IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

Therapeutic Potential Of *Allium Sativum* (L.) In Combating Urinary Tract Infections: An *In-Vitro* Study

Sowjanya Pulipati*, Lingineni Bhavitha, Shaik Umul Vara Mahenoor
Department of Pharmaceutics, Vignan Pharmacy College (Autonomous), Vadlamudi, Guntur (Dt), Andhra Pradesh, INDIA

Abstract

Urinary tract infections (UTIs) are among the most common bacterial infections, and the increasing prevalence of antimicrobial resistance necessitates the exploration of alternative therapeutic agents. Medicinal plants, particularly *Allium sativum* (garlic), have gained attention due to their rich phytochemical composition and broad-spectrum antimicrobial activity. The present study aimed to evaluate the antibacterial potential of different solvent extracts of *Allium sativum* against clinically isolated uropathogens.

Clinical isolates were identified using standard biochemical tests, including catalase, indole, methyl red (MR), Voges–Proskauer (VP), citrate utilization, and urease tests, confirming the presence of *Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Proteus vulgaris, Enterococcus faecalis,* and *Staphylococcus aureus.* Garlic extracts were prepared using chloroform, ethyl acetate, acetone, and ethanol by cold maceration. Antibacterial activity was evaluated using the agar well diffusion method, and minimum inhibitory concentration (MIC) was determined by microdilution technique, with amoxicillin used as the standard.

Among the extracts, the ethyl acetate extract (EAE) exhibited the highest antibacterial activity, with maximum zones of inhibition observed against *Staphylococcus aureus* (32 mm) and *Escherichia coli* (27 mm). Chloroform extract (CE) also showed notable activity, whereas acetone (AE) and ethanol (ETE) extracts demonstrated comparatively lower effects. MIC results further confirmed the superior efficacy of EAE, showing the lowest inhibitory concentration of 6.25 µg/ml against *Staphylococcus aureus* and 12.5 µg/ml against most other pathogens. The antibacterial activity of garlic extracts was found to be comparable to or greater than that of amoxicillin in certain cases.

In conclusion, *Allium sativum* exhibits significant antibacterial activity against a range of UTI pathogens, supporting its potential as a natural alternative in the management of urinary tract infections. Further studies are required to isolate active constituents and validate their clinical applicability.

Keywords: *Allium sativum*, Garlic, Urinary tract infections, Antibacterial activity, MIC, Agar well diffusion.

1. Introduction

Medicinal plants have played a crucial role in the discovery and development of therapeutic agents since ancient times. Natural products derived from plants have served as the foundation for many modern pharmaceuticals due to their diverse chemical structures and biological activities. It is estimated that a significant proportion of currently available drugs are either directly derived from plant sources or are synthetic analogues of naturally occurring compounds. The use of plant-based remedies is particularly significant in developing countries where traditional medicine remains an integral part of healthcare systems[1,2].

In recent decades, the emergence and rapid spread of antimicrobial resistance among pathogenic microorganisms have become a major global health concern. Excessive and inappropriate use of antibiotics has led to the development of resistant strains of bacteria, thereby reducing the effectiveness of conventional antimicrobial therapies. Urinary tract infections (UTIs) are among the most common bacterial infections affecting millions of individuals worldwide each year. The majority of UTIs are caused by Gram-negative bacteria such as *Escherichia coli* and *Pseudomonas aeruginosa*, as well as Gram-positive bacteria including *Staphylococcus aureus* and *Enterococcus faecalis*[3,4]. Increasing resistance among these pathogens has prompted researchers to explore alternative therapeutic strategies, particularly those derived from natural products.

Phytochemicals present in medicinal plants have gained considerable attention as potential antimicrobial agents. These bioactive compounds include alkaloids, flavonoids, tannins, terpenoids, phenolic compounds, and sulfur-containing compounds that exhibit significant antibacterial, antifungal, and antiviral activities[5]. Plant-derived antimicrobial compounds often exert their effects by disrupting microbial cell walls, inhibiting essential enzymes, interfering with nucleic acid synthesis, or altering membrane permeability[6]. Due to their complex chemical composition, plant extracts may also reduce the likelihood of microbial resistance compared with single-target synthetic antibiotics.

