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Antibacterial Activity of the Leaf and Root Extracts of *Sansevieria zeylanica* Against Strains of Methicillin - Sensitive and -Resistant *Staphylococcus aureus*.

Ugbomoiko, D.O.

Department of Medical Laboratory Science, College of Health Sciences, Igbinedion University Okada, Edo State, Nigeria.

Egunjobi, T.O.*

Department of Medical Microbiology, School of Basic Clinical Sciences, College of Health Sciences, Igbinedion University Okada, Edo State, Nigeria.

Omosigho, P. O.

Department of Medical Laboratory Science, College of Health Sciences, Igbinedion University Okada, Edo State, Nigeria.

Olley, M.

Department of Medical Laboratory Science, College of Health Sciences, Igbinedion University Okada, Edo State, Nigeria.

Osaiyuwu, C.

Department of Medical Laboratory Science, College of Health Sciences, Igbinedion University Okada, Edo State, Nigeria.

Asemota, P.A.

Department of Paediatric and Child Health, Igbinedion University/Teaching Hospital, Okada,Nigeria.

Omoruyi, Z.

Department of Medical Laboratory Science, School of Basic Medical Sciences, University of Benin, Edo State, Nigeria.

***Corresponding Author:**

Egunjobi, T O

Department of Medical Microbiology, School of Basic Clinical Sciences, College of Health Sciences, Igbinedion University Okada, Edo State, Nigeria.

Email: tunde.egunjobi@iuokada.edu.ng

Phone: +2347065615730

Abstract

Introduction: There is an increase in the incidence of treatment failure which could be attributed to an upsurge in antimicrobial resistance (AMR) amongst pathogenic bacteria. As a result, there is a search for the discovery and development of new antimicrobial agents with modes of action different from that of conventional antibiotics. Sansevieria species are used in traditional medicine in the treatment of cough, diarrhea, viral hepatitis, ear infections, and anuria. The purpose of this study was to evaluate the antimicrobial activity of the leaf and root extracts of *Sansevieria zeylanica* against strains of methicillin-sensitive (MSSA) and –resistant *Staphylococcus aureus* (MRSA).
Materials and Methods: Solvent extraction was carried out with acetone, ethyl acetate, and methanol using the Soxhlet apparatus. The antibacterial activity of the extracts against strains of MSSA and MRSA was investigated using the agar well diffusion method.
Results: The acetone leaf extract at a concentration of 200mg/ml showed better antibacterial activity compared to ethyl acetate and methanol extracts with zones of inhibition ranging from 19mm to 25mm. The lowest MIC (2mg/ml) was produced by acetone leaf extract against certain strain of MRSA.The leaf extract enhances the activity of Amoxicillin Clavulanate (Augmentin) against the selected strains. However, the gentamycin and root extract combination produced an antagonistic effect.
Conclusion: Extracts of *Sansevieria zeylanica* possess potent antimicrobial activity against strains of MSSA and MRSA and may be considered as a treatment option for infections caused by these pathogens where conventional antibiotics fail due to AMR.

Keywords: Antibacterial Activity, Antibiotics, Antimicrobial Resistance, MRSA, *Sansevieria zeylanica*, *Staphylococcus aureus*.

Introduction

In developing nations, infectious diseases account for a major cause of hospitalization (Febriani *et al*., 2019). *Staphylococcus aureus* is a pathogen that often causes infection in humans (Brooks *et al.*, 2013), with Methicillin-sensitive (MSSA) and -resistant *S. aureus* (MRSA) together associated with the rise in nosocomial infection. This is a major health concern worldwide (Gould, 2005) due to an increase in the cost of treatment, length of bed stay, and mortality. Methicillin-Resistant *S. aureus* (MRSA), a pathogen of public health concern, has also been linked with septic arthritis, endocarditis, pneumonia, skin and soft tissue infections (SSTIs), sepsis, endovascular infections, and osteomyelitis (Yao *et al.*, 2010).

For ages, medicinal plants have been used in the treatment and management of many diseases and health conditions (Obydulla, 2016). About 70-80% of people across the globe depend mainly on traditional medicine as their primary healthcare (Hamilton, 2004). In Africa, medicinal plants have been an alternative in the management of microbial infections (Ahmad*et al.*, 2021). Half of the drugs in clinical use are from medicinal plants (Tripathi and Mukherjee, 2003).Over the years, there had been an increase in the incidence of treatment failure owing to the rise of antibiotic resistance bacteria (WHO, 2017). As a result of this, the search for new antimicrobial drugs is ongoing. Plant species in Nigeria that have been used traditionally in the treatment of various ailments could be sources of these agents.

