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Antistaphylococcal effects of alcoholic extracts of *Tetrapleura tetraptera* (Schum and Thonn.) (Taub.) against multidrug methicillin resistant *Staphylococcus aureus*

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Background: *Staphylococcus aureus* is a pathogen causing life-threatening hospital and community-acquired infections with high morbidity and mortality rates requiring constant vigilance.

Aim: This study aimed at investigating the antistaphylococcal effects of *Tetrapleura tetraptera* against different strains of multidrug methicillin resistant *S. aureus* (MRSA) to indicate the need for its use in ethnomedicine in addition to its fruits being used in traditional medicine.

Methods: In this study, the susceptibilities of *S. aureus* were investigated using multi-disc antibiotics and extracts of *T. tetraptera* by agar diffusion and macrobroth dilution methods.

Settings: While attention has been focused on the fruits of this plant, it is necessary to investigate the pharmacological importance of its stem bark.

Results: The antibiogram showed that 70% of the isolates were multidrug resistant. Nitrofurantoin and gentamicin antibiotics were the most effective whilst amoxicillin and augumentin were the least effective. The susceptibility of the isolates was concentration dependent as inhibition zones decreased with decrease in the concentrations of each of the extracts. The minimum inhibitory concentrations (MICs) of acetone extract ranged between 0.019 mg/mL and 20 mg/mL whilst the minimum bactericidal concentrations (MBCs) ranged between 0.3125 mg/mL and 20 mg/mL. The MICs of the methanol extract ranged between 0.039 mg/mL and 5.0 mg/mL whilst the MBCs ranged between 0.3125 mg/mL and 10 mg/mL. Both extracts were more bactericidal than being bacteriostatic against all the isolates. The methanol extract was more active than the acetone extract as indicated by the varied inhibition zones and MICs obtained from the different extracts.

Conclusion: This study revealed the great therapeutic potentials of *T. tetraptera* and validated its use in ethnomedicine and would be effective in the treatment of multidrug and MRSA infections.

Keywords: antistaphylococcal activities; macrobroth dilution assay; methicillin resistant bacteria; nosocomial infections; pharmacological activity; *Tetrapleura tetraptera.*

Introduction

Staphylococcus aureus is one of the most prevalent Gram-positive cocci found as transient normal floral of human skin and mucosal surfaces in 20% – 90% of human population (Falugi et al. 2013). As an established cause of superficial and deep life-threatening infections (Moreillon, Que & Glauser 2005), it is also an opportunistic pathogen causing skin lesions, abscesses, endocarditis, septicaemia, post-operative wound infections, nosocomial bacteraemia and toxic shock syndrome (Grothe et al. 2009) because of production of enzymes, toxins and release of multiple redundant virulence factors (Lacey, Geoghegan & McLoughlin 2016). Although a leading cause of hospital and community-acquired infections are associated with high morbidity and mortality rates, the high mortality rate of 6.0% – 46.5% because of *S. aureus* infections (Nickerson et al. 2009) has made this pathogen an important subject of constant vigilance.

As a result of global abuse and misuse of antibiotics used in treating human infections, resistance of pathogenic microorganisms to commonly used antibiotics has become a worldwide problem (Khorvash et al. 2012). In several African countries, methicillin resistant *S. aureus* (MRSA) became a major problem (Ahmed et al. 2012). Globally, MRSA infections have emerged as a major public health problem and a leading cause of nosocomial infections (Zhang 2001) and community-based infections such as soft tissue infections, septic arthritis, bacteraemia, toxic shock syndrome, necrotising fasciitis and necrotising pneumonia (Gillet et al. 2002). Although MRSA has become an endemic pathogen worldwide and exhibits multidrug resistance especially to both quinolones

and aminoglycosides (Batt et al. 2015), accurate and quick identification of MRSA in clinical specimens are essential for timely decision on effective antimicrobial chemotherapy, especially through naturally derived phytochemicals or natural products.

