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ANTIBACTERIAL ACTIVITY OF ALLIUM SATIVUM (GARLIC) AGAINST NEISSERIA GONORRHOEAE AND TREPONEMA PALLIDUM

Nawaf Abubakar and Hafiz Surajo Sandamu

Affiliation:Department of Applied Biology. Federal University of Technology Babura. Jigawa State

*Corresponding authors' email: nawafpg@gmail.com

ABSTRACT

Background: The emergence of multidrug-resistant strains of Neisseria gonorrhoeae and Treponema pallidum, the causative agents of gonorrhea and syphilis, necessitates the search for novel therapeutic agents . Allium sativum (garlic) has a long history of use in traditional medicine for its broad-spectrum antimicrobial properties. This study aimed to investigate the in vitro antibacterial activity of garlic extracts against clinical isolates of N. gonorrhoeae and T. pallidum. Fresh garlic juice was extracted and its phytochemical constituents were analyzed using Gas Chromatography-Mass Spectrometry (GC-MS). The antibacterial activity was evaluated using the agar well diffusion method (6 mm well diameter) to determine the zones of inhibition, and the broth dilution method was used to determine the Minimum Inhibitory Concentration (MIC). Concentrations of 25, 50, 75, and 100 mg/mL were tested. GC-MS analysis revealed 27 bioactive compounds. The major constituents were n-Hexadecanoic acid (25.72%), Oleic Acid (21.56%), and Hexadecanoic acid, ethyl ester (10.87%). The garlic extract demonstrated significant dose-dependent antibacterial activity against both pathogens. The highest zone of inhibition (16 mm) was observed at 100 mg/mL for N. gonorrhoeae. The MIC results indicated that the extract completely inhibited the growth of N. gonorrhoeae at 75 mg/mL and T. pallidum at 100 mg/mL. The findings confirm that Allium sativum possesses potent antibacterial activity against Neisseria gonorrhoeae and Treponema pallidum. It represents a promising candidate for developing alternative or adjunctive therapies against these antibiotic-resistant sexually transmitted infections.

Keywords: Allium sativum, Garlic, Neisseria gonorrhoeae, Treponema pallidum, Antibacterial activity, Minimum Inhibitory Concentration (MIC), GC-MS, Phytochemicals

INTRODUCTION

Sexually transmitted infections (STIs) represent one of the most pervasive global health burdens, with bacterial agents like Neisseria gonorrhoeae and Treponema pallidum—responsible for gonorrhea and syphilis, respectively—posing significant clinical and public health management challenges (Centers for Disease Control and Prevention [CDC], 2019). These infections are associated with severe reproductive health complications, neonatal mortality, and increased susceptibility to other pathogens, thereby exerting considerable social and economic strain on healthcare systems worldwide. The persistent nature of these diseases underscores the critical need for effective, accessible, and sustainable treatment regimens to control their transmission and mitigate long-term health consequences. The situation is particularly dire in regions with limited healthcare infrastructure, where diagnosis and treatment are often delayed, leading to higher rates of complications and community spread.

Compounding this public health crisis is the alarming and rapid evolution of antimicrobial resistance (AMR) among these bacterial pathogens. Neisseria gonorrhoeae has demonstrated a remarkable capacity to develop resistance to every class of antibiotic introduced for its treatment, with recent strains exhibiting high-level resistance to ceftriaxone, the current cornerstone of therapy (Unemo & Shafer, 2014). This progression has prompted the U.S. Centers for Disease Control and Prevention to classify drug-resistant gonorrhea as an "urgent threat," signaling a potential return to an era of untreatable infection (Ohnishi et al., 2011). Similarly, although Treponema pallidum remains largely susceptible to penicillin, treatment failures and concerns about emerging resistance highlight the precariousness of relying on a single therapeutic agent. This escalating resistance landscape creates an urgent imperative to discover and develop novel antimicrobial agents with new mechanisms of action to stay ahead of pathogen evolution.

