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Antimicrobial Effects of Garlic Extracts against Multidrug Resistant *Staphylococcus aureus* Isolates from Clinical Specimens

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Authors' contributions

This work was carried out in collaboration between all authors. Authors VUO, RAO and VON designed and wrote the protocol and first draft of the manuscript. Author VUO managed the literature search and analysis of the study. Authors SSO, MOO and IO revised the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aim: To assess the antibacterial potentials of *Allium sativum* and their interaction with antibiotics against clinical isolates of multidrug resistant *Staphylococcus aureus*.
Study Design: The study is designed to investigate the antibacterial activity of *Allium sativum* extract and its interactive potential to enhance the activity of antibiotics against multidrug resistant *S. aureus*.
Place and Duration of Study: The study was conducted between May, 2015 and June, 2016. This

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study was performed at the Microbiology laboratory of the Akanu Ibiam Federal Polytechnic, Unwana, Ebonyi State, Nigeria.
Methodology: Extraction was done using sterile water, acetone and methanol. The extracts were evaporated to dryness using rotary evaporator. Antibacterial susceptibility testing was carried out using the agar well diffusion method. The macrobroth dilution method was used to determine the MIC of the methanol extracts. Checkerboard method was used to determine the interaction between extracts and antibiotics.
Results: Aqueous and methanol extracts of *Allium sativum* had inhibition zone range of 10 ± 1.50 to 17 ± 1.50 mm and 7 ± 1.05 to 14 ± 0.95 mm respectively, at concentration range of 25 to 200 mg/ml. Acetone extract had inhibition range of 5 ± 0.50 to 11 ± 0.50 mm. Methanol and acetone extracts had the same inhibition zone (14 ± 0.79 mm) diameter against the *S. aureus* isolates at 200 mg/ml. There were no significant difference found between inhibition zone diameter of methanol/acetone extract (P = 0.50), methanol/aqueous extracts (P = 0.97) and acetone/aqueous extracts (P =0.48) against the test bacteria. The correlation analysis done between inhibition zone diameter and concentration of *A. sativum* recorded a positive correlation (r) ranging between 0.84 and 0.89. The combination of methanol extracts of garlic (MEG) plus ceftazidime (Caz) and MEG plus erythromycin (Ery) showed synergistic interaction in three and four of the isolates respectively. Antagonistic interaction was recorded in the combination of MEG plus cefuroxime in one isolate whereas it was recorded in three isolates in the interaction of MEG and ciprofloxacin. The synergistic interaction stood at 28.0% and antagonism 16.0%. The combination of ½ x MIC of MEG plus ½ x MIC Caz against *S. aureus* showed bactericidal activity against the isolates at 12 hours resulting in a 4.25 $\log_{10}$ cfu/ml reduction whereas the combinations of ½ x MIC of MEG plus ½ x MIC of Ery showed bactericidal activity after 8 hours with a 3.13 $\log_{10}$ cfu/ml decrease. The net reduction in colony counts was observed consistently between 12 – 24 hours.
Conclusion: The result of this study showed that methanol extract of *A. sativum* possesses bactericidal activities against *S. aureus*. In addition, the methanol extract may be a potential source of resistance modifying compounds that can potentially improve the performance of antibiotics in the treatment of multidrug resistant *S. aureus* infections. There is need to isolate the specific active agent(s) involved in the potentiation for drug compounding.

Keywords: Garlic; Staphylococcus aureus; antibacterial; potentiation; compounding.

1. INTRODUCTION

Bacterial infections associated with high mortality in humans and animals have been associated with multidrug resistance (MDR) [1,2]. Increasing rates of infections caused by multidrug resistant (MDR) bacteria as well as emergence of new antibiotic resistant pathogens in clinical environments have been reported by the World Health Organization (WHO), the USA Centre for Disease Control (CDC), and several research laboratories [3,4]. The emergence of antimicrobial resistance, especially the multidrug resistance makes the treatment of bacterial infections difficult. This is because the resistant organisms fail to respond to first line treatment, hence, leading to high cost of treatment, prolonged illness and high risk of death with its concomitant financial burden and loss in man-hour to families and societies [5].

