Antibacterial Activity and Phytochemical Screening of Leaf Extracts of *Sida acuta* From North-Central Nigeria.

Isaac Olubunmi Oluleye
University of Ilorin
Faith Feranmi Folahan (ffolahan08@gmail.com)
University of Ilorin College of Health Sciences https://orcid.org/0000-0003-4223-9469
Chukwuemeka Prince Nnabuike
University of Ilorin
Motolani Mohammed Ahmed
University of Ilorin

Research Article

Keywords: Sida acuta, leaf extracts, antibacterial, phytochemicals, scopoletin vomifoliol

Posted Date: January 21st, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1206214/v1

Abstract

This study was carried out to examine the antibacterial activity and phytochemical compositions of extracts from the leaf of Sida acuta . Using water and 70% ethanol, active components of Sida acuta were extracted and tested against six authenticated clinical isolates: Eschericia coli , Pseudomonas aeruginosa , Bacillus subtilis , Staphylococcus aureus , Klebsiella pneumoniae and Salmonella typhi . The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of extracts were determined using broth dilution and recovery plate methods respectively, while Thin Layer Chromatography (TLC) and Gas Chromatography Mass Spectrometry (GC-MS) were used in determining the presence and quantity of phytochemicals in the extracts. All clinical isolates, except P. aeruginosa , were markedly susceptible to Sida acuta leaf extracts, with inhibition zones ranging from 6.0mm to 20.0mm. The least MIC (3.13 mg/ml) and MBC (3.13 mg/ml) was observed in ethanolic leaf extract against S. aureus , while the highest (150 mg/ml and 150.00mg/ml) were observed in the ethanolic leaf extract against K. pneumoniae . Phytochemical tests identified flavonoids as the most dominant compound while tannins was the least. TLC analysis showed Rf values ranging from 0.10 to 0.98, while the GC-MS identified scopoletin (45.8%) and vomifoliol (41.0%) as the most abundant compounds from the aqueous extract. Evofolin-A, 4-ketopinoresinol, assamicin I and assamicin II were also observed from the leaf extracts. The findings of this study reveals the potency of Sida acuta leaf extracts as an antibacterial, thus suggesting its possible use in treating common infections as well as a potential candidate for further research on antibacterial therapeutics. Keywords: Sida acuta , leaf extracts, antibacterial, phytochemicals, scopoletin, vomifoliol.

Introduction

Plants have been used from ancient times for various purposes such as dietary supplements and drug use, with very convincing results that made the use of plants for chemotherapy a popular practice. Traditional healers in the early centuries adopted the use of extracts and components of plants for treating divers infectious diseases. In recent years, various scientists, ranging from microbiologists, chemists, ethnopharmacologists, botanists, etc., are re-inventing the age-long practice and studying various plants for possible components that may be useful in the development of chemotherapy for infectious diseases.

The use of plant materials as curative agent against infections and diseases is an important part of African tradition and over 70% of the population make use of it for primary health care. The presence of a rich variety of secondary metabolites, which includes flavonoids, tannins, alkaloids and terpenoids, in plants have increased the search for antimicrobial potency of various plants against certain infectious disease.The leaf, stem, bark, roots, salp, etc. of plants have been tested and reported to have various degrees of antimicrobial activities (Adomi, 2008, Touati et al., 2018, Cos et al., 2016, Mohammed et al., 2020).

Sida acuta, (commonly known as "*Iseketu*" in South-Western Nigeria), belongs to the family Malvaceae. Indigenous to Mexico and Central America, this shrub now dominates the tropics and sub tropics (Holm *et al*., 1977). The major traditional use of this wild plant is its application in Nigerian folk medicine to cure

infections such as malaria, wound infections, diarrhea, ulcer, fever, etc. (Kayode, 2006; Edeoga *et al*., 2005). The leaf is widely used on wounds and injuries to prevent infection, promote quick healing and ease pains.

Hence, this study was carried out to evaluate the antibacterial potential, and to investigate the phytochemical composition of *Sida acuta,* against certain clinical isolates, in order to understand its potency for antimicrobial use.

Materials And Methods

Authentication of Isolates

The authentication of the clinical isolates were carried out using cellular morphology and Microbat^{TM} Kit identification system, using a total of 19 cellular morphological and biochemical tests.

Collection of Plant

The *Sida acuta* plant used for this study was obtained from Tanke in Ilorin, Kwara State, Nigeria and was identified and authenticated by Mr. Bolu Ajayi in the Department of Plant Biology, University of Ilorin, Nigeria. The plant was also deposited at the Department's Plant Biology Herbarium with Voucher Number: UILH/001/201/2021.

Extraction of plant materials

The leaf of the plant was washed under running tap to remove debris, after which they were left to dry at normal room temperature for seven days. The dried leaves were then grounded to powder using an electric blender. The grounded samples were thereafter soaked into two different solvents - 70% ethanol and distilled water - measuring 20 grams of the sample into 100ml of the extractants (ratio 1:5 w/v). The resultant mixture was shaken at 150 rpm for 48 hours and then filtered using the Whatman filter paper No. 1.

The filtrates were evaporated to semi-solid mass using vacuum rotatory evaporator to get a dark brown resinous mass. This was then stored at 4^{o}C, in a dark sample bottle and prepared for further use. The dry extracts were reconstituted with their respective solvents to give a concentration of 200mg/ml for the antibacterial activity evaluation, as described by Banso and Ayodele, (2001).