Tetrapleura tetraptera (Fabaceae Family) is a medium-sized deciduous plant with fern-like foliage. It is a perennial plant that grows in the lowland forest along the Western Coast of Africa. The dried fruit has a pleasant aroma (Aladesanmi 2007) and is popularly used as a seasoning spice in Southern and Eastern Nigeria (Okwu 2003). *It* has many medicinal uses involving the uses of its leaves, fruit, bark and pod. The bark of this plant contains triterpenes (Note et al. 2009) with antiplasmodial activity (Lekana-Douki et al. 2011). Aderibigbe et al. (2007) indicated that it has neuropharmacological activity whilst the fruits are used as antidiabetic and anti-inflammatory agents (Ojewole 2005). From literature search, it was observed that much attention has been focused on the fruits of this plant because of its use as spices whilst little effort has been directed towards investigating the pharmacological importance of its stem bark. Thus, investigating the antibacterial activities of the stem bark of this plant will add more knowledge to its pharmacological relevance. This study, therefore, investigated the antistaphylococcal effects of acetone and methanol stem bark extracts of *T. tetraptera* against different strains of multidrug MRSA to indicate the need for its use in ethnomedicine in addition to its fruits being used in ethnomedicine.

Materials and methods

Collection of plant material

The bark materials of *T. tetraptera* (Taub.) were collected from the plant growing within a rural settlement in Ogun State, Nigeria. The plant was authenticated ethnobotanically whilst voucher specimen was being prepared. The bark sample was air-dried at room temperature, pulverised with a milling machine and extracted as described by Olajuyigbe and Afolayan (2012). This study was carried out in the Department of Microbiology of our University.

Test organisms and inocula preparation

The bacteria used in this study included 18 clinical strains of *S. aureus* obtained from urinary tract infections and two typed strains including *S. aureus* (ATCC 6538 and NCTC 6571) used as control. Bacteriologically, each of the clinical strains of *S. aureus* was streaked on mannitol salt agar (MSA) and nutrient agar, which were incubated overnight at 37 °C for 24 h – 48 h. The bacterial colonies were subjected to Gram staining, microscopic appearance, colony morphology and biochemical tests such as tube coagulase test according to standard protocols (Cheesbrough 2002, 2009). The inocula of the test *S. aureus* strains were prepared using the colony suspension method (EUCAST 2000). Colonies picked from 24-h-old cultures grown on nutrient agar were used to make suspensions of the test organisms in saline solution to give an optical density of approximately 0.1 at 600 nm. The suspension was then diluted 1:100 by inoculating 9.9 mL of sterile nutrient broth with 100 µL of the bacterial suspension and thoroughly agitated before being used.

Antibiogram study to test the Staphylococcus aureus using multi-disc antibiotics

The antibacterial activity was determined using agar diffusion assay technique according to the modified Kirby–Bauer diffusion technique by swabbing the Mueller-Hinton agar (MHA) (Lab M, UK) plates with the adjusted suspension of each of the test strains. Multi-discs (Abtek) containing different antibiotics including augmentin (Aug) (30 µg), amoxycillin (Amx) (25 µg), cotrimazole (Cot) (25 µg), tetracycline (TET) (10 µg), gentamicin (Gen) (10 µg), ofloxacin (OFL) (5 µg), nalidixic acid (Nal) (30 µg) and nitrofurantoin (Nit) (200 µg) were aseptically placed on the inoculated agar plates before being incubated at 37 °C for 24 h. After 24 h of incubation, the plates were examined for inhibition zones (Bauer et al. 1966). The diameter of the inhibition zones produced by each antibiotic disk were measured to the nearest millimetre and recorded.

