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Antibacterial Activity and Synergism of Sapium ellipticum (Hochst.) Pax and Harungana madagascariensis (Lam. Ex Poir) Stem bark Extract against Methicillin Resistant Staphylococcus aureus

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Abstract

Background: The increase in infections involving anti-microbial resistant (AMR) bacteria like Methicillin-resistant Staphylococcus aureus (MRSA) has limited therapeutic options and has been consistently found to be among the top causes of threats to global health. Combining antibacterial agents and/or medicinal plants has minimized this AMR crisis worldwide.

Aim: This study aimed to investigate *in vitro* synergistic antibacterial activity of *Sapium ellipticum* (Hochst.) Pax and *Harungana madagascariensis* (Lam. Ex Poir) stem bark extracts against Methicillin resistant *Staphylococcus aureus* (MRSA).

Methods: The MRSA clinical isolates were identified phenotypically and genotypically before being used as test microorganisms. Stem bark plant extracts of S. ellipticum and H. madagascariensis were prepared using methanol and dichloromethane solvents. Screening of antibacterial activities of plant extracts was done using the agar-well diffusion method, and minimum inhibitory concentration (MIC) determination was done by serial microdilution technique.

Results: The MIC for individual extracts ranged from 1.56 - 6.25 mg/mL, while MIC for combined extracts ranged from 0.2 - 0.8 mg/mL. Combined extracts were significantly more active than individual extracts. The study discovered synergistic interaction when combining methanol or dichloromethane extracts of *S. ellipticum* and *H. madagascariensis* against MRSA isolates. The suitable combination ratio for methanol and dichloromethane extracts was 1:1, though a 3:1 ratio also resulted in synergistic interaction in the methanolic combination.

Conclusion: The differences in MIC range between the individual and combined extracts might be attributed to the concentration and composition of the extracts. These results provide promising information for using methanol or dichloromethane crude extracts of *S. ellipticum* and *H. madagarscariensis* stem barks in synergism against MRSA isolates.

Keywords: Synergistm, Antibacterial activity, Plant extracts, and Methicillin-resistant *Staphylococcus aureus*

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Introduction

Medicinal plants possess drug-comparable properties and thus have been used throughout the history of mankind as substantial sources for modern drugs (Kumar Shakya and Arvind Kumar Shakya 2016). Early drugs discovered from plants include aspirin isolated from willow bark and morphine isolated from opium poppy (Anand et al. 2019). Currently, drugs produced from medicinal plants include artemisinin, used as an antimalarial, bicyclol in the treatment of hepatitis and albicidin as an antibiotic (Anand et al. 2019).

Sapium ellipticum is a jumping seed tree (Macdonald et al. 2016). Scientific studies that have been carried out on *S. ellipticum* extracts showed significant anti-plasmodial, anti-oxidant, antifungal, antibacterial and anti-human immunodeficiency virus activities (Masalu et al 2020; Masalu and Mpinda 2021). *Harungana madagascariensis* is a bushy tree, and its fruits, stem bark, roots, and leaves have been reported in the literature as having antibacterial, antifungal, antiprotozoal and antioxidant activities (Oe and Me 2020). Literature reported these plants' phytochemical composition of different morphological parts (Mouthé et al. 2019, Oe and Me 2020 and Abogo Mebale et al. 2022). Other reports show these plants' use and status in managing opportunistic infections (Kisangau et al. 2007, Kisangau et al. 2009, Amenu 2014 and Mpinda et al. 2018).

Rising of infections involving anti-microbial resistant (AMR) bacteria like methicillin-resistant Staphylococcus aureus (MRSA) have limited therapeutic options and have been consistently found to be among the upper source of threat to human health globally, with WHO estimating about 70% of AMR-related deaths globally (Kehinde Peter et al. 2020). MRSA alone caused more than 100,000 deaths globally, according to a recent systematic analysis in the Lancet in 2019 (Masimen et al. 2022).

Normally, *Staphylococcus aureus* exhibits resistance to methicillin by expression of the mecA gene, which marks in production of modified penicillin-binding proteins (PBP2a) (Reichmann and Pinho 2017). Another gene known for its resistance to drugs is FEMA (Lozano et al. 2016). The resistance of bacteria to antibiotics may arise through different mechanisms, such as inhibiting membrane absorptivity to antibiotics and producing enzymes that neutralize antibiotics (Cheesman et al. 2017). For enzyme mutation example, the β-lactamase enzyme cleaves the β-lactam ring and inhibits it from attaching to the PBPs, such as peptidoglycan transpeptidase (Reichmann and Pinho 2017). Also, increasing the efflux of antimicrobial compounds and altering drug accessibility could offer the development of resistance to antimicrobials (Vestergaard et al. 2019). No report presented resistance of methicillin-resistant *Staphylococcus aureus* to medicinal plants, though it had been reported to be resistant to all beta-lactam and multiple antibiotics (Sharaf et al. 2021). Hence, developing new antibacterial agents that block resistance mechanisms is an urgent issue (Sharaf et al. 2021).

A combination of antibacterial agents and /or medicinal plants has become an effective option to minimize this crisis of AMR worldwide (Thereza et al. 2015; Donkor et al. 2023). Examples of fixed-dose combined (FDC) antibiotics currently known are trimethoprim-sulfamethoxazole, amoxicillin-clavulanate and ampicillin cloxacillin for bacterial infections treatment in several human body parts (Pallett et al., 2023). Varieties of strategies are offered by antibacterial combination, and they are interested in treatment, for example, stopping the development of antibiotic resistance, refining the efficacy, attaining high specificity for target pathogens or the rise of target spectrum and reviving target antibiotics (Gideon and Ladan 2023). Some studies reported that combining plant extracts produces a greater effect than their individual extracts (Feng et al. 2023; Duremdes et al. 2023; Masalu and Mpinda 2021).

Synergism is the most desired combination interaction in treatment, and it can result in a significant extra beneficial effect by evading resistance mechanisms and reducing toxicity to the host. However, the effect can sometimes be antagonism, either by reducing the activity or causing toxicity (Victor Nyangabo Mbunde et al. 2019).
The present study aims to investigate the antibacterial activity and synergism of crude extracts from *Sapium ellipticum* (Hochst.) Pax and *Harungana madagarscariensis* (Lam. ex Poir) stem bark extracts against Methicillin resistant *Staphylococcus aureus* (MRSA).

Materials
Plant Material
Plant materials of *Sapium ellipticum* and *Harungana madagascariensis* collected October 2018 from Bukoba rural and Misenyi districts in Kagera region, Tanzania were used. The plant materials were identified, and voucher specimens were coded and kept in the herbarium of the Department of Botany at UDSM as *Sapium ellipticum* stem bark (SN03) and *Harungana madagascariensis* stem bark (SN05) (Masalu et al 2020). These plants were selected based on their wide use by traditional medicine healers in Bukoba, Tanzania and their potential antimicrobial activities as well as its widely use by indigenous communities reported in different studies done in Africa especially Tanzania, Zambia, Burundi, Ethiopia, Cameroon, Nigeria and Kenya (Kisangau et al. 2007, Kisangau et al. 2009, Amenu 2014, Macdonald et al. 2016 , Mpinda et al. 2018 and Oe and Me 2020).

