ACTA SCIENTIFIC MICROBIOLOGY (ISSN: 2581-3226)

Volume 4 Issue 5 May 2021

Research Article

In Vitro Antimicrobial Activity of Crude Extracts from *Vetiveria nigritana* (benth.) Stapf, *Mitragyna inermis* (Willd.) Kuntze, *Kalanchoe crenata* (andr.) Haw. against Methicillin-resistant *Staphylococcus aureus*

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Received: March 12, 2021

Published: April 27, 2021

Abstract

The evolution of increasingly antimicrobial-resistant bacterial species in general and particularly. The emergence of strains of methicillin-resistant *Staphylococcus aureus* (MRSA) are currently a real threat to humanity. There is an urgent need for new efficient antibiotics. Medicinal plants can be the sources of effective new therapeutic agents. This study as performed to study the antimicrobial activity of three medicinal plants (*Mitragyna inermis, Vetiveria nigritana, Kalanchoe crenata*) from Burkina Faso against *Staphylococcus aureus* strains isolate from patients. The antibiotics susceptibility of *Staphylococcus aureus* strains and the antimicrobial activities of the plant extracts were evaluated using standard agar disc diffusion method. Determination of minimum inhibitory concentrations (MIC) and minimum Bactericidal Concentration (MBC) of the active extracts was done using the agar microdilution method. The highest antimicrobial activity was recorded with ethanol extracts of plants against MRSA. No antimicrobial activity was detected with decoction extracts. The MIC and MBC of the different extracts ranged from 0.625 to10 mg/ml for *Mitragyna inermis* and *Vetiveria nigritana* extracts and from 0.625 to 5 mg/ml for *Kalanchoe crenata* extracts.

Mitragyna inermis, Vetiveria nigritana, Kalanchoe crenata are some therapeutically potential plants to combat microbial infections due to MRSA.

Keywords: *Staphylococcus aureus*; Antimicrobial Resistance; MRSA; *Mitragyna inermis; Vetiveria nigritana; Kalanchoe crenata;* Burkina Faso

Abbreviations

MRSA: Methicillin-Resistant *Staphylococcus aureus;* MBC: Minimum Bactericidal Concentration; MIC: Minimum Inhibitory Concentrations; SAB*: Staphylococcus aureus* Bacteremia; AIDS: Acquired Immunodeficiency Syndrome; CLSI: Clinical Laboratory Standards Institute; DMSO: Dimethyl Sulfoxide; SPSS: Statistical Package for Social Services; DDM: Decoction Extract Dried of *M. inermis;* DDK: Decoction Extract Dried of *K. crenata;* DDV: Decoction Extract Dried of *V. nigritana;* EDV: Ethanol Extracts Dried of *V. nigritana;* EDM: Ethanol Extracts Dried of *M. inermis;* EDK: Ethanol Extracts Dried of *K. crenata;* ELK: Ethanol Extracts Lyophilized of *K. crenata;* ELV: Ethanol Extracts Lyophilized of *V. nigritana;* ELM: Ethanol Extracts Lyophilized of *M. inermis;* DLM: Decoction Extract Lyophilised of *M. inermis;* DLV: Decoction Extract Lyophilized of *V.*

nigritana; DLK: Decoction Extract Lyophilised of *K. crenata;* MRSA/MDR: Methicillin Resistant *Staphylococcus aureus*/Multidrug Resistant; DIZ: Diameter of Inhibition Zone; Amox: Amoxicillin; Com: Community-acquired *S. aureus;* Hosp: Hospital-acquired *S. aureus*

Introduction

Nowadays, *Staphylococcus aureus* has been identified as a dangerous and difficult-to-tackle pathogen. Indeed, *Staphylococcus aureus* is one of the pathogen bacteria with a great capacity to adapt to different environmental conditions and ability to cause a diverse array of life-threatening infections because of its important intrinsic virulence. Besides, it is now, with *Clostridium difficile*, considered the leading overall cause of hospital acquired infections and is an increasing concern in the community [1,2].

Staphylococcus aureus bacteremia (SAB) is one of the most the important infections due to this bacteria. The incidence of SAB is estimated from 20 to 50 cases/100,000 population per year [3]. Comparatively, the number of deaths due to SAB is greater than the number due to AIDS, tuberculosis, and viral hepatitis combined [3]. Rates of SAB depend from regions, development status or specific groups of population [4]. In the United States, SAB rate as estimated between 38.2 to 45.7 per 100,000 person-years [5,6]. In the industrialized world, the incidence is approximately 10 to 30 per 100,000 person-years [5]. Despite the lack of data, the incidence SAB can be supposed to be much higher in developing countries and in the poorest regions of the world. This probably could get worse with the rapid expansions of MRSA if nothing is done. The mortality of patients with SAB in the pre-antibiotic era exceeded 80% and over 70% developed metastatic infections [7,8]. To no longer return to this situation, there is an urgent need to find new antimicrobial agents to deal with antimicrobial resistance and particularly MRSA.

As there is an imperative need to develop new antimicrobial drugs for the treatment of infectious diseases, one approach is to screen local medicinal plants for possible antimicrobial properties [9]. For millennia, medicinal plants have been a valuable source of therapeutic agents [10-12]. Plant secondary metabolites have already demonstrated their potential as antibacterial when used alone and as synergists or potentiators of other antibacterial agents [13,14]. Plant extracts or compounds often demonstrate high-level activity against pathogens, and they rarely have severe side effects [15]. Screening local medicinal plants can lead to new efficient antibiotics or complementary and alternative medicine therapies which have been gaining popularity throughout the world [16,17].

The flora of Burkina Faso abounds in a large number of plants used in traditional medicine for the treatment of bacterial infections. Regarding to the traditional use of these plants, some of them could have important therapeutic properties to fight against the multidrug-resistant bacteria like MRSA.