The Sansevierians, with about 70 species are herbaceous plants with rhizomatous roots belonging to the subfamily of Nolinoideae of the Asparagaceae family, in the order Asparagales (Chase *et al.*, 2016). The plants are found growing in subtropical and tropical countries of the world (Staples and Herbst, 2005). There are more Sansevierians in Africa than anywhere in the world (Carlquist and Schneider, 2007). The leaves and roots of these plants are used in traditional medicine for the treatment of cough, diarrhea, hemorrhoids, sexual weakness, asthma, hypertension, anuria, jaundice, viral hepatitis, edema, (Giovannini and Howes, 2017; Kpodar *et al.*, 2016; Andhare *et al.*, 2012; Bero *et al.*, 2009), ear infections, abdominal pains, (Aliero *et al.*, 2008),etc. Although saponins and flavonoids are the major secondary metabolites in the

Sansevierans, other bioactive compounds that have been isolated from these plants include stilbenes and steroids (Thu *et al.*, 2020; Sun *et al.*, 2019).

There are remarkable handfuls of documentation on the medicinal benefits of Sansevieria species (Deepa *et al.*, 2011). In addition to their antimicrobial and antioxidant effects (Aliero *et al.*, 2008), this genus of plants also has analgesic (Sunilson *et al.*, 2009) and anti-inflammatory activities (Da Silva *et al.*, 2003).Ethanol extracts of certain species of Sansevieria have shown antibacterial activity against *Escherichia coli*. These species include *S. trifasciata, S. roxburghiana, S. aethiopica, S. francisii, S. cylindrica, S. caulescens, S. canaliculata, S. forskaliana, S. arborescens, S. metallica* and *S. kirkii* (Tkachenko *et al.*, 2017).

The focus is on research to provide scientific evidence for the efficacy of these plants in the treatment of various diseases and health conditions. Although the antimicrobial properties of many Sansevieria species have been reported, there is a paucity of data as regards those of *Sansevieria zeylanica*. Hence, the objective of this study is to investigate the antibacterial activity of the leaf and root extracts of *Sansevieria zeylanica* against strains of methicillin-sensitive and –resistant *Staphylococcus aureus*.

Materials and Methods

Collection of Plant Samples

Fresh leaves and roots of *S. zeylanica* were collected from Igbinedion University Okada (IUO) and authenticated at the Department of Biological Sciences of the same institution.

Collection of Test Organisms

Strains of *Staphylococcus aureus* were collected from the Medical Microbiology laboratory Unit of Igbinedion University Teaching Hospital (IUTH), Okada, Edo State. The test strains were confirmed by carrying out Gram staining, catalase, coagulase, and DNase tests and culturing on mannitol salt agar. Antimicrobial susceptibility of the bacterial test strains to Cefoxitin was carried out. Bacterial test strains susceptible to Cefoxitin were regarded as MSSA (zone of inhibition ≥ 22mm) and MRSA for strains resistant to Cefoxitin (zone of inhibition ≤ 21mm) according to the criteria of the Centre for Disease Control and Prevention (CDC, 2019).

Preparation of Extracts

About 132.23g of the dried leaves and 54.81g of roots were pulverized and extraction was carried out with the aid of the Soxhlet extractor apparatus using acetone, ethyl acetate, and methanol as solvent. The extracts were filtered using Whatman No 1 filter paper and the solvent was removed using rotary evaporator apparatus at 45^0C to give a 1g/ml concentration (Salehzadeh *et al.*, 2014). The extracts were stored at 4^0C until further use.

Antibacterial Activity Test

Agar well diffusion method was used to determine the antibacterial activity of the sample extracts (White and Reeves, 1987). Bacterial isolates stored on nutrient agar slants were sub-cultured at 37^0C for 24 hours. Suspension of each isolate was prepared in physiological saline and turbidity compared with 0.5 McFarland standard. A lawn culture was obtained by seeding the test inoculum on freshly prepared Muller-Hinton agar. A sterile cork borer of 6mm in diameter was used to make wells on each seeded medium. Different concentrations of the plant extracts were prepared using 5% Dimethyl Sulfoxide (DMSO) and 200µl of each was transferred to the wells. The agar plates were left to stand at room temperature for about 15 minutes to allow the diffusion of the sample extracts into the agar and incubated at 37^0C. After 24 hours, the zone of inhibition around each well was measured and recorded.