Test Bacteria.
The Methicillin-resistant *Staphylococcus aureus* (MRSA) clinical isolates used in this study were obtained from the Department of Microbiology and Immunology, Muhimbili University of Health and Allied Sciences (MUHAS). These bacteria had been isolated from wounds of the patient from Muhimbili hospital and then identified as Methicillin-resistant *Staphylococcus aureus* (MRSA) by different phenotypic approaches and preserved well as a reference microorganism.

Methodology
Preparation of Plant Extracts
Plant stem barks were ground into fine powder by using a mixer grinder. Solvents used were methanol (Fluka Chemie GmbH (Sigma-Aldrich®, Zwijndrecht, Netherlands) and dichloromethane (Merk KgaA, Darmstadt,German). During extraction of methanolic crude extract, stem bark powder (300g) was soaked into 1000 mL methanol solvents and maintained at room temperature for 72 hours with twice daily homogenization. The solution was then filtered using Whatman no.1 filter paper (pore size: 11 µm). During extraction of dichloromethane crude extract, stem bark powder (300g) was soaked in 1000 mL dichloromethane for 72 hours with twice daily homogenization. The solution was then filtered using Whatman no.1 filter paper (pore size: 11 µm). Thereafter, the extracts were concentrated using a rotary evaporator (R-210 BUCHI, Switzerland) at 55 °C, 100 m bar pressure. Then, all crude extracts yielded were weighed and stored in a refrigerator (4 °C) pending the day of use.

Identification of MRSA Clinical Isolates
Phenotypic Identification
Gram Staining

Each slide smeared with pure colonies was applied with crystal violate, then iodine, decolourized by ethanol, and counterstained with safranin. The isolates were identified based on their morphological shapes and colours, as seen in the microscope.

Biochemical Tests

A catalase and coagulase tests were done to the pure culture of the suspected MRSA isolate as follows:

Catalase Test

A small amount of bacteria isolate was smeared on a clean glass slide using a wire loop and then dried. A drop of hydrogen peroxide was placed on top of the isolate.

Coagulase Test

A small amount of bacteria isolate was smeared on two clean glass slides with a drop of normal saline using a wire loop, and then the slides were dried. A drop of human plasma was added to one of the slides, smeared with bacterial suspension, and mixed gently.

Antibiotic Resistance

Antibiotic resistance was determined by disc diffusion method and standards according to Clinical Laboratory Standard Institute (CLSI-2015) guidelines. A pure culture of MRSA confirmed by Gram's staining and biochemical tests identification procedures was grown in a nutrient agar plate 24 hours before being used as the test microorganism in the antibiotic resistance test. The antibiotic resistance test used standard oxacillin discs (1 µg/mL) and methicillin discs (1 µg/mL) as the reference. The positive control was vancomycin disc (2 µg/mL).

The pure colonies of MRSA detected by Gram's staining and biochemical test identification procedures were grown in a nutrient agar plate 24 hours before being used as the test microorganism. The test organism's suspension approximated to 0.5 McFarland standards, was prepared in a test tube using sterile normal saline water. 20 mL of fresh sterile nutrient agar was poured into a sterilized plate and allowed to solidify at room temperature. The test organism was spread aseptically on solidified nutrient agar by a decontaminated cotton swab. Oxacillin (1 µg) standard discs were aseptically placed in a solidified nutrient agar plate, and 6 mm vancomycin discs (2 µg) were placed as the positive control. Another plate with standard discs of methicillin (1 µg) was used as a reference. Then, plates were incubated for 24 hours at 37°C. Inhibition zones were measured in millimeter and interpreted according to (CLSI) guidelines.

Genotypic Identification

Extraction of DNA

DNA was extracted by Zymo-Research kit protocol procedures with minor modifications. The MRSA culture was transferred into a Spin-Away™ Filter1 in a collection tube and then centrifugated at 10,000x g for 15 seconds. After centrifugation, Spin-Away Filter1 was placed in a new collection tube, 400 µL of DNA Prep buffer was added to the column, allowing for centrifugation, and then the flow was discarded. 700 µL of DNA wash buffer was added to the column, allowing centrifugation, and then the flow was discarded. A 400 µL of DNA wash buffer was added to the column and then centrifuged for 2 minutes to ensure the wash buffer was obliterated, then the column was transferred into a nuclease-free tube. During DNA elution, 100 µL of DNase-free water was added to the column matrix; then, it was allowed to remain standing for 5 minutes, followed by centrifugation. Lastly, the DNA sample was stored in the refrigerator (-20 °C) before being used in PCR reactions.

Polymerase Chain Reaction (PCR)

The reactions were attained in a final volume of 25 µL containing 4 µL of DNA sample (template), 12.5 µL of Taq 2X master mix (New England Biolabs), 1 µL of specific primer set and 7.5 µL of nuclease-free water (Water for Molecular biology, Bio Concept). PCR amplification cycle for 16S rRNA primer started with an initial denaturation step at 95 °C after 24 minutes, then 35 cycles of denaturation at 95 °C for the 30 s, annealing at 55 °C for 35 s, extension for 50 s at 72 °C and a last extension at 72 °C for 7 minutes.

The amplification cycle for detection of mecA was performed beginning with a denaturation step at 95 °C for 4 minutes, then 40 cycles of denaturation at 95 °C for 30 s, annealing at 56 °C for 1 minute, and a final extension step at 72 °C for 7 minutes. Forward and reverse primers for the mecA used were.

MRSA1 - 5' AAAATCGATGGTAAAGGTTGGC 3'
MRSA2 - 3' AGTTCTGCAGTACCGGATTTGC 5'

Amplified products were verified by electrophoresis in agarose gels (1%) stained with Safe view™ and classic dye visualized in the gel. LUMINAX Gel documentation System—BioZEN Labs (Nagar, India). The Quick-load 1500 bp and 533 bp amplicons were used as the molecular weight markers for 16S rRNA and mecA primers, respectively.

Antibacterial Activity of Individual Plant Extracts

Screening Antibacterial Activity of Individual Plant Extracts

The agar well diffusion antibacterial assay of methanol and dichloromethane of *Sapium ellipticum* and *Harungana madagascariensis* crude extracts was determined according to the Clinical Laboratory Standard Institute (CLSI-2015). Inoculum containing 1 x 10^6 CFU/mL of MRSA bacterial culture was spread on Muller Hinton agar plates with a sterile cotton swab moistened with the bacterial suspension. Subsequently, wells of 6 mm diameter were punched into agar medium, filled with 20 µL of each desired concentration of each plant extract, and allowed to diffuse at room temperature for 2 hours. Positive control wells filled with 20 µL vancomycin (2 mg/mL) and negative control wells filled with 10% DMSO (Dimethyl sulfoxide) were used. The plates were incubated at 37 °C for 24 hours. Different concentrations of plant extracts (200, 100, 25 and 1.56 mg/mL) were tested during antibacterial screening to simplify the approximation of concentrations that would be used for MIC determination.

