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Phytochemical Synergy and Antibacterial Activity of Crude and Fractionated Extracts of *Senna occidentalis* and *Croton zambesicus* Targeting Multidrug-Resistant Pathogens

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ABSTRACT

Introduction: The escalating burden of multidrug-resistant (MDR) bacterial infections has intensified the search for alternative antimicrobial agents. Medicinal plants rich in bioactive metabolites provide a promising source of new therapeutic leads. This study evaluated the in vitro antimicrobial activity of crude and fractionated leaf extracts of *Senna occidentalis* and *Croton zambesicus* against selected MDR clinical bacterial isolates.

Methods: Crude extracts were obtained by maceration and fractionated using liquid–liquid partitioning. Antibacterial activity was assessed via agar well diffusion and broth dilution assays. Minimum inhibitory (MIC) and bactericidal concentrations (MBC) were determined, and phytochemical composition was characterized using Gas Chromatography–Mass Spectrometry (GC–MS).

Results: Crude extracts exhibited broad-spectrum activity, with inhibition zones ranging from 12.67 ± 0.67 mm to 18.00 ± 0.00 mm. Fractionation reduced overall potency, though select n-hexane and ethyl acetate fractions retained moderate activity. MIC and MBC values indicated a concentration-dependent transition from bacteriostatic to bactericidal effects. GC–MS analysis revealed major constituents including unsaturated fatty acids, long-chain aldehydes, phytol derivatives, and sesquiterpenes.

Conclusion: *S. occidentalis* and *C. zambesicus* demonstrate notable antimicrobial potential, supporting further exploration for plant-based antibacterial agents and targeted isolation of bioactive compounds.

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Introduction

Medicinal plants have been central to human healthcare for millennia, serving both preventive and curative roles through their teeming diversity of bioactive compounds (Dal Cero et al., 2022; Mayimele et al., 2025). These phytochemicals found in different plant organs exhibit antimicrobial, anti-inflammatory, haematopoietic, and other pharmacological activities that continue to support their use in traditional and modern medicine (Vaou et al., 2021; Oluyele et al., 2022; Agu et al., 2025). Although synthetic pharmaceuticals dominate contemporary therapeutics, medicinal plants remain indispensable, predominantly in economically constrained regions where they provide accessible, affordable, and culturally accepted remedies (Vlachogianni et al., 2014).

The escalating prevalence of multidrug-resistant (MDR) pathogens has triggered renewed scientific interest in plant-derived antimicrobials (Marino et al., 2025; Oluyele et al., 2025a). Plant-based formulations often exhibit broad-spectrum activity, synergistic interactions, and reduced toxicity compared to conventional antibiotics, making them attractive leads for drug discovery (Omutindo, 2025). Natural compounds have historically informed modern drug development, and continue to offer structurally diverse scaffolds with therapeutic potential (Chaachouay and Zidane, 2024).

Croton zambesicus (Euphorbiaceae), commonly known as bushveld croton and "Ajekobale" in Yoruba, and *Senna occidentalis* (Caesalpiniaceae), known as coffee senna or stinking weed, are widely used medicinal plants across tropical regions. *C. zambesicus* is a shrub or small tree traditionally valued not only for treating urinary infections, malaria, dysentery, diabetes, cancer, and hypertension but also for its cultural role as a protective plant reputed to ward off malevolent influences (Matenyane et al., 2021; Isyaka et al., 2024; Obende et al., 2024). Its diverse phytochemicals flavonoids, tannins, steroids, saponins, and phenolic compounds account for its broad pharmacological properties (Coy-Barrera et al., 2025). *S. occidentalis*, a semi-woody herb from Africa and Asia, is traditionally used for gastrointestinal, infectious, and systemic ailments and exhibits laxative, analgesic, febrifuge, diuretic, hepatoprotective, and vermifuge properties (Matsabisa et al., 2022). Its noted to possess anthraquinones, flavonoids, sterols, tannins, saponins, and oils (Ishaya et al., 2022; Yadav et al., 2025). Together, their ethnomedicinal significance and rich phytochemistry highlight both plants as promising sources of novel antimicrobial agents.