In this quest for novel therapeutics, the plant kingdom offers a vast and largely untapped reservoir of bioactive compounds, having served as the foundation of medicinal systems for millennia (Packer et al., 2016). Ethnobotanical knowledge provides a valuable starting point, guiding researchers toward species with documented therapeutic use. The rationale for exploring plant-derived medicines is multifaceted; they often contain complex mixtures of secondary metabolites that can act synergistically, potentially reducing the likelihood of resistance development while targeting multiple pathways within microbial cells. This approach aligns with the global "One Health" initiative, seeking sustainable solutions that bridge traditional knowledge and modern scientific validation.

Among the plethora of medicinal plants, Allium sativum (garlic) stands out due to its extensive historical use and a robust body of scientific evidence supporting its pharmacological potency. Revered across ancient civilizations, garlic is not merely a culinary staple but a well-documented therapeutic agent (Bayan et al., 2014). Modern research has corroborated its diverse bioactivities, which include potent antimicrobial, antioxidant, anti-inflammatory, and cardioprotective effects. This broad-spectrum activity positions garlic as a prime candidate for the development of phytopharmaceuticals, particularly in the context of infectious diseases where multi-target therapies are increasingly desirable.

The antimicrobial efficacy of garlic is principally ascribed to its rich content of organosulfur compounds, most notably allicin (diallyl thiosulfinate). Allicin is not present in intact garlic cloves but is rapidly enzymatically synthesized from the precursor alliin when the clove is crushed or damaged (Ankri & Mirelman, 1999). This compound and its

degradation products (e.g., diallyl disulfide, diallyl trisulfide) exhibit broad-spectrum activity against a wide array of pathogens. The proposed mechanisms include the inhibition of thiol-containing enzymes crucial for microbial metabolism, disruption of cell membrane integrity, and interference with biofilm formation (Deresse, 2010). Supporting this, in vitro studies have consistently demonstrated the inhibitory effects of garlic extracts against numerous Gram-positive and Gram-negative bacteria, including problematic pathogens like Escherichia coli, Staphylococcus aureus, and Salmonella species (Tsao & Yin, 2001; Jaber & Al-Mossawi, 2007).

Despite this compelling evidence for garlic's general antibacterial properties, a significant research gap exists concerning its specific activity against the key STI pathogens N. gonorrhoeae and T. pallidum. The unique biological and structural characteristics of these bacteria, such as the complex outer membrane of N. gonorrhoeae and the cryptic, lipid-rich envelope of T. pallidum, may influence their susceptibility to plant-derived compounds. Therefore, extrapolating activity from studies on other bacteria is insufficient. To address this gap, the present study was meticulously designed with a threefold objective: first, to scientifically evaluate the in vitro antibacterial potential of Allium sativum extracts against clinical isolates of Neisseria gonorrhoeae and Treponema pallidum; second, to characterize the phytochemical profile of the active extract using Gas Chromatography-Mass Spectrometry (GC-MS) to identify the principal bioactive constituents; and third, to determine the Minimum Inhibitory Concentration (MIC) to quantify the extract's potency. This research aims to provide a foundational scientific validation for garlic's potential role in combating these increasingly resistant sexually transmitted infections.Materials and Methods

Plant Material and Extract Preparation

Dried garlic cloves were purchased from a local market in Lokoja, Nigeria. A total of 100g of peeled cloves were crushed in a sterile mortar and pestle. The resulting mixture was filtered through sterile cheesecloth, and the filtrate was considered the stock solution (100% w/v fresh garlic extract). This stock solution was stored at -20°C and thawed before use. Serial dilutions were prepared using Dimethyl Sulfoxide (DMSO) to achieve final concentrations of 25 mg/mL, 50 mg/mL, and 75 mg/mL, using the dilution formula C1V1 = C2V2, where the stock solution (C1 = 100 mg/mL) was diluted to the desired concentrations.

