

# ANTIBACTERIAL ACTIVITIES OF GARLIC ON BACTERIA *Neisseria gonorrhoeae* AND *Treponema pallidum* ASSOCIATED WITH SEXUALLY TRANSMITTED DISEASES

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## Abstract

The devastating effects of sexually transmitted diseases on women and infants make them a major global health priority. Many of the available synthetic medications have a number of negative side effects and are losing their effectiveness. Therefore, it is more efficient to research and create the various plant-based compounds needed to satisfy the need for the creation of new medications with fewer adverse effects and greater effectiveness. In this study, the antibacterial activity of *Allium sativum* against the sexually transmitted disease-causing bacteria *Neisseria gonorrhoeae* and *Treponema pallidum* was assessed. Fresh garlic juice was tested using the disc diffusion method against the bacteria isolates. The results of the antibacterial test of *Allium sativum* juice extract against the growth of *Neisseria gonorrhoeae* and *Treponema pallidum* showed a significant inhibitory effect of the juice. The zone of inhibition against *Neisseria gonorrhoeae* was highest at  $16.00 \pm 0.05$ mm and lowest at  $10.00 \pm 0.02$ , while the zone of inhibition against *Treponema pallidum* was highest at  $14.00 \pm 0.30$ mm and lowest at  $8.00 \pm 0.04$ mm. The study concluded that garlic juice extract was found to be an effective antibacterial agent against tested isolates, which are linked to sexually transmitted infections and thus recommend the inclusion of garlic in daily diet for better health.

**Keywords:** Antimicrobial, *Neisseria gonorrhoeae*, *Treponema pallidum*, STDs

## Introduction

Sexually transmitted diseases (STDs) are infections that are spread through intercourse between an infected and an uninfected individual. Examples include syphilis, gonorrhea, genital herpes, HIV/AIDS, chlamydia, and human papillomavirus infection (CDC, 2019). According to Mayo (2018), sexually transmitted diseases (STDs) can be brought on by viruses like HPV, genital herpes, and HIV, parasites like trichomoniasis, or bacteria like *Gonorrhoeae*, *Syphilis*, and *Chlamydia*. Microorganisms continue to spread at a high rate in developing nations including Nigeria. Diseases persist in areas with poor nutrition and hygienic conditions, and new diseases pose a greater threat to these populations. According to WHO (2015), *Neisseria gonorrhoeae* and

*Treponema pallidum* are two bacterial infections that are sexually transmitted in Nigeria with sexual contact been the mode of transmission. *Neisseria gonorrhoeae* and *Treponema pallidum* are Gram-negative diplococci bacteria (O'Donnell *et al.*, 2019), which result in gonorrhea, a genitourinary infection that is sexually transmitted (Ryan *et al.*, 2014), as well as additional gonococcal illnesses such as septic arthritis, disseminated gonococemia, and gonococcal ophthalmia neonatorum. According to Ryan *et al.* (2014) they are aerobic, oxidase positive, and resistant to phagocytosis and also grow inside neutrophils. They demonstrate antigenic variation by means of genetic recombination of their pili and immune-system-interacting surface proteins (O'Donnell *et al.*, 2019).

It has long been known that higher plants and their preparations can be used to treat infections. The rise of strains of bacteria that are resistant to antibiotics has reignited interest in plants with antimicrobial qualities (Amagase, 2016). In addition to being used extensively as a culinary flavoring, *Allium sativum* has been used as a medicine for both ancient and modern times to prevent and treat a variety of illnesses. Notably, *Allium sativum* is one of the most well-known traditional herbal treatments for a variety of illnesses. To lower the risk of heart disease, it is used to increase blood circulation. Additionally, *Allium sativum* is believed to lower high blood pressure and cholesterol (Getahun, 2016). Additionally, *Allium sativum* contains antioxidants that aid in the removal of environmental pollutants and the normal blood pressure that results from it (Jonkers *et al.*, 2019). It has been discovered that oils extracted from plants and herbs are efficient at eliminating viruses, bacteria, and other pathogens. Heart and blood system disorders such as high blood pressure, high cholesterol, coronary heart disease, and arterial hardening are among the numerous conditions for which *Allium sativum* is also used (Lawson *et al.*, 2011).

