summarized (Table 2).

In this study, the acute toxicity of *Alchornea cordifolia* leaves ethanol extract was assessed to determine the lethal dose (LD_{50}). The results revealed that the maximum dose causing 0% mortality was 1500 mg/kg, while the minimum dose inducing 100% mortality was 2000 mg/kg (Table 3). Using the empirical formula based on Hodge and Sterner's toxicity scale, the LD_{50} was calculated to be 1732.05 mg/kg. This finding suggests that *A. cordifolia* leaves extract can be classified as slightly non-toxic when administered intraperitoneally, aligning with earlier studies (Gatsing *et al*., 2010; Eco-Goldex, 2019; Yadav and Rohane, 2021). Previous studies have similarly indicated that the LD_{50} of *Alchornea cordifolia* extract is classified as slightly or almost non-toxic when administered to both male and female mice (Gatsing *et al*., 2010; Djemeli *et al*., 2017). Clinical signs linked to high-dose exposure to test substances as well as the sequence and timing of events preceding mortality remains critical components of toxicological investigations (Olorunniyi *et al*., 2023). The toxicological manifestations of a substance are typically reflected in behavioural or biochemical changes observed in the treated animals (Ekong and Okoro, 2016; Olorunniyi *et al*., 2023).

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Table1: Microscopic, biochemical and phenotypic characterization/identification of MDR-SSI test Isolates

Cell Morphology	Cell Morphology	Gram reaction	MOT	CAT	COA	OXI	IND	MR	VP	CIT	URE	GLU	LAC	MAL	MAN	SOR	SLANT	BUTT	H_2S	GAS	Probable organism
SAUTHC 1	Rods	-	+	+	ND	-	-	-	+	+	-	+	-	+	+	+	A	A	-	+	*Enterobacter cloacae*
SAUTHC 2	Short rods	-	+	+	ND	-	-	-	-	+	-	-	-	+	+	-	A	A	-	+	*Escherichia coli*
SAUTHC 3	Rods	-	-	+	ND	-	-	-	+	+	+	+	+	+	+	+	A	A	-	+	*Klebsiella pneumoniae*
SAUTHC 4	Short rods	-	+	+	ND	-	-	+	-	+	+	+	-	-	-	-	K	A	+	+	*Proteus mirabilis*
SAUTHC 5	Coccobacillus	-	-	+	-	-	-	-	-	+	-	+	-	D	-	-	K	K	-	-	*Acinetobacter baumannii*
SAUTHC 6	Cocci in clusters	+	-	+	+	-	-	+	+	+	+	+	+	+	+	-	A	A	-	-	*Staphylococcus aureus*
SAUTHC 7	Long rods	-	-	+	-	-	-	-	-	+	-	+	-	D	-	-	K	K	-	-	*Pseudomonas aeruginosa*
SAUTHC 8	Cocci in clusters	+	-	+	-	-	-	-	+	-	+	+	+	+	-	-	A	A	+	+	*Staphylococcus epidermidis*

Keys: Mot- Motility, Cat- Catalase, Oxi- Oxidase, Coa- Coagulase, Ind- Indole, MR-Methyl Red, VP- Voges Proskeur, Cit- Citrate, Ure- Urease, Glu- Glucose, Lac-Lactose, Sor- Sorbitol, Man-Mannitol, Mal- Maltose, A-Acid, K- Alkaline, d- Variable, ND- Not Determined, +: Positive, -: Negative, UCTH- University of Calabar Teaching Hospital, GH-General Hospital, NNRH-Nigerian Navy Reference Hospital, NAC- Nigerian Airforce Clinic, BMC-Bakor Medical Centre

Table 2: Resistance pattern of MDR-SSI test isolates.

Isolate code	MDR-SSI test isolates	Antibiotics resistance pattern of isolates	MAR index
SAUTHC 1	*E. cloacae*	AMP, TOB, TE, CEF, CAZ, PIP, LEV, CIP, CRO	0.6
SAUTHC 2	*E. coli*	AMP, MEM, TOB, AMS, CRO, CZN, CEF, PIP, GM, TE, LEV	0.7
SAUTHC 3	*K. pneumoniae*	AMP, TOB, GM, CZN, CEF, PIP, TE, LEV, SXT	0.6
SAUTHC 4	*P. mirabilis*	AMP, TOB, SXT, CEF, CAZ, AK, TE, CIP	0.5
SAUTHC 5	*A. baumannii*	AMS, CEF, CAZ, PIP, TOB, TE, CIP	0.5
SAUTHC 6	*S. aureus*	AMP, OXA, CLN, MEM, PIP, AK, LEV, CIP, SXT	0.7
SAUTHC 7	*P. aeruginosa*	AK, CIP, CAZ, MEM, CZN, CEF	0.6
SAUTHC 8	*S. epidermidis*	AMP, OXA, CIP, SXT PIP, TOB, TE	0.7

Keys: AMP-Ampicillin, TOB-Tobramycin, TE-Tetracycline, LEV-Levofloxacin, CIP-Ciprofloxacin, AMS- Ampicillin-Sulbactam, CRO-Ceftriaxone, CEF-Cefepime, CAZ-Ceftazidime, PIP-Piperacillin, SXT-Trimethoprim-sulfamethoxazole, CZN-Cefazolin, CLN-Clindamycin, MEM-Meropenem, GM-Gentamicin, AK-Amikacin, OXA-Oxacillin, MAR index ≤ 0.2: Low risk resistant isolates, MAR index ≥ 0.2: High risk resistant isolates, Key: SAUTHC: SSI-Associated Unique Test Hospital-Isolate Code.

Table 3: Determination of the lethal dose (LD_{50}) of *A. cordifolia* leaves extract.