Determination of Minimum Inhibitory Concentration (MIC)

The MIC of the leaf and root extracts against the test strains was determined using the microdilution method described by Eloff(1998). Different concentrations of the extracts ranging

from 0.125 mg/ml to 32 mg/ml were prepared in Muller-Hinton broth medium. A 100µl of standardized suspension of each of the test strains was inoculated into each tube and incubated at 37^0C for 24 hours. Tubes containing the broth medium and extracts without inoculum were used as controls. The MIC is the tube with the lowest concentration without visible growth when compared with the controls.

Determination of the Synergistic Effect of the Extracts with Amoxicillin Clavulanate (Augmentin) and Gentamycin

The synergistic effect of the sample extracts was determined as described by Kingsley *et al*., (2013). Antibiotic discs impregnated with sample extracts were used and the zone of inhibition was measured after 24 hours of incubation and compared with those produced by sample extract alone and antibiotic alone. A synergistic effect was indicated when the zone of inhibition produced by the combination of sample extract and antibiotic is greater than that of sample extract alone or antibiotic alone.

Results

In this study, a total of 1,013.11grams and 306.12grams of fresh leaves and roots, respectively of *Sansevieria zeylanica* were collected, dried, and pulverized.

Antibacterial activity of the different solvent extracts of the leaf and root of *S. zeylanica* were tested against strains of MSSA and MRSA using the agar well diffusion method (Figure 1).

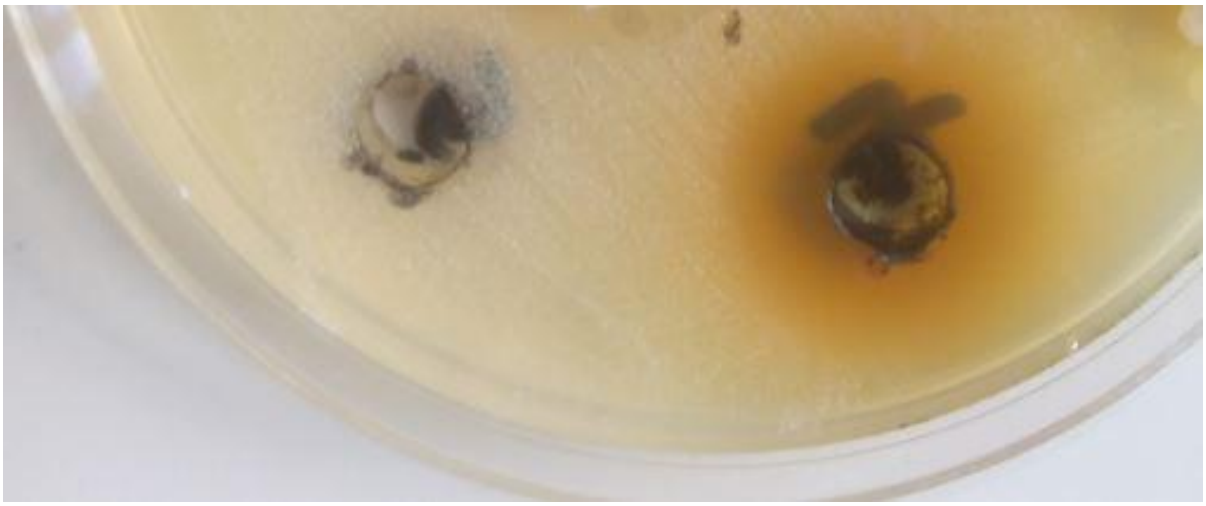

Figure 1: Antibacterial activity of *Sansevieria zeylanica* by Agar Well Diffusion.

The extracts showed varying degrees of antibacterial activities at concentrations between 100mg/ml and 400mg/ml with the zone of inhibition ranging from 7mm to 25mm. The acetone leaf extract particularly at a concentration of 200mg/ml (zone of inhibition of 19 – 25mm) showed greater antibacterial activity compared to ethyl acetate (0 – 21mm) and methanol extracts (0 – 24mm) (Table 1 - 3).

Table 1: Antimicrobial Activity of 100mg/ml extracts of *Sansevieria zeylanica* by Agar Well Diffusion Method (Zone of inhibition in mm).

