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Effects of Methanolic Ginger and Garlic Extracts on Biofilm Forming *Staphylococcus aureus* Isolated from Borehole Water

George, P. A. [a*], Akani, N. P. [a++] and Ugboma, C. J. [a]

[a] Department of Microbiology, Faculty of Science, Rivers State University, P.M.B.-5080, Nkpolu-Oroworukwo, Port Harcourt, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

This study investigated the effect of *Zingiber officinale* (Ginger) and *Allum sativum* (Garlic) on biofilm forming *Staphylococcus aureus* isolated from selected boreholes in Port Harcourt city Local Government Area of Rivers State. Twenty four water samples were collected from 4 randomly selected borehole. Borikiri had the highest number of isolates that produced biofilm, while Elelennwo had the lowest number. Out of 23 isolates of *Staphylococcus aureus*, 14 were positive for biofilm production. Hemolysis production range of these isolates was from alpha, beta and gamma. Filter paper disc were inoculated with various plant extract concentrations. Methanol garlic extract at 100mg/ml had the highest range of zone of inhibition at Borikiri when compared to methanol ginger extract while methanol garlic extract at 100mg/ml had no range at Elelenwo when compared to methanol ginger extract. This study suggests that methanolic ginger and garlic

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[++] *Professor;*
**Corresponding author: Email: georgephoebe00@gmail.com;*

extracts can be used as a potential anti-biofilm agent in borehole water, to reduce the risk of bacterial biofilm formation.
Surveillance systems should be increased for assessing risk factors of diseases and to provide strategies to prevent and protect public health.

Keywords: Borehole water; methanol; ginger; garlic; Staphylococcus aureus.

1. INTRODUCTION

A biofilm is a complex matrix of microbial communities made of polysaccharides, proteins, and other organic materials in which the cells adhere tightly to produce attachments to surfaces that are either biotic or abiotic. Microbes that adhere to a surface can survive under difficult conditions, such as the presence of innate host defenses and antimicrobial compounds, [1]. As a result, the development of biofilms is one of the indirect mechanisms by which bacteria become resistant to antibiotics, and biofilms are the sites where members of the biofilm microcommunity transfer resistance genes to one another. Biofilms could be involved in over 60% of microbial infections, while two-thirds of all human bacterial infections are caused by the biofilms. Antibiotic-resistant bacteria are those that are associated with biofilms. The complex biofilm structure and extracellular polymeric matrix may make it difficult for antibiotics to reach the bacterium [2]. The fundamental causes of many persistent and chronic infections are the development of these sessile communities and their innate resistance to antimicrobial drugs [3].

Ginger (*Zingiber officinale*) is a perennial herb with greenish yellow flowers that resemble orchids. It is a two-foot-tall perennial plant with thin leaves. The rhizome is white to yellowish to brown, horizontal, branching, meaty, and scented. The slender, linear-lanceolate leaves can reach a length of 20 cm and a width of 1.5–2 cm. The volatile oils, which are the medicinally active components and are also in charge of giving ginger its distinctive flavor and smell, can be found in between one and four percent of the dried rhizome. A dense spike of flowers, which are bright green with purple ends, is produced. South-Eastern Asia has a large distribution of this plant. The Liliaceae family includes the perennial bulbous plant *Allium sativum*, which is used medicinally. Numerous microorganisms are inhibited by it [4]. It has long been recognized that this plant contains antibacterial properties. Garlic has the potential to be a powerful antidermatophytic agent, as demonstrated by (Venugopal & Venugopal in [5]). Microorganisms with a high level of antibiotic resistance can be treated with garlic. Staphylococcus species are gram-positive, non-motile, spherical in shape, and come in a range of sizes. They can be discovered on their own, in pairs, or in random groups. Colonies are opaque and usually white or cream, however they may seem yellow or orange. It thrives between 30 and 37°C. *Staphylococcus aureus* is the most harmful species of *Staphylococci* that affects humans. Gram-positive, non-motile, facultative anaerobic *Staphylococcus aureus* might show up alone, in pairs, or in clusters that resemble grapes. *S. aureus* can enter the tissue by piercing the skin and mucous membrane barriers by traumatic inoculation or surgery. After penetrating the underlying tissue, it results in a characteristic local abscess lesion [6], and if it enters the lymphatic system, it may induce septicaemia [7]. *S. aureus* is regarded as a significant opportunistic pathogen that can infect both immunocompetent and immunocompromised individuals and cause a variety of infections, including food poisoning, boils, septicemia, pustules, soft tissue infections, urinary tract infections (UTIs), impetigo, osteomyelitis, mastitis, meningitis, bronchopneumonia, and wound infections [8]. A variety of multi-drug resistance genes are found on plasmids in Staphylococcus aureus strains, which encourages the spread of resistance even between distinct species [9]. Due to their potential to develop resistant genes, these pathogenic bacteria poses a challenge to the use of antibiotics in the effective treatment of diseases increasing the need for more expensive antibiotics, sophisticated medical equipment, and prolonged hospital stays for patients, particularly in developing nations like Nigeria.

