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Evaluation of the antibacterial activity of a combination of juice extracts of *Allium sativum bulbs* and *Curcuma longa* rhizomes against methicillin-resistant *Staphylococcus aureus*

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ABSTRACT

Methicillin-resistant *Staphylococcus aureus* (MRSA) is spreading around the world, with prevalence ranging from 23.3 % to 73 %. This study, therefore, evaluated the antibacterial activity of a combination of crude juice extracts of *Allium sativum* and *Curcuma longa* against methicillin-resistant *Staphylococcus aureus*. The combined crude juice extracts exhibited moderate antibacterial activity against methicillin-resistant *Staphylococcus aureus*, which was determined by the agar well diffusion method where the *Allium sativum* crude juice extract revealed a high level of antibacterial activity with the highest mean and standard error of the mean inhibition zone diameters of 21.67 ± 0.88 mm at concentration 100%v/v compared at a concentration of 50%v/v which showed a mean and standard error of the mean inhibition zone diameter of 21.33 ± 0.67 mm. On the other hand, *Curcuma longa* crude juice extract didn't show any antibacterial activity against the test organism. The minimum inhibitory concentration (MIC) of the combination of the crude juice extracts of *Allium sativum* and *Curcuma longa* crude juice extracts was 25%v/v, *Allium sativum* was 25%v/v w and *Curcuma longa* was 0%v/v. The minimum bactericidal concentration (MBC) results that the study extract was bacteriostatic since all used concentrations showed visible growth after overnight incubation. The FIC of the extract was 1 hence there was an additive antibacterial effect of the extract combination.

Keywords: *Allium sativum*, *Curcuma longa*, *Staphylococcus aureus*, methicillin-resistant.

INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) is spreading around the world, with prevalence ranging from 23.3 %to 73 %. In 1996, an international multicenter study found that among the countries studied, South Africa and Malaysia had some of the highest rates of MRSA, but in Africa, the prevalence ranged from 5% to 45%, with one of the first cases reported in the continent in 1978 [1]. In the United States in 1995, nosocomial infections cost $4.5 billion and resulted in over 88,000 deaths—one every 6 minutes [2]. MRSA is any *Staphylococcus aureus* strain that has acquired or developed multiple drug resistance to beta-lactam antibiotics, including methicillin, by natural selection or horizontal gene transfer. Methicillin and the more routinely administered penicillin, amoxicillin, oxacillin, fluoroquinolones, and macrolides are all resistant [3-5]. According to a Baylor College of Medicine study, MRSA is classified based on where it was obtained. The first form, healthcare-acquired MRSA (HA-MRSA), is obtained from hospitals and healthcare facilities and has been recognized since the 1960s and is more prevalent in people who have had surgery, had medical devices installed, or have impaired immune systems. The second type of MRSA, which appeared in the 1990s, is known as community-acquired MRSA (CA-MRSA), which occurs outside of

hospital settings and usually manifests itself as a skin infection in healthy individuals but can develop into a more serious, life-threatening illness. It tends to occur in situations where people are in close physical proximity, such as in childcare and long-term care facilities, soldiers, prisoners, and athletes who have skin-to-skin contact [6, 7]. MRSA is prevalent in hospitals, jails, and nursing homes, where persons with open wounds, intrusive equipment such as catheters, and weaker immune systems are more susceptible to healthcare-associated illness [8, 9]. MRSA strains have emerged as a source of nosocomial infections that can lead to life-threatening pneumonia, necrotizing fasciitis, endocarditis, osteomyelitis, severe sepsis, and toxic shock syndrome [10, 11]. According to Garoy *et al.* [12], the risk factors for MRSA that have been reported in the literature as immunosuppression, hemodialysis, peripheral malperfusion, advanced age, prolonged in-hospital stays, residency in long-term care facilities, the inadequacy of antimicrobial therapy, indwelling devices, insulin-requiring diabetes, and decubitus ulcers.

Allium sativum is a plant from the family of Alliaceae. It is an herbaceous plant with a height of 20-40 cm, a bulb with a strong odor and pungent taste. *Allium sativum* contains organosulphur compounds that are responsible both for their strong smell and for their medicinal properties and has long been known as an effective plant species in the treatment of bacterial infections [13-16]. It has been used as a flavoring ingredient and a medicinal herb for thousands of years. *Allium sativum* bulbs are used as seasonings, and the liquid collected from them is also medicinally beneficial and its extracts were also found to be effective against Gram-negative *(E. coli*, *Salmonella sp*., *and Citrobacterenterobacter*, *Pseudomonas Klebsiella*) as well as Gram-positive (S. aureus) bacteria [17]. The properties of *Allium sativum* are associated with its extremely rich compositions as it contains approximately 33 sulfur compounds (some are diallyl thiosulfate, diallyl sulfide, diallyl disulfide and alliin), 17 amino acids, enzymes, mineral salts (e.g. germanium, selenium, phosphates, calcium, and iron salts), vitamins (e.g. ascorbic acid, riboflavin, niacin, thiamine, folic acid. *Allium sativum* is estimated to contain over two hundred chemical substances that can protect the human body against various diseases [15]. Aside anti-microbial effect, *Allium sativum* possesses anti-diabetic and liver-protective effects [13, 14, 18].