Garlic (*Allium sativum* L.), a member of the family Amaryllidaceae (formerly Liliaceae), is one of the most widely used medicinal plants and culinary spices in the world. Garlic has been used for thousands of years in traditional systems of medicine including Ayurveda, Chinese medicine, and Egyptian medicine for the treatment of various diseases. Historically, garlic has been used to treat infections, wounds, respiratory disorders, gastrointestinal ailments, and cardiovascular diseases[7,8]. The medicinal properties of garlic are attributed to its rich content of organosulfur compounds, vitamins, minerals, and other phytochemicals.

The characteristic odor and many of the therapeutic properties of garlic are primarily associated with allicin, a biologically active sulfur compound produced when garlic cloves are crushed or chopped. Allicin is formed from the precursor compound alliin through the enzymatic action of alliinase. Allicin and its related sulfur-containing derivatives, such as diallyl sulfide, diallyl disulfide, and ajoene, have been shown to exhibit broad-spectrum antimicrobial activity against numerous bacterial species[9,10]. These compounds can inhibit microbial growth by interfering with thiol-containing enzymes and disrupting essential metabolic pathways in microorganisms.

Several studies have demonstrated that garlic extracts possess strong antibacterial activity against both Gram-positive and Gram-negative bacteria. Garlic-derived compounds have been shown to inhibit pathogens responsible for respiratory infections, gastrointestinal infections, and urinary tract infections[11,12]. The antimicrobial activity of garlic has also been attributed to its ability to penetrate bacterial cell membranes and inhibit RNA synthesis, thereby suppressing microbial proliferation[13]. In addition to its antibacterial properties, garlic also exhibits antioxidant, anti-inflammatory, anticancer, and immunomodulatory activities, making it a valuable medicinal plant with multiple therapeutic applications.

Urinary tract infections remain a significant public health problem due to their high prevalence and recurrent nature. The development of antibiotic-resistant strains of uropathogens has further complicated the management of UTIs. Consequently, there is a growing interest in identifying plant-based antimicrobial agents that may serve as alternative or complementary therapies. Garlic, with its well-documented antimicrobial properties and long history of medicinal use, represents a promising candidate for the development of novel antimicrobial agents.

Therefore, the present study aims to investigate the antibacterial activity of different solvent extracts of Allium sativum against common uropathogenic bacteria using the agar well diffusion method. The findings of this study may contribute to the development of plant-based antimicrobial agents for the management of urinary tract infections.

Materials & Methods:

***Plant material*:** The garlic bulbs were purchased from market, identified and authenticated by Botanist, Acharya Nagarjuna University, Guntur.

Extraction: Fresh garlic bulbs were peeled, cleaned, and surface sterilized. The cloves were homogenized and subjected to cold maceration using different solvents (chloroform, ethyl acetate, acetone and ethanol). Extracts were filtered, concentrated, dried, and stored in a desiccator.

Test bacteria: In the study two Gram positive bacteria *Staphylococcus aureus, Enterococcus faecalis* and four Gram negative bacteria *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Proteus vulgaris* isolated from subjects suffering with urinary tract infections.

Phytochemical screening: The phytochemical screening for the crude extracts of *Allium sativum* was carried out by standard protocols[14]. The presence of alkaloids, glycosides, saponins, carbohydrates, proteins, aminoacids, flavonoids, steroids, tannins was analysed and results were reported in table 1.

Isolation and Identification of UTI bacteria: The microorganisms present in urine samples of UTI infected patients were cultured in the nutrient broth and using gram staining procedure gram positive and gram negative organisms were differentiated.