Collection and Authentication of the test isolates

The organisms used for this study were clinical isolates obtained from the Department of Medical Microbiology and Parasitology, University of Ilorin Teaching Hospital, Ilorin, Kwara state. They include *E. coli, Salmonella typhi, Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. The isolates were further authenticated using cellular morphology, biochemical tests and the Microbact^{TM} kit identification system. The isolates were maintained on nutrient and MacConkey agar slants at 4^{o}C prior to use.

Antibacterial Examination of Extracts

One gram of each extract was dissolved in 5ml of sterile distilled water and 70% ethanol, to give the desired concentration of the extract in milligram (200mg/ml). 0.5 ml of McFarland standard of 18hour culture of each isolate was mixed with 20ml of Mueller-Hinton agar in a Petri dish. The medium was allowed to solidify. 5 wells (6mm in diameter) were punched in the agar medium using sterile stainless cork borer, after which 0.5ml of plant extracts or solvent (negative control) was introduced and allowed to diffuse for 2hours. The plates were incubated for 18- 24 hours at 37^{o}C and the diameters of any resulting zones of inhibition were measured. Ciprofloxacin (10µg) was used as a positive control. The tests were performed in quadruplicate as described by Adomi, (2008).

Determination of Minimum Inhibitory Concentration (MIC) using broth dilution method

The standard solution (200mg/ml) of the extract was diluted to obtain concentrations ranging from 1.56mg/ml - 200mg/ml. One milliliter each of the concentrations of the extracts was mixed with 9ml of the Mueller- Hinton broth in test tubes. The contents were thoroughly mixed and the tubes will be inoculated with 0.lml of McFarland standard of the test isolates. The tubes were incubated at 37^{0}C and examined for growth after 24hours. The least concentration of the extracts that did not permit any visible growth of the inoculated test isolates in the broth medium was regarded as the MIC in each case. Test tubes inoculated with the test isolates without the extracts will serve as controls according to the procedures described by Black, (1996).

Determination of Minimum Bactericidal Concentration (MBC) by recovery plate method

The concentration of the extracts used for the MIC which do not permit any visible growth were inoculated on plates containing the respective growth medium and incubated for 24 hours. The least concentration which showed no growth after incubation was taken as the minimum bactericidal concentration (Black, 1996).

Phytochemical Screening of the plant extracts

Phytochemical tests and estimations were carried out on the aqueous and ethanolic extracts of the leaf and stem of the plant samples using standard procedures to determine the presence of phytochemicals and to identify the various constituents. We synergized and followed the procedures earlier described by Harbone (1973), Trease and Evans (1989), Boham and Kocipai, (1994), Obadoni and Ochuko 2001; and Sofowora (1993).

Thin Layer Chromatography(TLC).

TLC was performed on 5cm × 20cm TLC plates that were pre-coated with 0.25mm layers of silica gel 60. After drying, the extracts were applied to the TLC plate with a 1µL microfuge tubes. The plate was then developed in a glass chamber. Two chromatography solvents used were n- hexane: ethanol (2:3 and 1:4)

and n- hexane: ethylacetate (3:2 and 1:4). After running for sufficient time, visualization of the bands was achieved by spraying the plate with iodine and exposing to UV light (Sherma and Fried, 1996). The Rf values were calculated by dividing the distance travelled by the spots by the distance travelled by the solvents.

Rf = distance travelled by spots / distance travelled by solvents

Gas Chromatography- Mass Spectrometry (GC-MS)

The compounds were extracted using the direct solvent extraction method described by Ibrahim *et al*., (2011). Three grams of each plant extract was weighed into bottles and saturated with 15ml of ethanol and water. They were allowed to stand at room temperature for 24hours, filtered using Whatman No.1 filter paper and the filtrate was evaporated to semi-solid mass using vaccum rotatory evaporator to give a dark brown resinous mass and stored in at $4^{o}C$ in a dark sample bottle prior to use. Ten grams of the evaporated samples was extracted by 10ml of ethyl acetate and concentrated to 1ml prior to injection into GC-MS.

GC-MS analysis was performed using GC-MS-QP2010 plus (Shmadzu, Japan) equipped with flame ionization detector (FID). The injection was conducted in split less mode at $250^{o}C$ for 3mins by using an inlet of 0.75mm i.e. to minimize peak broadening. Chromatographic separations was performed by using DB-WAX analytical column 30m by 0.32m by 0.25m (J&W scientific, Folsom C.A) with helium as carrier gas at constant flow rate of 0.8ml/min. The oven temperature was programmed at $60^{o}C$ for 5min, followed by an increase (held for 5min), and finally at $10^{o}C$/min to $310^{o}C$ (held for 10min). The temperature of the FID was set to 250 ^{o}C, under an optimum Mass spectrometry operating conditions (electron impact ionization mode) with ion source temperature of $200^{o}C$, ionization voltage of 70eV and mass scan range of m/z 23-450 at 2.76scans/s (Ibrahim *et al*., 2011). The chromatographic peak identification was carried out by comparing their mass spectra with those of the bibliography data of unknown compounds from NIST library mass spectra database on the basis of the criterion similarity (SI)>800 (the highest value is 1,000). Following the procedures described by Wanakhachornkrai and Lertsiri, (2003), approximate quantification of volatile compounds were estimated by the integration of peaks on the total ion chromatogram using Xcalibur software (Vienna, VA), and the results were presented as the peak area normalized.