*Neisseria gonorrhoeae* and *Treponema pallidum* have been recognized by the US Centers for Disease Control as one of the most urgent threats to antibiotic resistance due to their successive development of antimicrobial resistance to several classes of antibiotics over several decades (Goire *et al.*, 2018). According to Ohnishi *et al.* (2011), reported cases of severe ceftriaxone resistance have raised concerns and accelerated efforts to find new alternative treatments. Searching for newer antibiotics has therefore become crucial. The majority of antibiotics are naturally occurring and plants create defenses against microbiological threats (Chanda *et al.*, 2016), which may be investigated in order to produce more potent antibiotics against bacterial isolates linked to STDs like *Treponema pallidum* and *Neisseria gonorrhoeae*. Numerous synthetic medications currently on the market have a number of negative side effects and are losing their effectiveness in treating STDs. Therefore, it is more efficient to research and create the various plant-based compounds needed to satisfy the need for the creation of new medications with fewer adverse effects and greater effectiveness.

## Materials and Methods

### Sample Collection

Dried *Allium sativum* cloves were bought from Lokoja's local markets, stored in an airtight container, and then transported to the Department of Biology laboratory at Federal University Lokoja for analysis.

Strains of *Treponema pallidum* and *Neisseria gonorrhoeae* were acquired from the Federal Teaching Hospital Lokoja. Before being brought to the lab for studies, the strains were kept in transport media (an ice box) at 4°C.

## Culture Media

The media used in this study were Nutrient agar and Nutrient broth. The media were prepared according to the manufacturers' instructions.

### Nutrient agar

Following the manufacturer's instructions, 28g of nutrient agar (NA) (Biotech Laboratories Ltd) was dissolved in 1000mL of distilled water, and the suspension was stirred until it was fully homogenized. Cotton wool was used to plug the conical flask holding the media, and aluminum foil was used to cap it. After being sterilized for 15 minutes at 121°C in a lender autoclave, the flask was cooled and transferred into sterile petri dishes (Cheesebrough, 2006).

### Nutrient broth

Using a weighing balance, 25g of nutrient broth agar (NBA) was weighed. The powder was then dissolved in 1000 milliliters of distilled water that had been sterilized. To guarantee a homogenous mixture, the mixture was then heated and gently stirred. 10ml of the agar solution were moved from the volumetric flask into a test tube of 20ml volume. To stop air from entering or leaving each test tube during the 15 minutes autoclaving process at 121°C, each test tube was wrapped in cotton wool and cupped with aluminum foil (Cheesebrough, 2006).

## Preparation of Fresh Garlic Juice

Cloves of *Allium sativum* were cleaned, weighed (100g), and peeled. A sterile cheesecloth was used to filter the mixture after clean cloves had been crushed in a sterile mortar and pestle. This filtrate was regarded as a pure juice extract of fresh garlic. Before being used, the filtrate was thawed and kept at -20°C. After that, the thawed juice extracts were dissolved in DMSO and weighed in different concentrations.

## Preparation of working concentration

5g of the solid plant extracts were dissolved in 10ml of regular saline to create a stock solution with 500mg/ml. The stock solution was diluted using the formula below to create the working concentration.

$$C_1V_1 = C_2V_2$$

where:  $C_1$  = present concentration,  $V_1$  = Volume to use,  $C_2$  = required concentration

$V_2$  = Required volume

A working solution containing 25mg/ml, 50mg/ml, 75mg/ml, and 100mg/ml was employed in testing the antimicrobial activity of the juice extracts

## Antimicrobial Sensitivity Test

Using the Agar Well diffusion method (Bauer and Tittel 1995), the plant extracts' antimicrobial properties were evaluated. The isolates of *Treponema pallidum* and *Neisseria gonorrhoeae* were added to the culture plates that had been prepared. On the agar surface, wells were created using a 14mm cork borer. A sterile syringe was then used to pour the juice extracts into the well, and the plates were then incubated. Each inoculation plate was labeled with the microbial culture's name. Zones of inhibition were checked on each plate. The zones of inhibition of microbial growth in each plate were used to read the results.

## Determination of Minimum Inhibitory Concentration

Using the tube method (Chanda *et al.*, 2016), the lowest concentration that demonstrated an inhibitory effect on test organisms was identified as the Minimum Inhibitory Concentration (MIC). Using nutrient broth, two-fold serial dilutions were created. 5ml of a 200mg/ml juice extract solution was aseptically added to 5ml of double-strength medium, and the mixture was shaken. 5ml of the mixture was moved to a second test tube, which also held 5ml of the single strength medium, using a brand-new pipette. This was also shaken, and 5ml were aseptically transferred to a third test tube and shaken again. The control was the ninth tube, which contained no test compound. Lastly, 0.2ml of the test organism inoculum was aseptically added to each tube. After 21 days of incubation at 37°C with cotton wool covering it, the test tube was checked for turbidity. The MIC was defined as the lowest concentration that prevented the test organism from growing.

## Data Analysis

Measured and recorded zones of inhibition were presented in tabular format indicating the concentrations, mean inhibition zones. Tabulated data was analyzed using ANOVA package at  $p < 0.05$ .