Aim of the Study

Therefore, the aim of this study was to screen the antibacterial properties of different extracts from three medicinal plants (*Mitragyna inermis*, *Vetiveria nigritana*, *Kalanchoe crenata*) of Burkina Faso against *Staphylococcus aureus* isolate including MRSA.

Materials and Methods

Microbial samples and their susceptibility to antimicrobial

The bacteria (40 strains of *Staphylococcus aureus*) were isolated from patient specimens as described previously [18]. Kirby-Bauer method was performed for antibiotic susceptibility testing according to the guidelines of the Clinical Laboratory Standards Institute [19]. Briefly, fresh bacterial strain with 0.5 McFarland turbidity was swabbed onto the Mueller-Hinton agar (Oxoid, UK) surface using sterile swab sticks. Antimicrobial discs (HIMEDIA, India,) were evenly embedded onto the inoculated agar incubated at 37°C overnight. The antibiotics discs used for identification of antibiotic sensitivity pattern of MRSA isolates were: erythromycin (15 µg), tetracycline (30 g), gentamicin (10 µg), clindamycin (2 µg), ciprofloxacin (5 µg), levofloxacin (30 µg), tobramycin (30 µg), kanamycin (30 µg), cotrimoxazole (1.25 + 23.75 µg), fusidic acid (10 µg), and chloramphenicol (30 µg).

Collection and identification of plants

Plants samples were collected from Loumbila, a village located at the north of Ouagadougou in March 2020. These samples were identified at the Biodiversity Information Centre of University Joseph KI-ZERBO. These plant materials were collected on the basis of their traditional uses by the population as medicines in Burkina Faso. Table 1 show common names and general uses of the three plant species used in this study.

Plants names	Common name	Family	Part tested	Traditional uses
Vetiveria nigritana (Benth.) Stapf	Vetiver	*Gramineae*	Roots	Boils, epilepsy, burns, snakebites, scorpion stings, fever, headache, as a tonic for weakness, rheumatism.
Mitragyna inermis (Willd.) Kuntze	False abura	*Rubiaceae*	Leaves	Malaria, syphilis, bilharzia, gonorrhea, parasitosis, dysentery and cholera
Kalanchoe crenata (Andr) haw	Neverdie	*Crassulaceae*	Leaves	Epilepsy, wounds, ear infections, odontalgia, burns, ulcers

Table 1: Selected medicinal plants the parts used and their traditional uses.

After harvesting, the samples were washed under running water to remove dust. Subsequently, they were dried in the shade, and afterwards the dried plant materials were finely grounded by mechanical grinders. The powder was stored in tightly closed glass containers in the dark at room temperature**.**

Preparation of plant extracts

The ethanol extracts were prepared by soaking 25g of the fine powder of each plant part was in 250 ml of ethanol (70%) with stirring for 48h. Then, the extracts were firstly filtered through double layers of compress and finally filtered again through Whatman no. 1 filter paper. The ethanol was evaporated using rotatory vacuum evaporator and each filtrate was divided into 2 parts. A part was concentrated then dried in an oven at 40°C under ventilation. The other part was freezed and lyophilized. For each plant, a dried extract and a lyophilized extract were obtained.

The water extracts were obtained by boiling 25g of the powder of each plant part in distilled water for 30 minutes. This process follows approximately that of traditional healer's method. Each decoction is then cooled, filtered and concentrated with a rotary evaporator under vacuum. Each filtrate was treated as described above to obtain dried decocted extract and lyophilized decocted extract.

Crude extracts were weighted and stored in small bottles in fridge at 5^0C and their yield calculated using the following equation:

$$\text{Yield (g/100 g)} = \frac{W1 \times 100}{W2}$$

Where W1 is the weight of the extract residue obtained after drying of lyophilisation and W2 is the weight of the plant part powder used for the extraction.

Plants antibacterial activity evaluation

In the antibacterial tests, Kirby-Bauer method was performed according to the Clinical Laboratory Standards Institute guidelines [19]. Extracts were dissolved in dimethyl sulfoxide (DMSO) to obtain final concentration of 100 mg/ml and sterilized through Millipore filter (0.22 mm). Then 10 µl were loaded over sterile filter paper discs (6 mm in diameter) Disks are left under the hood for a few minutes at room temperature.

An overnight culture of each strain with 0.5 McFarland turbidity was swabbed onto the Mueller-Hinton agar (Oxoid, UK) surface using sterile swab sticks. The paper discs prepared with the extracts were then placed onto the inoculated agar incubated at 37°C for 24h. Antibiotic discs (Amoxicillin) was used as positive control and paper discs soaked in DMSO without extract were used as negative control (DMSO didn't show inhibition effects to the growth of bacterial strains used).

Antibacterial activity is recorded when an inhibition zone diameter more than 9 mm is observed around the paper disk [20]. The inhibition zone diameters were measured with vernier calliper.

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of active extracts

Determination of minimum inhibitory concentrations (MIC) and minimum Bactericidal Concentration (MBC) of the extracts was done using the agar microdilution method [21]. The extracts that showed antimicrobial activity were selected to determine the MIC and the MBC for *Staphylococcus aureus* strains. Seven strains (1 SASM, 2 SARM, 3 SARM/MDR, and 1 SR) were selected and grown in nutrient broth for theses assays.

