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Comparative Antimicrobial Activity of *Securidaca longipedunculata* (Fresen) Extracts on Selected Human Pathogens

Hassan L. G.[1], Umar K. J.[2], Yusuf A. J.[3], Rabah A. B.[4], Umar A. U.[5*]

[1]Department of Pure and Environmental Chemistry, Usmanu Danfodiyo University, Sokoto, Nigeria
[2]Department of Pure and Industrial Chemistry, Federal University Birnin Kebbi, Nigeria
[3]Department of Pharmaceutical and Medicinal Chemistry, Usmanu Danfodiyo University, Sokoto, Nigeria
[4]Department of Microbiology, Usmanu Danfodiyo University, Sokoto, Nigeria
[5]Department of Chemistry, Sokoto State University, Sokoto, Nigeria

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***Corresponding author:** Umar A. U.
Department of Chemistry, Sokoto State University, Sokoto, Nigeria

Abstract

The escalating trend of microbial resistance has become a pressing global concern, challenging the effectiveness of antimicrobial agents in treating infections. This study was aimed at evaluating the antimicrobial activity of the n-hexane, ethyl acetate, and methanol extracts of *Securidaca longipedunculata* against selected human pathogens. The antimicrobial screening against bacterial pathogens- (e.g., *Methicillin-resistant staphylococcus aureus, Vancomycin-resistant Enterococci, Staphylococcus aureus, Staphylococcus feacalis, Escherichia coli, Salmonella typhimurium, Pseudomans fluorescens* and *Klebsiella pneumonieae*) and fungal pathogens (which includes *Candida albicans, Candida krusei, Aspergillus niger, Aspergillus fumigates, and Microsporum canis*) was performed using agar well diffusion and broth dilution methods. The results demonstrated significant antimicrobial activity, with higher efficacy against *S. aureus.* Susceptibility test results showed that the extract inhibited the growth of all the test organisms (bacteria and fungi) with a mean zone of inhibition range of 18-29 mm, with the exception of *VRE, S. typhimurium, P. fluorescens, C. krusei and M. canis* in all the three extracts, while Hexane extract showed no inhibition against *E. coli and A. niger*. The standard drugs sparfloxacin and ciprofloxacin (500 µg/cm^3) had zone of inhibition ranging from 28 - 40 mm. While the antifungal drugs fluconazole and fulcin exhibited zone of inhibition ranging from 27 mm to 34 mm. The Minimum inhibitory concentration (MIC) for the extracts ranges from 62.5 - 250µg/cm^3, while the Minimum Bactericidal/Fungicidal Concentrations (MBC/MFC) range from 250 - 500 µg/cm^3, respectively. The ethyl acetate extracts possessed higher antimicrobial activity among the three extracts tested against *S. Aureus*. The findings suggest that *S. longipedunculata* leaves hold promise as a natural source of antimicrobial agents, warranting further exploration for potential therapeutic applications.

Keywords: *Securidaca longipedunculata*, Antimicrobial resistance, phytochemicals, Microbial infection, mechanisms of action.

INTRODUCTION

Antimicrobial resistance (AMR) is a growing global health crisis that poses a significant threat to synthetic medicine, patient outcomes, and public health (Ndamitso *et al.,* 2013). AMR occurs when microorganisms, such as bacteria, fungi, and parasites develop the ability to withstand the effects of antimicrobial agents, rendering them ineffective in treating infection (Gupta and Kumar, 2017). This phenomenon undermines the effectiveness of antibiotics and antifungal drugs that have been critical in controlling infectious diseases for decades (Walsh and Ames, 2004). Addressing the challenge of Antimicrobial resistance requires a comprehensive approach involving healthcare, agriculture, policy-making, and public awareness. It's important to promote appropriate use of antibiotics, invest in research for new treatments, improve infection prevention and control, and encourage international collaboration to combat this global health threat (Yusuf *et al.,* 2018). In addition, widespread of diseases such as cancer, tuberculosis, influenza, cardio-vascular diseases, skin rashes, typhoid fever and malaria coupled with high level poverty in Africa and other developing countries like Nigeria has made savings and investigations on herbal plants an attractive endeavor in human healthcare. This is because most of the synthetic medicinal products

are expensive and usually difficult to get among low income earners (WHO, 2014). Plants have been used as traditional medicine for centuries to treat various diseases (Yusuf *et al.,* 2023), and are free from unpleasing side-effects (Abdullahi *et al.,* 2015). Many plant compounds have antimicrobial properties that can help alleviate symptoms or aid in the recovery process (Kebede *et al*., 2021).