The aim of the study is to determine the antimicrobial effect of *Allium sativum* (Garlic) and *Zingiber officinale* (Ginger) on biofilm forming *Staphylococcus aureus* and isolated from borehole water.

2. MATERIALS AND METHODS

2.1 Collection of Water Samples

The study was carried out in 4 different locations in Rivers State, Nigeria. The coordinates of the locations includes; Rivers State University (4° 47'32"N, 6° 58′56"E), Borikiri (4 ° 52′15.46"N, 6 ° 59′55"E) Rumuolumeni (4° 48′13"N, 6° 50'45"E), Elelenwo (4° 50′33"N, 7° 4′41"E. Standard procedures were adopted in water sample collection and laboratory analysis in line with requirement specified by WHO. The collected samples were placed in ice pack container (Sabino *et al*., 2014) and sent to the microbiology laboratory of the department of Microbiology, Rivers State University for analysis.

2.2 Isolation and Characterization of Staphylococcus species

Direct plating and serial dilution (10-2) was carried out before the samples were inoculated on Mannitol Salt Agar. The plates were spread evenly using sterile bent glass rod and were incubated at 37°c for 24-48 hours. After incubation, plates were observed for microbial growth. Colonies appear yellowish, slightly raised and smooth, counts were made for the respective plates and colonies were characterized morphologically and were subcultured on freshly prepared nutrient agar plates.

2.3 Preservation of Isolates

Pure cultures of the bacterial isolates were preserved in bijou bottles containing 10% freshly prepared glycerol, the bottles were kept frozen in the refrigerator [10].

2.4 Characterization and Identification of Bacterial Isolates

The morphological and biochemical characteristics of the bacterial isolates were determined using the method of Cheesbrough [11]. The morphological and biochemical test includes; Gram staining, motility, indole, sugar fermentation, citrate utilization, methyl red, vogees proskauer test.

2.5 Sugar Fermentation Test

With a straight inoculation needle, an isolated colony of organisms from a pure culture was streaked onto the surface of Triple Sugar Iron Agar Slant (TSI). The medium was first stabbed through the middle to the bottom of the tube. The tube's cap was left loose, and it was incubated for 18 to 24 hoursat 35 to 37 °c.

2.6 Methyl Red Test

A colony of the test organism was aseptically inoculated into recently made MRVP broth. Each test tube was filled with five (5ml) of MRVP broth before being autoclaved at 121°C for 15 minutes. Forty eight hours were spent incubating the broth after a loopful of the test organism was added. After incubation, the culture received 5 drops of methyl red indicator [12].

2.7 Citrate Utilization Test

Simmons citrate agar was made, put into test tubes, and autoclaved. After being angled, the tubes were given time to cool and solidify. By contacting the surface of the slant with cultures of 18–24 hour-old organisms, the slant was infected. For 24 to 48 hours, the tubes were incubated at 37°c. Alkalization- related blue color development was noticed and noted as beneficial, otherwise, as negative [11].

2.8 Biofilm Screening

The tube approach was employed for detection of biofilm. A loop of inoculum was incubated in 10 ml of nutrient glucose broth for 24 hours. The tubes content were discarded and they were given a single wash in 9 ml of phosphate buffer saline before being thrown away. Each tube was rinsed with 10 ml of crystal violet and left to sit at room temperature for 30 minutes before being discarded. After being cleaned with water, the tubes were dried at room temperature while being turned upside down. Clear film on the tube walls and bottom served as a biofilm production indicator [13].