Diameters of the zone of growth inhibition were measured using a transparent ruler calibrated in millimetres. Results were presented as means and standard deviations mean ± SD from the three independent experiments. Then, the activity Index (AI) was calculated by using this formula.

AI = $ZOI\ of\ sample/ZOI\ of\ positive\ control$

Minimum Inhibitory Concentrations of the Individual Plant Extracts

The minimum inhibitory concentration (MIC) of plant crude extracts that exhibited antibacterial activities was determined by using the broth microdilution method in the 96-well microtiter plate according to (Mpinda et al. 2018) with minor modification. The MRSA inocula used were prepared from 24 hours-grown cultures. A 50 µL of sterile Muller Hinton broth was put in each well, and 50 µL of 50 mg/mL of crude extract was diluted by serial dilution to obtain the following concentrations along the column: 25, 12.5, 6.25, 3.12, 1.56, 0.8, 0.4 and 0.2 mg/mL as explained below. To each well of the first row, 50 µL of the crude extracts were added to the 50 µL broth. After thorough mixing, 50 µL of the mixture was drawn and transferred to the second well. This procedure was repeated until the last well in each column.

Then, 50 µL of the mix was discarded from each previous well of the column. Then 50 µL of inoculated bacteria approximated to 0.5 McFarland test organisms were added to each well to make 100 µL per well. Microtiter plates with the same scheme but with no test organisms inoculated in Muller Hinton broth were included as a reference. Then, microtiter plates were incubated at 37 °C for 24 hours. The positive control column contained vancomycin (6 µg/mL), broth and inoculated bacteria, the negative control column contained broth and inoculated bacteria and the blank (sterility control) column contained broth only. The MIC endpoints were determined using a SPECTRO star Nano® plate reader (BMG LABTECH) at 560 nm. The overall results were taken from three independent experiments and were interpreted by the relationship between the absorbance and concentration.

Investigation of Synergism

Screening for Antibacterial Activity of Combined Plant Extracts

The agar well diffusion assay screened the antibacterial activity of combined extracts of *Sapium ellipticum* and *Harungana madagascariensis*. Inoculum containing 1 x 10^6 CFU/mL of MRSA bacterial culture was spread on Muller Hinton agar plates with a sterile cotton swab moistened with the bacterial suspension. Subsequently, wells of 6 mm diameter were punched into agar medium and for the case of combining the extracts, the total 20 µL of extracts were filled in each well by 1:1 v/v (*Sapium ellipticum*: *Harungana madagascariensis)* combination ratio for each desired concentration. They were allowed to diffuse at room temperature for 2 hours. Positive control wells filled with 20 µL vancomycin (2 mg/mL) and negative control wells filled with 10% DMSO (Dimethyl sulfoxide) were used. The plates were incubated at 37 °C for 24 hours.

Minimum Inhibitory Concentration of Combined Plant Extracts

The minimum inhibitory concentration assay for combined plant extracts was accomplished using 96 wells of a microtiter plate by broth microdilution method as previously described. Extracts combination was performed so that methanol crude extracts were mixed in pairs among themselves. The same was done among dichloromethane crude extracts. A sterile 50 µL of Muller Hinton broth was put in each well. To each well of the first row, 50 µL of crude extract was added to 50 µL of broth. 50 µL of crude extract was put in combination, and the suggested (v/v) ratios were 3:1, 1:1 and 1:3 (*S. ellipticum*: *H. madagarscariensis*) for methanol and dichloromethane extract combinations. After thorough mixing, 50 µL of the mixture was moved to the next well.

In each column, the procedure was constant to the last well. 50 µL of the mixture was drawn and discarded to the previous well. The extracts were serially diluted to obtain 25, 12.5, 6.25, 3.12, 1.56, 0.8, 0.4 and 0.2 mg/mL concentrations. Serial dilution of crude extract concentrations was done along the columns in triplicate for the combined crude extracts. Then 50 µL of inoculated MRSA approximated to 0.5 McFarland test organisms were added to each well to make a total of 100 µL per well. Microtiter plates used as a reference were prepared with the same filling profile but with no inoculation of test MRSA. All plates were put in an incubator at 37 °C for 24 hours. The positive control column contained vancomycin (6 µg/mL), broth and inoculated bacteria, the negative control column contained broth and inoculated bacteria and the blank (sterility control) column contained broth only. The MIC endpoints were determined using the SPECTRO star Nano® plate reader (BMG LABTECH) at 560 nm. The overall results were taken from three independent experiments and were interpreted by the relationship between the absorbance and concentration. The suggested combination ratios have also been used in the study reported by Johnson and Ayoola (2015).

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Fractional Inhibitory Concentration (FIC)

The synergistic antibacterial activity of plant extracts was determined by the checkboard method. The equations below calculated the Fractional activity index from the minimum inhibitory concentration (MIC) of the individual and combined crude extracts of the two selected plants.

$FIC_1 = (MIC_{1+2} / MIC_1)$

$FIC_2 = (MIC_{1+2} / MIC_2)$

$FIC = FIC_1 + FIC_2$

MIC_1= minimum inhibitory concentration of *H. madagascariensis* alone, MIC_2= minimum inhibitory concentration of *S. ellipticum* alone, MIC_{1+2}= minimum inhibitory concentration of combined crude extract, FIC_1= fraction inhibitory concentration of *H. madagascariensis* alone, FIC_2= fraction inhibitory concentration of *S. ellipticum* alone and FIC = fraction inhibitory concentration of the combined extract. The criteria used in the interpretation of the FIC Index concerning the mode of plant extract interactions were.

≤ 0.5 Synergism, > 0.5 to 1 Additive, > 1 to ≤ 4 Indifference and > 4 Antagonism.

Statistical Analysis

Antimicrobial susceptibility of plant crude extracts data obtained from agar well diffusion assay was represented by mean ± standard deviation. One-way ANOVA analyzed the comparisons in activeness of extracts; $p < 0.05$ were considered statistically significant. The MIC values in the tables are interpreted according to standard breakpoints described by Clinical and Laboratory Standard Institute (CLSI) criteria for antibacterial agents. FIC values were analyzed by checkboard data analysis depending on the model of deviations from theories of synergism, additive, no interaction and antagonist interactions.

Results

Identification of MRSA Clinical Isolates

Purple-coloured and cocci-shaped bacterial cells were observed on the slides spread with pure bacteria colonies. The bacteria isolates were catalase-positive and coagulase-positive. The results of antibiotic resistance showed that the tested bacteria were resistant to oxacillin and methicillin while were intermediate to vancomycin as shown in (Table 1).

Table 1: Antibiotic Resistance Inhibition Zone Diameters (ZOI) and Interpretation

Antibiotics	ZOI (mm)	Interpretation
Oxacillin (1µg/mL)	6.5 ± 0.1	Resistant
Methicillin (1µg/mL)	9.0 ± 0.5	Resistant
Vancomycin (2µg/mL)	20.0 ± 0.0	Intermediate

In this study the DNA sample of MRSA isolate were positively detected as bacteria by 16S rRNA PCR amplification as shown in (figure 1). The mecA resistant genes detection was used to confirm if the bacterial sample was methicillin resistant. The DNA sample of the MRSA clinical isolate tested was found to be mecA positive as shown in (figure 2).

