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# Time-Kill Effect of Crude Extracts of *Garcinia kola* Seeds on Methicillin-Resistant *Staphylococcus aureus* from the Anterior Nares of Healthcare Workers at a Tertiary Hospital in Nigeria

**C. C. Egwuatu[1], T. O. Egwuatu[2], A. A. Iwuafor[3], C. N. Akujobi[1], A. U. Nnachi[4*], I. N. Aghanya[1], F. T. Ogunsola[5] and O. O. Oduyebo[5]**

*[1]Department of Medical Microbiology and Parasitology, Faculty of Medicine, Nnamdi Azikiwe University, Nnewi Campus, Nigeria.*
*[2]Department of Medical Microbiology and Parasitology, Faculty of Science, University of Lagos, Nigeria.*
*[3]Department of Medical Microbiology and Parasitology, Faculty of Medicine and Dentistry, College of Medical Sciences, University of Calabar, Calabar, Nigeria.*
*[4]Department of Immunology, Faculty of Medicine, Nnamdi Azikiwe University, Nnewi Campus, Nigeria.*
*[5]Department of Medical Microbiology and Parasitology, College of Medicine, University of Lagos, Nigeria.*

***Authors' contributions***

*This work was carried out in collaboration among all authors. Author CCE designed the study and served as the principal investigator. Author TOE participated in sample processing and analysis. Author AAI participated in study design and sample collection. Author CNA participated in data acquisition and analysis. Author AUN participated in the study design and sample processing and drafted the manuscript for publication. Author INA participated in sample collection and processing. Author FTO participated in study design and served as the principal supervisor. Author OOO participated in sample processing and drafting of study protocol. All authors read and approved the final manuscript.*

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**Corresponding author: E-mail: nnachiau@gmail.com;*

## ABSTRACT

**Aims:** This study investigated the time-kill effects of crude methanol and aqueous extracts of *Garcinia kola* (*Bitter kola*) against isolates of *Staphylococcus aureus* obtained from the anterior nares of healthcare workers in a tertiary hospital in Nigeria.
**Study Design:** This was a cross-sectional study.
**Place and Duration of Study:** Lagos University Teaching Hospital (LUTH), Lagos, Nigeria between June 2013 and January 2014.
**Methodology:** Nasal swab samples were aseptically collected from two hundred and fifty (250) health workers using sterile swab sticks and analyzed using standard microbiological techniques. The isolates were identified using standard techniques and subjected to susceptibility testing that categorized them into oxacillin-resistant, β-lactamase producing and oxacillin-sensitive strains. The isolates were further subjected to agar well dilution to determine the minimum inhibitory concentration (MIC) and time-kill assays of the extracts.
**Results:** The overall carriage rate of 35.6% (89 of 250) was observed and out of the 89 *Staphylococcus aureus* isolates recovered, 26 were from laboratory workers and 16 from those in labour ward. Also, 34 (38.2%) of the isolates were oxacillin-resistant (MRSA), 32 (36%) were oxacillin-sensitive (MSSA) while the remaining 23 (25.8%) were β-lactamase producers. The overall MRSA presence among the healthcare workers was 13.6%. Both the methanol and aqueous extracts of *Garcinia kola* seeds showed intense activity with complete inhibition of most strains at 25 µg/ml (including all β-lactamase strains). However, thirty-two out of the thirty-four of the oxacillin-resistant (MRSA) strains showed sensitivity to the extracts at higher minimum inhibitory concentrations (MIC) of 50 µg/ml while most strains that were oxacillin-sensitive (including *S. aureus* ATCC 25923) were sensitive at lower MIC of 12.5 µg/ml. At 200 µg/ml of the extracts, all isolates under study were killed within 4 minutes whereas 100 µg/ml killed (oxacillin-sensitive and β-lactamase producers) within 10 minutes and MRSA within 30 minutes.
**Conclusion:** Extracts of *Garcinia kola* seeds possessed good antibacterial activity against isolates of *Staphylococcus aureus*. Therefore, using this plant extract as an ointment for nasal decolonization will go a long way in reducing the transmission of staphylococcal infections from health workers to patients. The study also reveals that nasal decolonization using these extracts greatly depend on concentration and contact time. Abiding by standard infection control methods and judicious use of antibiotics could greatly reduce transmission and antimicrobial resistance respectively among health workers.