2.9 Preparation of Plant Extracts

Zingiber officianle (Ginger) and *Allum sativum* (Garlic) were dried and blended into powder using a blender. 100 g of dried ginger and garlic powder were combined with 400 mL of pure methanol to create the methanolic extract. For 24 hours, the created solution was kept at room temperature. The mixture was agitated once more, filtered through Whatman filter paper, and the solvent was then evaporated out of the mixture [14]. The disc diffusion method was used

to conduct the antimicrobial susceptibility test [15]. Filter paper disks, made as described by Ochie and Kolhatker [16] were impregnated with concentrations of extracts

3. RESULTS

3.1 Distribution of *Staphylococcus aureus* among Study Location

14 isolates of *Staphylococcus aureus* were positive for biofilm production. Borikiri had the highest prevalence occurrence of 50%, Rivers State University had 28.6%, Rumuolumeni had 14.2% and Elelenwo had 7.1%. The result is shown in Fig. 1.

3.2 Range of Zones on Inhibition of Plant Extracts

The results of methanol ginger extracts on biofilm forming *Staphylococcus aureus* as revealed in Table 1 shows that at 100mg/ml, Borikiri ranged from 9-19mm, Rivers State University ranged from 15- 17mm, Rumuolumeni ranged from 0-16mm and Elelenwo had 17mm. At 50mg/ml, Borikiri ranged from 8-18mm, Rivers State University ranged from 12-14mm, Rumuolumeni ranged from 0-15mm and Elelenwo had 14mm. At 25mg/ml, Borikiri ranged from 0-13mm, Rivers State University ranged 10-13mm, Rumuolumeni ranged from 0-14mm and Elelenwo had 13mm. At 12.5mg/ml Borikiri ranged from 0-11mm, Rivers State University ranged from 8-11mm, Rumuolumeni ranged from 0- 11mm and Elelenwo had 11mm.

The results of methanol garlic extracts on biofilm forming *Staphylococcus aureus* as revealed in Table 2 shows that at 100mg/ml, Borikiri ranged from 0-22mm, Rivers State University ranged from 0- 20mm, Rumuolumeni ranged from 0-13mm and Elelenwo had no zones of inhibition. At 50mg/ml, Borikiri ranged from 0-19mm, Rivers State University ranged from 0-19mm, Rumuolumeni ranged from 0-11mm and Elelenwo had no range. At 25mg/ml, Borikiri ranged from 0-17mm, Rivers State University ranged from 0-17mm, Rumuolumeni ranged from 0-11mm and Elelenwo had no range. At 12.5mg/ml Borikiri and Rivers State University ranged from 0-16mm, Rumuolumeni ranged from 0- 8mm while Elelenwo had no range.

