Int.J.Curr.Microbiol.App.Sci (2022) *11*(08): 274-282

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 11 Number 08 (2022)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2022.1108.029

Antibacterial Activity and Phytochemical Screening of *Sida acuta* Leave Extracts on *Staphylococcus aureus* and *Escherichia coli* Associated with Urinary Tract Infection

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Keywords

Sida acuta, Phytochemical, Antimicrobial, *S. aureus*, *E. coli*, Urinary tract infection

Article Info

Received:
05 July 2022
Accepted:
29 July 2022
Available Online:
10 August 2022

A B S T R A C T

Medicinal plants have been used as herbal remedies against many infectious diseases throughout the history of mankind. They contain secondary metabolites which have great therapeutic potential in the treatment of diseases. This study was carried out to investigate the antimicrobial activity of *Sida acuta* leave extract against *Staphylococcus aureus* and *Escherichia coli* isolated from urinary tract infections. The ethanol, methanol and chloroform extracts of *Sida acuta* leaves were prepared using cold extraction method. Antimicrobial sensitivity test was carried out on the different extracts using Agar-well diffusion method. The zones of inhibition were measured after 24 hours incubation. The results obtained from the study showed that the ethanol extract had more inhibitory activity on *S. aureus* with 24mm as the zone of inhibition at the concentration of 200mg/ml and a lower inhibitory effect on *E. coli* with 19mm as the zone of inhibition at the same concentration. Also, the methanol extract (21mm at 200mg/ml) and chloroform extract (19mm at 200mg/ml) had lower inhibitory effect on *S. aureus* with 20mm as the zone of inhibition and 19mm as the zone of inhibition on *E. coli* at the same concentration. The MIC of the ethanol, methanol and chloroform extracts of *Sida acuta* leave were determined using the broth dilution method. The MIC of the ethanol and methanol extracts on the test organisms were 12.5 mg/ml and 25mg/ml on *S. aureus* and *E. coli* respectively while the MBC of the extracts ranged between 12.5mg/ml and 100mg/ml respectively. The chloroform extracts showed no bactericidal effect on the test organisms. The phytochemical analysis carried out on the plant extracts showed the presence of alkaloids, tannins, flavonoids, saponins, steroids and terpanoids. These secondary metabolites are responsible for the antimicrobial activities which aids in the treatment of diseases. This study therefore encourages the use of this plant extract in the treatment of human diseases caused by the test organisms.

Introduction

The emergence and spread of antibiotic resistance, as well as the evolution of new strains of disease causing agents, are of great concern to the global health community. Antibiotic resistance is increasing among urinary pathogens, resulting in worse clinical and economic outcomes. (Sarita *et al.,* 2019; Marion *et al.,* 2021). Antibiotic resistance has been on the rise globally due to antibiotics being prescribed unnecessarily or inappropriately (Joanna, 2020). Effective treatment of a disease entails the development of new pharmaceuticals or some potential source of novel drugs (Sarita *et al.,* 2019). The use of plant extracts in the treatment of diseases has become an important interest over the years. This is because microorganisms are developing resistance to many antimicrobial drugs and as such created situations where some of the common and less expensive antimicrobial agents are losing their effectiveness (Malairajan *et al.,* 2014). The importance of medicinal plant in drug development is very essential and humans have used them for different diseases from the beginning of human history (Rhaman *et al.,* 2017).

A vast number of medicinal plants have been recognized as valuable resources of natural antimicrobial compounds as an alternative that can potentially be effective in the treatment of these problematic bacterial infections (Sarita *et al.,* 2019) due to their antimicrobial properties. Phytochemical evaluation is one of the tools for quality assessment, which includes preliminary phytochemical screening and evaluation of the quality of plant medicines (Senthilkumar *et al.,* 2018). Phytochemicals are bioactive compounds found in plants that work with nutrients and dietary fibre to protect against diseases. Many phytochemicals have antioxidant activity and reduce the risk of many diseases (Agbafor and Nwachukwu, 2011).