Antibacterial assay by agar diffusion (inhibition zones) methods

For the initial determination of the antibacterial activities of the crude acetone and methanol extracts of *T. tetraptera*, the susceptibility screening of the test strains to the extracts was determined using the modified Kirby–Bauer diffusion technique involving swabbing sterile MHA (Lab M Ltd., Quest Park, UK) plates with the resultant saline suspension of each adjusted strain of *S. aureus*. Different concentrations of each of the extracts were prepared by dissolving known weights of the extracts in the extracting solvents. Wells, later filled with 100 µL of different concentrations (C1 = 20 mg/mL, C2 = 40 mg/mL, C3 = 60 mg/mL, C4 = 80 mg/mL and C5 = 100 mg/mL) of each of the extracts taking care not to allow spillage of the extracts onto the agar surface, were bored into the agar medium with a heat sterilised 6-mm-cork borer. The culture plates were allowed to stand on the laboratory bench for 1 h to allow proper diffusion of these solutions before incubating at 37 °C for 24 h. Wells in blank MHA containing 5% of each of the extracting solvents representing the final concentration in the test plates without the extract served as positive control. The determinations were performed in duplicates. After 24 h of incubation, the plates were examined for the presence of inhibition zones. Whilst the diameters of the inhibition zones produced by each concentration of each of the extracts were measured in millimetres (Clinical and Laboratory Standard Institute [CLSI] 2007) and interpreted using the CLSI zone diameter interpretative standards (CLSI 2008), the break point with an inhibition zone of diameter ≥ 11 was chosen for bacterial susceptibility to the plant extracts (Nyenje & Ndip 2011).

Macrobroth dilution for determining minimum inhibitory concentration

Minimum inhibitory concentration (MIC) defined as the lowest concentration, which resulted in maintenance or

reduction of inoculums' viability was determined by macrobroth tube dilution technique for the *S. aureus* strains. Different concentrations ranging from 0.0048 mg/mL to 20 mg/mL of each of the crude extract were prepared by serial dilutions in double strength Mueller-Hinton broth. Each tube was then inoculated with 100 µL of each of the adjusted strains. Two blank Mueller-Hinton broth tubes, with and without bacterial inoculation, were used as the growth and sterility controls. Assays were carried out in duplicate. The bacteria containing tubes were incubated at 37 °C for 24 h. The first tubes in the series with no visible growth after the incubation period were taken as the MICs.

Determination of minimum bactericidal concentrations

As the clinical occurrences of tolerance usually necessitate bactericidal testing, the minimum bactericidal concentration (MBC) was determined by sampling all the macroscopically clear tubes and the first turbid tube in the MIC series. Before being sampled, the tubes were gently mixed by flushing them with a sterile pipette and a 100 µL aliquot was removed. Each aliquot was placed on a single antibiotic-free nutrient agar plate in a single streak down the centre of the plate in accordance with the method of Shanholtzer et al. (1984). The samples were allowed to be absorbed into the agar until the plate surface appeared dry (after 30 min). The aliquot was then spread over the plate by making a lawn of the bacterial culture with sterile cotton swab. In many studies on microbial susceptibility, this subculturing method has been found satisfactory in eliminating the problem of antimicrobial agent carryover from the 100 µL subcultured volume (Fasching et al. 1990). The growth and sterility controls were sampled in the same manner. The MBC determining lawned plates were incubated for 24 h at 37 °C. After the incubation periods, the lowest concentrations of the extract that did not produce any bacterial growth on the solid medium were regarded as the MBC values for each of these crude extracts. This observation was matched with the MIC test tube that did not show evidence of growth after 48 h of incubation.

Determination of mechanisms of antibiosis (bactericidal or bacteriostatic)

The mechanism of antibiosis of the extracts was calculated using the ratio of MBC/MIC or MIC_{index} as described by Shanmughapriya et al. (2008) to elucidate whether the observed antibacterial effects were bactericidal or bacteriostatic. When the ratio of MBC/MIC was ≤ 2.0, the extract was considered bactericidal or otherwise bacteriostatic. If the ratio is ≥16.0, the extract was considered ineffective.

Statistical analysis

Data expressed as mean ± standard deviation (SD) of results obtained from duplicate determinations were analysed using Statistical Programme for Social Sciences (SPSS) V.16 (Statistical Programme for Social Sciences, SPSS Corporation, Chicago, IL). One way analysis of variance (ANOVA) were used to determine the differences amongst the mean and $p < 0.05$ were regarded to be significant.