The worldwide escalation of antimicrobial resistance (AMR) represents a critical public health challenge, leading to higher rates of treatment failure, extended illness duration, and increased healthcare expenditures (Oluyele et al., 2023; Ahmed et al., 2024). Infections caused by multidrug-resistant (MDR) bacteria frequently necessitate the use of last-resort antibiotics, which are expensive, less widely available, and often accompanied by significant side effects (Bharadwaj et al., 2022; Macesic et al., 2025). These circumstances highlight the pressing need to discover novel, effective, and affordable antimicrobial agents, with natural products offering particularly promising avenues.

Given their long-standing ethnomedicinal use and diverse phytochemical profiles, *C. zambesicus* and *S. occidentalis* are promising candidates for developing new antibacterial agents. Despite their traditional applications, there is limited comparative evidence on their efficacy against clinically confirmed MDR bacterial isolates, and the impact of systematic fractionation on their activity remains largely unexplored. This study addresses these gaps by evaluating, the in-vitro antimicrobial activity of both crude and fractionated leaf extracts of *S. occidentalis* and *C. zambesicus* against selected MDR clinical bacterial strains, providing insights into the role of phytochemical synergy in plant-based antibacterial activity.

Materials and Methods

Test Organisms and Inoculum Preparation

Multidrug-resistant (MDR) clinical isolates employed in this study were sourced from the Microbiology Laboratory of our institution. The test panel comprised *Klebsiella aerogenes*, *Escherichia coli*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. Inoculum preparation followed standard procedures. A 0.5 McFarland turbidity standard was produced by combining 0.05 mL of 1% barium chloride dihydrate ($BaCl_2{\cdot}2H_2O$) with 9.95 mL of 1% sulfuric acid (H_2SO_4) to generate a barium sulfate suspension equivalent to 1.0% w/v. Each bacterial isolate was subcultured on nutrient agar and incubated at 37 °C for 18 hours, after which colonies were suspended in sterile normal saline and adjusted visually to the 0.5 McFarland benchmark. The resulting inocula corresponded to approximately 1×10^6 CFU/mL, which served as the working concentration for antimicrobial assays (Oluyele et al., 2023).

Plant Specimen Collection and Verification

Newly harvested leaves of *S. occidentalis* (Linn.) (family Fabaceae, subfamily Caesalpinoideae) and *C. zambesicus* were collected from the local environment and authenticated by taxonomists at

the Plant Science and Biotechnology Department, Adekunle Ajasin University, Akungba-Akoko. The verified specimens were subsequently deposited in the departmental herbarium under voucher numbers PSB-253 (*S. occidentalis*) and PSB-259 (*C. zambesicus*) for future reference.

Preparation and Crude Extraction

Leaves were rinsed thoroughly, shade-dried at 25–28 °C under ambient humidity (55–65%) and protected from direct sunlight, then pulverized into fine powder using an electric blender. Crude extraction was carried out by maceration: 500 g of *S. occidentalis* powder was soaked in 3.5 L of ethanol for 96 h, while 600 g of *C. zambesicus* powder was soaked in 4 L of 70% ethanol for the same duration, with intermittent agitation to enhance solute–solvent interaction. The mixtures were filtered through muslin cloth followed by Whatman No. 1 filter paper, and the resulting filtrates were concentrated under reduced pressure using a rotary evaporator (Heidolph Laborota 4000, Germany) at 40 °C; any remaining aqueous residues were dried using a freeze dryer (Labconco FreeZone 2.5, USA). All crude extracts were stored in airtight containers at 4 °C until further use.

Fractionation of Crude Extracts

Fractionation of the crude extracts was carried out using a liquid–liquid partitioning protocol in which each crude extract was reconstituted in distilled water at a 1:10 (w/v) ratio and transferred into a separatory funnel for successive partitioning with n-hexane and ethyl acetate. The process yielded two n-hexane fractions (F1–F2), two ethyl acetate fractions (F3–F4), and an aqueous fraction (F5) for *S. occidentalis*, while *C. zambesicus* produced two n-hexane fractions (F1–F2), one ethyl acetate fraction (F3), and an aqueous fraction (F4). All organic fractions were concentrated under reduced pressure using a rotary evaporator, and aqueous fractions were freeze-dried before being stored at 4 °C.