Securidaca longipedunculata which belongs to the family *polygalaceae* is a shrub which grows up to 10 cm high, 2 to 9 cm long and 0.5 to 2.5 cm broad leaves. *S. longipedunculata* is locally known as "uwar magunguna" in Hausa, "ipeta" in Yaruba and "ezeogwu" in Igbo language (Namadina *et al.,* 2020). It grows in varying climatic conditions, from hot arid to humid climates and in broad vegetation range, from hillside grassland in Guinea to Northern Nigeria, Western Cameroon and Central Africa to Angola (Anjarwalla *et al.,* 2015). The plant is commonly used in Hausa-speaking communities in Nigeria as flavour, food (sauces) and medicine. It is used as sexual enhancer for men (Musa *et al.,* 2013). It was reported to be used against tuberculosis, cancer, venereal diseases, headache, rheumatism, diabetes as well as abortifacient (Avhurengwi and Walter, 2006). This study is therefore aimed at evaluating the antimicrobial activity of the n-hexane, ethyl acetate and methanol leaves extract of *S. longepedunculata* against selected pathogens with the view to identify the most potent extract that can serve as lead for development of novel antimicrobial agent.

MATERIALS AND METHODS

Sample Collection and Identification

The samples of *S. longipedunculata* were collected from the Zuru local government area of Kebbi State in August, 2021. The sample was then transported to the laboratory and identified by a taxonomist at the Department of Pharmacognosy and Ethnopharmacy, Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University, Sokoto. The voucher specimen with the number PCG/UDUS/POLY/0001 was prepared and deposited for future reference at the herbarium of the department.

Sample Preparation

Fresh leaves of *S. longipedunculata* were properly washed to remove earthy impurities. The leaves were shade dried and pulverized using a wooden pestle and mortar. The powdered sample was then stored in an air-tight container until it was needed for analysis.

Extraction Procedure

In accordance with the maceration and exhaustive percolation method described by Nweze *et al.,* (2004), 200 g of the milled sample, *S. longipedunculata* leaves was soaked in about 2500 cm^3 of n-hexane (plant material to solvent ratio was 1:10, w/v) in a glass beaker. The beaker was tightly sealed and kept for 72 hours with regular agitation to release the soluble metabolites into the solvent. The mixture was filtered and pre-concentrated by evaporating the solvent. The same extraction procedures were repeated using ethyl acetate and methanol respectively. The respective extracts were dried and stored for further analysis.

Test Organisms

Clinical isolates of bacterial pathogens (e.g., *Methicillin-resistant staphylococcus aureus, Vancomycin-resistant Enterococci, Staphylococcus aureus, Staphylococcus feacalis, Escherichia coli, Salmonella typhimurium, Pseudomans fluorescens* and *Klebsiella pneumonieae*) and fungal pathogens (which includes *Candida albicans, Candida krusei, Aspergillus niger, Aspergillus fumigates, and Microsporum canis*) was obtained from Department of Medical Microbiology of Ahmadu Bello University Teaching Hospital Zaria. All the isolates were checked for purity and maintained in Mueller Hinton agar (for bacteria) and in slants of Sabouraud Dextrose Agar for fungi.

