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# Phytochemical Screening and Antimicrobial Evaluation of *Alchornea cordifolia* (Schumach. & Thonn.) Müll.Arg. Leaves Extracts Against Multi-Drug-Resistant Bacterial Isolates from Post-Operative Wound Infections

Sylvanus Akpak Upula\*<sup>1</sup>, Ubong Samuel Ekong<sup>2</sup>.

1. Department of Microbiology, University of Cross River State, P.M.B 1123, Calabar-Nigeria.
2. Department of Pharmaceutical Microbiology and Biotechnology, University of Uyo, P.M.B 1017, Uyo, Nigeria.

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**ABSTRACT:** *The treatment of post-operative wound infections has been exacerbated by frequent evolvment of multi-drug resistant (MDR) pathogens. Consequently, this study evaluated the phytochemical and antibacterial properties of methanol, ethanol, and aqueous crude extracts of Alchornea cordifolia leaves for their efficacy against selected MDR bacterial isolates from patients with surgical site infection (SSI). Bacterial isolates obtained within 12 months from patients clinically diagnosed of SSI in five specialist hospitals in Calabar-Nigeria were analyzed and identified using standard techniques. Among the MDR-isolates, eight highly resistant bacterial isolates (Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Enterobacter cloacae, Acinetobacter baumannii, and Staphylococcus epidermidis) were selected for further evaluation. The susceptibility profiles of these isolates were assessed against crude extracts of Alchornea cordifolia leaves. Additionally, the minimum inhibitory concentrations (MIC), minimum bactericidal concentrations (MBC), and phytochemical properties of the extracts were determined to evaluate their antimicrobial potential. The ethanol crude extract of A. cordifolia leaves demonstrated superior broad-spectrum activity against the MDR-SSI isolates compared to the aqueous and methanol extracts, even at the lowest tested concentration of 62.5 mg/mL, and also exhibited an MBC/MIC ratio of  $\leq 4$  mg/mL, indicating bactericidal properties. Further qualitative phytochemical analysis of the extracts revealed the presence of bioactive compounds including alkaloids, flavonoids, tannins, cardiac glycosides, terpenoids, saponins, phenolics, and anthraquinones in varying concentrations. A. cordifolia leaves possesses potent antimicrobial properties and various phytochemical constituents and is therefore recommended for further studies towards potential drug development in order to enhance therapeutic options against MDR bacterial pathogens associated with SSI.*

**Keywords:** bacteria, antibiotics, multi-drug resistance, *alchornea cordifolia*, post-operative wounds, infections, phytochemicals.

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## INTRODUCTION

Post-operative wound infections represent a significant challenge in healthcare, contributing to patient morbidity, prolonged hospital stay, increased healthcare costs, and, in severe cases, mortality (Olowo-Okere *et al.*, 2019; Owusu *et al.*, 2021). These infections occur at the site of surgical incisions and are typically caused by microbial contamination which usually manifest after surgery (Pal *et al.*, 2019; Owusu *et al.*, 2021; Upula *et al.*, 2022). The global incidence of post-operative wound infections varies widely, ranging from 2% to 20%, depending on the surgical procedure, healthcare setting, and patient demographics (Allegranzi *et al.*, 2016; Olowo-Okere *et al.*, 2019; Owusu *et al.*, 2021). While many cases can be managed with appropriate second or third generation antibiotic therapy, the rise of multidrug-resistant (MDR) pathogens has made treatment increasingly complex, limiting the efficacy of conventional antibiotics and necessitating the search for novel therapeutic options (Magill *et al.*, 2014; Villari and Gentle, 2022).

Among the most common MDR pathogens implicated in post-operative wound infections are *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, and *Pseudomonas aeruginosa*. (Pal *et al.*, 2019; Upula *et al.*, 2019; Owusu *et al.*, 2021). These organisms exhibit resistance to multiple classes of antibiotics, including beta-lactams, fluoroquinolones, and aminoglycosides, posing a critical threat to patient treatment outcomes (WHO, 2017; Upula *et al.*, 2022; Villari and Gentle, 2022). In light of this growing challenge, the exploration of alternative antimicrobial agents, particularly from natural sources, has gained momentum.