S. aureus, a common cause of human infections have been recognized as pathogen of high public health significance [6]. *S. aureus* is a normal inhabitant of the human skin and the respiratory tract; and, nasal presence of *S. aureus* plays a key role in the development of *S. aureus* infections [7]. The bacterium has been noted as a major risk for the development of infection in patients undergoing haemodialysis, continuous ambulatory peritoneal dialysis, surgical patients, and patients with intravascular devices [8]. According to Onemu et al. [9], *S. aureus* is important in human infections ranging from minor skin-infections to serious life threatening infections that may include endocarditis, deep seated abscesses, septicaemia, catheter-associated bacteraemia, ventilator-associated pneumonia, food borne-illness, toxic shock syndrome (TSS) among other infections. *S. aureus* ability to acquire antimicrobial resistance mechanisms and its advantageous pathogenic determinants has contributed to emergence of infections in both nosocomial and community settings [8]. Antimicrobial resistance among nosocomial pathogens is a significant problem in many countries, and, it is with severe consequences including increased medical costs, morbidity and mortality of patients [8].

The problem of bacterial resistance to commonly used antibiotics and the high cost of conventional drugs particularly in developing countries has led to the increased use of plants as an alternative for the treatment of infectious diseases. Nwakaeze et al. [10] reported that, about 80% of individuals from developing countries rely on traditional medicine that incorporates one herb to another as their main therapeutic agent.

Garlic (*Allium sativum*) that belongs to the family of *Alliaceae* is widely used in culinary and medicine [11]. Aqueous, methanol and ethanol extracts of *A. sativum* has been reported to possess antimicrobial effect against drug resistant organisms such as *Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis, S. aureus, Klebsiella pneumoniae, Shigella sonnei, Staphylococcus epidermidis* and *Salmonella typhi* [12,13,14]. *A. sativum* is primarily a bacteriostatic agent that destroys sulfhydryl groups necessary for bacterial growth [15].

Multidrug therapy involving the combination of conventional drugs has become a practice in the fight against multidrug resistant microbial strains. For instance; without the current multidrug approach used to treat tuberculosis (isoniazid, rifampicin, pyrazinamide, and ethambutol), the mortality of infected patients could reach global epidemic proportions [16]. Another antimicrobial agent having a significant synergistic effect in combination is amoxicillin (a β-lactam antibiotic) and clavulanic acid. Clavulanic acid binds to β-lactamase producing microorganisms, which protects amoxicillin from β-lactamase attack, which in turn results in an extended spectrum of activity for amoxicillin [16].

However, new antimicrobial combination drugs which include natural product combinations have recently become a research priority [16]. Although, plants has been reported as a potential sources of direct antibacterial drugs, researchers have shown that some secondary metabolites of plants with no intrinsic antimicrobial activity are useful in sensitizing bacterial cells to antimicrobial agents [17]. Recently, suggestions has it that such compounds can potentially be used to improve the efficacy of antibiotics against bacterial pathogens.

This study is aimed at investigating the antibacterial potentials of *Allium sativum* extract and their interactions with some antibiotics against multidrug resistant clinical isolates of *S. aureus*.

2. MATERIALS AND METHODS

2.1 Sample Collection

The *Allium sativum* (Garlic) used in this study was purchased from dealers in Eke market, Afikpo, Ebonyi State, and identified by plant taxonomist at National Root-Crop Research Institute (NRCRI), Umudike, Abia State, Nigeria.

2.2 Bacteria Used in the Study

The multidrug resistant *S. aureus* isolates used in this study was isolated from wound, nose and ear swabs and confirmed based on their cultural and biochemical characteristics [18]. Multidrug resistance was taken in this study as resistance to at least three classes of antibiotics [19].

