



Antimicrobial Potentials of *Sida acuta* Leaf Extracts on Some Resistant Bacterial Isolates

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ABSTRACT

The quest for a solution to antimicrobial resistance issues has recently led to a series of research activities, focusing on plant options which are believed to be accessible and cheaper. *Sida acuta* leaf extracts were assayed in this study. The powdered leaf samples were subjected to extraction using ethanol, n-hexane, and water as solvents. A phytochemical analysis of the crude extract was performed. In addition, the potency of each extract was tested against six (6) clinical resistant strains of bacteria and their respective minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs). Phytochemical analyses revealed the presence of phenols, saponins, steroids, tannins, flavonoids, and alkaloids. The data obtained from this study were analysed using SPSS version 23. Data are represented as mean $\pm$ standard error of the mean (SEM), while the difference between means was evaluated by analysis of variance (ANOVA) at $P \leq 0.05$. The ethanol extract showed the greatest antibacterial activity against the tested isolates, whereas the n-hexane extract had the lowest. The extracts also had different minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) against the isolates, with some having bactericidal effects and others having bacteriostatic effects. The results of this study indicate that *S. acuta* leaf could be an excellent candidate in the search for plant alternatives that will serve as an effective and accessible antibacterial compound.

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Introduction

The development of drug-resistant, multi-drug-resistant, and drug-resistant strains of bacteria has become a serious concern, making it difficult to treat infections and diseases caused by pathogens harbouring resistance. These strains are commonly referred to as 'superbugs', which can be normal human commensal flora that has acquired antibiotic resistance and increased virulence, such as Multidrug Resistant *Staphylococcus aureus* (MRSA) strains and vancomycin-resistant enterococci (VRE), or intrinsically resistant environmental bacteria that can become opportunistic pathogens, such as *Pseudomonas aeruginosa* and *Acinetobacter baumannii* [1]. The ability to successfully treat infectious diseases worldwide is threatened by antimicrobial resistance [2] which is caused by factors such as overuse, abuse, and unnecessary prescription of antimicrobials [3]. The extensive application of antimicrobials in agriculture is also a contributing factor to the resistance and spread of antimicrobial resistance [4].

Interestingly, the development of new antibiotics by the pharmaceutical industry, a strategy that has been effectively used in the past to combat resistant bacteria, has halted due to economic and regulatory obstacles [5,6]. Of the 18 largest pharmaceutical companies, 15 abandoned the field [6]. In addition, mergers between pharmaceutical companies have substantially reduced the number and diversity of research teams, while antibiotic research conducted in academia has drastically reduced owing to insufficient funding as a result of the global economic crisis [7]. Despite the continuous popularity of herbal medicine worldwide, traditional antibiotics have overshadowed the exploration of plant-based products as therapeutics. However, owing to the growing need for new antimicrobial agents, many scientists have expanded their search to include novel plants and other environmental sources [8,9]. Indeed, mainstream medicine is increasingly receptive to the use of plant-derived drugs, especially those for which antimicrobial resistance is more difficult or unlikely to develop. Notably, 26% of all newly approved drugs and 33% of all new small-molecule approved drugs between 1981 and 2014 were botanical drugs, unaltered natural products, or derivatives thereof [10].

Sida acuta (broom weed) is a flowering shrub that belongs to the Malvaceae family [11] and has antibacterial, antifungal, antiprotozoal, antiplasmodial, and antioxidant activities. It has been traditionally used for the treatment of wounds, ulcers, haemorrhage, abortion, fever, malaria, gonorrhoea, abortion, dysentery, poisoning, blood disorders (to stop bleeding), and urinary diseases [12]. It is used traditionally to treat dysentery, skin diseases, asthma, renal inflammation, headache, hypertension, measles, haemorrhoids, elephantiasis, erectile dysfunction, colds, rheumatism, bronchitis, insect bites, preparation of drinks, ointments, and external baths to protect against snake bites. The roots of *S. acuta* are used traditionally as coolant, to treat bowel complaints, and to expel worms; when boiled in gingelly oil, both the roots and the leaves are used on testicular swellings and elephantiasis, and to hasten suppuration [11].