Antimicrobial Screening

The antimicrobial sensitivity assay was carried out against bacterial pathogens (e.g., *Methicillin-resistant staphylococcus aureus, Vancomycin-resistant Enterococci, Staphylococcus aureus, Staphylococcus feacalis, Escherichia coli, Salmonella typhimurium, Pseudomans fluorescens* and *Klebsiella pneumonieae*) and fungal pathogens (which includes *Candida albicans, Candida krusei, Aspergillus niger, Aspergillus fumigates, and Microsporum canis*) by the agar well diffusion method (Yusuf *et al.,* 2015; Ekambaram and Santhy, 2023). The stock concentration of the extracts (500 µg/cm^3) was prepared by dissolving 0.005 mg of the extracts in 10 cm^3 dimethyl sulfoxide (DMSO). Mueller Hinton agar and the growth medium, was prepared according to manufacturer's instructions, and sterilized for 15 minutes at 121 °C. Nutrient broth and Sabouraud dextrose broth were used for antibacterial and antifungal evaluations. The test organisms were inoculated and incubated for 24 hours for bacteria and 48 hours for fungi. The solidified sterile medium contained in petri dish was seeded with 0.1 cm^3 standard inoculum of the test microbe at 45 °C. Wells were bored into the solidified inoculated nutrient agar plates using cork borer of 6 mm diameter. The wells were filled with 0.1 cm^3 DMSO solution of the extracts. Discs containing blank extraction solvents served as control. The reference antibacterial drug (Sparfloxacin and Ciprofloxacin) and (Fluconazole, and Fulcin) antifungal drug were used as a positive control. The extracts and standard drugs were allowed to diffuse into the agar and incubated overnight. All incubations were done at 37 and 25 °C for bacteria and fungi respectively. At the end of incubation period, diameter of inhibition zone was measured using transparent ruler and recorded. The zones of inhibition of microbial growth were tested in duplicates and the mean of the results was recorded in millimeters (mm).

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal/Fungicidal Concentration (MBC/MFC)

Minimum inhibitory concentration (MIC) of *S. longipedunculata* extracts was determined using broth dilution method (Gupta and Kumar, 2017; Muhammad *et al.*, 2019). Mueller Hinton broth was prepared according to manufacturer's instructions; 2.0 cm^3 of the media was dispensed in screwcapped test tubes and sterilized at 121 °C for 15 minutes and allowed to cool; Mc-Farland's standard turbidity scale number 0.5 was prepared. Dilution of the organism suspension was done continuously using sterile normal saline until the turbidity matched that of Mc-Farland's scale by visual comparison. At that point, the concentration of the test microbe was about 1.5 x 10^8 cfu/cm^3. Two-fold serial dilution of the extract in the sterile broth was made to obtain the concentrations of 500, 250, 125, 62.5 and 31.25 µg/ cm^3, respectively; 0.1 cm^3 of the standard inoculum of the test microbe was then inoculated into the different concentrations of the extract in the broth. The tubes were incubated aerobically at 37 °C for 24 hours (for bacteria) and 25 °C for 48 hours (for fungus). The lowest concentration (highest dilution) of the extract that produced no visible growth (no turbidity) in the first 24 hours when compared with the control tubes was considered as initial MIC. The dilutions that showed no turbidity were incubated further for 24 hours at 37 °C. The lowest concentration that produced no visible turbidity after a total incubation period of 48 hours was considered as final MIC.

The MBC/MFC was carried out to determine whether there is complete death of test microbes or just growth inhibition. Mueller Hinton agar broth was prepared, sterilized at 121 °C for 15 minutes, and transferred into sterile petri dishes to cool and solidify. The contents of the MIC in the serial dilution were sub-cultured into the prepared medium and incubated at 37 °C for 24 hours; the plates were observed for colony growth; the MBC/MFC was the plate with lowest concentration of the extract in serial dilution without colony growth (Gupta and Kumar, 2017; Muhammad *et al.*, 2019).

RESULTS AND DISCUSSION

Results

Extraction

The percentage yield of the extracts used for the analysis is represented in Table 1. The methanol extract has the highest yield of 13.51 % while n-hexane yielded the least percent of 4.81.

Determination of antimicrobial activity of *S. longipedunculata* leaves extracts

The results of the antimicrobial activity of *S. longipedunculata* leaves extracts are presented in Table 2 - 4.

