https://chemclassjournal.com/
ChemClass Journal Vol. 9 Issue 3 (2025); 326 - 333
e-ISSN:3092-8214 p-ISSN:3092-8206
doi.org/10.33003/chemclass-2025-0903/25

Phytochemical Constituents and Synergistic Antibacterial Effects of *Securidaca longepedunculata*

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Abstract

Phytochemical screening revealed the presence of various bioactive compounds in the samples, including alkaloids, flavonoids, tannins, saponins, glycosides, terpenoids, and carbohydrates, depending on the plant part analyzed. This research aimed to investigate the phytochemical constituents, antibacterial activity, and potential combination effects of *Securidaca longepedunculata* extracts with a standard antibiotic drug. The antibacterial activity was assessed using broth dilution method to determine the minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) against three selected Gram-negative bacterial strains: *Escherichia coli*, *Salmonella typhi*, and *Klebsiella pneumoniae*. The root extracts demonstrated the strongest antibacterial activity, with MIC values consistently at 50 mg/ml across all tested bacterial strains, while the MBC values were all above 200 mg/ml. Fractionation of the most active extract (root extract) via column chromatography yielded three major fractions: F1, F2, and F3. Among these, fraction F1 exhibited the highest activity, with MIC values of 25 mg/ml against the tested organisms. The study further explored the potential combination effects of the plant extracts with a standard antibiotic using a checkerboard assay, which revealed synergistic, additive, and indifferent interactions. Overall, the findings suggest that *Securidaca longepedunculata* extracts possess promising potential as natural antimicrobial agents.

Keywords: *Escherichia coli*, Fractional Inhibitory Concentration, *Klebsiella pneumoniae*, Minimum Inhibitory Concentration, Minimum Bactericidal Concentration, *Salmonella typhi*, Synergy

Introduction

Medicinal plants have been used in various places for treatment of numerous pathogenic infections, promoting general wellbeing, as culinary additives, and sometimes for spiritual purposes [1]. Many African medicinal plants have been traditionally and scientifically connected to treating vast human diseases [2]. The therapeutic properties of plants are connected to different classes of compounds contained [1,2].

Securidaca longepedunculata is a small plant mostly found in Nigeria and many parts of Africa [3]. It is a small tree with a pale grey, smooth bark and hairless alternate leaves, which are different in size and in shape. Its flower is smaller, mostly pink or purple in color, sweet scented typically blooming in early summer [4]. It is commonly

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known as violet tree in English Language, In Nigeria, it's called in different names such as Uwar magunguna/Sanya in Hausa, Ezeogwu in Igbo, Aaalali in Fulfulde and Ipeta in Yoroba, respectively. The root of this plant has been utilized to treat fever, malaria, gonorrhea, headaches, rheumatism, diabetes, sexual impotence, fungal infections, epilepsy, cancer, convulsions, constipations, pneumonia, blood purification, sexually transmitted diseases, skin infections, tuberculosis, venereal diseases, syphilis, toothache, pains, epilepsy, backache, and aphrodisiac among other uses [3].

It was also reported that the leaves of the plant are employed for treating epilepsy, headaches, stomach ache, infertility, snakebite, toothache, cancer, skin infections, dislocated jaw, and contraceptive purposes and to facilitate placenta expulsion [5]. Similarly, the stem bark is traditionally used for treating venereal diseases, skin diseases, dysentery, malaria, typhoid, inflammation, chest complaints, abortion, constipation, viral infections, snake bites and infertility problems [6].

Materials and Method

Sample Collection and Identification

The fresh samples of *Securidaca longepedunculata* plant were collected from its natural habitat in Shira Local Government Area, Bauchi State, Nigeria. The plant sample were identified and Authenticated by the traditional herbalist.

Sample preparation

The sample plants were cleaned, air dried and ground to coarse powder using mortar and pestle to reduce its surface area. The resulting powder was stored in airtight containers for further use [7,8].

Extraction of sample

A 100g of the powdered sample were extracted using a Soxhlet extraction apparatus. Methanol solvent of high analytical grade was used for the extraction. The obtained extracts were concentrated to dryness using water bath and the percentage yield of the extracts was determined using the relationship:

Percentage yield (%) = ((Dry weight of extracts))/((Dry weight of sample)) X 100

Collection of pathogenic microorganisms

The following stock culture organisms were obtained from postgraduate laboratory, Department of Microbiology, Modibbo Adama University Yola, Adamawa state, Nigeria.