2.3 Preparation of Plant Extract

The method of Iram et al. [12] was adopted for the extraction with slight modification. The garlic was washed with sterile distilled water, peeled, cut into pieces and sun-dried for a week. After drying, the garlic slices were ground into powder using electric blender. Exactly 20 g of the ground garlic were mixed with 100 ml of sterile distilled water, methanol and acetone separately. The flasks containing each extract and solvent were allowed to stand for 72 hours at room temperature with intermittent shaking. The crude extracts were centrifuged at 3000 rpm for 10 minutes. The supernatant was then filtered using Whatman No. 1 filter paper to further remove undissolved residues. The methanol and acetone extracts were evaporated to dryness at 50℃ while the aqueous extract was evaporated at 80℃ in rotary evaporator. The extracts were stored in the refrigerator for further analysis. Exactly 2.0 g of the evaporated extracts were re-suspending in 10 ml of 10% dimethylsulphuroxide (DMSO) to produce a stock solution of 200 mg/ml. Thereafter, two fold serial dilutions were carried out using the 10% DMSO to get concentrations of 100, 50 and 25 mg/ml used in the study.

2.4 Antibacterial Susceptibility Testing of Plant Extract

The antibacterial susceptibility testing of *A. sativum* extracts against isolates of *S. aureus* was carried out using the agar well diffusion method [18]. A sterile Pasteur pipette was used to drop 0.2 ml standardized inoculums equivalent to 0.5 McFarland turbidity standards on the

surface of already prepared and dry Mueller-Hinton agar. The inoculum was evenly spread using Hockey stick shaped glass rod. Six wells were carefully bored into each agar plate after standing for about 5 minutes with heat sterilized 6 mm diameter cork borer and labeled. The first four wells were filled with 0.1 ml volume each of the 200 mg/ml, 100 mg/ml, 50 mg/ml and 25 mg/ml concentrations of the extracts prepared with 10% DMSO. The other two wells were filled with 0.1 ml of 4 mg/L ciprofloxacin as positive control and 10% DMSO as negative control. Care was taken not to allow spillage of the solutions onto the surface of the agar. The plates were allowed to stand for about 30 minutes for proper diffusion of the solutions before being incubated at 37℃ for 24 hours [20]. This experiment was conducted in duplicates. After 24 hours, antibacterial activity was evaluated by measuring the diameter of the zones of inhibition produced by the extracts against the test organisms in millilitres.

2.5 Determination of Minimum Inhibitory Concentration (MIC) of Plant Extract and Antibiotics

The macrobroth dilution method according to CLSI [20] was used to determine the MIC of the methanol extract of *A. sativum* and antibiotics against MDR isolates of *S. aureus.* Different concentration ranging from 1.563-200 mg/ml of the plant extract and 0.0156 -128 µg/ml of each antibiotic (ceftazidime, cefuroxime, erythromycin, cloxacillin and ciprofloxacin) were prepared by two fold serial dilution in nutrient broth. Combination of different concentrations ranging from 0.25 – 2 x MIC of the extract and antibiotics were used to determine the MIC of their combination effect. The range of extract and antibiotic concentration used in this study was chosen so that the MIC of the extract and antibiotic will fall within the dilution range. Each tube was inoculated with 0.1 ml each of the standardized bacterial isolates. An un-inoculated nutrient broth tube was used as control. The tubes were incubated at 37℃ for 24 hours. The MIC was taken as the lowest concentration of the methanol extract, antibiotic or their combination that showed no visible growth after 24 hours incubation at 37℃. The combination analysis was carried out in duplicate.

2.6 Combination Studies (The Checkerboard Method)

The antibacterial activity of the combination of methanol extract of *A. sativum* and antibiotic (Ceftazidime (Caz), Cefuroxime (Cxm), Erythromycin (Ery), Ciprofloxacin (Cip) and Cloxacillin (Clx) against MDR isolates of *S. aureus* were investigated using the checkerboard method previously described by Jayaraman et al. [21].

The Fractional Inhibitory Concentrations (FICs) were derived from the MIC of extract alone, MIC of antibiotic alone, and MIC of extract plus antibiotic combination permitting no visible growth of the test organism after incubation at 37℃ for 24 hours. The FIC values for the combinations were calculated using the following formula:

FIC (antibiotic) = (MIC of antibiotic in combination with extract / MIC of antibiotic alone).

FIC (extract) = (MIC of extract in combination with antibiotic / MIC of extract alone).

The interactions between the antibiotics plus extracts were evaluated using the FIC indices which was calculated using the formula;

FIC Index = ΣFIC = FIC (antibiotic) + FIC (plant extract).