Curcuma longa is a perennial herbaceous plant in the ginger family (Zingiberaceae), whose tuberous rhizomes, or underground stems, have been used as a food preservative, a textile color, and a medical stimulant since ancient times. *Curcuma longa* is a spice and scent that is native to southern India and Indonesia. It is taken as a pill for a variety of ailments, including arthritis and intestinal problems. *Curcuma longa* plants grow to a height of approximately 1 meter (3.3 feet) and have long simple leaves with long petioles, which emerge from branching rhizomes close below the soil surface. Young rhizomes are pale yellow to brown-orange, whereas older rhizomes are scaly and brown [19]. It has antioxidant properties and thus can be useful in the management of oxidative stress-induced diseases [20, 21]. MRSA is still a major global health issue. The increased occurrence of side effects for example ototoxicity, hematopoietic effects, allergic reactions, and low blood pressure associated with the overuse of antibiotics and other conventional drugs creates a need for developing alternative safer remedies [22-35]. More so, the use of plant-based products in the management of various diseases including bacterial infections has been an age-long practice, especially among rural dwellers [35-40]. In addition, the rapid resistance to numerous classes of antibiotics, including linezolid creates a need of developing alternative safer remedies [12]. Furthermore, *S. aureus* bacteremia, which has a high rate of morbidity and mortality and can lead to metastatic or complex infections such as infective endocarditis or sepsis, is of particular concern requiring alternatives for the treatment of MRSA [27]. However, there is scarcely any study that has been done to evaluate the effectiveness of antimicrobial activity of a combination of juice extracts of *Curcuma longa* and *Allium sativum* against MRSA. Hence, this study aims to bridge the gap by evaluating the effectiveness of the antimicrobial activity of a combination of juice extracts of *Curcuma longa* and *Allium sativum* against MRSA.

METHODOLOGY

Study Design

The study is an *in-vitro* experimental study that was done to evaluate the antibacterial activity of a combination of juice extracts of *Allium sativum and Curcuma longa against* Methicillin-resistant *Staphylococcus aureus* and was carried out in the microbiology and pharmacognosy laboratory of Kampala International University western campus from Feb to March 2023

Study Area

The experiment was carried out at the Microbiology and Pharmacognosy laboratories of Kampala international university western campus and the fresh samples of *Allium sativum* bulbs and *Curcuma longa* rhizomes were bought from the Ishaka market.

Materials used

The materials and equipment used in this study were *Allium sativum*, *Curcuma longa* and methicillin-resistant *Staphylococcus aureus*, Mueller-Hinton agar, masks, sterile diffusion disc, filter papers, sterile cotton swabs, agar plates, distilled water, an incubator, a ruler, sterile gloves, an autoclave, McFarland standard solution, vancomycin, syringe, tubes, mannitol salt agar, BHI broth, foil paper, Petri dishes, mortar and pestle, refrigerator, measuring cylinder, pen, calibrated ruler, water source, sterile wire loop, tube racks vials

Preparation of the Plant Extracts

Collection of plant materials

The *Curcuma longa* rhizomes and *Allium sativum* bulbs were collected from the Ishaka market and authenticated by the Botanist.

Plant extraction

This was performed according to Marhajan [28] method where *Curcuma long* rhizomes, *Allium sativum* bulbs were collected, washed thoroughly with clean distilled water and later disinfected with 75% alcohol then dried and cut into small pieces which were later pound by use of a sterile mortar and pestle to squeeze juice from it. Later the juice was collected using a pipette into a clean sterile microcentrifuge tubes and spinned at 5000rpm for five minutes. The centrifuged samples were later filtered 0.45µm syringe filters.

Identification of Staphylococcus aureus

The test microorganism used in this study was a clinical isolate of methicillin-resistant *Staphylococcus aureus* that was obtained from the microbiology laboratory. The isolate was initially subcultured on blood agar to observe the beta-hemolytic pattern. This was later sub-cultured on mannitol salt agar to examine the formation of golden yellow colonies of typical *Staphylococcus aureus*.

Antimicrobial resistance pattern of *Staphylococcus aureus*

To assess for the methicillin resistance of the confirmed *Staphylococcus aureus* above, Kirby Bauer disk diffusion was performed according to the method described by Hudzicki [29] where an overnight culture was suspended into a test tube containing normal saline and later picked by use of a sterile cotton swab and spread onto Mueller-Hinton agar supplemented by 0.5% blood agar. This was allowed time to dry for ten minutes before antibiotic discs that is Cefoxitin, Oxacillin were introduced. This was then incubated overnight at 37℃. The zones of inhibition of growth were measured by use of a meter rule and interpreted using a standard chart of the Clinical and laboratory standards institute (CLSI) 2018.

Preparation of the culture Media

Mueller-Hinton agar

15.2g of Mueller Hinton agar powder was dissolved in 400ml of distilled water in one conical flask of 500ml, and mix completely. It was then sterilized by autoclaving at 121°C for 15 min, then pour the liquid onto the petri dish and wait for the medium to solidify. Ensuring that the agar is prepared in the clean environment to avoid contamination and after solidifying it.

Brain heart infusion broth

3.7g of Brain heart infusion broth was suspended in 100ml of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize at 121°C for 15 minutes. To prepare a selective medium for fungi, the sterilized and melted should be cooled at 45-50°C before adding the appropriate antibiotics. Occasionally a small amount of sediment may appear which should be re-suspended with a gentle swirl before dispending

Antibacterial susceptibility of extracts of *Allium sativum, Curcuma longa,* and their combination.

The antibacterial activity of extracts was tested using the agar well diffusion method. The agar plate surface was inoculated by thin streaking of inoculum of Methicillin-Resistant *Staphylococcus aureus* uniformly over the entire agar surface then five holes with a diameter of 6mm were punched aseptically with a sterile cork borer in 6 agar plates.

And a volume of 50µl of *Allium sativum*, *Curcuma Longa*, and a combination of extract solution at a concentration of 100%v/v and 50%v/v was introduced into the wells, and 50µl of 30µg/ml of positive control of vancomycin and 50µl of 0.85% of negative control (normal saline) were introduced as well in wells. Then agar plates were incubated under suitable conditions at 37°C for 24 hours. The resulting zone of inhibition was measured using a meter rule

Determination of the minimum inhibitory concentration of *Allium sativum and Curcuma longa* and their combination against methicillin-resistant *S. aureus*