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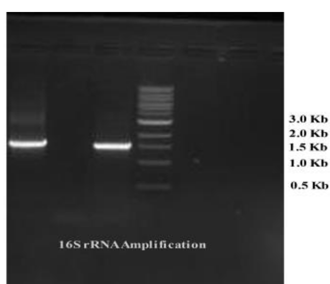


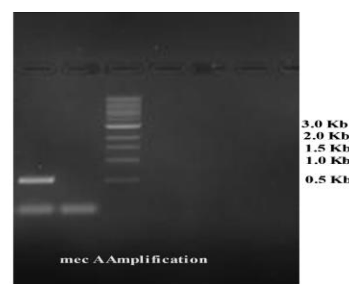
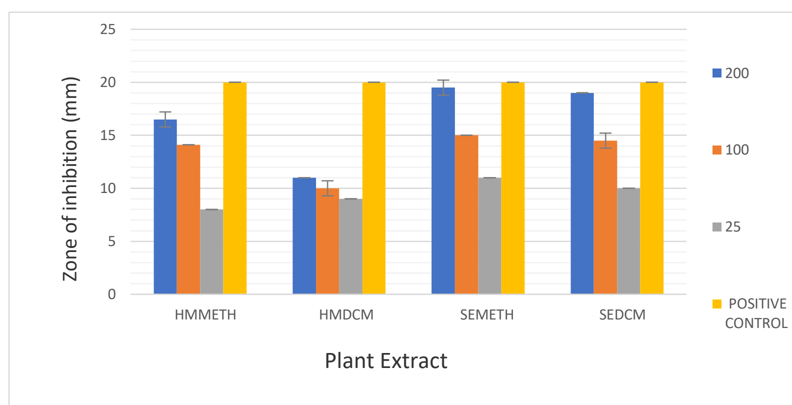


Figure 1: **Figure 2:**
Agarose Gel for 16S rRNA Gene Amplicon (Figure 1) and for mecA Resistant Gene (Figure 2) Observed for MRSA Isolate in which there was positive genotypic/phenotypic concordance. M: Molecular Marker (1kb+DNA ladder). Lane 1: PCR amplicon of 16S rRNA gene (1500 bp) in (Figure 1) and of mecA (533 bp) in (Figure 2). NC: Negative control and PC: Positive control.



Antibacterial Activity of Individual Plant Extracts

All extracts at different concentrations exhibited antibacterial effects against MRSA isolate. Generally, the values of the zone of inhibition decreased with the decrease in concentrations of the extracts. ZOI was measured and represented in mean ± SD (mm) for triplicate data and the results were analyzed by using a graph chart presented in (Figure 3).

The concentrations of plant extracts tested were 200, 100, 25 and 1.56 mg/mL. For the individual extracts, there was no clear ZOI when concentration was 1.56 mg/mL. The values of ZOI for individual extracts ranged from (8 mm - 19.5 mm) for the concentrations of (200, 100 and 25 mg/mL) as shown clearly in the following clarification: At 200 mg/mL ZOI were; SEMETH= 19.5mm, SEDCM= 19mm, HMMETH= 16.5mm and HMDCM= 11mm. At 100 mg/mL ZOI were; SEMETH= 15mm, SEDCM= 14.5mm, HMMETH= 14mm and HMDCM= 10mm. At 25 mg/mL*, ZOI were SEMETH= 11mm, SEDCM= 10mm, HMMETH= 8mm,* and HMDCM= 9mm. *S. ellipticum* methanolic extract showed the highest effect in concentrations tested while *H. madagascariensis* dichloromethane extract showed less effect in almost all concentrations tested.

Figure 3: Zones of Inhibition (mm) for Individual Extracts in Different Concentrations against MRSA Isolates by Agar Well Diffusion. Error Bar Implies Standard Deviation for Triplicate Experiments. HMMETH and SEMETH are *H. madagascariensis* and *S. ellipticum* methanolic extracts while the HMDCM and SEDCM are *H. madagascariensis* and *S. ellipticum* Dichloromethane extracts

Determination of the AI values as presented in (Table 2) formed the foundation of distinguishing antibacterial activeness of the plant extracts used in this study. Generally, the results shown in (Table 2) provide the information that the activeness of the plant extract was increased by increase in concentration and differences in activity of plant extracts was depending on the extract composition and type of solvent used. Results for determination of activity index (AI) for individual extracts showed that *S. ellipticum* methanolic extract was more active than other individual extracts for all concentrations tested while the least active extract was *H. madagascariensis* dichloromethane extracts for the concentration of 200 mg/mL and 100 mg/mL except for concentration of 25 mg/mL where *H. madagascariensis* methanolic extract showed less activity than its Dichloromethanolic counterpart. Methanolic extracts showed to be more active than dichloromethane extracts but not significantly ($p > 0.05$) in almost all concentrations except for the concentration of 25 mg/mL where *H. madagascariensis* methanolic extract showed less activity than all extracts. *S. ellipticum* extract showed to be active than *H. madagascariensis* but not significantly ($p > 0.05$).

Table 2: Activity Index (AI) of Individual Extracts against MRSA Isolate

Concentration (mg/mL)	HM METH	HM DCM	SE METH	SE DCM
200	0.83	0.55	0.98	0.95
100	0.7	0.5	0.75	0.73
25	0.4	0.45	0.55	0.5

HM METH= *H. madagascariensis* methanolic extract, HM DCM= *H. madagascariensis* dichloromethane extract, SE METH= *S. ellipticum* methanolic extract and SE DCM= *S. ellipticum* dichloromethane extract.

Minimum inhibitory concentrations for individual extracts against MRSA isolate were determined and the average results for triplicate experiments were recorded in (table 3). The MIC values ranged from 1.56 - 6.25 mg/mL for individual extracts. MIC values for all single extracts showed that *S. ellipticum* methanolic extract was very active extract (MIC = 1.56 mg/mL) while *S. ellipticum* dichloromethane extract was least active (MIC = 6.25 mg/mL).

Table 3: Minimum Inhibitory Concentration M(mg/mL) for Individual Plant Extracts

Extracts	HMMETH	HMDCM	SEMETH	SEDCM
MIC (mg/mL)	3.125	3.125	1.56	6.25

HM METH= *H. madagascariensis* methanolic extract, HM DCM= *H. madagascariensis* dichloromethane extract, SE METH= *S. ellipticum* methanolic extract and SE DCM= *S. ellipticum* dichloromethane extract.

Investigation of Synergism.