Results

The isolates authentication, using Microbat^{TM} Kit identification system, yielded percentage probability which ranged from 85.3% for *P. aeruginosa* to 99.87% for *S. typhi*. All isolates were authenticated above 85%.

The susceptibility test of test organisms against leaf extracts of *Sida acuta* showed varying inhibition zones which ranged from 6.70mm - 20.0mm. The widest zone of inhibition was obtained in the ethanolic extract against *S. aureus* while the least zone of inhibition was obtained in aqueous extract against *B. subtilis*. On the other hand, *Pseudomonas aeruginosa* showed no zone of inhibition in all the two extracts

while *Klebsiella pneumoniae* and *Salmonella typhi* showed no zone of inhibition in the aqueous extract (Table 1). These microbial susceptibility were further compared with standard antibiotics (Table 2).

Table 1: Antibacterial activities of leaf extracts of *S. acuta*.

Zones of inhibition (mm)		
Test organisms	**Aqueous leaf extract**	**Ethanolic leaf extract**
E. coli	11.8±0.2	14.7±0.5
K. pneumoniae	NZ	4.8±0.2
P. aeruginosa	NZ	NZ
B. subtilis	6.7±0.2	17.6±0.2
S. aureus	12.0±0.6	20.0±0.9
S. typhi	NZ	NZ

S. aureus was observed to exhibit the least MIC (3.13mg/ml and 6.25mg/ml) and MBC (3.13mg.ml and 25.0mg/ml) in both ethanolic leaf extracts (ELE) and aqueous leaf extract (ALE) of *Sida acuta* respectively. *E coli* also showed an average level of MIC and MBC to both the ethanolic and aqueous extracts. However, *K. pneumoniae* showed the highest MIC and MBC in ethanolic extracts, with no inhibition in the aqueous leaf extracts. Overall, the ethanolic leaf extracts had an higher MIC and MBC across the test isolates than the aqueous leaf extract (Table 3).

Table 2: Activity Index of the test isolatess to the *S. acuta* leaf extracts

Activity index

Test organisms	Aqueous extracts	Ethanolic extracts	Standard
E. coli	0.48	0.59	24.80
K. pneumoniae	ND	0.21	22.60
P. aeruginosa	ND	ND	19.33
B. subtilis	0.31	0.84	21.00
S. aureus	0.55	0.80	22.00
S. typhi	ND	0.32	21.00

Key: *ND = Not determined since there was no antibiotic inhibitory activity*

$$Activity\ index = \frac{Inhibition\ Zone\ of\ the\ test\ sample}{Inhibition\ Zone\ of\ the\ standard}$$

Standard = Inhibition zones of ciprofloxacin (10 µg).

Table 3: Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of *S.acuta* leaf extracts.

	MIC (mg/ml)		MBC (mg/ml)	
Test organisms	**ALE**	**ELE**	**ALE**	**ELE**
E. coli	50.0	12.5	80.0	12.5
K. pneumoniae	ND	150.0	ND	150.0
P. aeruginosa	ND	ND	ND	ND
B. subtilis	50.0	6.25	50.0	6.25
S. aureus	6.25	3.13	25.0	3.13
S. typhi	ND	160.0	ND	NB

Key: ND = Not determined since there was no inhibition zone. NB = Not bactericidal.

ALE= Aqeuous leaf extract. ELE= Ethanolic leaf extract.

The qualitative phytochemical analysis of both aqueous and ethanolic leaf extracts of *S. acuta* showed the presence of alkaloids, flavonoids, cardiac glycosides, saponins, tannins, phenols and terpenoids (Table 4). The quantitative phytochemical analysis of the extracts showed that phenol was the most predominant in all the two extracts followed by saponins and flavonoids while tannin was the least predominant (Figure 1).

Table 4: Qualitative phytochemical screening of *Sida acuta* leaf extracts.

Phytochemical content							
Plant Extracts	**Alkaloids**	**Saponins**	**Tannins**	**Flavonoids**	**Phenols**	**Cardiac Glycosides**	**Terpennoids**
Aqueous Leaf Extract	**+**	**++**	**+**	**++**	**++**	**+**	**+**
Ethanolic Leaf Extract	**+**	**++**	**+**	**++**	**++**	**+**	**+**

Key : ++ = strongly present + = present in small amount.

Figure 1: Quantitative phytochemical screening of *S. acuta* leaf extracts.

The thin layer chromatography indicates the Rf values of various phytocomponents of both cold aqueous and ethanolic extracts of leaf of *S. acuta* using ethanol and ethylacetate. Highest Rf value (0.98cm) was

observed in cold aqueous leaf extract with ethylacetate, while lowest Rf value (0.10cm) was observed in ethanolic leaf extract with the same solvent (Table 5 & 6).

The GC-MS showed that ten compounds were identified in ethanolic leaf extract and eight in the aqueous leaf extrcat. The most abundant compounds were scopoletin, vomifoliol and evofolin-B while dispacus and saponin were minute (Tables 7 & 8).

Table 5: Rf values of phytocomponents identified in the extracts of *S.acuta* by TLC with n-hexane: ethanol.

Extracts	Spots	Visualization		Rf (cm)	hRf (%)	Solvent system ratio.
		Iodine	UV			
Ethanolic leaf extracts	I	Brown	Florescence	0.33	33	2:3
Cold aqueous leaf extract	I	Brown	Florescence	0.43	43	2:3
	II	Brown	Florescence	0.93	93	1:4

Table 6: Rf values of phytocomponents identified in the extracts of *S.acuta* by TLC with n-hexane: ethylacetate.

