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Antibacterial activity of *Sarcocephalus latifolius* and *Acacia sieberiana*, and the effect of their association with antibiotics against multidrug-resistant *Staphylococcus aureus*

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Abstract

Background: Infectious diseases continue to wreak havoc around the world causing directly or indirectly 17 million deaths per year. The present study was carried out to evaluate the in vitro antibacterial activity of the methanol extracts from the root of *Sarcocephalus latifolius* and from the leaves of *Acacia siberiana*, and their ability to potentiate the action of antibiotics against multidrug-resistant (MDR) *Staphylococcus aureus*.

Methods: The antibacterial assays were performed using the broth microdilution method, and the extracts were screened for phytochemicals using standard qualitative methods. The effect of *Sarcocephalus latifolius* root extract on the functioning of H^{+}/ATPases proton pumps of ATCC25923 *Staphylococcus aureus* was determined using a standard qualitative method.

Results: The extracts of *Sarcocephalus latifolius* and *Acacia siberiana* had minimum inhibitory concentrations (MIC) ranging from 16 to 1024 µg/mL and 32 to 1024 µg/mL, respectively, with action spectra of 93.75% and 87.5% against the 16 strains of *Staphylococcus aureus* tested. The *S. latifolius* root extract contained alkaloids, phenols, and terpenoids as the main classes of secondary metabolites. In contrast, the *A. siberiana* leaf extract contained phenols and terpenoids as the major classes of secondary metabolites. The *S. latifolius* extract enhanced the activity of penicillin against 100% of the isolates tested. On the other hand, the *A. siberiana* extract enhanced the activity of cefixime against 85.71% of the isolates tested with a 128-fold increase in activity.

Conclusion: The results obtained in this study provide significant data that could potentially support the use of *Sarcocephalus latifolius* and *Acacia siberiana* in combating bacterial infections caused by multidrug-resistant Gram-positive *Staphylococcus aureus*.

Keywords: *Acacia siberiana*; Antibacterial; modes of action; multidrug resistance; *Sarcocephalus latifolius*.

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Background

Infectious diseases are illnesses caused by microorganisms such as bacteria, viruses, fungi, and parasites [1]. They are a major cause of death worldwide, particularly in low-income countries and among children. Infectious diseases create a significant global burden of disease, impacting public health systems and economies worldwide, and disproportionately affecting vulnerable populations [2]. They were ranked among the top 10 causes of death in the world in 2020. Approximately half of the mortality rate is in developing countries, especially in South Asia and sub-Saharan Africa [1]. They are responsible for 17 million deaths per year worldwide, roughly 30% of the global mortality rate. Among infectious diseases, those caused by bacteria are primarily responsible for global morbidity and mortality, with 2.7 million neonatal deaths and 560,000 deaths each year attributed to bacterial infections. In 2019, 4.95 million deaths were associated with bacterial anti-microbial resistance (AMR), with 1.27 million deaths attributable to bacterial AMR [3].

The inappropriate use of antibacterial drugs in recent years has led to the emergence of resistance to these antibiotics by various mechanisms, including inactivation of enzymes, change of target site, reduction of membrane permeability, formation of biofilms, and overexpression of efflux pumps [4]. This resistance has resulted in more bacteria expressing multidrug-resistant phenotype [5]. Bacterial multi-resistance is becoming more linked to Gram-positive bacteria, particularly *S. aureus* (WHO, 2024 BPPL). Methicillin-resistant *Staphylococcus aureus* caused more than 100,000 deaths in 2019 [3]. *Staphylococcus aureus* is resistant to many families of antibiotics such as β-lactams (imipenem), fluoroquinolone (ciprofloxacin), and tetracycline [6-12] leading to therapeutic failures. The increase in this multidrug resistance has propelled scientists to intensify the search for new antibacterial substances as an alternative to these antibiotics that have become ineffective. Some African natural medicinal plants showed their effectiveness on the *S. aureus* species [13-15]. Medicinal plants are widely used in African communities to treat bacterial infections [16, 17], and a huge portion of these pharmaceutical products are plant-derived [18-20]. These medicinal plant extracts made up of several secondary metabolites, have shown activity on both Gram-negative and Gram-positive bacteria [21, 22] and may serve as potential natural antibacterials for the treatment of bacterial infections which can be cheaper, safer, and more effective. Recent studies have shown the effectiveness of some medicinal plants acting as anti-staphylococcal agents and potentiating the effects of usual antibiotics [23-25].