Medicinal plants have long been recognized for their potential as sources of bioactive compounds with antimicrobial properties (Ogungbe *et al.*, 2013). In particular, *Alchornea cordifolia* (Schumach. & Thonn.) Müll.Arg., a member of the Euphorbiaceae family, has been traditionally used in ethnomedicine to treat infections, wounds, and inflammatory conditions (Akinmoladun *et al.*, 2018; Owusu *et al.*, 2021). The leaves of *A. cordifolia* are known to contain a diverse array of phytochemicals, including alkaloids, flavonoids, tannins, saponins, and phenolic compounds, many of which have demonstrated antimicrobial efficacy against bacterial pathogens, including antibiotic-resistant strains (Ogungbe *et al.*, 2013; Akinmoladun *et al.*, 2018).

Despite the documented antimicrobial potential of *A. cordifolia*, limited studies have evaluated its efficacy specifically against MDR pathogens associated with surgical site infections. Additionally, a comprehensive understanding of the phytochemical properties of its extracts is necessary to assess their therapeutic potential and suitability for formulation into clinical applications. Key phytochemical properties play a crucial role in the bioavailability, efficacy, and shelf-life of plant-based antimicrobial agents (Khan *et al.*, 2020).

This study aimed to address these gaps by evaluating the antimicrobial susceptibility of methanol, ethanol, and aqueous extracts of *A. cordifolia* leaves against selected MDR clinical isolates from post-operative wound infections. The investigation is complemented by an analysis of the physicochemical properties of the extracts. By integrating antimicrobial

susceptibility testing with physicochemical characterization, this research also sought to establish a scientific foundation for the use of *A. cordifolia* as a potential source of novel therapeutic agents for the management of MDR post-operative wound infections.

## MATERIALS AND METHODS

### Macroscopic Examination and Extraction of Medicinal Plant Materials

The ethnomedicinal plant used in this study was collected and identified at the Herbarium unit of the Department of Pharmacognosy and Natural Medicine, University of Uyo, and authenticated at the Department of Botany and Ecological Studies University of Uyo. The plant and its corresponding voucher number was: *Alchornea cordifolia*; UUPH 31(b). *Alchornea cordifolia* leaves (120.0 g) was examined macroscopically for the presence of any foreign matter or contaminants including insects, molds, and undesirable material. Thereafter the plant material was washed with distilled water and air-dried at room temperature (28–32 °C) for 14 days in a shed. The completely dried medicinal plant leaves were subsequently pulverized.

For both methanol and ethanol extraction, 120.0 g of pulverized, *A. cordifolia* leaves was accurately weighed using an analytical balance. The material was then combined with 3.5 L of 70% v/v methanol and allowed to macerate at room temperature (28–32 °C) for four days with occasional stirring. Following maceration, 70% of the solvent mixture was decanted into corresponding containers and filtered through Whatman No. 1 filter paper. The resulting extract was concentrated using a drying oven at a relatively low temperature of 38 °C for 24 h and subsequently stored at 4 °C in a refrigerator. The extraction process followed previously established methodologies (Ngoupayo *et al.*, 2015; Ogie-Odia *et al.*, 2019; Olayemi *et al.*, 2019; Nigussie *et al.*, 2021).

For aqueous extraction, 120.0 g of pulverized, dried plant material (*A. cordifolia* leaves) was boiled in 3.5 L of distilled water for approximately 1.5 h and then strained. The aqueous extract was filtered using Whatman No. 1 filter paper and allowed to cool for 45 min. The extract was then concentrated in a drying oven at 38 °C for 24 h and stored at 4 °C in a refrigerator. This method also followed protocols reported in earlier studies (Ngoupayo *et al.*, 2015; Ogie-Odia *et al.*, 2019; Olayemi *et al.*, 2019).