*Keywords: Garcinia kola; Staphylococcus aureus; MRSA; time-kill; healthcare workers.*

## 1. INTRODUCTION

The ever-present threat of antimicrobial resistance and the associated health and economic implications have led to a continuous search for novel treatment alternatives, especially among plants, that would meet the therapeutic need of the global community. *Staphylococcus aureus* is an opportunistic pathogen often carried asymptomatically on the bodies of both humans and animals, and has been implicated as causing severe morbidity and mortality worldwide [1]. It is a leading cause of both community acquired and nosocomial infections [2,3], ranging from relatively benign superficial skin and soft tissue infections to severe and life-threatening conditions, including deep tissue abscesses, joint and bone infections, nosocomial pneumonia, bacteriemia, and endovascular infections [4,5]. Methicillin-resistant *S. aureus* (MRSA) include those strains of *Staphylococcus aureus* that have acquired a gene (*mec* A) giving them resistance to methicillin and essentially all other beta-lactam antibiotics [1]. Methicillin-Resistant *Staphylococcus aureus* (MRSA) has raised a global concern both in the hospital and the community over the past decades [6]. Following its first recognition in the early 1960s, the increasing incidence of nosocomial and community-associated methicillin-resistant *Staphylococcus aureus* (MRSA) infections has become a global problem [7]. Currently, MRSA is causing a significant morbidity and mortality worldwide [8,9].

Both Methicillin-sensitive and Methicillin-resistant strains can be found as normal commensals on the skin (especially the axillae and perineum), the nasopharynx and anterior nares of some of

the population [10]. Colonization with *S. aureus* can occur any time after birth; carriage may be transient or persistent and may or may not lead to symptomatic infections [10,11]. Nasal carriage is known to be a means of persistence and spread of multi-resistant staphylococci, especially methicillin-resistant *Staphylococcus aureus* (MRSA) from health workers to patients [12,13]. In general, nasal carriage of *Staphylococcus aureus* plays a key role in the epidemiology and pathogenesis of infection and is a major risk factor for the development of both community-acquired and nosocomial infections [14].

Transmission of *S. aureus* (including MRSA) usually occurs by direct contact, often via the hands, with colonized or infected people [15,16]. In human hospitals, colonized and infected human patients are the main reservoir for MRSA, and this organism is typically spread from patient to patient on the hands of staff [10,17]. Aerosol transmission was reported in one hospital outbreak [18]. Community-acquired MRSA has been reported to spread by direct contact, on fomites and in aerosol [10,15]. The elimination of this pathogen from the anterior nares using antibacterial agents is one of the primary strategies for the prevention of these life-threatening infections which has risen to the level of public health threat. However, it is an unrealisable goal due to the increasing menace of antimicrobial resistance.

Given the high mortality rates of staphylococcal infections against a background of antibiotic resistant strains, it becomes imperative to explore the medical importance of plant remedies and their potentials in curbing antibiotic resistance. It has been hypothesized that, in addition to the production of intrinsic antimicrobial compounds, plants also produce multi-drug resistance (MDR) inhibitors which enhance the activity of the antimicrobial compounds [19]. This hypothesis was tested by Tegos et al. [20], who showed that the activity of putative plant antimicrobials against gram positive and gram negative organisms was significantly enhanced by synthetic MDR inhibitors of MDR efflux proteins. Those findings provided a base to believe that plants can be potential sources of natural MDR inhibitors that can potentially improve the performance of antibiotics against resistant strains.

In recent times, *Garcinia kola*, a commonly eaten natural seed in West Africa has been attracting a lot of interest as natural alternatives to synthetic compounds due to its contents of benzophenones and flavonones [21,22]. *Garcinia kola* is a traditional medicinal plant which has been used since time immemorial for its medicinal prowess mainly in Central and West Africa [23]. Almost every part of the plant has been found to be of medical importance; the nut is used for nervous alertness, induction of insomnia and also as a masticatory; the root of the plant is used as bitter chewing sticks; the stem bark is used as a purgative; the latex is externally applied to fresh wounds to prevent sepsis, thereby assisting in wound healing [24].