Extracts	Spots	Visualization		Rf (cm)	hRf (%)	Solvent system ratio.
		Iodine	UV			
Ethanolic leaf extracts	I	Brown	Florescence	0.10	10	3:2
	II	Brown	Florescence	0.26	26	3:2
	III	Brown	Florescence	0.79	79	3:2
	IV	Brown	Florescence	0.81	81	3:2
Cold aqueous leaf extract	I	Brown	Florescence	0.12	12	1:4
	II	Brown	Florescence	0.98	98	1:4
	III	Brown	Florescence	0.38	38	1:4

Table 7: Phytocomponents identified in the aqueous leaf extract of *Sida acuta* by GC-MS

Retention time (min.)	Phytocomponents	Peak area (%)
5.016	Evofolin-B	15.1
7.150	Loliolid	2.8
7.533	4-Ketopinoresinol	9.9
7.816	Assamicin 11	10.9
8.600	Assamicin 1	12.3
9.200	Scopoletin vomifoliol	45.8
11.016	Dipsacus Saponin	1.1
11.483	Momordin 1c	2.2

Table 8: Phytocomponents identified in the ethanolic leaf extract of *Sida acuta* by GC-MS.

Retention time (min.)	Phytocomponents	Peak area (%)
4.033	Evofolin-A	2.0
5.016	Evofolin-B	19.3
7.150	Loliolid	3.0
7.533	4-Ketopinoresinol	9.5
7.816	Assamicin 11	10.4
8.600	Assamicin 1	11.1
9.200	Scopoletin vomifoliol	41.0
11.016	Dipsacus Saponin	1.0
11.843	Momordin 1c	2.6

Discussion

Sida acuta is a perennial environmental weed which originated in Central America, and has spread to the tropics. Having good tolerance, the weed has adapted to different growing conditions and has been

established in pastures and fields worldwide. This plant is widely used in sub-Saharan Africa as a wound-healing substance, and for decades it has been effective and the practice still in vogue. In this study, we examined the antimicrobial activity and phytochemical presence in different extracts of *S. acuta*.

In this study, the aqueous and ethanolic extracts from *S. acuta* leaves used in testing for its antimicrobial activity were observed to possess various levels of antimicrobial potency against different clinical isolates. Overall, the ethanolic extracts showed the most potent antimicrobial ability against *S. aureus,* where the lowest MIC (3.13mg/ml) and MBC (3.13mg/ml) were observed. Low MIC and MBC indicates that the ethanolic extracts only required a smaller dose to inhibit the growth and to kill *S. aureus* respectively.

Studies by Lim et al., (2006) and Najiah et al., (2011) have also reported similar MIC and MBC values of 3.13mg/ml against *S. aureus* from *Rhizopora apiculat*a bark and extracts from Malaysian edible herbs respectively. Their reports conforms with that of other works (Zeouk et al, 2019, Manilal et al., 2020, Low, 2021) which supports the findings of this study. However, lower values have been observed from other studies on plant extracts, with Helal et al., (2019) observing MIC values of 0.05mg/ml and MBC values 0.05mg/ml against *S. aureus* while Nath et al, (2019) recorded MIC 0.02mg/ml and MBC 0.04mg/ml against the same organism.

The varying degrees of MIC and MBC values may be due to the differences in the crude plant extracts, because of the presence of different phyto-constituents which has divers compositions. The MIC of crude extracts could also be dependent on the level of susceptibility of the isolates used. However, a crude extract with about 5.0mg/ml MIC value may contain active compounds with high antibacterial potency and a very low MIC that is lesser than 0.2mg/ml. Hence, purification of extracts, using bioassay guided fractionation, may be used to isolate fractions with high antimicrobial potency from crude extracts.

Pseudomonas aeruginosa and *Klebseilla pneumoniae* were the only organisms in this study that showed no signs of inhibition or MIC/MBC and a very high MIC/MBC value respectively.This translates that these organisms are not susceptible to extracts from *S. acuta* leaves, and cannot be used as an antibacterial candidate against such. Similar observations of resistance or very high MIC/MBC values in *P. aeruginosa* and/or *K. pneumoniae* against plant extracts with antimicrobial potency were also reported by Al-Sa'ady (2020) and Atata et al., (2003). However, some studies have reported antimicrobial activities of various plant extracts in different locations around the globe (Jahani et al., 2006; Gangoue-Pieboji et al., 2009; Pallavali et al., 2019). It may be accepted that despite its high antimicrobial potential against *S. aureus*, *Sida acuta* have very weak or no antimicrobial potential against *P. aeruginosa* and/or *K. pneumoniae.* This may be as a result of the high resistance-ability of these organisms, as various reports suggest that both organisms develop multi-drug resistance to well known antibiotics than other gram-negative bacteria (Al-Mawlah et al., 2017; Sah et al., 2020).