Bacterial Strains

Clinical isolates of Neisseria gonorrhoeae and Treponema pallidum were obtained from the Federal Teaching Hospital, Lokoja. The strains were transported in an ice box and stored at 4°C until use.

Antibacterial Susceptibility Testing

The antibacterial activity was assessed using the agar well diffusion method (Bauer et al., 1966). Nutrient agar plates were inoculated with a standardized suspension of the test organisms. Wells of 6 mm diameter were bored into the agar using a sterile cork borer. A volume of 100 µL of each garlic extract concentration (25, 50, 75, and 100 mg/mL) was introduced into the respective wells. DMSO served as the negative control. The plates were incubated at 37°C for 24-48 hours, and the zones of inhibition (ZOI) were measured in millimeters (mm).

Determination of Minimum Inhibitory Concentration (MIC)

The MIC was determined using the broth dilution method. Two-fold serial dilutions of the garlic extract were prepared in nutrient broth to achieve a concentration range from 100 mg/mL downwards. A 0.2 mL inoculum of each test organism was added to the tubes. The tubes were incubated at 37°C for 24-48 hours. The MIC was defined as the lowest concentration that showed no visible turbidity, indicating complete inhibition of bacterial growth.

Phytochemical Analysis by GC-MS

The phytochemical profile of the garlic extract was analyzed using an Agilent Intuvo 9000 GC system coupled with a 5977B MSD detector. A DB-5 MS capillary column was used with helium as the carrier gas. The identification of compounds was achieved by comparing their mass spectra with the NIST library.

Statistical Analysis

All experiments were performed in triplicate. Data were presented as mean ± standard error and analyzed using one-way Analysis of Variance (ANOVA) followed by Tukey's post-hoc test. A p-value of < 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Phytochemical Composition

The GC-MS analysis identified 27 bioactive compounds in the garlic extract. The major constituents were n-Hexadecanoic acid (25.72%), Oleic Acid (21.56%), Hexadecanoic acid, ethyl ester (10.87%), 9,17-Octadecadienal, (Z)- (6.01%), and 9-Octadecenoic acid (3.66%). The complete phytochemical profile is detailed in Table 1, and the representative chromatogram is presented in Figure 1.

Table 1: Chemical Composition of Allium Sativum Extract as Determined by GC-MS Analysis

Peak	RT	Area (%)	Name of the compound	Molecular Formula	Molecular Weight
1	5.450	0.70	Trisulfide, di-2-propenyl	$C_6H_{10}S_3$	178
2	5.709	1.58	Trisulfide, di-2-propenyl	$C_6H_{10}S_3$	178
3	5.840	0.98	Trisulfide, di-2-propenyl	$C_6H_{10}S_3$	178
4	6.003	4.26	5-Methylthiophen-3-ylamine	C_5H_7NS	113
5	13.008	0.58	9-decenoate	$C_{12}H_{22}O_2$	198
6	19.110	0.53	Carbonic acid, allyl pentadecyl ester	$C_{19}H_{36}O_3$	300
7	19.193	0.28	6-Methylheptyl 2-methylbutanoate	$C_{13}H_{26}O_2$	214
8	19.377	0.12	12 2-Butyl-3-methyl-5-(2-methylprop-2-enyl) cyclohexanone	$C_{15}H_{26}O$	222
9	19.709	0.10	2H-Azepin-2-one, hexahydro-1-	$C_6H_{11}NO$	113
10	20.314	0.34	Cyclononene	C_9H_{16}	124
11	20.530	1.19	Octatriacontyl pentafluoropropionate	$C_{41}H_{77}F_5O_2$	681

