Bima Journal of Science and Technology, Vol. 7 (3) Sept, 2023 ISSN: 2536-6041

DOI: 10.56892/bima.v7i3.490

Antibacterial Evaluation of *Alium sativum* on Gram-Negative Urinary Tract Bacteria

*Garba L., Muhammad R., Adamu M.T., Yarma A.A, Jauro H. A. and Isa S.

Department of Microbiology, Faculty of Science, Gombe State University, PMB 127, Tudun Wada Gombe, Gombe State, Nigeria

*Correspondence Author: lagarpak@gsu.edu.ng

ABSTRACT

Bacterial pathogens of the Urinary Tract have been prevalent in many settings particularly the community or hospital. The Urinary Tract Infections may be symptomless or symptomatic and classified as bladder infection (cystitis), kidney infection (pyelonephritis), and urine infection (bacteriuria). The emergence of antibiotic resistance particularly among bacterial pathogens has necessitated the need to search medicinal plants for bioactive compounds as possible alternative source of potent antimicrobial agents. Garlic is a plant that is well-known as hardy perennial member of onion family. Previous reports have confirmed the antimicrobial activity of Garlic extracts, however, assessment of garlic *in vitro* inhibitory activity appears to be the first in the study area to the best of our knowledge. One hundred (100) mid-stream urine samples were collected from pregnant women in Liji primary healthcare and brought to Microbiology laboratory of Gombe State University for analysis. The samples were cultured on Nutrient and MacConkey agar plates prepared according manufacturers recommendation. The plates were incubated at 37 ^{o}C for 24 hours. After incubation, the bacterial colonies were identified based on cultural characteristics, Gram staining and biochemical tests. Garlic extract was prepared in methanol using percolation method. The extract recovered was screened for phytochemicals and tested against the bacteria identified. The results showed that, among the Gram-negative bacteria isolated, *Escherichia coli* had the highest occurrence of 24(40%) isolates followed by *Proteus mirabilis,* 15(25%), *Klebsiella pneumoniae,* 9(15%), *Enterobacter aerogenes,* 8(13%) and *Citrobacter freundii,* 4(7%) isolates. Various concentrations (50-400 mg/mL) of garlic methanolic extract tested showed that *E. aerogenes* was the most sensitive bacterium while the least sensitive organism was *E. coli*. The phytochemicals detected include saponins, tannins, flavonoid, and steroid. In conclusion, the garlic extract was found to be effective against all the Gram-negative bacteria tested.

Keywords: Garlic extract, antibacterial activity, Gram-negative bacteria, Sensitivity test, phytochemicals.

INTRODUCTION

Bacterial Infections of the Urinary Tract have been very prevalent in many settings such as the community or hospital (Tajbakhsh et al., 2015). These infections could be asymptomatic or symptomatic, categorised as bladder infection (cystitis), kidney infection (pyelonephritis), and urine infection (bacteriuria) (Piranfar et al., 2014). Globally, the symptomatic urinary tract infections (UTIs) result in several million visits to emergency departments, outpatient clinics and hospitalizations each year (Tajbakhsh et al., 2015). Prevalence of infection varies with respect to gender, age and other predisposing factors (Noor et al., 2013).

Among the urinary tract pathogens, *Escherichia coli* has the highest prevalence of 75–90% in both community and nosocomial cases. Other uropathogenic bacteria include *S. saprophyticus*, *P. mirabilis*, *P. aeruginosa*, *E. faecalis*, and *K. pneumoniae* (Sheerin and Glover, 2019; Kasper et al., 2018; Bazzaz et al., 2021). Fimbrial adhesions play a crucial role for

DOI: 10.56892/bima.v7i3.490

bacterial attachment to the glycolipids and glycoproteins associated with the epithelial surface to survive urine flow. Additionally, other virulence factors produced by the bacterial pathogens include hemolysin, toxins and colony-necrotizing factors which directly affect epithelial integrity promoting invasiveness and the risk of infection (Bazzaz et al., 2021; Behzadi, 2020).