Each previously prepared and sterilized plant extract which showed antibacterial activity in the previous test was transferred in sterile 96 wells-plates (Nunc) previously filed with sterile nutrient broth to obtain a serial dilutions ranging from 20 to 0.0975 mg/ml. Then plates were inoculated with microbial suspensions diluted from the same 0.5 MacFarland standards to have 1 to 2 x 10^6 CFU/ in each well. Some wells were reserved in each 96 wells plate for sterility control (no microorganism added), inoculums vi-

ability (no extract added) and the DMSO inhibitory effect. The final volumes in wells were 200 µL. Afterwards 24h at 37°C, the MIC of each sample was appreciated visually by appreciating the growth of the microorganisms in each well (presence or absence of turbidity and/or a pellet in the well) with comparison to sterile and non-inoculated nutrient broth. The MIC is the lowest concentration of antimicrobial agent that completely inhibits growth of the studied microorganism. The experiment was replicated 3 times.

To determine the MBC, the microbial suspension was taken from the wells of concentration greater than or equal to MIC and inoculated on the Mueller-Hinton agar and then incubated for 24 hours at 37°C. The lowest extracts concentration at which no growth was observed on the agar after 24h of incubation is considered as MBC. The MBC was defined as the lowest extract concentration at which 99.9% of the bacteria have been killed. The experiment was replicated 3 times. The ratio MBC/ MIC was used to determine the intrinsic activity (bactericidal or bacteriostatic) of plant extracts considering that:

- When MBC/MIC = 1 or 1 <MBC/MIC ≤ 4, it shows absolute bactericidal activity.
- When 8 < MBC/MIC < 16, it shows bacteriostatic activity.

Data analysis

The data were described as mean standard deviation (S.D.) using SPSS for Windows version 20. (Statistical Package for Social Services, Chicago, IL, USA).

Results

Extraction yields

The yields of the studied extracts are summarised in table 2. There was no significant difference between yields of aqueous extracts and ethanol extracts. Yields varied with plant used. The extracts yielded from 0.25 to 2.72%. The highest yield of plant extract was obtained from *Kalanchoe crenata* residue dried (2.72%) followed by *Mitragyna inermis* (2.63%) while *Vetiveria nigritana* give the lowest extract yield respectively (Table 2).

Susceptibility to antibiotics of tested *Staphylococcus aureus* strains

Out of the 40 *S. aureus* strains selected and tested, 9 (22.5%) were MRSA and 20 (50%) were *Staphylococcus aureus* Methicillin-Resistant-Multidrug resistant (MRSA/MDR) (Table 3). Multidrug resistance was defined as a resistance to at least one agent in three or more antimicrobial. It can be noticed the high level of MRSA/ MDR among tested *S. aureus* strains.

Plant extracts	Yield(%)
DDM	1.70
DDK	2.72
DDV	0.27
EDV	0.72
EDM	2.63
EDK	2.17
ELK	1.18
ELV	0.87
ELM	1.92
DLM	1.54
DLV	0.85
DLK	2.59

Table 2: Plant extracts yield percentage.

DDM: Decoction Extract Dried of *M. inermis;* DDK: decoction extract dried of *K. crenata*; DDV: Decoction Extract Dried of *V. nigritana;* EDV: Ethanol Extracts Dried of *V. nigritana*; EDM: Ethanol Extracts Dried of *M. inermis*; EDK: Ethanol Extracts Dried of *K. crenata*; ELK: Ethanol Extracts Lyophilized of *K. crenata*; ELV: Ethanol Extracts Lyophilized of *V. nigritana*. ELM: Ethanol Extracts Lyophilized of *M. inermis*; DLM: Decoction Extract Lyophilised of *M. inermis*; DLV: Decoction Extract Lyophilized of *V. nigritana*; DLK: Decoction Extract Lyophilised of *K. crenata.*

Antibiotic sensitivity	Number of strain (N (%))
MSSA	11(27.5)
MRSA	9(22.5)
MRSA/MDR	20(50)

Table 3: Antibiotic sensitivity pattern of *Staphylococcus aureus* isolates.

MRSA: Methicillin Resistant *Staphylococcus aureus*; MRSA/ MDR: Methicillin Resistant *Staphylococcus aureus*/Multidrug Resistant.

Antibacterial activity of plant extracts

The results of our investigations to evaluate the antibacterial activity of the 3 medicinal plants against *Staphylococcus aureus* strains showed variable antimicrobial activity. Al the 3 plants exhibited inhibitory effect against studied microorganisms but this effect depend on the type of extract.

It can be seen that ethanol extract of *Mitragyna inermis* gave larger diameter of inhibition zone (DIZ) than aqueous extract (Table 4). Decocted and oven-dried extract did not show antibacterial

