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Full Length Research Paper

In vitro antibacterial activities of crude extracts of *Garcinia kola* seeds against wound sepsis associated *Staphylococcus* strains

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Extracts of *Garcinia kola* seeds were evaluated for their activity against four *Staphylococcus* strains isolated from wound sepsis specimens. Three of the isolates were identified by 16S rDNA sequencing as *Staphylococcus aureus* with the GenBank Accession numbers, EU244633, EU244634, EU244636, and another was identified as *Staphylococcus sciuri* (Accession number EU244635). The aqueous methanol and acetone extracts of *Garcinia kola* seeds showed activity against all four isolates at 30 mgml^{-1} (aqueous extract) and 10 mgml^{-1} (acetone and methanol extracts). The MIC values for the aqueous extract were the same (10 mgml^{-1}) for all the isolates. The acetone and methanol extracts had lower MIC values in the ranges of 0.3125 - 0.625 mgml^{-1}. The acetone extract showed strong bactericidal activity against *S. aureus* strain OKOH3 resulting in a 2.70 Log_{10} reduction in counts at 1.25 mgml^{-1} (2 × MIC) within 4 h of exposure and a complete elimination of the organism after 8 h. The same extract was weakly bactericidal against *S. aureus* strain OKOH1, achieving only a 2.92 Log_{10} reduction in counts at 1.25 mgml^{-1} (4 × MIC) in 24 h. The interactions between the acetone extract and antibiotics were largely additive and indifferent with no combinations showing classical synergistic interactions. We conclude that extracts of *Garcinia kola* seeds can potentially be useful in the treatment of staphylococcal wound infections.

Key words: *Staphylococcus*, wound sepsis, *Garcinia kola*, antistaphylococcal activity, antibiotic potentiation.

INTRODUCTION

The genus *Staphylococcus* is widely distributed in nature being part of the indigenous microflora of the skin and nasal cavities of healthy persons. This association with the skin presents the organisms with an opportunity to cause local infection of wounds. Among members of this genus, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus saprophyticus* and *Staphylococcus haemolyticus* are some of the species causing community and nosocomial human infections (Oliveira et al., 2006). In particular, *S. aureus* is the causative agent of a wide range of diseases, ranging from carbuncles and food poisoning, through more serious wound-related infections, to life threatening conditions, such as bacteremia, necrotizing pneumonia, and endocarditis (Holden et al., 2004). This species is capable of expressing a variety of virulence factors that it is almost always considered medically relevant when encountered in clinical specimens.

In addition to strains of *Staphylococcus* being signifycant pathogens in terms of the variety of infections that they cause, the organisms have been recognized as having the ability to develop changes in their sensitivity to antimicrobials (Oliveira et al., 2006). In the case of *S. aureus*, strains resistant to methicillin (MRSA) were first identified following the introduction of the antibiotic in clinical use in the 1960s (Lowy, 2003). At present MRSA strains are a common occurrence and are now virtually resistant to all beta-lactam antibiotics (Cook, 1998; Archer, 1998). In addition to the problem of treatment failure in MRSA infections, the infections have often been

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associated with severe illnesses resulting in increased hospitalization periods and mortality (Rello et al., 1994; Engemann et al., 2003). Until recently, the glycopeptide antibiotic vancomycin has been used as the drug of last resort for many MRSA infections, but recent trends show that vancomycin resistant strains have also emerged (Tenover et al., 2004). Reports of isolated cases of resistance to the newest anti-staphylococcal agent linezolid (a member of the oxazolidinone group that has been heralded as a solution to MRSA infections) have also been noted (Wilson et al., 2003).

Owing to their popular use in traditional medicine for the treatment of various ailments including infectious diseases, interest in medicinal plants as a source of novel antimicrobial compounds has been growing. A number of studies have validated the use of plants in the treatment of disease conditions (Samie et al., 2005; Akinpelu and Onakoya, 2006). A typical example is *Garcinia kola*, a tropical plant of the African Continent which has been a subject of investigation as a potential source of antimicrobial compounds (Madubunyi, 1995; Han et al., 2005).