The minimum inhibitory concentration of *Allium sativum* and *Curcuma longa* was determined by broth dilution method by use of 96 well microtiter plate as described by Eve [11]. Brain heart infusion broth was prepared according to the manufacturer's instructions after which 200µl was dispensed into wells of the microtiter plate. Test organism suspension containing $1.5x10^8$CFU/ml was prepared using normal saline by dissolving fresh colonies while comparing with 0.5 Mc Farland standard solution. A two-fold serial dilution was used where 200µl (50%v/v) of the individual stock solution of *Curcuma longa* and *Allium sativum* extracts were serially diluted by picking 200µl and subsequently transferring it into the second well containing 200µl of the broth. This was performed continuously by transferring 200µl until in the last well where the 200µl was discarded to maintain a uniform volume. Similarly, a combination of *Allium sativum* and *Curcuma longa* extracts were serially diluted by a two -fold serial dilution. Here, 200µl of the combination of the stock solution of *Allium sativum* and *Curcuma longa* extracts were put into the first well of the microtiter plate and then transferred into the second well. This was repeated until the last well where 200µl of the extract was discarded to maintain a uniform volume. 50µl of the diluted bacteria equivalent to $1.0x10^6$CFU/ml added into each tube containing individual and combined serially diluted extracts. Two controls were prepared where control one (positive control) contained broth and bacteria but without extract to find out whether the broth supported the growth of methicillin resistant *Staphylococcus aureus*. Control two (negative control) contained only broth without bacteria but with extract to find out if it was not contaminated by other organisms. The microtiter plate was then incubated at 37°C overnight. After incubation 50 µl of 0.2 mg/ml resazurin was added in all the wells of the microtiter and incubated at 37°C for 2 hours. The development of pinkish-red color in the respective tubes was indicative of bacterial growth and blue solution was suggestive of no growth. The lowest concentration of the individual and combined extracts that showed no color change compared to the next well dilution was taken as the minimum inhibitory concentration (MIC) of the individual and combined juice extracts against the test clinical methicillin-resistant *S. aureus*.

Minimum bactericidal concentration (MBC) of *Curcuma longa and Allium sativum* and their combination against methicillin-resistant *S. aureus*

Minimum bactericidal concentration (MBC) is described as the lowest concentration of medicinal plant extract required to kill 99.9% of microorganisms on the agar plate assessed by picking the MIC contents in the microtiter plate by use of a sterilized wire loop and inoculating them on the surface of Muller Hinton Agar plate. Wells without visible growth were picked out and the solutions were subcultured onto freshly prepared Mueller Hinton Agar by streaking on the agar surface after which incubated at 37°C for 24 hours. The concentrations of *Curcuma longa* and *Allium sativum* that didn't show visible growth on Muller Hinton agar plates were considered as the MBC Vuong *et al.* [30].

Determination of FIC (Fractional Inhibitory Concentration)

FIC value was determined using the standard formula according to Akinyele [31].

$$\text{FIC (extract)} = \frac{\text{MIC of the extract in combination}}{\text{MIC of the extract alone}}$$

Therefore, the sum of the FIC of the individual extracts gave the FIC index as shown in the equation; FIC index = FIC of ***Allium sativum*** + FIC of ***Curcuma longa.***

The effect of the combination was classified as synergism, additive, indifference and antagonistic if the FIC (index) is <1, $=1$, >1but ≤ 2 and >2 respectively.

Ethical Considerations

The used culture, inoculum and plates were disposed in a way that does not cause harm to the environment and minimizes the risk of infection in healthy individuals. They were autoclaved at 121° C for 20 min and then disposed of in the bins in the laboratory.

Data Analysis

The measurements for inhibition zone diameters and the respective concentrations used were entered into Microsoft Excel and then exported to Statistical Package for social science version-20 (SPSS-20) software for analysis. One-way ANOVA was used to compute descriptive statistics of mean and standard error of inhibition zone diameters in mm. Tukey's post hoc test was used to obtain multiple comparisons between the antibacterial activity of plant extract, positive control and negative control. Statistical significance was considered at $P \geq 0.05$ No significance. $P \leq 0.05$ there is significance. Data was also analyzed and graphs drawn with Graph Pad prism version 4.

RESULTS

Identification of the bacterial isolate. (MRSA)

This was done on mannitol salt agar. The bacterial isolate was sub cultured on Mannitol salt agar to observe the small yellow golden colonies after which the isolate was spread on blood agar plate for sensitivity testing to confirm for methicillin resistance by use of the Kirby-Bauer method as seen in the figure below.

Figure 1: Showing MRSA on Mannitol salt agar plate after an overnight incubation

Antibacterial activity of *Allium sativum, Curcuma longa* juice extracts and their combination against MRSA

The susceptibility of Methicillin-resistant *Staphylococcus aureus* to *Allium sativum* extract, *Curcuma longa* extract, and their combination was determined by agar well diffusion method where the diameter zones of inhibition in mm were measured after 24 hours of incubation at 37°C and it was done in triplicate (3times), the final results were reported as mean ±SEM. The *Allium sativum* extract showed a high level of antibacterial activity with the highest mean and standard error of the mean inhibition zone diameters of 21.67 ± 0.88 mm at a concentration 100%v/v compared at a concentration of 50%v/v which showed a mean and standard error of the mean inhibition zone diameter of 21.33 ± 0.67 mm. However, the combined extracts showed a moderate level of activity with the moderate mean ±SEM inhibition zone diameters of 16.67 ± 1.67 mm at concentrations 100%v/v and 50%v/v. Furthermore, *Curcuma longa* had a lowest level of activity with the lowest mean ±SEM inhibition zone diameters of 7.00 ± 00 mm at a concentration 100%v/v and 6.67 ±0.33mm at a concentration of 50%v/v. The antibacterial activity of *Allium sativum extract at* (100%v/v) gave an inhibition diameter of (21.67 ± 0.88 mm) which was statistically significantly different (p= 0.00) from that of *Curcuma longa extract* at a concentration of 100%v/v of which had a lower inhibition diameter of 7.00± 0. 00mm. There was a statistically significant difference (p= 0.02) between the antibacterial activity of

combination extracts and *Allium sativum* extract at concentrations of 100%v/v and 50%v/v. The positive control (vancomycin, 30mcg/ml) had an inhibition which was statistically significant difference (p= 0.02) from that of *Allium sativum* extract and the combination showed a lower mean and standard error of the mean inhibition zone diameter of 14.67 ± 0.67 mm compared to *Allium sativum* extract 21.67 ± 0.88mm and the combination 16.67 ± 1.67mm. For concentrations of the extracts used, there was a significant difference (p=0.000) between the extracts and the negative control (Normal saline) which showed no inhibition zone diameter(0.0mm).