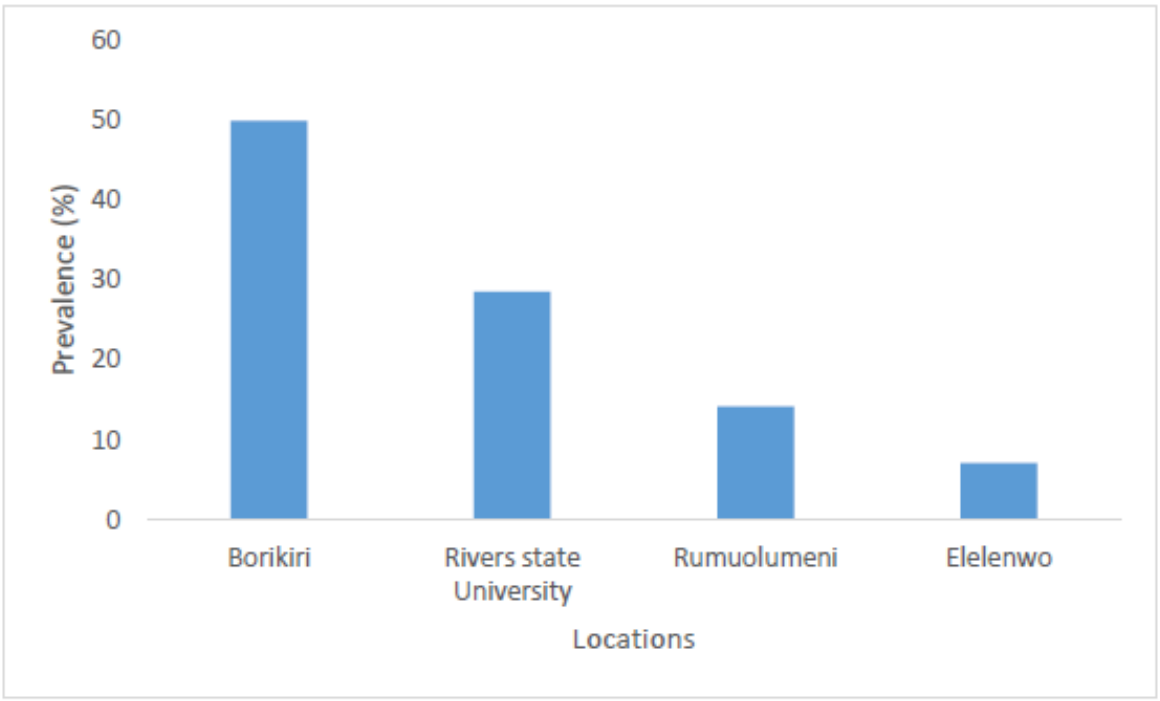

Fig. 1. Prevalence of biofilm forming *Staphylococcus aureus* in different locations

Table 1. Range and means of zones of inhibition (mm) of different concentration of methanol ginger extract on biofilm forming *Staphylococcus aureus* isolated from differentborehole locations

Locations	100 mg/ml	50 mg/ml	25 mg/ml	12.5 mg/ml	Gentamicin (10µg/ml)
Borikiri	9-19 (13.4)	8-18(11.7)	0-13 (9.7)	0-11 (8.3)	20-23 (28)
Rivers StateUniversity	15-17(16.3)	12-14 (13.3)	10-13 (12)	8-11 (10)	20-27 (30.5)
Rumuolumeni	0-16 (8)	0-15 (7.5)	0-14 (6.5)	0-11 (5.5)	28-33 (23.5)
Elelenwo	17	14	13	11	29

Table 2. Range and means of zones of inhibition (mm) of different concentration of methanol garlic extract on biofilm forming *Staphylococcus aureus* isolated from differentborehole locations

Locations	100 mg/ml	50 mg/ml	25 mg/ml	12.5 mg/ml	Gentamicin (10µg/ml)
Borikiri	0-20 (13.7)	0-19 (13)	0-17 (11.7)	0-16 (10.9)	20-23 (28)
Rivers StateUniversity	0-20 (10)	0-19 (9.5)	0-17 (8.5)	0-16 (8)	20-27 (30.5)
Rumuolumeni	0-13 (6.5)	0-11 (5.5)	0-10 (5)	0-8 (4)	28-33 (23.5)
Elelenwo	0	0	0	0	0