The plant seeds are routinely used by the local community in the prevention and treatment of gastric ulcers and wound infections [25,26]. The antiulcerogenic and gastric lowering effect of *Garcinia kola* was also reported by Okunji and Iwu [27]. The aqueous extract decreased the mycelial weight of *Aspergillus parasiticus* yeast extract. Also Aflatoxin production was most effectively decreased by *Garcinia kola* extract showing some antimicrobial activity [28]. In a Nigerian study, ten isolates of *Clostridium difficile* were studied to determine their susceptibility to both 70% methanol and aqueous extracts of *Garcinia kola* using agar well diffusion method. It was found out that the methanol extract showed antimicrobial activity at minimum inhibitory concentration of 3.125 mg/ml while the aqueous extract was at 12.5 mg/ml. In conclusion, their study demonstrated that the plant *Garcinia kola* has activity against *Clostridium difficile* [29].

Various studies on *Garcinia kola* plant have focused on its antimicrobial and therapeutic potentials [30-34]; however, there is still lack of information on the time-kill effect of the crude extracts of the seeds on *Staphylococcus aureus* (including MRSA), hence this study.

## 2. MATERIALS AND METHODS

### 2.1 Study Design/Population

This was a cross-sectional study in which a total of two hundred and fifty healthcare workers from various sections of the Lagos University Teaching Hospital (LUTH), Lagos, Nigeria, were screen for the presence of different strains of *Staphylococcus areus* in their nostrils. The isolates were exposed to the methanol and aqueous extracts of *Garcinia kola* seeds to determine their antimicrobial and time-kill effects.

## 2.2 Sample Collection

A total of 250 health workers from the Lagos University Teaching Hospital, Lagos were recruited for this study. Two nasal swabs were collected from each participant using sterile swab sticks and transported to the Department of Medical Microbiology, College of Medicine, University of Lagos, Nigeria for immediate processing. The control strains; ATCC 38591 (beta-lactamase susceptible) and ATCC 25923 (for oxacillin susceptible) were obtained from the Department of Medicine, College of Medicine, University of Lagos, Nigeria.

## 2.3 Isolation and Identification of *Staphylococcus aureus*

The two nasal swabs collected from the participants were aseptically inoculated on previously prepared media; one on blood agar and the other on chocolate agar. The plates were incubated at 37℃ for 24 hours after which suspicious colonies were sub-cultured on mannitol salt agar (OXOID, UK). Pure cultures of each isolate were subjected to Gram reaction and Gram positive isolates were further identified using catalase and coagulase tests [1]. ATCC 38591 (beta-lactamase susceptible) and ATCC 25923 (for oxacillin susceptible) were used as control.

## 2.4 Identification of Methicillin-Resistant *Staphylococcus aureus*

Oxacillin-Resistance Screening Agar Base (ORSAB), supplemented with Oxacillin (2g/l) was used to confirm isolates as methicillin-resistant *Staphylococcus aureus* [1]. All isolates identified as *Staphylococcus aureus* were streaked on ORSAB medium plates. These were incubated at 37℃ for 24 hours. After incubation, the plates were examined for growth. Blue-coloured colonies indicated presence of MRSA, while isolates that showed absence of growth were interpreted as Methicillin Sensitive *Staphylococcus aureus* (MSSA). ATCC 38591 (beta-lactamase susceptible) and ATCC 25923 (for oxacillin susceptible) were used as control.

## 2.5 Preparation of *Garcinia kola* Seeds Extracts

*Garcina kola* seeds were purchased from a local market in Lagos city, and were identified by the Department of Pharmacognosy, College of Medicine, School of Pharmacy of the University of Lagos, Idi-Araba. All extracts were obtained using Soxhlet extractor as described by Irobi et al. [35]. The *Garcina kola* seed were thinly sliced and dried over a period of 5-7 days at room temperature, and then crushed into powder in a mortar. Both aqueous and methanol extracts of the seeds were obtained. In this, a 50 g portion of the powdered plant materials was loaded into a Soxhlet extractor containing 250 ml of methanol, and exhaustively extracted at 60℃ for 24 hours. The solvent (methanol) was evaporated using a rotary evaporator to leave sticky extracts. Also, 50 g of the powdered plant materials was loaded into a Soxhlet extractor containing 300 ml of deionized water and extracted at 100℃ for 4 hours. The extracts were filtered and subjected to gentle evaporation over a hot plate at 60℃ until it became concentrated.