Strains code	Origin	Strain type	Decoction extracts		Ethanol extracts		Amox
			Oven-dried	lyophilised	Oven-dried	lyophilised	
SA1	com	MRSA/MDR	6 ± 0.00	7 ± 1.44	9.5 ± 0.72	11 ± 0.72	14 ± 1.44
SA2	hosp	MRSA/MDR	6 ± 0.00	7 ± 1.44	10 ± 0.00	11.5 ± 0.72	9 ± 1.44
SA3	hosp	MRSA/MDR	6 ± 0.00	8 ± 0.00	11 ± 0.00	12 ± 0.00	11.5 ± 0.72
SA4	com	MRSA/MDR	6 ± 0.00	9 ± 0.00	10.5 ± 0.72	12 ± 0.00	8.5 ± 0.72
SA5	hosp	MRSA/MDR	6 ± 0.00	9 ± 0.00	10 ± 0.00	12 ± 0.00	14.5 ± 0.72
SA6	hop	MRSA/MDR	6 ± 0.00	9 ± 0.00	11 ± 0.00	12 ± 0.00	10.5 ± 0.72
SA7	com	MRSA/MDR	6 ± 0.00	6 ± 0.00	10.5 ± 0.72	12.5 ± 0.72	14.5 ± 0.72
SA8	com	MRSA/MDR	6 ± 0.00	6 ± 0.00	10 ± 0.00	10.5 ± 0.72	10 ± 0.00
SA9	com	MRSA/MDR	6 ± 0.00	7 ± 0.00	10 ± 0.00	12 ± 0.72	9.5 ± 0.72
SA10	com	MRSA/MDR	6 ± 0.00	9 ± 0.00	11 ± 0.00	12.5 ± 0.72	8 ± 0.00
SA11	com	MRSA/MDR	6 ± 0.00	8.5 ± 0.72	10.5 ± 0.72	11 ± 0.00	15 ± 1.4
SA12	hosp	MRSA/MDR	6 ± 0.00	6 ± 0.00	10 ± 0.00	11.5 ± 0.72	13 ± 1.4
SA13	com	MRSA/MDR	6 ± 0.00	9 ± 0.00	11 ± 0.00	11.5 ± 0.72	14.5 ± 0.72
SA14	com	MSSA	6 ± 0.00	6 ± 0.00	10.5 ± 0.72	12 ± 0.00	6 ± 0.00
SA15	com	MSSA	6 ± 0.00	9 ± 0.00	11 ± 0.00	11.5 ± 0.72	10.5 ± 0.72
SA16	com	MRSA/MDR	6 ± 0.00	8.5 ± 0.72	10 ± 0.00	12.5 ± 0.72	14 ± 1.44
SA17	com	MRSA/MDR	6 ± 0.00	6 ± 0.00	10 ± 0.00	11.5 ± 0.72	12 ± 0.00
SA18	com	MSSA	6 ± 0.00	9.5 ± 0.72	10 ± 0.00	11.5 ± 0.72	13.5 ± 0.72
SA19	hosp	MRSA	6 ± 0.00	6 ± 0.00	10 ± 0.00	12.5 ± 0.72	14.5 ± 0.72
SA20	hosp	MSSA	6 ± 0.00	6.5 ± 0.72	11.5 ± 0.72	11 ± 0.00	14 ± 0.00
SA21	com	MSSA	6 ± 0.00	9.5 ± 0.72	11.5 ± 0.72	12.5 ± 0.72	10.5 ± 0.72
SA22	hosp	MRSA/MDR	6 ± 0.00	6.5 ± 0.72	10.5 ± 0.72	12.5 ± 0.72	23.5 ± 0.72
SA23	hosp	MRSA/MDR	6 ± 0.00	6.5 ± 0.72	9 ± 1.44	11.5 ± 0.72	14.5 ± 0.72
SA24	com	MRSA	6 ± 0.00	9.5 ± 0.72	10.5 ± 0.72	11.5 ± 0.72	30 ± 0.00
SA25	com	MRSA	6 ± 0.00	8 ± 0.00	10 ± 0.00	12.5 ± 0.72	12 ± 0.00
SA26	hosp	MSSA	6 ± 0.00	9.5 ± 0.72	11.5 ± 0.00	11.5 ± 0.72	12.5 ± 0.72
SA27	hosp	MSSA	6 ± 0.00	7 ± 0.00	9 ± 0.00	12 ± 0.00	32 ± 1.44
SA28	com	MRSA	6 ± 0.00	8 ± 0.00	11 ± 1.44	11.5 ± 0.72	13 ± 1.44
SZ29	com	MRSA	6 ± 0.00	9 ± 1.44	11.5 ± 0.72	12 ± 0.00	145 ± 0.72
SA30	com	MRSA	6 ± 0.00	7 ± 0.00	12 ± 0.00	11.5 ± 0.72	11 ± 1.44
SA31	hosp	MSSA	6 ± 0.00	6 ± 0.00	10.5 ± 0.72	11.5 ± 0.72	11 ± 1.44
SA32	hosp	MRSA	6 ± 0.00	7 ± 0.00	9 ± 0.00	10.5 ± 0.72	6 ± 0.00
SA33	hosp	MRSA	6 ± 0.00	6 ± 0.00	10 ± 0.00	10.5 ± 0.72	15.5 ± 0.72
SA34	hosp	MRSA	6 ± 0.00	6 ± 0.00	10.5 ± 0.72	10.5 ± 0.72	13.5 ± 0.72
SA35	hosp	MRSA/MDR	6 ± 0.00	7 ± 0.00	9.5 ± 0.72	11.5 ± 0.72	14.5 ± 0.72
SA36	hosp	MSSA	6 ± 0.00	6 ± 0.00	10 ± 0.00	12.5 ± 0.72	15.5 ± 0.72
SA37	com	MSSA	6 ± 0.00	7 ± 0.00	8.5 ± 0.72	11.5 ± 0.72	29.5 ± 0.72
SA38	com	MSSA	6 ± 0.00	7 ± 0.00	10 ± 1.44	12.5 ± 0.72	13 ± 1.44
SA39	hosp	MRSA/MDR	6 ± 0.00	6.5 ± 0.72	11.5 ± 0.72	12 ± 0.00	14.5 ± 0.72
SA40	hosp	MRSA/MDR	6 ± 0.00	6 ± 0.00	9 ± 0.00	10 ± 0.00	11 ± 1.44
S. aureus ATCC 25923	-	MSSA	6 ± 0.00	6.5 ± 0.72	10.5 ± 0.72	12 ± 1.44	31 ± 1.44

Table 4: Diameters of inhibition zone (mm) of *Mitragyna inermis* extracts.

Amox: Amoxicillin; com: Community-acquired *S. aureus*; hosp: Hospital-acquired *S. aureus.*

activity in this study. Lyophilized extracts (ethanol and aqueous) showed better activity against MRSA/MDR and MRSA. The higher DIZ obtained with *M. inermis* ethanol extract on either MRSA/MDA and MSSA was 12 mm.