Results

In this study, the susceptibility of different strains of *S. aureus* was investigated using multi-disc antibiotics and different extracts of *T. tetraptera* by agar diffusion assay whilst the degree of the antibacterial activities of the extracts was determined by macrobroth dilution methods. From the susceptibility studies using multi-disc antibiotics, 70% of the isolates exhibited multidrug resistance whilst they exhibited varied degree of susceptibility to different antibiotics used. For all the isolates, Nit and Gen inhibited the highest number of isolates with inhibition zones of 20–37 ± 1.0 mm for Nit and between 15 and 33 ± 1.0 mm for Gen. Nit and Gen both inhibited 13 of the isolates. Whilst TET inhibited 12 isolates with inhibition zones between 13 and 40 ± 1.0 mm and cotrimoxazole inhibited 11 isolates with inhibition zones between 18 and 40 ± 1.0 mm. Ofloxacin inhibited 10 of the isolates and had inhibition zones ranging between 20 and 46 ± 1.0 mm, Nal inhibited eight isolates with inhibition zones between 24 and 34 ± 1.0 mm and amoxicillin inhibited two isolates and augumentin inhibited one of these two isolates to produce higher inhibition zone of 28 mm ± 1.0 mm against 16 mm ± 1.0 mm produced by amoxicillin as shown in Table 1.

The susceptibility of the different strains of *S. aureus*, used in this study, to the two extracts of *T. tetraptera* is presented in Table 2. The susceptibility of the isolates was concentration dependent as it decreased with decrease in the concentrations of each of the extracts. A total of 100 µL of different concentrations of each of the extracts produced varied degree of inhibition zones. At the lowest concentration of 20 mg/mL of acetone extract, 100 µL produced inhibition zones that ranged between 12 mm ± 1.0 mm and 18 mm ± 1.0 mm. At the highest concentration of 100 mg/mL of this extract, 100 µL produced inhibition zones that ranged between 17 mm ± 1.0 mm and 25 mm ± 1.0 mm. At the lowest concentration of 20 mg/mL of the methanol extract, 100 µL produced inhibition zones that ranged between 13 mm ± 1.0 mm and 20 mm ± 1.0 mm. At the highest concentration (100 mg/mL) of this extract, the inhibition zones ranged between 18 mm ± 1.0 mm and 26 mm ± 1.0 mm. Other concentrations in between the highest and the lowest concentrations also produced varied sizes of the inhibition zones.

The degrees of the antibacterial activities of *T. tetrapleura* against the clinical isolates of *S. aureus* as determined by the macrobroth dilution assay are presented in Table 3. The MICs of the acetone extract ranged between 0.019 mg/mL and 20 mg/mL whilst the MBC ranged between 0.3125 mg/mL and 20 mg/mL. Eight of the isolates had their MICs at concentrations less than 1 mg/mL. Three of the isolates had their MICs at concentrations equal to 1.25 mg/mL whilst the rest of the isolates had their MICs at concentrations greater than 2 mg/mL. Although it was

bacteriostatic against eight of the isolates, MIC_{index} showed that the extract was bactericidal against more of the isolates. For two of the isolates, SA13 and SA16, having their MBCs equal to 0.3125 mg/mL, the MBCs of the acetone extract were > 1 mg/mL.

Considering the antibacterial activities of the methanol extract, this extract inhibited all the isolates at concentrations ranging between 0.039 mg/mL and 5.0 mg/mL whilst the MBCs, generally, ranged between 0.3125 mg/mL and 10 mg/mL. Of the nine isolates that had their MICs at concentrations < 1 mg/mL, six isolates had their MICs at 0.625 mg/mL. With the exception of SA11 and SA14 having MBCs equal to 0.3125 mg/mL and those of SA1 and SA15 being 0.625 mg/mL, the MBCs of other isolates ranged between 1.25 mg/mL and 10 mg/mL. Whilst the MIC_{index} indicated that the extract was ineffective against SA1 and SA2 and bacteriostatic against six isolates, both extracts were

TABLE 1: Antibiotics resistance profile of different strains of *Staphylococcus aureus* as determined by disc diffusion assay using different antibiotic discs.