	Extracts					
	Acetone		Ethyl acetate		Methanol	
Test Isolate	LE	RE	LE	RE	LE	RE
MSSA-1	11	9	7	0	9	0
MSSA-2	10	0	0	0	9	0
MSSA-3	9	9	0	0	7	0
MRSA-1	21	18	16	13	19	17
MRSA-2	13	11	8	0	10	9
MRSA-3	12	8	8	0	11	0

MSSA = Methicillin-Sensitive *Staphylococcus aureus*, MRSA = Methicillin-Resistant *Staphylococcus aureus*, LE = Leave extract, RE = Root extract (Values are the mean of three replicates)

Table 2: Antimicrobial Activity of 200mg/ml extracts of *Sansevieria zeylanica* by Agar Well Diffusion Method (Zone of inhibition in mm).

	Extracts					
	Acetone		Ethyl acetate		Methanol	
Test Isolate	LE	RE	LE	RE	LE	RE
MSSA-1	23	21	16	17	20	20
MSSA-2	21	23	17	19	19	20
MSSA-3	20	18	13	11	17	17
MRSA-1	19	18	16	17	20	18
MRSA-2	25	19	21	15	24	17
MRSA-3	23	21	17	15	21	20

MSSA = Methicillin-Sensitive *Staphylococcus aureus*, MRSA = Methicillin-Resistant *Staphylococcus aureus*, LE = Leave extract, RE = Root extract (Values are the mean of three replicates)

Table 3: Antimicrobial Activity of 400mg/ml extracts of *Sansevieria zeylanica* by Agar Well Diffusion Method (Zone of inhibition in mm).

	Extracts					
	Acetone		Ethyl acetate		Methanol	
Test Isolate	LE	RE	LE	RE	LE	RE
MSSA-1	14	12	11	7	13	9
MSSA-2	17	11	12	10	16	11
MSSA-3	18	12	13	9	16	10
MRSA-1	11	11	10	7	11	9
MRSA-2	12	15	12	15	13	18
MRSA-3	15	19	11	15	15	20

MSSA = Methicillin-Sensitive *Staphylococcus aureus*, MRSA = Methicillin-Resistant *Staphylococcus aureus*, LE = Leave extract, RE = Root extract (Values are the mean of three replicates)

The MIC of the extracts against the test strains is shown in Tables 4 and 5. Growth of MRSA-1 was inhibited by acetone leaf extract at a MIC of 2mg/ml. All the test isolates except MRSA-1 showed the highest MIC value of >32mg/ml with ethyl acetate root extract.

Table 4: Minimum Inhibitory Concentration (MIC) of leaf extract of *S. Zeylanica*

	Extracts (mg/ml)		
Test Isolate	**Acetone**	**Ethyl acetate**	**Methanol**
MSSA-1	8	>32	8
MSSA-2	4	16	4
MSSA-3	16	>32	16
MRSA-1	2	4	4
MRSA-2	4	16	8
MRSA-3	8	>32	4

MSSA = Methicillin-Sensitive *Staphylococcus aureus*, MRSA = Methicillin-Resistant *Staphylococcus aureus*. (Values are the mean of three replicates)

Table 5: Minimum Inhibitory Concentration (MIC) of root extract of *S. zeylanica*

	Extracts (mg/ml)		
Test Isolate	**Acetone**	**Ethyl acetate**	**Methanol**
MSSA-1	8	>32	16
MSSA-2	8	>32	16
MSSA-3	16	>32	>32
MRSA-1	8	8	4
MRSA-2	8	>32	16
MRSA-3	8	>32	8

MSSA = Methicillin-Sensitive *Staphylococcus aureus*, MRSA = Methicillin-Resistant *Staphylococcus aureus*. (Values are the mean of three replicates)

The effects of the combination of the leaf and root extracts with Amoxicillin-Clavulanate (Augmentin) and Gentamycin were investigated against the test strains using the disk diffusion method. The zone of inhibition produced by the antibiotics with and without the extracts against the isolates is shown in Table 6. The antibacterial activity of Amoxicillin-Clavulanate (Augmentin) and Gentamycin increased in the presence of the leaf extract (171.43% and 50.00% fold increase, respectively) against the selected MSSA strain. Similarly, the activity of Amoxicillin-Clavulanate (Augmentin) increased against the tested MRSA strain in the presence of the leaf and root extracts (fold increase of 33.33% each). There was a marked decrease in the zone of inhibition produced by Gentamycin and root extract combination compared with Gentamycin alone in both MSSA and MRSA strains tested(Figure 2), with negative fold increase (-10.00% and -50.00%, respectively) showing an antagonistic relationship (Table 6).

Table 6: Zone of inhibition of Amoxicillin-Clavulanate (Augmentin) and Gentamycin with and without 200mg/ml Acetone extracts of *Sansevieria zeylanica*.

