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Characterization and Evaluation of the Synergistic Potency of Securidaca Longepedunculata Plant Extracts with Standard drug Against Some Pathogenic Organisms as Alternative Source of Chemotherapy

Manu, J. M[1]; Mohammed Muftahu Beli[2], Ahmed Sarkin Aska[2], Babai Mahdi[2], Aminu Muktari[3]

[1]*Department of Science Laboratory Technology, Modibbo Adama University, Yola.*

[2]*Department of Chemistry, Nigerian Army University Biu.*

[3]*Collage of Education, Azare.*

Abstract

The emergence of antimicrobial resistance among pathogenic bacteria has intensified the search for plant-derived bioactive compounds as alternative or adjunct therapeutic agents. This study investigated the antimicrobial activity of *Securidaca longepedunculata* root extracts and evaluated their synergistic potential with a standard antibiotic against Gram-positive bacterial pathogens. Phytochemical screening revealed the presence of alkaloids, flavonoids, tannins, saponins, glycosides, terpenoids, steroids, and carbohydrates, indicating a rich reservoir of bioactive constituents. The crude root extract exhibited a minimum inhibitory concentration (MIC) of 50 mg/mL against *Bacillus pyrogens*, *Staphylococcus aureus*, and *Streptococcus aureginosis*. Fractionation by column chromatography yielded fraction X1 (F1), which demonstrated enhanced antibacterial activity with MIC values ranging from 12.5 to 25 mg/mL. Synergistic evaluation using the checkerboard assay revealed significant interactions between fraction X1 and the standard antibiotic against *Bacillus pyrogens* and *Staphylococcus aureus*, with a fractional inhibitory concentration index (FICI) of 0.50, corresponding to a four-fold reduction in the effective antibiotic dose. Fourier Transform Infrared (FTIR) spectroscopy identified characteristic O–H (3339 cm^{-1}), C=O (1636 cm^{-1}), and C–O (1012 cm^{-1}) functional groups, confirming the presence of oxygenated phytochemicals. Gas Chromatography–Mass Spectrometry (GC–MS) analysis of fraction X1 identified nine compounds, predominantly ethane, 1-ethoxy-1-methoxy (100%), pholedrine (15.9%), and phenol (6.93%). The combination sample contained twenty-three compounds, with polygalitol (28.77%), benzene methanesulfonamide (12.08%), and dimethyl sulfoxide (8.33%) as the major constituents, suggesting complex molecular interactions responsible for the observed synergistic antibacterial effects. These findings demonstrate that *Securidaca longepedunculata* root extracts possess promising antibacterial properties and may serve as effective adjuvant agents for enhancing aminoglycoside antibiotic efficacy against Gram-positive bacterial infections.

Keywords

Securidaca longepedunculata,
Synergism,
Antibiotic,
Spectrometric,
Antimicrobial resistance

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Faculty of Science, ATBU Bauchi

Corresponding Author: Manu, J. M., [1]Department of Science Laboratory Technology, Modibbo Adama University, Yola.
Email: jomaitandu@yahoo.com

1.0 INTRODUCTION

The challenge of increasing drug resistance, inefficacy of many modern chemotherapeutic drugs for the management, attendant side effects of several antibiotics and cost of drugs, the use of medicinal plants is commonly adopted amongst rural and urban dwellers. Antimicrobial resistance is reported to threaten the efficacy of conventional antibiotics with Gram-positive bacteria emerging as significant contributors to healthcare-associated infections (Ewansiha, 2020).

The global economic burden exceeds $20 billion annually in direct healthcare costs. Medicinal plants offer potential sources of novel antimicrobial agents and adjuvants to restore antibiotic efficacy **(**Ewansiha 2020). ***Securidaca*** *longepedunculata* Fresen (Polygalaceae), commonly known as violet tree, is widely distributed across African regions. Traditional medicine practitioners utilize its roots for treating fever, malaria, infections, and inflammatory conditions **(**Abubakar et al, 2022).

Previous phytochemical studies revealed bioactive compounds including alkaloids, flavonoids, saponins, and terpenoids. However, investigations into synergistic interactions between plant extracts and conventional antibiotics remain limited (Baladrin et al, 2016). Combination therapy represents a promising approach to combat resistance by targeting multiple bacterial pathways simultaneously while reducing required dosages (Uperty et al, 2010). This study aimed to: (1) identify phytochemical constituents in *Securidaca longepedunculata* extracts; (2) evaluate antimicrobial activity against Gram-positive bacteria; (3) investigate synergistic effects with gentamicin; and (4) characterize chemical composition using FTIR and GC-MS analysis.