While it is common practice that standard reference cultures of test isolates are used in evaluating the antimicrobial activity of plant extracts, the use of clinical isolates of pathogenic organisms may provide a more relevant and accurate prediction of the therapeutic potentials of plant extracts (Rios and Recio, 2005). While the antibacterial activity of *Garcinia kola* seed extracts has been demonstrated against reference strains of *Staphylococcus* (Ezeifeka et al., 2003; Akoachere et al., 2003), their efficacy against clinical strains of this organism particularly those associated with wound sepsis has not been documented.

In this paper, we report the antibacterial activity of extracts of *Garcinia kola* seeds against clinical isolates of Staphylococcus obtained from cases of wound sepsis as well as the potentials of the plant extracts in combination with six selected antibiotics.

MATERIALS AND METHODS

Isolation and identification of *Staphylococci* from wound sepsis specimens

Pus swabs obtained from three patients presenting with septic abrasion of the hip, wrist and elbow respectively were suspended in sterile physiological saline. One milliliter of the suspension was inoculated into 50 ml of sterile nutrient broth and incubated aerobically at 37°C for 24 h.

At the end of the incubation period, the broth cultures were streaked for isolation on mannitol salt agar. Presumptive *Staphylococcus* colonies were identified as golden yellow, circular, convex colonies that ferment mannitol. One colony from each specimen displaying the above morphology was purified by subculturing onto nutrient agar. Where more than one type of colonies displaying the cultural characteristics of *Staphylococcus* were observed on each plate, one colony from each colony type was selected and purified by subculturing onto nutrient agar. The presumptive *Staphylococcus* isolates were processed for molecular identification by 16S rRNA gene amplification and sequencing.

Amplification, sequencing and analysis of the 16S rRNA gene of the bacterial isolates

Total genomic DNA was isolated from LB-grown bacterial cultures using the QIAamp DNA miniprep kit, following the manufacturer's instructions and used directly as template for PCR amplification. The 16S rRNA genes of the bacterial isolates were amplified with the oligonucleotide primers: 63f (5'–CAGGCCTAACACATGCAAGTC-3') and 1387r (5'–GGGCGG(A/T)GTGTACAAGGC-3') described by Marchesi et al. (1998). The amplification reaction mixture contained standard Taq amplification buffer, 100 µM (each) deoxyribonucleotide triphosphate, 0.5 µM (each) primers, genomic DNA and 2.5 U of Taq DNA polymerase in a 50 µl reaction volume. The cycling parameters were 94°C for 2 min followed by 30 cycles of 92°C for 30 s, 55°C for 30 s, and 75°C for 45 s, with a final elongation step of 75°C for 5 min.

Amplification products were directly cycle sequenced using the Spectrumedix SCE2410 genetic analysis system with 24 capillaries. Big Dye version 3.1 dye terminator cycle sequencing kit (Applied Biosystems) was used for the sequencing reactions. The analysis of the 16S rRNA gene sequences of the bacterial isolates were carried out by comparing them with those in the GenBank database (http://www.ncbi.nlm.nih.gov) by using BLAST (Altschul et al., 1997) to determine the most similar sequences.

Preparation of plant extracts

The extracts of the plant were prepared in accordance with the description of Basri and Fan (2005). One hundred grams of seed powder was steeped in 500 ml of the respective solvent (water, acetone and methanol) for 24 h with shaking. The resultant extract was centrifuged at 3000 rpm for 5 min at 4°C. The supernatant was then filtered through a Whatman No.1 filter paper while the residue was used for a second extraction with 300 ml of the respective solvents. After the second extraction process, the aqueous extract was freeze-dried at -50°C under vacuum whereas the acetone and methanol extracts were concentrated under reduced pressure using a rotary evaporator at 50 and 65°C respectively. The concentrated extracts were then allowed to dry at room temperature to a constant weight.

Preparation of bacterial inocula

The inocula of the test organisms were prepared using the colony suspension method (EUCAST, 2003). Colonies picked from 24 h old cultures grown on nutrient agar were used to make suspensions of the test organisms in saline solution (0.85% NaCl) to give an optical density of approximately 0.1 at 600 nm. The suspension was then diluted 1:100 by transfer of 0.1 ml of the bacterial suspension to 9.9 ml of sterile nutrient broth before use.

Antibiotics used in this study

The following antibiotics which were available as powders were used in this study: Penicillin G sodium (Duchefa); Amoxycillin (Duchefa); Chloramphenicol (Duchefa); Tetracycline hydrochloride (Duchefa); Erythromycin (Duchefa) and Ciprofloxacin (Fluka).