	Zone of Inhibition (mm)			Zone of Inhibition (mm)		
Test Isolate	**AUG (a)**	**AUG+LE (b)**	**fold increase (%) = ((b - a) / a) x 100**	**GEN (a)**	**GEN+LE (b)**	**fold increase (%) = ((b - a) / a) x 100**
MSSA-2	7	19	171.43%	10	15	50.00%
MRSA-1	9	12	33.33%	22	22	0.00%

	Zone of Inhibition (mm)			Zone of Inhibition (mm)		
Test Isolate	**AUG (a)**	**AUG+RE (b)**	**fold increase (%) = ((b - a) / a) x 100**	**GEN (a)**	**GEN+RE (b)**	**fold increase (%) = ((b - a) / a) x 100**
MSSA-2	7	7	0.00%	10	9	-10.00%
MRSA-1	9	12	33.33%	22	11	-50.00%

MSSA = Methicillin-Sensitive *Staphylococcus aureus*, MRSA = Methicillin-Resistant *Staphylococcus aureus*, AUG = 30µg/disc Amoxicillin-Clavulanate (Augmentin), GEN = 25µg/disc Gentamycin, AUG+LE = 30µg/disc Amoxicillin-Clavulanate (Augmentin) and 200mg/ml leave extract combination, , GEN+LE = 25µg/disc Gentamycin and 200mg/ml leave extract combination, AUG+RE = 30µg/disc Amoxicillin-Clavulanate (Augmentin) and 200mg/ml root extract combination,GEN+RE = 25µg/disc Gentamycin and 200mg/ml root extract combination.

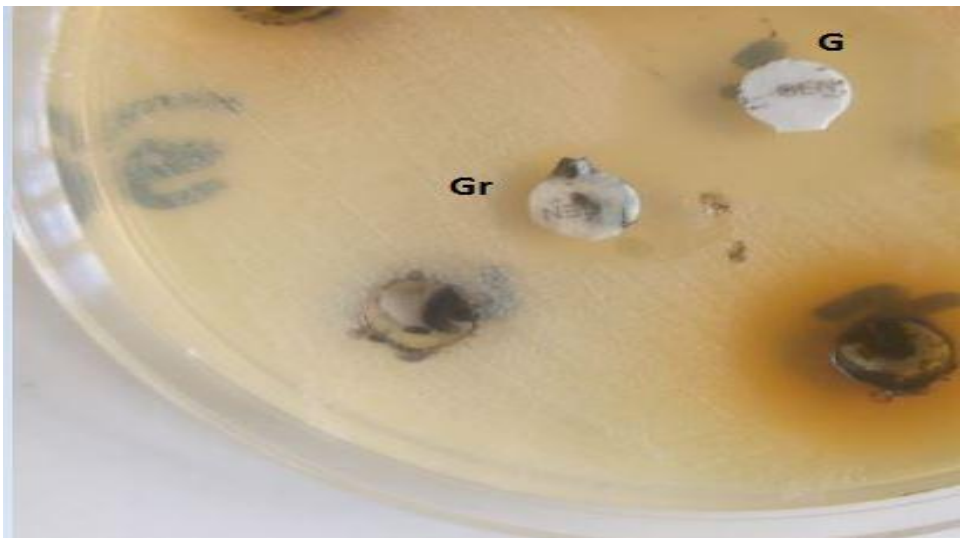

Figure 2: Antagonistic relationship between Gentamycin and the Root extract of *Sansevieria zeylanica*. Gentamycin and Root Extract combination (Gr) showed decreased zone of inhibition compared to Gentamycin alone (G).

Discussion

The use of medicines of plant origin in the treatment and management of microbial infection is widespread (Salehzadeh *et al*., 2014). In this study, the antibacterial activity of different solvent extracts of the leaf and root of *S. zeylanica* against strains of MSSA and MRSA was investigated *in vitro*. Since there are no reports available exclusively on *S. zeylanica*, the antibacterial activity of *S. zeylanica* in the present study was comparable with available studies on the antibacterial activity of related species such as *S. liberica*, S. *trifasciata* and *S. roxburghiana* (Deepa *et al.*, 2011; Ikewuchi *et al.*, 2010; Sunilson *et al.*, 2009; Ogukwe *et al.*, 2004).