1. *Escherichia coli* (ATCC 25922)

2. *Salmonella typhi* (ATCC 6539)

3. *Klebsiella pneumonia* (ATCC 700603)

All assays were conducted as three independent experiments; each performed in duplicate. Statistical significance of the MICs and MBCs of methanol root, stem and leaf extracts of *Securidaca longepedunculata* against selected

Bacterial Strains compared with gentamicin was determined by one-way ANOVA with $p < 0.05$ considered significant.

Results and Discussion

Phytochemical evaluation

The results of the phytochemical analysis from the root, stem, and leaf methanol crude extracts of Securidaca longepedunculata are presented in Table 1.

Table 1: Phytochemical screening of the root, stem back and leave extracts

Phytochemical Species	Root	Stem back	Leaf
Alkaloids	+	+	-
Flavonoids	+	+	+
Tannins	+	+	+
Saponins	+	+	-
Glycosides	+	+	+
Terpenoids	+	+	-
Anthraquinone	-	+	+
Steroids	+	+	+
Carbohydrate	+	+	+

The phytochemical screening of methanolic extracts from the root, stem, and leaf extract of *Securidaca longepedunculata* revealed the presence of several bioactive compounds. As shown in the table above. The root extracts were found to contained Alkaloids, Flavonoids, Saponins, Tannins, and steroids, Anthraquinone, Glycosides and Carbohydrates. Similarly, the stem extract contained alkaloids, flavonoids, tannin, saponins, glycoside, terpenoids, anthraquinones, steroids and carbohydrate while the leaf extracts showed the presence of flavonoids, tannins, glycosides steroids, anthraquinones, and carbohydrates but lacked alkaloids, saponin, and terpenoids respectively.

These finding are consistent with previous research [7,9,10], although some discrepancies exist, likely due to environmental factors, geographical location, harvesting period, and soil compositions [11]. For example, variation in the presence of tannin, carbohydrate, flavonoids and anthraquinone have been reported by difference researchers [8,12] such phytochemical diversity underscores the influence ecological and methodological factors on the chemical profile of *Securidaca longepedunculata* [13].

Antibacterial Analysis

Table 2: MICs and MBCs of methanol root, stem and leaf extracts of *Securidaca longepedunculata* against selected Bacterial Strains

Test organisms	MIC (mg/ml) Root extract	MIC (mg/ml) Stem Extract	MIC (mg/ml) Leave extract	MBC (mg/ml)	MIC (mg/ml) Gentamicin	MBC (mg/ml) Gentamicin
Escherichia coli	50	50	100	200	2	4
Salmonella typhi	50	100	100	200	4	8
Kle. Pneunoniae	50	50	50	200	4	8

The table presents the antibacterial activity of methanol extracts derived from the root, stem, and leaf extracts of *Securidaca longepedunculata* against three clinically tested Gram-negative *bacteria: Escherichia coli, Salmonella typhi, and Klebsilla pneunoniae* respectively. The root extract showed consistent antibacterial inhibition across all the test organisms (MIC 50mg/ml), indicating a strong activity compared to other part. The stem extracts showed variable activity, requiring 100mg/ml to inhibit *Salmonella typhi.* Leaf extract showed weakest activity overall, except against *Klebsilla pneunoniae. MBC* values where uniform (200mg/mL) for all extracts and organisms, indicating that higher concentrations are needed to achieve bactericidal effects. These findings support the traditional use of *Securidaca longepedunculata* and highlight its potential for further phytomedicinal developments.

Antibacterial Studies of the Fractions

After successfully, obtaining the three fractions from the column chromatographic analysis the next stage was to determine the MICs and MBCs activity these fractions. The results of the MICs and MBCs of the three fractions were presented in the table below:

Table 3: MICs and MBCs of the fractions obtained from column chromatography

Test organisms	MIC (mg/ml) Fraction 1	MIC (mg/ml) Fraction 2	MIC (mg/ml) Fraction 3	MBC (mg/ml)	MIC (mg/ml) Gentamicin	MBC (mg/ml) Gentamicin
Escherichia coli	25	50	200	200	2	4
Salmonella typhi	25	100	200	200	4	8
Klebsiella pneumonia	25	50	200	200	4	8

Susceptibility Test

In order to achieve the combination of the most potent fraction (F1) with a standard antibiotic drug a susceptibility test on a wide range of antibiotic drugs was conducted and the results of this test were categorized in to three classes viz: resistance antibiotics, intermediate antibiotics and susceptible antibiotics using clinical laboratory science method. In which the chosen antibiotic for this study falls under the intermediate antibiotics (gentamicin). This is because the selected antibiotics fells within a middle range where they neither performed better nor worse, the results of the antibiotics for both gram-positive and gram-negative bacteria were presented in the tables below:

