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Original Research Article

Extracts of *Bryophyllum pinnatum* and *Vincetoxicum rossicum* synergize with standard antibiotics against multidrug-resistant gram-negative bacteria

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ABSRTACT

The increasing prevalence of antibiotic-resistant Gram-negative bacteria has intensified the need for alternative antimicrobial agents. This study evaluated the antibacterial activities of ethanolic extracts of *Bryophyllum pinnatum* and *Vincetoxicum rossicum* against clinical isolates of *Escherichia coli*, *Klebsiella pneumoniae*, and *Salmonella typhi*. The leaves were extracted using 95% ethanol, and their antibacterial efficacy was assessed using the agar well diffusion method on Mueller-Hinton agar, with standard antibiotics (Amoxicillin-Clavulanate, Cefuroxime, and Ceftazidime) as controls. Zones of inhibition were measured, and data were analyzed using two-way ANOVA with a $P < 0.05$ significance level. The ethanolic extract of *V. rossicum* at 100% concentration demonstrated significant antibacterial activity against *E. coli* (18.33 ± 0.33 mm), *K. pneumoniae* (15.33 ± 0.66 mm), and *S. typhi* (13.33 ± 0.33 mm). In *B. pinnatum, the* ethanolic extract at 100% concentration was active against *E. coli* (14.67 ± 0.33 mm) and *K. pneumoniae* (14.67 ± 0.90 mm) but showed no effect on *S. typhi*. Among the antibiotics, Ceftazidime produced the highest inhibition against *E. coli* (34.00 ± 0.58 mm), followed by Cefuroxime (29.75 ± 0.86 mm) and Amoxicillin–Clavulanate (28.25 ± 0.75 mm). Notably, synergistic effects against the bacterial isolates were observed between the ethanolic extract of *V. rossicum* and Ceftazidime (53.00 mm), and between Cefuroxime and Ceftazidime (45.00 mm). These findings suggest that ethanolic extracts of *B. pinnatum* and *V. rossicum* may serve as complementary agents in the treatment of infections caused by Gram-negative bacteria.

Keywords: Bryophyllum pinnatum, *Vincetoxicum rossicum*, Ethanolic extract, Gram-negative bacteria, Antimicrobial resistance, Synergistic effect.

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Introduction

The global rise in antimicrobial resistance has significantly compromised the effectiveness of conventional antibiotics, particularly in treating infections caused by Gram-negative pathogens. Organisms such as *Escherichia coli*, *Klebsiella pneumoniae*, and *Salmonella typhi* have developed resistance to multiple antibiotics, rendering standard treatments increasingly ineffective.[1–2] Factors contributing to this crisis include the widespread misuse and overuse of antibiotics, the high cost and limited availability of next-generation drugs, and the declining development of novel antimicrobial agents.[3–4] Third-generation cephalosporins, such as Ceftazidime and Cefuroxime, are routinely used to manage Gram-negative infections. Still, their efficacy is waning due to increasing resistance.[5–6] One promising strategy involves combining conventional antibiotics with plant-derived bioactive compounds, which may exert direct antimicrobial effects or potentiate existing drugs through synergistic mechanisms.[7]

In Nigeria, traditional medicine leverages a rich diversity of indigenous plants known for their therapeutic potential.[8–9] Numerous studies have demonstrated that extracts from medicinal plants possess antibacterial properties against a broad range of pathogens.[10]

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Among these plants, *Bryophyllum pinnatum* (Crassulaceae), commonly referred to as the "miracle leaf" or "life plant," has been widely used in Nigerian ethnomedicine. Its antimicrobial activity has been attributed to diverse phytochemicals, including flavonoids, alkaloids, and phenolic compounds. [11–13]

Vincetoxicum rossicum (Apocynaceae), commonly referred to as European swallow-wort, is primarily recognized as an invasive species. 'Nevertheless, emerging reports indicate the presence of biologically active secondary metabolites with antimicrobial and insecticidal properties.[8, 14–16] Despite limited documentation of its medicinal application in Nigeria, its phytochemical profile suggests potential antibacterial relevance.

In Nigeria, some of the common infectious bacterial agents of clinical significance include *S. typhi,* which is responsible for typhoid fever,[15] with an incidence rate estimated to be between 3.9 % and 18.9 %, especially in rural populations with limited access to potable water.[17] Meanwhile, *K. pneumoniae* and *E. coli* are major etiological agents of pneumonia, urinary tract infections, and gastroenteritis.[18] Reports from the World Health Organization indicate growing resistance of these pathogens to commonly used antibiotics in several regions of the country, resulting in suboptimal clinical outcomes.[19] In view of these concerns, the present study evaluated the antibacterial activity of ethanolic extracts of *Bryophyllum pinnatum* and *Vincetoxicum rossicum* against clinical isolates of *E. coli*, *K. pneumoniae*, and *S. typhi*. Additionally, the study investigated the potential synergistic interactions between the extracts and selected standard antibiotics to explore their potential application in complementary treatment strategies against antibiotic-resistant Gram-negative pathogens.