Combinations were classified as synergistic, if the FIC indices were ≤ 0.5, additive/indifferent if the FIC indices were > 0.5 – ≤ 4.0 and antagonistic if the FIC indices were > 4.0 [21].

2.7 Time-Kill Assay

Time-kill assay was performed using the identified synergistic combinations by the checkerboard method. The assay was carried out on methanol extract of garlic (MEG) plus ceftazidime and MEG plus erythromycin against a single *S. aureus.* The macrobroth dilution technique described by Olajuyigbe and Afolayan, [22] with little modification was used in this analysis.

The extracts and antibiotics were incorporated into 10 ml nutrient broth in a screw-capped test tube at a concentration of ½ x MIC. The tubes containing the individual antibiotic, extract and their combinations were inoculated with 1.0 ml of the bacterial cultures diluted to $x10^8$ cfu/ml. Growth control tubes consists of extract/antibiotic free nutrient broth inoculated with test organisms. Immediately after inoculation, aliquots (0.1 ml) of the control tube was taken, serially diluted in sterile normal saline and plated on nutrient agar

in order to determine the zero hour counts. The inoculated tubes were then incubated in a shaking water bath at 37℃ for 24 hours. Aliquots of 0.1 ml of the culture were withdrawn at 0, 4, 8, 12 and 24 hours of incubation and serial 10-fold dilutions were prepared in sterile normal saline and the dilutions plated on nutrient agar in duplicates. After incubating at 37℃ for 24 hours, emergent bacterial colonies were counted and compared with the culture control without extract or antibiotic. The results were expressed in $\log_{10}$ cfu/ml. Synergy was defined as decrease of ≥ 2 $\log_{10}$ cfu/ml in colony counts [21] and bactericidal activity defined as a ≥ 3 $\log_{10}$ cfu/ml (99.9%) decrease in the bacterial counts for each of the indicated times [23].

2.8 Statistical Analysis

Statistical analysis was carried using the statistical package for social sciences (SPSS) version 15. All the inhibition zones of the plants extracts were given as mean ± SD (standard deviation). $P < 0.05$ was considered significant.

3. RESULTS AND DISCUSSION

Our result indicates that the *A. sativum* extract has antibacterial activity with variable degree of sensitivity of the test organisms (MDR *S. aureus*). The aqueous and methanol extracts of *A. sativum* were more effective against MDR *S. aureus* than acetone extract. Aqueous and methanol extracts had inhibition zone range of 10 ± 1.50 to 17 ± 1.50 and 7 ± 1.05 to 14 ± 0.95 mm respectively at concentration range of 25 to 200 mg/ml (Table 1). Acetone extract had inhibition range of 5 ± 0.50 to 11 ± 0.50 mm. Methanol and acetone extracts had the same inhibition zone (14 ± 0.79 mm) diameter against the *S. aureus* isolates at 200 mg/ml. However, there were no significant difference found between inhibition zone diameter of methanol/acetone extracts (P = 0.50), methanol/aqueous extracts (P = 0.97) and acetone/aqueous extracts (P =0.48) against the test bacteria.

The result of the correlation analysis conducted between inhibition zone size and concentrations of garlic extracts revealed a positive correlation (r) that ranged between 0.84 and 0.89. Generally, inhibition zone diameter of the plant extracts against *S. aureus* increased with increase in concentration of the extracts.

Table 2 shows the MIC values of antibiotics and methanol extract of garlic singly and in combination against five MDR *S. aureus* isolates.

Combination therapies have been used with the aim of better efficacy and improved treatment option [24]. This study recorded synergistic interaction between methanol extract of garlic and ceftazidime in three MDR *S. aureus* isolates. Also, the combination of methanol extract of garlic and erythromycin yielded synergistic interaction in four isolates (Table 3). The synergistic interaction stood at 28.0%. The combination of methanol extract of garlic (MEG) plus cefuroxime showed antagonistic interaction in one isolate whereas it yielded antagonistic interaction in three isolates in the combination of MEG with ciprofloxacin representing 16.0% antagonism.