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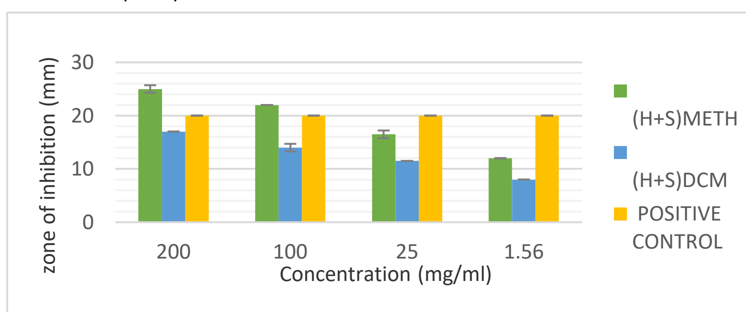
The zone of inhibition (ZOI) for the concentrations of 200, 100, 25 and 1.56 mg/mL ranged from 25 mm - 8 mm for combined extracts as shown in (Figure 4). In combined extracts 1.56 mg/mL concentration showed the inhibition zone diameter. Methanolic combined extract showed the greater value of inhibition zone diameter than dichloromethane extracts in all concentrations tested and at 200mg/mL the methanolic combined extract (ZOI=25 mm) showed to be larger than even the positive control (ZOI=20mm).

Figure 4: Zones of Inhibition (mm) for Combined Plant Extracts in Different Concentrations Against MRSA



Isolates. Error Bars Represent Standard Deviation for Triplicate Experiments.

Determination of activity index (AI) for combined extracts shown in (Table 4) showed that methanolic combined extract has the higher AI value than dichloromethane combined extracts (but not significantly) in all concentrations tested. Using one way ANOVA test the results concluded that: The combined extracts were statistically significant active than the individual extracts ($p < 0.05$).

Table 4: Activity Index (AI) of Combined Extracts against MRSA Isolate

Concentration(mg/mL)	200	100	25	1.56
(H+ S) METH	1.75	1.35	0.95	0.7
(H + S) DCM	1	0.8	0.58	0.5

Minimum inhibitory concentration (MIC) for combined extracts ranged from 0.2 - 0.8 mg/mL. MIC for combined extracts showed that methanolic combination ratio 3: 1 (*S. ellipticum*: *H. madagascariensis*) was the most active with MIC of 0.2 mg/mL while methanolic combination ratio 1: 3 (*S. ellipticum*: *H. madagascariensis*) and dichloromethane combination ratio 1: 1 (*S. ellipticum*: *H. madagascariensis*) were the least active with MIC of 0.8 mg/mL as shown (Table 5).

Table 5: Minimum Inhibitory Concentration (mg/mL) of combined plant extracts against MRSA.

Combined Extract	Ratios	MIC (mg/mL)
	3: 1	0.2
S + H METH	1: 1	0.4
	1: 3	0.8
	3: 1	0.4
S + H DCM	1: 1	0.8
	1: 3	0.4

By using a one-way ANOVA test, MIC values of combined extracts were significantly lower than those of the individual extracts ($p < 0.05$), as shown in the comparative analysis (Table 6). This analysis concluded that combined extracts showed higher activity than individual extracts.

Table 6: Comparative Analysis between the MIC (mg/mL) of Combined Extracts and that of Individual Extracts

Individual extracts	MIC (mg/mL)
SE METH	1.56
SE DCM	6.25
HM METH	3.125
HMDCM	3.125
Combined Extracts	
S + H METH (3: 1)	0.2
(1: 1)	0.4
(1: 3)	0.8
S + H DCM (3: 1)	0.4
(1: 1)	0.8
(1: 3)	0.4

In this study the synergism among methanol and dichloromethane extracts of *S. ellipticum* and *H. madagascariensis* was successfully investigated against MRSA clinical isolate. The results showed a synergistic effect in these combinations as follows; methanolic combination ratio 3:1 (*S. ellipticum*: *H. madagascariensis*) (FICI= 0.192) and 1:1 (*S. ellipticum*: *H. madagascariensis*) (FICI= 0.384) as well as in dichloromethane combination ratio 1:1 (*S. ellipticum*: *H. madagascariensis*) (FICI= 0.384). The 1:1 ratio showed synergistic effect in both methanol and dichloromethane combinations while with the other combinations, additive effect and non-interactive interactions were observed as shown in (table 7). In all combinations, no antagonism interaction was observed.

Table 7: FIC Index of Combined Plant Extracts in (v/v) Ratio against MRSA Isolates.

Extract	Ratio	FIC- Index	Interpretation
S + H (METH)	3: 1	0.192	Synergistic
	1: 1	0.384	Synergistic
	1: 3	0.768	Additive
S + H (DCM)	3: 1	3.589	Non-interactive
	1: 1	0.384	Synergistic
	1: 3	3.589	Non-interactive

The results of this study showed that the combination of *S. ellipticum* and *H. madagascariensis* has a synergistic effect as the beneficial antibacterial activity against MRSA, and the suggested ratios in this study observed no antagonism (reduction of activity). By using the test for association, there was a significant association ($r = 0.801$) in antibacterial activity screened (AI) with the results of FIC Index ($p < 0.05$)

Discussion

Identification of MRSA Clinical Isolates

In the present study, gram staining, catalase, coagulase and antibiotic resistance tests were the important phenotypic identifying markers of *S. aureus* as similarly reported (Karmakar et al. 2016). Clinical isolate isolated from the patient's wound was catalase positive and coagulase positive,

which are among the characteristics of S. aureus, as similarly reported by (Guan et al. 2022). The antibiotic resistance test used oxacillin disc diffusion as the sensitive method for MRSA detection and the result showed that the isolate was resistant to oxacillin. This is similarly reported by (Salas et al. 2020). PCR for 16 rRNA specific primer assisted to confirm if the bacterial species was *S. aureus* before performing the mecA gene detection (Guan et al. 2022) .

In this study, phenotypically identified MRSA possessed mecA gene during PCR for mecA specific primer. It has been reported that the resistance in MRSA is commonly conferred by mecA gene encoding a modified penicillin-binding protein (PBP2a) with reduced affinity for methicillin and other beta-lactam antibiotic (Singh et al. 2023). mecA gene carried horizontal transfer of MGE known as staphylococcal cassette chromosome mec (SCCmec) (Singh et al. 2023). Molecular and phenotypic identification result was in concordance as similarly reported that the clinical strains of *S. aureus* from infected wounds might be MRSA (Koupahi1 et al. 2016). Confirmation of the addressed clinical isolate as the MRSA approved using the isolate as the intended microorganism in this study.

Antibacterial Activities of Individual Plant Extracts

In this study, the selected plant extracts employed were found to be susceptible to MRSA isolate. The antibacterial activity increased with increased concentrations, as Odongo et al. (2023) reported. Determination of AI established the base for describing the activeness of extracts. In the present study, S. ellipticum methanolic extract was more active (AI= 0.98) than other individual extracts.

The activity index (AI) for *H. madagarscariensis* dichloromethane extract at 200 mg/mL against MRSA isolate in this study was (AI= 0.55) comparable to that of methanol leaf extract of *H. madagascariensis* at 25 mg/mL against *Escherichia coli* reported by (B et al. 2020). In contrast, (Onajobi et al. 2020) reported the activity (AI=0.51) for *H. madagascariensis* ethanol stem bark extract against *S. aureus*.