After complete drying, the yield of each extract was measured individually and stored at 4 °C for subsequent analysis. The percentage yield of all extracts was calculated following established protocols as previously described (Ogie-Odia *et al.*, 2019; Olayemi *et al.*, 2019; Nigussie *et al.*, 2021).

$$\text{Percentage yield (\%)} = \frac{\text{Dry weight of extract}}{\text{Dry weight of plant material}} \times 100$$

### Qualitative Phytochemical Screening of Bioactive Crude Extract

Phytochemical analysis was conducted using established standard procedures to identify the active constituents in the extracts. The analysis included tests for alkaloids, saponins, phenols, tannins, anthraquinones, terpenoids, flavonoids, glycosides, cardiac glycosides, and steroids.

The methodologies followed were consistent with those reported in previous studies (Pandey *et al.*, 2014; Ogie-Odia *et al.*, 2019; Srivastava *et al.*, 2021).

### **Preparation of Extract Concentrations and Sterility Testing**

All extracts used in this study were reconstituted in the polar aprotic solvent dimethyl sulfoxide (DMSO) to prepare solutions at concentrations of 500.0 mg/mL, 250.0 mg/mL, 125.0 mg/mL, and 62.5 mg/mL. For aqueous extracts, water was used as the diluent. Sterility testing was performed following the method described by Owusu *et al.*, (2021). Specifically, 1 mL of each extract was inoculated into 5 mL of nutrient broth and incubated at 37 °C for 24 hours. The absence of turbidity in the broth confirmed the sterility of the extracts.

### **Identification of Bacterial Isolates**

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

### **Antimicrobial Susceptibility Evaluation of Crude Extracts**

The agar well diffusion method was used to evaluate the antibacterial activity of *A. cordifolia* extracts against MDR surgical site infection isolates as described in previous studies (Ekong *et al.*, 2013; Mordi *et al.*, 2016; Adeonipekun *et al.*, 2018). A single colony from each pure culture of the test isolates was collected from the 18-24 h agar plates, using a sterile loop and inoculated into 4 mL of peptone broth. The turbidity of each inoculum was adjusted to 0.5 McFarland standard, equivalent to a suspension of  $1.5 \times 10^8$  CFU. Mueller Hinton agar plates were seeded by spreading 100.0 µL of the standardized bacterial suspension(s) evenly, followed by a 5-minute drying period. Using a sterile 6 mm cork borer, four equidistant wells were created on each plate, with a fifth well positioned at the center. To prevent the extracts from diffusing beneath the agar, one drop of sterile molten agar was used to seal the bottom of each well. Subsequently, 100.0 µL of various extract concentrations (62.5 mg/mL, 125.0 mg/mL, 250.0 mg/mL, and 500.0 mg/mL) was dispensed into the labelled wells, with the fifth well serving as a control (positive or negative). The plates were refrigerated for one hour to facilitate diffusion of the extracts into the medium, then incubated at 37 °C for 24 h. Zones of inhibition were measured in millimetres and analyzed. All experiments were conducted in duplicates.

### **Minimum Inhibitory Concentration Determination**

The Minimum Inhibitory Concentration (MIC) was determined using the broth dilution method, following protocols outlined in previous studies (Ekong *et al.*, 2013; Adeonipekun *et al.*, 2018; Ekong *et al.*, 2019). The crude extract of *A. cordifolia* leaves was diluted in nutrient broth to prepare a range of concentrations: 500.0 mg/mL, 250.0 mg/mL, 125.0 mg/mL, 62.5 mg/mL, 31.2 mg/mL, 15.6 mg/mL, 7.8 mg/mL, and 3.9 mg/mL. Using a standard micropipette, 100 µL of the standardized bacterial suspension was added to each tube containing a specific extract concentration. Negative controls consisted of tubes with only sterile growth medium, while positive controls contained growth medium inoculated with each test isolate. All setups were

incubated at 37 °C for 18-24 h. The MIC was recorded as the lowest concentration of the crude extract that inhibited visible growth of the test isolates, consistent with previously reported methods (Adeonipekun *et al.*, 2018; Ekong *et al.*, 2019).