Table 4: Antibiogram profile of Gram-Negative Bacterial test organisms

Bacteria	AUG	CTX	IMP	OFX	GN	NA	NF	CXM	CRO	ACX	ZEM	LBC
E.Coli	R(0)	R(0)	R(0)	S(17)	R(12)	S(16)	R(0)	R(0)	R(0)	R(0)	R(6)	I(15)
K.pneu	R(0)	R(0)	R(0)	S(19)	R(9)	S(19	R(0)	R(0)	S(18)	R(0)	R(0)	S(16)
S.Typhi	R(0)	R(0)	I(15)	S(18)	S(16)	I(14	R(0)	R(0)	I(15)	R(0)	R(7)	S(18)

KEY56u: R: Resistant (0-12mm) I: Intermediate(13-15mm) S: Susceptible (16mm& above)
AUG: Amoxicillin clavulanate CTX: Cefolamine IMP: Imipenem/cilastatin
OFX: Floxacin GN: Gentamycin NF: Nitrofurantolin
CXM: Cefuroxime NA: NalidixiCacid CRO: Ceft ACX: Ampiclox
LBC: Levofloxacin LBC: Levofloxacin

Synergistic Interactions

Table 5: Synergistic interactions between the plant extract (drug B) and the chosen antibiotic (drug A)

Bacteria	FIC of A ug/mg	FIC of B mg/ml	FIC index mg/ml	Remarks
Escherichia coli	0.50	0.25	0.75	Additive
Salmonella typhi	0.25	0.25	0.50	Synergy
Kleb. Pneumonia	0.50	0.50	1.00	Indifference

The checkerboard method, frequently employed to assess interactions between Antibiotics and plant extracts, was utilized in this study. Specifically, synergistic interactions involving the plant extracts (refers to as drug A) combining with antibiotic (referred to as drug B) were investigated. The concentrations of the agents used were initially set as twice their minimum inhibitory concentration (MIC) and then serially diluted in fivefold step. The combined effects were assessed by calculating the

Fraction Inhibitory Concentrations (FIC) index for each combination using the formula:

$$FIC\ of\ drug\ A\ (FICA) = \frac{MIC\ of\ compound\ A\ in\ combination}{MIC\ of\ compound\ A\ alone}$$

$$FIC\ of\ drug\ B\ (FICB) = \frac{MIC\ of\ compound\ B\ in\ combination}{MIC\ of\ compound\ B\ alone}$$

FIC index = FICA + FICB

Synergism was defined as an FIC index ≤ 0.5, while an FIC index above 4.00 indicated antagonism between the two agents. FIC index between 4 and 1 suggested indifference, and those between 1 and 0.5 were considered additive, based on the Loewe additivity zero interaction theory [14]. *E. coli* (additive Effect): the combination of the plant extracts (F1) and standard antibiotic drug (gentamicin) against this bacteria specie resulted in an FIC index of 0.75 mg/ml, indicating an additive effect.

This suggested that the combination of the plant extracts and gentamicin exhibited a combined effect that was greater than the effects of either agent alone, albeit not synergistic. *Salmonella typhi* results (synergistic effects): the combination of the F1 fraction and gentamicin against Salmonella typhi showed an FIC index of 0.5mg/ml, indicating synergistic effects. This suggest that the combined action of the fraction (F1) and standard drug (gentamicin) was greater than the sum of their individual effects, implying potential enhanced efficacy against *Salmonella typhi. Klebsiella* results (indifference): in the case of klebsiella*,* the combination of the plant with gentamicin resulted in an FIC index of 1.0 mg/ml, indicating indifference. This implies that the combined effect of the plant and gentamicin is similar to the effect of either agent alone, suggesting no significant between them against *klebsiella* [15,16,17]. In summary, the overall results indicate varying degrees of interaction between the plant (F1) and standard drug (gentamicin) across different bacterial strains. While, the combination exhibited an additive effect against *E. coli,* it demonstrated synergy against *Salmonella typhi,* and indifference against *klebsiella pneumoniae* respectively [18,19,20]. These findings highlight the importance of considering bacterial strains specificity when assessing the efficacy of combination therapies.

Conclusion

Results from study reveal that *Securidaca longepedunculata,* especially the root extract, possesses significant antibacterial potential owing to its rich composition of phytochemical species. Fraction 1 (F1) exhibited the most potent effects, and enhanced the activity of gentamicin, particularly against *salmonella typhi* (synergistic) and *Escherichia coli* (Additive). These findings validate the plant's traditional use.

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