In our continuous search for new drugs to combat resistant staphylococcal infections, this study aims to evaluate the anti-staphylococcal activity of methanol extracts of *Sarcocephalus latifolius* JE Sm.) EA Bruce (Rubiaceae) and *Acacia siberiana* var. vermoesenii (De Wild.) Keay & Brenan (Fabaceae). The effect of the association between the plant extracts and antibiotics against *S. aureus* was further determined, as well as the effect of the most active plant extract on the most sensitive strain at the level of the H+/ATPase-dependent proton pumps. *Sarcocephalus latifolius* is a plant found in tropical Africa and Asia and is seen growing as a shrub with green leaves and multiple stems ([26]. It is traditionally used to treat pathologies such as yellow fever, gonorrhea, diarrhea, measles, malaria, HIV/AIDS, typhoid fever, helminthiasis, and leprosy [27, 28] and is also used in the treatment of non-infectious ailments such as digestive disorders, disorders of cardiovascular and metabolic functioning, reproductive disorders, skin disorders, pain, eye conditions, and respiratory disorders [29-31]. The antibacterial activity of *S. latifolius* using the root ethanolic extract and the root methanol extract was seen to be effective on *S. aureus* using the macrodilution methods [32]; also using the method of agar plate diffusion method, the leaf was shown to have antimicrobial effects [33]. *Acacia siberiana*, is commonly known as paperbark acacia, and is mostly found growing in the Savanna and the Sahel in Africa. It has been seen to contain a wide range of secondary metabolites responsible for its antibacterial effects and these secondary metabolites include saponins, tannins, phenols, flavonoids, cardiac glycosides, and anthraquinones [34]. This plant has also been shown to have anti-proliferative properties on cancer cells [35]. The antibacterial effect of this plant was shown with the agar well diffusion method using its stem bark and leaves on the *S. aureus* ATCC25923 [36].

Methods

Plant material and extraction

On October 31, 2023, in the town of Laï in the Tandjilé Region of Chad, *Sarcocephalus latifolius* was harvested, and in February 2017, in the town of Kaele in the Far North region of Cameroon, *Acacia sieberiana* was harvested. They were identified in Yaoundé at the National Herbarium of Cameroon by the botanists Mr. Eric Ngansop Tchatchouang and Victor Mr. Nana, respectively, with the reference numbers 67005/HNC and 49882/HNC. The roots of *S. latifolius* and the leaves of *A. sieberiana* were harvested, dried away from direct sunlight, and then crushed. The powder obtained from the roots of *S. latifolius* and the leaves of *A. sieberiana* was soaked in methanol (in a 1:3 weight/volume ratio) at room temperature for 48 h. The mixture was stirred four times a day to maximize the yield. After the soaking period, the mixture was filtered using Wattman No. 1. The filtrate obtained was evaporated using a rotary evaporator at 65°C. The resulting crude extract was collected in a sterile bottle, dried in an oven at 40°C to remove any remaining solvent, and then stored at 4°C for future use.

Chemicals and culture media

Dimethyl sulfoxide (DMSO) served to solubilize plant extracts. Eight antibiotics, namely doxycycline (DOX), levofloxacin (LEV), penicillin (PEN), cefixime (CFX), tetracycline (TET), ciprofloxacin (CIP), imipenem (IMI), and ceftriaxone (CTX) were used. Mueller Hinton Agar (MHA) was used for the activation of bacteria; Mueller Hinton Broth (MHB) was used for microdilution as a nutrient medium for bacteria. All chemicals were purchased from Sigma-Aldrich (St. Quentin Fallavier, France).

Tested bacteria

The *S. aureus* tested included both reference strains (ATCC 25923) and the resistant clinical isolates MSSA A1, MRSA A4, MRSA A6, MRSA A9, MRSA A11, and MRSA A12 [37], DO 21SA, DO 31SA, DO 49SA, DO 57SA, DO 58SA, DO 74SA, DO 94SA, DO 96SA, and DO 09SA [12]. Their bacterial features were previously reported [12, 37].