### Minimum Bactericidal Concentration Determination

The Minimum Bactericidal Concentration (MBC) of the crude extract of *A. cordifolia* leaves was determined using established protocols as described in previous studies (Ekong *et al.*, 2013; Adeonipekun *et al.*, 2018; Ekong *et al.*, 2019). Following the MIC assay, samples from tubes with no visible growth (turbidity) were sub-cultured onto freshly prepared Mueller-Hinton agar plates devoid of the extract. The plates were incubated at 37 °C for 24 h. The MBC was defined as the lowest concentration of the crude extract, as determined from the MIC tubes, that completely inhibited bacterial growth on the agar plates. This procedure aligns with methodologies reported in earlier studies (Adeonipekun *et al.*, 2018; Ekong *et al.*, 2019).

### Ethical Consideration

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

### Data and Statistical Analysis

Data was analyzed statistically using SPSS (v.25), and Microsoft Excel software. Comparative analyses of continuous variables were analyzed using t-test for two groups, and one-way analysis of variance (ANOVA) for more than two groups. *P*-value of 0.05 was considered statistically significant.

## RESULTS AND DISCUSSION

For any ethnomedicinal plant to be pragmatically considered as an effective treatment option against pathogenic infections, they must be scientifically assessed (Siwe-Noundou *et al.*, 2016, Hemeg *et al.*, 2020). In the present study, the percentage yield of crude extracts revealed that the aqueous crude extract of *A. cordifolia* leaves generated a higher extract yield (23.23 %) compared to the percentage yield of methanol extract (20.78 %), and ethanol crude extract (18.32 %) (Table 1). This corroborates the findings of Adam *et al.*, (2019), that the yield of plant extracts varies based on method of extraction and the plant material.

**Table 1: Percentage yield of ethanol and aqueous crude extract of *A. cordifolia* leaves.**

| S/N | Extracts | Weight of plant sample<br>(g) | Weight of extract<br>(g) | Percentage yield<br>(%) |
|-----|----------|-------------------------------|--------------------------|-------------------------|
| 1   | Ethanol  | 120                           | 21.98                    | 18.32                   |
| 2   | Aqueous  | 120                           | 27.87                    | 23.23                   |
| 3   | Methanol | 120                           | 24.93                    | 20.78                   |

Result from the phytochemical screening revealed that the crude plant extracts of *A. cordifolia* leaves contained phytoconstituents including alkaloid, flavonoid, phenolic, saponins, tannins, cardiac glycosides, anthraquinones, and terpenoids at varying concentrations (Table 2). Similar

studies have also documented the pharmaceutical and medicinal importance of the aforementioned phytochemical components (Hackman *et al.*, 2020; Owusu *et al.*, 2021; Bisso *et al.*, 2022). For instance, tannin have been documented to possess inhibitory effects against enzymes of major wound pathogens due to its protein precipitation activities, thereby facilitating the formation of protective coatings when topically applied on wounds, and optimizing its efficacy in the local treatment of skin sores and contaminated wounds (Hackman *et al.*, 2020; Owusu *et al.*, 2021).

Additionally, *A. cordifolia* leaves which is a saponin containing plants, has been reported to contain haemolytic and anti-inflammatory properties (Siwe-Noundou *et al.*, 2016; Bisso *et al.*, 2022). Tannins are also medicinally significant due to their astringent properties as they have been shown to promote rapid healing and the formation of new tissues on wounds and inflamed mucosa (Nghaha *et al.*, 2016). Furthermore, the presence of flavonoids and phenolic compounds suggests that it can be used as an anti-spasmodic and antimicrobial agent, thereby affirming its medicinal potential for the treatment of microbial infections, including wound healing properties as reported in previous studies (Hackman *et al.*, 2020; Owusu *et al.*, 2021). Additionally, flavonoids have been considered to possess anti-inflammatory, anti-oxidative, anti-mutagenic and anti-carcinogenic properties including its ability to modulate vital cellular enzyme functions (Panche *et al.*, 2016).

