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PHYTOCHEMICAL AND *IN VITRO* ANTIMICROBIAL STUDIES OF CRUDE METHANOL AERIAL PART EXTRACTS OF *POLYGONUM SENEGALENSIS* MEISN. (POLYGONACEAE)

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ABSTRACT

The aerial part of *Polygonum senegalensis* was extracted with methanol using the Soxhlet method and evaluated for its phytochemical composition and *in vitro* antimicrobial activity. Extraction gave a 19.43% (w/w) yield of a brownish, powdery crude extract. Phytochemical screening revealed the presence of terpenoids, flavonoids, carbohydrates, tannins, cardiac glycosides, steroids, cardenolides and saponins, while alkaloids and anthraquinones were absent. The hole-diffusion antimicrobial assay showed weak activity at 20 mg/hole (zones of inhibition ranging from 8.00 to 13.00 mm) against most Gram-positive and Gram-negative bacteria, with moderate-to-high activity at 40 and 80 mg/hole. At 160 mg/hole the extract was active against all test organisms except *Aspergillus niger*, producing zones of inhibition between 20.00 and 27.67 mm that were comparable to, and in the case of *Pseudomonas aeruginosa* exceeded, the reference antibiotic (ciprofloxacin, 10 µg/hole). Antimicrobial activity increased in a concentration-dependent manner across all organisms tested. These findings support the ethnomedicinal use of *Polygonum senegalensis* and indicate that its phytoconstituents, particularly flavonoids, tannins, saponins and terpenoids, may act synergistically against clinically relevant pathogens.

KEYWORDS: Phytochemical screening; *Polygonum senegalensis*; Antimicrobial susceptibility; Disc/hole diffusion; Antimicrobial resistance.

1. INTRODUCTION

Plants have been used as sources of medicine since antiquity, and this practice remains especially prominent in African societies, where researchers continue to investigate medicinal flora in the search for safe, effective, and affordable therapeutic agents (Aliu & Nwude, 1982). The World Health Organization estimates that a substantial majority of the world's population still relies on traditional, plant-based medicine for primary healthcare, and roughly a quarter of conventional pharmaceuticals are derived directly or indirectly from plant constituents (Adnan et al., 2010).

Interest in medicinal plants has intensified in recent years because of the accelerating global crisis of antimicrobial resistance (AMR). Recent regional analyses estimate that resistant bacterial infections were associated with more than one million deaths in the World Health Organization African Region alone in a single reference year, a burden exceeding that of HIV/AIDS or malaria in the same region (Sartorius et al., 2024). Commentary accompanying this analysis stresses that under-resourced surveillance systems mean the true burden of AMR in Africa is still likely underestimated (Essack & Lenglet, 2024), a concern reinforced by continent-wide reviews calling for strengthened genomic and laboratory surveillance capacity (Kajumbula et al., 2024), improved public understanding of resistance (Fuller et al., 2023), and a coordinated One Health response tailored to the Nigerian context specifically (Alabi et al., 2025). Rising resistance among common Gram-negative pathogens such as *Escherichia coli* and *Klebsiella pneumoniae* has been documented across sub-Saharan Africa (Kitaba et al., 2024), underscoring the urgency of identifying new, plant-derived antimicrobial leads that can complement or extend the useful life of existing antibiotics.

Nigeria's flora, and that of Borno State in particular, remains comparatively under-documented despite continued reliance on traditional healers and herbal remedies among rural communities (Adeniran & Akindele, 2024). *Polygonum* (family Polygonaceae) is one such under-investigated genus. It comprises perennial herbs found along watercourses, drains, and canal borders, and species within the genus have long been used across different cultures to manage hypertension, gastrointestinal complaints, dysuria, skin conditions, and haemostatic disorders (Idoudi et al., 2024). Phytochemical surveys of the genus have identified phenolic acids, flavonoids, tannins, stilbenes, terpenes, and fatty acids as recurring constituents responsible for antioxidant, antimicrobial, anti-inflammatory, and other pharmacological activities (Idoudi et al., 2024). Species-specific investigations of *Polygonum hydropiper* (Kong et al., 2023), *Polygonum cognatum* (Erol & Kutlu, 2025), and other Turkish and Asian *Polygonum* taxa (Büyükyıldırım et al., 2025) have similarly reported

antibacterial, antidiabetic, and antioxidant bioactivities linked to their flavonoid and phenolic content, yet *Polygonum senegalensis*, a species native to West African wetlands, has received very little attention in the phytochemical or pharmacological literature.