The organisms were transferred to CLED agar medium for further identification. Six biochemical tests were performed for each organism. Catalase test, Indole production test, Citrate utilization test, Urease test, Methyl red, Voges Proskauer's test and results were reported in table 2.

Antibacterial activity: The antibacterial activity of garlic clove extracts was evaluated using the agar well diffusion method[15]. The collected clinical isolates were cultured in Mueller-Hinton broth (HiMedia, Mumbai) and incubated at 37°C for 24 hours under shaking conditions. Following incubation, the bacterial cultures were aseptically spread onto sterile Mueller-Hinton agar plates using sterile cotton swabs.

Wells of 6 mm diameter were created in the inoculated agar plates using a sterile cork borer. Subsequently, 100 µL of methanolic extract (50 mg/mL prepared in dimethyl sulfoxide) was introduced into the designated wells. For comparison, 50 µL of the positive control (amikacin, 10 mg/mL in dimethyl sulfoxide) and 50 µL of the negative control (dimethyl sulfoxide) were also added into separate wells.

The plates were then incubated at 37°C for 24 hours in an upright position. After incubation, the zones of inhibition were measured and recorded. All experiments were performed in triplicate, and the average values were presented in Table 3.

Determination of MIC by Microtitre plate assay: The minimum inhibitory concentration (MIC) of the test sample was determined using a microtiter plate assay under aseptic conditions[16, 17]. A stock solution of the test material (10% w/v) was prepared in dimethyl sulfoxide (DMSO). An aliquot of 100 µL of this solution was added to the first row of the microtiter plate. Subsequently, 50 µL of sterile nutrient broth was dispensed into the remaining wells.

Serial two-fold dilutions of the test sample were performed by transferring the solution from one well to the next. To each well, 30 µL of resazurin indicator solution (0.02%) was added. Finally, 10 µL of bacterial suspension (1×10^8 CFU/mL) was inoculated into all wells.

Control wells were also included: a positive control containing all components except the test sample, and a negative control containing all components except the bacterial inoculum (replaced with sterile nutrient broth). The plates were then incubated at 37°C for 24 hours.

Following incubation, a color change of resazurin from purple to pink indicated bacterial growth. The MIC was determined as the lowest concentration of the test sample that prevented this color change. The MIC values for each bacterial strain were recorded and presented in Table 4.

Results & Discussion:

Table 1. Preliminary phytochemical screening of leaves of *Allium sativum*

S.No	Name of the test	Chloroform Extract	Ethyl Acetate Extract	Acetone Extract	Ethanol Extract
1	Carbohydrates	+	+	+	+
2	Proteins	+	+	+	+
3	Aminoacids	+	+	+	+
4	Steroids	+	+	+	+
5	Cardiac glycosides	+	+	+	+
6	Flavonoids	+	+	+	+
7	Alkaloids	+	+	+	+
8	Tannins	+	+	+	+

Note: "+" indicates positive.

Table-2: Biochemical tests of recovered clinical isolates

S.No	Catalase	Indole	MR	VP	Citrate	Urease	Organism confirmed
1	+	+	+	-	-	-	*E. coli*
2	+	+	-	-	+	+	*Pseudomonas*
3	+	+	-	-	+	+	*Klebsiella*
4	+	+	+	-	+	+	*Proteus*
5	+	+	+	-	-	-	*Enterococcus*
6	+	-	-	-	+	+	*Staphylococcus*

Table-3: Antibacterial activity of different extracts of *Allium sativum* & Amoxycillin

S. No	Microorganisms	Zone of Inhibition in mm				
		CE 25µg/ml	EAE 25µg/ml	AE 25µg/ml	ETE 25µg/ml	Amoxicillin 10µg/ml
1.	*Staphylococcus aureus*	28	32	22	17	12
2.	*Enterococcus faecalis*	29	20	18	15	10
3.	*Escherichia coli*	26	27	21	13	10
4.	*Klebsiella pneumoniae*	24	26	20	14	12
5.	*Pseudomonas aeruginosa*	24	25	19	13	11
6.	*Proteus vulgaris*	22	23	18	12	11

(Note: CE - Chloroform Extract, EAE – Ethyl Acetate Extract, AE - Acetone Extract, ETE – Ethanol Extract)

Table-4: Minimum inhibitory concentration (MIC) of different extracts of Allium sativum & Amoxycillin against selected UTI pathogens.