Peak	RT	Area (%)	Name of the compound	Molecular Formula	Molecular Weight
12	20.722	1.66	Oleic Acid	$C_{18}H_{34}O_2$	282
13	20.801	1.28	Oleic Acid	$C_{18}H_{34}O_2$	282
14	20.983	0.96	Oleic Acid	$C_{18}H_{34}O_2$	282
15	21.412	10.87	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284
16	21.616	25.72	n-Hexadecanoic	$C_{16}H_{32}O_2$	256
17	21.923	6.21	n-Hexadecanoic	$C_{16}H_{32}O_2$	256
18	22.069	3.66	Octadecenoic acid	$C_{18}H_{34}O_2$	282
19	22.167	1.46	trans-13-Octadecenoic acid	$C_{18}H_{34}O_2$	282
20	22.207	1.66	cis-Vaccenic acid	$C_{18}H_{34}O_2$	282
21	22.251	2.69	Lactose	$C_{12}H_{22}O_{11}$	342
22	22.326	2.43	Z-8-Methyl-9-tetradecenoic acid	$C_{15}H_{28}O_2$ 240	240
23	22.393 2.13	2.13	Melezitose $C_{18}H_{32}O_{16}$	$C_{18}H_{32}O_{16}$	504
24	23.555	21.56	Oleic Acid	$C_{18}H_{34}O_2$ 2	282
25	24.491	1.73	Palmitoleic acid	$C_{16}H_{30}O_2$	245
26	24.655	6.01	9,17-Octadecadienal, (Z)-		
27	38.293 -	0.67	9-Octadecenoic acid, (E)-		

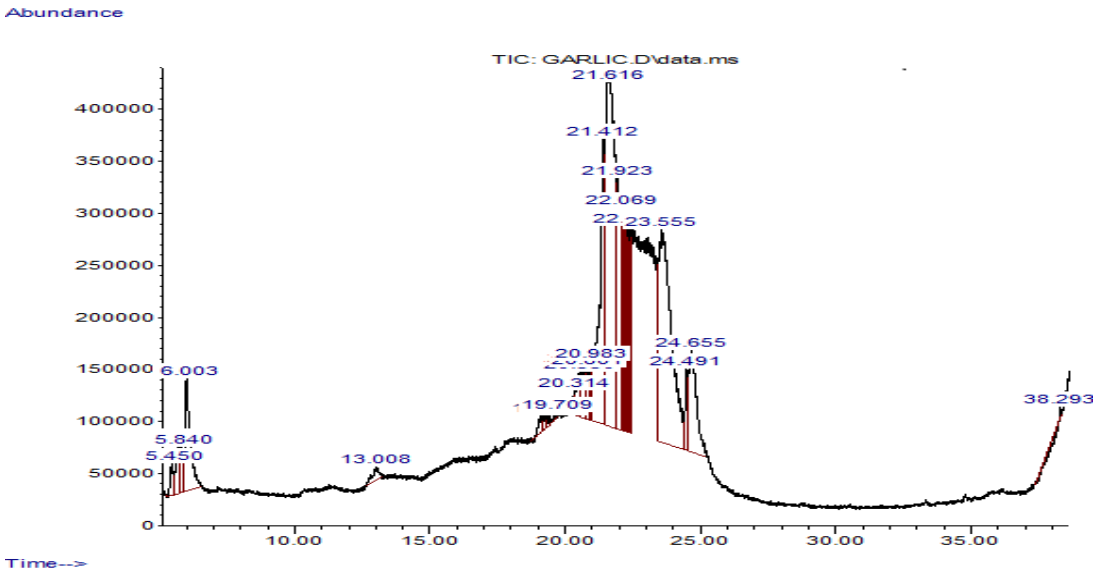

Figure 1: GC-MS Chromatogram of the Bioactive Compounds Present in Allium Sativum)

Antibacterial Activity

The garlic extract exhibited concentration-dependent antibacterial activity against both test organisms (Table 2). For Treponema pallidum, the zones of inhibition ranged from 8.00 ± 0.04 mm at 25 mg/mL to 14.00 ± 0.30 mm at 100 mg/mL. For Neisseria gonorrhoeae, the ZOIs were larger, ranging from 10.00 ± 0.02 mm at 25 mg/mL to 16.00 ± 0.05 mm at 100 mg/mL. The negative control (DMSO) showed no activity. Statistical analysis confirmed that the differences in ZOIs between concentrations were significant ($p < 0.05$).