The antimicrobial relevance of individual phytochemical classes is increasingly well characterized. Flavonoids disrupt bacterial membranes, inhibit nucleic acid synthesis, and suppress efflux-pump activity, giving them broad-spectrum activity against Gram-positive and Gram-negative pathogens (Thebti et al., 2023; Zhang et al., 2025). Structurally related flavonoids isolated from other medicinal plants have shown comparable inhibition-zone and transcriptomic evidence of membrane and cell-wall disruption in *Staphylococcus aureus* (Zhou et al., 2023). Saponins act as amphiphilic, detergent-like molecules that compromise microbial membrane integrity and can modulate host immune clearance of pathogens (Pikhtirova et al., 2023). Tannins bind microbial proteins and disrupt cell membranes, conferring broad antibacterial, antifungal, and antiviral effects (Huang et al., 2024), including demonstrated activity against cariogenic and other Gram-positive bacteria (Czerkas et al., 2024). Terpenoids, meanwhile, interfere with microbial oxygen uptake, oxidative phosphorylation, and efflux-pump function (Siddiqui et al., 2024), with marine- and plant-derived terpenoids increasingly explored as leads against drug-resistant pathogens (Liu et al., 2024). Because the crude extract examined in this study contains several of these constituent classes simultaneously, synergistic rather than single-compound mechanisms are plausible.

Given this background, the present study was designed to (i) determine the phytochemical profile of the crude methanol extract of the aerial part of *Polygonum senegalensis*, and (ii) evaluate its *in vitro* antimicrobial activity against clinically relevant Gram-positive bacteria, Gram-negative bacteria, and fungi, in order to generate baseline evidence for its potential as a source of novel antimicrobial leads.

2. MATERIALS AND METHOD

2.1 Sample Collection and Identification

The aerial part of *Polygonum senegalensis* was collected from Zabarmari village, Jere Local Government Area, Borno State, Nigeria. The sample was identified by a taxonomist in the Department of Botany, University of Maiduguri, and a voucher specimen (no. 20259004) was deposited at the Herbarium of the Department of Biological Sciences, University of Maiduguri.

2.2 Chemicals and Reagents

All chemicals, solvents, and reagents were of analytical grade and used without further purification. Reagents were prepared in accordance with S.I. units.

2.3 Sample Preparation and Extraction

The aerial part was air-dried under shade and pulverized into a coarse powder using a wooden mortar and pestle. Five hundred grams (500 g) of powdered material was exhaustively extracted with methanol using the Soxhlet apparatus. Soxhlet extraction was selected because continuous solvent recirculation through the sample bed generally gives higher recovery of phytochemicals than simple maceration, despite its longer processing time and greater solvent demand (Bitwell et al., 2023). The extract was filtered and concentrated to dryness under monitored conditions, and the percentage yield was calculated. The dried extract was then subjected to phytochemical evaluation and antimicrobial testing.

2.4 Preliminary Phytochemical Screening

Qualitative phytochemical screening was carried out using standard colour-reaction and precipitation procedures to detect the major classes of secondary metabolites: alkaloids, anthraquinones (free and combined), carbohydrates, flavonoids, tannins, saponins, glycosides (including cardiac glycosides and cardenolides), steroids, and terpenoids/terpenes.

2.5 Antimicrobial Studies

2.5.1 Test organisms. The Gram-positive organisms used were *Bacillus subtilis*, *Staphylococcus aureus*, *Streptococcus pyogenes*, and *Corynebacterium* spp.; the Gram-negative organisms were *Escherichia coli*, *Salmonella Typhi*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*, and the fungal strains were *Candida albicans* and *Aspergillus niger*. All isolates were clinical laboratory isolates obtained from the Department of Medical Microbiology and the Department of Veterinary Medicine, University of Maiduguri, Nigeria.