The plant *Sida acuta* is a very common weed which belongs to the Malvaceae family (Senthilkumar *et al.,* 2018; Jimoh *et al.,* 2021). *S. acuta* is a shrub indigenous to pantropical areas, widely distributed in these regions (Damintoti *et al.,* 2006). It is commonly known as stubborn weed (Ezeabara and Egenti, 2018). It is a shrub that grows commonly in different parts of Nigeria. It is perennial in nature, surviving different seasons. It appears to be stubborn specie with a high capacity to thrive in harsh environmental conditions. It is a shrub with multiple stems and often seen growing along road sides and in bushes, waste areas, grazing and abandoned farm lands (Ezeabara and Egenti, 2018; Enemor *et al.,* 2013).

Sida acuta Burm.f (Malvaceae) is one of those plants currently used in folklore for the treatment and management of some health problems (Olivier *et al.,* 2017). It is widely used in traditional medicine (Enemor *et al.,* 2013; Senthilkumar *et al.,* 2018). The whole plant is reported to have many biological activities such as antimicrobial, antihelmintic, antiemetic, diuretic, aphrodisiac, diaphoretic, antipyretic, anti fertility and wound healing properties (Senthilkumar *et al.,* 2018; Ezeabara and Egenti, 2018). It is used as a remedy for inflammation of respiratory tract, bronchitis, stomach ache and gastritis (Mridha *et al.,* 2012). It is also used as a tonic and astringent and for treatments of urinary, bile, hepatic and nervous disorders (Daniel *et al.,* 2020; Ezeabara and Egenti, 2018) asthma, renal inflammation, colds, fever, headache, ulcers and worm infections (Enemor *et al.,* 2013; Senthilkumar *et al.,* 2018; Jimoh *et al.,* 2021) gastroenteritis, intestinal parasites, insect bites, snake bites and infection on wounds. It has been used as a sedative (Amusa *et al.,* 2016) posses anti cancer properties and it has also been recommended for dysentery diarrhea, urinary disease (Senthilkumar *et al.,* 2018) blood disorders, wounds, heart disease, tuberculosis, asthma, toothache, rheumatic infections and cold cough(Swathi, 2021).

Urinary tract infections (UTI) are the inflammatory disorder of the urinary tract caused by the abnormal growth of pathogens (Martin *et al.,* 2019). It is a collective term for infections that involve any part of the urinary tract (Chee and Maciej, 2016). It is one of the most common entities in medicine and

affected patients are presented daily in a typical family medicine practice (Thomas, 2018). It is known to cause short-term morbidity in terms of fever, dysuria and lower abdominal pain and may result in permanent scarring of the kidney (Martin *et al.,* 2019). UTIs are among the most frequent infectious diseases affecting humans, and at least one in two women and one in ten men will experience a UTI in their lifetime (Lisa, 2019; Annarita, 2017). It represents an important public health problem with a substantial economic burden.

Due to the high empiric use of antibiotics for the treatment of UTI, antibacterial resistance of Enterobacteriaceae, specifically the main uropathogens *Escherichia coli* and *Klebsiella pneumonia*, has significantly increased worldwide (Annarita, 2017). A UTI can be caused by bacteria resistant to common antibiotics. This makes it more difficult to treat and can lead to complications (Joanna, 2020).

Materials and Methods

Collection and Processing of Plant Material

The leaves of the plant *Sida acuta* were collected locally from gardens and road sides in Achara Layout, Enugu South Local Government Area of Enugu State. It was identified by a taxonomist in the Department of Applied Biology and Biotechnology of the Enugu State University of Science and Technology (ESUT). The leaves were washed with distilled water and dried for 7days at room temperature. The dried leaves were blended to powder with a sterile blender and were stored in an air tight container until required for the analysis.

Preparation of Plant Extracts

A modified method of Abdulrahman *et al.,* (2014) was used. Fifty (50) grams of the ground leaves of *Sida acuta* was weighed into three conical flasks containing 200mls of solvent extractants (Ethanol, Methanol and Chloroform) respectively. The conical flasks were covered tightly and left for 48 hours to extract at a room temperature with intermittent shaking. The extracts were filtered aseptically into sterile conical flasks using what-man no 1 filter paper. The ethanol, methanol and chloroform extracts were evaporated at 50ºC using a rotary evaporator and the crude extracts were stored at 4ºC in a refrigerator.