Pregnant women are amongst the group at risk of acquiring UTIs with infection prevalence of approximately 2–10% (Garnizov, 2016; Santoso et al., 2017). Reduced immunity of pregnant women promotes colonization of both pathogenic and non-pathogenic microorganisms which lead to asymptomatic bacteriuria (Santoso et al., 2017; Matuszkiewicz-Rowińska et al., 2015). Almost 40% of untreated cases of asymptomatic bacteriuria in pregnancy are likely to progress to acute morbidity and even death of the mother and fetus. Prompt treatment is always essential to stop prenatal complications such as bacteremia, premature birth as well as low birth weight (Rosana et al., 2020). Choice of treatment depends on pathogens encountered, evidence, clinician consensus, susceptibility patterns, and antimicrobial costs amongst others (Alkhyat and Al-Maqtari, 2014; Angami et al., 2015).

Widespread use of antibiotics for the treatment of urinary tract pathogens has led to a high incidence of antibiotic resistant organisms throughout the world. Over 80% of bacterial strains causing urinary tract infections are now resistant to many potent drugs in underdeveloped countries (Duffa et al., 2018). Such incidences of drug resistant microorganisms have intrigued many researchers to search for novel antibiotics. Thus, huge amount of money and time have been invested on plants natural products to formulating cost-effective remedies that are affordable. On this course, many medicinal plants including *A. sativum*) have been used all over the globe (Lionel et al., 2020).

Antimicrobial activity of various garlic extracts has been tested on several human pathogenic organisms such as bacteria, fungi, and viruses. Garlic is a plant that is well-known as hardy perennial member of onion family. Originally, the garlic plant was from Asia, but now adopted all over the globe. "*Allium sativum*" is the scientific garlic name derived from Celtic word "all" denoting burning or stinging, while the Latin "sativum" referred to as planted or cultivated (Wolde et al., 2018). A large percentage of populace meet their health care needs through traditional medical practitioners as well as medicinal plants in the most underdeveloped countries. The garlic plants have been greatly examined for many years (Wolde et al., 2018). Although many reports have confirmed the antimicrobial activity of Garlic against several organisms from varied sources of infections including the urinary tract, the present study evaluated the *in vitro* inhibitory effect of garlic extract on Gram-negative urinary tract bacteria isolated from pregnant women appears to be the first in the study area.

MATERIALS AND METHODS

Source of Urine Samples

A total of one hundred (100) mid-stream urine samples of pregnant women were collected in sterile disposable universal containers from primary health care Liji and transported immediately to microbiology laboratory at the department of Microbiology of Gombe State University for processing (Ezeigbo et al., 2016).

Isolation of Bacteria

The urine samples were cultured on Nutrient agar and MacConkey agar plates prepared according to manufacturers' recommendations, followed by incubation for 24 hours at 37 ^{o}C (Cheesbrough, 2018).

Identification of Gram-Negative Bacteria

Following the overnight incubation, the

DOI: 10.56892/bima.v7i3.490

bacterial colonies were identified based on culture and morphological characteristics as well as a series of phenotypic tests including citrate utilization test, Indole test, oxidase test, motility test, and urease test, Kligler iron agar test (Cheesbrough, 2018).

Collection and Preparation of Garlic

The *Allium sativum* was bought from the main market in Gombe State, Nigeria and authenticated at Botany Department of Gombe State University. Subsequently, the garlic bulbs were crushed, dried in shade for 7 days and ground into fine powder using clean laboratory pestle and mortar (Yusha'u et al., 2008).

Extraction of Garlic

The extraction was carried out by percolation method in which fifty grams (50g) powder of the garlic was soaked in methanol (200 mL) for three days with regular shaking. Whatman no 1 filter paper was used to filter the mixture. The Garlic extract was obtained from the filtrate with the aid of a rotary evaporator at 40 ^{o}C. The extract was kept at 4 ^{o}C for further use (Yusha'u et al., 2008; Alyasari et al., 2018).

Phytochemical Analysis of Garlic Extracts

The phytochemical analyses of the Garlic extract were performed using standard procedures as demonstrated by Garba et al. (2019), under the following subheadings;

Tannins test

Ferric chloride (5%) (two drops) were mixed with 2 mL of the extract.

Test for saponins

The Garlic extract (1 g) was emulsified in distilled water (10 mL) and thoroughly shaken.

Test for Steroid

Chloroform (2-3 drops) was mixed with 2 mL of the extract and added concentrated sulphuric acid (H_2SO_4) prior to detection of steroids.