Time-kill assays were performed on the methanol extracts plus antibiotic combinations found to be synergistic by the checkerboard method. The time-kill analysis showed synergy in the combinations of MEG plus ceftazidime, and MEG plus erythromycin against *S. aureus* at 8 and 4 hours respectively (Figs. 1 and 2). The combination of ½ x MIC of MEG plus ½ x MIC of ceftazidime against *S. aureus* (Fig. 1) showed bactericidal activity against the isolates at 12 hours resulting in a 4.25 $\log_{10}$ cfu/ml reduction. Similarly, bactericidal activity was recorded in the combinations of ½ x MIC of MEG plus ½ x MIC

Table 1. Antibacterial activity of *Allium sativum* (Garlic) extracts against MDR *S. aureus* isolates

Extract	Mean ± SD zones of inhibition (mm) at different concentrations					
	200 mg/ml	100 mg/ml	50 mg/ml	25 mg/ml	+ve Control Cip.(4mg/L)	-ve Control 10% DMSO
Methanol	14 ± 0.95	13 ± 0.75	10 ± 1.15	7 ± 1.05	25 ± 0.50	0
Aqueous	17 ± 1.50	16 ± 1.05	14 ± 1.50	10 ± 1.50	25 ± 0.50	0
Acetone	11 ± 0.50	9 ± 0.72	5 ± 0.50	0 ± 0.00	26 ± 0 .50	0

Key: MDR = Multidrug Resistant; Cip = Ciprofloxacin; DMSO = Dimethylsulfoxide; +ve = Positive; -ve = Negative; SD = Standard Deviation

Table 2. MIC values of antibiotics and methanol extract of garlic singly and in combination against five MDR *S. aureus* isolates

Antibiotics / Plant extract	MIC (µg/ml) values of antibiotics and plants extract against MDR *S. aureus*				
	mmw13	**mfn15**	**ffw24**	**mfw68**	**ffn38**
Caz	2	2	4	2	2
Cxm	16	16	8	8	8
Ery	0.5	0.5	0.5	0.5	0.25
Clx	32	16	16	16	8
Cip	0.5	0.5	0.5	0.5	0.5
MEG	50000	50000	50000	50000	25000
MEG + Caz	1	1	1	0.5	0.5
MEG + Cxm	32	32	32	16	16
MEG + Ery	0.125	0.125	0.125	0.125	0.125
MEG + Clx	32	16	16	16	16
MEG + Cip	1	1	2	2	2

Key: MDR = Multidrug Resistant; MEG = Methanol extract of garlic.
mmw13, mfn15, ffw24, mfw68 and ffn38 = Code for S. aureus isolates.
MIC = Minimum inhibitory concentration
Caz = Ceftazidime; Cxm = Cefuroxime; Ery = Erythromycin; Clx = Cloxacillin; Cip = Ciprofloxacin

Table 3. Effect of combination of methanol extracts of garlic and some antibiotics against five MDR *S. aureus* isolates by the checkerboard FIC index

Plant extract + Antibiotic combination	Effect of combination of methanol extract garlic and antibiotics against MDR *S. aureus* by the checkerboard FIC index					
		mmw13	**mfn15**	**ffw24**	**mfw68**	**ffn38**
MEG + Caz	FICE	0.00002	0.00005	0.00002	0.00001	0.00002
	FICA	0.5	0.5	0.25	0.25	0.25
	FICI	0.50002	0.50002	0.25002	0.25001	0.25002
	Rem	**Add**	**Add**	**Syn**	**Syn**	**Syn**
MEG + Cxm	FICE	0.00064	0.00064	0.00064	0.00032	0.00064
	FICA	2	2	4	2	2
	FICI	2.00064	2.00064	4.00064	2.00032	2.00064
	Rem	**Add**	**Add**	**Ant**	**Add**	**Add**
MEG + Ery	FICE	0.0000025	0.0000025	0.0000025	0.0000025	0.000005
	FICA	0.25	0.25	0.25	0.25	0.5
	FICI	0.2500025	0.2500025	0.2500025	0.2500025	0.500005
	Rem	**Syn**	**Syn**	**Syn**	**Syn**	**Add**
MEG + Clx	FICE	0.00064	0.00032	0.00032	0.00032	0.00064
	FICA	1	1	1	1	2
	FICI	1.00064	1.00032	1.00032	1.00032	2.00064
	Rem	**Add**	**Add**	**Add**	**Add**	**Add**
MEG + Cip	FICE	0.00002	0.00002	0.00004	0.00004	0.00008
	FICA	2	2	4	4	4
	FICI	2.00002	2.00002	4.00004	4.00004	4.00008
	Rem	**Add**	**Add**	**Ant**	**Ant**	**Ant**