Sida acuta has been reported to exhibit activities against diverse microorganisms. However, there is a dearth of information on the potential of this medicinal plant to treat multidrug-resistant strains of bacteria. Hence, further research is needed.

Materials and Methods

Plant Collection and Identification

Sida acuta was collected from Warrah community (Latitude 8.437664916; Longitude 4.48999804), Ilorin, Kwara State, Nigeria, and taken to the Department of Plant Biology, University of Ilorin for identification by Mr. Ajayi Boluwatife, and a voucher number (UILH/001/766/2024) was assigned. The leaves were air-dried, ground, sieved with a mesh, and stored securely for further use.

Test Microorganisms

The test organisms (*Staphylococcus aureus*, *Acinetobacter baumannii*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Proteus mirabilis*) were obtained from the Microbiology Department, University of Ilorin Teaching Hospital, Ilorin. They were subjected to morphological, biochemical, and antibiotic susceptibility tests. Bacterial samples were stored on agar slants in a refrigerator for further use.

Preparation of Extract

Powdered plant materials (100 g each) were soaked in 600 ml of water, ethanol, and n-hexane. The respective mixtures were thoroughly stirred for homogeneity and then placed on an auto shaker with continuous shaking for 48 h. The samples were removed from the autoshaker and the supernatant layers were filtered into flasks. The filtrate from the aqueous solution was evaporated at 40 °C in a water bath, while the filtrates from ethanol and n-hexane were concentrated using a rotary evaporator. The recovered extracts were refrigerated until further use. Crude extracts were reconstituted by dissolving the extract in 3% dimethyl sulfoxide (DMSO). From the stock, serial dilutions of equal volumes of extract and diluent were mixed to successively prepare concentrations of 500 mg/ml, 250 mg/ml, and other dilutions [13].

Sample Preparation and Phytochemical Analyses

Sida acuta leaf powder was poured into a conical flask, soaked, and shaken well. The solution was left to stand for about

10 minutes and then filtered. Phytochemical analyses of the crude extracts of this plant were performed according to standard methods [14,15].

Determination of Sensitivity Test

Mueller Hinton agar was prepared into Petri dishes and allowed to gel. An inoculum suspension from an 18-hour old culture of the test organisms that was previously incubated at 37 °C was prepared and standardised with a McFarland Standard of turbidity of 0.5. Cotton swabs were used to pick the standardised inoculum, and the surfaces of agar plates were swabbed and allowed to dry for a few minutes. Holes were bored on the inoculated plates with a flamed 5 mm cork borer. Extracts of varying concentrations were dispensed into the wells. Streptomycin and DMSO were used as positive and negative controls respectively. The plates were then incubated at 37 °C for 24 h. Zones of inhibition in millimetres (mm) were measured using the metre rule [16]. Resistance of test bacteria to antibiotics was determined using standard antibiotic-impregnated discs.

Assessment of Minimum Inhibitory and Bactericidal Concentrations (MIC and MBC)

Equal volumes (50:50 ratio) of the extract and sterilised nutrient broth were dispensed into the microwells. Standardised inoculum was poured into each well. A well without extract but containing inoculum and nutrient broth was used as a positive control, whereas a well without inoculum but containing nutrient broth and extract was used as a negative control. Another well with only nutrient broth but without extract and inoculum was used as a negative control. The wells were incubated at 37°C for 24 h. The lowest concentration of the extract without turbidity was recorded as the minimum inhibitory concentration. Wells without turbidity from the minimum inhibitory concentration wells were subcultured and incubated at 37°C for 18 to 24 h. After incubation, a plate without growth was recorded at its corresponding minimum bactericidal concentration [17,18].

Data Analysis

Data obtained from this research was analyzed using SPSS version 23. Data are presented as mean $\pm$ standard error of the mean (SEM), while the difference between means was evaluated by analysis of variance (ANOVA) at $P \leq 0.05$.