Determination of minimal inhibitory and bactericidal concentrations

The bacterial inoculum was prepared as previously described [25, 38-43] in comparison to the turbidity of a standard McFarland 0.5 ($1.5x10^8$ CFU/mL). The various plant extracts and the reference drug (imipenem) were dissolved in DMSO-MHB. Plant extracts were prepared at 8192 µg/mL, and antibiotics at 1024 µg/mL. PAβN was prepared at the concentration of 100 µg/mL. The minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) of test extracts were determined using a 96-well broth microdilution method combined with the rapid INT colorimetric method [15, 44, 45]. Imipenem was used as a positive antibacterial control, whereas DMSO 2.5%+MHB and MHB alone were used as negative controls. MIC was considered the lowest concentration of plant extract which produced complete inhibition of bacterial growth after 18 to 24 hours of incubation at 37°C, whereas MBC was considered the lowest concentration of a sample that did not induce a color change with the addition of INT following additional 48 hours of incubation [46-48]. Each experiment was repeated three times in triplicate.

Evaluation of the effect of S. latifolius root extract on the functioning of H+/ATPases proton dependent pumps of S. aureus ATCC 25923

The effects of *S. latifolius* root were assessed on the H^+-ATPase-mediated proton pumping of *S. aureus ATCC* 25923 at 0.5×MIC, MIC, and 2×MIC as earlier described [43]. The action on H^+-ATPase-mediated proton pumping was done by controlling the acidification of the bacterial growth medium over 60 min following the procedures previously described [49, 50].

Determination of the antibiotic-potentiating effects of the botanicals

The effects of the association of the botanicals with antibiotics were determined against the MSSA A1, MRSA A4, MRSA A11, and MRSA A12, DO 57SA, DO 96SA, and DO 09SA. At first, extracts were used at the sub-inhibitory concentrations of MIC/2, MIC/4, MIC/8, and MIC/16 for a preliminary assay on DO 74SA, which then allowed the selection of appropriate sub-inhibitory concentrations of MIC/2 and MIC/4 for further combination testing (Data not shown). Antibiotic-resistance modulating factor (AMF) was calculated as the ratio of the MIC of the antibiotic alone versus MIC in combination with the plant extract. The potentiation effect was considered for AMF ≥ 2 [51].

Phytochemical screening of botanicals

Phytochemical screening was done following the standard methods described for alkaloids, anthocyanins, flavonoids (Shinoda test), phenols, saponins, and triterpenes (Liebermann-Burchard test) [16, 52].

Interpretation of antibacterial data

Several cutoff points are available for the interpretation of the antibacterial activity of plant products including extracts from edible plants [53, 54]. According to Kuete [54], the following threshold values are applied to botanicals: significant activity (MIC <100 µg/mL), moderate (100 <MIC ≤ 625 µg/mL), and low or negligible (MIC> 625 µg/mL). However, updated and rationally defined cutoff points of the antibacterial botanicals have been defined, considering the various bacterial species [55-58]. For Gram-positive bacteria: Outstanding activity: minimal inhibitory concentration (MIC) ≤ 8 µg/mL; Excellent activity: 8 < MIC ≤ 40 µg/mL; Very good activity: 40 < MIC ≤ 128 µg/mL; Good activity: 128 < MIC ≤ 320 µg/mL; Average activity: 320 < MIC ≤ 625 µg/mL; Weak activity: 625 < MIC ≤ 1024 µg/mL; Not active: MIC values> 1024 µg/mL [58]. Bactericidal activities are considered when the ratios MBC/MIC are below or equal to 2 [47, 48, 59, 60].

Results

Phytochemistry

Phytochemical screening of methanol extract from the roots of *S. latifolius* revealed the presence of Alkaloids, phenols, flavonoids, terpenoids, saponins and anthocyanins; That of the methanol extract from the leaves of *A. siberiana* highlighted the presence of phenols, flavonoids, and saponins (Table 1).

In vitro antibacterial activity of crude extracts of S. latifolius and A. sieberiana