Flavonoid test

Few drops of concentrated sodium hydroxide (NaOH) were mixed with 2 mL of the extract and added a few drops of dilute hydrochloric acid prior to detection of flavonoids.

Glycosides test

Two (2 mL) of the extract was boiled and added to it a few drops of concentrated hydrochloric acid (HCl). The mixture was subjected to the second boiling and adjusted to alkaline pH using a few drops of aqueous ammonia solution. The mixture (Five drops) was added to 2 mL of Benedict's reagent and boiled.

Evaluation of *In vitro* Antibacterial Activity of Garlic extract

Standardization of inoculum

Individual colonies of the bacterial isolates were standardized by using 0.5 McFarland standards (Garba et al., 2020). Briefly, a single colony of each organism was picked from the stock culture using a sterile wire loop, inoculated on a prepared nutrient agar plate and incubated for 24 hours at 37 ^{o}C. Following the incubation, a loopful of the colony was picked and emulsified in 5 mL of sterile normal saline to match the 0.5 McFarland turbidity standards.

Extract preparation

A 0.4g of the extract was dissolved in 1 mL of dimethyl sulfoxide (DMSO) to obtain a 400 mg/mL stock solution. Then, three more concentrations of 200,100 and 50 mg/mL were derived from the stock solution by two-fold serial dilution (Garba et al., 2021).

Bioassay procedure

Fresh Mueller Hinton Agar plates were prepared according to manufacturer's recommendation. Sterile swab stick was immersed into a test tube containing the standardized inoculum of each bacterium. Excess fluid was removed by pressing the

DOI: 10.56892/bima.v7i3.490

swab stick against wall of the test tube and swabbed the surface of the Mueller Hinton Agar plates by streaking. Sterile cork borer was used to cut off the inoculated Mueller Hinton agar plates to produced 6 mm wells. Various concentrations (100 µL) of the Garlic extract were added to their respective wells. A 100 µL of DMSO was used alongside to serve as a negative control. The plates were left at room temperature for 15 minutes for pre-diffusion of the extract and incubated for 24 hours at 37 ^{o}C. The zones of growth inhibition of the extract against the bacterial organisms were measured and recorded (Yusha'u et al., 2008; Alyasari et al., 2018).

Statistical Analysis

The results were analysed using two-way analysis of variants (ANOVA) test (Jauro et al., 2021).

RESULTS AND DISCUSSION

Physical properties and phytochemical analysis of Garlic extract

The *A. sativum* extract was found to be brown yellow in colour, jelly in texture and with a yield of 6%. The secondary metabolites present in the extract include saponins, tannins, flavonoid, steroid, but there was no any evidence of glycoside (Table 1).

Table 1: Extract properties and phytochemicals

Phytochemicals		Properties	
Saponins	+	Percentage yield	6%
Tannins	+	Texture	Jelly
Flavonoid	+	Colour	Brown yellow
Steroid	+		
Glycoside	-		

Key: +=detected, -=Not detected

Occurrence of Gram-Negative Bacteria in the Urine Samples

Sixty (60) Gram negative bacteria were isolated from the urine samples. *Escherichia coli* had the highest occurrence of 24(40%) isolates followed by *Proteus mirabilis,* 15 isolates (25%), *Klebsiella pneumoniae,* 9(15%) isolates, *Enterobacter aerogenes,* 8(13%) isolates and then *Citrobacter freundii,* 4(7%) isolates (Table 2).

Table 2: Occurrence of Gram negative bacteria in the urine samples

Organisms	Occurrence	Percentage (%)
Escherichia coli	24	40
Proteus mirabilis	15	25
Klebsiella pneumoniae	9	15
Citrobacter freundii	4	7
Enterobacter aerogenes	8	13
Total	**60**	**100**

The pattern of occurrence of the Gram-negative bacteria observed in this study, particularly the *E. coli, P. mirabilis* and *K. pneumoniae* suggests their role in causing urinary tract infections, precisely the uncomplicated cystitis and pyelonephritis as confirmed by the previous reports (Mazzariol and Bazaj,2017; Flores-Mireles et al.,2015; Gupta et al., 2011). However, the overall percentage of occurrence (60%) of the Gram-negative bacteria observed in this study was very high contrary to many reports such as the reports of Onu et al., 2015(24.7%) in Abakaliki, Nigeria, Labi et al., 2015(5.5%) in a teaching hospital in Ghana, Bizuwork et al., 2021 (15.7%), Tadesse et al., 2014 (18.8%) , Tadesse et al., 2018 (21.2%), and Tadesse et al., 2007(9.8%), all from Ethiopia. The difference of bacterial occurrence from place to place could be correlated to several factors such as manner of urine samples collection, socio-economic status and genital hygiene practices of the pregnant women (Bizuwork et al., 2021; Fatima and Ishrat, 2006; Enayat et al., 2008).