Assay for antistaphylococcal activity

The antistaphylococcal activities of the crude extracts were carried out using the agar dilution method (Afolayan and Meyer, 1997). The extracts were incorporated directly into molten nutrient agar at 50°C

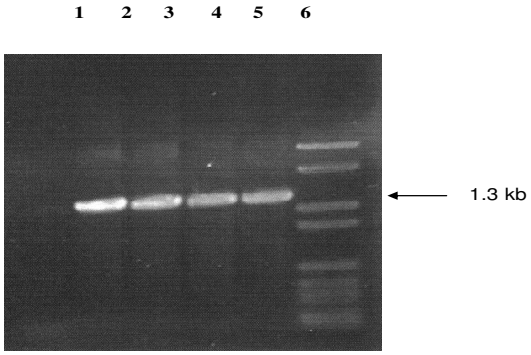

Figure 1. Gel picture of the 16S rRNA gene amplicon for OKOH1 (Lane 2), OKOH2A (Lane 3), OKOH2B (Lane 4) and OKOH3 (Lane 5). Lane 1 represents the negative control and Lane 6 is a molecular weight ladder.

to achieve concentrations of 30 mgml^{-1} for the aqueous extract and 10 mgml^{-1} for the acetone and methanol extracts. Dilutions of the acetone and methanol extracts were done in such a way that the final solvent concentration was 5% in the media. Standardised bacterial suspensions were used to inoculate the agar plates by streaking in duplicates. The inoculated plates were incubated under aerobic conditions at 37°C for 24 h. Positive controls consisted of extract free plates of nutrient agar inoculated with the test organisms. For the acetone and methanol extracts, controls consisted of nutrient agar plates with 5% of the respective solvent (which represented the final solvent concentration in the test plates). The absence of growth on the test plates compared with the positive controls was used to indicate the inhibitory activity of the extracts (Afolayan and Meyer, 1997).

Determination of the minimum inhibitory concentrations (MIC)

The minimum inhibitory concentrations of the extracts and antibiotics were determined using the agar dilution method following the standard protocol of the European Committee for Antimicrobial Susceptibility Testing (EUCAST, 2000a). The extracts and antibiotics were incorporated into molten nutrient agar at 50°C and allowed to solidify at room temperature. The dilutions of the extract ranged from 0.039 - 10 mgml^{-1} (acetone and methanol) and 0.625 - 20 mgml^{-1} (aqueous). The antibiotic test plates had concentrations ranging from 0.004 - 512 mgl^{-1}. Standardised inocula of test strains were used to seed the test plates in duplicates by streaking. Plates were incubated at 37°C for 24 h. The controls consisted of extract-free and antibiotic- free nutrient agar plates. The MIC value was taken as the lowest concentration of the extract or antibiotic showing complete lack of growth after the incubation period (EUCAST, 2000a).

Rate of kill experiment of the crude acetone extract

The rate of kill determination for the acetone extract was done by assaying of bacterial cell death over time following the description of Okoli and Iroegbu (2005). The assay was carried out using two of the *S. aureus* strains, OKOH1 and OKOH3. The assay method was a broth macrodilution based technique, with the extract incurporated into 50 ml nutrient broth in flasks, at concentrations of 1, 2 and 4 times the MIC values. Control flasks consisted of extract-free nutrient broth. The flasks were inoculated with standardized bacterial suspensions to a final cell density of approximately 10^5 cfuml^{-1}. The inoculated flasks were immediately incubated at 37°C with shaking. At intervals of 1, 2, 4, 8, 12 and 24 h, samples (100 µl) were withdrawn from each flask, diluted in tenfold series and plated out in duplicates on nutrient agar. Plates were incubated at 37°C for 24 h after which the number of survivors was enumerated.

Extract - antibiotic combination studies

The effect of combinations of the acetone extract of *Garcinia kola* seeds and antibiotics was evaluated by the use of the rate of kill assay following the descriptions of White et al. (1996) and Pankey et al. (2005) with modifications. The extract and antibiotics were incorporated into 50 ml of nutrient broth in flasks at concentrations equivalent to their respective MIC values for each test strain. Positive controls, consisting of the extract and antibiotic alone at the test concentrations were included in each experiment. The negative controls consisted of antibiotic- and extract-free broth.