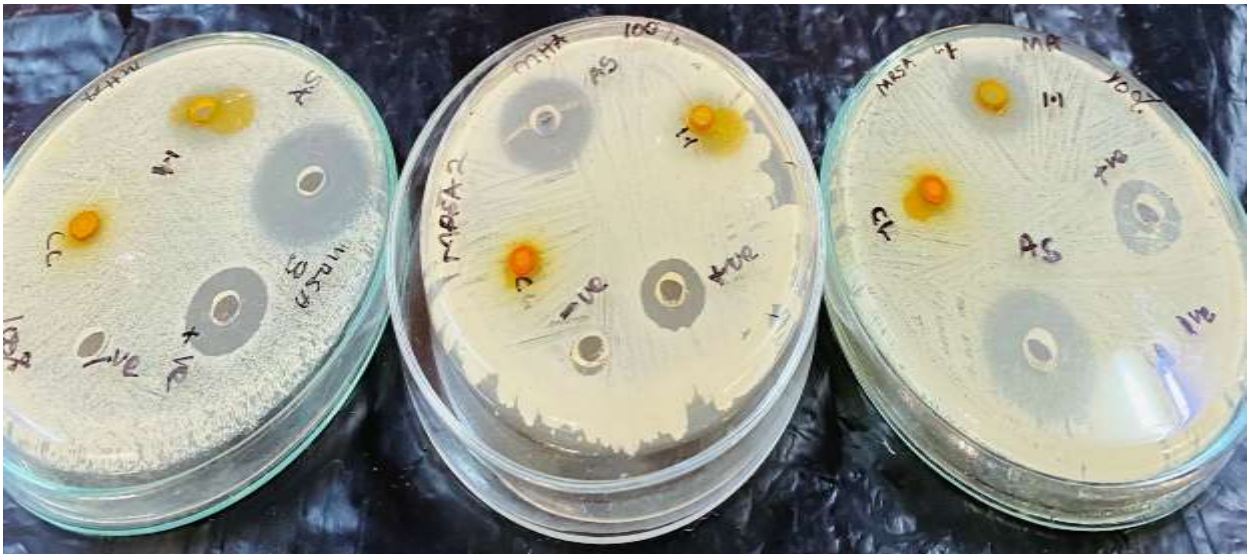

Figure 2: Showing the antibacterial activity of *Curcuma longa* and *Allium sativum* against MRSA

Table 1: Mean and standard deviation of inhibition zone diameters (mm) of *Allium sativum, Curcuma longa* juice extracts and their combination against MRSA

Mean± SEM inhibition zone of diameter(mm)

	Extracts Concentrations	
Extracts	**100%v/v**	**50%v/v**
Allium sativum	21.67± 0.88	21.67±0.33
Curcuma longa	7.00± 0.00	6.67± 0.33
Combination (1:1)	16.67±1.67	16.67±1.67
Controls		
Positive control		
Vancomycin	14.67± 0.67	
30 µg/ml		
Negative control	0.000± 0.000	
Normal saline		

Table 1 above shows the Mean and standard deviation of inhibition zone diameters (mm) of *Allium sativum*, *Curcuma longa* juice extracts and their combination against MRSA. Value ≥0.05 is not significant and value ≤ 0.05 is significant.

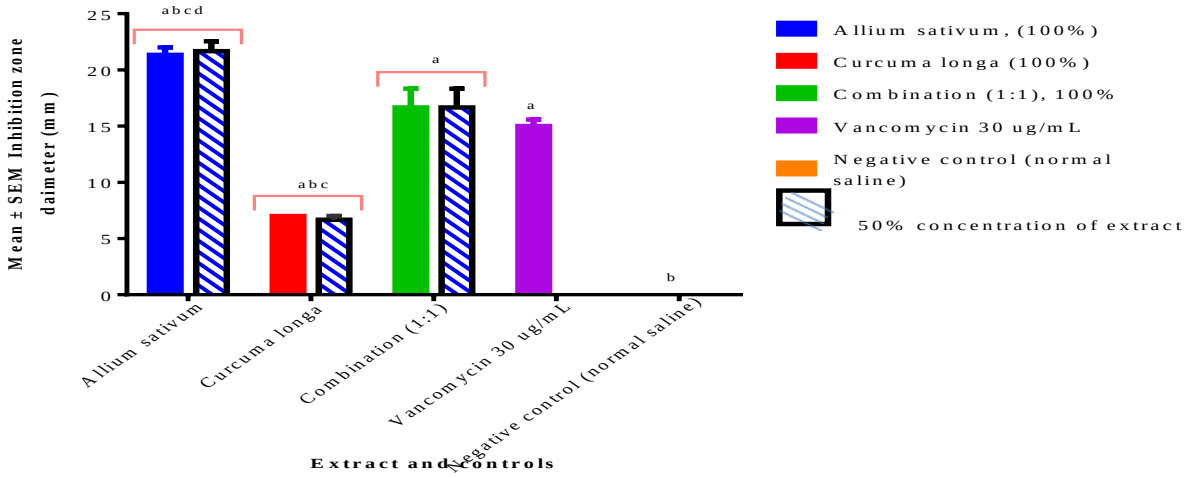

Figure 3: Comparison of mean inhibition zone diameters of *Allium sativum, Curcuma longa* juice extracts and their combination of 100%v/v and 50%v/v concentrations against MRSA.

a- Statistical significance Vs negative control
b- Statistical significance Vs Positive control
c- Statistical significance Vs Combination (1:1)
d- Statistical significance between *Allium sativum* and *Curcuma long*

The minimum inhibitory concentration of *Allium sativum* and *Curcuma longa* juice extracts and their combinations

The study determined the minimum inhibitory concentration of *Allium sativum* and *Curcuma longa* juice extracts and their combination against methicillin-resistant *S. aureus*, where MIC of *Allium sativum was 25%v/v* and for the combination was 25%v/v and *Curcuma longa* had no MIC and Vancomycin had 3.73µg/ml as shown in figure 4 below.