In vitro Antibacterial Activity of *A. sativum* Extract

Table 3 shows the inhibitory effects of the garlic extract on the Gram-negative bacteria. The overall growth zone diameters of the

DOI: 10.56892/bima.v7i3.490

inhibitory effect showed that *E. aerogenes* was the most sensitive to the extract followed by *K. pneumoniae, P. mirabilis, C. fruendii* and then *E. coli.* The antibacterial evaluation of the garlic extract against the isolated bacteria demonstrated remarkable effects at all concentrations (100-400mg/mL) against *E. aerogenes, K. pneumoniae, P. mirabilis, C. freundii* and *E. coli*.

Table 3: Sensitivity of Gram-negative bacteria to methanolic extract of *A. sativum*

Organisms	Concentration (mg/mL)/zones of inhibition (mm)				
	400	**200**	**100**	**50**	**0**
E. coli	20	16	13	9	0
P. mirabilis	22	19	15	12	0
K.pneumoniae	22	16	16	13	0
E. aerogenes	23	20	17	13	0
C. freundii	21	19	16	9	0

Key: mg= milligram, mL= millitre, mm= millimetre, Co=Control.

The observed extract activity against the bacteria was found to increase with every increase in concentration. Saponins, tannins, and flavonoid were detected in this study as already confirmed to be amongst the key phytochemical constituents responsible for antibacterial activity of Garlic (Gibbons, 2003). Moreover, the phytochemicals detected are similar to those reported by Lionel et al., (2020), except glycosides. However, despite the extractability difference of the extraction solvents and peculiarity of the test bacterial organisms, the antibacterial activity results demonstrated by Lionel et al. (2020) showed garlic extracts to be active against *E. coli* and Proteus spp. as observed in this study.

CONCLUSION

The garlic methanol extract tested against the Gram-negative bacteria isolated from the urine samples of pregnant women showed a remarkable *in vitro* inhibitory effect, which support the plant's traditional medicinal claims. Qualitative phytochemical screening of the garlic extract revealed the presence of saponins, tannins, flavonoid, and steroid.

Acknowledgements

The authors acknowledge the technical support of Laboratory technologists of Microbiology Department, Gombe State University.

REFERENCES

Alkhyat, S. H., & Al-Maqtari, M. A. (2014). Prevalence of microorganisms isolates from urinary tract infections at some hospitals in Sana'a city, Yemen. *International Journal of Current Microbiology and Applied Sciences*, *3*(6), 876-885.

Alyasari, H. F., Al-khafaji, J. K., & Al-Masoudi, H. K. (2018). Inhibitory effects of garlic extract on uropathogenic escherichia coli; proteus mirabilis and trichomonas vaginalis isolated from urogenital tract cases. *Research Journal of Pharmacy and Technology*, *11*(3), 1071-1077.

Angami, S., Jamir, N., Sarma, P. C., & Deka, A. C. (2015). Urinary tract infection, its causative microorganism and antibiotic susceptibility in Nagaland. *Archives of Medicine and Health Sciences*, *3*(1), 40-43.

Behzadi, P. (2020). Classical chaperone-usher (CU) adhesive fimbriome: uropathogenic Escherichia coli (UPEC) and urinary tract infections (UTIs). *Folia microbiologica*, *65*(1), 45-65.

Bizuwork, K., Alemayehu, H., Medhin, G., Amogne, W., & Eguale, T. (2021). Asymptomatic bacteriuria among pregnant women in Addis Ababa, Ethiopia: prevalence, causal agents, and their antimicrobial susceptibility. *International Journal of Microbiology*, *2021*.

Cheesbrough M(2018).District Laboratory Practice in Tropical Countries. Internat'nal J. of Medic Sci., 1(1),65-68.