Standardization of Inoculum

The test organisms used were *Staphylococcus aureus* and *Escherichi coli* that were isolated from the urinary tract of infected patients attending clinic in University of Nigeria Teaching Hospital (UNTH) Enugu, Enugu State. Confirmatory tests were carried out on each of the organisms using biochemical characterizations tests.

McFarland equivalent turbidity standard was prepared. The 0.5McFarland turbidity standard was used to adjust the turbidity of the inocula that was used for antimicrobial susceptibility test.

Antimicrobial susceptibility test using the extracts

Agar well diffusion method was used to determine the antibacterial activity of the extracts.

To test for this, 1g of each of the extracts were dissolved in 5mls of DMSO respectively and then varying concentrations of the extract (200mg/ml, 100mg/ml, 50mg/ml, 25mg/ml, 12.5mg/ml, 6.25mg/ml, 3.125mg/ml, 1.562mg/ml and 0.76mg/ml) were obtained.

A standard inoculum of 1.5 x 10^8 cells (*Staphylococcus aureus* and *Escherichia coli*) which is equivalent to 0.5 McFarland standards were spread on the surface of sterile Mueller Hinton agar plates in duplicates. A sterile 6mm cork borer was used to make holes on the Mueller Hinton agar plates in which 0.1ml of various concentrations of the extracts were added. The plates were then incubated at 37ºC for 24hours and the zones of inhibition were measured.

Determination of the Minimum Inhibitory Concentrations (MIC)

This was determined using broth dilution method. 0.1 ml of the inoculum (*Staphylococcus aureus* and *Escherichia coli*) was spread on petri dishes containing Muller Hinton agar. Wells (6 mm diameter) were pouched into the already inoculated Muller Hinton agar plates using sterile cork borer and 0.1ml of each of the extracts (200mg/ml, 100mg/ml, 50mg/ml, 25mg/ml, 12.5mg/ml, 6.25mg./ml, 3.125mg/ml, 1.562mg/ml and 0.76mg/ml) were added into each of the wells respectively and they were incubated at 37ºC for 24 hours. After the incubation, the MIC was determined as the least concentration of the extract that inhibited the growth of the organism.

Determination of Minimum Bactericidal Concentration (MBC)

In this technique, test tubes containing the various concentrations of the extracts were inoculated with 0.1ml of the standardized organisms respectively and were incubated at 37°C for 24 hours.

Test tubes with no visible growth were streaked on various plates containing Mueller Hinton agar and incubated at 37°C for 24 hours. They were observed for the absence or presence of any visible growth. The MBC was taken as the concentration of the plant extract that did not exhibit any bacterial growth after the incubation.

Phytochemical Screening of the Plant extract

The leaf extracts were screened for their phytochemical activity using the standard method. The phytochemical component analysed were alkaloids, saponins, flavonoid, tannins, reducing sugar, steroids and glycosides.

Statistical analysis

Analysis of variance (ANOVA) and the t-test were used for data analysis.

Results and Discussion

The antimicrobial susceptibility pattern of the ethanol extract of *Sida acuta* leaves showed that the ethanol extract had more effect on *Escherichia coli* and *Staphylococcus aureus* than the methanol and chloroform extracts but at varying concentrations.

At the concentration of 200mg/ml, *Staphylococcus aureus* showed the highest susceptibility (24mm) while *Escherichia coli* showed 18mm as the zone of inhibition at the same concentration. *Staphylococcus aureus* had the least susceptibility (6mm) and *Escherichia coli* (3mm) at the concentration of 1.56mg/ml (Table 1 and 2).

Staphylococcus aureus and *Escherichia coli* were susceptible to the methanol extract but at varying concentrations. At the concentration of 200mg/ml *Staphylococcus aureus* showed the highest zone of inhibition (21mm) and *Escherichia coli* (19mm) as the zone of inhibition.

Though, *Staphylococcus aureus* and *Escherichia coli* had the least zone of inhibition (3mm and 2mm) at the concentrations of 1.56mg/ml and 3.125mg/ml respectively (Table 1 and 2).

The chloroform extract also showed an appreciable effect on the test organisms. *Staphylococcus aureus* showed the highest zone of inhibition (19mm) at the highest concentration of 200mg/ml while *Escherichia coli* showed 17mm zone of inhibition at the same concentration. At lower concentrations, no inhibition was recorded (Table 1 and 2).