Materials and Methods

Plant material Collection and authentication

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Fresh leaves of *Bryophyllum pinnatum* and *Vincetoxicum rossicum* (Fig 1) were collected from Calabar municipality and Akpabuyo Local Government Area, Cross River State, Nigeria. Botanical identification was conducted at the Department of Plant and Ecological Studies, University of Calabar, where voucher specimens were deposited.

Bacterial isolates and antibiotics
Clinical isolates of *Escherichia coli*, *Klebsiella pneumoniae*, and *Salmonella typhi* were obtained from the Microbiology Laboratory of the University of Calabar Teaching Hospital. Commercial antibiotic formulations of Amoxicillin–Clavulanate (Augmentin), Cefuroxime, and Ceftazidime were procured from a certified pharmaceutical outlet in Calabar.

(Willie *et al*., 2021)

Fig 1: Morphological appearance of a) *Bryophyllum pinnatum* plant, b) *Vincetoxicum rossicum* plant collected in Cross River State, Nigeria.

Plant extraction and dilutions
Leaves were rinsed, air-dried at ambient temperature, and pulverized. Two hundred grams of powdered material were macerated in 500 mL of 95% ethanol for 72 hours with periodic agitation. Filtration was performed using Whatman No. 1 filter paper, and the filtrate was concentrated at 40 °C using a water bath. The dried extract was dissolved in 5% dimethyl sulfoxide (DMSO) to obtain a stock concentration of 1000 mg/mL, followed by serial dilution to prepare 75%, 50%, and 25% concentrations.

Media preparation
Mueller-Hinton agar, MacConkey agar, and nutrient agar were prepared according to the manufacturer's instructions and sterilized at 121 °C for 15 minutes. Prepared media were poured into sterile Petri dishes under aseptic conditions.

Standardization of inoculum
Bacterial suspensions were adjusted to 0.5 McFarland turbidity standard (approximately 10^8 CFU/mL) using sterile saline, in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines. [20–22]

Antibacterial assay
Agar well diffusion assays were performed on Mueller-Hinton agar plates seeded with standardized bacterial suspensions. Wells (6 mm diameter) were filled with different concentrations of the extracts and incubated at 37 °C for 24 hours. Zones of inhibition were measured in millimeters.

Prepared antibiotic discs (30 µg/disc) were applied to inoculated plates and incubated under identical conditions.

Synergistic interaction assessment
Synergy was evaluated by combining plant extracts with antibiotics in agar wells. The bacterial inocula were uniformly spread on sterile petri dishes containing MHA. The plant extracts and the antibiotics were added to wells created in the agar and incubated for 24 hours at room temperature.[23] An interaction was considered synergistic when the inhibition zone of the combination exceeded that of individual agents.

Statistical analysis
All experiments were factorial (2x5 factorial for antibacterial assay; 3x3 factorial for antibiotic susceptibility assay) and conducted in triplicate. Data were analyzed using two-way ANOVA at $p < 0.05$, and mean separation was performed using the least significant difference (LSD) test.

Results and Discussion

The antibacterial activity of the ethanolic extracts of *Bryophyllum pinnatum* and *Vincetoxicum rossicum* was evaluated against clinical isolates of *Escherichia coli*, *Klebsiella pneumoniae*, and *Salmonella typhi*. Two-way ANOVA revealed that both plant species and extract concentration significantly influenced inhibition zones ($p < 0.05$), with a significant interaction effect between species and concentration (Fig. 2). These findings indicate that antibacterial performance depended not only on extract potency but also on the intrinsic susceptibility of the test organisms.

Fig.2: Antibacterial response of clinical isolates following exposure to graded concentrations of ethanolic plant extracts: (a) *Klebsiella pneumoniae*, (b) *Salmonella typhi*, and (c) *Escherichia coli*. Values represent mean inhibition diameters (mm).

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The ethanolic extract of *V. rossicum* produced a maximum inhibition zone of 18.33 ± 0.33 mm against *E. coli* at full concentration, followed by progressive reduction at lower concentrations (Table 1). A similar concentration-dependent trend was observed for *B. pinnatum*, although with considerably lower inhibition values.