The antibacterial activity of the crude extracts was assessed by determining the Minimum Inhibitory Concentrations (MICs) and Minimum Bactericidal Concentrations (MBCs) against 16 bacterial isolates and strains. The bactericidal and bacteriostatic effects were determined by calculating the MBC/MIC ratios. The specific results for MICs, MBCs, and MBC/MIC ratios can be found in Table 2. Table 2 demonstrates that the antibacterial activity varies based on the plant extract and the specific isolates and strains studied with MICs ranging from 16 to 2048 µg/mL. The root extract of *S. latifolius* was the most effective, with a MIC of 16 µg/mL against the *S. aureus* strain ATCC 25923, 32 µg/mL against the MRSA A9, and a MIC of 64 µg/mL against the MRSA A12. This extract showed activity ranging from 128 µg/mL to 1024 µg/mL for the remaining isolates, except for the *S. aureus* MRSA A6, which had a MIC value of 2048 µg/mL. This plant extract exhibited the highest activity spectrum against 93.75% of the isolates and strains tested. The extract from the leaves of *A. sieberiana* was most active against the *S. aureus* MSSA A1 with a MIC of 32 µg/mL and against the MRSA A4 with an MIC of 128 µg/mL. The leaf extract of *A. sieberiana* showed activities ranging from 256 µg/mL to 1024 µg/mL for the rest of the isolates. This extract showed no activity against two of the isolates, with MIC values of 2048 µg/mL. This extract demonstrated an activity spectrum against 87.5% of the bacteria tested. The extract from the roots of *S. latifolius* exhibited a bactericidal effect with a MBC/MIC ≤4 against 75% (12/16) of the tested bacteria, while the extract from the leaves of *A. sieberiana* showed a bactericidal effect with MBC/MIC ≤4 against 62.5% (10/16) (Table 2).

Effects of the combination of antibiotics and plant extracts

To determine the most suitable sub-inhibitory concentrations (MIC/2, MIC/4, MIC/8, and MIC/16) of plant extracts, a preliminary test against the bacterial isolate DO 74SA, which exhibited the highest resistance to the reference antibiotic was conducted. As a result, the methanol plant extract from the roots of *S. latifolius* at MIC/2 and MIC/4 potentiated 100% of the antibiotics against *S. aureus* DO 74SA. MIC/8 and MIC/16 potentiated 50% and 37.5% respectively of the antibiotics tested against the DO 74SA isolate. The extract from the leaves of *A. siberiana* potentiated 100% of the antibiotics at MIC/2 and 87.5% at MIC/4 against the *S. aureus* DO 74SA. At MIC/8 and MIC/16, the extract from the leaves of *A. siberiana* potentiated 12.5% of the antibiotics used against DO

74SA. The methanol extracts of *S. latifolius* and *A. siberiana* at sub-inhibitory concentrations of MIC/2 and MIC/4 showed a better potentiating effect of the antibiotics against DO 74SA and were therefore selected to continue testing the associations between botanicals (*S. latifolius* and *A. siberiana*) and the antibiotics. The results are shown in Tables 3 and 4. These tables demonstrate that the extracts improved the effectiveness of antibiotics on at least one tested bacterial isolate, with activity enhancement ranging from 2 to 128. The methanol extracts from the roots of *S. latifolius* enhanced the activity of penicillin against 100% and 85.71% of the tested isolates at MIC/2 and MIC/4, respectively (Table 3). The extracts also enhanced the activity of certain antibiotics (IMI, CTX, CFX, and TET) against 71.42% of the isolates at MIC/2, and MII, CTX, and TET against at least 57.14% of the isolates at MIC/4. Additionally, the extracts potentiated the activity of other antibiotics (LEV, CFX, and DOX) against 42.85% of the isolates at their MIC/2 concentrations and showed minimal potentiated effect on ciprofloxacin against 14.28% of the isolates (Table 3). It is also observed that methanol extracts from the leaves of *A. siberiana* enhanced the activity of cefixime at MIC/2 and MIC/4 concentrations against 85.71% of the tested isolates (Table 4). CIP at MIC/2 was potentiated against 14.28% of the isolates. Furthermore, increased activity was observed with PEN, CTX, and IMI against at least 71.42% of the isolates at their MIC/2 and MIC/4. Similarly, DOX and LEV showed increased activity at MIC/2 against 42.85% and 57.14% of the isolates, respectively, and against 28.57% of the isolates at MIC/4. Additionally, the activity of TET was enhanced at MIC/2 and MIC/4 against 71.42% and 42.85% of the isolates, respectively (Table 4).