## 2.6 Determination of Minimum Inhibitory Concentration (MIC)

The hard pellets of the extracts were further grinded into powdery form. The minimum inhibitory concentrations for the organisms were tested using the agar dilution method [36]. Different weights of both powdery methanol and aqueous extracts ranging from 5 g, 2.5g, 1.25 g, 0.625 g, 0.3125 g, 0.15625 g, and 0.07813 g were each respectively incorporated with 25mls of Muller-Hinton agar to make final concentrations of 200 µg/ml, 100 µg/ml, 50 µg/ml, 25 µg/ml, 12.5 µg/ml, 6.25 µg/ml, and 3.125 µg/ml, respectively and mixed thoroughly [37]. Two control plates were prepared, one without extract and the other with methanol only and were run with each batch. A 0.1 ml aliquot of the bacterial suspension standardized to 0.5 McFarland was spot-inoculated on each of the plates containing the different concentrations of extract and controls and allowed to diffuse for one hour. All the plates were incubated at 37℃ for 24 hours after which they were examined for growth. All plates showing zones of inhibition were considered sensitive and all plates with no zones inhibition were considered resistant to the extract.

## 2.7 Determination of Time-kill Effect

Broth macrodilution method was performed by dispensing 1 ml of the different concentrations of the extract starting from the minimum inhibitory concentration (25 µg/ml) to the highest concentration onto each sterile universal bottles container [38]. With a 2 mls syringe, 0.1 ml of 0.5

McFarland turbidity standard of the test organism was inoculated into each of the universal bottles. All the bottles were incubated at 37℃ and at different intervals of 1 minute, 2 minutes, 4 minutes, 5minutes, 8 minutes, 10 minutes, 30 minutes, 40 minutes, and 60 minutes. Each bottle was brought out at each time interval and a loopful of the broth solution was streaked onto freshly prepared dried blood agar (OXOID, UK) plates, then incubated at 37℃ for 18-24 hrs. The number of viable count was determined.

## 3. RESULTS

The screening of two hundred and fifty health workers in Lagos University Teaching Hospital (LUTH) showed that they were nasally-colonized with *Staphylococcus aureus* at the overall prevalence rate of 35.6% (89 of 250). Out of the 89 *Staphylococcus aureus* isolates recovered, 26 were from laboratory workers, 16 from those in labour ward and 15 were from the theatre (Table 1). Also, of the recovered isolates, 34 (38.2%) were oxacillin-resistant (MRSA), 32 (36%) were oxacillin-sensitive while the remaining 23 (25.8%) were β-lactamase producers. The overall MRSA presence among the health workers was 13.6% (34 of 250) (Table 2).

The minimum inhibitory concentration (MIC) of the thirty-two out of thirty-four of the oxacillin resistant strains otherwise referred to as MRSA was 50 µg/ml while the remaining two isolates and all the twenty-three β-lactamase producers were inhibited at 25 µg/ml concentration. Thirty-two strains that were oxacillin-sensitive including the control *S. aureus* ATCC 25923 strain were inhibited at 12.5 µg/ml concentration. The result of the aqueous extract was similar to that of methanol (Table 2).

The rate at which different concentrations of *G. kola* (both methanol and aqueous extracts) inhibited different strains of *S. aureus* varied. At the concentration of 200 µg/ml, *G. kola* inhibited all strains of *S. aureus* within 4 minutes. This was prolonged to 10 minutes for β-lactamase producers and oxacillin-sensitive strains and 30 minutes for MRSA when the concentration was halved to 100 µg/ml. At a concentration of 50 µg/ml, oxacillin-sensitive strains of *S. aureus* were killed within 40 minutes while MRSA and β-lactamase producers were killed at 80 minutes (Figs. 1-6).