AS/AS/AS CL/CL/CL COM/COM/COM POS NEG

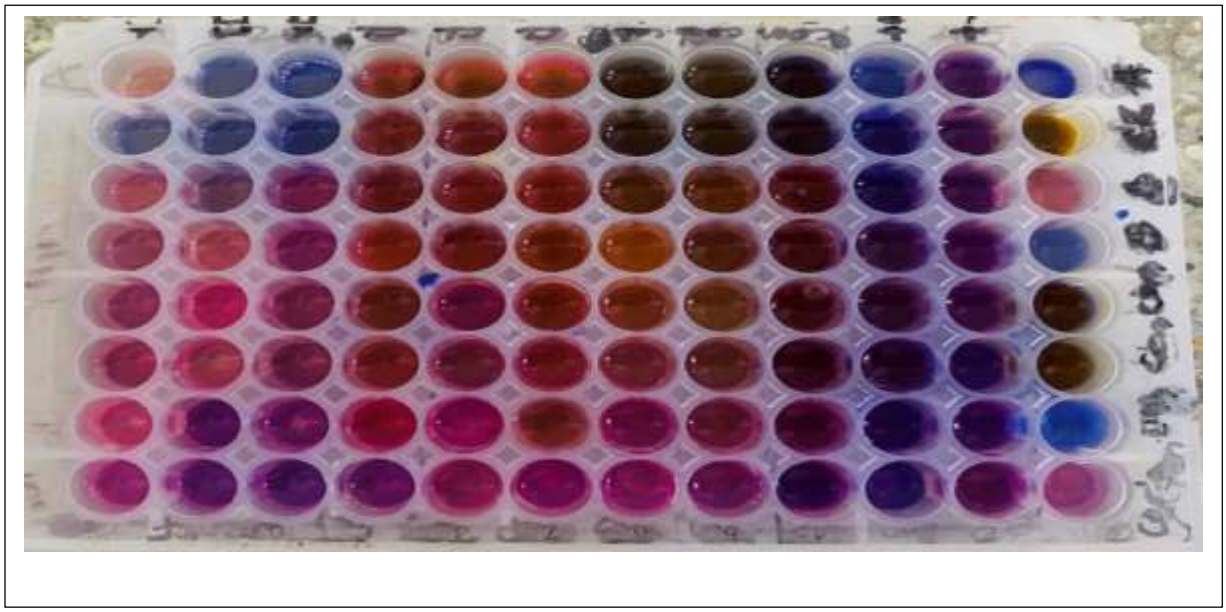

Figure 4: showing Minimum Inhibitory Concentration (MIC) of *Allium sativum* (AS), *Curcuma longa* (CL) juice extracts and their combination (COM), Positive control (pos), Negative control (Neg) against methicillin resistant *S. aureus*

Table 2: Showing MIC values of the Allium sativum and Curcuma longa and their combinations against MRSA

	Plant extracts		
	Allium sativum	***Curcuma longa***	**Combination**
(%v/v)	**25**	**0**	**25**

Minimum Bactericidal Concentration (MBC) of *Allium sativum* and *Curcuma longa* juice extracts and their combination against MRSA

All concentrations that were considered for MBC had visible growth of bacteria MRSA as shown in figure below

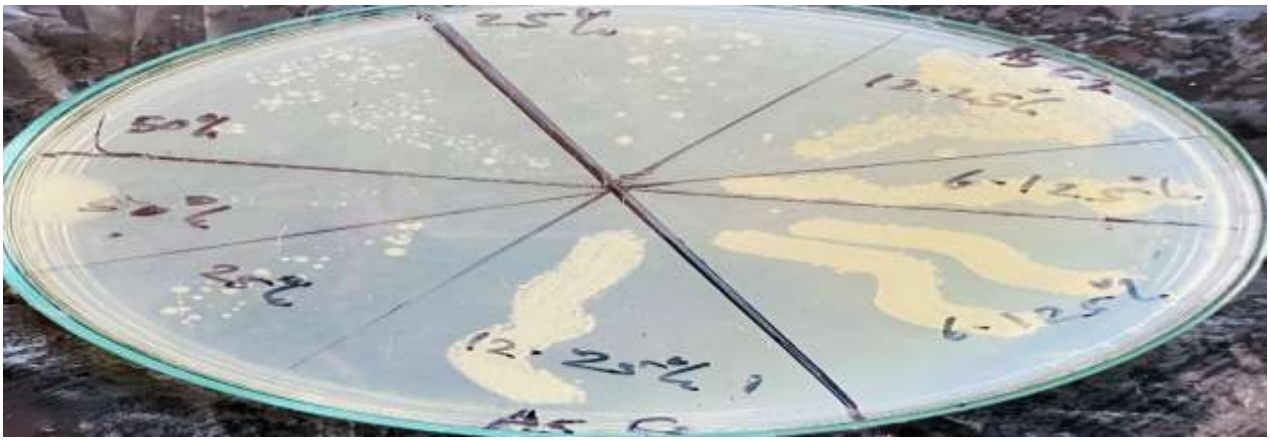

Figure 5: Showing the MBC of *Curcuma longa* and *Allium sativum* against MRSA on Muller Hinton agar plate

Determination of FIC (Fractional Inhibitory Concentration)

FIC value was determined using the standard formula:

FIC (extract) = MIC of the extract in combination
MIC of the extract a lone.