S. No	Microorganisms	Minimum Inhibitory Concentration (mcg/ml)				
		CE	EAE	AE	ETE	Amoxicillin
1.	*Staphylococcus aureus*	12.5	6.25	25	25	12.5
2.	*Enterococcus faecalis*	12.5	12.5	25	25	12.5
3.	*Escherichia coli*	12.5	12.5	12.5	50	12.5
4.	*Klebsiella pneumoniae*	12.5	12.5	12.5	50	12.5
5.	*Pseudomonas aeruginosa*	12.5	12.5	25	50	12.5
6.	*Proteus vulgaris*	25	12.5	25	50	12.5

(Note: CE - Chloroform Extract, EAE – Ethyl Acetate Extract, AE - Acetone Extract, ETE – Ethanol Extract)

Discussion:

Urinary tract infections (UTIs) are among the most prevalent bacterial infections worldwide, affecting individuals across all age groups and posing a significant burden on healthcare systems. The increasing incidence of antimicrobial resistance among uropathogens has reduced the effectiveness of conventional antibiotics, necessitating the exploration of alternative and complementary therapeutic agents. In this context, medicinal plants such as *Allium sativum* (garlic) have gained considerable attention due to their well-documented antimicrobial properties and long history of traditional use.

Biochemical characterization plays a crucial role in the accurate identification and confirmation of uropathogens. In the present study, a series of standard biochemical tests including catalase, indole, methyl red (MR), Voges–Proskauer (VP), citrate utilization, and urease tests were performed to confirm the identity of the isolated microorganisms.

All isolates showed positive catalase activity, indicating their ability to decompose hydrogen peroxide into water and oxygen, a common feature among many aerobic and facultative anaerobic bacteria. This result supports the presence of metabolically active organisms capable of surviving oxidative stress.

The indole test was positive for most isolates, particularly confirming the presence of Escherichia coli, which is well known for its ability to produce indole from tryptophan. The combination of indole positive and MR positive results strongly supports the identification of *E. coli*, as it follows mixed acid fermentation.

The isolate identified as *Pseudomonas* showed citrate and urease positivity with negative MR and VP tests, which aligns with its known metabolic profile as a non-fermenter capable of utilizing citrate as a carbon source. Similarly, *Klebsiella* exhibited citrate and urease positivity, which is characteristic of this genus, although it typically shows VP positivity; slight variations may occur due to strain differences or experimental conditions.

The biochemical profile of *Proteus* demonstrated positive MR, citrate, and urease tests, which is consistent with its strong urease activity and its role in urinary tract infections, particularly in catheter-associated cases. Urease production contributes to increased urine pH and stone formation, enhancing pathogenicity.

The identification of *Enterococcus* based on the given biochemical pattern (catalase positive and indole positive) may indicate variability, as Enterococci are generally catalase negative; this could be due to weak pseudocatalase activity or experimental deviation. Further confirmatory tests such as bile esculin hydrolysis or salt tolerance would strengthen the identification.

The isolate identified as *Staphylococcus* showed catalase positivity and indole negativity, which is a key distinguishing feature separating Staphylococci from Streptococci. Additional tests such as coagulase test would further differentiate species like *S. aureus*.

Overall, the biochemical test results are largely consistent with standard microbial identification patterns and confirm the presence of common uropathogens including *E. coli, Pseudomonas, Klebsiella, Proteus, Enterococcus, and Staphylococcus.* These organisms are frequently implicated in urinary tract infections and are known for their varying degrees of antibiotic resistance.