This study also revealed that ethanolic leaf extracts had higher MIC and MBC across the test isolates than the aqueous leaf extracts, which is consistent with the findings of Bussmann et al., (2010), Sen and Batra (2012) and Debalke et al., (2018). It had been earlier highlighted by Mostafa et al. (2018) and Al-Hashimi, (2012), that the MIC values of plant extracts may vary due to the chemical constituents and their

components volatility. Hence, the use of ethanolic solvents for extraction gives most plant extracts the ability to exhibit higher antimicrobial potency than aqueous extracts or other solvents. Also, the presence of a large number of chemical compounds (such as phenols, terpenes, etc,) in ethanolic extract increases its antimicrobial potency, as these compounds elicit multiple target site actions against the bacterial cells than in other solvents (Gonelimali et al., 2018)

The active antibacterial potential of *Sida acuta* extracts against *S. aureus* in this study indicates that the plant is a viable antibiotics alternative, considering that majority of the common clinical symptoms caused by *S. aureus*, such as boil, breast abscess, surgical wound infection, etc. are on the rise, as well as also being persistently recalcitrant to first-line antibiotics in developing countries. Mohammed et al., (2020) also opined that the use of plant-derived antibiotics may be adopted in the face of a rising antibiotics-resistance across the world.

The qualitative phytochemical analysis from this research identified alkaloids, flavonoids, cardiac glycosides, saponins, tannins, phenols and terpenoids from aqueous and ethanolic leaf extracts of *S. acuta,* which is also similar to the findings of Mujeeb et al., (2014), Kebede et al., (2021), Mansoori et al., (2020), and Muhammad et al., (2021). The presence of these components in the extracts also play a major role in the overall antimicrobial activity of the extracts. Phenols were most predominant in all the two extracts and tannin was the least. Phenolic compounds exhibit certain actions such as membrane damage, blocking of enzymes and toxins production, as well as suppression of biofilm formation, on bacterial cells, thus aiding the antibacterial potency of crude substances in which they are found. Also, these compounds have been observed to carry out synergistic actions alongside other chemotherapeutic antimicrobials against resistant bacteria, and as such a promising candidate for alternative antimicrobial therapeutic administrations (Miklasińska-Majdanik et al., 2018).

Scopoletin, vomifoliol, evofolin-B, assamicin11, assamicin1 and 4-ketopinoresinol were also identified as major phytoconstituents present in extracts of *S. acuta*. Karou et al., (2007), Altemimi et al., (2017) and Aminah et al., (2021) have also reported the presence, bioactivities and antimicrobial importance of these components derived from *Sida acuta*, while Jang et al., (2003) highlighted the effects of these constituents in inducing quinone reductase and in inhibiting 7,12-dimethylbenz[a]anthracene-induced preneoplastic lesions in mouses. Scopoletin and vomifoliol compounds have been reported to significantly increase lipoprotein lipase activity 3T3-L1 adipocytes in dose and time dependent manners (Jang et al., 2003). Likewise, Yang et al., (2007) found scopoletin to partially reverse tumor necrosis factor alpha- induced suppression of lipoprotein lipase activity. Studies have reported this component as a potential regulator of inflammatory reactions mediated by mast cells. It does not release enzyme from the adipocyte membrane in organisms, but instead, decreases the level of mRNA, suggesting a post transcriptional control in bacterial cells. Thus, it is said to act as a facilitator of plasma triglyceride clearance (Yang *et al.*, 2007, Moon *et al.*, 2007). Scopoletin has also been found to inhibit leukemia cell proliferation (Manuele *et al.*, 2006). Apart from the antimicrobial activities of these constituents, their bioactive potential and remediation in pathological sites are also useful for an overall therapeutic use against infections and their impacts on certain organs. Nevertheless, there is need for more findings into the bioactivities of phytoconstituents from plant extracts.

Conclusion

The findings of this study concludes that *S. acuta* has good antibacteria potential and the presence of certain phytocompounds in the plant does not only make it a good antibactrial agent, but a promising remedial therapeutic agent. If explored and well studied, extracts from *S. acuta* could be designed for use in rural tropical regions where majority rely on herbal therapy and are very sceptical about chemical-based therapeutics. Besides, majority of the population in these regions live below the poverty-line and cannot afford the potent antibiotics that are currently being advised for use. For a plant such as *S. acuta,* that has its leaf, stem and roots as potential antimicrobial candidate, there are very few studies on its antimicrobial properties. Hence, there is need for further and broader studies on this plant, its antimicrobial properties and bioactive remediation effect on the pathological impact of infection/diseases.

Declarations

Acknowledgments

We acknowledge the Departments of Medical Microbiology and Parasitology (UITH), Microbiology and Plant Biology (School of Life Sciences), University of Ilorin, Ilorin, Nigeria, for supporting and making possible the acquisition, authentication and identification of specimens used in this research work.

Statements and Declarations

Financial Interest: All authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript.

Ethics approval and consent to participate: Not applicable.

Competing interest: The authors declare that they have no competing interest.

Funding: Not applicable.

Statement of Human and Animal Rights: Not Applicable.

References

Adomi, O.P. (2008). Screening of the leaves of three Nigerian medicinal plants for antibacterial activity. *African Journal of Biotechnology.* 7(15): pp 2540- 2542.

Al-Hashimi, A. G. (2012). Antioxidant and antibacterial activities of *Hibiscus sabdariffa* L. extracts. *Afr. J. Food Sci.* 6, 506–511.

Al-Mawlah Y.H, Alrubaei H.A, Aljelawi S.O. (2017). Antibacterial activity of three plant extracts against multidrug resistance Pseudomonas Aeruginosa. *Asian Journal of Pharmaceutical and Clinical Research* 10 (12) : 193. doi:10.22159/ajpcr.2017.v10i12.21071

Al-Sa'ady A.T. (2020). Antibacterial screening for five local medicinal plants against nosocomial pathogens: Klebsiella pneumoniae and Staphylococcus epidermidis. *Eurasian Journal of Biosciences*, Vol. 14: 1, pp. 553-559. http://www.ejobios.org/article/antibacterial-screening-for-five-local-medicinal-plants-against-nosocomial-pathogens-klebsiella-7528

Atata R.F, Sani A, Ajewole S.M. (2003). Effect of Stem Bark Extracts of Enantia chloranta on Some Clinical isolates. *Biokemistri* Vol. 15: 2. eISSN: 0795-8080

Banso, A. and Ayodele, O.P. (2001). Activities of extracts of *Garcina kola* against *Escherichia coli* and *Aspergillus niger*. *Journal of Applied Science and Management*. 5: 58-65.