The test and control flasks were inoculated with standardised suspensions of the test organisms to a final inoculum density of approximately 10^5 cfuml^{-1}. Immediately after inoculation, aliquots (100 µl) of the negative control flasks were taken, serially diluted in sterile saline and plated on nutrient agar in order to determine the zero hour counts. The test flasks were then incubated at 37°C with shaking at 120 rpm. After 24 h of incubation, aliquots (100 µl) were withdrawn from each test and control flask, serially diluted in sterile saline and plated (100 µl) on nutrient agar in duplicates. To improve the visual observation of colonies in the agar, 1 ml of a 0.5% aqueous solution of 2, 3, 5 triphenol tetrazolium chlorides (Neugebauer and Gilliland, 2005) were added to 100 ml of molten agar at 50°C before plating. The plates were then incubated at 37°C for 24 h under aerobic conditions after which, the number of colonies were enumerated.

The interactions between the extract and antibiotics were considered synergistic if there was a decrease of ≥ 2 Log_{10} cfuml^{-1} in colony counts at 24 h by the combination compared to the most active single agent (Pankey et al., 2005). Additivity or indifference was defined as a < 2 Log_{10} cfuml^{-1} change in the average of viable counts at 24 h for the combination, in comparison with the most active single drug. Antagonism was defined as a ≥ 2 Log_{10} cfuml^{-1} increase in colony counts at 24 h by the combination compared with that by the most active single agent (Pankey et al., 2005; Lee et al., 2006).

RESULTS

The polymerase chain reaction amplification of the 16S rRNA gene of the bacterial isolates resulted in the expected 1.3 kb amplicons (Figure 1). Partial sequencing of the amplified DNA revealed that three of the isolates had high (≥ 98%) sequence homology to *S. aureus* and have since been deposited in GenBank with Accession numbers EU244633, EU244634 and EU244636, while one of the isolates had a 100% sequence homology to *Staphylococcus sciuri* and has also been deposited in GenBank with Accession number EU244635 (Table 1).

The susceptibility profiles of the Staphylococcus isolates to the extracts of *Garcinia kola* seeds revealed that all the strains showed susceptibility to the aqueous extract at 30 mgml^{-1} as well as to the methanol and acetone extracts at 10 mgml^{-1}. All the isolates had identical MIC values for the aqueous extract (10 mgml^{-1}) and the

Table 1. Identity of bacterial isolates based on 16 rDNA sequencing.

Specimen source	Isolate identity	GenBank accession number
Septic abrasion (Hip)	*S. aureus* OKOH1	EU244633
Septic abrasion (Wrist)	*S. aureus* OKOH2A	EU244634
Septic abrasion (Wrist)	*S. sciuri* OKOH2B	EU244635
Septic abrasion (Elbow)	*S. aureus* OKOH3	EU244636

Table 2. Minimum inhibitory concentration (MIC) values for the crude extracts of *Garcinia kola* seeds and antibiotics against the *Staphylococcus* isolates.

Test Isolate	Extract MIC values ($mgml^{-1}$)			Antibiotics MIC values (mgl^{-1})					
	Aq	Met	Ace	Amx	PenG	Chlo	Tet	Ery	Cip
S. aureus OKOH1	10	0.312	0.312	1	0.25	4	0.25	0.25	0.5
S. aureus OKOH2A	10	0.312	0.312	1	0.5	4	0.25	0.5	0.5
S. sciuri OKOH2B	10	0.312	0.625	0.25	0.06	4	0.25	0.5	1
S. aureus OKOH3	10	0.312	0.625	1	1	4	0.25	0.5	0.5

Key: Aq - aqueous; Met - methanol; Ace - Acetone; Amx- amoxycillin; PenG- penicillin G; Tet-tetracycline; Chlo-chloramphenicol; Ery- erythromycin; Cip- ciprofloxacin

Table 3. The effect of combinations of acetone extract of *G. kola* seeds and antibiotics on Staphylococcus isolates.