As observed with the extracts of *Mitragyna inermis*, the aqueous extracts of *Vetiveria nigritana* (Table 5) and those of *Kalanchoe crenata* (Table 6) did not show antibacterial activity against *S. aureus* strains in this study. On the other hand, their ethanol extracts

Strains	Origin	Strain type	Decoction extracts		Ethanol extracts		Amox
			Oven-dried	Lyophilised	Oven-dried	Lyophilised	
SA1	com	MRSA/MDR	6 ± 0.00	6 ± 0.00	12.5 ± 0.72	9 ± 0.00	14 ± 1.44
SA2	hosp	MRSA/MDR	6 ± 0.00	6 ± 0.00	10 ± 1.44	8 ± 1.44	9 ± 1.44
SA3	hosp	MRSA/MDR	6 ± 0.00	6 ± 0.00	10 ± 0.00	7 ± 0.00	11.5 ± 0.72
SA4	com	MRSA/MDR	6 ± 0.00	6 ± 0.00	10.5 ± 0.72	7 ± 0.00	8.5 ± 0.72
SA5	hosp	MRSA/MDR	6 ± 0.00	6 ± 0.00	9.5 ± 0.72	6.5 ± 0.72	14.5 ± 0.72
SA6	hop	MRSA/MDR	6 ± 0.00	6 ± 0.00	9.5 ± 0.72	9 ± 0.00	10.5 ± 0.72
SA7	com	MRSA/MDR	6 ± 0.00	6 ± 0.00	10 ± 0.00	7 ± 0.00	14.5 ± 0.72
SA8	com	MRSA/MDR	6 ± 0.00	6 ± 0.00	10 ± 0.00	7 ± 0.00	10 ± 0.00
SA9	com	MRSA/MDR	6 ± 0.00	6 ± 0.00	10 ± 0.00	9.5 ± 0.72	9.5 ± 0.72
SA10	com	MRSA/MDR	6 ± 0.00	6 ± 0.00	10 ± 0.00	9 ± 0.00	8 ± 0.00
SA11	com	MRSA/MDR	6 ± 0.00	6 ± 0.00	9.5 ± 0.72	9 ± 0.00	15 ± 1.4
SA12	hosp	MRSA/MDR	6 ± 0.00	6 ± 0.00	11 ± 0.00	9.5 ± 1.72	13 ± 1.4
SA13	com	MRSA/MDR	6 ± 0.00	6 ± 0.00	10 ± 0.00	9 ± 0.00	14.5 ± 0.72
SA14	com	MSSA	6 ± 0.00	6 ± 0.00	12.5 ± 0.72	11.5 ± 0.72	6 ± 0.00
SA15	com	MSSA	6 ± 0.00	6 ± 0.00	11.5 ± 0.72	10 ± 0.00	10.5 ± 0.72
SA16	com	MRSA/MDR	6 ± 0.00	6 ± 0.00	10.5 ± 0.72	9.5 ± 0.72	14 ± 1.44
SA17	com	MRSA/MDR	6 ± 0.00	6 ± 0.00	10.5 ± 0.72	7.5 ± 2.1	12 ± 0.00
SA18	com	MSSA	6 ± 0.00	6 ± 0.00	10.5 ± 0.72	6.5 ± 0.72	13.5 ± 0.72
SA19	hosp	MRSA	6 ± 0.00	6 ± 0.00	8 ± 0.00	8.5 ± 0.72	14.5 ± 0.72
SA20	hosp	MSSA	6 ± 0.00	6 ± 0.00	9.5 ± 0.72	11.5 ± 0.72	14 ± 0.00
SA21	com	MSSA	6 ± 0.00	6 ± 0.00	10 ± 0.00	10 ± 0.00	10.5 ± 0.72
SA22	hosp	MRSA/MDR	6 ± 0.00	6 ± 0.00	8.5 ± 0.72	10.5 ± 0.72	23.5 ± 0.72
SA23	hosp	MRSA/MDR	6 ± 0.00	6 ± 0.00	9 ± 0.00	9.8 ± 0.72	14.5 ± 0.72
SA24	com	MRSA	6 ± 0.00	6 ± 0.00	11 ± 0.00	10 ± 2.88	30 ± 0.00
SA25	com	MRSA	6 ± 0.00	6 ± 0.00	8 ± 0.00	11 ± 0.00	12 ± 0.00
SA26	hosp	MSSA	6 ± 0.00	6 ± 0.00	13.5 ± 0.72	10 ± 1.44	12.5 ± 0.72
SA27	hosp	MSSA	6 ± 0.00	6 ± 0.00	11 ± 2.88	12 ± 1.44	32 ± 1.44
SA28	com	MRSA	6 ± 0.00	6 ± 0.00	6.5 ± 0.72	7 ± 1.44	13 ± 1.44
SZ29	com	MRSA	6 ± 0.00	6 ± 0.00	10 ± 0.00	11.5 ± 0.72	145 ± 0.72
SA30	com	MRSA	6 ± 0.00	6 ± 0.00	11 ± 0.00	9.5 ± 0.72	11 ± 1.44
SA31	hosp	MSSA	6 ± 0.00	6 ± 0.00	10.5 ± 0.72	10.5 ± 0.72	11 ± 1.44
SA32	hosp	MRSA	6 ± 0.00	6 ± 0.00	10.5 ± 0.72	12.5 ± 0.72	6 ± 0.00
SA33	hosp	MRSA	6 ± 0.00	6 ± 0.00	7.5 ± 0.72	12.5 ± 2.16	15.5 ± 0.72
SA34	hosp	MRSA	6 ± 0.00	6 ± 0.00	8.5 ± 0.72	10.5 ± 0.72	13.5 ± 0.72
SA35	hosp	MRSA/MDR	6 ± 0.00	6 ± 0.00	8.5 ± 0.72	10.5 ± 0.72	14.5 ± 0.72
SA36	hosp	MSSA	6 ± 0.00	6 ± 0.00	8 ± 0.00	10.5 ± 0.72	15.5 ± 0.72