Black, J. G. (1996). Antimicrobial therapy. In: *Microbiology, Principles and Application,* 3rd edition. Prentice Hall, Upper Saddle River, New Jersey. pp. 255, 356-358.

Boham, B.A., Kocipai, A.R (1974). Flavonoids, phenol and condensed tannins from leaves of *Hawaiian vaccinium vaticulatum* and *V. calycinium. Pacific Sci*. 48: 458-463.

Bussmann, R. W., Malca-García, G., Glenn, A., Sharon, D., Chait, G., Díaz, D., Pourmand, K., Jonat, B., Somogy, S., Guardado, G., Aguirre, C., Chan, R., Meyer, K., Kuhlman, A., Townesmith, A., Effio-Carbajal, J., Frías-Fernandez, F., & Benito, M. (2010). Minimum inhibitory concentrations of medicinal plants used in Northern Peru as antibacterial remedies. *Journal of ethnopharmacology*, *132*(1), 101–108. https://doi.org/10.1016/j.jep.2010.07.048

Cos, P., Vlietinck, A. J., Berghe, D. V. & Maes, L. Anti-infective potential of natural products: how to develop a stronger in vitro proof-of-concept'. *J. Ethnopharmacol.* 106, 290–302. https://doi.org/10.1016/j.jep.2006.04.003 (2006).

Debalke D, Birhan M, Kinubeh A, Yayeh M. (2018). Assessments of Antibacterial Effects of Aqueous-Ethanolic Extracts of Sida rhombifolia's Aerial Part. *The Scientific World Journal. V*ol. 2018, Article ID 8429809, 8 pages. https://doi.org/10.1155/2018/8429809

Doughari, J. H. 2006. Antimicrobial activity of *Tamarindus indica* Linn. *Tropical J. Pharm. Res.* 5(2):592-603.

Edeoga, H.O., Okwu, D.E. and Mbaebie, B.O. (2005). Phytochemical constituents of some *Nigeria medicinal plants.* Afri. J. Biotechnol. 4:685-688.

Gangoue-Pieboji J, Eze N, Ngongang Djintchui A, Ngameni B, Tsabang N, Pegnyemb DE, Biyiti L, Ngassam P, Koulla-Shiro S, Galleni M (2009) The in-vitro antimicrobial activity of some medicinal plants against β-

lactam-resistant bacteria. J Infect Dev Ctries 3:671-680. doi: 10.3855/jidc.77

Gonelimali F.D., Lin J, Miao W, Xuan J, Charles F, Chen M, Hatab S.R.(2018). Antimicrobial Properties and Mechanism of Action of Some Plant Extracts Against Food Pathogens and Spoilage Microorganisms.*Frontiers in Microbiology.* Vol. 9. pp.1639 https://www.frontiersin.org/article/10.3389/fmicb.2018.01639. doi:10.3389/fmicb.2018.01639

Harborne, J.B. (1973). Phytochemical methods, London Chapman and Hall, Ltd. Pp.49-188.

Helal IM, El-Bessoumy A, Al-Bataineh E, Joseph M.R.R, Rajagopalan P, Chandramoorthy HC, Hadj SB Ahmed. (2019). Antimicrobial Efficiency of Essential Oils from Traditional Medicinal Plants of Asir Region, Saudi Arabia, over Drug Resistant Isolates, BioMed Research International, vol. 2019: 9 pages, https://doi.org/10.1155/2019/8928306

Holm, L.G., Plucknett, D.C., pancho, J.V. and Herberger, J.P. (1997). The world worst weeds; distribution and biology university press of Hawai, Honolulu, USA pp. 632-667. ISBN: 01-8248-0295-0.

Ibrahim, A.D., Sani, A., Manga, S.B., Aliero, A., Joseph, R.U., Yakubu, S.E. and Ibafidonn, H. (2011). Microorganisms associated with volatile metabolite product in soft rot of sweet pepper fruits (Tattase). *Int. J. Biotech and Biochem*. 7(2):217-227.

Jahani S, Saeidi S, Javadian F, Akbarizadeh Z, Sobhanizade, A.(2006). Investigating the Antibacterial Effects of Plant Extracts on Pseudomonas aeruginosa and Escherichia coli. International Journal of Infection. 3 (2); e34081 DOI : 10.17795/iji-34081

Jang, D.S., Park, E.J., Kang, Y.H., Su, B.N., Hawthorne, M.E., Vigo, J.S, Graham,J.G., Cabieses, F., Fong, H., Mehta, R.G., Pezzuto, J.M. and Kinghorn, A.D (2003). Compounds obtained from *Sida acuta* with the potential to induce quinone reductase and to inhibit 7,12- dimethylbenz[a]anthracene- induced reneoplastic lesions in a mouse mammary organ culture model. *Arch. Pharmacol. Res*. 26: 585-590.