Results

The percentage yield of extracts revealed that the aqueous extract had the highest recovery of 16.90%, the ethanolic extract had 14.85%, and n-hexane extract with 12.52 % was the lowest percentage yield recovered (Table 1). Phytochemical analysis of *S. acuta* has revealed the presence of phenols, saponins, tannins, alkaloids, flavonoids, steroids, triterpenoids, and glycosides. No proteins and reducing sugars were detected (Table 2). Antibiotic susceptibility patterns (Table 3) revealed that all the test bacteria were resistant to three of the test antibiotics, namely cotrimoxazole, sulfatriad, and cephalothin, whereas they were susceptible to the antibiotics in different patterns. Out of the total eight different standard antibiotics discs used on the test bacteria, *P. mirabilis*, *K. pneumoniae* and *E. coli* were susceptible to five and resistant to three of the antibiotics and thus the least resistant isolates. *A. baumannii* and *P. aeruginosa* were both susceptible and resistant to four of the antibiotics used while *S. aureus* was susceptible to just two of the antibiotics and resistant to the remaining six reference antibiotics making it the isolate with the highest degree of resistance.

Tables 4, 5, and 6 show the antibacterial activities of *S. acuta* against the test bacteria. The extracts showed varying degrees of activity, with the ethanolic extract showing the highest zones of inhibition. All the organisms have some degrees of susceptibilities towards the extracts with *S. aureus* and *P. aeruginosa* highly susceptible while *A. baumannii* was moderately susceptible towards the extracts. *P. mirabilis*, *K. pneumoniae* and *E. coli* were however resistant to the extracts.

The minimum inhibitory and bactericidal concentrations of the *S. acuta* extracts (Table 7) revealed that the ethanolic extract exhibited the greatest efficacy, with the lowest MICs and MBCs for all the extracts. *P. aeruginosa* and *S. aureus* were sensitive to the least MIC of 31.5 mg/ml each and 62.5 mg/ml and 125 mg/ml MBC values respectively in ethanolic extract. *E. coli* and *P. mirabilis* were sensitive to an MIC of 250 mg/ml each for ethanolic extract and having 500 mg/ml and no MBC respectively for *E. coli* and *P. mirabilis* meaning that *P. mirabilis* was bacteriostatic for the ethanolic extract. In addition, for the aqueous and n-hexane extracts, all the test bacterial species showed varying degrees of differences in their MIC and MBC sensitivities. The extracts showed bactericidal and bacteriostatic effects against certain species.

Table 1. Percentage recovery of *S. acuta* extracts

Extracts	Sample weight (g)	% Recovery
N -Hexane	100	12.52
Aqueous	100	16.90
Ethanol	100	14.85

Table 2: Phytochemical analysis of *S. acuta* crude extract

Phytochemicals	Observations
Saponin	++
Phenol	+++
Steroids	++
Alkaloids	+++
Triterpenoids	++
Tannins	+++
Flavonoids	+
Glycosides	+++
Proteins	-
Reducing sugar	-

*+++ = abundantly present, ++ = moderately present, + = present in trace quantities, - = absent.

Table 3: Standard Antibiotics Susceptibility on Test Bacterial Samples

Test Bacteria	Zone of Inhibition (mm)							
	CO	T	S	GM	AP	TS	ST	KF
<i>P. mirabilis</i>	20.00	10.00	25.00	28.00	15.00	0.00	0.00	0.00
<i>S. aureus</i>	12.00	0.00	22.00	0.00	0.00	0.00	0.00	0.00
<i>K. pneumoniae</i>	14.00	9.00	21.00	12.00	9.00	0.00	0.00	0.00
<i>A. baumannii</i>	13.00	8.00	20.00	14.00	0.00	0.00	0.00	0.00
<i>P. aeruginosa</i>	15.00	9.00	23.00	16.00	0.00	0.00	0.00	0.00
<i>E. coli</i>	17.00	8.00	20.00	26.00	8.00	0.00	0.00	0.00

CO= Colistin Sulphate (25µg); T= Tetracycline (25µg); S= Streptomycin (10µg); GM= Gentamycin (10µg); TS= Cotrimoxazole (25µg); AP= Ampicillin (10µg); ST= Sulphatriad (200µg); KF = Cephalothin (25µg).