## Results

### Antibacterial Activity of *Allium sativum* Extracts

Table 1 shows the outcome of the antibacterial test conducted on juice extracts from *Allium sativum* as it relates to the growth of *Treponema pallidum* and *Neisseria gonorrhoeae*. At 100% concentration, the highest zone of inhibition, measuring  $16.00 \pm 0.05$ mm was observed against *Neisseria gonorrhoeae*, while the least zone of inhibition ( $10.00 \pm 0.02$ mm) was observed at 25% concentration. Zone of inhibition was highest against *Treponema pallidum* at 100% concentration with  $14.00 \pm 0.03$ mm inhibitory zone and least at 25% concentration with  $8.00 \pm 0.04$ mm inhibitory zone. Table 2 demonstrated that the plant material extract prevented the tested bacterial isolates from growing. For both sensitive isolates, the maximum growth inhibition was observed at 100% concentration. All of the results were similar, indicating that the result is significant at  $p < 0.05$ .

**Table 1: Antibacterial activity of *Allium sativum* juice extract against test organisms**

Test Organisms	Concentrations/ Zones of Inhibition $\pm$ Standard error (mm)				
	25%	50%	75%	100%	Control
<i>Treponema pallidum</i>	$8.00 \pm 0.04$	$10.00 \pm 0.08$	$12.00 \pm 0.05$	$14.00 \pm 0.30$	$16.00 \pm 0.20$
<i>Neisseria gonorrhoeae</i>	$10.00 \pm 0.02$	$12.00 \pm 0.04$	$14.00 \pm 0.11$	$16.00 \pm 0.05^a$	$16.00 \pm 0.01^a$

**Values are mean  $\pm$  standard error. Mean values with the same superscript in a column are not significantly different ( $p < 0.05$ ) for Tukey-Kramer Multiple Comparisons Test**

**Table 2: MIC of juice extract of *Allium sativum* extract against test organisms**

Test Organisms	Concentrations				Control
	25%	50%	75%	100%	
<i>Treponema pallidum</i>	+++	+	+	-	-
<i>Neisseria gonorrhoeae</i>	++	+	-	-	-

**Keys: +++ = Highly Turbid; ++ = Moderately Turbid; + = Slightly Turbid; - = No Turbidity**

## Discussion

Many diseases in developing nations are treated by the rural populace using traditional ethno-medical practices. The careless use of chemical products and antibiotics in disease management and control has thus raised concerns. Due to the careless use of chemicals and antibiotics, they have entered the food chain, developed drug resistance, and posed major health risks to human health (Mayo, 2018). Due to human health concerns, several nations have outlawed the use of antibiotics and other chemicals for the treatment of animals. About 74% of medicines made from plants have a contemporary indication that corresponds with their traditional, cultural, and occasionally ancient uses (Wynn *et al.*, 2016). Therefore, an alternative to traditional methods of disease control is the use of ethano-medicinal practices. The presence of phytochemical compounds in the plant material under study (Lanzotti, 2016), as well as the inhibitory zone and concentrations that were effective on the organisms under test, demonstrated that the study plant’s antibacterial potency varied.

The variance in sensitivity may also be explained by variations in the inoculum size, temperature, nutritional needs, and growth rate of the tested isolates (Khalimuthu *et al.*, 2010). The maximum zone of inhibition was found when *Allium sativum* juice extracts were used against *Salmonella typhi*, *Lactobacillus*, and *Escherichia coli*, according to similar findings by Contreras and Molina (2019). The extracts show that the inhibitory activity of the aqueous extract at varying concentrations varies significantly ( $p < 0.05$ ). Higher concentrations of extracts seemed to have more antibacterial activity than lower concentrations because they may contain a higher dose of active ingredients (Kaur *et al.*, 2011). This study found that the efficiency of the garlic juice extract increased with its concentration, which in turn reduced the inhibition and growth of the test bacteria. Thus, garlic may have both bactericidal and bacteriostatic properties.

## Conclusion

The findings of this study suggest that garlic may be used as a potent antibacterial agent against the sexually transmitted diseases-causing *Neisseria gonorrhoeae* and *Treponema pallidum*, which are known to be developing resistance to current antibiotics. It could be produced as a tablet with the most effective concentrations and reasonably priced dosages to treat these two harmful bacteria. Alternative medications with a long history of preventing such newly emerging diseases and that are readily available and reasonably priced must be prioritized in the age of drug-resistant bacteria. For improved health, this study suggests including garlic in one's daily diet, especially fresh garlic. Purified *Allium sativum* extract ought to be taken into consideration as a possible active component of traditional antibacterial medications. Because the plant extracts are

readily available, reasonably priced, and have less of an eye-effect, the results of this study may actually be adopted by STD-infected people.

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