Table 1: Percentage yield of *S. longipedunculata* leaves extracts

Extracts	Yield (g)	Yield (%)
n-Hexane	9.62	4.81
Ethyl acetate	16.41	8.21
Methanol	27.01	13.51

Table 2: Zone of inhibition of *S. longipedunculata* leaves extracts against the test organisms

Test organisms	Mean zone of inhibition (mm)						
	HE	EAE	ME	SF	CF	FZ	FC
MRSA	20	27	24	35	0	0	0
VSE	0	0	0	0	28	0	0
S. aureus	21	29	22	31	0	0	0
S. feacalis	18	25	21	30	30	0	0
E. coli	0	26	20	34	37	0	0
S. typhimurium	0	0	0	0	40	0	0
P. fluorescens	0	0	0	31	0	0	0
K. pneumonieae	20	26	23	0	31	0	0
C. albicans	19	24	20	0	0	32	0
C. krusei	0	0	0	0	0	34	32
A. niger	0	23	20	0	0	0	27
A. fumigates	20	25	22	0	0	0	31
M. canis	0	0	0	0	0	0	30

Key: HE = Hexane extract, EAE = Ethyl acetate extract, ME = Methanol extract, SF = Sparfloxacin, CF = Ciprofloxacin, FZ = Fluconazole, FC = Fulcin

Table 3: MIC of *S. longipedunculata* extracts against the test organisms

Extracts	Test organisms	Concentration (µg/mL)				
		500.00	250.00	125.00	62.50	31.25
HE	MRSA	-	-	0*	+	++
	S. aureus	-	-	0*	+	++
	S. feacalis	-	0*	+	++	+++
	K. pneumonieae	-	-	0*	+	++
	C. albicans	-	0*	+	++	+++
	A. fumigates	-	-	0*	+	++
EAE	MRSA	-	-	-	0*	+
	S. aureus	-	-	-	0*	+
	S. feacalis	-	-	0*	+	++
	E. coli	-	-	0*	+	++
	K. pneumonieae	-	-	0*	+	++
	C. albicans	-	-	0*	+	++
	A. niger	-	-	0*	+	++
	A. fumigates	-	-	0*	+	++
ME	MRSA	-	-	0*	+	++
	S. aureus	-	-	0*	+	++
	S. feacalis	-	-	0*	+	++
	E. coli	-	-	0*	+	++
	K. pneumonieae	-	-	0*	+	++
	C. albicans	-	-	0*	+	++
	A. niger	-	-	0*	+	++
	A. fumigates	-	-	0*	+	++

Key: HE = Hexane extract, EAE = Ethyl acetate extract, ME = Methanol extract, - = No turbidity, 0* = Minimum inhibitory concentration, + = turbid (light growth), ++ = Moderate turbidity. +++ = High turbidity

Table 4: Minimum bactericidal and fungicidal concentration of *S. longipedunculata* leaves extracts against the test organisms

Extracts	Test organisms	Concentration (µg/mL)				
		500.00	250.00	125.00	62.50	31.25
HE	MRSA	0*	+	++	+++	++++
	S. aureus	0*	+	++	+++	++++
	S. feacalis	0*	+	++	+++	++++
	K. pneumonieae	0*	+	++	+++	++++
	C. albicans	0*	+	++	+++	++++
	A. fumigates	0*	+	++	+++	++++
EAE	MRSA	-	0*	+	++	+++
	S. aureus	-	0*	-	++	+++
	S. feacalis	-	0*	+	++	+++
	E. coli	-	0*	+	++	+++
	K. pneumonieae	-	0*	+	++	+++
	C. albicans	-	0*	+	++	+++
	A. niger	-	0*	+	++	+++
	A. fumigates	0*	+	++	+++	++++
ME	MRSA	-	0*	+	++	+++
	S. aureus	0*	+	++	+++	++++
	S. feacalis	0*	+	++	+++	++++
	E. coli	0*	+	++	+++	++++
	K. pneumonieae	0*	+	++	+++	++++
	C. albicans	0*	+	++	+++	++++
	A. niger	0*	+	++	+++	++++
	A. fumigates	0*	+	++	+++	++++

Key: HE = Hexane extract, EAE = Ethyl acetate extract, ME = Methanol extract, - = No turbidity (no growth), 0* = Minimum bactericidal and fungicidal concentration, + = light colony growth, ++ = Moderate colony growth, +++ = Heavy colony growth

DISCUSSION

The results of the solvent extraction of the *S. longipedunculata* leaves showed comparable outline yield in the various solvents used for the extraction Table 1. The percentage yield of the extracts showed that methanol had the highest percentage yield, followed by ethyl acetate and then n-Hexane extracts, this variation could be due to the polarity of methanol which is superior to the other solvents. This is in line with the report of Hassan *et al.* (2018). The plant is more abundant in polar compounds hence methanol extracts more bioactive polar compounds which are useful in ethno medicine.