The AI of *S. ellipticum* dichloromethane extract at 200 mg/mL was (AI=0.72) similar to that shown by *Beta vulgaris* aqueous extract against *P. aeruginosa* reported by (Kousar et al. 2023). In contrast, a study reported by (Octavie Merveille 2017) showed (AI=0.84) of *S. ellipticum* methanol extract against *Staphylococcus saprophiticus* at 50 mg/mL. These differences of activity index possibly were due to the difference in sensitivity of tested microorganisms, environmental influences to the used plant, the plant part used, type of solvent used in extraction as well as the methods used (Ugboko et al. 2020). The susceptibility of MRSA to the selected plant extracts in this study correlates with other studies which have reported the presence of antibacterial activity on single or combined plant extracts and/or antibiotics against MRSA (Anyanwu and Okoye 2017, Aqil et al. 2006 (Voravuthikunchai and The Kitpipit The 2005). However, no study has been reported on synergistic effect between *S. ellipticum* and *H. madagarscariensis* against the MRSA.

Minimum Inhibitory Concentration of the Individual Extracts

In the present study, the range of MIC (1.56 - 6.25 mg/mL) for individual extracts was comparable to that exhibited by *Psidium guaja* against *Staphylococcus aureus* reported by (Qaralleh et al. 2020). This was in contrast with the range of MIC (6.2 – 100 mg/mL) for aqueous extracts of *S. ellipticum* and *H. madagarscariensis* stem barks against *Streptococcus lactis* reported by (Mpinda et al. 2018). However, the MIC value range (0.02 - 1.56 mg/mL) is shown in findings reported by (Jatin Chadha et al. 2021).

The MIC value (6.25 mg/mL) of *S. ellipticum* dichloromethane extract in the present study was comparable that of *P. mirabilis* methanol extract against *Klebsiella pneumonia* and *Escherichia*

coli reported by (Ahmed et al. 2023). Also, was nearly comparable with the MIC (6.3 mg/mL) of *S. ellipticum* dichloromethane stem bark extract against *Aspergillus niger* reported by (Kisangau_2009). In contrast, activity of *S. ellipticum* methanol extract (MIC < 1 mg/mL) against *S. saprophiticus* was reported by (Octavie Merveille 2017).

Results showed the activity of *H. madagascariensis* was (MIC=3.125 mg/mL) comparable with that of *Croton macrostachyus* and *Calpurina aurea* methanol extract reported by (Teshale et al. 2023). In contrast, *H. madagascariensis* aqueous leaf extract showed (MIC=1.56 mg/mL) against Salmonella (Kengni et al. 2013).

The results explored that methanol extracts were more active against MRSA isolate than dichloromethane extracts (intermediate). Differences in MIC range might be attributed to the solvent used, a starting concentration during serial microdilution and the sensitivity of test microorganisms. *S. aureus* has been reported to be the most inhibited isolate by plant crude extracts (Dahiya and Purkayastha et al. 2021). Also, reports showed that MRSA is more susceptible to different plant extracts than other microorganisms (Amenu 2014 and Zakaria et al. 2014).

Based on the report described by (Octavie Merveille 2017), the antimicrobial potential of the various extracts is categorized as follows: MIC < 1 mg/mL; very active, 1 ≥ MIC ≤ 8 mg/mL; moderately active, 8 > MIC ≤ 64 mg/mL; less active or negligible and MIC > 64 mg/mL; not active. Therefore, in this study, the results of MIC ranges (1.56 - 6.25 mg/mL) showed that all individual extracts were moderately active.

Investigation of Synergism

Zones of inhibition for combined extracts in a particular concentration exceeded those obtained from individual extracts. This is related to what has been reported by (Kehinde Peter et al. 2020). The AI of the methanolic combination at 200 mg/mL (AI=1.75) and 100 mg/mL (AI=1.35) in the present study showed its activity exceeded that expressed by a positive control (AI=1). The information revealed here is that, combination of extracts as well as an increase in concentration improves the effectiveness of extracts as similarly reported (Odongo et al. 2023).

Comparison analysis between MIC for combined and individual extracts revealed that all combined extracts were active against MRSA, and there was a significant difference in ($p < 0.05$). The range of MIC (0.2 - 0.8 mg/mL) for combined extracts in this study was not comparable to the range of MIC (3.125 – 25 mg/mL) for two combined extracts reported by (Teshale et al. 2023) and that of MIC range (0.5 – 1 mg/mL) for three combined extracts reported by (Donkor et al. 2023). There was no significant difference between methanolic and dichloromethane extract in their activeness ($p > 0.05$). The study showed the methanolic combination has higher activity in the 3:1 and 1:1 ratios, which was in contrast with the results with higher activity in the methanolic combination in 1: the 3 and 3:1 ratios reported by (Johnson and Ayoola 2015).

FIC Indices in this study discovered the novel information that there is a potential synergism interaction among combined extracts of *S. ellipticum* and *H. madagascariensis* stem bark extracts, which had not yet been reported in the literature before. Therefore, it validates the relevance of combining these plants in treating MRSA infections by traditional medicine practitioners and explores the information of discovering new and effective antibacterial agents using a combination of these plants.

Methanolic combinations showed two ratios with synergistic interaction and one ratio with additive interaction. Dichloromethane combinations showed only one ratio with synergistic interaction and two ratios with non-interactive effects. This indicates that in combinations of

extracts, polar compounds like methanol interact more synergistically than non-polar compounds, as reported by Johnson and Ayoola (2015; Odongo et al., 2023).

In this study, the best ratio that possessed a synergistic effect in both methanol and dichloromethane combination was 1:1. Many studies done on the combination of plant extracts have reported a synergistic effect in a 1:1 combination ratio (Karmegam et al. 2019, Donkor et al. 2023). However, there is insufficient reported information about the extract–extract combination effect for comparison.

Conclusion

The results demonstrate that combined extracts from selected plants exhibit significantly higher antibacterial activity than individual extracts. This study revealed a synergistic interaction when methanol or dichloromethane extracts of *S. ellipticum* and *H. madagascariensis* were combined against MRSA isolates. The optimal ratio for achieving this synergism in both methanol and dichloromethane combinations was 1:1. In conclusion, these findings provide promising evidence for the potential use of methanol or dichloromethane crude extracts of *S. ellipticum* and *H. madagascariensis* stem barks in synergistic treatments for MRSA infections.

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References

Abogo Mebale A-J, Massimba Dibama H, Grace OMBOUMA J, Martial BOUROBOU Y, Mefouet Abessolo D-D & Aubin ONDO J. (2022) Phytochemical Constituents and Evaluation of Total Polyphenols and Total Flavonoids content of the Ethanolic Extract of *Harungana madagascariensis* Trunk bark from Gabon. *Article in IOSR Journal of Applied Chemistry* 15: 1–05.

Ahmed J, Abdu A, Mitiku H & Ataro Z. (2023) In vitro Antibacterial Activities of Selected Medicinal Plants Used by Traditional Healers for Treating Urinary Tract Infection in Haramaya District, Eastern Ethiopia. *Infectious Drug Resistance* 16: 1327–1338.

Amenu D. (2014) Antimicrobial Activity of Medicinal Plant Extracts and Their Synergistic Effect on Some Selected Pathogens. *American Journal of Ethnomedicine* 1: 018-029.