The resistance profile and MAR-index of the selected eight (8) MDR-SSI bacterial isolates are as shown (Table 3). Result from the *in-vitro* susceptibility analysis of the methanol, ethanol, and aqueous crude extracts of *A. cordifolia* leaves against the MDR-SSI test isolates indicated that the ethanol extract exhibited the most potent inhibitory activity by demonstrating superior broad-spectrum antibacterial activity against the MDR-SSI test isolates (Table 3). The ethanol crude extract demonstrated clear zones of inhibition in all concentrations against the MDR-SSI test isolates (Figure 1). Statistical analysis affirmed that the antimicrobial activity of the ethanol crude extract, measured by the zone of inhibition, was significantly greater ( $P < 0.05$ ), compared to the methanol and aqueous extracts. Furthermore, the antimicrobial efficacy of all extracts was concentration-dependent, with higher concentrations producing significantly greater inhibition ( $P < 0.05$ ) than lower concentrations of the same extracts against the MDR-SSI test isolates.

Further comparative susceptibility analysis of the methanol, ethanol, and aqueous crude extracts of *A. cordifolia* leaves, showed that the ethanol extract of *A. cordifolia* leaves exerted the highest antibacterial inhibitory activity against the MDR-SSI test isolates at all concentrations (Table 4). This finding corroborates results from similar studies which reported that ethanol extract of *A. cordifolia* leaves exerted highest inhibition to the growth of bacterial MDR pathogens isolated from post-operative wounds (Hackman *et al.*, 2020; Owusu *et al.*, 2021).

The values of the diameters of inhibition, MICs and MBCs revealed that the degree of activity of *A. cordifolia* varied among the test isolates (Table 5). Furthermore, the MIC/MBC index against the MDR-SSI test isolates in the present study were within the range of 2 to 4 suggesting possible bactericidal activity. Similar (MIC/MBC) ratio had been previously reported against both Gram positive and Gram-negative pathogenic isolates including *S. aureus*, *E. coli*,

*Klebsiella spp*, *Pseudomonas aeruginosa* (Okwu and Ukanwa, 2010; Ekong *et al.*, 2015). Antimicrobial substances are considered as bactericidal agent when the ratio MIC/MBC  $\leq$  4 and bacteriostatic when the ratio MBC/MIC is  $>$  4 (Ngoupayo *et al.*, 2015; Ekong *et al.*, 2015; Ekong *et al.*, 2019).

This antimicrobial activity variation has been reported in a previous study to be due to factors including the active compounds present in each solvent and their interactions, the solubility of active constituents in each solvent, the nature of each solvent, and the cell composition of the tested isolates (Gatsing *et al.*, 2010). These results are close to those obtained in similar studies, that the MIC and MBC of ethanol extracts of *A. cordifolia* leaves against most MDR bacterial pathogens including *Pseudomonas aeruginosa*, *Escherichia coli*, *Proteus mirabilis*, *Klebsiella pneumonia* and *Staphylococcus aureus* were of lesser concentrations when compared to the MIC and MBC of aqueous and methanol extracts of *A. cordifolia* leaves (Ngoupayo *et al.*, 2015; Owusu *et al.*, 2021).

Additionally, in this study, the MIC obtained for the ethanol extract of *A. cordifolia* leaves, contradicts the notion that the effectiveness of plant extracts is higher against Gram-positive than Gram-negative bacteria, as both Gram-positive and Gram-negative MDR-SSI isolates tested against the *A. cordifolia* extract were inhibited at concentrations ranging between 32.25 – 125mg/mL (Table 5). This result corroborates findings from similar studies which reported equal effectiveness based on MIC of plant extracts against Gram-positive than Gram-negative bacteria (Nmema *et al.*, 2010; George *et al.*, 2010; Ngoupayo *et al.*, 2015; Osuntokun *et al.*, 2019; Owusu *et al.*, 2021).