Table 1: Diameter of growth inhibition (mm) caused by graded concentrations of ethanolic extracts of *Bryophyllum pinnatum* and *Vincetoxicum rossicum* against selected Gram-negative bacteria.

Microbes	***Bryophyllum pinnatum***					***Vincetoxicum rossicum***					
	100%	**75%**	**50%**	**25%**	**0%**	**100%**	**75%**	**50%**	**25%**	**0%**	**LSD**
E. coli	14.67^a±0.33	11.00^b±0.58	7.00^c±0.58	3.33^d±0.33	0.00^e±0.00	18.33^a±0.33	12.67^b±0.9	10.33^b±0.33	4.67^d±0.66	0.00^e±0.00	4.27
K. pneumoniae	14.67^a±0.9	13.33^a±0.33	11.00^b±0.58	6.00^d±0.58	0.00^e±0.00	15.33^a±0.67	11.00^b±0.58	9.30^c±0.33	5.00^d±0.58	0.00^e±0.00	4.18
S. typhi	0.00^c±0.00	0.00^c±0.00	0.00^c±0.00	0.00^c±0.00	0.00^c±0.00	13.33^a±0.33	10.33^a±0.33	8.00^b±0.58	3.67^b±0.33	0.00^c±0.00	6.51

*Values are expressed as mean ± standard deviation of three independent determinations.
Within each row, means bearing different superscript letters differ significantly at $p < 0.05$ (LSD test).

Significant differences observed across concentrations indicate a dose-dependent antibacterial effect of the ethanolic extracts. The high susceptibility of *E. coli* in this study supports previous findings by McMurray *et al*.[24] and Elisha *et al*.[25] who reported that *E. coli* is highly responsive to plant-derived antimicrobial agents. Ethanol, being a moderately polar solvent, likely facilitated the extraction of phenolic compounds, flavonoids, terpenoids, and alkaloids, which are known to disrupt bacterial membrane integrity and interfere with metabolic pathways.[8, 26–31]

Both plant extracts inhibited *K. pneumoniae*, with *V. rossicum* again demonstrating slightly superior activity (15.33 ± 0.66 mm) at higher concentrations. Notably, inhibition decreased proportionally with dilution for both extracts, reinforcing the dose-dependent pattern observed across organisms (Table 1). The interaction effect between plant species and bacterial isolates confirms differential susceptibility patterns among the organisms tested. *K. pneumoniae* is known for its polysaccharide capsule and multiple resistance determinants, which often reduce antibiotic penetration. The ability of the extracts to generate measurable inhibition zones suggests that certain phytochemicals may bypass or compromise protective bacterial barriers. Membrane-disrupting compounds and efflux pump modulators may have contributed to the observed activity.[32-33] The differential inhibition among plant species suggests variability in phytochemical composition, highlighting *V. rossicum* as potentially richer in antibacterial constituents under the extraction conditions employed.

A distinct response pattern was observed for *S. typhi*. While *V. rossicum* exhibited measurable inhibition across concentrations (13.33 ± 0.33 mm (100%), 10.33 ± 0.33 mm (75%), 8.00 ± 0.58 mm (50%), and 3.67 ± 0.33 mm (25%)), *B. pinnatum* showed no detectable activity at any tested concentration (Table 1).

The lack of activity of *B. pinnatum* against *S. typhi* contrasts with some previous findings,[34-36] and may reflect differences in strain susceptibility, geographic variation in plant phytochemistry, extraction efficiency, or seasonal influences on metabolite concentrations. Additionally, *S. typhi* possesses well-characterized resistance mechanisms, including active efflux systems and permeability barriers, which may reduce intracellular accumulation of plant-derived compounds.[37-38]

Among the antibiotics tested, Ceftazidime produced the largest inhibition zones across all bacterial isolates, followed by Cefuroxime, while Amoxicillin–Clavulanate exhibited comparatively lower activity (Table 2).

Table 2: Antibacterial activity of selected antibiotics against clinical isolates, expressed as mean inhibition diameter (mm).

Antibiotics	**Test Organisms**		
	E. Coli	***K. Pneumoniae***	***S. Typhi***
AUG	28.25±0.75	16.00±1.08	20.25±2.66
CFU	29.75±0.86	20.00±1.23	21.00±1.23
CFT	34.00±0.58	23.25±1.11	24.00±1.08

Data represent mean ± standard deviation of triplicate assays.

These findings are consistent with established clinical data indicating the broad-spectrum efficacy of third-generation cephalosporins against Gram-negative pathogens.[5–6] However, the need for enhanced therapeutic strategies remains pressing due to the increasing resistance patterns.