Anand U, Jacobo-Herrera N, Altemimi A & Lakhssassi N. (2019) A comprehensive Review on Medicinal Plants as Antimicrobial Therapeutics: Potential Avenues of Biocompatible Drug discovery. *Metabolites* 9: 11.

Anyanwu MU & Okoye RC. (2017) Antimicrobial Activity of Nigerian Medicinal Plants. *Journal of Intercultural Ethnopharmacology* 6 (2): 240–259.

Aqil F, Ahmad I & Owais M. (2006) Evaluation of Anti-Methicillin-Resistant *Staphylococcus aureus* (MRSA) Activity and Synergy of Some Bioactive Plant Extracts. *Biotechnology Journal* 1 (10): 1093–1102.

B OI, AAA & C OF. (2020) Antibacterial effect of five selected Nigerian medicinal plant extract. 2408.

Beda Mpinda C, Meyer D & Lyantagaye SL. (2018) Antimicrobial Activity of Extracts of Some Plants Species Used in the Management of HIV/AIDS in Tanzania. *Tanzania Journal of Science* 44 (1): 179–190.

Chanda S, Rakholiya KD & Rakholiya K. (2011) Combination therapy: Synergism between Natural Plant Extracts and Antibiotics against Infectious Diseases Toxicity. *FUW Trends in Science & Technology Journal* 160-67.

Cheesman MJ, Ilanko A, Blonk B & Cock IE. (2017) Developing New Antimicrobial Therapies: Are Synergistic Combinations of Plant Extracts/Compounds with Conventional Antibiotics the Solution? *Pharmacognosy Reviews 11 (22): 57–72.*

Dahiya P & Purkayastha S. (2012) Phytochemical Screening and Antimicrobial Activity of Some Medicinal Plants against Multi-drug Resistant Bacteria from Clinical Isolates. *Indian Journal of Pharmaceutical Sciences* 125, 201- 303

Donkor MN, Donkor AM & Mosobil R. (2023) Combination Therapy: Synergism Among Three Plant Extracts Against Selected Pathogens. *BMC Research Notes* 16: 83.

Duremdes KN, Lorenz Revil T, Millen Iral J, Grace A & Bingcang F. (2023) An In Vitro Study on the Synergistic Effect of the *Musa Acuminata* and *Citrofortunella Microcarpa* Peel Extract Against *Escherichia coli*. *ISBN* 978-623-99026-0-5.

Feng M, Xu B, Nahidul Islam M, Zhou C, Wei B, Wang B, Ma H & Chang L. (2023) Individual and Synergistic Effect of Multi-frequency Ultrasound and Electro-infrared Pretreatments on Polyphenol Accumulation and Drying Characteristics of Edible Roses. *Food Research International* 163: 20-25.

Gideon M & Ladan Z. (2023) Synergistic Combinatorial Strategy for Combating Antimicrobial Resistance (AMR) in Clinical Bacteria by Combining Antibiotics with Plant Extracts. *Fine Chemical Engineering*: 1–12.

Guan S, Zhong L, Yu H, Wang L, Jin Y, Liu J, Xiang H, Yu H, Wang L & Wang D. (2022) Molecular Docking and Proteomics Reveals the Synergistic Antibacterial Mechanism of Theaflavin with β-lactam Antibiotics Against MRSA. *Frontier Microbiology* 13: 993430.

Jatin Chadha, Manish Gupta, Nishtha Nagpal, Madhav Sharma, Tarun Adarsh, Vaibhav Joshi, Vidhi Tiku, Tamanna Mittal, Vaibhav Kumar Nain, Ashish Singh, SK Snigdha, Nidhi S. Chandra, Salome John & Prerna Diwan (2021) Antibacterial Potential of Indigenous Plant Extracts Against Multidrug-Resistant Bacterial Strains Isolated from New Delhi Region. *GSC Biological and Pharmaceutical Sciences* 14 (2): 185–196.

Johnson OO & Ayoola GA. (2015) Antioxidant Activity Among Selected Medicinal Plants Combinations (Multi-Component Herbal Preparation). *International Journal of Pharma Research and Health Sciences* 3 (1): 526–532.

Jouda MM, Elbashiti T, Masad A & Albayoumi M. (2016) The Antibacterial Effect of some Medicinal Plant Extracts and their Synergistic Effect with Antibiotics. *Journal of Pharmacy and Pharmaceutical Science,* 2: 23-33.

Karmakar A, Dua P & Ghosh C. (2016) Biochemical and Molecular Analysis of *Staphylococcus aureus* Clinical Isolates from Hospitalized Patients. *Canadian Journal of Infectious Diseases and Medical Microbiology* 2016: ID 9041636.

Karmegam N, Karuppusamy S, Prakash Mani, Jayakumar M & Kuppuraj Rajasekar (2019) Antibacterial Potency and Synergistic Effect of Certain Plant Extracts against Food-Borne Diarrheagenic Bacteria. *International Journal of Biomedical and Pharmaceutical Science*, 2(2), 88-93.

Kehinde Peter A, Paul A, Olabisi L & Clement A. (2020) Synergistic Evaluation of *Moringa oleifera*, *Hunteria umbellate* and *Azadirachta indica* with Antibiotics Against Environmental MRSA Isolates. *American Journal of Bioscience* 8 (4): 91.

Kengni F, Tala D, Djimeli M, Fodouop S, Kodjio N, Magnifouet H & Gatsing D. (2013) *In vitro* Antimicrobial Activity of *Harungana madagascriensis* and *Euphorbia prostrata* Extracts Against Some Pathogenic *Salmonella* Sp. *International Journal of Biological and Chemical Sciences* 7 (3): 1106.

Kisangau DP, Hosea KM, Lyaruu HVM, Joseph CC, Mbwambo ZH, Masimba PJ, Gwandu CB, Bruno LN, Devkota KP & Sewald N. (2009) Screening of Traditionally Used Tanzanian Medicinal Plants for Antifungal Activity *Pharmaceutical Biology*. pp. 708–716.

Kisangau DP, Lyaruu HVM, Hosea KM & Joseph CC. (2007) Use of Traditional Medicines in the Management of HIV/AIDS Opportunistic Infections in Tanzania: A case in the Bukoba rural district. *Journal of Ethnobiology and Ethnomedicine 3:29.*

Kousar F, Khanem A, Ullah I & Younas F. (2023) Phytochemical Analysis and Synergistic Antimicrobial Potential of Extracts from *Carica papaya* and *Beta vulgaris*. *Kuwait Journal of Science* 50 (2023) 307–312.

Kumar Shakya A & Arvind Kumar Shakya C. (2016) Medicinal Plants: Future Source of New Drugs. *International Journal of Herbal Medicine* 4 (4): 59–64.

Kyany'a C, Nyasinga J, Matano D, Oundo V, Wacira S, Sang W & Musila L. (2019) Phenotypic and Genotypic Characterization of Clinical *Staphylococcus aureus* Isolates from Kenya. *BMC Microbiol* 19 (1).