Test Organism	Changes in bacterial counts (Log_{10} $cfuml^{-1}$) for the combination compared with the two agents used alone					
	Amx	PenG	Chlo	Tet	Ery	Cip
S. aureus OKOH1	-1.35(I)	-1.38(I)	-0.64(I)	-1.66(I)	-0.78(I)	-0.62(I)
S. aureus OKOH2A	-0.19(I)	0.40(I)	0.44(I)	0.37(I)	0.51(I)	1.27(I)
S. sciuri OKOH2B	0.13(I)	0.37(I)	0.26(I)	-0.88(I)	0.13(I)	0.04(I)
S. aureus OKOH3	-1.00(I)	-3.90(S)	-0.62(I)	-0.73(I)	-1.30(I)	-0.82(I)

Key: Amx - amoxycillin; PenG - penicillin G; Tet - tetracycline; Chlo - chloramphenicol; Ery - erythromycin; Cip - ciprofloxacin. (S) - Synergy; (I) - indifference/additivity

methanol extract (0.3125 $mgml^{-1}$) (Table 2). Susceptibility to the acetone extract was slightly higher for the *S. aureus* strains, OKOH1 and OKOH2A with MIC values of 0.3125 $mgml^{-1}$, while *S. aureus* OKOH3 and *S. sciuri* OKOH2B, had MIC values of 0.625 $mgml^{-1}$ (Table 2). Two of the isolates, *S. aureus* OKOH1 and OKOH3 were used to investigate the bactericidal activity of the acetone extract by time-kill assays. The extract was strongly bactericidal against isolate OKOH3 resulting in a 2.70 Log_{10} reduction in counts at 1.25 $mgml^{-1}$ (2× MIC) within 4 h of exposure (Figure 2).

A complete elimination of the test organism was achieved after 8 h of exposure. In contrast, the extract was weakly bactericidal against isolate OKOH1 achieving only a 2.92 Log_{10} reduction in counts at 1.25 $mgml^{-1}$ (4× MIC) in 24 h (Figure 3). At the MIC (0.3125 $mgml^{-1}$), the extract exhibited limited bactericidal activity during the first 8 h but the organism showed evidence of regrowth as the exposure time was extended to 24 h (Figure 3).

The effect of combinations of the acetone extract and antibiotics on the susceptibility of the Staphylococcus isolates is shown in Table 3. The efficacy of all the antibiotics against isolates OKOH1 and OKOH3 was marginally improved in the presence of the extract. The extract-antibiotic combinations achieved decreases in bacterial counts ranging from 0.62 - 1.66 Log_{10} $cfuml^{-1}$ (Table 3). In contrast, extract-antibiotic combinations involving isolates OKOH2A and OKOH2B produced slight increases in bacterial counts for all but two antibiotics, with increases ranging from 0.04 to 1.27 Log_{10} $cfuml^{-1}$ (Table 3). Only the combinations involving amoxycillin (on isolate OKOH2A) and tetracycline (on isolate OKOH2B) showed marginal potentiation of antibiotic activity. The interaction between penicillin G and the extract on isolate OKOH3, was the only one to produce a synergistic effect with a 3.90 Log_{10} (> 1000 times) potentiation (Table 3).

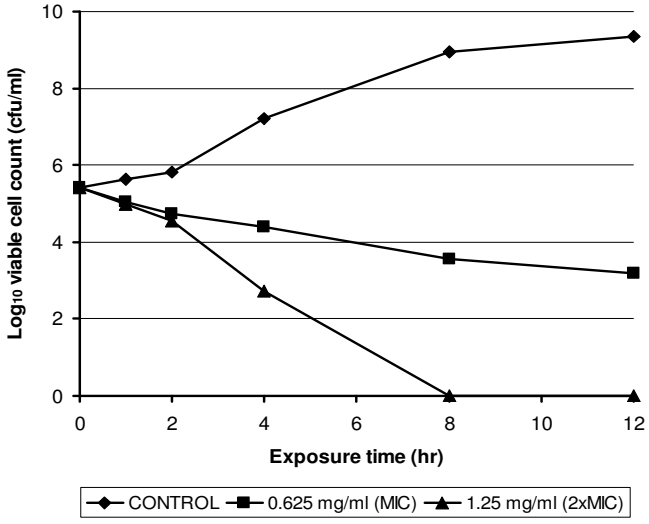

Figure 2. Effect of exposure to the acetone extract of *Garcinia kola* seeds on the viability of *Staphylococcus aureus* OKOH3.