**Table 2: Phytochemical screening of ethanol, aqueous and methanol crude extracts of *A. cordifolia* leaves.**

| Phytochemical Compounds |                                  |         |          |         |            |           |          |            |            |                       |                |
|-------------------------|----------------------------------|---------|----------|---------|------------|-----------|----------|------------|------------|-----------------------|----------------|
| S/N                     | <i>A. cordifolia</i><br>Extracts | Tannins | Saponins | Phenols | Terpenoids | Alkaloids | Steroids | Glycosides | Flavonoids | Cardiac<br>Glycosides | Anthraquinones |
| 1                       | Ethanol                          | ++      | ++       | +       | +          | +         | +        | ++         | +          | -                     | -              |
| 2                       | Aqueous                          | +++     | ++       | -       | -          | -         | ++       | -          | +          | -                     | -              |
| 3                       | Methanol                         | ++      | ++       | +       | -          | +         | +        | +          | +          | -                     | ++             |

Keys: +++: Abundantly present, ++: Moderately present, +: Trace amount, -: Absent.

**Table 3: Multi-drug resistance profile of selected SSI-MDR test isolates.**

| S/N | Isolate code | SSI MDR<br>Isolates   | Antibiotics resistance pattern of isolates (Antibiotype) | Number of antibiotics<br>resisted (a) | Total number of<br>antibiotics tested (b) | MAR index<br>(a/b) |
|-----|--------------|-----------------------|--|---------------------------------------|---|--------------------|
| 1   | SAUTHC 1     | <i>E. cloacae</i>     | CRO, CEF, CAZ, PIP, AMP, TOB, TE, LEV, CIP               | 9                                     | 15  | 0.6                |
| 2   | SAUTHC 2     | <i>E. coli</i>        | CRO, CZN, CEF, PIP, AMP, MEM, TOB, GM, TE, LEV, AMS      | 11                                    | 15  | 0.7                |
| 3   | SAUTHC 3     | <i>K. pneumoniae</i>  | CZN, CEF, PIP, AMP, TOB, GM, TE, LEV, SXT                | 9                                     | 15  | 0.6                |
| 4   | SAUTHC 4     | <i>P. mirabilis</i>   | CEF, CAZ, AMP, TOB, AK, TE, CIP, SXT                     | 8                                     | 15  | 0.5                |
| 5   | SAUTHC 5     | <i>A. baumannii</i>   | CEF, CAZ, PIP, TOB, TE, CIP, AMS                         | 7                                     | 13  | 0.5                |
| 6   | SAUTHC 6     | <i>S. aureus</i>      | PIP, AMP, OXA, AK, LEV, CIP, SXT, CLN, MEM               | 9                                     | 13  | 0.7                |
| 7   | SAUTHC 7     | <i>P. aeruginosa</i>  | CZN, CEF, CAZ, MEM, AK, CIP                              | 6                                     | 10  | 0.6                |
| 8   | SAUTHC 8     | <i>S. epidermidis</i> | PIP, AMP, OXA, TOB, TE, CIP, SXT                         | 7                                     | 10  | 0.7                |