Table 2: Zones of Inhibition (mm, mean ± SE) of Allium Sativum Extract against Test Organisms

Test Organisms	25mg/ml	50mg/ml	75mg/ml	100mg/ml	Control (DMSO)
Treponema pallidum	8.00 ± 0.04^a	10.00±0.08^a	12.00±0.05^a	14.00±0.30^a	0
Neisseria gonorrhoeae	10.00±0.02^bc	12.00±0.04^bc	14.00±0.11^bc	16.00±0.05^a	0

Values are mean ± standard error (n=3). Mean values with different superscripts in a row are significantly different ($p < 0.05$). The well diameter was 6 mm.

Minimum Inhibitory Concentration (MIC)

The MIC results are summarized in Table 3. The extract showed a stronger inhibitory effect on N. gonorrhoeae, with complete inhibition (no turbidity) observed at 75 mg/mL. For T. pallidum, complete inhibition was achieved at 100 mg/mL.

Table 3: Minimum Inhibitory Concentration (MIC) of Allium Sativum Extract

Test Organisms	25mg/ml	50mg/ml	75mg/ml	100mg/ml	Control
	+++	+	+	-	-
	++	+	-	-	-

Test Organism 25 mg/mL 50 mg/mL 75 mg/mL 100 mg/mL Control
Treponema pallidum +++ + + - -
Neisseria gonorrhoeae ++ + - - -
Key: +++ = Highly Turbid; ++ = Moderately Turbid; + = Slightly Turbid; - = No Turbidity (Inhibition)

Discussion

This study provides compelling evidence for the potent in vitro antibacterial activity of Allium sativum (garlic) extract against two significant STI pathogens, Neisseria gonorrhoeae and Treponema pallidum. The efficacy observed aligns with the plant's renowned history in ethnomedicine (Bayan et al., 2014; Block, 2010) and underscores its potential as a source of novel therapeutic agents in an era of escalating antibiotic resistance (Unemo & Shafer, 2014; Ventola, 2015).

The most salient finding was the dose-dependent antibacterial response, a hallmark of bioactive antimicrobial agents (Kaur et al., 2011; Cowan, 1999). The significantly larger zones of inhibition (ZOIs) and lower Minimum Inhibitory Concentration (MIC) for N. gonorrhoeae (75 mg/mL) compared to T. pallidum (100 mg/mL) are noteworthy. This differential susceptibility can be attributed to fundamental differences in cellular biology. N. gonorrhoeae is a Gram-negative diplococcus with a complex cell envelope containing porins and lipooligosaccharides, which can be targeted and disrupted by the lipophilic compounds found in garlic (Nikado, 2003; Shafer & Qu, 2013). In contrast, T. pallidum is a spirochete with a unique outer membrane that is lipid-rich and contains a paucity of transmembrane proteins, often making it less permeable to external agents and more difficult to eradicate (Radolf & Kumar, 2018; Cox & Radolf, 2001). Our results are consistent with the broader trend that spirochetes often exhibit higher intrinsic resistance to antimicrobials compared to many other bacteria (Stamm, 2010).