Lozano C, Gharsa H, Ben Slama K, Zarazaga M & Torres C. (2016) *Staphylococcus aureus* in Animals and Food: Methicillin Resistance, Prevalence and Population Structure. A Review in the African continent. *Microorganisms* 4 (1).

Macdonald Ighodaro O, Adeboye Akinloye O, Ighodaro OM, Akinloye OA, Popoola JA & Adebodun SO. (2018) Phytochemical Distribution and Antimicrobial Sensitivity of *Sapium ellipticum* (Hochst.) Pax Leaf Extracts Optimization and Production of Ascorbic Acid by Fusant Cell of *Aspergillus flavus* and *Aspergillus tamarii*. *Complete Peer review History*, EJMP.8252.

Masalu RJ, Ngassa S, Kinunda GA & Mpinda CB. (2020) Antibacterial and Anti-HIV-1 Reverse Transcriptase Activities of Selected Medicinal Plants and Their Synthesized Zinc Oxide Nanoparticles. *Tanzania Journal of Science* 46 (3): 597–612.

Masalu RJ & Mpinda CB. (2021) Antibacterial and Anti-HIV-1 GP 120 Activities of Selected Medicinal Plants. *Journal of Education, Humanities and Science (JHHS),* 1O (5).

Masimen MAA, Harun NA, Maulidiani M & Ismail WIW. (2022) Overcoming Methicillin-Resistance *Staphylococcus aureus* (MRSA) Using Antimicrobial Peptides-Silver Nanoparticles. *Antibiotics* 11 (7).

Mukeba FB, Mukoko JB, Mayangi MM, Ngondo MM, Ngelinkoto PM, Bamba J V., Kitwa F, Bongo GN, Mutwale PK, Ngbolua K-N, Ngombe NK, Mpiana PT & Mbemba TFM. (2020) Microscopic Features, Mineral Contents, Anti-sickling, Antioxidant and Antibacterial Activities of Stem Bark of *Harungana madagascariensis* Lam. Ex Poiret (Hypericaceae). *European J Med Plants*: 33–47.

Nana O, Momeni J, Tepongning RN & Ngassoum MB. (2013) Phythochemical Screening, Antioxydant and Antiplasmodial Activities of Extracts from *Trichilia roka* and *Sapium ellipticum*. *The Journal of Phytopharmacology* 2, 4.

Nichol KA, Adam HJ, Golding GR, Lagacé-Wiens PRS, Karlowsky JA, Hoban DJ & Zhanel GG. (2019) Characterization of MRSA in Canada from 2007 to 2016. *Journal of Antimicrobial Chemotherapy* 74: IV55–IV63.

Octavie Merveille T. (2017) Antimicrobial Activities of Some Medicinal Plants Against Multi-resistants Microorganisms Causing Urogenital Tract Infections in Cameroon. *Journal of Diseases and Medicinal Plants* 3 (2): 33.

Odongo EA, Mutai PC, Amugune BK, Mungai NN, Akinyi MO & Kimondo J. (2023) Evaluation of the Antibacterial Activity of Selected Kenyan Medicinal Plant Extract Combinations Against Clinically Important Bacteria. *BMC Complement Med Ther* 23 (1)

Oe A & Me U. (2020) Evaluation of Some Antioxidant Indices of the Fresh Flowers of *Harungana madagascariensis* Lam. Ex Poiret (Hypericaceae). *The Pharma Innovation Journal* 9(4): 142-146.

Pallett S, Charani E, Hawkins L, Mazzella A, Anton-Vazquez V, Banerjee R, Evans T, Patterson B, Subbarao S, Alqahtani S, Basarab M, Breathnach A, Mughal N & Moore L. (2023) National Action Plans for Antimicrobial Resistance and Variations in Surveillance Data Platforms. *Bull World Health Organ* 101 (08): 501-512F.

Qaralleh H, Al-Limoun MO, Khlaifat A, Khleifat KM, Al-Tawarah N, Alsharafa KY & Abu-Harirah HA. (2020) Antibacterial and Antibiofilm Activities of a Traditional Herbal Formula Against Respiratory Infection Causing Bacteria. *Tropical Journal of Natural Product Research* 4 (9): 527–534.

Reichmann NT & Pinho MG. (2017) Type in Resistance to the Synergistic Activity of Oxacillin and Cefoxitin in MRSA. *Sci Rep* 7 (1)

Salas M, Wernecki M, Fernández L, Iglesias B, Gutiérrez D, Álvarez A, García L, Prieto E, García P & Rodríguez A. (2020) Characterization of clinical MRSA Isolates from Northern Spain and Assessment of their Susceptibility to Phage-derived Antimicrobials. *Antibiotics* 9 (8): 1–18.

Sharaf MH, El-Sherbiny GM, Moghannem SA, Abdelmonem M, Elsehemy IA, Metwaly AM & Kalaba MH. (2021) New Combination Approaches to Combat Methicillin-Resistant *Staphylococcus aureus* (MRSA). *Scientific Report* 11(1): 4240.

Singh I, Roshan M, Vats A, Behera M, Gautam D, Rajput S, Rana C & De S. (2023) Evaluation of Virulence, Antimicrobial Resistance and Biofilm Forming Potential of Methicillin-Resistant *Staphylococcus aureus* (MRSA) Isolates from Bovine Suspected with Mastitis. *Curr Microbiol* 80 (6).

Teshale A, Berhane N & Sendeku W. (2023) Combined Antibacterial Effect of Croton macrostachyus and Calpurina aurea Against Selected Clinical and Standard Pathogenic Bacteria. *European Journal of Clinical and Biomedical Sciences* 9(3): 38-46.

Thereza A, Ferreira T, Cláudio L, Bezerra C, Fernando A, Oliveira M De, Araújo JM, Tereza M, Lúcia V & Lima DM. (2015) Organic Extracts from *Indigofera Suffruticosa* Leaves have Antimicrobial and Synergic Actions with Erythromycin Against *Staphylococcus aureus*. *Frontier in Microbiology* 6; 1–7.

Vestergaard M, Frees D & Ingmer H. (2019) Antibiotic Resistance and the MRSA Problem. *Microbiology Spectrum* 7: 2.

Victor Nyangabo Mbunde M, Mabiki F, Andersson PG, Mourice Victor Nyangabo Mbunde C & Innocent E. (2019) Antifungal Activity of Single and Combined Extracts of Medicinal Plants from Southern Highlands of Tanzania. *Journal of Pharmacognosy and Phytochemistry* 8 (1): 181–187.

Voravuthikunchai SP & Kitpipit L. (2005) Activity of Medicinal Plant Extracts Against Hospital Isolates of Methicillin-Resistant *Staphylococcus aureus*. *Clinical Microbiology and Infection* 11 (6): 510–512.

Zakaria ZA, Suhaili Z, Nasir M, Desa M, Chuah EL, Zakaria ZA, Suhaili Z, Abu Bakar S & Desa MNM. (2014) Antimicrobial Activities of Plant Extracts against Methicillin-Susceptible and Methicillin-Resistant *Staphylococcus aureus* Molecular Characterization of MRSA. *Journal of Microbiol Research* 1: 6–13.