FIC (*Allium sativum* extract) = $\frac{25}{25}$ =1

The FIC of Curcuma longa was =0

FIC index = FIC of ***Allium sativum*** + FIC of ***Curcuma longa.***

FIC index = 1 + 0 = 1 Therefore the combination of the two extracts exhibited antagonist antibacterial effect

DISCUSSION

Bacterial infections are considered one of the most contributors to human illness and death in developed and developing worlds. Various microbes have evolved over and over again to gain resistance to a number of available antimicrobials evidenced by methicillin-resistant *Staphylococcus aureus* which accounts for about 50,000 deaths every year in the united states [32]. These resistant strains are a major threat to human health which requires an urgent intervention to combat such a challenge, especially with the use of nature as a potential source for drug discovery [32]. Therefore, this study aimed at evaluating the synergistic antibacterial effects of crude juice extracts of *Allium sativum* and *Curcuma longa* against Methicillin-resistant *Staphylococcus aureus.* The antibacterial effects of the juice extract against *Staphylococcus aureus* were determined by agar well diffusion method as described by [11]. The present study revealed that *Allium sativum* crude juice extract exhibited high antibacterial activity with the highest mean and standard error of the mean of inhibition zone diameters of 21.67±0.88mm at (100%v/v) compared to 21.33±0.67mm at (50%v/v). The high antibacterial activity of *Allium sativum* is attributed to the bioactive compound allicin found in fresh garlic [15].

On the other hand, the study revealed no activity of *Curcuma longa* against MRSA when used alone due to sequential mutations resulting in the thicker cell wall and the production of a protein called PBP2a which is able to avoid the inhibitory effects of the antibiotics by MRSA [2]. Some studies however, have shown the antibacterial effect of the juice extract of *Curcuma longa* against *Staphylococcus aureus* despite the fact that a small zone of the diameter of inhibition was recorded at a high concentration of 500mg/ml. This is different from the current study where a crude juice extract of *Curcuma longa* was used. Secondly, our study used Methicillin-resistant *Staphylococcus aureus*

compared to Kasta [33-40] who reported the activity of *Curcuma longa* against methicillin-sensitive *Staphylococcus aureus*.

The 1:1 combination of *Allium sativum* and *Curcuma longa* crude juice extracts (100%v/v and 50%v/v) showed a lower mean and standard error of the mean inhibition zone diameter of 16.67 ± 0.67 mm compared to *Allium sativum* extract 21.67 ± 0.88mm due to antagonism interactions between bioactive compounds. The results obtained in this study are contrary to the results obtained by Maharjan et al. [28] due to the difference in extraction methods used. Secondly, this could be due to different mechanisms of action of the two extracts which would interfere with the other's activity. The negative control 0.85% normal saline did not have any antibacterial effect against Methicillin-resistant *Staphylococcus aureus* indicative that does not have bioactive compounds. Nevertheless, this study revealed a high-level activity of the extract (100%v/v) (*Allium sativum*) against MRSA compared to the standard drug (vancomycin) (30µg/ml). This is attributed to the organosulphur compound allicin which has been shown to inhibit bacterial growth by disrupting the cell membrane and inhibiting DNA replication of MRSA [16, 34]. The minimum inhibitory concentration of *Allium sativum* was 25%v/v against the test organism which is in line with results obtained by Magryś *et al.* [15]. However, his study revealed a lower MIC value of 6.25% against MRSA compared to our study which reported a higher MIC value of 25%. Nonetheless, Magryś *et al.* [15] reported a higher MIC value of 50%v/v against vancomycin-resistant *Enterococcus faecalis* which is contrary to the present study that reported a lower MIC of 25%v/v against MRSA. Similarly, the MIC of the combination of the crude juice extracts of the *Allium sativum* and *Curcuma longa* was 25% v/v which is in line with results obtained by Maharjan et al. [28].*Curcuma longa* also contains bioactive compounds with antibacterial activity, such as curcumin, which has been shown to inhibit the growth of a range of bacteria. However, the antibacterial activity of turmeric extract in this study was lower due to mutations in the thicker cell wall and the production of a protein called PBP2a which is able to avoid the inhibitory effects of the *Curcuma longa* juice extracts against MRSA [2]. The (FIC value=1) of the combination of the extracts revealed an antagonist effect as reported by Maharjan et al. [28].

This study revealed no bactericidal action of the extract against the test organism (MRSA) which was not in line with the MIC results where an inhibitory effect of the extract against the test organism was visibly seen. This is indicative of the bacteriostatic nature of the used crude juice extract. This is contrary to the results obtained by Magryś et al. [15] where their study revealed a bactericidal action against the test organisms.

CONCLUSION

The combined effect of the study extracts (*Allium sativum* and *Curcuma longa*) was an indication of the additive effect of such a combination against the test organism. The extract only exhibited a bacteriostatic action against the test organism at all concentrations hence the test organism is likely to develop resistance against such extract. The *in vivo* study of the combination of the test extract together with its toxicity is advised.

REFERENCES

1. Bustamante, C., De La Vega, F. & Burchard, E. Genomics for the world. *Nature*. 2011; **475:** 163–165.
2. Gupta, A., Mahajan, S., & Sharma, R. Evaluation of antimicrobial activity of Curcuma longa rhizome extract against Staphylococcus aureus $. *Biotechnology Reports*, 2015; 6: 51–55.
3. Asogwa, F. C., Okoye, C. O. B., Ugwu, O. P. C., Edwin, N., Alum, E. U. and Egwu, C. E. Phytochemistry and Antimicrobial Assay of *Jatropha curcas* Extracts on Some Clinically Isolated Bacteria - A Comparative Analysis. *European Journal of Applied Sciences*,2015; **7**(1): 12-16.
4. Abimana, J. B., Kato, C. D., & Bazira, J. Methicillin-resistant Staphylococcus aureus nasal colonization among healthcare workers at Kampala international University Teaching Hospital, Southwestern Uganda. *Canadian Journal of Infectious Diseases and Medical Microbiology*. 2019; Article ID 4157869.
5. Alum, E. U., Uti, D. E., Agah, V. M., Orji, O. U., Ezeani, N. N., Ugwu, O. P., Bawa, I., Omang, W. A. and Itodo, M. O. Physico-chemical and Bacteriological Analysis of Water used for Drinking and other Domestic Purposes in Amaozara Ozizza, Afikpo North, Ebonyi State, Nigeria. *Nigerian Journal of Biochemistry and Molecular Biology*, 2023; 38(1): 1-8.
6. Asogwa, F. C., Okechukwu, P. U., Esther, U. A., Chinedu, O. E., & Nzubechukwu, E. Hygienic and sanitary assessment of street food vendors in selected towns of Enugu North District of Nigeria. *American-Eurasian Journal of Scientific Research*, 2015; 10(1): 22-26.