## 4. DISCUSSION

The recent years have witnessed the increasing resistance of *Staphylococcus aureus* to many antimicrobial agents. The most notable example is the emergence of Methicillin-resistant *Staphylococcus aureus* (MRSA), which was reported just one year after the launch of methicillin [39]. This study revealed that heath workers in Lagos University Teaching Hospital (LUTH) at the time of this study had

**Table 1. Prevalence rate of *S. aureus* isolates among healthcare workers**

| Outcome | Wards | | | | | | Total |
|---|---|---|---|---|---|---|---|
| | Laboratory | Labour ward | Neonatal ward | Post natal ward | Surgical ward | Theatre | |
| Positive | 26(33.77%) | 16(22.85%) | 10(47.61%) | 10(50%) | 12(42.85%) | 15(44.11%) | 89 |
| Negative | 51(66.23%) | 54(77.14%) | 11(52.38%) | 10(50%) | 16(57.14%) | 19(55.88%) | 161 |
| **Total** | **77** | **70** | **21** | **20** | **28** | **34** | **250** |

**Table 2. Antimicrobial activity of crude methanol and aqueous extracts of *Garcina kola* on different strains of *S. aureus* isolates**

| Strain | Minimum inhibitory concentration (MIC) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Methanol extracts (µg/ml) | | | | | | Aqueous extract (µg/ml) | | | | | |
| | 6.25 | 12.5 | 25 | 50 | 100 | 200 | 6.25 | 12.5 | 25 | 50 | 100 | 200 |
| Oxacillin-resistant (MRSA) | + | + | + | - | - | - | + | + | + | - | - | - |
| β-Lactamase Producer | + | + | - | - | - | - | + | + | - | - | - | - |
| Oxacillin-sensitive (MSSA) | + | - | - | - | - | - | + | - | - | - | - | - |
| Control | + | - | - | - | - | - | + | - | - | - | - | - |

*Key: + =Growth is present, - =No growt*

*Staphylococcus aureus* in their nostrils with overall recovery rate of 35.6%. This finding is comparatively higher than those of Rongpharpi et al. [39] in Assam, Vinodhkumaradithyaa et al. [40] in India, and Shobha et al. [41] in India who report 22.22%, 15% and 0% respectively. This higher prevalence may have resulted to improper practice of infection control strategies among health workers in our study area. Among these staphylococcal isolates was revealed an overall MRSA presence of 13.6%. This study is in agreement with other previous studies by Kaur and Narayan [14] who reported 14.24% in India and Golia et al. [42] who reported 13.37% in Bangalore. Other workers reported the prevalence of MRSA of 11.43% [40]. However, Mathanraj et al. [43] reported only 1% prevalence for MRSA.

Also, this study reveals that crude methanol and aqueous *G. kola* seed extracts possess *in vitro* antibacterial activities at varying concentration against the staphylococcal isolates. This is in conformity with the findings as reported by Esimone et al. [44] that the seed of *G. kola* is believed to possess many medicinal properties which include anti-inflammatory, antibacterial, anti-microbial, antiviral, antidiabetic, purgative, and antihe-patotoxic [23,45-48]. Muanya [49] also identified *G. kola* to have strong antibiotic activities and found the plant to be very effective against disease-causing microorganisms such as *E. coli, S. aureus, P. aeruginosa, Salmonella spp., Streptococcus spp., Candida albicans, Vibrio cholera* and *Neisseria gonorrhoea*. The methanol and aqueous extracts of *Garcinia kola* were tested for their inhibitory effect on various categories of *S. aureus* from very antibiotic susceptible strain (oxacillin-sensitive) to β-lactamase producer and MRSA. It was clear that oxacillin-sensitive strain was also very sensitive to *G. kola* at a minimum inhibitory concentration (MIC) of 25 µg/ml while the multiple resistant MRSA had MIC of 50 µg/ml.