Key: MDR = Multidrug Resistant; MEG = Methanol extract of garlic;
mmw13, mfn15, ffw24, mfw68 and ffn38 = Code for S. aureus isolates.
FIC = Fractional inhibitory concentration
Caz = Ceftazidime; Cxm = Cefuroxime; Ery = Erythromycin; Clx = Cloxacillin; Cip = Ciprofloxacin
FICE = Fractional inhibitory concentration of extract; FICA = Fractional inhibitory concentration of antibiotic;
FICI = Fractional inhibitory concentration index; Rem = Remark;
Syn = Synergism; Add = Additive/indifferent; Ant = Antagonism

of erythromycin against *S. aureus* (Fig. 2) after 8 hours with a 3.13 $\log_{10}$ cfu/ml decrease. The net reduction in colony counts was observed consistently between 12-24 hours with a 3.13 - 4.25 $\log_{10}$ cfu/ml decrease. However, re-growth of the organisms was observed between 12 and

24 hours incubation for garlic extract and antibiotics alone.

The present finding corroborates earlier reports that garlic has antimicrobial potency [13,25,26]. The antimicrobial activity of garlic has been attributed to its phytochemical component, allicin (a thiosulfonate) whose removal renders garlic ineffective against microorganisms [25]. In the present study, garlic aqueous extracts had the highest antibacterial activity of 17 ± 1.50 mm against MDR *S. aureus* whereas Hamza [13] reported 30 mm as the maximum inhibition zone of garlic extract against *S. aureus*. Similarly, Muhsin and Al-Mossawi [27] found that *E. coli* and *S. aureus* showed promising sensitivity to water extracts of garlic. Belguith et al. [28], Bakht et al. [29] and Iram et al. [12] also reported a higher inhibition zone with aqueous extracts of garlic against *S. aureus*. The finding of this study agreed with the reports of Esimone et al. [30] and Hindi [31] that aqueous garlic extracts are active against *S. aureus*. Ameh et al. [32] found methanol extracts of garlic to be active against Gram positive and Gram negative bacteria in agreement with the present finding.

The correlation analysis results showed that zone size increases with increase in concentration of the extracts. Similar result was reported by Vishal and Trivedi [5] in their study.

The result of this study indicates that methanol extract of garlic contains chemical compounds that can potentiate the activity of antibiotics against MDR bacteria. A number of studies have reported the ability of plant extracts to potentiate antibiotics against *S. aureus.* In a study of some Jordanian plants, results showed that sub-inhibitory levels (200 µg/ml) of the methanol extracts yielded synergistic interactions in combination with chloramphenicol, gentamicin, erythromycin and penicillin G against resistant and sensitive *S. aureus* [33]. The present study recorded synergistic interaction between MEG and ceftazidime (an inhibitor of cell wall synthesis) and erythromycin (an inhibitor of protein synthesis) against MDR *S. aureus*