Effects of S. latifolius root extract on the functioning of H+/ATPases proton pumps of S. aureus ATCC 25923

The study aimed to determine whether the root extract of *S. latifolius* could interfere with the functioning of H+-proton-dependent pumps and ATPases in *S. aureus* ATCC 25923. To test this, the pH of the medium containing S. aureus ATCC 25923 was measured at various time intervals (0 to 60 minutes) in the presence and absence of the extract. Figure 1 illustrates the pH curves over time (in minutes) in the presence of different concentrations of the extract (MIC/2, MIC, 2MIC) and a positive control (ATB), as well as a negative control (absence of extract). The pH curve of S. aureus ATCC 25923 in the presence of *S. latifolius* root extract at half of the MIC shows a significant decrease compared to the curve in the absence of the extract. The initial pH of 6.7 sharply decreases to 6.2 within the first minute, indicating a drop of 0.5 pH units. On the other hand, the pH curve in the presence of the root extract at MIC shows a less pronounced increase in pH, reaching a value of 6.7. Meanwhile, for the pH curve in the presence of the root extract of *S. latifolius* at 2MIC, there is a very slight increase in pH during the first 20 minutes, followed by constancy throughout the experiment, reaching a final pH of 6.8. This is an indication that the root extract of *S. latifolius* slightly inhibit the functioning of H+/ATPases proton pumps of *S. aureus* ATCC 25923.

Discussion

The fight against bacterial infections continues to be a major global challenge due to the rise of resistance and multidrug resistance in bacteria. *Staphylococcus*, the most pathogenic genus of Gram-positive bacteria, and its most virulent species, *S. aureus*, are responsible for a wide range of hospital and community infections [1]. This bacterium is the cause of conditions such as sepsis, pneumonia, and toxinosis. Over the years, numerous studies have shown that medicinal plants are rich sources of potential antimicrobial agents due to the many secondary metabolites they contain [61]. Therefore, this study aimed to assess the antibacterial properties of the methanol root extract of *S. latifolius* and the methanol leaf extract of *A. sieberiana*, as well as their ability to enhance the effectiveness of known antibiotics against strain and isolates of *S. aureus* tested. As per the established classification scale [58], the root extract of *S. latifolius* demonstrated the following antimicrobial activities: Excellent activity on the S. aureus ATCC 25923 strain with a MIC value of 16 µg/mL and on the MRSA A9 isolate with a MIC value of 32 µg/mL; Very good activity on the MRSA A12 and the DO 31SA isolates of S. aureus with a MIC value of 64 µg/mL, and on the MRSA A11 and DO 49SA isolates with a MIC value of 128 µg/mL; Good activity on the DO 21SA, DO 58SA, DO 94SA, and DO 96SA with a MIC value of 256 µg/mL; Average activity on the DO 57SA, MRSA A1, and MRSA A4 with a MIC value of 512 µg/mL; Weak activity on the DO 74SA and DO 09SA with a MIC value of 1024 µg/mL; Not active on MRSA A6 as it showed a MIC value >1024µg/mL. The results support the findings of Oluremi et al. [62], who demonstrated the antibacterial activity of the leaf and stem bark extracts of *S. latifolius*. The lowest MIC value of 0.782 mg/mL (782 µg/mL) was observed on the *S. Typhi* UCH02 using the leaf extract, indicating weak activity based on our scale. This variation may be due to differences in the testing methods. Furthermore, the leaf extract was tested on a Gram-negative bacterium, while the present extract was tested on a Gram-positive bacterium using the root extracts. Ekamgue et al. also reported the anti-staphylococcal activity of this plant extract with the lowest MIC of 64 µg/mL against S. aureus ST135 [15]. On the other hand, the leaf extract of *A. sieberiana* showed excellent activity on MRSA A1 with a MIC value of 32 µg/mL, very good activity on MRSA A4. This extract showed good activity on MRSA A6, MRSA A11, DO 31SA, DO 49SA, DO 58SA, and DO 96SA. This result went in the same line as the work shown by Kirabo et al. [36] which showed an effective antibacterial activity on *S. aureus ATCC* 25923. The lowest MIC value of 160 µg/mL was obtained on the Gram-negative bacteria *P.aeruginosa* on the ATCC27853 strain, while a MIC value of 630 µg/mL was seen against *S. aureus* ATCC25923. The difference in activity observed can be a result of the extraction solvent used; in this case, ethanol instead methanol like in the present study. The phytochemical analysis of the tested botanicals reveals the presence of plant metabolite with potential antibacterial effects [53].

The H+/ATPase proton pumps are transmembrane proteins involved with the regulation of bacterial cytoplasmic pH and also play a role in the supply of energy in the form of ATP to the bacteria [63]. These two elements are necessary for the growth of the bacteria [64] since they expel H+ ions from the cytoplasm while ATP enters. An increase in the environmental pH in the presence of an antibacterial substance can cause this substance to inhibit the H+/ATPase-dependent proton pumps. Inhibiting these pumps would lead to the death of the bacteria because the extracellular environment would lose these protons and become less acidic, and the quantity of energy produced would be

insufficient for the growth, metabolism, and multiplication of the bacteria [65]. S. aureus has an optimum growth pH of 6 to 7. According to the results obtained, *S. latifolius* only slightly inhibited the H+/ATPase-dependent proton pumps in *S. aureus* ATCC25923 when compared to the negative control, indicating that they are not the primary target of *S. latifolius* antibacterial action.