Assay for Antibacterial Activity of the Extracts

The antibacterial activity of the extracts was evaluated using the agar well diffusion method (Oluyele et al., 2025a). Sterile Mueller–Hinton agar plates were inoculated with 1 mL of standardized bacterial suspension (0.5 McFarland) and allowed to air-dry. Wells of 6 mm diameter were aseptically created and filled with 100 µL of extract solution (100 mg/mL in 5% dimethyl sulfoxide). Amoxicillin, gentamicin, and ofloxacin served as reference antibiotics. Plates were pre-diffused at room temperature for 15 min and then incubated at 37 °C for 24 h, after which zones of inhibition were measured in millimeters. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values were determined using standard broth dilution and subculture procedures. Serial two-fold dilutions of the extracts were prepared in Mueller–Hinton broth, inoculated with bacterial suspension, and incubated at 37 °C for 24 h. The MIC was recorded as the lowest concentration showing no visible turbidity, while the MBC was defined as the lowest concentration producing no bacterial growth upon subculture.

Data Analysis

All experiments were performed in triplicate (n = 3), and results were presented as mean ± standard deviation (SD). Data analysis was conducted using the Statistical Package for the Social Sciences (SPSS) version 22.0 (IBM Corp., Armonk, NY, USA). One-way analysis of variance (ANOVA) was used to assess differences among groups, and significant variations between means were identified using Duncan's New Multiple Range Test, with statistical significance set at $p \leq 0.05$.

Gas Chromatography Mass spectrometric analysis of Extracts

The phytochemical constituents of the samples were profiled using a Shimadzu QP2010 Ultra GC–MS system (Kyoto, Japan) fitted with an Rtx-5MS fused-silica capillary column (30 m × 0.25 mm, 0.25 µm film thickness). Helium of high purity (99.999%) served as the mobile phase, flowing at a constant rate of 1.0 mL/min. Each diluted extract (0.5 µL in n-hexane) was introduced in split mode at a ratio of 1:5, with the injector temperature maintained at 250 °C. The oven program began at 60 °C (2-min hold), increased to 180 °C at 3 °C/min, and then progressed to 300 °C at 10 °C/min with a final 10-min hold. The interface and ion source were both kept at 280 °C, and ionization occurred under electron-impact conditions at 70 eV. The mass spectrometer scanned ions between m/z 40 and 500 using a quadrupole analyzer coupled to an electron multiplier detector. Compounds were identified by matching their mass spectra with reference spectra in the NIST 14 and Wiley databases and by comparing calculated retention indices with those derived from a C7–C28 n-alkane series analyzed under the same conditions. Only constituents exhibiting a match factor of at least 85% together with consistent retention times and RI values were considered reliable identifications. Data acquisition and interpretation were performed using Shimadzu LabSolutions GC–MS Postrun Analysis software (Oluyele et al., 2025a).

Results

Antibacterial Activity of *C zambesicus* Extracts

The inhibitory activity of crude versus fractionated plant extracts of *C. zambesicus* is presented in Table 1. The crude extract demonstrated broad-spectrum activity against all MDR pathogens tested, with inhibition zones ranging from 15.00 ± 0.58 mm against *E. coli* to 18.00 ± 0.00 mm against *K. aerogenes*. Fractionation led to a marked decrease in antibacterial potency. The n-hexane fraction (F1) retained moderate activity, inhibiting *E. coli*, *S. aureus*, and *K. aerogenes*, whereas fractions F2, F3, and F4 exhibited no detectable activity against most organisms. Statistical analysis indicated significant differences between crude extract and fractions ($p \leq 0.05$). The MIC and MBC of *C. zambesicus* extracts are presented in Table 2. MIC values for the crude extract ranged from 3.125 ± 0.00 mg/mL to 12.5 ± 0.00 mg/mL, and MBC values spanned from 12.5 ± 0.00 mg/mL to 50 ± 0.00 mg/mL. Statistical comparisons indicated that MIC and MBC values of crude extracts were significantly different from fractions ($p \leq 0.05$).

Antibacterial Activity of *S. occidentalis* Extracts

The antibacterial effects of *S. occidentalis* extracts are shown in Table 3. The unfractionated extract exhibited potent inhibitory activity against all test organisms, with inhibition zones between 12.67 ± 0.67 mm and 16.67 ± 0.67 mm. Activity decreased substantially after fractionation, with both n-hexane fractions showing minimal or no inhibition. Moderate activity was recorded in the ethyl acetate and aqueous fractions for selected pathogens, particularly *K. aerogenes*. Statistical analysis confirmed significant differences between crude extract and fractions ($p \leq 0.05$). MIC and MBC results are presented in Table 4. MIC values for the crude extract ranged from 3.125 ± 0.00 to 6.25 ± 0.00 mg/mL, and MBC ranged from 25 ± 0.00 to 100 ± 0.00 mg/mL, showing statistically significant differences between crude and fractionated extracts ($p \leq 0.05$).