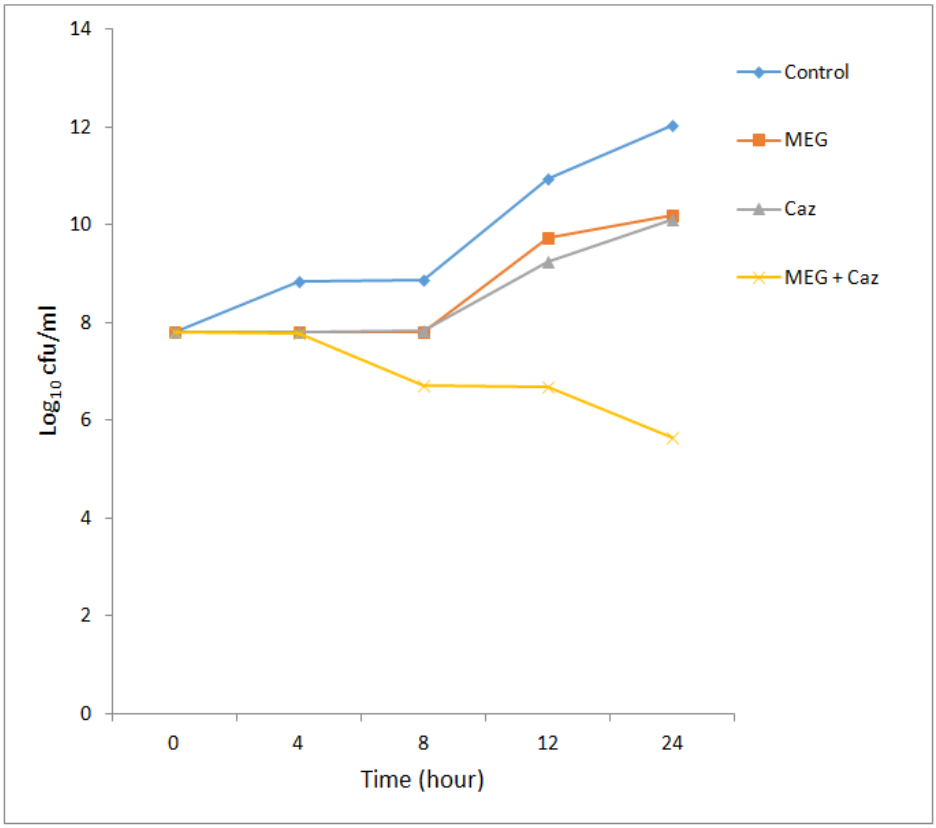

Fig. 1. Time-kill curve for the synergistic interaction of Methanol extract of garlic (MEG) and Ceftazidime (Caz) at ½ x MIC against MDR *S. aureus* isolate