The botanicals from the roots of *S. latifolius* and the leaves of *A. sieberiana* were found to significantly increase the effectiveness of antibiotics by 2 to 128 times. This finding is consistent with a study by Ekamgue et al. [15], which demonstrated the ability of certain medicinal plants to enhance the action of established antibiotics against MDR *S. aureus*. Although the plant extracts used in our study differed from those in Ekamgue's work, both studies showed that medicinal plants have the potential to amplify the effects of antibiotics against MDR *S. aureus*. The resistant *S. aureus* strains and isolates, as well as the six antibiotics used, were the same in both studies. Consequently, our findings indicate that our plants contain compounds that can work in synergy with antibiotics and serve as a valuable approach in combating bacterial drug resistance. This study strengthens the hypothesis that the African flora has the potential to alleviate various human ailments, as previously documented [66-85].

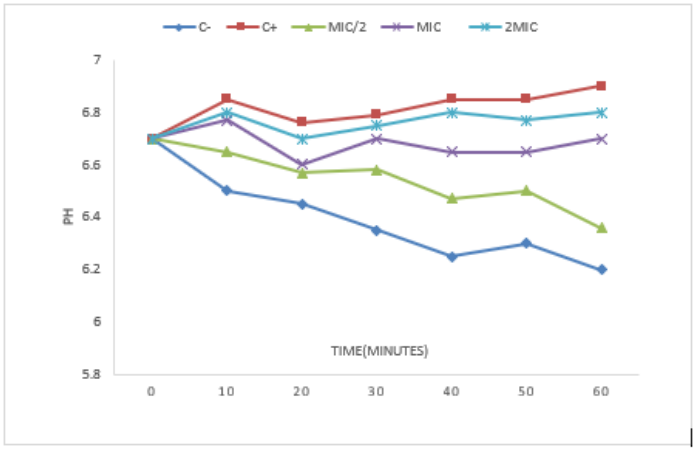

Figure 1. Effect of the root extract of *S. latifolius* on the H+ proton pumps/ATPases of *S. aureus* ATCC 2593

Table 1. Phytochemical comosition of methanol extracts from the roots of *Sarcocephalus latifolius* and the leaves of *Acacia siberiana*

Secondary metabolites	***Sarcocephalus latifolius* roots extract**	***Acacia siberiana* leaves extract**
Alkaloids	+	-
Phenols	+	+
Flavonoids	+	+
Terpenoids	+	-
Saponins	+	+
Anthocyanins	+	-

+: Present -: Absent

Table 2. MICs and MBCs in µg/mL of botanicals and imipenem against *S. aureus*

Strain and isolates	***Tested samples, MIC, MBC values, and MBC/MIC ratio***								
	***Sarcocephalus latifolius* roots extract**			***Acacia sieberiana* leaves extract**			***Imipenem***		
	MIC	**MBC**	**R**	**MIC**	**MBC**	**R**	**MIC**	**MBC**	**R**
ATCC 25923	16	512	32	1024	1024	1	1	32	64
S.aureus MSSA A1	512	>2048	Nd	32	1024	32	8	>2048	Nd
S.aureus MRSA A4	512	>2048	Nd	128	1024	8	128	512	4
S.aureus MRSA A6	2048	2048	1	256	>2048	Nd	16	512	32
S.aureus MRSA A9	32	32	1	512	2048	4	128	512	4
S.aureus MRSA A11	128	128	1	256	256	1	128	512	4
S.aureus MRSA A12	64	256	4	2048	2048	1	128	256	2
DO 21SA	256	256	1	1024	2048	2	64	256	4
DO 31SA	64	64	1	256	512	2	16	>2048	Nd
DO 49SA	128	128	1	256	256	1	64	>2048	Nd
DO 57SA	512	512	1	1024	>2048	Nd	2	128	64
DO 58SA	256	512	1	256	2048	8	32	32	1
DO 74SA	1024	1024	1	2048	2048	1	2	64	32
DO 94SA	256	>2048	Nd	512	512	1	16	>2048	Nd
DO 96SA	256	1024	4	256	2048	8	2	64	32
DO 09SA	1024	1024	1	1024	Nd	1	2	>2048	Nd

MIC: Minimum inhibitory concentration, MBC: Minimum bactericidal concentration, Nd: not determined, R: Ratio of MBC/MIC.