**Table 3. Percentage distribution of different strains of *Staphylococcus aureus* from the anterior nares of health workers in Lagos University Teaching Hospital (LUTH)**

| Strains | Ward n(%) | | | | | | Total n(%) |
|---|---|---|---|---|---|---|---|
| | Laboratory | Labour ward | Neonatal ward | Post natal ward | Surgical ward | Theatre | |
| Oxacillin-sensitive (MSSA) | 10(38.46%) | 6(37.5%) | 4(40%) | 2(20%) | 4(33.33%) | 6(40%) | 32(36) |
| Oxacillin-resistant (MRSA) | 9(34.62%) | 6(37.5%) | 3(30%) | 7(70%) | 5(41.67%) | 4(26.67%) | 34 (38.2) |
| β-lactamase producers | 7(26.92%) | 4(25%) | 3(30%) | 1(10%) | 3(25%) | 533.33%) | 23(25.8) |
| **Total** | **26** | **16** | **10** | **10** | **12** | **15** | **89(35.6)** |

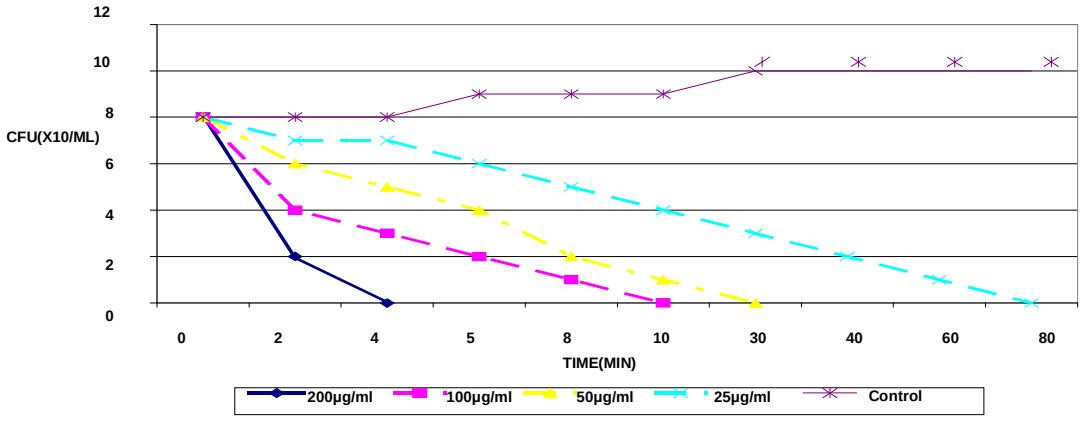


**Fig. 1. Time-kill effect of the methanol extract of *Garcinia kola* on oxacillin-sensitive *S. aureus***

However, these concentrations are relatively high and cannot be achieved *in-vivo* where the activity needed is to be in concentrations of µg/ml. Nevertheless, as a topical agent, the concentration of 50 µg/ml is achievable. In addition, the time-kill effect was determined at various concentrations of *G. kola* to determine how much contact time will be required before