The Minimum Inhibitory Concentration (MIC) of the ethanol extract on *Staphylococcus aureus and Escherichia coli* were 12.5Mmg/ml and 25mg/ml respectively, while the MIC of the methanol extract on *Staphylococcus aureus* and *Escherichia coli* were 50mg/ml and 100mg/ml respectively. Also, the MIC of the chloroform extract on *Staphylococcus aureus* and *Escherichia coli* were 100mg/ml and 200mg/ml respectively (Table 3, 4, 5).

Table.1 Zones of Inhibition of Extracts of *Sida acuta* Leaves on *Staphylococcus aureus.*

Concentrations (mg/ml)	Zones of Inhibition (mm)		
	Ethanol	Methanol	Chloroform
200	23.917±0.083	21.75 ± 0.25	19.167 ± 2.167
100	21.369 ± 0.631	19.464 ± 0.536	16.226 ± 0.226
50	18.821 ± 0.179	17.179 ± 0.179	13.288 ± 1.714
25	16.274 ± 0.274	14.893 ± 0.107	10.345 ± 2.655
12.5	13.726 ± 0.726	12.607 ± 1.607	7.405 ± 2.595
6.25	11.179 ± 1.179	10.321 ± 0.321	4.464 ± 4.464
3.125	8.631 ± 0.369	8.036 ± 0.964	1.524 ± 1.524
1.56	6.083 ± 0.917	3.75 ± 0.25	1.417 ± 1.417

Table.2 Zones of Inhibition of Extracts of *Sida acuta* Leaves on *Escherichia coli.*

Concentrations (mg/ml)	Zones of Inhibition (mm)		
	Ethanol	Methanol	Chloroform
200	19.5 ± 0.5	18.417 ± 0.417	17 ± 1
100	16.921 ± 1.321	16.583 ± 0.417	14.286 ± 0.714
50	14.143 ± 0.143	13.75 ± 0.78	11.571 ± 1.429
25	11.964 ± 1.036	10.917 ± 0.917	8.857 ± 0.143
12.5	9.786 ± 1.214	8.083 ± 0.917	6.1423 ± 0.857
6.25	7.607 ± 1.393	5.25 ± 1.75	3.429 ± 3.429
3.125	5.429 ± 1.571	2.417 ± 2.417	0.714 ± 0.714
1.56	3.25 ± 3.25	0.417 ± 0.417	2 ± −2

Table.3 Minimum Inhibitory Concentration (MIC) of *Sida acuta* Ethanol Extract on the Test Organisms.

	Concentrations								
Test Organisms	200	100	50	25	12.5	6.25	3.125	1.56	MIC
S. aureus	-	-	-	-	-	+	+	+	12.5
E. coli	-	-	-	-	+	+	+	+	25

KEY: - = no visible growth
+ = visible growth

Table.4 Minimum Inhibitory Concentration(MIC) 0f Methanol Extract of *Sida acuta* Leave on *S. aureus* and *E. coli*

	Concentrations								
Test Organisms	200	100	50	25	12.5	6.25	3.125	1.56	MIC
S. aureus	-	-	-	+	+	+	+	+	50
E. coli	-	-	+	+	+	+	+	+	100

KEY: - = no visible growth
+ = visible growth

Table.5 Minimum Inhibitory Concentration (MIC) of chloroform extract of *Sida acuta* Leaves on *S. aureus* and *E. coli.*

	Concentrations								
Test Organisms	200	100	50	25	12.5	6.25	3.125	1.56	MIC
S.aureus	-	-	+	+	+	+	+	+	100
E.coli	-	+	+	+	+	+	+	+	200

KEY: - = no visible growth
+ = visible growth

Table.6 Minimum Bactericidal Concentration (MBC) of *Sida acuta* leave Extracts on the Test Organisms

	Minimum bactericidal concentration (mg/ml)		
Test Organisms	Ethanol	Methanol	Chloroform
S. aureus	12.5	25	-
E. coli	25	100	-