2.0 MATERIALS AND METHODS

2.1 Sample Collection and Identification

Fresh *Securidaca longepedunculata* plant materials (root, stem bark, leaves) were collected from Shira Local Government Area, Bauchi State, Nigeria. A botanist at Modibbo Adama University, Yola, authenticated the specimens.

2.2 Sample Preparation

Plant materials were cleaned, air-dried at room temperature (25-30°C) for two weeks, and ground to coarse powder using mortar and pestle. Powders were stored in airtight containers until extraction.

2.3 Sample Extraction

Based on the method reported by Herbane, et al, 1973. One hundred grams of each powdered material underwent Soxhlet extraction with methanol (200 ml) for 6 hours. Extracts were concentrated at 60°C using a water bath. Percentage yield was calculated as: (Dry weight of extract/Dry weight of sample) × 100. Column chromatography separated the most active crude extract using silica gel (100 g) with gradient elution: n-hexane (100%), hexane:ethyl acetate (70:30, 50:50, 30:70), ethyl acetate (100%), ethyl acetate:methanol (70:30, 50:50, 30:70), and methanol (100%). Fractions with similar TLC profiles were pooled, yielding three major fractions (F1, F2, F3).

2.4 Collection of Pathogenic Microorganisms

Gram-positive bacterial strains: *Bacillus pyrogens, Staphylococcus aureus,*

Streptococcus aureginosis were obtained from the Postgraduate Laboratory, Department of Chemistry, Modibbo Adama University. Cultures were maintained on Mueller-Hinton agar at 37°C.

2.5 Antimicrobial and Synergism Assays

Phytochemical screening followed standard procedures. MIC and MBC determinations employed broth microdilution method with standardized inocula (5×10^5 CFU/ml) in 96-well microplates. Concentrations tested ranged from 200 to 6.25 mg/ml.

Antibiotic susceptibility testing against twelve antibiotics identified gentamicin as suitable for combination studies based on intermediate activity profiles **CLSI (2012).** Checkerboard assay evaluated F1-gentamicin combinations with two-fold serial dilutions. Fractional inhibitory concentration index (FICI) was calculated as: (MIC_combination F1/MIC_alone F1) + (MIC_combination gentamicin/MIC_alone gentamicin). FICI ≤0.5 indicated synergy, 0.5-1.0 additive, 1.0-2.0 indifference, and >2.0 antagonism (Isidora, 2022).

2.6 Spectroscopic Analysis

FTIR spectroscopy (PerkinElmer Spectrum Version 10.4.3) analyzed functional groups in F1 and F1-gentamicin combination (400-4000 cm^{-1} range).

GC-MS analysis (Agilent 7890B GC with 5977A MSD) identified chemical constituents. Compounds were characterized by comparing mass spectra with NIST library database (Mahdi, 2020; Fair, J.D and C.M. Kormas (2008)).

3.0 RESULTS AND DISCUSSION

3.1 Phytochemical Screening

Phytochemical Species	Root	Stem	Leave
Alkaloid	+	+	-
Flavonoid	+	+	+
Tanins	+	+	+
Saponins	+	+	+
Glycosides	+	+	+
Typenoids	+	+	-
Anthraquinone	-	+	+
Steroid	+	+	+
Carbohydrate	+	+	+

Root extracts tested positive for alkaloids, Flovonoids, tannins, saponons, glycosides, terpenoids, steroids, and carbohydrates. Stem bark showed similar constituents plus anthraquinones (Sani et al 2023). Leaf extracts contained flavonoids, tannins, glycosides, steroids, anthraquinones, and carbohydrates but lacked alkaloids, saponins, and terpenoids (Junaid, 2015). These secondary metabolites contribute to antimicrobial properties through membrane disruption, protein denaturation, and enzyme inhibition mechanisms.

3.2 Antibacterial Activity of Fractions

Table 1: Antimicrobial activity of extracts and fractions

Bacterial Spp	MIC mg/ml F1	MIC mg/ml F2	MIC mg/ml F3	MBC mg/ml
Bacillus Pyroge	12.5	50	200	200
Strept Aureg	25	50	200	200
Staps aureu	25	50	200	200

Root extracts exhibited superior activity with uniform MIC (50 mg/ml) against all tested organisms. Fractionation enhanced potency, with F1 demonstrating two-fold to four-fold improvement (MIC: 12.5-25 mg/ml). MBC values exceeded 200 mg/ml for all samples, indicating primarily bacteriostatic effects, superior to reports in Auwal, 2022 and Atsu et al, 2024.