Test *Staphylococcus* strains	Inhibition zones produced by different antibiotics (±1.0 mm)							
	OFL (5 µg)	AUG (30 µg)	TET (25 µg)	AMX (25 µg)	COT (25 µg)	NIT (200 µg)	NAL (30 µg)	GEN (10 µg)
SA 1	40(S)	6(R)	40(S)	20(S)	34(S)	20(S)	32(S)	28(S)
SA 2	20(S)	6(R)	6(R)	6(R)	28(S)	6(R)	30(S)	16(S)
SA 3	29(S)	6(R)	6(R)	6(R)	26(S)	20(S)	28(S)	6(R)
SA 4	6(R)	6(R)	6(R)	6(R)	6(R)	6(R)	6(R)	6(R)
SA 5	6(R)	6(R)	16(S)	6(R)	6(R)	28(S)	6(R)	18(S)
SA 6	6(R)	6(R)	6(R)	6(R)	6(R)	6(R)	6(R)	6(R)
SA 7	24(S)	6(R)	6(R)	6(R)	6(R)	6(R)	25(S)	21(S)
SA 8	6(R)	6(R)	6(R)	6(R)	6(R)	6(R)	6(R)	6(R)
SA 9	6(R)	6(R)	6(R)	6(R)	6(R)	6(R)	6(R)	6(R)
SA 10	35(S)	6(R)	32(S)	6(R)	18(S)	33(S)	24(S)	23(S)
SA 11	29(S)	6(R)	32(S)	6(R)	23(S)	25(S)	30(S)	22(S)
SA 12	27(S)	6(R)	15(S)	6(R)	22(S)	30(S)	6(R)	32(S)
SA 13	46(S)	28(S)	28(S)	16(S)	40(S)	26(S)	34(S)	24(S)
SA 14	29(S)	6(R)	32(S)	6(R)	33(S)	23(S)	24(S)	27(S)
SA 15	6(R)	6(R)	6(R)	6(R)	6(R)	6(R)	6(R)	6(R)
SA 16	6(R)	6(R)	13(I)	6(R)	18(S)	35(S)	6(R)	33(S)
SA 17	6(R)	6(R)	14(I)	6(R)	20(S)	37(S)	6(R)	33(S)
SA 18	6(R)	6(R)	15(S)	6(R)	6(R)	30(S)	6(R)	17(S)
SA 19 ATCC 6538	25(S)	6(R)	34(S)	6(R)	23(S)	25(S)	6(R)	6(R)
SA 20 NCT 6571	6(R)	6(R)	16(S)	6(R)	6(R)	30(S)	6(R)	15(S)

S, *Staphylococcus aureus*; OFL, Ofloxacin; AUG, Augmentin; TET, Tetracycline; AMX, Amoxicillin; COT, Cotrimazole; NIT, Nitrofurantoin; NAL, Nalidixic acid; GEN, Gentamicin; R, Resistant; S, Sensitive; I, Intermediate susceptible.

TABLE 2: Antistaphylococcal activities of *Tetrapleura tetraptera* against *Staphylococcus aureus* by agar diffusion assay.