Phytochemical Profile of *C. zambesicus* and *S. occidentalis* Crude Extracts

The phytochemical constituents identified in the GC–MS analysis of *C. zambesicus* are presented in Table 5. The extract contained nineteen compounds dominated by unsaturated fatty acids, including cis-13-octadecenoic acid and 9-octadecenoic acid, cis-vaccenic acid. Sesquiterpenes such as caryophyllene and copaene were also detected. Similarly, *S. occidentalis* yielded thirty compounds as depicted in Table 6. The major constituents were long-chain fatty acids, aldehydes, phytol derivatives, and other hydrocarbons, many of which are documented antimicrobial agents.

Table 1: Antibacterial potentials of *Croton zambesicus* leaf extract against multidrug resistant bacteria

Organism	Crude Extract (mm)	Fraction 1 (mm)	Fraction 2 (mm)	Fraction 3 (mm)	Fraction 4 (mm)
Escherichia coli	15.00±0.58b	17.00±0.00c	0.00±0.00a	0.00±0.00a	0.00±0.00a
Acinetobacter baumannii	17.00±0.00c	10.67±0.67b	0.00±0.00a	0.00±0.00a	0.00±0.00a
Staphylococcus aureus	16.67±0.33c	18.00±0.00a	15.33±0.67b	0.00±0.00a	0.00±0.00a
Klebsiella aerogenes	18.00±0.00e	11.33±0.67d	7.33±0.67c	0.00±0.00a	2.67±0.33b
Pseudomonas aeruginosa	17.33±0.67b	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00a
Klebsiella pneumoniae	15.33±0.67c	12.00±0.00b	0.00±0.00a	0.00±0.00a	0.00±0.00a

F1=n-hexane fraction 1, F2 =n-hexane fraction 2, F3= Ethyl acetate fraction, F4= Aqueous Fraction. Values with different letters within each column are significantly different ($p \leq 0.05$).

Table 2: Minimum inhibitory and bactericidal concentrations of *Croton zambesicus* crude extract against multidrug resistant bacteria

Organism	MIC (mg/mL)	MBC (mg/mL)
Escherichia coli	12.5 ± 0.00	50 ± 0.00
Acinetobacter baumannii	3.125 ± 0.00	25 ± 0.00
Staphylococcus aureus	12.5 ± 0.00	50 ± 0.00
Klebsiella aerogenes	6.25 ± 0.00	50 ± 0.00
Pseudomonas aeruginosa	3.125 ± 0.00	12.5 ± 0.00
Klebsiella pneumoniae	12.5 ± 0.00	50 ± 0.00

MIC = minimum inhibitory concentration, MBC = minimum bactericidal concentration

Table 3: Antibacterial activity of *Senna occidentalis* leaf extracts against multidrug resistant bacteria

Test organisms	MIC (mg/mL)	MBC (mg/mL)
Escherichia coli	3.125 ± 0.00	25 ± 0.00
Acinetobacter baumannii	6.25 ± 0.00	100 ± 0.00
Staphylococcus aureus	3.125 ± 0.00	100 ± 0.00
Klebsiella aerogenes	3.125 ± 0.00	50 ± 0.00
Pseudomonas aeruginosa	6.25 ± 0.00	50 ± 0.00
Klebsiella pneumoniae	6.25 ± 0.00	50 ± 0.00

Fraction 1=n-hexane fraction A, Fraction 2 =n-hexane fraction B, Fraction 3= Ethyl acetate fraction A, F4= ethyl acetate Fraction B, F5= Aqueous Fraction. Values with different letters within each column are significantly different ($p \leq 0.05$).