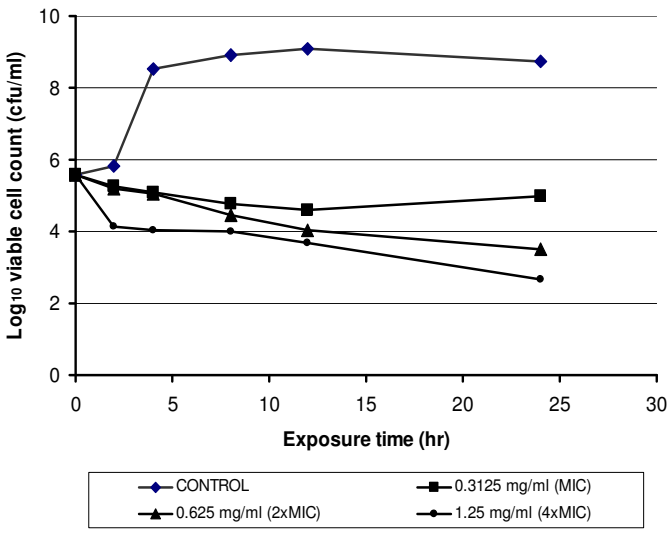

Figure 3. Effect of exposure to the acetone extract of *Garcinia kola* seeds on the viability of *Staphylococcus aureus* OKOH1.

DISCUSSION

S. aureus is a prominent pathogen in hospital and community acquired infections as a major cause of wound suppuration (Archer, 1998). It is therefore always relevant when encountered in clinical specimens, particularly those of wound infections. The detection of *S. aureus* in all the three specimens tested in this study underlines its importance as a cause of wound infections and is in agreement with the findings of other researchers such as Styers et al. (2006) and Moran et al. (2005) confirming that *S. aureus* as a common pathogen frequently encountered in clinical specimens. In addition to *Staphylococcus aureus*, the coagulase negative *S. sciuri* was also isolated from one of the specimens. *S. sciuri* is a common inhabitant of the skin of rodents and other mammals such as dogs (Stepanovic et al., 2001). The organism may also be found as a colonizing bacterium in humans, with low carrier rates in the nasopharynx, skin and urogenital tract (Stepanovic et al., 2005). Occasionally, the organism has been isolated from patients with boils and wounds (Marsou et al., 1999). While *S. sciuri* is not so frequently encountered in clinical specimens, its presence is significant as the organism has been reported to carry a number of resistance plasmids (Schwarz et al., 2002). Its co-infection with *S. aureus* could therefore present problems of transmission of resistance genes to a true pathogen like *S. aureus*.

The observation that all the strains of *S. aureus* were susceptible to the crude extracts of *Garcinia kola* at concentrations as low as 0.3125 $mgml^{-1}$ supports the idea that extracts of this plant can be of value in the treatment of staphylococcal infections. The activity of the acetone and methanol extracts at concentrations of 0.3125 $mgml^{-1}$, were comparable to that of other plant extracts reported to possess antistaphylococcal activity (Palombo and Semple, 2002; Voravuthikunchai and Kitpipit, 2005). *Garcinia kola* seeds have been known to possess compounds such as biflavonoids, xanthones and benzophenones (Iwu et al., 1999). Some of these compounds such as Kolanone, hydroxybiflavononol (GB1) have been reported to have good antibacterial activity (Madubunyi, 1995: Han et al., 2005). The antistaphylococcal activity particularly against clinical isolates is a very important finding as it demonstrates the potential of this plant in the treatment of problematic infections.

The killing rates experiments showed that the acetone extract possesse strong bactericidal activity against isolate OKOH3 at 2 × MIC; achieving a > 99.9% (3 Log_{10}) reduction in counts after 8 h. A greater than 99.9% killing activity in 24 h is generally used as a standard of measurement of bactericidal efficacy (EUCAST 2000b). However, the same extract exhibited a relatively weaker bactericidal activity against isolate OKOH1 achieving a 2.92 Log_{10} reduction after 24 h at 4 × MIC.

The antibacterial activity of the extract against isolate OKOH1 at MIC level is noteworthy as the isolate began to regrow after the 10^{th} hour of interaction. Emergence of resistant sub-populations may account for the re-growth as has been suggested elsewhere (Pankey and Ashcraft, 2005).