7. Orji, O. U., Awoke, J. N., Aja, P. M., Aloke, C., Obasi, O. D., Alum, E. U., Udu-Ibiam, O. E. and Oka, G. O. Halotolerant and metalotolerant bacteria strains with heavy metals biorestoration possibilities isolated from Uburu Salt Lake, Southeastern, Nigeria, *Heliyon*, 2021; **7**(7): e07512.
8. Aliero, A. A., Emmanuel, E., Josephat, M. N., Sambo, H. A., Matilda, A. O., & John, O. Antibacterial Activity of Actinomycetes Isolated from Waste Dump Soil from Western Uganda. *Microbiology Research Journal International*, 2017; 21(5): 1-14.
9. Arthur, M., Aliero, A.A., & Odda, J. ANTIBACTERIAL ACTIVITY OF ETHANOL CRUDE EXTRACTS OF WHOLE PLANT OF THE UGANDAN PHYLLANTHUS AMARUS SCHUMACH. & THONN AGAINST SHIGELLA DYSENTERIAE. *Bacterial Empire*.2019; *2*(2), 33–36.
10. Nalwoga, J., Tirwomwe, M., Onchweri, A. N., Maniga, J. N., Nyaribo, C. M., & Miruka, C. O. Drug resistant Staphylococcus aureus in Clinical Samples at Kampala International University-teaching Hospital, Bushenyi District, Uganda. *American Journal of Biomedical Research*, 2016; *4*(4): 94-98.
11. Eve, A., Aliero, A. A., Nalubiri, D., Adeyemo, R. O., Akinola, S. A., Pius, T., ... & Ntulume, I. In Vitro Antibacterial Activity of Crude Extracts of Artocarpus heterophyllus Seeds against Selected Diarrhoea-Causing Superbug Bacteria. *The Scientific World Journal*, 2020; 9813970.
12. Garoy EY, Gebreab YB, Achila OO, Tekeste DG, Kesete R, Ghirmay R, Kiflay R, Tesfu T. Methicillin-Resistant *Staphylococcus aureus* (MRSA): Prevalence and Antimicrobial Sensitivity Pattern among Patients-A Multicenter Study in Asmara, Eritrea. Can J Infect Dis Med Microbiol. 2019; 2019:8321834.
13. Offor, C. E., Nkasi, D. and Alum, E. U. Effects of Ethanol Bulb-extract on *Allium sativum* on Serum Transaminase and Alkaline Phosphatase Activities in Albino Rats. *World Engineering and Applied Sciences Journal*, 2014; **5(1):** 17-19.
14. Offor, C. E., Ugwu, O. P. C. and Alum, E. U. The Anti-Diabetic Effect of Ethanol Leaf-Extract of *Allium sativum* on Albino Rats. *International Journal of Pharmacy and Medical Sciences*, 2014; **4(1):** 01-03.
15. Magryś, A., Olender, A., & Tchórzewska, D. Antibacterial properties of Allium sativum L . against the most emerging multidrug - resistant bacteria and its synergy with antibiotics. *Archives of Microbiology*, 2021; *203*(5): 2257–2268.
16. Mustapha, A., Chidiebere, O., Victor, F. I., Bata, M. M., Tanko, N., Yakubu, M. N., ... & David, A. S. Antibacterial activity of Nigerian medicinal plants as panacea for antibiotic resistance: A systematic review. *Journal of Medicinal Herbs*. 2022; *13*(3): 11-21.
17. Yousufi, M. K. To Study Antibacterial Activity of Allium Sativum , Zingiber Officinale and Allium Cepa by Kirby-Bauer Method. *IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS)*, 2012; 4(5): 6–8.
18. Bassey, E. O., Oyebadejo, S., & Ikanna, A. Histopathological assessment of the kidney of alloxan induced diabetic rat treated with macerated Allium sativum (garlic). *Asian Journal of Biomedical and Pharmaceutical Sciences*, 2014; 4(35): 13-17.
19. Britannica, T. Editors of Encyclopaedia (2023, May 18). turmeric. Encyclopedia Britannica. https://www.britannica.com/plant/turmeric.
20. Kim H, Ban I, Choi Y, Yu S, Youn SJ, Baik MY, Lee H, Kim W. Puffing of Turmeric (*Curcuma longa* L.) Enhances its Anti-Inflammatory Effects by Upregulating Macrophage Oxidative Phosphorylation. Antioxidants (Basel). 2020; 9(10):931.
21. Nwali, B. U., Ogbanshi, M. E., Nwaze, C. P., Alum, E. U., Ebenyi, L. N., Ominyi, M. C. and Ali, F. U. Ameliorative Effect of Curcuma Zedoaria (White Turmeric) on Mercury Chloride Induced Oxidative Stress in Wistar Albino Rat. *International Journal of Basic Clinical Toxicology*, 2022; 1 (2): 8-17.
22. Alum, E. U., Ibiam, U. A., Ugwuja, E. I., Aja, P. M., Igwenyi, I. O., Offor, C. E., Orji, O. U., Aloke, C., Ezeani, N. N., Ugwu, O. P. C. and Egwu, C. O. Antioxidant Effect of *Buchholzia coriacea* Ethanol Leaf Extract and Fractions on Freund's Adjuvant-induced Arthritis in Albino Rats: A Comparative Study. *Slovenian Veterinary Research* 2022; 59 (1): 31–45.
23. Alum, E. U., Umoru, G. U., Uti, D. E., Aja, P. M., Ugwu, O. P., Orji, O. U., Nwali, B. U., Ezeani, N., Edwin, N. and Orinya, F. O. Hepato-protective effect of Ethanol Leaf Extract of *Datura stramonium* in Alloxan-induced Diabetic Albino Rats. *Journal of Chemical Society of Nigeria*, 2022; 47 (3): 1165 – 1176.