2.5.2 Culture preparation. Nutrient agar (28 g/L) was used for bacterial culture and Sabouraud dextrose agar (30 g/L) for fungal culture. Media were sterilized by autoclaving at 121 °C for 15 minutes, cooled to 45–50 °C, and poured into Petri dishes to gel.

2.5.3 Hole/disc diffusion assay. Antimicrobial activity was evaluated by the hole-diffusion technique. A maximum of 12 wells were cut per 150 mm plate, spaced at least 24 mm apart, centre to centre. Stock concentrations of 400, 200, and 100 mg/mL were prepared by dissolving 4 g, 2 g, and 1 g of extract, respectively, in 5 mL of 80% methanol, giving final test concentrations of 160, 80, 40, and 20 mg/hole after serial dilution. Approximately 0.2 mL of extract solution was introduced into each well on plates seeded with the test organism; plates were incubated at 37 °C for 24 hours for bacteria and for 2–3 days for fungi. The

diameter of the clear zone showing no visible growth was measured and recorded as the zone of inhibition.

2.6 Data Analysis

Data were analysed using one-way analysis of variance (ANOVA), with statistical significance set at $P < 0.05$. Results are presented as mean ± standard error of the mean (mean ± SEM). Values within the same row bearing different superscript letters differ significantly ($P \leq 0.05$).

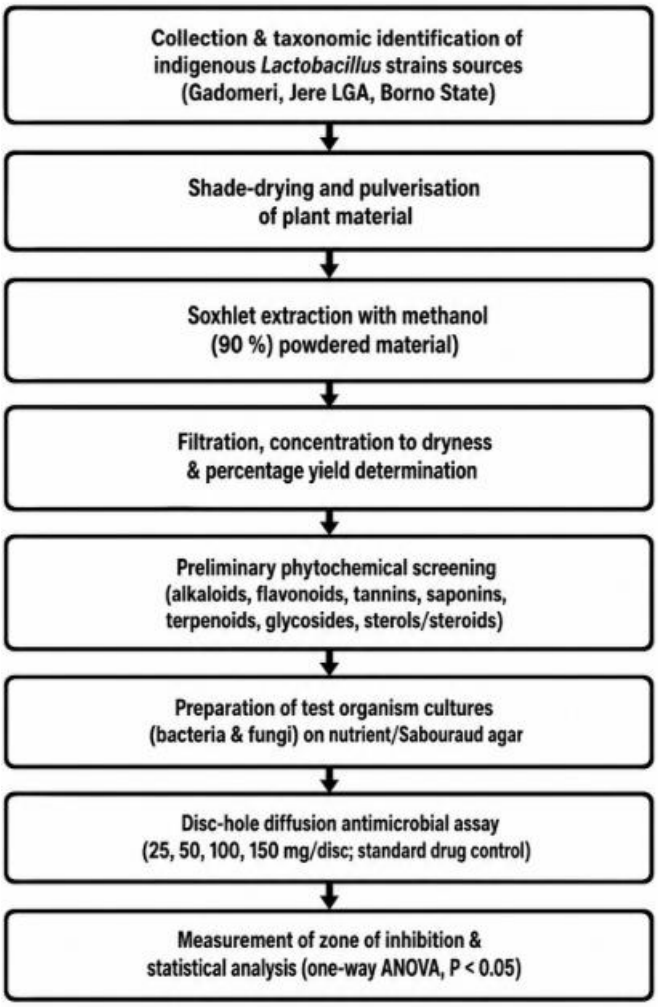

Figure 1. Schematic overview of the experimental workflow, from sample collection through antimicrobial assay and statistical analysis (illustrative diagram; not a photograph of laboratory procedure).

3. RESULTS AND DISCUSSION

3.1 Extraction Yield

Soxhlet extraction of 500 g of powdered aerial-part material with methanol yielded 97.17 g of a brownish, powdery crude extract, corresponding to a percentage yield of 19.43% w/w (Table 1, Figure 2).

Table 1. Nature, yield, and percentage yield of the crude methanol extract of Polygonum senegalensis.