S/N	Experimental Groups	Dose (mg/kg)	Survival rate (a/n)	Percentage Death (%)
1	One	500	$0/6$	100
2	Two	1000	$6/6$	0
3	Three	1500	$6/6$	0
4	Four	2000	$0/6$	100
5	Five	3000	$0/6$	100
6	Six	4000	$0/6$	100
7	Seven	5000	$0/6$	100

Keys: (a/n): a = number of survivals, n = number of mice.

Findings also demonstrated a dose-dependent reduction in locomotion, as well as in the response to noise and pinch stimuli across all experimental animal groups. These observations are consistent with prior studies reporting similar side effects, including reduced sensitivity to stimuli, decreased mobility, and softer feces, potentially attributed to the extract's effect on nociceptors, inhibition of algogenic substances, or interference with pain transmission (Djimeli *et al*., 2017). Furthermore, reduced mobility and responsiveness to noise in treated mice have been linked to the sedative or depressant effects of the extract, potentially exerting a tranquilizing influence on the central nervous system (CNS) and motor neurons (Njateng *et al*., 2010; Djimeli *et al*., 2017).

Ethanol extracts of *A. cordifolia* leaves have also been reported to exhibit sedative or depressant effects on the CNS at high doses in both male and female mice (Gatsing *et al*., 2010; Panche *et al*., 2016). These effects may function as myorelaxants or tranquilizers acting on the nervous system or motor fibers (Gatsing *et al*., 2010; Wang *et al*., 2018). Plants containing bioactive compounds such as flavonoids are known to exhibit CNS depressant activities, which could explain the observed effects (Panche *et al*., 2016). The impact of the extract on pain perception may result from inhibiting the production of algogenic substances like prostaglandins and histamines or blocking the transmission of pain signals at the CNS level (Wang *et al*., 2018). Flavonoids, known for targeting prostaglandins involved in the late phase of acute inflammation and pain perception, may contribute to the depressant effects observed in *A. cordifolia* ethanol extract (Gatsing *et al*., 2010; Wang *et al*., 2018).

The study also recorded variations in food and water intake among mice administered different concentrations of the ethanol extract of *A. cordifolia* leaves. Mice given higher concentrations exhibited reduced food and water intake, whereas lower concentrations appeared to stimulate increased food consumption. This observation aligns with findings from related studies (Gatsing *et al*., 2010). Additionally, excrement texture varied between groups: mice given lower doses produced granular excrement, whereas those administered higher doses had sticky or liquid excrement, indicative of mild diarrhoea. This suggests that higher doses of the extract may irritate the intestinal mucosa, leading to increased permeability of mucosal cells and altered electrolyte transport kinetics (Gatsing *et al*., 2010). Previous research has also

highlighted that saponins, a significant phytochemical component of *A. cordifolia*, may cause gastrointestinal side effects, including appetite suppression, weight loss, and gastroenteritis or diarrhea, as observed in other plant studies (Kengni *et al*., 2013; Djimeli *et al*., 2017). These findings underscore the potential dose-dependent physiological and biochemical impacts of *A. cordifolia* ethanol extract and provide a basis for further toxicological evaluation.

The survival rate and percentage mortality of infected but treated mice were monitored at intervals of 24 h, 48 h, 72 h, 96 h, and 120 h. Mice infected with *Enterobacter cloacae*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Acinetobacter baumannii*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* and subsequently treated with the bioactive ethanol extract of *Alchornea cordifolia* exhibited 100% survival throughout the 5-day post-treatment observation period (Table 4). Similarly, mice infected with *Escherichia coli* and *Staphylococcus epidermidis* showed a survival rate of 50% within the first 24 hours post-treatment, which remained consistent over the entire 5-day monitoring period (Table 4).

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Isolate code	MDR-SSI test isolates	Number of animals used	Number of deaths recorded within five days					Total number of deaths per group	Percentage death per group (%)	Percentage protection per group (%)
			1	2	3	4	5			
SAUTHC1	*E. cloacae*	6	-	-	-	-	-	0	0.0	100.0
SAUTHC2	*E. coli*	6	1	2	-	-	-	3	50.0	50.0
SAUTHC3	*K. pneumoniae*	6	-	-	-	-	-	0	0.0	100.0
SAUTHC4	*P. mirabilis*	6	-	-	-	-	-	0	0.0	100.0
SAUTHC5	*A. baumannii*	6	-	-	-	-	-	0	0.0	100.0
SAUTHC6	*S. aureus*	6	-	-	-	-	-	0	0.0	100.0
SAUTHC7	*P. aeruginosa*	6	-	-	-	-	-	0	0.0	100.0
SAUTHC8	*S. epidermidis*	6	1	1	1	-	-	3	50.0	50.0

Table 4: Mouse protection profile of *A. cordifolia* leaves ethanol extract against establishment of infection by MDR-SSI isolates

Keys: SAUTHC: SSI-Associated Unique Test Hospital-Isolate Code.

These findings align with earlier studies reporting no mortality within 46 hours after a single oral dose of *A. cordifolia* leaf extract administered to infected mice (Gatsing *et al*., 2010). Additional corroboration is provided by other research demonstrating the therapeutic efficacy of plant-based extracts in enhancing survival rates in treated animal models of bacterial infections (Ekong *et al*., 2015; Ekong and Okoro, 2016).

CONCLUSION

A. cordifolia exhibits potent antimicrobial activity, with promising applications in alternative medicine. These results underscore the potential of *A. cordifolia* ethanol extract as a protective therapeutic agent against multi-drug resistant bacterial pathogens, particularly those implicated in surgical site infections.

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CONFLICT OF INTEREST
The authors have no conflict of interest.

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