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



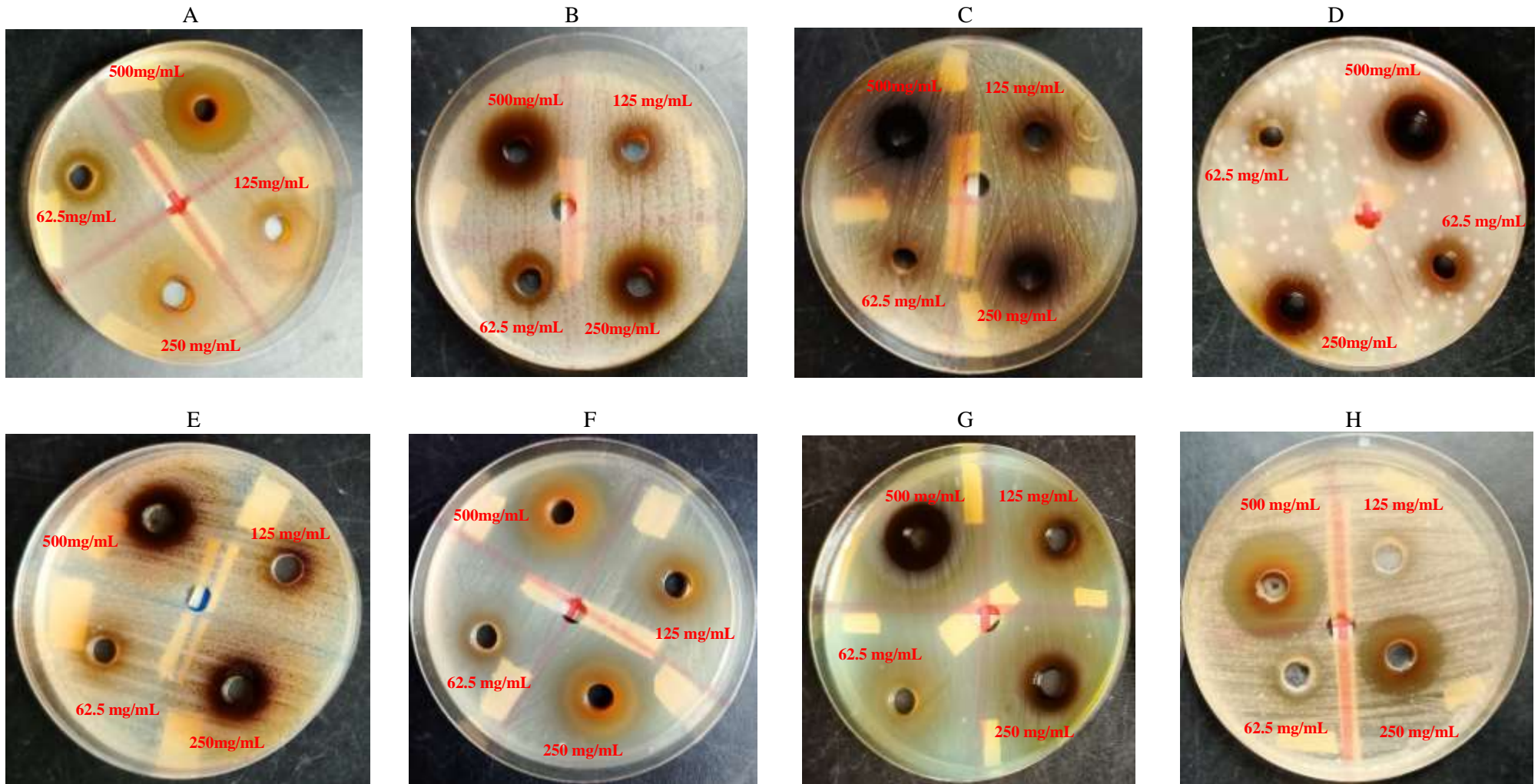
Website: <https://www.eajournals.org/>

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**Table 4: Comparative mean antibacterial susceptibility profile of *A. cordifolia* leaves crude extracts against MDR-SSI isolates**

| Isolate Code | Test isolate          | <i>A. cordifolia</i> leaves extracts mean inhibition zone diameter (mm) |            |            |
|--------------|-----------------------|---|------------|------------|
|              |                       | Ethanol   | Aqueous    | Methanol   |
| SAUTHC1      | <i>E. cloacae</i>     | 26.0 ± 1.41   | 23.5 ± 2.1 | 12.0 ± 0.0 |
| SAUTHC2      | <i>E. coli</i>        | 22.0 ± 0.0  | 20.5 ± 0.7 | 13.5 ± 2.1 |
| SAUTHC3      | <i>K. pneumoniae</i>  | 18.5 ± 0.7  | 13.0 ± 0.0 | 20.0 ± 1.4 |
| SAUTHC4      | <i>P. mirabilis</i>   | 19.0 ± 0.0  | 20.0 ± 0.0 | 18.0 ± 0.0 |
| SAUTHC5      | <i>A. baumannii</i>   | 17.0 ± 1.4  | 0.0 ± 0.0  | 11.0 ± 0.7 |
| SAUTHC6      | <i>S. aureus</i>      | 28.5 ± 0.7  | 22.5 ± 0.7 | 19.0 ± 0.0 |
| SAUTHC7      | <i>P. aeruginosa</i>  | 25.0 ± 1.4  | 24.0 ± 1.4 | 16.0 ± 0.0 |
| SAUTHC8      | <i>S. epidermidis</i> | 27.0 ± 0.0  | 26.5 ± 0.7 | 20.0 ± 0.0 |

Key: SAUTH: SSI-Associated Unique Test Hospital isolate code.