Table 4: Antibacterial Activities of Ethanolic Extract of *S. acuta* on Test Bacteria

Concn.(mg/ml)	Zones of Inhibitions on Test Bacteria (mm)					
	<i>P. mirabilis</i>	<i>S. aureus</i>	<i>K. pneumoniae</i>	<i>A. baumannii</i>	<i>P. aeruginosa</i>	<i>E coli</i>
500	5.38 ± 0.92	17.73 ± 0.42	6.57 ± 0.15	10.85 ± 0.63	17.05 ± 0.35	9.30 ± 0.87
250	-	16.32 ± 0.35	5.50 ± 0.25	9.30 ± 0.91	15.13 ± 0.47	7.52 ± 0.17
125	-	15.05 ± 0.11	-	8.45 ± 0.61	13.50 ± 0.13	6.25 ± 0.17
62.5	-	12.33 ± 0.73	-	7.35 ± 0.33	11.35 ± 0.53	-
31.25	-	9.15 ± 0.45	-	-	8.56 ± 0.54	-
15.63	-	7.55 ± 0.68	-	-	6.83 ± 0.31	-

Values are means of triplicate measurements, while values after (±) represent standard error of means of triplicate readings. Concn. = concentration; - = no zone of inhibition.

Table 5: Antibacterial Activities of Aqueous Extract of *S. acuta* on Test Bacteria

Concn.(mg/ml)	Zones of Inhibitions on Test Bacteria (mm)					
	<i>P. mirabilis</i>	<i>S. aureus</i>	<i>K. pneumoniae</i>	<i>A. baumannii</i>	<i>P. aeruginosa</i>	<i>E. coli</i>
500	12.23 ± 0.22	14.32 ± 0.21	6.78 ± 0.15	10.05 ± 0.18	15.13 ± 0.25	10.25 ± 0.91
250	10.62 ± 0.91	12.45 ± 0.63	5.46 ± 0.32	9.33 ± 0.12	14.15 ± 0.35	8.20 ± 0.11
125	8.32 ± 0.56	11.48 ± 0.33	-	-	12.75 ± 0.73	6.52 ± 0.17
62.5	-	12.33 ± 0.73	-	-	10.33 ± 0.15	-
31.25	-	9.84 ± 0.42	-	-	8.28 ± 0.21	-
15.63	-	8.95 ± 0.82	-	-	-	-

Values are means of triplicate while values after (±) represent standard error of means of triplicate readings.
 Concn. = concentration; - = no zone of inhibition.

Table 6: Antibacterial Activities of n-hexane Extract of *S. acuta* on Test Bacteria

Concn. (mg/ml)	Zones of Inhibitions on Test Bacteria (mm)					
	<i>P. mirabilis</i>	<i>S. aureus</i>	<i>K. pneumoniae</i>	<i>A. baumannii</i>	<i>P. aeruginosa</i>	<i>E. coli</i>
500	5.23 ± 0.22	15.40 ± 0.15	6.30 ± 0.65	10.21 ± 0.18	16.25 ± 0.17	14.50 ± 0.16
250	-	14.45 ± 0.33	5.13 ± 0.74	9.87 ± 0.24	14.41 ± 0.25	13.20 ± 0.11
125	-	12.15 ± 0.17	-	6.55 ± 0.29	13.35 ± 0.46	10.25 ± 0.91
62.5	-	9.40 ± 0.35	-	-	12.30 ± 0.21	-
31.2 5	-	8.95 ± 0.82	-	-	9.26 ± 0.51	-
15.63	-	7.73 ± 0.21	-	-	8.14 ± 0.33	-

Values are means of triplicate while values after (±) represent standard error of means of triplicate readings.
 Concn. = concentration; - = no zone of inhibition.