Table 4: Minimum inhibitory and bactericidal concentrations of *Senna occidentalis* against multidrug resistant bacteria

Test Organisms	Crude extract (100 mg/mL)	Fraction 1 (100 mg/mL)	Fraction 2 (100 mg/mL)	Fraction 3 (100 mg/mL)	Fraction 4 (100 mg/mL)	Fraction 5 (100 mg/mL)
Escherichia coli	16.67 ± 0.67c	0.00 ± 0.00a	0.00 ± 0.00a	1.33 ± 0.67a	7.33 ± 0.67b	8.67 ± 0.67b
Acinetobacter baumanni	14.33 ± 0.58d	0.00 ± 0.00a	0.00 ± 0.00a	2.00 ± 0.00b	2.67 ± 0.33b	4.67 ± 0.33c
Staphylococcus aureus	15.33 ± 0.33c	0.00 ± 0.00a	0.00 ± 0.00a	3.33 ± 0.89b	3.00 ± 0.57b	3.0 ± 0.57b
Klebsiella aerogenes	15.33 ± 0.67d	5.00 ± 0.57a	8.33 ± 0.33b	4.33 ± 0.33a	10.00 ± 0.67c	9.00 ± 0.57b
Pseudomonas aeruginosa	12.67 ± 0.67c	0.00 ± 0.00a	0.00 ± 0.00a	4.67 ± 0.67b	0.00 ± 0.00a	0.00 ± 0.00a
Klebsiella pneumoniae	14.33 ± 0.33d	0.00 ± 0.00a	4.33 ± 0.33c	0.00 ± 0.00a	0.00 ± 0.00a	2.00 ± 0.00b

MIC = minimum inhibitory concentration, MBC = minimum bactericidal concentration

Table 5: GC-MS identified compounds of *croton zambesicus* crude extract

Peak #	Retention time	Area (%)	Detected compounds
1	3.220	0.28	2-Acetylcyclopentanone
2	3.560	0.21	Copaene
3	3.630	0.54	1H-Pyrrole, 1-butyl-
4	3.829	0.46	Caryophyllene
5	3.942	0.74	(1S,4aR,7R)-1,4a-Dimethyl-7-(prop-1-en-2-yl)-1,2,3,4,4a,5,6,7-octahydronaphthalene
6	4.055	1.72	1H-Cycloprop[e]azulene, 1a,2,3,4,4 a,5,6,7b-octahydro-1,1,4,7-tetramethyl-, [1aR-(1a.alpha.,4.alpha.,4a.beta.,7b.alpha.)]-
7	4.126	0.51	(1R,9R,E)-4,11,11-Trimethyl-8-methylenebicyclo[7.2.0]undec-4-ene
8	4.183	0.77	1H-Cyclopropa[a]naphthalene, decahydro-1,1,3a-trimethyl-7-methylene-, [1aS-(1a.alpha.,3a.alpha.,7a.beta.,7b.alpha.)]-
9	4.282	0.85	Selina-3,7(11)-diene
10	4.437	0.56	Benzene, 1-fluoro-2-methoxy-
11	4.551	0.74	Bicyclo[6.1.0]nonane, 9-(1-methylethylidene)-
12	6.773	0.20	Hexadecanoic acid, ethyl ester
13	7.013	3.41	1-Naphthalenemethanol, decahydro-5 -(5-hydroxy-3-methyl-3-pentenyl)-1,4a-dimethyl-6-methylene-, [1S-[1. Alpha., 4a.alpha., 5.alpha. (E) alpha.,8a.beta.]]-
14	7.240	10.06	n-Hexadecanoic acid
15	7.396	5.83	cis-13-Octadecenoic acid
16	7.622	24.06	9-Octadecenoic acid
17	7.707	21.12	cis-Vaccenic acid
18	7.962	23.95	cis-13-Octadecenoic acid
19	8.882	3.97	4-Hexadecen-6-yne, (E)-