Two notable synergistic interactions were identified (Table 3; Fig. 3). The combination of Ceftazidime with 100% ethanolic extract of *V. rossicum* produced a markedly larger inhibition zone (53 mm) against *E. coli* than either agent alone. Additionally, the combination of Cefuroxime and Ceftazidime generated enhanced inhibition against *S. typhi* (45 mm).

Table 3: Inhibition diameters (mm) obtained from combined treatments demonstrating synergistic antibacterial interactions.

Microbes	**Visible Synergistic effect**	**Zone of inhibition**
Salmonella typhi	CFT – CFU	45mm
Escherichia coli	CFT – 100% Conc. of *V. rossicum*	53mm

The enhanced inhibition observed in these combinations suggests plant-derived compounds may enhance antibiotic penetration, suppress resistance mechanisms, or disrupt protective cellular structures. Potential mechanisms include increased membrane permeability, efflux pump inhibition, or interference with β-lactamase activity.[39,7]

Overall, the findings demonstrate that both plants possess notable antibacterial activity, particularly *V. rossicum*, which exhibited broad-spectrum efficacy against all tested pathogens. This may be attributed

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to phytochemicals previously identified in these plants, including Phenols, flavonoids, and terpenoids.[8, 12-13] The statistically significant dose-dependent effects ($p < 0.05$) further confirm the biological relevance of the extracts. The observed synergistic interactions reinforce the potential of integrating plant-based agents into combination therapy strategies for the management of multidrug-resistant Gram-negative infections. The stronger and broader activity profile of *V. rossicum* suggests that it may warrant further phytochemical characterization and mechanistic investigation.

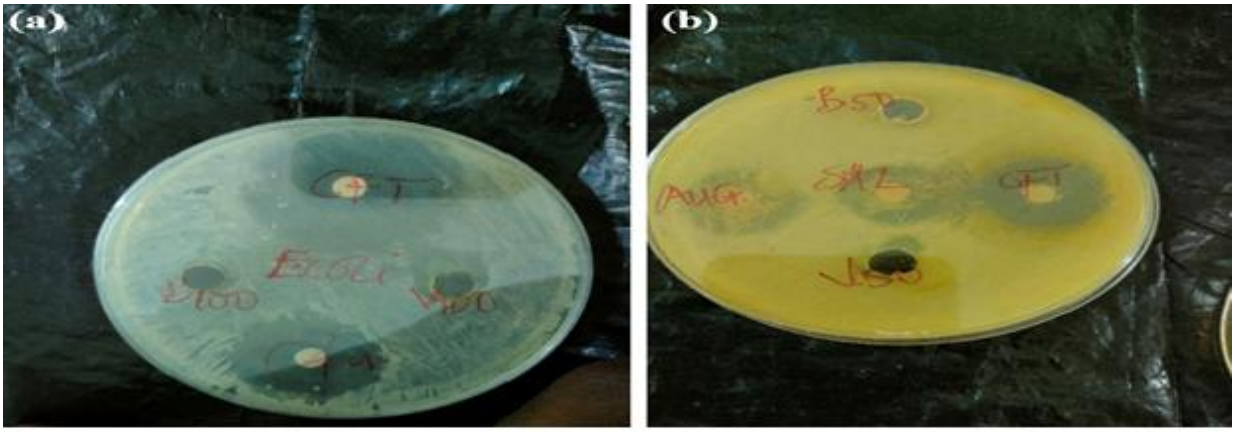

Fig. 3: Increased inhibition diameter resulting from synergistic combinations of antibiotics and *V. rossicum* extract compared with individual treatments

Conclusion

This study demonstrates that ethanolic extracts of *Vincetoxicum rossicum* possess consistent and concentration-dependent antibacterial activity against clinically relevant Gram-negative pathogens, whereas *Bryophyllum pinnatum* exhibited selective inhibition. The broader efficacy profile of *V. rossicum*, particularly against *Escherichia coli* and *Salmonella typhi*, highlights its potential as a source of bioactive compounds with antimicrobial relevance.

Importantly, the pronounced enhancement observed when *V. rossicum* extract was combined with Ceftazidime suggests that plant-derived metabolites may function as antibiotic potentiators. Such interactions may offer a practical approach for improving therapeutic outcomes against resistant bacterial strains.

These findings support further phytochemical characterization and mechanistic studies to identify the specific constituents responsible for antibacterial and synergistic effects. Integrating plant-based bio-actives into combination therapy strategies may represent a complementary approach to addressing the escalating challenge of multidrug-resistant Gram-negative infections.

Conflict Of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article are original and any liability for claims relating to the content of this article will be borne by them.

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