Table 7: Minimum Inhibitory and Bactericidal Concentrations of Extracts on Test Bacteria

Test Isolates	Ethanol		Aqueous		n-Hexane	
	MIC	MBC	MIC	MBC	MIC	MBC (mg/ml)
<i>P. mirabilis</i>	250	-	125	250	500	-
<i>S. aureus</i>	31.25	125	62.5	125	250	500
<i>K. pneumoniae</i>	250	500	125	500	500	-
<i>A. baumannii</i>	62.5	125	125	250	250	500
<i>P. aeruginosa</i>	31.25	62.5	31.25	125	125	250
<i>E. coli</i>	250	500	250	500	125	250

Discussion

The polarity of the extractant used in extraction determines the yield of the extract [19]. Hence, the aqueous extract, which had the highest yield in this study, indicated that *Sida acuta* contains more polar biomolecules than others. Yield is also dependent on the extraction method, seasonal variations, and chemical properties [20].

The antimicrobial activities of the *Sida acuta* leaf extracts were analysed in this study. It was revealed from the outcome that extracts of ethanol had the greatest effects on the test organisms, even though the organisms had different levels of sensitivity towards the extracts. Bacterial resistance or sensitivity to a biological compound could result from strain differences [8,19], changes in the genetic makeup of the organism, cell wall structure, plant extract composition, and mode of action [21]. The sensitivity variability observed in this study could be due to any of the aforementioned reasons, coupled with the fact that the bacterial species used were all resistant strains.

The highest activity was recorded for the ethanolic leaf extract, followed by the aqueous extract, while the lowest activity was recorded for the n-hexane extract. This is because the potency of extracts is influenced by the type of solvent used for extraction and the composition and concentration of bioactive molecules present [22]. Collection time, pretreatment method, and geographical location also determine the concentrations and composition of phytochemicals in plants [12]. The presence of bioactive metabolites in plants has been linked to microbial cell death through various mechanisms such as cell wall and protein disruption coupled with enzyme inactivation [23].

In addition, the bacterial species exhibited varying degrees of susceptibility to the extracts. *S. aureus* and *P. aeruginosa* were highly susceptible while *A. baumannii* was moderately susceptible towards the extracts. *E. coli*, *P. mirabilis* and *K. pneumoniae* were however relatively resistant to the extracts. The difference in susceptibilities could be a result of the strain of bacteria used, the extract's mechanism of action on the test isolates [19], the extraction method and solvent used, the concentration and composition of the extract used, geographical location of the plant, and time of collection [19,23]. Thus, the disparities in the efficacy of the extract used in this study could be due to any of these factors. The outcomes of the antimicrobial studies in this study were in accordance with those reported by various researchers [24,25].

Conclusion

This study examined the antibacterial potential of *S. acuta* leaf extracts against some resistant bacterial species. The organisms tested were sensitive to the extracts at different concentrations. Although some bacterial species were susceptible, some were resistant to the extracts at varying concentrations. Comparably, the greatest activities and susceptibilities were found with the ethanolic extract, followed by the aqueous extract, while n-hexane had the least activities. The effectiveness of *S. acuta* extracts in this study indicates that it could be an excellent candidate for further research on antimicrobial drug inclusion.

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Declarations

Authors' Contributions

ASA and AAK conceptualised the study. ASA and AAK designed the study. ASA, AAE, and AKA participated in fieldwork and data collection. ASA, AKA, and ZRF performed data analysis; ASA, AKA, and KMO interpreted the data. ASA prepared the first draft of the manuscript, which was reviewed by AAK, ASA, and KMO. All the authors contributed to the development of the final manuscript and approved its submission.

Disclosure of Conflict of Interest

None

Ethics Approval and Informed Consent

This study did not involve either human or animal subjects. Therefore, ethical considerations were not applicable.

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