The accurate identification of these pathogens is essential for evaluating the antibacterial efficacy of *Allium sativum* extracts, as different organisms may exhibit variable susceptibility patterns.

The antibacterial activity of different solvent extracts of *Allium sativum* was evaluated against selected uropathogens using the agar well diffusion method. The results demonstrate that all extracts exhibited varying degrees of antibacterial activity, with notable differences depending on the solvent used and the microorganism tested.

Among the extracts, the ethyl acetate extract (EAE) showed the highest antibacterial activity overall, particularly against *Staphylococcus aureus* (32 mm), followed by *Escherichia coli* (27 mm) and *Klebsiella pneumoniae* (26 mm). This suggests that moderately polar phytoconstituents present in the ethyl acetate fraction may be primarily responsible for the observed antimicrobial effects.

The chloroform extract (CE) also exhibited strong activity, especially against *Enterococcus faecalis* (29 mm) and *S. aureus* (28 mm), indicating that non-polar compounds contribute significantly to antibacterial action. In contrast, acetone (AE) and ethanol extracts (ETE) showed comparatively lower activity across all tested organisms.

Notably, all garlic extracts demonstrated greater zones of inhibition than the standard antibiotic amoxicillin, highlighting the potent antibacterial potential of *Allium sativum*. The activity against both Gram-positive (*S. aureus, Enterococcus faecalis*) and Gram-negative bacteria (*E. coli, K. pneumoniae, P. aeruginosa, Proteus vulgaris*) indicates a broad-spectrum effect.

The observed antibacterial activity can be attributed to bioactive sulfur-containing compounds such as allicin, which disrupt bacterial enzyme systems and metabolic pathways. Overall, the findings support the potential of garlic extracts as effective natural alternatives for the management of urinary tract infections, warranting further studies on isolation and characterization of active constituents.

The minimum inhibitory concentration (MIC) results of *Allium sativum* extracts against selected uropathogens indicate significant antibacterial efficacy, with variations depending on the solvent system and organism tested.

Among the extracts, the ethyl acetate extract (EAE) demonstrated the lowest MIC values, particularly against *Staphylococcus aureus* (6.25 µg/mL), indicating the highest potency. For most other organisms, including *Enterococcus faecalis, Escherichia coli, Klebsiella pneumoniae,* and *Pseudomonas aeruginosa,* EAE exhibited MIC values of 12.5 µg/mL, comparable to the standard antibiotic amoxicillin.

The chloroform extract (CE) also showed consistent activity with MIC values of 12.5 µg/mL against most organisms, though slightly less effective against *Proteus vulgaris* (25 µg/mL). The acetone extract (AE) displayed moderate activity, with MIC values ranging from 12.5 to 25 µg/mL, while the ethanol extract (ETE) showed the least activity, with higher MIC values (25–50 µg/mL), indicating lower antibacterial potency.

Overall, the MIC findings corroborate the agar diffusion results, confirming that ethyl acetate extract possesses the most potent antibacterial activity, likely due to the presence of optimally extracted bioactive compounds such as allicin and related sulfur constituents. The comparable MIC values of garlic extracts with amoxicillin highlight their potential as effective natural antimicrobial agents against both Gram-positive and Gram-negative uropathogens.

These results support further investigation into the isolation, characterization, and clinical applicability of active phytoconstituents from *Allium sativum* for the treatment of urinary tract infections.

Conclusion:

The present study successfully demonstrated the significant antibacterial potential of Allium sativum (garlic) extracts against major uropathogens associated with urinary tract infections. The results obtained from biochemical characterization confirmed the presence of clinically relevant microorganisms, including *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Proteus vulgaris*.

Among the different solvent extracts evaluated, the ethyl acetate extract consistently exhibited superior antibacterial activity, as evidenced by larger zones of inhibition and lower minimum inhibitory concentration (MIC) values. The chloroform extract also showed appreciable activity, whereas acetone and ethanol extracts were comparatively less effective. Notably, the antibacterial efficacy of garlic extracts was found to be comparable to or greater than the standard antibiotic amoxicillin against several tested organisms.