Test *Staphylococcus* strains	Average inhibition zones produced by 100 µL of different concentrations of the extract (±1.0 mm)									
	Acetone (mg/mL)					Methanol (mg/mL)				
	C5 = 100	C4 = 80	C3 = 60	C2 = 40	C1 = 20	C5 = 100	C4 = 80	C3 = 60	C2 = 40	C1 = 20
SA 1	22 ± 1.23	20 ± 0.58	18 ± 1.23	16 ± 0.58	15 ± 1.00	23 ± 0.00	20 ± 0.58	18 ± 0.58	16 ± 1.23	13 ± 0.00
SA 2	18 ± 0.58	17 ± 0.00	15 ± 0.58	15 ± 0.00	13 ± 0.00	20 ± 0.58	18 ± 0.58	17 ± 0.00	15 ± 0.58	13 ± 0.00
SA 3	18 ± 1.00	15 ± 0.58	13 ± 0.58	14 ± 1.00	13 ± 0.00	18 ± 1.23	15 ± 0.00	14 ± 0.00	13 ± 0.00	12 ± 0.00
SA 4	20 ± 0.58	18 ± 0.58	17 ± 0.00	15 ± 0.00	14 ± 0.00	22 ± 0.58	21 ± 0.58	19 ± 0.58	17 ± 0.00	15 ± 0.58
SA 5	22 ± 0.58	20 ± 1.00	18 ± 0.00	18 ± 1.00	16 ± 0.58	26 ± 0.58	23 ± 0.58	21 ± 0.58	20 ± 0.58	20 ± 0.58
SA 6	20 ± 0.58	17 ± 0.00	15 ± 0.58	14 ± 0.00	14 ± 0.00	22 ± 0.58	20 ± 1.00	17 ± 0.00	15 ± 1.00	14 ± 1.00
SA 7	19 ± 1.00	16 ± 0.58	15 ± 1.23	18 ± 0.58	13 ± 0.58	24 ± 0.58	21 ± 0.58	19 ± 1.00	17 ± 1.23	16 ± 0.58
SA 8	17 ± 1.00	16 ± 0.00	15 ± 0.58	15 ± 0.58	14 ± 0.00	22 ± 0.58	20 ± 0.00	17 ± 0.58	15 ± 0.00	12 ± 0.00
SA 9	18 ± 0.00	17 ± 0.00	15 ± 0.00	15 ± 0.00	13 ± 0.00	22 ± 0.58	20 ± 0.00	18 ± 0.58	15 ± 0.58	14 ± 0.58
SA 10	19 ± 0.58	16 ± 0.58	13 ± 0.00	18 ± 0.00	16 ± 0.58	18 ± 0.00	20 ± 0.58	18 ± 0.00	17 ± 1.00	15 ± 0.00
SA 11	21 ± 0.58	20 ± 0.58	18 ± 0.58	16 ± 0.58	15 ± 0.00	21 ± 0.58	18 ± 0.58	17 ± 1.23	15 ± 0.58	13 ± 0.58
SA 12	18 ± 1.23	18 ± 1.00	17 ± 0.58	16 ± 0.58	15 ± 0.00	19 ± 0.00	18 ± 0.00	19 ± 0.58	17 ± 0.00	15 ± 0.00
SA 13	20 ± 1.00	18 ± 0.00	16 ± 0.00	15 ± 0.00	14 ± 0.00	23 ± 0.58	23 ± 0.00	20 ± 0.58	18 ± 0.58	16 ± 0.58
SA 14	17 ± 0.58	15 ± 0.58	14 ± 0.00	13 ± 0.00	13 ± 1.00	21 ± 0.58	18 ± 0.58	17 ± 0.00	14 ± 0.00	12 ± 0.00
SA 15	20 ± 0.58	18 ± 1.00	17 ± 0.58	16 ± 1.00	14 ± 0.58	22 ± 1.23	20 ± 0.00	18 ± 0.58	16 ± 0.00	15 ± 0.00
SA 16	25 ± 0.58	23 ± 0.00	21 ± 0.00	20 ± 0.58	18 ± 0.58	26 ± 1.00	25 ± 0.58	23 ± 0.58	22 ± 0.58	20 ± 0.00
SA 17	25 ± 0.58	24 ± 0.58	20 ± 0.58	17 ± 0.00	15 ± 0.00	20 ± 0.58	18 ± 0.58	16 ± 0.00	15 ± 0.00	15 ± 0.58
SA 18	21 ± 0.58	20 ± 0.58	19 ± 0.58	17 ± 0.00	16 ± 1.23	22 ± 0.58	20 ± 1.23	19 ± 0.58	17 ± 0.58	15 ± 0.00
SA 19 ATCC 6538	15 ± 0.00	14 ± 0.00	15 ± 0.00	15 ± 1.23	12 ± 0.00	20 ± 0.00	18 ± 0.58	16 ± 1.00	15 ± 0.00	14 ± 0.58
SA 20 NCT 6571	21 ± 0.58	20 ± 0.58	18 ± 1.00	16 ± 0.00	13 ± 0.00	22 ± 0.58	21 ± 0.00	19 ± 0.58	17 ± 0.00	15 ± 1.00