3.3 Susceptibility Testing and Synergistic Interactions

Table 2: Antibiogram and synergism results

Gram-positive bacteria	AUG	CTX	IMP	OFX	GN	ERY	AZN	CXM	CRO	CIP	ZEM	LBC
Staph.Aures	R(0)	R(6)	R(11)	S(21)	R(12)	S(16)	R(0)	R(11)	R(0)	S(28)	R(0)	S(22)
Bacillus Pyrogenosis	R(0)	R(0)	R(6)	S(19)	S(16)	R(6)	R(0)	R(0)	R(7)	S(27)	R(0)	S(19)
Strep. Auregenosis	R(0)	R(0)	I(13)	R(0)	S(16)	S(17)	R(0)	R(9)	R(8)	S(29)	R(0)	S(18)

Gentamicin demonstrated variable activity (12-16 mm zones). Checkerboard assay revealed synergistic interactions (FICI = 0.50) against B. pyrogens and S. aureus, with FIC values of 0.25 for both agents, indicating four-fold concentration reduction potential. Indifference against S. aureginosis (FICI = 1.50) highlights strain-specific responses. The synergism likely involves multiple mechanisms: membrane permeabilization enhancing gentamicin penetration, efflux pump inhibition increasing intracellular drug accumulation, and multi-target attack overwhelming bacterial defenses (Silva, 2019). For *Staphlocuccus. aureus*, the combination restored efficacy against a resistant organism.

3.4 FTIR Analysis

Major FTIR peaks

FTIR spectroscopy identified hydroxyl groups (3339 cm^{-1}), carbonyl groups (1636 cm^{-1}), and ether/alcohol groups (1012-1099 cm^{-1}). The combination sample exhibited nearly doubled fingerprint region complexity (18 vs. 10 peaks), suggesting formation of new molecular species and coordination complexes. Minimal shifts in major functional groups indicate preserved core structures with enhanced molecular interactions.

3.5 GC-MS Analysis

Table 4: Major compounds in F1 and combination

S/N	Proposed Compound	Molecular Formula	Molecular Mass g/mol	Retention Time	Area %
1	Methyl Chloroformate	$C_2H_3ClO_2$	93.98	1.429	4.52
2	Ethanamine,N,N-Dimethyl-2-(Phenylmethoxy)	$C_{11}H_{17}NO$	179.13	2.029	35.89
3	Dimethyl Sulfoxide	C_2H_6OS	78.13	3.542	8.33
4	Carbonic Acid, Phenyl Ester	$C_8H_8O_2$	136.O2	2.542	100
5	Phenol	C_6H_6O	94.11	3.64	3.99
6	Methyl Salicylate	$C_8H_8O_3$	152.05	6.569	1.57
7	M-Guaiacol	$C_7H_8O_2$	124.05	7.433	1.25
8	Acetophenone,2[(PNitrophenyl)Imino]	$C_{14}H_{10}N_2O_3$	254.06	7.718	1.02
9	2- Methyl - 2-Hydroxy-Decalin-4a-Carboxylic Acid, 2,4a- Lactone	$C_{12}H_{18}O_2$	194.14	8.685	1.11
10	Tetradecene	$C_{14}H_{18}$	**196.36**	**9.798**	**14.3**
11	3,5- Methano- 2,3,4 (1h) - tetrahydrobenzo(b) azepine	$C_{11}H_{13}N$	159.10	10.691	7.71
12	methyl 4- methoxysalicylate	$C_9H_{10}O_4$	182.05	10.947	16.02
13	Phthalic acid, methyl 2-nitrophenyl ester	$C_{15}H_{11}N\ O_6$		11.13	1.62
14				11.431	1.6
15	Acetamide,2-(4-hydroxy-3-methoxyphenyl)	$C_9H_{11}N\ O_3$	181.07	13.107	4.69
16	Polygalitol	$C_6H_{12}O_5$	164.06	13.774	28.77
17	Tetradecene	$C_{19}H_{38}$	266.49	16.439	1.02
18	Methylpentadecanoate	$C_{17}H_{38}O_2$	244.41	18.408	2.57
19	Undecanoic acid	$C_{11}H_{22}O_2$	186.06	19.039	2.13
20	phenol, 2,6-dimethyl- 4-nitro	$C_8H_9N\ O_3$	167.05	20.649	6.66
21	methyl 4- methyltridecenoate	$C_{13}H_{26}O_2$	214.34	20.803	2.13
22	Benzenemethanesulfonamide	$C_7H_9N\ O_2S$	171.03	21.066	12.08
23	1,6,10,14 tetramethyl 6- hydroxy - pentadecane	$C_{15}H_{40}O$	236.00	21.689	2.22

GC-MS analysis of F1 identified nine compounds. The dominant component, ethane 1-ethoxy-1-methoxy, likely contributes membrane-disrupting effects. Pholedrine (15.9%), a phenethylamine derivative, and phenol (6.93%) possess established antimicrobial properties. Three fatty acid methyl esters (8.63% combined) disrupt bacterial membranes through lipid bilayer integration.