SA37	com	MSSA	6 ± 0.00	6 ± 0.00	9.5 ± 2.16	11 ± 1.44	29.5 ± 0.72
SA38	com	MSSA	6 ± 0.00	6 ± 0.00	8.5 ± 0.7	11.5 ± 0.72	13 ± 1.44
SA39	hosp	MRSA/MDR	6 ± 0.00	6 ± 0.00	9 ± 0.00	12.5 ± 0.72	14.5 ± 0.72
SA40	hosp	MRSA/MDR	6 ± 0.00	6 ± 0.00	8 ± 1.44	11.5 ± 0.72	11 ± 1.44
S. aureus ATCC 25923	-	MSSA	6 ± 0.00	6 ± 0.00	9 ± 0.00	10 ± 1.44	31 ± 1.44

Table 5: Diameters of inhibition zone (mm) of *Vetiveria nigritana* extracts.

Amox: Amoxicillin; com: Community-acquired *S. aureus*; osp: hospital-acquired *S. aureus.*

showed moderate antibacterial activity. There was no significant difference in the antibacterial activity for MRSA/MDR, MRSA or MSSA. The DIZ obtained with plant extracts were more or less comparable to those obtained with the reference antibiotics used. However, it should be noted that the extract disc loads were of the order of milligrams while those of the antibiotics had loads of the order of micrograms.

Minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) of the active plant extracts

The MIC and the MBC of the most effective plant extracts (ethanol extracts) were determined by microdilution method as described above. For *Mitragyna inermis* ethanol extracts, MIC ranged from 0.625 to 2.5 mg/ml and MBC from 5 to 10 mg/ml (Table 7). Similar results were recorded with the ethanol extracts of *Vetiveria*

Strains	Origin	Strain type	Decoction extracts		Ethanol extracts		Amox
			Oven-dried	Lyophilised	Oven-dried	Lyophilised	
SA1	com	MRSA/MDR	6 ± 0.00	9 ± 0.00	12 ± 1.44	10.5 ± 0.72	14 ± 1.44
SA2	hosp	MRSA/MDR	6 ± 0.00	9.5 ± 0.00	11.5 ± 0.72	11.5 ± 0.72	9 ± 1.44
SA3	hosp	MRSA/MDR	6 ± 0.00	9.5 ± 0.72	11 ± 0.00	12 ± 0.00	11.5 ± 0.72
SA4	com	MRSA/MDR	6 ± 0.00	10.5 ± 0.72	12 ± 0.00	12.5 ± 0.72	8.5 ± 0.72
SA5	hosp	MRSA/MDR	6 ± 0.00	7.5 ± 2.16	12 ± 0.00	10.5 ± 0.72	14.5 ± 0.72
SA6	hosp	MRSA/MDR	6 ± 0.00	9 ± 0.00	11.5 ± 0.72	12 ± 0.00	10.5 ± 0.72
SA7	com	MRSA/MDR	6 ± 0.00	10 ± 1.44	12 ± 0.00	11.5 ± 0.72	14.5 ± 0.72
SA8	com	MRSA/MDR	6 ± 0.00	6 ± 0.00	11.5 ± 0.72	11 ± 0.72	10 ± 0.00
SA9	com	MRSA/MDR	6 ± 0.00	7 ± 1.44	11 ± 0.00	11.5 ± 0.72	9.5 ± 0.72
SA10	com	MRSA/MDR	6 ± 0.00	9.5 ± 0.72	11 ± 0.00	12.5 ± 0.72	8 ± 0.00
SA11	com	MRSA/MDR	6 ± 0.00	8.5 ± 0.72	11.5 ± 0.72	11 ± 0.00	15 ± 1.4
SA12	hosp	MRSA/MDR	6 ± 0.00	7 ± 0.00	12 ± 0.00	11 ± 0.00	13 ± 1.4
SA13	com	MRSA/MDR	6 ± 0.00	9 ± 0.00	11 ± 0.00	12 ± 0.00	14.5 ± 0.72
SA14	com	MSSA	6 ± 0.00	8 ± 0.00	12.5 ± 0.72	12 ± 1.4	6 ± 0.00
SA15	com	MSSA	6 ± 0.00	7 ± 0.00	10.5 ± 0.72	11 ± 0.00	10.5 ± 0.72
SA16	com	MRSA/MDR	6 ± 0.00	8.5 ± 0.00	11.5 ± 0.72	11.5 ± 0.72	14 ± 1.44
SA17	com	MRSA/MDR	6 ± 0.00	9 ± 0.00	11 ± 0.00	11.5 ± 0.72	12 ± 0.00
SA18	com	MSSA	6 ± 0.00	6.5 ± 0.72	18 ± 0.00	11.5 ± 0.72	13.5 ± 0.72
SA19	hosp	MRSA	6 ± 0.00	10 ± 0.00	13.5 ± 2.1	12.5 ± 0.72	14.5 ± 0.72
SA20	hosp	MSSA	6 ± 0.00	10 ± 0.00	11.5 ± 0.72	11 ± 1.4	14 ± 0.00
SA21	com	MSSA	6 ± 0.00	8.5 ± 0.72	11.5 ± 0.72	12.5 ± 0.72	10.5 ± 0.72
SA22	hosp	MRSA/MDR	6 ± 0.00	9 ± 0.00	11.5 ± 0.72	11.5 ± 0.72	23.5 ± 0.72
SA23	hosp	MRSA/MDR	6 ± 0.00	6.5 ± 0.72	9.5 ± 0.72	11.5 ± 0.72	14.5 ± 0.72
SA24	com	MRSA	6 ± 0.00	10 ± 0.00	11.5 ± 0.72	12.5 ± 0.72	30 ± 0.00
SA25	com	MRSA	6 ± 0.00	8.5 ± 0.72	12.5 ± 0.72	11.5 ± 0.72	12 ± 0.00