Table 3. Effects of the association test between antibiotics and the extract from the roots of *S. latifolius* against the tested bacteria.

ATB	Extracts (SL)	MIC of antibiotics in the presence of root-extracts of SL at sub-inhibitory concentrations and AEF									POF (%)
		MSSA		MRSA		*S. aureus*					
		A1	A4	A11	A12	DO 96SA	DO	09SA	DO	57SA	
CIP	0	4	1	1	1	<1/2	<1/2		<1/2		
	MIC/2	1**(4)**	1(1)	1(1)	1(1)	<1/2(1)	<1/2(1)		<1/2(1)		14.28%
	MIC/4	1**(4)**	1(1)	1(1)	1(1)	<1/2(1)	<1/2(1)		<1/2(1)		14.28%
LEV	0	2	4	1	1	<1/2	1		2		
	MIC/2	1**(2)**	1**(4)**	1(1)	1(1)	<1/2(1)	1(1)		<1/2**(4)**		42.85%
	MIC/4	1(**2**)	1(**4**)	1(1)	1(1)	<1/2(1)	2(1/2)		2(1)		28.57%
PEN	0	128	256	1024	64	128	128		1024		
	MIC/2	<8(**16**)	<8(**32**)	<8(**128**)	32(**2**)	16(**8**)	32(**4**)		<8(**128**)		100%
	MIC/4	<8(**16**)	<8(**32**)	16(**64**)	32(**2**)	16(**8**)	128(1)		<8(**128**)		85.71%
TET	0	2	64	64	1	<1/2	1		1		
	MIC/2	1(**2**)	1(**64**)	1(**64**)	1(1)	<1/2(1)	<1/2(**2**)		<1/2(**2**)		71.42%
	MIC/4	1(**2**)	1(**64**)	1(**64**)	1(1)	<1/2(1)	1(1)		<1/2(**2**)		57.14%
CTX	0	32	16	64	32	<8	<8		16		
	MIC/2	<8(4)	<8(2)	16(4)	16(2)	<8(1)	<8(1)		<8(2)		71.42%
	MIC/4	<8(4)	<8(2)	16(4)	32(1)	<8(1)	16(1/2)		<8(2)		57.14%
CFX	0	64	256	32	16	32	32		<8		
	MIC/2	<8(8)	<8(32)	32(1)	<8(2)	<8(4)	<8(4)		<8(1)		71.42%
	MIC/4	<8(8)	<8(32)	32(1)	16(1)	<8(4)	32(1)		32(1/4)		42.85%
DOX	0	2	1	1	1	<1/2	4		4		
	MIC/2	1(2)	1(1)	1(1)	1(1)	<1/2(1)	<1/2(8)		1(4)		42.85%
	MIC/4	1(2)	1(1)	1(1)	1(1)	<1/2(1)	<1/2(8)		2(2)		42.85%
IMI	0	8	128	>128	128	2	2		2		
	MIC/2	1(**8**)	1(**128**)	>128(1)	128(1)	1(**2**)	1(**2**)		1(**2**)		71.42%
	MIC/4	1(**8**)	1(**128**)	>128(1)	128(1)	1(**2**)	2(1)		1(**2**)		57.14%

SL*: Sarcocephalus latifolius*; MIC: Minimal inhibitory concentration; (): Activity enhancement factor; POF (%): Percentage of potentialization; ATB: Antibiotics; CIP: ciprofloxacin; LEV: levofloxacin; PEN: penicillin; TET: tetracycline; CTX: ceftriaxone; CFX: cefixime; DOX: doxycycline; IMI: imipenem

Table 4. Effects of the association test between antibiotics and the extract from the roots of Acacia siberiana (AS) against the tested bacteria.