Extract	Colour (nature)	Yield (g) / Percentage yield (%)
Crude methanol	Brownish (powdery)	97.17 g / 19.43%

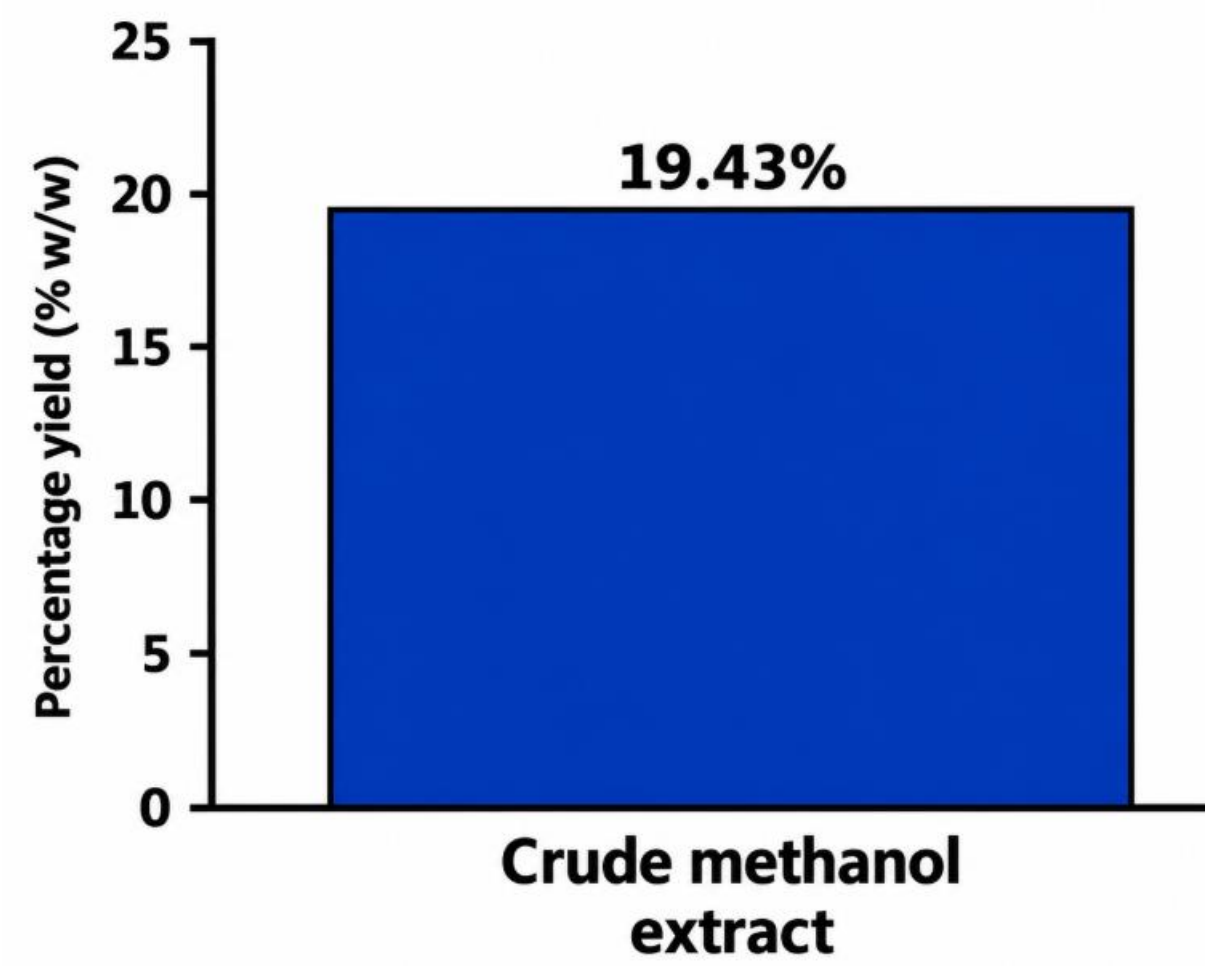

Figure 2. ***Percentage yield of the crude methanol extract of Polygonum senegalensis.***

3.2 Phytochemical Composition

Phytochemical screening of the crude methanol extract revealed the presence of terpenoids, tannins, flavonoids, carbohydrates, cardiac glycosides, saponins, and cardenolides, while alkaloids and both free and combined anthraquinones were absent (Table 2). This profile is

broadly consistent with phytochemical surveys of other *Polygonum* species, which similarly report flavonoid-, tannin-, and phenolic-acid-rich profiles as the dominant bioactive classes across the genus (Idoudi et al., 2024; Kong et al., 2023).