SA26	hosp	MSSA	6 ± 0.00	9.5 ± 0.72	10.5 ± 0.72	11.5 ± 0.72	12.5 ± 0.72
SA27	hosp	MSSA	6 ± 0.00	8 ± 0.00	12.5 ± 2.16	11 ± 0.00	32 ± 1.44
SA28	com	MRSA	6 ± 0.00	10 ± 0.00	11 ± 0.00	13 ± 1.4	13 ± 1.44
SZ29	com	MRSA	6 ± 0.00	10.5 ± 0.72	12.5 ± 0.72	14.5 ± 0.72	145 ± 0.72
SA30	com	MRSA	6 ± 0.00	10 ± 0.00	11 ± 0.00	12.5 ± 0.72	11 ± 1.44
SA31	hosp	MSSA	6 ± 0.00	6.5 ± 0.72	11.5 ± 0.72	11.5 ± 0.72	11 ± 1.44
SA32	hosp	MRSA	6 ± 0.00	7.5 ± 0.72	12.5 ± 0.72	12.5 ± 0.72	6 ± 0.00
SA33	hosp	MRSA	6 ± 0.00	8.5 ± 0.72	11.5 ± 0.72	11.5 ± 0.72	15.5 ± 0.72
SA34	hosp	MRSA	6 ± 0.00	8.5 ± 0.72	10.5 ± 0.72	11.5 ± 0.72	13.5 ± 0.72
SA35	hosp	MRSA/MDR	6 ± 0.00	8.5 ± 0.72	12 ± 1.44	12 ± 1.4	14.5 ± 0.72
SA36	hosp	MSSA	6 ± 0.00	8.5 ± 0.72	9.5 ± 0.72	12.5 ± 0.72	15.5 ± 0.72
SA37	com	MSSA	6 ± 0.00	8 ± 0.00	10.5 ± 0.72	11.5 ± 0.72	29.5 ± 0.72
SA38	com	MSSA	6 ± 0.00	8.5 ± 0.72	11 ± 0.00	13 ± 0.00	13 ± 1.44
SA39	hosp	MRSA/MDR	6 ± 0.00	10.5 ± 0.72	12 ± 0.00	13 ± 0.00	14.5 ± 0.72
SA40	hosp	MRSA/MDR	6 ± 0.00	9 ± 0.00	11 ± 0.00	11 ± 0.00	11 ± 1.44
S. aureus ATCC 25923	SR	MSSA	6 ± 0.00	8.5 ± 0.72	10.5 ± 0.72	12 ± 1.44	31 ± 1.44

Table 6: Diameters of inhibition zone (mm) of *Kalanchoe crenata* extracts.

Amox: Amoxicillin; com: Community-acquired *S. aureus*; hosp: hospital-acquired *S. aureus.*

nigritana (Table 9). The MIC ranged from 0.625 to 2.5 mg/ml and MBC from 2.5 to 5 mg/ml for *Kalanchoe crenata* extracts (Table 8). According to the results, *Kalanchoe crenata* seems to have bactericidal effects toward all the strains (1 <MBC/MIC≤ 4). For *Mitragyna inermis* and *Vetiveria nigritana,* the intrinsic activity of the extracts was variable. A bacteriostatic effect was recorded with some strains (8 < MBC/MIC< 16) while bactericidal effect was observed with other strains (1 <MBC/MIC≤ 4).

Discussion

Antimicrobial susceptibility testing showed that most of the strains (50%) of the MRSA were multidrug-resistant. These results confirm the preponderance of antibacterial resistance of *Staphylococcus aureus* strains. Similar results have been observed by Gupta., *et al*. [22], Kengne., *et al*. [23], Garoy., *et al*. [24], Ibrahim., *et al*. [25] and Sinda., *et al* [26]. Nowadays, MRSA has become a

Strains	Strain type	*M. inermis* extracts	MIC (mg /ml)	MBC (mg /ml)	MBC/MIC
S. aureus ATCC 25923	MSSA	Oven-dried extract	1.25	10	8
		Lyophilized extract	0.625	10	16
SA1	MRSA/MDR	Oven-dried extract	1.25	5	4
		Lyophilized extract d	2.5	5	2
SA3	MRSA/MDR	Oven-dried extract	2.5	5	2
		Lyophilized extract	2.5	5	2
SA35	MRSA/MDR	Oven-dried extract	1.25	5	4
		Lyophilized extract	1.25	5	4
SA19	MRSA	Oven-dried extract	1.25	5	4
		Lyophilized extract	0.625	5	8
SA24	MRSA	Oven-dried extract	1.25	5	4
		Lyophilized extract	0.625	5	8
SA38	MSSA	Oven-dried extract	1.25	5	4
		Lyophilized extract	1.25	5	4

Table 7: MIC and MBC obtained with *Mitragyna inermis* extracts against *S. aureus strains.*

Strains	Strain type	*K. crenata* extracts	MIC	MBC	MBC/MIC
S. aureus ATCC 25923	MSSA	Oven-dried extract	1.25	2.5	2
		Lyophilized extract	1.25	2.5	2
SA1	MRSA/MDR	Oven-dried extract	2.5	5	2
		Lyophilized extract	1.25	5	4
SA3	MRSA/MDR	Oven-dried extract	0.625	2.5	4
		Lyophilized extract	0.625	2.5	4
SA35	MRSA/MDR	Oven-dried extract	1.25	2.5	2
		Lyophilized extract	1.25	5	4
SA19	MRSA	Oven-dried extract	2.5	5	2
		Lyophilized extract	1.25	2.5	2
SA24	MRSA	Oven-dried extract	2.5	5	2
		Lyophilized extract	1.25	5	4
SA38	MSSA	Oven-dried extract	1.25	5	4
		Lyophilized extract	1.25	5	4

Table 8: MIC and MBC obtained with *Kalanchoe crenata* extracts against *S. aureus* strains.