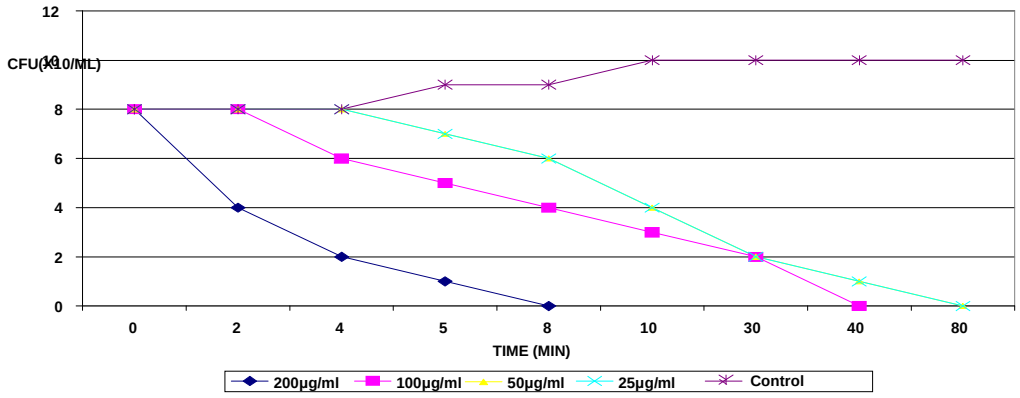


**Fig. 2. Time-kill effect of the methanol extract of *Garcinia kola* on oxacillin-resistant *S. aureus***

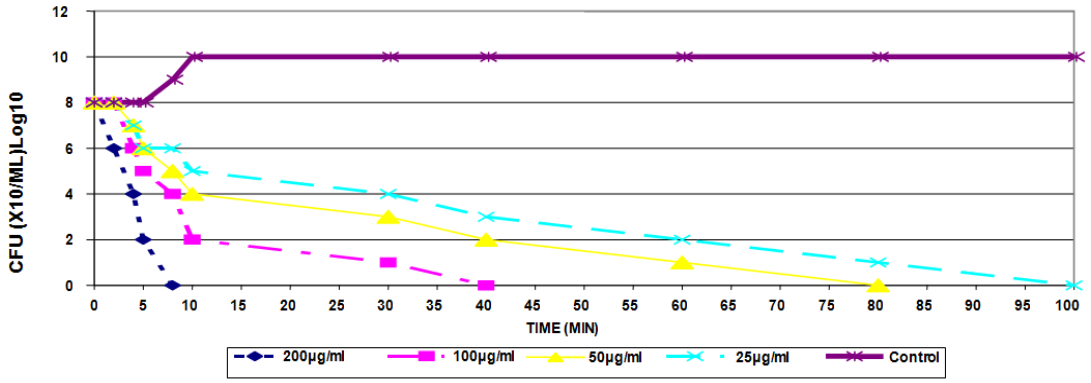


**Fig. 3. Time-kill effect of the methanol extract of *Garcinia kola* on beta-lactamase producing *S. aureus***

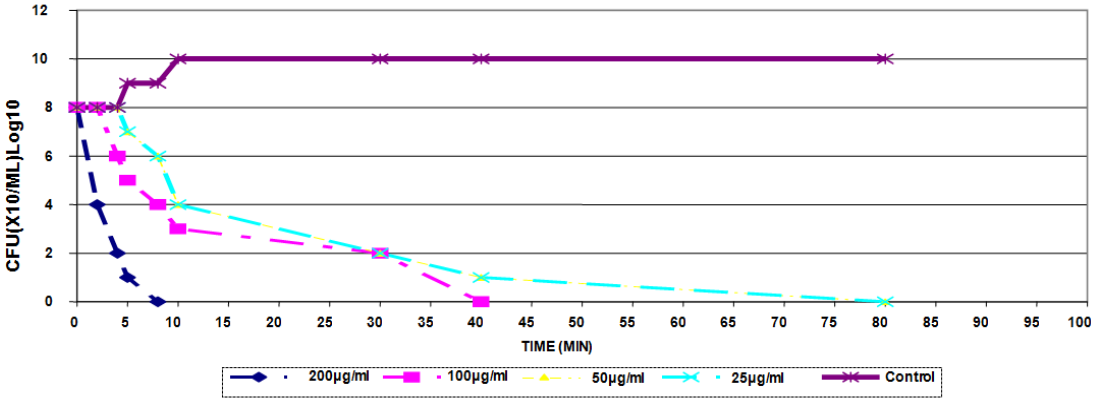


**Fig. 4. Time-kill effect of the aqueous extract of *Garcinia kola* on oxacillin-sensitive *S. aureus***

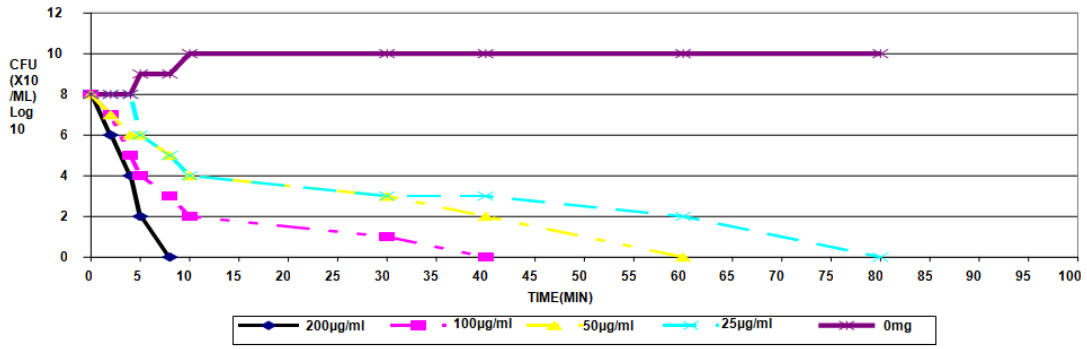


**Fig. 5. Time-kill effect of the aqueous extract of *Garcinia kola* on oxacillin-resistant *S. aureus***

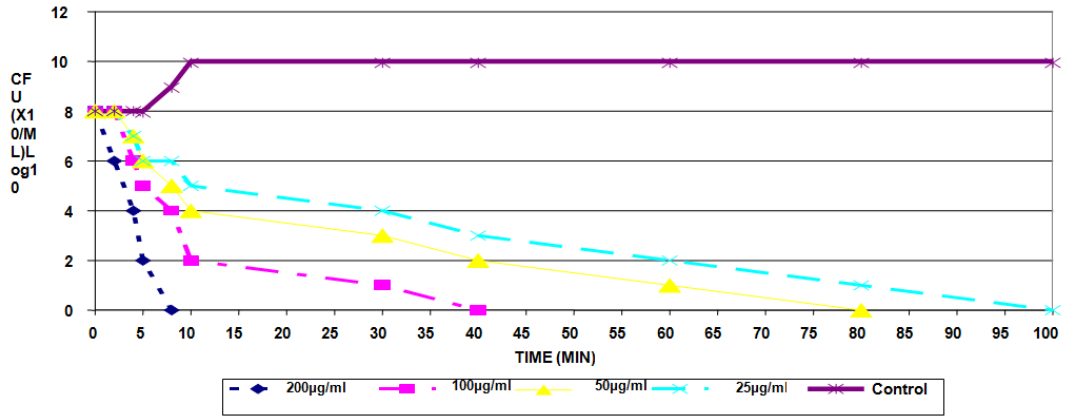


**Fig. 6. Time-kill effect of the aqueous extract of *Garcinia kola* on beta-lactamase producing *S. aureus***

MRSA is completely eradicated. At 50 mg/ml, all categories of *S. aureus* (oxacillin-sensitive, β-lactamase, and MRSA) were completely killed within 30 minutes. The effectiveness of an antibacterial agent is measured by its ability to inhibit and kill bacteria [50]. *In vitro* time-kill assays expressed as the rate of killing by a fixed concentration of an antimicrobial agent are one of the most reliable methods for determining tolerance [50]. Generally, the effects of the crude extracts of *G. kola* on the test bacteria in this experiment was time and concentration dependent, as evident from the data presented. At higher concentration and longer duration of interaction, 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 [51-53]. The result also support the suggestion that extracts of this plant can be valuable in the treatment of some staphylococcal infections.