The acetone leaf extract of *S. zeylanica* was remarkably active at a concentration of 200mg/ml with zones of inhibition ranging from 19mm to 25mm against the test strains. In the present study, the lowest MIC (2mg/ml) was produced by acetone leaf extract against MRSA-1,this is comparable to MIC reported for *S. roxburghiana* against *S. aureus* by Deepa *et al*., (2011). The highest MIC (>32mg/ml) was produced by ethyl acetate extracts which correspond with the MIC reported in a previous study for other species of Sansevieria against *S. aureus* (Febriani *et al*., 2019). The better antimicrobial activity of the leaf extracts compared to the root extracts observed in this study could be due to certain bioactive substance(s) present in the leaf extracts but absent in the root extracts (Deepa*et al.*, 2011).

Flavonoids, saponins, tannins, and alkaloids in plants have been reported to have varying antimicrobial activities (Ibrahim *et al.*, 1997, Levan *et al.*, 1979). The activity of alkaloids against *S. aureus* has been reported (Prajapati, 2007). Also, flavonoid-containing preparations have been used extensively in the treatment of various diseases (Essiett and Ukpong, 2009). Specifically, the presence of glycosides, alkaloids, steroids, tannins, terpenoids, and acidic compounds in Sansevierians justifies the medicinal use of these plants (Ikewuchi *et al.*, 2011; Ikewuchi *et al.*, 2010).

According to Mpila *et al*., (2012), plant extracts strength can be categorized based on the zone of inhibition: ≤ 5mm as weak, 6mm-10mm as moderate, 11mm-19mm as strong, and ≥ 20mm as very strong. Also, the Indonesian Pharmacopoeia (Directorate General of Drug and Food Control, 1995) regarded plant extracts with a zone of inhibition of at least 14 mm as effective. From the foregoing, the antibacterial activity of the extracts of *S. zeylanica* against the tested strains of MSSA and MRSA can be categorized as strong/very strong and effective.

Although both selected strains showed resistance to Amoxicillin-Clavulanate(Augmentin) (7mm – 9mm) and the MSSA-2 isolate also to Gentamycin (10mm), both were found to be susceptible to the leaf and root extracts of *S. zeylanica* (up to 23mm). This indicated that the extracts from this plant might have modes of action different from the selected antibiotics. This is in agreement with the report of Eloff (1998) who suggest that extracts from plants with target sites different from those targeted by conventional antibiotics could be effective against drug-resistant bacteria.

In tandem with the results from the present study, previous studies have reported the antimicrobial activity of Sansevieria species against various strains of *S. aureus* (Febriani *et al*., 2019; Dewatisari *et al*., 2017). The activity of the extracts against strains of MRSA is noteworthy as these bacteria are known to be a major cause of morbidity and mortality worldwide.

Although there was no clear synergistic effect between the extracts and the selected antibiotics, it was observed that the acetone extracts potentiated the activity of Amoxicillin-Clavulanate (Augmentin) against the selected strains with a fold increase between 33.33% and 171.43%. In addition, the activity of Gentamycin against MSSA-2 was doubled by the leaf extract. Garvey *et al*., (2011), and Rakholiya & Chanda (2012), all reported that certain plants contain biologically active compounds that can enhance the antibacterial activity of antibiotics against

drug-resistant pathogens. These bioactive compounds have been referred to as resistance -modifying, -reversal, or -modulating agents. This enhancement of antibiotics activity by plant extracts may be due to increased permeability, inhibition of drug efflux, or inhibition of β-lactamase (Garvey *et al*., 2011; Smith *et al*., 2007). In addition, the reversal of antimicrobial resistance observed in this study could also be due to the inhibition of ABC transporters that are often overexpressed in multidrug-resistant eukaryotic and prokaryotic cells (Lage, 2009).The Gentamycin and root extract combination produced an antagonistic effect. This effect could be attributed to the presence of certain compounds in the root extract inhibitory to the protein synthesis-inhibition action of gentamycin.

Conclusion

To the best of our knowledge, this is the first time the antibacterial activity of *S. zeylanica* against strains of MSSA and MRSA is reported. The results of this study showed the potency of different solvent extracts of the leaf and root of *S. zeylanica* against strains of MSSA and MRSA. The leaf and root extracts of *S. zeylanica* possess effective antibacterial agents that can be used in the treatment and management of various infections especially those associated with MSSA and MRSA. Also, the result of this present study showed that the acetone extracts of *S. zeylanica* can enhance the antimicrobial activity of Amoxicillin-Clavulanate(Augmentin) against strains of MSSA and MRSA. Findings in this present study provide substantial evidence that supports the traditional use of this plant in the treatment and management of microbial infections.

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Authors' Contribution: This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Conflict of Interest: There is no conflict of interest.

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