ATB	Extracts (SL)	MIC of antibiotics in the presence of root-extracts of SL at sub-inhibitory concentrations and AEF									POF (%)
		MSSA		MRSA		*S. aureus*					
		A1	A4	A11	A12	DO 96SA	DO	09SA	DO	57SA	
CIP	0	4	1	1	1	<1/2	<1/2		<1/2		
	MIC/2	1(**4**)	1(1)	2(1/2)	1(1)	<1/2(1)	<1/2(1)		<1/2(1)		14.28%
	MIC/4	4(1)	1(1)	1(1)	1(1)	<1/2(1)	<1/2(1)		<1/2(1)		0%
LEV	0	2	4	1	1	<1/2	1		2		
	MIC/2	1(**2**)	1(**4**)	1(1)	1(1)	<1/2(1)	<1/2(**2**)		1(**2**)		57.14%
	MIC/4	2(1)	1(**4**)	1(1)	1(1)	<1/2(1)	<1/2(**2**)		2(1)		28.57%
PEN	0	128	256	1024	64	128	128		1024		
	MIC/2	32(**4**)	<8(**32**)	<8(**128**)	256(1/4)	<8(**16**)	128(1)		16(**64**)		71.42%
	MIC/4	128(1)	<8(**32**)	<8(**128**)	256(1/4)	16(**8**)	32(**4**)		16(**64**)		71.42%
TET	0	2	64	64	1	<1/2	1		1		
	MIC/2	1(**2**)	1(64)	1(64)	1(1)	<1/2(1)	<1/2(2)		<1/2(2)		71.42%
	MIC/4	1(**2**)	1(**64**)	2(**32**)	1(1)	<1/2(1)	1(1)		1(1)		42.85%
CTX	0	32	16	64	32	<8	<8		16		
	MIC/2	16(**2**)	<8(**2**)	<8(**8**)	<8(**4**)	<8(1)	<8(1)		<8(**2**)		71.42%
	MIC/4	16(**2**)	<8(**2**)	<8(**8**)	<8(**4**)	<8(1)	<8(1)		<8(**2**)		71.42%
CFX	0	64	256	32	16	32	32		<8		
	MIC/2	<8(**8**)	<8(**32**)	<8(**4**)	<8(**2**)	<8(**4**)	<8(**4**)		16(1/2)		85.71%
	MIC/4	16(**4**)	<8(**32**)	<8(**4**)	<8(**2**)	<8(**4**)	<8(**4**)		64(1/8)		85.71%
DOX	0	2	1	1	1	<1/2	4		4		
	MIC/2	1(**2**)	2(1/2)	1(1)	1(1)	<1/2(1)	<1/2(**8**)		<1/2(**8**)		42.85%
	MIC/4	1(**2**)	1(1)	1(1)	1(1)	<1/2(1)	8(1/2)		<1/2(**8**)		28.57%
IMI	0	8	128	>128	128	2	2		2		
	MIC/2	<1(**8**)	1(**128**)	>128(1)	1(**128**)	1(**2**)	2(1)		1(**2**)		71.42%
	MIC/4	2(**4**)	1(**128**)	>128(1)	128(1)	1(**2**)	1(**2**)		1(**2**)		71.42%

AS: *Acacia siberiana;* MIC: Minimal inhibitory concentration; (): Activity enhancement factor; POF (%): Percentage of potentialization; ATB: Antibiotics; CIP: ciprofloxacin; LEV: levofloxacin; PEN: penicillin; TET: tetracycline; CTX: ceftriaxone; CFX: cefixime; DOX: doxycycline; IMI: imipenem

Conclusion

The present study aimed to evaluate the effectiveness of 2 medicinal plant extracts in fighting *Staphylococcus aureus* infections, particularly those that are resistant to antibiotics. We also aimed to assess whether these plant extracts can enhance the activity of common antibiotics. The research focused on the roots of *S. latifolius* and the leaves of *A. sieberiana*. The obtained data suggest that these plant parts contain bioactive compounds that can combat multidrug-resistant *S. aureus* infections. Our next steps involve isolating the active substances in these plants and studying their effectiveness and safety in live organisms.

Abbreviations

ATCC: American-type culture collection
MBC: Minimum Bactericidal Concentration
MIC: Minimal inhibitory Concentration
DMSO: Dimethylsulfoxide
HNC: National Herbarium of Cameroon
INT: Iodonitrotetrazolium chloride
MDR: Multidrug resistant
MHA: Mueller Hinton agar
MHB: Mueller Hinton broth
WHO: World Health Organization
CFU: Colony Forming Unit

Authors' Contribution

LM, AWBY, DJA, EC, VYM, and JFM carried out the study; ATM and VK supervised the study; All authors read and approved the final version of the manuscript.

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Conflict of interest

The authors declare no conflict of interest.

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