SA, *Staphylococcus aureus*; C1 – C5, different concentrations of the extracts.

TABLE 3: Antistaphylococcal activities of *Tetrapleura tetraptera* against *Staphylococcus aureus* by macrobroth dilution assay.

Test *Staphylococcus* strains	Acetone				Methanol			
	MIC (mg/mL)	MBC (mg/mL)	MIC_{index}	Remarks	MIC (mg/mL)	MBC (mg/mL)	MIC_{index}	Remarks
SA 1	0.625	2.5	4	B'static	0.039	0.625	16	Ineffective
SA 2	2.5	2.5	1	B'cidal	0.625	10.0	16	Ineffective
SA 3	0.625	1.25	2	B'cidal	2.5	2.5	1	B'cidal
SA 4	20.0	20.0	1	B'cidal	5.0	5.0	1	B'cidal
SA 5	0.625	5.0	8	B'static	1.25	1.25	1	B'cidal
SA 6	> 20.0	> 20.0	1	B'cidal	5.0	10	2	B'cidal
SA 7	2.5	5.0	2	B'cidal	2.5	5.0	2	B'cidal
SA 8	> 20.0	> 20.0	1	B'cidal	5.0	10	2	B'cidal
SA 9	> 20.0	> 20.0	1	B'cidal	1.25	2.5	2	B'cidal
SA 10	2.5	5.0	2	B'cidal	2.5	10.0	4	B'static
SA 11	0.625	1.25	2	B'cidal	0.078	0.3125	4	B'static
SA 12	1.25	5.0	4	B'static	0.625	1.25	2	B'cidal
SA 13	0.019	0.3125	8	B'static	1.25	2.5	2	B'cidal
SA 14	1.25	5.0	4	B'static	0.019	0.3125	8	B'static
SA 15	2.5	20.0	8	B'static	0.625	0.625	1	B'cidal
SA 16	0.3125	0.3125	1	B'cidal	0.625	2.5	4	B'static
SA 17	0.156	1.25	8	B'static	0.625	2.5	4	B'static
SA 18	0.156	1.25	8	B'static	0.625	2.5	4	B'static
SA 19 ATCC 6538	> 20.0	> 20.0	1	B'cidal	5.0	5.0	1	B'cidal
SA 20 NCT 6571	1.25	2.5	2	B'cidal	5.0	10	2	B'cidal

SA, Different strains of *Staphylococcus aureus*; B'static, Bacteriostatic; B'cidal, Bacteriocidal; MIC, Minimum inhibitory concentrations; MBC, Minimum bactericidal concentrations.

bactericidal against 12 different isolates. The methanol extract was more active than the acetone extract as indicated by the varied inhibition zones produced from the least to the highest concentrations and ranges of MICs obtained from the different extracts.

Discussion

To effectively decrease the usage of vancomycin and discover an alternative therapeutic agent for treating infections caused by MRSA, interest in traditional and nonconventional medical treatment has increased tremendously. As a result, mixed and severe infections have been treated effectively and emergence of resistant microorganisms have been prevented whilst attention has been focused simultaneously on discovering new antimicrobial compounds of plant origin (Ncube, Afolayan & Okoh 2008). Eventually, natural products were regarded as interesting alternatives for the treatment of infections because they are rich in varieties of secondary metabolites with antimicrobial properties (Moglad 2021).