**Figure 1: Antimicrobial susceptibility profile of ethanol crude extract of *A. cordifolia* leaves against MDR-SSI isolates at different concentrations. (A) *E. cloacae*, (B) *E. coli*, (C) *K. pneumoniae*, (D) *P. mirabilis*, (E) *A. baumannii*, (F) *S. aureus*, (G) *P. aeruginosa*, (H) *S. epidermidis*.**

**Table 5: MIC/MBC of *A. cordifolia* leaves crude extracts on MDR-SSI isolates.**

| Isolate Code | Bacteria              | <i>A. cordifolia</i> leaves (ethanol) |             |               | <i>A. cordifolia</i> leaves (aqueous) |             |               | <i>A. cordifolia</i> leaves (methanol) |             |               |
|--------------|-----------------------|---------------------------------------|-------------|---------------|---------------------------------------|-------------|---------------|--|-------------|---------------|
|              |                       | MIC (mg/mL)                           | MBC (mg/mL) | MIC/MBC Index | MIC (mg/mL)                           | MBC (mg/mL) | MIC/MBC Index | MIC (mg/mL)                            | MBC (mg/mL) | MIC/MBC Index |
| SAUTHC1      | <i>E. cloacae</i>     | 62.5                                  | 250         | 4             | 62.5                                  | 250         | 4             | 500                                    | 1000        | 2             |
| SAUTHC2      | <i>E. coli</i>        | 62.5                                  | 250         | 4             | 62.5                                  | 250         | 4             | 500                                    | 1000        | 2             |
| SAUTHC3      | <i>K. pneumoniae</i>  | 62.5                                  | 250         | 4             | 62.5                                  | 250         | 4             | 500                                    | 1000        | 2             |
| SAUTHC4      | <i>P. mirabilis</i>   | 31.25                                 | 125         | 4             | 62.5                                  | 250         | 4             | 500                                    | 1000        | 2             |
| SAUTHC5      | <i>A. baumannii</i>   | 62.5                                  | 125         | 2             | 62.5                                  | 125         | 2             | 500                                    | 1000        | 2             |
| SAUTHC6      | <i>S. aureus</i>      | 62.5                                  | 125         | 2             | 62.5                                  | 125         | 2             | 500                                    | 1000        | 2             |
| SAUTHC7      | <i>P. aeruginosa</i>  | 62.5                                  | 250         | 4             | 62.5                                  | 250         | 4             | 500                                    | 1000        | 2             |
| SAUTHC8      | <i>S. epidermidis</i> | 62.5                                  | 125         | 2             | 62.5                                  | 250         | 4             | 500                                    | 1000        | 2             |

\*Antimicrobial substances are considered as bactericidal agent when the ratio of MIC/MBC  $\leq 4$ , and bacteriostatic when the ratio of MBC/MIC is  $> 4$ .

## CONCLUSION

This study affirmed that the *A. cordifolia* leaves represents a novel source of bioactive compounds employable as an antimicrobial agent against MDR-SSI bacterial pathogens, and is a rich source of relevant bioactive phytochemicals that do not only enhance antibacterial properties, but also ascertain vital health-promoting qualities. Therefore, the broad antibacterial potentials embedded in the extracts of *A. cordifolia* leaves can be exploited to produce novel broad-spectrum antibiotics to circumvent the ever-emerging trend of multiple drug-resistant pathogenic microorganisms causing wound infections including SSIs.

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## Completing Interests

No competing interest exists.

## Authors' Contributions

All authors were involved in the design and execution of the study, including reading and approving the final manuscript.

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