The effectiveness of an antibacterial agent is measured by its ability to inhibit and kill bacteria (Nostro et al., 2001). In vitro time-kill assays are expressed as the rate of killing by a fixed concentration of an antimicrobial agent and are one of the most reliable methods for determining tolerance (Nostro et al., 2001). Generally, the effects of the crude extracts of *G. kola* on the test bacteria in this experiment is time and concentration dependent, as it is evident from the data presented. At

higher concentration (2 × MIC or 4 × MIC) and longer duration of interaction (12 or 24 h), more bacteria were killed. The in vitro data corroborates the reported efficacies of the several different crude extracts of *G. kola* on a wide range of microorganisms and this support the folkloric uses of this plant in treatment of different topical ailments among the traditional people. The result also support the suggestion that extracts of this plant can be valuable in the treatment of some staphylococcal infections.

According to the MIC breakpoint values recommended by the British Society for Antimicrobial Chemotherapy (BSAC) and (EUCAST) (2005), two of the *S. aureus* isolates (OKOH2A and OKOH3) had MIC values (Table 3) higher than the breakpoints for penicillin G (breakpoint MICs; 0.25 mgl^{-1}). This could be a reflection of the general trend in beta-lactam resistance among strains of *S. aureus* in hospital and community settings. On the other hand, *S. sciuri* exhibited high sensitivity to the beta-lactam antibiotics (MIC values; 0.06 and 0.25 mgl^{-1} for penicillin G and amoxycillin respectively). Susceptibility to tetracycline and erythromycin was high with MIC values for all the isolates, lower than the breakpoints (2 mgl^{-1} tetracycline and 1 mgl^{-1} erythromycin), thus suggesting that therapy by these drugs could be effective. However the closeness of the erythromycin MIC values to the breakpoint for isolates OKOH2A, OKOH2B and OKOH3 (MIC values; 0.5 mgl^{-1}) could be a sign of emerging low level resistance to this drug. *S. sciuri* was the only isolate to be classified as resistant to ciprofloxacin. This could be confirming the quinolone resistance gene carrying capacity that organisms of this group are known of (Schwarz et al., 2002).

The level of interaction between the acetone extract of *Garcinia kola* seeds and antibiotics was investigated by the time-kill assay. In this method, concentrations of the extract and antibiotics equivalent to the MIC values were used in combination. Results from this study revealed a lack of synergistic interactions (that is > 100 times potentiation) between the extract and antibiotics on most of the isolates. However, results still showed a positive picture of the interactions with marginal improvements (additive interactions) in the bactericidal activity of some antibiotics particularly against isolates OKOH1 and OKOH3. The varying levels of enhancement of antibiotic activity by the extracts is consistent with previous findings involving combinations of plant extracts and antibiotics on *S. aureus* strains such as Darwish et al. (2002) and Yang et al. (2005). The likely reason for the observed lack of synergy could be the concentrations of the extract and antibiotics used in this study. Den-Hollander et al. (1997) emphasized that when determining synergy between two drugs, one of the drugs should have a concentration which does not affect the bacterial growth of the test organism. We also observed that some studies on the combinational effects of plant extracts and antibiotics such as Al-hebshi et al. (2006) and Braga et al. (2005) employed sub-inhibitory concentrations of the extracts and antibiotics. In this study, both the extract and the antibiotics at the MIC had some level bactericidal activity on the test isolates (data not shown) and this is likely to have interfered with synergy detection.

The enhancement in the killing effect (additivity) of the antibiotics suggests that extracts of *Garcinia kola* seeds could be containing compounds that can improve the efficacy of antibiotics. Such compounds are likely to be broad-spectrum resistance modifiers considering that this was observed across all classes of antibiotics tested.

Conclusion

Strains of Staphylococcus are important pathogens causing wound infections. Most clinical isolates of *S. aureus* are at present resistant to a number of antibiotics necessitating the search for alternative treatment options using medicinal plants. Extracts of *Garcinia kola* seeds possessed good antibacterial activity against clinical isolates of Staphylococcus. The combinations of the acetone extract of this plant with six first-line antibiotics exhibited a marginal ability to improve the bactericidal potency of the antibiotics. The findings of this study are significant as they demonstrate the potential of obtaining some valuable compounds from this plant that can be combined with common antibiotics in the treatment of drug resistant staphylococcal infections. It is therefore necessary to carry out a bioassay directed fractionation of the acetone extract so as to isolate and identify the compounds responsible for the antistaphylococcal as well as the enhancement of antibiotic activity. Such compounds could be useful in the development of new antistaphylococcal drugs.

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