Duffy, L., De Wilde, L. O., Spellman, K., Dunlap, K., Dainowski, B.,

DOI: 10.56892/bima.v7i3.490

McCullough, S., ... & van Muelken, M. Resilience and Adaptation: Yukon River Watershed Contaminant Risk Indicators. *Scientifica*, *2018*.

Enayat, K., Fariba, F., & Bahram, N. (2008). Asymptomatic bacteriuria among pregnant women referred to outpatient clinics in Sanandaj, Iran. *International braz j urol*, *34*, 699-707.

Ezeigbo, O. R., Nnadozie, R. I. A., Asuoha-Chuks, N., Ojiako, V. U., Awurum, I. N., & Ugochukwu, M. G. (2016). Incidence of urinary tract infection (UTI) among pregnant women attending antenatal clinics at some selected hospitals in Aba, southeastern Nigeria. *Int. J. Curr. Microbiol. App. Sci*, *5*(1), 193-199.

Fatima, N., & Ishrat, S. (2006). Frequency and risk factors of asymptomatic bacteriuria during pregnancy. *Journal of the College of Physicians and Surgeons--pakistan: JCPSP*, *16*(4), 273-275.

Fazly Bazzaz, B. S., Darvishi Fork, S., Ahmadi, R., & Khameneh, B. (2021). Deep insights into urinary tract infections and effective natural remedies. *African Journal of Urology*, *27*(1), 1-13.

Flores-Mireles, A. L., Walker, J. N., Caparon, M., & Hultgren, S. J. (2015). Urinary tract infections: epidemiology, mechanisms of infection and treatment options. *Nature reviews microbiology*, *13*(5), 269-284.

Garba, L., Abba, H., Adamu, Z. Y., Muhammed, I., Adamu, M. T., Aisami, A., ... & Tahir, F. (2021). Isolation and Evaluation of In vitro Antibacterial Potential of Endophytic Fungi from the leaves of Psidium guajava (Guava). *Bayero Journal of Pure and Applied Sciences*, *13*(1), 20-24.

Garba, L., Lawan, H. S., Puma, H. U., Abdullahi, M. M., Yusuf, I., & Mukhtar, M. D. (2019). Phytochemical Screening and in vitro Bacteriostatic Effects of Syzigium aromaticum (Clove) Extracts on Clinical Bacterial Isolates. *Journal of Biochemistry, Microbiology and Biotechnology*, *7*(1), 5-9.

Garba, L., Muhammad, B., Adamu, M. T., Isa, S., Abdullahi, M. M., & Yarma, A. A. (2021). Potential in vitro inhibitory effects of Moringa oleifera leaf extracts on extended-spectrum β-lactamase-producing bacteria. *Journal of Fundamental and Applied Sciences*, *13*(1), 137-150.

Garnizov, T. M. (2016). Asymptomatic bacteriuria in pregnancy from the perspective of public health and maternal health care: review and case report. *Biotechnology & Biotechnological Equipment*, *30*(3), 443-447.

Gibbons, S. (2003). An overview of plant extracts as potential therapeutics. *Expert Opinion on Therapeutic Patents*, *13*(4), 489-497.

Glover, E. K., & Sheerin, N. S. (2023). Urinary tract infection. *Medicine*, 47(9),546-550. doi.org/10.1016/j.mpmed.2019.06.008.

Gupta K., Hooton T.M., Naber K.G., Wullt B., Colgan R., Miller L.G., and Soper, D. E(2011). Infectious Diseases Society of America European Society for Microbiology and Infectious Diseases. International clinical practice guidelines for the treatment of acute uncomplicated cystitis and pyelonephritis in women: A 2010 update by the Infectious Diseases Society of America and the European Society for Microbiology and Infectious Diseases. Clin. Infect. Dis. 52(5), 103-120. doi.org/10.1093/acid/ciq257.

Jauro, A. H., Shuâ, I., Lawan, G., Adamu, M. T., Iliyasu, M. Y., & Umar, A. F. (2021). Inhibitory Effect of Sodium Citrate, Sodium Nitrite and Cinnamaldehyde on Biofilm-forming

DOI: 10.56892/bima.v7i3.490

Escherichia coli O157: H7. *Journal of Environmental Bioremediation and Toxicology*, *4*(2), 11-16.