Karou, D., Savadogo, A., Kanini, A., Yameogo, S., Montesano, C., Simpore, J., Colizzi,V. and Traore, A.S. (2006). Antibacterial activity of Alkaloids from *S. acuta* Burm f. *Afri. J. Biotech*. 5(2):195-200.

Kayode, J. (2006). Conservation of indigenous medicinal botanicals in Ekiti state, Nigeria. J. heijiang University Sci. b, 7: 713-718 DO: 10. 6311jzus BO713 PMCI559802.

Kebede, T., Gadisa, E., & Tufa, A. (2021). Antimicrobial activities evaluation and phytochemical screening of some selected medicinal plants: A possible alternative in the treatment of multidrug-resistant microbes. *PloS one*, *16*(3), e0249253. https://doi.org/10.1371/journal.pone.0249253

Lim, S. H., Darah, I., & Jain, K. (2006). Antimicrobial Activities Of Tannins Extracted From Rhizophora Apiculata Barks. Journal of Tropical Forest Science, 18(1), 59–65. http://www.jstor.org/stable/43594647

Low Y.X, Toh H.M., Puteri N.L., Mohd Z, Thiruchelvi P., Jimmy N.A, Thaigarajan P. (2021). Antibacterial potential of Malaysian ethnomedicinal plants against methicillin-susceptible Staphylococcus aureus

(MSSA) and methicillin-resistant Staphylococcus aureus (MRSA). *Saudi Journal of Biological Sciences,*Vol. 28 (10) Pages 5884-5889.https://doi.org/10.1016/j.sjbs.2021.06.036.

Manilal, A., Sabu, K. R., Shewangizaw, M., Aklilu, A., Seid, M., Merdikios, B., & Tsegaye, B. (2020). In vitro antibacterial activity of medicinal plants against biofilm-forming methicillin-resistant Staphylococcus aureus: efficacy of Moringa stenopetala and Rosmarinus officinalis extracts. *Heliyon,* 6(1), e03303. https://doi.org/10.1016/j.heliyon.2020.e03303

Mansoori A, Singh N, Dubey S.K, Thakur T.K, Alkan N, Das S.N, Kumar A. (2020). Phytochemical Characterization and Assessment of Crude Extracts From Lantana camara L. for Antioxidant and Antimicrobial Activity. *Frontiers in Agronomy.* Vol. 2. https://www.frontiersin.org/article/10.3389/fagro.2020.582268. doi: 10.3389/fagro.2020.582268.

Manuele, M.G., Ferraro, G.B., Arcos, M.L., López, P., Cremaschi, G. and Anesini, C. (2006). Comparative immunomodulatory effect of scopoletin on tumoral and normal lymphocytes. *Life Sci*. 79: 2043- 2048.

Miklasińska-Majdanik, M., Kępa, M., Wojtyczka, R. D., Idzik, D., & Wąsik, T. J. (2018). Phenolic Compounds Diminish Antibiotic Resistance of *Staphylococcus Aureus* Clinical Strains. *International journal of environmental research and public health*, *15*(10), 2321. https://doi.org/10.3390/ijerph15102321

Mohamed, E.A.A., Muddathir, A.M. & Osman, M.A. (2020) Antimicrobial activity, phytochemical screening of crude extracts, and essential oils constituents of two *Pulicaria spp.* growing in Sudan. *Sci Rep* **10,** 17148. https://doi.org/10.1038/s41598-020-74262-y

Moon, P.D., Lee, B.H., Jeong, H.J., An, H.J., Park, S.J., Kim, H.R., Ko, S.G., Um, J.Y., Hong, S.H., Kim, H.M. (2007). Use of scopoletin to inhbit the production of inflammatory cytokine through inhibition of Ikappa B/NF-Kappa Bsignal cascade in the human mast cell line HMC-1. *Eur. J.Pharmacol*. 555: 218-225

Mostafa, A. A., Al-Askar, A. A., Almaary, K. S., Dawoud, T. M., Sholkamy, E. N., and Bakri, M. M. (2018). Antimicrobial activity of some plant extracts against bacterial strains causing food poisoning diseases. *Saudi J. Biol. Sci.* 25, 361–366. doi: 10.1016/j.sjbs.2017.02.004

Muhammad I.R, Syed H.A.N, Nazakat H.M, Muhammad I, Farhatullah K, Navish L.P, Md-Nazirul I.S, Muhammad A.B. (2021). Determination of antimicrobial and phytochemical compounds of Jatropha curcas plant, *Saudi Journal of Biological Sciences.* Vol. 28:5. pp.2867-2876. ISSN 1319-562X. https://doi.org/10.1016/j.sjbs.2021.02.019.

Mujeeb, F., Bajpai, P., & Pathak, N. (2014). Phytochemical evaluation, antimicrobial activity, and determination of bioactive components from leaves of Aegle marmelos. *BioMed research international*, *2014*, 497606. https://doi.org/10.1155/2014/497606

Najiah, M., Nadirah, M., Arief, Z., Zahrol, S., Tee, L. W., Ranzi, A. D., ... & Aida, R. J. (2011). Antibacterial activity of Malaysian edible herbs extracts on fish pathogenic bacteria. Research Journal of Medicinal Plant, 5(6), 772-778.

Nath, K., Talukdar, A.D., Bhattacharya, M.K. et al. Cyathea gigantea (Cyatheaceae) as an antimicrobial agent against multidrug resistant organisms. BMC Complement Altern Med 19, 279 (2019). https://doi.org/10.1186/s12906-019-2696-0

Obadoni, B.O and Ochuko, P.O. (2001). Phytochemical studies and comparative efficacy of the crude extracts of some Homostatic plants in Edo and Delta States of Nigeria. Global *J. Pure Appl. Sci*. 8(11):203-208.