Table 6: Identified compounds by GCMS of *Senna occidentalis* crude extract

Peak #	Retention time	Area (%)	Detected compounds
1	2.187	1.47	Acetic acid, hexyl ester, hexadecanoic acid, butyl ester, 1,3-Hexanediol, 2-ethyl-
2	2.654	0.10	Benzene, 1,3-dimethyl-, Ethylbenzene
3	2.739	0.43	Benzene, 1,3-dimethyl-, o-Xylene, p-Xylene
4	2.979	0.22	Benzene, 1,3-dimethyl-, o-Xylene, 1,3-dimethyl-
5	8.740	0.25	8-Heptadecene, E-14-Hexadecenal, 1-Heptadecene
6	8.967	0.39	8-Heptadecene, Acetic acid, chloro-, hexadecyl ester
7	10.014	0.07	Cyclodecene, 1-methyl-, 1,2-15,16-Diepoxyhexadecane, cis-5-Decen-1-yl acetate
8	11.175	0.53	n-Hexadecanoic acid
9	11.458	0.78	Hexadecanoic acid, ethyl ester
10	12.024	1.36	n-Hexadecanoic acid, 2-Methyl-Z,Z-3,13-octadecadienol
11	12.477	1.91	n-Hexadecanoic acid, Cyclopropaneoctanal, 2-octyl-
12	12.534	0.47	n-Hexadecanoic acid
13	12.619	0.33	n-Hexadecanoic acid, cis-Vaccenic acid, Oleic acid
14	13.001	4.03	Phytol, Hexadecyl pentyl ether
15	13.185	1.11	Oleic Acid, Octadec-9-enoic acid, n-Hexadecanoic acid
16	13.609	31.19	9-Octadecenoic acid, cis-13-Octadecenoic acid, 9-Octadecenoic acid, (E)-
17	13.808	7.64	cis-Vaccenic acid, 9,17-Octadecadienal, (Z)-, cis-13-Octadecenoic acid
18	14.091	7.16	cis-13-Octadecenoic acid, trans-13-Octadecenoic acid, 9-Oxabicyclo[6.1.0]nonane, cis-
19	15.124	2.38	Oleic acid, 9,17-Octadecadienal, (Z)-,
20	15.917	1.43	Cyclopropaneoctanal, 2-octyl-, 5-Eicosene, (E)-, 2-Methyl-Z,Z-3,13-octadecadienol
21	17.134	1.64	9-Octadecenamide, (Z)-, Mannosamine
22	18.408	0.43	13-Octadecenal, (Z)-, Cyclopropaneoctanal, 2-octyl-, 12-Methyl-E,E-2,13-octadecadien-1-ol
23	19.314	1.79	9-Octadecenal, (Z)-, Cyclopropaneoctanal, 2-octyl-, 9,17-Octadecadienal, (Z)-
24	20.800	0.39	9-Eicosenoic acid, (Z)-, Cyclopropaneoctanal, 2-octyl-, 9-Octadecenoic acid (Z)-, 2,3-dihy, droxypropyl ester
25	23.419	1.78	cis-13-Octadecenoic acid, Cyclopropaneoctanal, 2-octyl-, trans-13-Octadecenoic acid
26	23.857	5.88	9-Octadecenal, (Z)-, Oleic acid, cis-7,cis-11-Hexadecadien-1-yl acetate
27	23.956	4.06	9-Octadecenal, (Z)-, 9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester, 9-Oxabicyclo[6.1.0]nonane, cis-
28	24.310	3.32	Oleic acid, 6-Octadecenoic acid, (Z)-, 9-Octadecenoic acid, (E)-
29	25.924	8.14	7-Pentadecyne, 2,3-Dihydroxypropyl elaidate, 9-Octadecenoic acid, (E)-
30	26.150	12.28	cis-13-Octadecenoic acid, trans-13-Octadecenoic acid, 9,17-Octadecadienal, (Z)-

Discussion

The findings of this study demonstrate that the crude extracts of *C. zambesicus* and *S. occidentalis* exhibit significant antibacterial activity against multidrug-resistant (MDR) pathogens, whereas their fractionated counterparts show markedly reduced efficacy. This pattern underscores the importance of preserving phytochemical complexity in plant extracts, as antimicrobial activity is often the result of multiple metabolites acting in concert. The superior performance of crude extracts in this study suggests that synergistic interactions among structurally diverse compounds enhance membrane disruption, interfere with metabolic processes, and contribute to either bacteriostatic or bactericidal effects.

Evidence from previous investigations further supports this interpretation. Crude extracts of Piper guineense, Sphenostylis stenocarpa and Euphorbia dumalis have consistently demonstrated greater antimicrobial activity than their solvent-partitioned fractions, with fractionation frequently reducing or redistributing efficacy (Oluyele et al., 2025b; Dagne et al., 2025). Collectively, these studies reinforce the observation that separating metabolites based on solvent polarity disrupts essential interactions among active constituents. In the present work, the marked decline in activity of most fractions suggests that antimicrobial compounds were either dispersed across multiple fractions or present at sub-inhibitory concentrations once separated.