Kasper, D. L., Fauci, A. S., Hauser, S. L., Longo, D. L., Jameson, J. L., & Loscalzo, J. (2018). Urinary tract infections, pyelonephritis, and prostatitis. *Harrison's principles of internal medicine, 20th edn. McGraw-Hill Education, New York.*

Labi, A. K., Yawson, A. E., Ganyaglo, G. Y., & Newman, M. J. (2015). Prevalence and associated risk factors of asymptomatic bacteriuria in ante-natal clients in a large teaching hospital in Ghana. *Ghana medical journal*, *49*(3), 154-158.

Lionel, O. O., Adegboyega, I. P., Ezekiel, A. O., & Olufunke, B. C. (2020). Antimicrobial activity of garlic (Allium sativum) on selected uropathogens from cases of urinary tract infection. *Annals of Tropical Pathology*, *11*(2), 133.

Matuszkiewicz-Rowińska, J., Małyszko, J., & Wieliczko, M. (2015). State of the art paper Urinary tract infections in pregnancy: old and new unresolved diagnostic and therapeutic problems. *Archives of Medical Science*, *11*(1), 67-77.

Mazzariol, A., Bazaj, A., & Cornaglia, G. (2017). Multi-drug-resistant Gram-negative bacteria causing urinary tract infections: a review. *Journal of Chemotherapy*, *29*(sup1), 2-9.

Noor, A. F., Shams, F., Munshi, S. K., Hassan, M., & Noor, R. (2013). Prevalence and antibiogram profile of uropathogens isolated from hospital and community patients with urinary tract infections in Dhaka City. *Journal of Bangladesh academy of sciences*, *37*(1), 57-63.

Onu, F. A., Ajah, L. O., Ezeonu, P. O., Umeora, O. U. J., Ibekwe, P. C., & Ajah, M. I. (2015). Profile and microbiological isolates of asymptomatic bacteriuria among pregnant women in Abakaliki, Nigeria. *Infection and drug resistance*, 231-235.

Piranfar, V., Mirnejad, R., & Erfani, M. (2016). Incidence and antibiotic susceptibility pattern of most common bacterial pathogen causing urinary tract infection (UTI) in Tehran, Iran, 2012-2013. *International Journal of Enteric Pathogens*, *2*(1), 2-15490.

Rosana, Y., Ocviyanti, D., Halim, M., Harlinda, F. Y., Amran, R., Akbar, W., ... & Akhmad, S. R. P. (2020). Urinary tract infections among Indonesian pregnant women and its susceptibility pattern. *Infectious Diseases in Obstetrics and Gynecology*, *2020*, 1-7.

Santoso, B. I., Surya, R., Yasmin, F. A., & Irwinda, R. (2018). The awareness of urinary tract infection management in pregnant women. A qualitative study. *Majalah Obstetri & Ginekologi*, *25*(3), 92-96.

Tadesse, A., Negash, M., & Ketema, L. S. (2007). Asymtomatic bacteriuria in pregnancy: assesment of prevlence, microbial agents and ther antimicrobial sensitivty pattern in Gondar Teaching Hospital, north west Ethiopia. *Ethiopian Medical Journal*, *45*(2), 143-149.

Tadesse, E., Teshome, M., Merid, Y., Kibret, B., & Shimelis, T. (2014). Asymptomatic urinary tract infection among pregnant women attending the antenatal clinic of Hawassa Referral Hospital, Southern Ethiopia. *BMC research notes*, *7*(1), 1-5.

Tajbakhsh, E., Tajbakhsh, S., & Khamesipour, F. (2015). Isolation and molecular detection of Gram negative bacteria causing urinary tract infection in patients referred to Shahrekord hospitals, Iran. *Iranian Red Crescent Medical Journal*, *17*(5).

Wolde T., Kuma H., Trueha K., and Yabeker

DOI: 10.56892/bima.v7i3.490

A (2018). Anti-bacterial activity of garlic extract against human pathogenic bacteria. Journal of Pharmacy, 6(253), 2-8. doi.org/10.4172/2329-6887.1000253.

Yusha'u, M., Garba, L., & Shamsuddeen, U. (2021). In vitro inhibitory activity of garlic and ginger extracts on some respiratory tract isolates of gram-negative organisms. *International Journal of Biomedical and Health Sciences*, *4*(2).