Table 2. Phytochemical contents of the crude methanol extract of Polygonum senegalensis.

Phytochemical	Crude Methanol Extract
Terpenoids	+
Alkaloids	−
Tannins	+
Flavonoids	+
Carbohydrate	+
Cardiac glycosides	+
Saponins	+
Free anthraquinones	−
Combined anthraquinones	−
Cardenolides	+

Key: (+) = present, (−) = absent.

3.3 Antimicrobial Susceptibility

At the lowest test concentration (20 mg/hole), the extract showed weak activity, with zones of inhibition of 8.00–13.00 mm against *Streptococcus pyogenes*, *Corynebacterium* spp., *Escherichia coli*, *Salmonella Typhi*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Candida albicans*, while *Staphylococcus aureus*, *Bacillus subtilis*, and *Aspergillus niger* showed no inhibition at this dose. Activity increased in a clear, concentration-dependent manner at 40 and 80 mg/hole (moderate-to-high activity across nearly all organisms), and at the highest concentration tested (160 mg/hole) the extract was active against every organism except *Aspergillus niger*, with zones of inhibition ranging from 20.00 mm (*S. aureus*) to 27.67 mm (*P. aeruginosa*) (Table 3, Figures 1–3). Notably, the 160 mg/hole zone of inhibition against *P. aeruginosa* (27.67 ± 0.33 mm) exceeded that of the reference antibiotic ciprofloxacin (25.00 mm), and activity against *Klebsiella pneumoniae* and *Streptococcus pyogenes* matched the standard drug exactly (Figure 2). Across all tested organisms, the extract's action was concentration-dependent, with the strongest inhibition consistently recorded at the highest concentration (Figure 3). *Aspergillus niger* was resistant at every

concentration tested, a pattern also seen with several other filamentous-fungus-focused extract studies and suggesting that fungal cell-wall composition in this species limits penetration of the extract's active constituents.

Table 3. Susceptibility of test organisms to the crude methanol extract at different concentrations (mg/hole) and their diameter zone of inhibition (mm, mean ± SEM).

Organism	160	80	40	20	CPX (10)
Staphylococcus aureus	20.00±0.00^{a}	14.33±0.33^{b}	9.67±0.33^{c}	0.00±0.00^{d}	23.00±0.00^{e}
Streptococcus pyogenes	25.00±0.00^{a}	19.67±0.33^{b}	15.00±0.00^{c}	9.67±0.33^{d}	25.00±0.00^{a}
Bacillus subtilis	21.00±0.00^{a}	19.67±0.33^{a}	15.00±0.00^{c}	9.67±0.33^{d}	26.00±0.00^{e}
Corynebacterium spp.	21.67±0.33^{a}	15.33±0.33^{b}	10.00±0.00^{c}	0.00±0.00^{d}	26.00±0.00^{e}
Escherichia coli	24.00±0.00^{a}	19.00±0.00^{b}	14.00±0.00^{c}	8.67±0.33^{d}	28.00±0.00^{e}
Salmonella Typhi	23.00±0.00^{a}	18.33±0.33^{b}	13.00±0.00^{c}	8.00±0.00^{d}	27.00±0.00^{e}
Klebsiella pneumoniae	25.00±0.00^{a}	20.00±0.00^{b}	15.00±0.00^{c}	9.33±0.33^{d}	23.00±0.00^{a}
Pseudomonas aeruginosa	27.67±0.33^{a}	22.67±0.33^{b}	18.00±0.00^{c}	13.00±0.00^{d}	25.00±0.00^{a}
Candida albicans	25.00±0.00^{a}	20.00±0.00^{b}	15.00±0.00^{c}	10.00±0.00^{d}	0.00±0.00^{e}
Aspergillus niger	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}

Key: values within the same row bearing different superscript letters differ significantly at $P \leq 0.05$; CPX = ciprofloxacin (positive control).