Pallavali R.R, Avula S, Degati V.L, Penubala m, Damu A.G, Prasad-Durbaka V.R. (2019) Data of antibacterial activity of plant leaves crude extract on bacterial isolates of wound infections. Data in brief. Vol. 24. 103896,https://doi.org/10.1016/j.dib.2019.103896.

Panda, S. and Kar, A. (2006). Evaluation of antithyroid, antioxidative and antihyperglycemic activity of scopoletin from *Aegle marmelos* leaves in hyperthyroid rats. *Phytother. Res*. 20: 1103-1105.

Sah S.K, Rasool U, Hemalatha S. (2020). Andrographis paniculata extract inhibit growth, biofilm formation in multidrug resistant strains of Klebsiella pneumoniae. *Journal of Traditional and Complementary Medicine*. Vol. 10:6. pp. 599-604. https://doi.org/10.1016/j.jtcme.2019.02.006.

Sen A, and Batra A. (2012). Evaluation Of Antimicrobial Activity Of Different Solvent Extracts Of Medicinal Plant: *Melia azedarach L. International Journal Of Current Pharmaceutical Research.* Vol 4, Issue 2. https://innovareacademics.in/journal/ijcpr/Issues/Vol4Issue2/488.pdf

Sherma, J. and Fried, B (1996) ”Handbook of Thin Layer Chromatography. Marcel Dekker Inc., New York.

Sofowora, A. (1993). Medicinal plants and traditional medicine in Africa.Spectrum books Ltd. Ibadan, Nigeria. p.289.

Touati, N., Saidani, K., Boudries, H., Hammiche, H., Ouazene, N. and Bedjou, F. (2018). Antibacterial activity of phenolic compounds of Pulicaria odora, wild plant in northern *Algeria. Int. Food Res*. J. 25(5), 2121–2130.

Yang, J.Y., Koo, J.H., Lee, J.H., Park, B.H., Kim, J.S., Chi, M.S. and Park, J.W. (2007). Effect of scopoletin on lipoprotein lipase activity in 3T3-L1 adipocytes. *Int. J. Mol. Med*. 20: 527-231.

Zeouk I, Balouiri M, Bekhti K. (2019). Antistaphylococcal Activity and Phytochemical Analysis of Crude Extracts of Five Medicinal Plants Used in the Center of Morocco against Dermatitis. *International Journal of Microbiology,* Vol. ‘19 (7) ID 1803102 https://doi.org/10.1155/2019/1803102

Figures

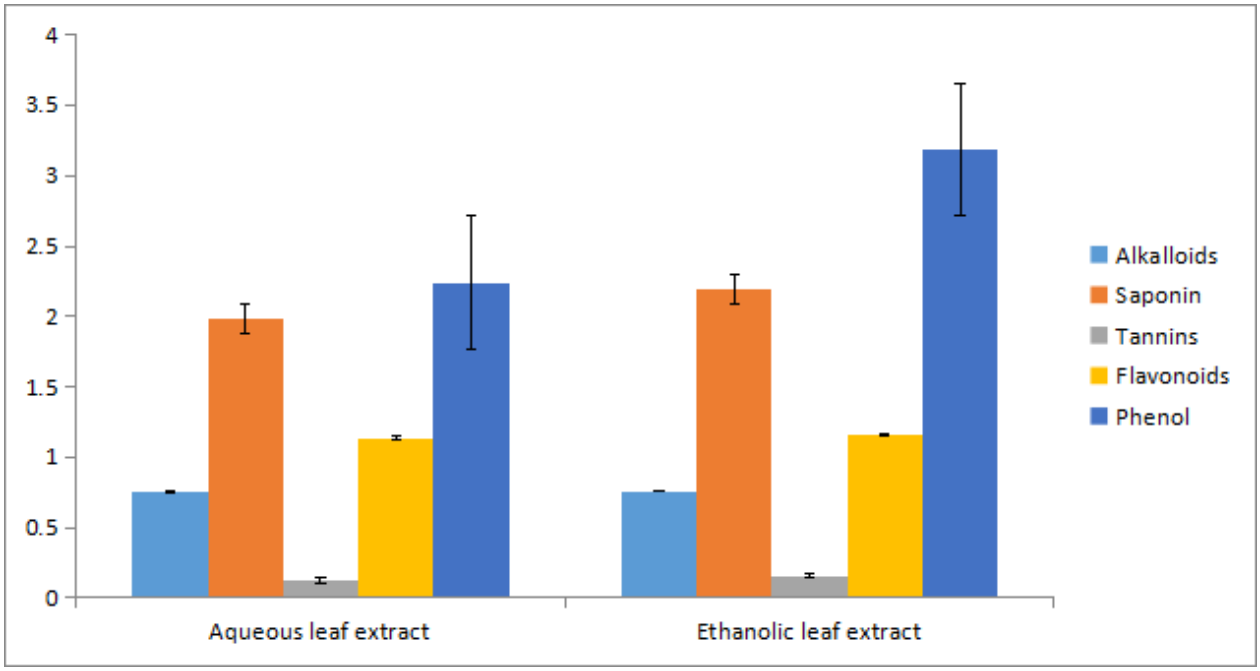

Figure 1

Quantitative phytochemical screening of *S. acuta* leaf extracts.