Table.7 Result of the Phytochemical Screening of *Sida acuta* Leave Extracts

Plant extract	Alkaloids	Tannins	Saponins	Terpanoids	Glycosides	Flavonoids	Steroids
Ethanol	+	+	+	+	-	+	+
Methanol	+	+	+	+	-	+	+
Chloroform	+	+	+	+	-	+	+

KEY: + = Presence of Constituent
- = Absence of constituents

The Minimum Bactericidal Concentration MBC of the ethanolic extract on *Staphylococcus aureus* and *Escherichia coli* were 12.5mg/ml and 25mg/ml respectively while the MBC of the methanolic extract of *Staphylococcus aureus* and *Escherichia coli* were 25mg/ml and100mg/ml respectively. The chloroform extract of *Sida acuta* leave had no bactericidal effect on the test organisms (Table 6).

The results of the phytochemical screening showed the presence of some essential phyto chemicals such as: alkaloids, tannins, saponins, steroids, flavonoids, and glycosides (Table 7).

The present study was carried out to determine the antimicrobial activity of *Sida acuta* leave extracts against *Escherichia coli* and *Staphylococcus aureus* isolated from urinary tract of infected patients. The ethanol, methanol and chloroform extracts of *Sida acuta* leaves were evaluated for their antimicrobial activity. It was observed that the ethanol extract had more inhibitory effect on the test organisms (24mm and 19mm at 200mg/ml respectively) when compared to the methanol and chloroform extracts which had 21mm; 18mm and 19mm; 17mm respectively as their zones of inhibition at the same concentration. This agrees with the work of Akilandeswari *et al.,* (2010) who assessed the anti bacterial activity for both the chloroform and ethanolic extract, and observed that appreciable antibacterial activity was found against all the selected bacteria with the maximum activity recorded against gram positive *Staphylococcus aureus* and gram negative *Escherichia coli* respectively.

The MIC of the ethanol, methanol and chloroform extracts were determined for *Staphylococcus aureus*

and *Escherichia coli* at varying concentrations of 200, 100, 50, 25, 12.5, 6.25, 3.125 and 1.56mg/ml. The result showed that the MIC of the ethanolic extracts of *Sida acuta* leaves on *Staphylococcus aureus* and *E. coli* were 12.5mg/ml and 25mg/ml respectively while the least MIC was shown by the chloroform extract at 100mg/ml and 200mg/ml respectively.

This shows that the ethanol extract was more effective against the test organisms than other extracts. This is in conformity with Mbajiuka *et al.*, (2014) who observed that the minimum inhibition concentration of *Staphylococcus aureus* against the ethanolic extracts was 0.125 while *Escherichia coli* had the lowest minimum inhibition concentartion of 0.0625 on ethanolic extracts of *Sida acuta*.

The MBC result for ethanolic extracts of *Sida acuta* leaves on *S. aureus* and *E. coli* were 12.5mg/ml and 25mg/ml respectively while the methanol extract were 25mg/ml and 100mg/ml for *S. aureus* and *E. coli* respectively. The Chloroform extract had no bactericidal effect on the test organisms. The phytochemical screening of *Sida acuta* leave extract revealed the presence of alkaloids, tannins, saponins, terpanoids, steroids and flavonoids. The results obtained from phytochemicals and micronutrients screening of Sida acuta gives credence to the medicinal benefits that this herb have been used for, in the past years and supports its traditional use for the management of various health problems (Olivier *et al.*, 2017). These results also suggest that the antibacterial activity shown by the extracts against the test organisms might be due to natural occurring bioactive phytochemicals present in the plant (Akilandeswari *et al.*, 2010). This suggests that the plant could serve as a remedy to fight against infections caused by these pathogens.

Acknowledgements

I acknowledge everyone who made this research work a success especially the staff of the Department of Applied Microbiology, ESUT Enugu, Nigeria.

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How to cite this article:

Ezugwu Roseline Ifeyinwa, Walter, Binyelu Walter and Ugwu Celestina Chibuzor. 2022. Antibacterial Activity and Phytochemical Screening of *Sida acuta* Leave Extracts on *Staphylococcus aureus* and *Escherichia coli* Associated with Urinary Tract Infection. *Int.J.Curr.Microbiol.App.Sci.* 11(08): 274-282. doi: https://doi.org/10.20546/ijcmas.2022.1108.029