24. Ugwu, O. P.C., Alum, E. U., Okon, M. B., Aja, P. M., Obeagu, E. I. and Onyeneke, E. C. Ethanol root extract and fractions of *Sphenocentrum jollyanum* abrogate hyperglycemia and low body weight in Streptozotocin-induced diabetic Wistar albino Rats, *RPS Pharmacy and Pharmacology Reports*, 2023; rqad010.

25. Alum, E. U., Famurewa, A. C., Orji, O. U., Aja, P. M., Nwite, F., Ohuche, S. E., Ukasoanya, S. C., Nnaji, L. O., Joshua, D., Igwe, K. U. and Chima, S. F. Nephroprotective effects of *Datura stramonium* leaves against methotrexate nephrotoxicity via attenuation of oxidative stress-mediated inflammation and apoptosis in rats. *Avicenna J Phytomed*, 2023;13(4): 377-387.

26. Alum, E. U., Inya, J. E., Ugwu, O. P. C., Obeagu, I.E., Aloke, C., Aja, P. M., Okpata, M. G., John, E. C., Orji, M. O. and Onyema, O. Ethanolic leaf extract of *Datura stramonium* attenuates Methotrexate-induced Biochemical Alterations in Wistar Albino rats. *RPS Pharmacy and Pharmacology Reports*, 2023; 2(1):1–6.

27. Hassoun, A., Linden, P.K. & Friedman, B. Incidence, prevalence, and management of MRSA bacteremia across patient populations—a review of recent developments in MRSA management and treatment. *Crit Care*. 2017; **21**: 211.

28. Maharjan, R., Thapa, S., & Acharya, A. *Evaluation of Antimicrobial Activity and Synergistic Effect of Spices against Few Selected Pathogens*. 2019; 10–18.

29. Hudzicki, J. Kirby-Bauer Disk Diffusion Susceptibility Test Protocol Author Information. *American Society For Microbiology*, *December 2009*, 2012; 1–13.

30. Vuong, T.D., Sonah, H., Meinhardt, C.G. *et al.* Genetic architecture of cyst nematode resistance revealed by genome-wide association study in soybean. *BMC Genomics*2015; **16**: 593.

31. Akinyele, T. A., Igbinosa, E. O., Akinpelu, D. A., & Okoh, A. I. In vitro assessment of the synergism between extracts of Cocos nucifera husk and some standard antibiotics. *Asian Pacific Journal of Tropical Biomedicine*, 2017; 7(4): 306–313.

32. Jubair N, Rajagopal M, Chinnappan S, Abdullah NB, Fatima A. Review on the Antibacterial Mechanism of Plant-Derived Compounds against Multidrug-Resistant Bacteria (MDR). Evid Based Complement Alternat Med. 2021; 2021:3663315.

33. Kasta, G. Antimicrobial Activity of Ethanol Extract of Rhizome Turmeric (Curcuma Longa L.) For Growth of Escherichia coli, Staphylococcus aureus and Candida albicans. *Asian Journal of Pharmaceutical Research and Development*, 2020; *8*(3): 5–8.

34. Tibyangye, J., Okech, M. A., Nyabayo, J. M., & Nakavuma, J. L. In vitro Antibacterial Activity of Ocimum suave Essential Oils against Uropathogens Isolated from Patients in Selected Hospitals in Bushenyi District, Uganda. *British microbiology research journal*, 2015;8(3): 489-498.

35. Ilozue NM, UP Ikezu and PC Ugwu Okechukwu (2014). Anti-microbial and phytochemical screenin g of the seed extracts of Persea americana (Avocado pear). IOSR Journal of Pharmacy and Biological Sciences,9(2): 23-25.

36. Onyeze RC, MC Udeh Sylvester, C Okwor Juliet, PC Ugwu Isolation and characterisation of bacteria that is associated with the production and spoilage of ogi (Akamu)(2013). International Journal of Pharma Medcine and Biological Sciences,2(3): 79-85.

37. Ikeyi Adachukwu P*, Ogbonna Ann O., Ibekwe Rita O and Ugwu Okechukwu P C(2013). Antimicrobial activity of Xylopia aethiopica (UDA) on Escherichia coli and Staphylococcus aureus isolates from gastroenteritic patients. International Journal of Life Sciences Biotechnology and Pharma Research,2(3): 330-338.

38. Chukwuezi Fabian O and P Ugwu Okechukwu (2014). Antimicrobial effects of bitter kola (Garcinia kola) nut on Staphylococcus aureus, Eschererichia coli and Candida alibicans. Journal of Dental and Medical Sciences, 13(4): 29-32.

39. Epila Haron, Albert Nyanchoka Onchweri, Maniga Josephat, Tenywa Mercy Jacqueline Njeri Muchiri and Ugwu Okechukwu Paul-Chima (2023). Evaluation of the anti-bacterial activity of aqueous leaf extract of Phyllanthus amarus on Streptococcus pyogenes for the treatment of tonsillitis. IDOSR JOURNAL OF BIOLOGY, CHEMISTRY AND PHARMACY,8(2): 47-62.

40. CHUKWUEZI FABIAN O* and UGWU OKECHUKWU P.C.(2013). DISTRIBUTION OF MYCOBACTERIUM BACILLI IN ONITSHA METROPOLIS AND ITS RELATIONSHIP WITH HIV INFECTION. PHARMANEST An International Journal of Advances in Pharmaceutical Sciences, 4(5):902-906.

Kaitesi Jolly (2023). Evaluation of the antibacterial activity of a combination of juice extracts of *Allium sativum bulbs* and *Curcuma longa* rhizomes against methicillin-resistant *Staphylococcus aureus*. EURASIAN EXPERIMENT JOURNAL OF SCIENTIFIC AND APPLIED RESEARCH, 4(1):31-43