The observed antimicrobial activity can be attributed to bioactive phytoconstituents, particularly sulfur-containing compounds such as allicin, which interfere with bacterial metabolic pathways and enzyme systems. The ability of garlic extracts to inhibit both Gram-positive and Gram-negative bacteria highlights their broad-spectrum antimicrobial potential.

In conclusion, *Allium sativum* represents a promising natural source for the development of alternative or adjunct therapies in the management of urinary tract infections, especially in the context of rising antibiotic resistance. However, further studies involving isolation of active compounds, mechanistic investigations, toxicity evaluation, and in vivo validation are necessary to establish its clinical applicability.

References:

1. Newman DJ, Cragg GM. Natural products as sources of new drugs over the last 25 years. J Nat Prod. 2007;70:461–77.
2. Fabricant DS, Farnsworth NR. The value of plants used in traditional medicine for drug discovery. Environ Health Perspect. 2001;109:69–75.
3. Flores-Mireles AL, Walker JN, Caparon M, Hultgren SJ. Urinary tract infections: epidemiology, mechanisms of infection and treatment options. Nat Rev Microbiol. 2015;13:269–84.
4. Foxman B. Epidemiology of urinary tract infections: incidence, morbidity, and economic costs. Dis Mon. 2003;49:53–70.
5. Cowan MM. Plant products as antimicrobial agents. Clin Microbiol Rev. 1999;12:564–82.
6. Cushnie TPT, Lamb AJ. Antimicrobial activity of flavonoids. Int J Antimicrob Agents. 2005;26:343–56.
7. Rivlin RS. Historical perspective on the use of garlic. J Nutr. 2001;131:951S–4S.
8. Lawson LD, Gardner CD. Composition, stability, and bioavailability of garlic products used in research studies. J Nutr. 2005;135:716S–25S.
9. Ankri S, Mirelman D. Antimicrobial properties of allicin from garlic. Microbes Infect. 1999;1:125–9.
10. Borlinghaus J, Albrecht F, Gruhlke MCH, Nwachukwu ID, Slusarenko AJ. Allicin: chemistry and biological properties. Molecules. 2014;19:12591–618.
11. Ross ZM, O'Gara EA, Hill DJ, Sleightholme HV, Maslin DJ. Antimicrobial properties of garlic oil against human enteric bacteria. J Appl Microbiol. 2001;91:711–8.
12. Nidadavolu P, Amor W, Tran PL, Dertien J, Colmer-Hamood JA, Hamood AN. Garlic ointment inhibits biofilm formation by bacterial pathogens from burn wounds. J Med Microbiol. 2012;61:662–71.
13. Feldberg RS, Chang SC, Kotik AN, Nadler M, Neuwirth Z, Sundstrom DC, et al. In vitro mechanism of inhibition of bacterial cell growth by allicin. Antimicrob Agents Chemother. 1988;32:1763–8.
14. Evans WC, Trease ET (2005). Pharmacognosy, 15th ed., W.B. Saunders Company Ltd., London, pp. 191-393.
15. Bauer AW, Kirby WMM Sherries M (1966). Antibiotic susceptibility testing by a standardized single disk method. Am. J. Clin. Pathol. 45: 493-496.

16. Sowjanya Pulipati, Srinivasa Babu P. and Lakshmi Narasu M. Phytochemical analysis and antibacterial efficacy of *Amaranthus tricolor* (L) methanolic leaf extract against clinical isolates of urinary tract pathogens. African Journal of Microbiology Research. May, 2015; 9(20), 1381-1385.
17. Sowjanya Pulipati, Srinivasa Babu. P and B. Sri Devi. Preliminary phytochemical and antibacterial Investigations of the methanol extract of *Alternanthera philoxeroides* (Mart.) Griseb. *ejpmr, 2015,2(4), 347-356*