S. longipedunculata leaves extracts, exhibited varying grade of antimicrobial effect against the test organisms. Susceptibility test result showed zone inhibition ranging from 18 - 29 mm (Table 2) against all the test organisms, except for VRE, *S. typhimurium*, *P. fluorescens*, *C. krusei* and *M. canis* in all the three extracts, while hexane extract showed no inhibition against *E. coli* and *A. niger*. The ethyl acetate extract recorded the highest sensitivity against *S. aureus* (29 mm), and the least sensitive organism was *S. feacalis* (18 mm) on hexane extract. The ethyl acetate extract of *S. longipedunculata* showed a significant activity compared to the other two extracts, which may be explained by the extraction process's ability to concentrate an active compound in some extracts more than the other due to the varying solubility of the phytochemicals (Abdullahi *et al.*, 2017). The antimicrobial activity of the extracts of *S. longipedunculata* was in the order ethyl acetate extract > methanol extract > hexane extract which indicating that the active phytochemical constituents in the plant with antimicrobial properties are resides in the moderately polar solvent (ethyl acetate).

The appreciable value of the zone inhibition has also revealed that, the extracts has excellent antimicrobial activity against the susceptible organisms, as the extract with diameters of zone inhibition greater than 10 mm are considered to have strong antimicrobial agents or compounds (Sharma *et al.*, 2012; Namadiana *et al.*, 2020). The standard drugs, sparfloxacin and ciprofloxacin at concentration of 500 µg/cm3, indicated zone of inhibition ranging from 31 – 35 nm and 28 - 40 mm, respectively against the bacterial isolates; while the standard antifungal agents, fluconazole and fulcin (at 500µg/cm^3) had 32 -34 nm and 27 – 32 nm against the test organisms (Table 2 - 3). The antimicrobial activity of the extracts is evident from the minimum inhibition concentration (MIC) values, which range from 62.5 – 250 µg/cm^3; while the minimum bactericidal concentration (MBC) or minimum fungicidal concentration (MFC) ranges from 250 – 500 µg/cm^3. The broad-spectrum antimicrobial activity observed from *S. longipedunculata* leave extracts is in line with previous studies by Namadina *et al.*, (2020). Musa *et al.*, (2013) also reported that the crude methanol extract from the root of *S. longipedunculata* exhibited anti-diarrhea activity in mice through the inhibition of prostaglandin biosynthesis and the reduction of osmotic pressure.

Interestingly, *S. longipedunculata* extract has been found to possess broad-spectrum activity against numerous pathogens. This means the extract demonstrates inhibitory effects against various microorganisms, including bacteria and fungi. The observed antimicrobial activity in this study can be attributed to the presence of various secondary metabolites present in the plant extracts. These phytoconstituents, such as alkaloids, tannins, flavonoids, and saponins, have been established to have activity against multiple organisms and therefore aid the antimicrobial activities of *S. longipedunculata* and support their conventional use for the treatment of microbial infection (Usman and Osuji, 2007; Sharma *et al.*, 2012). The presence of soluble phenolic and polyphenolic compounds on *S. longipedunculata* (Aiyelaagbe *et al.*, 2007) could attribute the used of the plant in the treatment of sexually transmitted infection. These phytochemical compounds contribute to the antimicrobial properties of *S. longipedunculata* leaves through diverse mechanisms, including disrupting cell membranes, intercalating DNA, inactivating microbial adhesions, and enzymes (Abdullahi *et al.*, 2017; Namadiana *et al.*, 2020). These findings support the traditional use of the plant in treatment of microbial infections.

CONCLUSION

The hexane, ethyl acetate and methanol extracts of *S. longipedunculata* leaves exhibited broad-spectrum of antimicrobial activity against the test organism; this means the extracts demonstrates inhibitory effects against some microorganisms. Some of these properties confirmed that the extracts of *S. longipedunculata* has a wider spectrum of antibacterial and antifungal activity as such the plant products is crucial to mitigate the emergence of multi-drug resistance and ensure their long-term effectiveness.

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