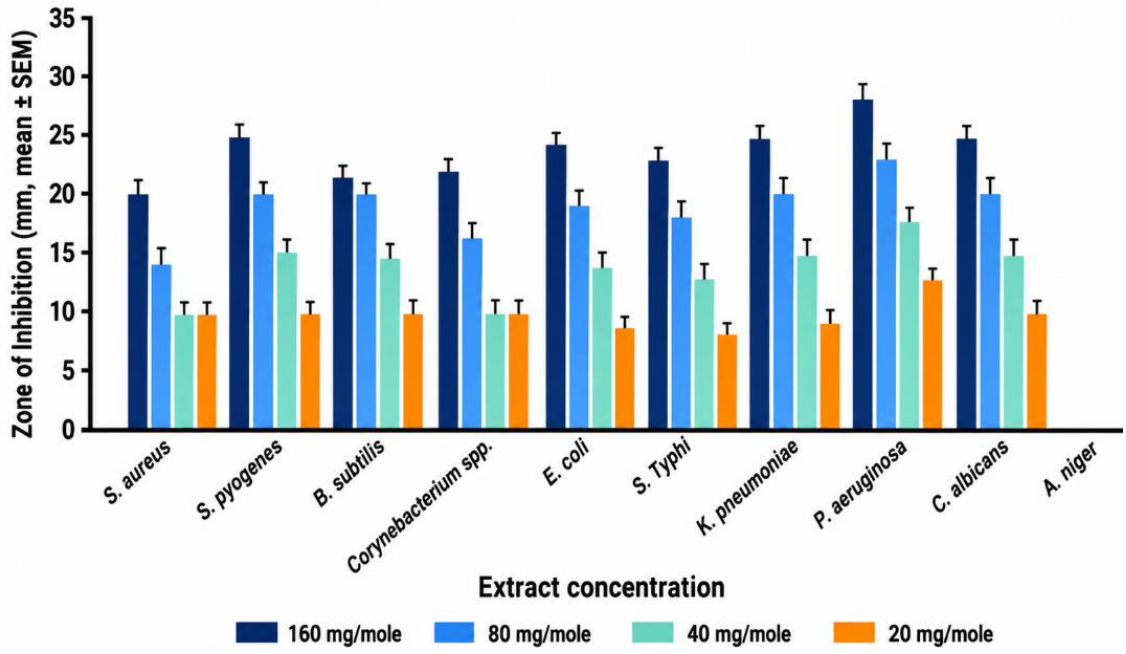

Figure 3. ***Concentration-dependent antimicrobial activity of the crude methanol extract of Polygonum senegalensis against test organisms.***