Strains	Strain type	*V. nigritana* extracts	MIC	MBC	MBC/MIC
S. aureus ATCC 25923	MSSA	Oven-dried extract	1.25	2.5	2
		Lyophilized extract	1.25	2.5	2
SA1	MRSA/MDR	Oven-dried extract	1.25	10	8
		Lyophilized extract	1.25	5	4
SA3	MRSA/MDR	Oven-dried extract	2.5	10	4
		Lyophilized extract	1.25	5	4
SA35	MRSA/MDR	Oven-dried extract	0.625	2.5	4
		Lyophilized extract	1.25	5	4
SA19	MRSA	Oven-dried extract	1.25	5	4
		Lyophilized extract	1.25	2.5	2
SA24	MRSA	Oven-dried extract	0.625	5	8
		Lyophilized extract	1.25	10	8
SA38	MSSA	Oven-dried extract	1.25	10	8
		Lyophilized extract	1.25	5	4

Table 9: MIC and MBC obtained with *Vetiveria nigritana* extracts against *S. aureus*.

pathogen bacterium with alarming therapeutic problems. This is probably related to its rapid spread and its capacity to acquire resistance to commonly used antibiotics. It would be necessary to search for new active molecules that could limit the diffusion of these MRSAs and ensure the inhibition of their growth without developing resistance to them. Medicinal plants have been widely used to treat a variety of infectious and non-infectious ailments. According to one estimate, 25% of the commonly used medicines contain compounds isolated from plants [27]. Most of medicinal plants, are well known for their antibacterial, antifungal, antiviral properties [28]. These medicinal plants could be the sources of the treatment of SARM related infections and solve the problem of staphylococcal resistance to current antibacterial agents.

The present study was designed to evaluate the antistaphylococcal properties of extracts from three plants medicinal plants.

The highest yield of plant extract was obtained from *Kalanchoe crenata* (2.72%) followed by *Mitragyna inermis* (2.63%) while *Vetiveria nigritana* gave the lowest extract yield. These yields were similar to those obtained by Djoko., *et al*. [29] with *Kalanchoe crenata* and those obtained by [29,30] and [31] respectively with *Vetiveria nigritana*. The differences between yields could be due to the differences of extraction methods, the extraction time, the nature of the extract or the part of the plant used.

In the present study, the antimicrobial activity of extracts of three diverse medicinal plants prepared in different solvents was evaluated against *S. aureus* strains. The extracts showed zones of inhibition which prove the existence of antimicrobial activity against all the MRSA tested. Prior studies have showed the antibacterial activities of *Mitragyna inermis*, *Vetiveria nigritana*, *Kalanchoe crenata* against *S. aureus strains* [30,32,33]. The highest antimicrobial activity was observed with ethanol extracts of the three plants. No antimicrobial activity was observed the decoction extracts in this study. These results suggest that antimicrobials activity depend of the solvent used for the extraction. Previous research has shown that ethanol was a better solvent than water for active compounds extraction [34]. In fact, the organic solvents have better results as compared to water [35,36]. The overall findings supported the existence of antibacterial metabolites in the crude extract of *Mitragyna inermis, Vetiveria nigritana, Kalanchoe crenata* against MRSA strains and MRSA/MDR strains.

The range of MIC and MBC of the different extracts observed was 0.625 to 10 mg/ml for *Mitragyna inermis* extracts. Similar MIC (0.625 mg/ml) were obtained with the alkaloids of Mitragyna inermis against *Staphylococcus aureus* strains [32]. Similar results were recorded with the ethanol extracts of *Vetiveria nigritana*. However, MIC obtained with essential oils from *Vetiveria nigritana* (800 ug/ml) was lower than MIC obtained with solvent extracts [37]. However, the extracts of *Mitragyna inermis* and *Vetiveria nigritana* showed bacteriostatic and bactericidal effects against the studied strains. The extracts of *Kalanchoe crenata* seem to have bactericidal effects on the studied strains. The variation in the antimicrobial activities may be attributed to differences in the time of harvest [38], the developmental stage of plants and the method of extraction [39]. The study have shown that all plants used have overall bactericidal effects on MRSA and that would justify their use in the traditional against infections.

Conclusion

Results of this study shows *in vitro* antimicrobial activity of *Mitragyna inermis*, *Vetiveria nigritana* and *Kalanchoe crenata* against *Staphylococcus aureus* isolates including MRSA and MRSA/MDR. Ethanol extracts of these medicinal plants were found to have the strongest and broadest action spectrum. This study brings scientific evidence of the use of these medicinal plants traditionally to combat microbial infections. These plants can be used to formulate a traditional ameliorated herbal medicine against staphylococcal infections and also sources of new actives molecules to fight emerging MRSA.

Acknowledgments

The authors would like to acknowledge the Biodiversity Information Centre of University Joseph KI-ZERBO for their assistance in plant identification. They sincerely thank the association of traditional healers of Ouagadougou for their collaboration.

Conflict of Interest

The authors declare that they have no conflicts of interest regarding the data published in this article.

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