The phytochemical profile revealed by GC-MS analysis offers a chemical basis for the observed antibacterial activity. While allicin, the classic antimicrobial compound in crushed garlic, is often cited (Ankri & Mirelman, 1999; Borlinghaus et al., 2014), our extract's dominant components were various fatty acids. n-Hexadecanoic acid (Palmitic acid), the most abundant compound (25.72%), has documented antibacterial properties. Studies have shown it can disrupt the cell membrane of Gram-positive bacteria like Staphylococcus aureus (Sun et al., 2003), and its efficacy against Gram-negatives, though sometimes requiring higher concentrations, is linked to its ability to perturb the outer membrane (Zheng et al., 2005; Desbois & Smith, 2010). Oleic Acid (21.56%), another major constituent, is known to increase cell membrane permeability and has been shown to synergize with traditional antibiotics, potentially reversing resistance mechanisms (Huang et al., 2011; Rouse et al., 2007). The presence of Hexadecanoic acid, ethyl ester further suggests a complex mixture where esterification might influence the bioavailability and activity of the fatty acids (McGaw et al., 2002). This composition differs from studies that focus on steam-distilled garlic oils, which are richer in diallyl disulfides and trisulfides (Tsao & Yin, 2001; Lawson & Hughes, 1992). The potent activity of our fresh juice extract, dominated by fatty acids, highlights that garlic's antimicrobial power is not reliant on a single compound but is a result of the synergistic action of multiple bioactive constituents (Ankri & Mirelman, 1999; Lanzotti et al., 2014).

Our findings find strong support in the existing literature, yet they also provide a crucial extension. For instance, Tsao and Yin (2001) demonstrated that diallyl sulfides from garlic oil were effective against a range of foodborne pathogens like E. coli and Salmonella. Similarly, Jaber and Al-Mossawi (2007) reported significant ZOIs for garlic extract against multi-drug resistant clinical isolates of Pseudomonas aeruginosa and Klebsiella pneumoniae. Our study directly builds upon this by confirming that this broad-spectrum activity extends to highly relevant and resistant STI pathogens. The ZOIs we recorded (up to 16 mm for N. gonorrhoeae at 100 mg/mL, measured from a 6 mm well) are comparable to, and in some cases superior to, those reported in other studies using plant extracts against similar pathogens. For example, a study on the antibacterial activity of Moringa oleifera against N. gonorrhoeae reported a maximum ZOI of 14 mm (Nweze & Eze, 2009). Furthermore, the MIC results, showing complete inhibition of N. gonorrhoeae at 75 mg/mL, indicate a potent bactericidal effect, which is more therapeutically desirable than a bacteriostatic one (Pankey & Sabath, 2004).

The clinical implication of this research is substantial. The relentless rise of ceftriaxone-resistant N. gonorrhoeae and the challenges in treating late-stage syphilis demand innovative solutions (Unemo & Shafer, 2014; Ohnishi et al., 2011; Hook, 2017). While garlic extract is not proposed as an immediate replacement for first-line antibiotics, it presents a promising candidate for several applications: as an adjunct therapy to enhance efficacy and reduce antibiotic doses (Hemaiswarya et al., 2008; Liu et al., 2010); as a topical formulation for localized infections (Cutler & Wilson, 2004); or as a prophylactic agent in high-risk populations. Its multi-component nature also suggests a lower risk of bacteria developing resistance compared to single-target antibiotics (Hemaiswarya et al., 2008; Gibbons, 2004).

However, this study has limitations. The use of a crude extract, while ethnobotanically relevant, makes it difficult to pinpoint the exact compound(s) responsible for the activity (Cos et al., 2006). The in vitro environment cannot fully replicate the complex conditions of a human infection. Furthermore, the stability of the active compounds in the extract over time and under different storage conditions was not assessed (Iberl et al., 1990).

CONCLUSION

The results of this study conclusively demonstrate that Allium sativum extract, at concentrations ranging from 25 to 100 mg/mL, possesses significant in vitro antibacterial activity against Neisseria gonorrhoeae and Treponema pallidum. The presence of potent bioactive compounds, including n-Hexadecanoic acid and Oleic Acid, supports its traditional use in treating infections. Given the rising threat of antimicrobial resistance, garlic presents a promising, naturally derived candidate for further investigation as an alternative or complementary therapeutic agent against these sexually transmitted infections. Future research should focus on isolating garlic's active compounds and conducting in vivo studies to validate its therapeutic potential, while also

exploring its use as an adjunct therapy and promoting its dietary benefits to combat antimicrobial resistance and improve public health.

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