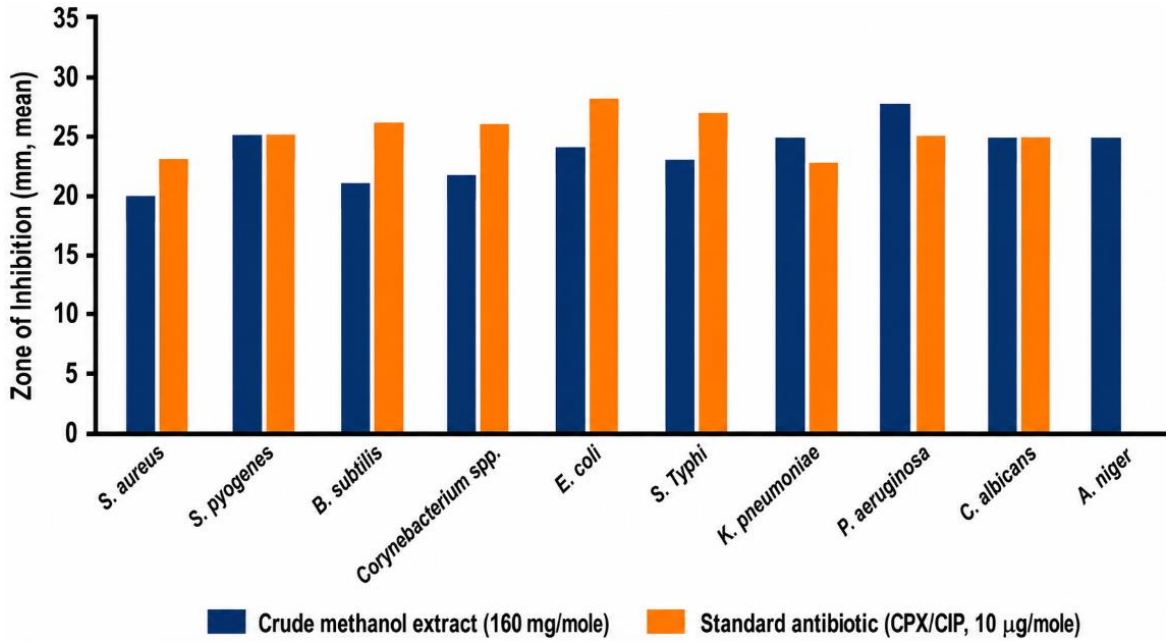

Figure 2. ***Zone of inhibition produced by the highest extract concentration (160 mg/hole) compared with the reference antimicrobial standard (ciprofloxacin, 10 µg/hole).***

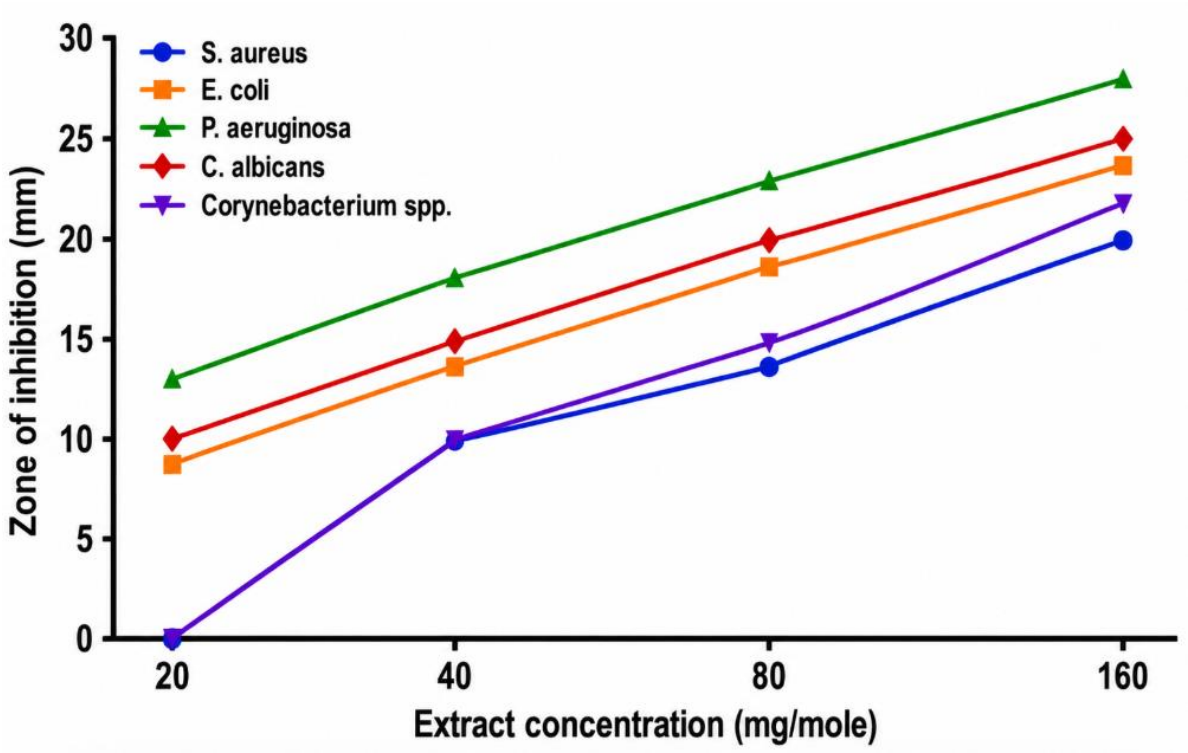

Figure 4. ***Dose–response relationship of the crude methanol extract against selected test organisms.***