The combination sample exhibited twenty-three compounds, indicating extensive chemical transformations. Key observations include: Polygalitol (28.77%) emerged as a major component, potentially imposing osmotic stress and enhancing membrane permeability for gentamicin entry. Benzenemethanesulfonamide (12.08%) represents a significant finding as sulfonamides inhibit bacterial folate synthesis. Its formation during combination adds a second antimicrobial mechanism complementing gentamicin's protein synthesis inhibition. Dimethyl sulfoxide (8.33%) functions as a penetration enhancer, facilitating drug uptake across bacterial membranes.

Carbonic acid phenyl ester (100%) and ethanamine derivative (35.89%) suggest esterification and amination reactions occurred, possibly catalyzed by gentamicin's amino groups. The dramatic increase in chemical diversity (23 vs. 9 compounds) provides molecular evidence for synergism. New nitrogen-containing (26% of compounds), sulfur-containing (9%), and modified phenolic compounds likely contribute through: enhanced membrane permeabilization, efflux pump inhibition, multi-target bacterial pathway disruption, and prodrug formation releasing active antimicrobials (Umar et al, 2026).

3.6 Discussion

This investigation demonstrated that S. longepedunculata root extracts possess significant antimicrobial activity, enhanced through fractionation, with synergistic potential when combined with gentamicin. The four-fold dose reduction achieved through synergism has important clinical implications, potentially minimizing gentamicin's nephrotoxicity and ototoxicity while maintaining or enhancing antibacterial efficacy. (Ghadi et al, 2018; Varghhese, 2019).

The chemical characterization revealed multi-component activity. Phenolic compounds, fatty acid esters, and aromatic hydrocarbons in F1 provide complementary mechanisms to gentamicin's ribosomal binding. The formation of novel compounds during combination (particularly benzene-methane-sulfonamide and polyols) represents a unique finding suggesting chemical synergism alongside pharmacological synergism.

Strain-specific responses underscore the complexity of combination therapy. While B. pyrogens and S. aureus showed synergism, *S. aureginosis* exhibited indifference, likely reflecting different resistance mechanisms or cell envelope structures. This highlights the need for pathogen-specific susceptibility testing before clinical application.

The integrated FTIR and GC-MS data provide complementary information: FTIR confirmed functional group preservation with enhanced

molecular interactions, while GC-MS identified specific bioactive compounds and transformation products. Together, these techniques elucidate the chemical basis of observed biological activities (Mahdi, 2020).

Compared to previous studies reporting MICs of 26-31.5 mg/ml for *Securidaca longepedunculata* extracts, this study achieved comparable or enhanced activity through systematic fractionation. The synergism findings extend earlier work on antimalarial combinations to antibacterial applications, suggesting broad applicability of this plant's adjuvant potential.

Limitations include the in vitro nature of the study and testing against a limited bacterial panel. Future investigations should include: in vivo efficacy studies in animal models, mechanistic studies elucidating efflux pump inhibition and membrane effects, testing against clinically relevant resistant strains including MRSA, toxicological assessments, isolation and individual testing of major compounds, and investigation of synergism with other antibiotic classes.4.0 **(**Olaoye et al, 2021).

CONCLUSION

This study validates the antimicrobial properties of *Securidaca longepedunculata* root extracts and demonstrates significant synergistic interactions with gentamicin against Gram-positive bacteria. Fraction F1 exhibited enhanced activity (MIC: 12.5-25 mg/ml) compared to crude extracts (MIC: 50 mg/ml).

Synergistic effects (FICI = 0.50) against B. pyrogens and S. aureus enable four-fold dose reduction of both agents. Chemical characterization identified nine compounds in F1 and twenty-three in the combination, including newly formed benzenemethanesulfonamide and polygalitol, providing molecular evidence for multi-mechanism synergism.

These findings support the potential development of *Securidaca longepedunculata* extracts as adjuvant therapy to restore aminoglycoside efficacy, reduce toxicity through dose reduction, and combat antimicrobial resistance. Clinical translation requires in vivo validation, mechanistic studies, and safety assessments.

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