Although several studies have focused on the antibacterial activity of different plant extracts (Govindappa et al. 2011; Olajuyigbe & Afolayan 2018) and acetone and methanol solvents have been referred to as junk extractor pulling out more phytoconstituents than other solvents (Eloff 1998), attention is rarely focused on the comparative analysis of their antibacterial or therapeutic potentials whilst some school of thought has indicated that these two solvents should not be used together in extraction because of the closeness in their polarity. However, this study, using a single bacterial species considered having similar intrinsic morphological and physiological features and exhibiting significant multidrug resistant patterns, has focused on comparing the antibacterial activity of the extracts of these two solvents to either justify their use as extractors or agree to the above-mentioned acclaimed reasoning.

In this study, the antibacterial potential of the acetone and methanol extracts of *T. tetraptera* was significant even though the varied sensitivity observed may be attributed to different resistance levels between the strains (Ahmad & Aqil 2007). Whilst Rios and Recio (2005) suggested that MIC > 1 mg/mL of crude extracts should be disregarded and Simões, Bennett and Rosa (2009) reported that phytochemicals are routinely classified as antimicrobials when susceptibility tests had MICs in the range of 0.1 mg/mL – 1.0 mg/mL, Fabry, Okemo and Ansorg (1998) defined active crude extracts as those having MIC values < 8 mg/mL. For acetone extract, eight of the isolates had MICs < 1 mg/mL and 15 of the isolates had MICs < 8 mg/mL. For methanol extract, nine of the isolates had MICs < 1 mg/mL whilst MICs for all the isolates were ≤ 5 mg/mL. Hence, as lower MIC and MBC values indicate higher efficacy (Cowan 1999), the extracts were considered effective bactericidal agents as indicated by their mechanisms of antibiosis or MIC_{index}. By inference from data obtained, the methanol extract, in this study, was more effective and showed better antistaphylococcal activity than the acetone extract. The antistaphylococcal activity exhibited by the methanol extract may be attributed to the fact that more phytochemicals with higher therapeutic potentials may have been extracted by the methanol than what were extracted by the acetone. This is in agreement with the previously reported ethanol extract of *T. tetraptera* indicating MICs ranging between 0.0078 mg/mL and 10.0 mg/mL (Olajuyigbe & Afolayan 2018).

This study is in agreement with many studies that indicated that Gram-positive bacteria are often more inhibited by plant extracts than Gram-negative bacteria (Duraipandiyan & Ayyanar 2006), that the Gram-positive multidrug and MRSA strains were susceptible to both extracts showed that the extracts were able to overcome the permeability barrier provided by the cell wall and the membrane accumulated resistance mechanisms including efflux of antibacterial agents (Kim et al. 2013). Considering that most of these isolates were multidrug resistant, the ability of the extracts to modulate and overcome resistance is implied by the degree of antibacterial activities exhibited by the two extracts as Aburjai et al. (2001) and Darwish et al. (2002) indicated that many plants are known for their action as a resistance-modifying agent. The MBC values appeared to be more reliable than the MIC values because it indicated that the extracts are bactericidal (Olajuyigbe & Afolayan 2011) coupled with the resistance modulation effects of the extracts, however, further affirmed the tendency to achieve effective treatment of bacterial infection with these extracts when used against bacteria, which have become grossly resistant.

Conclusion

In conclusion, this study signified that methanol extract of the stem bark showed a better antibacterial activity at lower concentrations than the acetone extract and this may be attributed to the fact that methanol extracted more phytochemicals than the acetone. The present study indicated the potential effectiveness of this plant in the treatment of infections, which may involve multidrug MRSA and justify its use in ethnomedicine. This could help researchers to form the basis for selecting this plant species for isolating, identifying and comparing the bioactive constituents of these two extracts to justify the variation in the degree of the antibacterial activities of the plant.

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Competing interests

The authors declare that they have no financial or personal relationships that may have inappropriately influenced them in writing this article.

Authors' contributions

All authors contributed equally to this work.

Ethical considerations

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Data created or analysed in this study were included in this manuscript.

Disclaimer

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