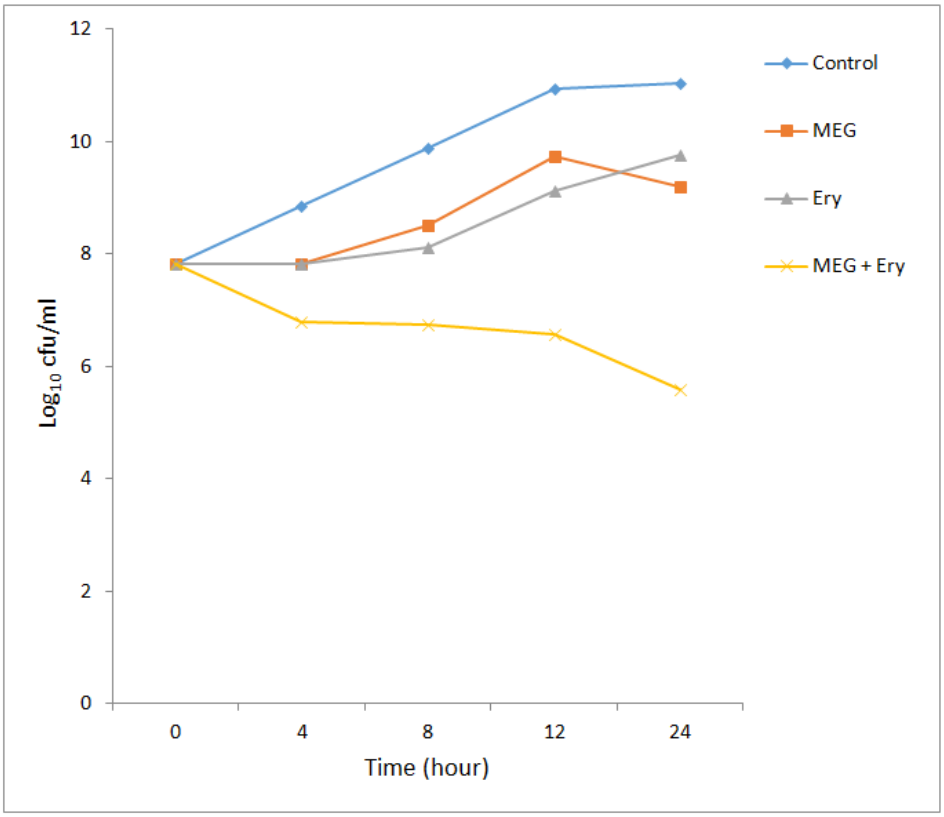

Fig. 2. Time-kill curve for the synergistic interaction of Methanol extract of garlic (MEG) and Erythromycin (Ery) at ½ x MIC against MDR *S. aureus* isolate

isolates. This finding agrees with the report of Betoni et al. [34], who observed synergistic interactions between extracts of guaco (*Mikania glomerata*), guava (*Psidium guajava*), clove (*Syzygium aromaticum*), garlic (*Allium sativum*), lemongrass (*Cymbopogon citratus*), ginger (*Zingiber officinale*), carqueja (*Baccharis trimera*), and mint (*Mentha pieria*) from Brazil and some antibiotics which represented inhibitors of protein synthesis, cell wall synthesis, nucleic acid synthesis and folic acid synthesis against *S. aureus*. Similar result was reported by Zafar et al. [35] when they combined *Salvadora persica* extracts, tetracycline and penicillin against *S. aureus.* In a similar study, Ahmed et al. [36] investigated inhibitory effect of penicillin and tetracycline against *S. aureus* individually and in combination with ethanol extract of leaf and stem of *Salvadora persica.* The highest synergistic effect was observed when *S. aureus* was exposed to tetracycline with stem extract of *S. persica*. It was followed by tetracycline with leaf extract of *S. persica*. Eja et al. [25] reported a synergistic interaction between the combination of garlic and ampicillin against *S. aureus* and an additive effect between the combination of garlic and ciprofloxacin opposed to the present study that found additive/indifferent and antagonistic effects with ciprofloxacin. Souto de Oliveira et al. [37] investigated the synergistic activity of norfloxacin, tetracycline and erythromycin with ethanol extract of *Mangifera indica* L. peel against *S. aureus* strains, and reported a four-fold reduction in the MIC values for tetracycline and erythromycin. In the investigation of the *in vitro* combined effects of erythromycin and methanol extract of leaves of *Euphorbia hirta* against clinical isolates of *S. aureus* using the Checkerboard technique by Adikwu et al. [38], synergistic effect was obtained by a combination of erythromycin and *E. hirta* against *S. aureus* in the ratios (9:1, 8:2, 7:3, 6:4, 3:7, 2:8, 1:9) while others (5:5, 4:6) showed indifference. This study recorded additive/indifferent and antagonistic effect between the combination of MEG and ciprofloxacin against *S. aureus* similar to Salah et al. [39], who reported additive effect between the combination of aqueous garlic extract and ampicillin or ciprofloxacin.

The result of the time-kill assay confirmed the synergy obtained with the MIC checkerboard method.

4. CONCLUSION

In this study, *A. sativum* extracts exhibited varying levels of antibacterial activity against MDR *S. aureus.* Synergistic interaction was recorded between methanol extract of garlic and ceftazidime against three MDR *S. aureus* isolates. Also, the combination of methanol extract of garlic and erythromycin yielded synergistic interaction in four MDR *S. aureus* isolates. The result of the time-kill assay confirmed the synergy obtained with the MIC checkerboard method. Bactericidal activity was observed between the combination of ½ x MIC of MEG plus ½ x MIC of ceftazidime against *S. aureus* and in the combinations of ½ x MIC of MEG plus ½ x MIC erythromycin against *S. aureus*. This study provides baseline information for possible use of *A. sativum* extracts in the treatment of bacterial infections involving MDR *S. aureus.* The extracts of this plant could be used in association with antibiotics to combat multidrug resistant *S. aureus.* Hence, there is the need to isolate the specific active agent(s) involved in the potentiation for drug compounding.

CONSENT

All authors declare that written informed consent was obtained from the patients for publication of this paper.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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