The chemical basis for these differences likely lies in the consequences of solvent partitioning, which narrows the chemical diversity within each fraction. While crude extracts retain the full complement of fatty acids, terpenoids, aldehydes, esters, hydrocarbons, and related compounds identified through GC–MS analysis, individual fractions contain only subsets of these components. Because many plant-derived antimicrobials act synergistically, reducing the number of co-occurring metabolites diminishes their capacity to disrupt bacterial membranes, potentiate enzyme inhibition, or enhance intracellular penetration. The moderate activity observed in the ethyl acetate fraction of S. occidentalis indicates that mid-polar metabolites contribute meaningfully to antibacterial activity, although they remain insufficient on their own to match the potency of the crude extract.

Synergistic interactions among phytochemicals likely play a central role in the observed differences between crude and fractionated samples. Compounds such as unsaturated fatty acids can increase membrane permeability, enabling terpenoids, aldehydes, or phenolic derivatives to more effectively reach intracellular targets. Other metabolites may act on distinct pathways simultaneously, producing a cumulative antimicrobial effect that exceeds the activity of individual constituents. The inactivity of several fractions in this study provides indirect but strong support for the presence of such synergy, as separation removes the cooperative interactions necessary for maximal activity.

Variation in bacterial susceptibility aligns with established differences in cell wall structure and intrinsic resistance mechanisms (Torrens and Cava, 2024). Both crude extracts exhibited strong inhibitory effects against A. baumannii and P. aeruginosa, pathogens known for robust outer-membrane barriers. The presence of unsaturated fatty acids such as oleic, linoleic, and vaccenic acids in both plants may account for this potency, as these compounds are capable of integrating into and destabilizing Gram-negative membranes (Das, 2018; Casillas-Vargas et al., 2021). In *C. zambesicus*, terpenoids including caryophyllene and selina derivatives may further enhance antibacterial action by disrupting membrane integrity or inhibiting key metabolic enzymes (Masyita et al., 2022; Câmara et al., 2024; Khanam et al., 2025). This aligns with the lower MBC values recorded for *C. zambesicus*, reflecting its predominantly bactericidal effect.

In contrast, *S. occidentalis* displayed lower MIC values, suggesting strong inhibitory effects at minimal concentrations, yet exhibited higher MBC values indicative of a largely bacteriostatic mechanism. This pattern may reflect its phytochemical profile, which includes long-chain aldehydes, phytol derivatives, and mixed unsaturated fatty acids compounds known to slow microbial proliferation without necessarily inducing rapid cell death (Aljaafari et al., 2021; Casillas-Vargas et al., 2021). These compositional differences between the two plants therefore likely account for their distinct antibacterial profiles.

The GC–MS results further support these interpretations by revealing that both plants contain complex mixtures of fatty acids, terpenoids, esters, phytol derivatives, and hydrocarbons with documented antibacterial effects. Their combined actions provide a plausible explanation for the broad-spectrum activity observed, as these compounds may concurrently affect membrane integrity, enzyme function, cell wall synthesis, and intracellular metabolic processes.

Study Limitations

This study was limited to in-vitro assays, which may not fully reflect in-vivo efficacy. Fractionation could have led to the loss or dilution of active compounds, and GC–MS primarily detected

volatile and semi-volatile constituents, leaving non-volatile metabolites uncharacterized. Additionally, only a limited number of MDR bacterial isolates were tested.

Conclusion

The superior activity of crude extracts compared to fractionated samples underscores the crucial role of phytochemical synergy in plant-based antimicrobial activity. Preserving the full array of interacting metabolites allows crude extracts to achieve greater potency than isolated fractions. These findings highlight the need to identify the specific combinations of metabolites responsible for synergistic effects, characterize their mechanisms of action, and determine whether recombining isolated compounds can restore or enhance activity under controlled conditions. Future investigations should extend these findings by evaluating in-vivo antimicrobial efficacy, characterizing non-volatile bioactive compounds, and experimentally testing synergistic interactions among phytochemicals. Additionally, assessing a broader spectrum of multidrug-resistant bacterial strains will help validate the wider therapeutic potential of *C. zambesicus* and *S. occidentalis*.

Declarations

Conflict of Interest

The authors declare no conflicts of interest.

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Ethical Considerations

The authors have fully adhered to ethical standards, ensuring no issues related to plagiarism, misconduct, data fabrication, falsification, duplicate publication or submission, or redundancy. The authors declare that no artificial intelligence tools were used in the preparation, writing, analysis, or editing of this manuscript.

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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