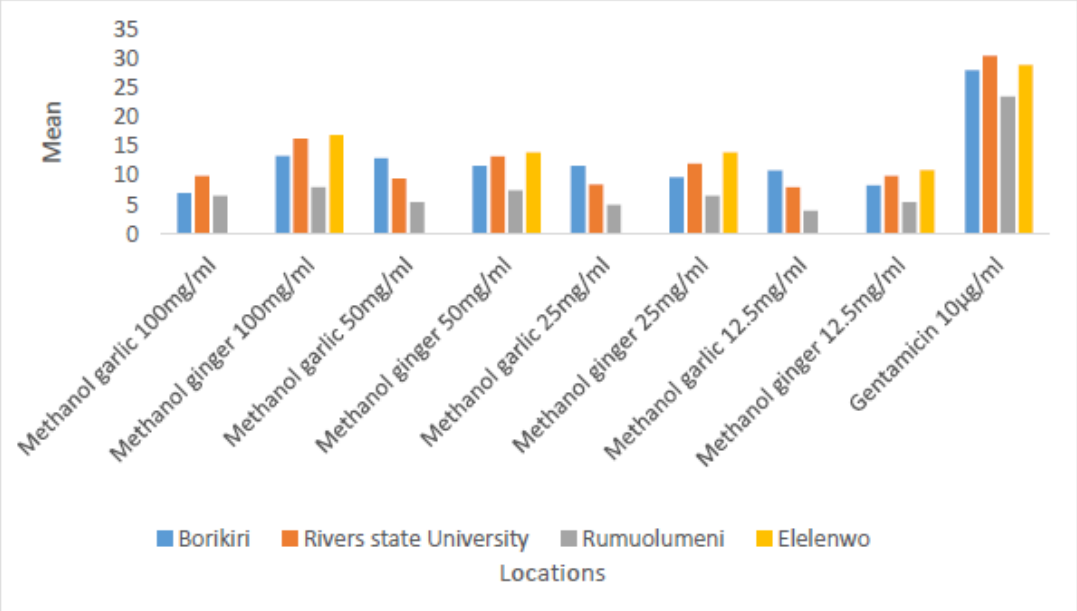

Fig. 2. Means of different concentrations of methanolic garlic, ginger extracts andgentamicin as control respectively using disc diffusion method

4. DISCUSSION

Biofilms are bacterial aggregates attached to various biotic and abiotic surfaces which can interact with each other and adapt themselves to environmental stressors [17]. According to previous studies [18], biofilms tend to become more resistant to antibiotics and disinfectants thereby become a reservoir for spread of pathogenic organisms. Borikiri had the highest number of prevalence of biofilm forming *Staphylococcus aureus* while Elelenwo had the lowest. Out of 23 isolates of *Staphylococcus aureus*, 14 isolates were positive for biofilm production, 5 isolates that produced biofilm had gamma hemolysis, 8 isolates had beta hemolysis and 1 isolate had alpha hemolysis. Hemolysis test indicates that these microorganisms are capable of lysing the red blood cells which is harmful to human. Hemolysis refers to the disruption of erythrocyte membranes that causes the release of hemoglobin which could result in decrease of erythrocyte life span [19,20]. For metahnolic garlic extract at 100mg/ml Rivers state university had the highest mean while Elelenwo had no value, for methanol ginger

extract at 100 mg/ml Elelenwo had the highest mean while Rumuolumeni had the lowest, for Methanol garlic extract at 50 mg/ml Borikiri had the highest mean while Elelenwo had no value, for methanol ginger extract at 50 mg/ml Elelenwo had the highest mean while Rumuolumeni had the lowest, for Methanol garlic extract at 25 mg/ml Borikiri had the highest mean while Elenwo had no value, for methanol ginger extract at 25 mg/ml Elelenwo had the highest mean while Rumuolumeni had the lowest, for methanol garlic extract at 12.5 mg/ml Borikiri had the highest mean while Elelenwo had no value, for methanol ginger extract at 12.5 mg/ml Elelenwo had the highest mean while Rumuolumeni had the lowest. Rivers State University had the highest mean value for gentamicin (Control) while Rumuolumeni had the lowest mean value [21]. Methanol garlic extract at 100mg/ml had the highest range of zone of inhibition at Borikiri when compared to methanol ginger extract while methanol garlic extract at 100mg/ml had no range at Elelenwo when compared to methanol ginger extract.

5. CONCLUSION AND RECOMMENDATION

The discovery of *Staphylococcus aureus* in borehole water suggested that food poisoning is a significant reason for public health action. Poor sanitation practices, such as refuse dump site, septic system and human activities close to these boreholes, are a sure pollution contributing factor. Biofilm producer organisms cause nosocomial and recurrent infections. To overcome chronic and recurrent infections, it is important to detect biofilms of microorganisms. The safety of borehole waters is an important and timely issue, especially as regards to public health sustainable water management.

Surveillance systems should be increased for assessing risk factors of diseases and to provide strategies to prevent and protect public health. Proper purification and treatment of domestic water sources in the study area should be ensured before being used. Methanolic ginger and garlic extracts can be used as a potential anti-biofilm agent in borehole water.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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