3.4 DISCUSSION

Selection of *Polygonum senegalensis* for antimicrobial investigation was based on its ethnomedicinal use. The plant's aerial part was exhaustively extracted with methanol using the Soxhlet method, giving a yield of 97.17 g (19.43% w/w) of brownish, powdery crude extract (Table 1). Phytochemical evaluation confirmed the presence of terpenoids, flavonoids, carbohydrates, tannins, cardiac glycosides, steroids, cardenolides, and saponins, while alkaloids and anthraquinones were absent (Table 2), consistent with the phytochemical trends reported for the wider genus (Idoudi et al., 2024).

The observed antimicrobial activity is plausibly explained by the combined action of several of the identified constituent classes rather than any single compound. Flavonoids are known to disrupt bacterial membrane integrity, inhibit nucleic acid synthesis, and suppress efflux-pump activity, mechanisms that broaden their spectrum of action against both Gram-positive and Gram-negative organisms (Thebti et al., 2023; Zhang et al., 2025); comparable membrane- and transcription-level disruption has been documented for flavonoids from other medicinal plants tested directly against *S. aureus* (Zhou et al., 2023). Saponins act through detergent-like disruption of microbial membranes and can additionally modulate host immune clearance of pathogens, an activity consistent with the pronounced inhibition observed here even at moderate concentrations (Pikhtirova et al., 2023). Tannins contribute by binding microbial surface proteins and destabilizing cell membranes, giving broad-spectrum antibacterial and antifungal effects that have been demonstrated against both oral and systemic pathogens (Huang et al., 2024; Czerkas et al., 2024). Terpenoids, meanwhile, are reported to interfere with microbial oxidative phosphorylation and efflux-pump function, extending activity even to fungal targets in some systems (Siddiqui et al., 2024; Liu et al., 2024). The near-complete resistance of *Aspergillus niger* across all tested concentrations suggests that this filamentous fungus's cell-wall architecture may limit penetration by these constituents, a resistance pattern also reported for other crude plant extracts tested against the same organism.

The concentration-dependent increase in zone of inhibition observed across all susceptible organisms (Figure 4), together with activity at 160 mg/hole that matched or exceeded the reference antibiotic against several Gram-negative pathogens (Figure 2), indicates that the crude extract of *Polygonum senegalensis* merits further investigation as a candidate source of antimicrobial leads. This is particularly relevant given the scale of the regional AMR burden documented for the WHO African Region (Sartorius et al., 2024) and the specific antimicrobial stewardship and surveillance gaps highlighted for Nigeria (Alabi et al., 2025;

Kajumbula et al., 2024). At the same time, the crude nature of the extract, the absence of minimum inhibitory concentration (MIC) determination, and the lack of bioassay-guided fractionation are limitations that should be addressed before any translational claims can be made.

4. CONCLUSION

The crude methanol extract of the aerial part of *Polygonum senegalensis* contains terpenoids, flavonoids, tannins, cardiac glycosides, carbohydrates, and saponins, and exhibits concentration-dependent *in vitro* antimicrobial activity against a broad panel of Gram-positive bacteria, Gram-negative bacteria, and *Candida albicans*, with activity at the highest tested concentration comparable to, and in some cases exceeding, a standard reference antibiotic. *Aspergillus niger* was resistant at all concentrations tested. These findings support the plant's traditional medicinal use and its potential as a source of novel antimicrobial compounds at a time when antimicrobial resistance is placing an increasing burden on health systems in Nigeria and across the WHO African Region. Future work should prioritize solvent-partitioning and bioassay-guided fractionation to isolate and characterize the specific active compound(s), determination of minimum inhibitory and minimum bactericidal concentrations, mechanistic studies of the mode of antimicrobial action, and preliminary toxicological evaluation before any pharmaceutical application is considered.

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