## 5. CONCLUSION

This high MRSA presence recorded in this study among healthcare workers in such tertiary hospital is alarming since they can serve as reservoir for frequent transmission of this multi-drug resistant *Staphylococcus aureus* in the hospital. The high MRSA presence must have been associated with the fact that these healthcare workers are constantly exposed to antimicrobial agents. Also, being healthcare workers, there is possibility of self-prescription of drugs when ill without proper laboratory examinations. These two factors can encourage selection pressure that leads to the development of drug resistance. The results also support the claim that extracts of this plant can be valuable in the treatment of some staphylococcal infections. It suggests a possible role for *G. kola* as a topical chemotherapeutic agent for *S. aureus* especially MRSA and this natural product is locally available and is likely to be cheap. However, a lot is needed to be done especially on toxicity studies and its effect on the flora of the nasal

cavity. It will also be important to identify the active agent. The study also reveals that nasal decolonization using these extracts greatly depend on concentration and contact time. Abiding by standard infection control methods and judicious use of antibiotics could greatly reduce transmission and antimicrobial resistance respectively among health workers.

## ETHICAL APPROVAL

All authors hereby declare that this study was examined and approved by the Research and Ethics Committee of the Lagos University Teaching Hospital (LUTH) in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki. All patients were required to sign an informed consent and those that gave their consent were recruited. Samples, data and results obtained were treated with utmost confidentiality and used for the purpose of this study only.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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