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Antibacterial Activity of Ethanolic and Aqueous Extracts of *Allium Sativum* (Garlic) on Selected Ocular Bacterial Isolates

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ABSTRACT

Background: *Allium sativum*, commonly known as "garlic", is a widely used plant with a rich history of medicinal applications across various cultures. This study assessed the antibacterial activity of the ethanolic and aqueous extracts of *Allium sativum* on selected ocular bacterial isolates.

Materials and Methods: This study was a prospective laboratory study whereby ocular bacteria swabs samples from infected patients were taken to the Microbiology Laboratory for culturing and identification of microorganisms. Ethanolic and aqueous extracts of *Allium sativum*, and ciprofloxacin which served as a control were prepared at various concentrations and their antibacterial activity was tested against the selected bacteria which include *Staphylococcus aureus*, *Streptococcus viridans*, *Escherichia coli*, and *Pseudomonas aeruginosa*.

Results: The ethanolic extract of *Allium sativum* produced zones of inhibition for all bacteria isolated at concentrations of 100, 50, and 25 mg/ml. No zones of inhibition were observed at 12.5 mg/ml and 6.25 mg/ml. The aqueous extract of *Allium sativum* produced zones of inhibition at concentrations of 100 mg/ml and 50 mg/ml against all the bacteria isolated. No zones of inhibition were observed at concentrations of 25, 12.5, and 6.25 mg/ml. Ciprofloxacin, which served as a control, produced zones of inhibition at all concentrations against the selected ocular bacterial isolates and showed a significantly higher mean zone of inhibition ($P < 0.05$) than the ethanolic and aqueous extracts of *Allium sativum*.

Conclusion: *Allium sativum* showed antibacterial activity against the selected ocular bacterial isolates, highlighting its potential as an alternative ophthalmic medication for the treatment of bacterial infections.

Keywords: *Allium sativum*, Ciprofloxacin, Ethanolic extract, Aqueous extract, Zone of Inhibition, Bacteria

How to cite this article

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INTRODUCTION

Allium sativum, commonly known as "garlic", is an angiosperm classified under the order Asparagales, and belongs to the Amaryllidaceae family under the genus Allium.[1] Its close relatives include the onion, shallot, leek, chive, Welsh onion and Chinese onion. It is widely cultivated for both its culinary and medicinal applications with a history of several thousand years of human consumption and use.[2] Originally native to Central Asia, garlic is now grown globally in temperate regions. Garlic is a herbaceous plant, typically reaching a height of around 60 cm. The leaf blade is flat, linear, solid, and approximately wide, with an acute apex.[3] The bulb is odoriferous and contains outer layers of thin sheathing leaves surrounding an inner sheath that encloses the clove. Often the bulb contains 10 to 20 cloves that are asymmetric in shape, except for those closest to the center. It is pollinated by bees, butterflies, moths, and other insects.[4] The pungent odor and flavor of garlic come from sulfur-containing compounds, particularly allicin. These compounds exhibit antimicrobial, antioxidant, and anti-inflammatory properties.[5] A study[6] on its pharmacological properties revealed that garlic has significant antibacterial, antifungal, antiviral, and heart disease activity. It is known to be hepatoprotective, antihelmintic, anti-inflammatory, and an antioxidant.[7] In traditional medicine, garlic has been used for treating conditions such as infections, high blood pressure, and cardiovascular diseases.[7] It is known for its ability to reduce cholesterol levels and blood pressure, and is rich in essential nutrients such as vitamins C, B6, manganese, and selenium.[8] Modern research supports its role in promoting a good heart health, combating oxidative stress, and providing various therapeutic benefits.[2]

Allium sativum is a widely used plant with a rich history of traditional and medicinal applications across various cultures. Its uses span from culinary enhancements to therapeutic interventions, showcasing its versatility and significance in traditional medicine. In ancient civilizations, *Allium sativum* was used extensively not only as a culinary ingredient but also as a therapeutic agent. Historical records indicate that in ancient Egypt, garlic was valued for its potential to enhance physical strength and endurance.[9] It was commonly used to treat infections and digestive issues, showcasing its early application in health management. Similarly, in ancient Greece, garlic was noted for its health benefits, including its use to support overall vitality and longevity.[9] *Allium sativum* (Garlic) contains several biologically active compounds including allicin, sulfides (diallyl sulfide, diallyl disulfide, and diallyl trisulfide), flavonoids, phenolic compounds, and saponins.[10] The nutritional composition of garlic per 100 g, edible portion, is: water 65 - 70 g, energy 149 kJ (35 kcal), protein 1.8 g, fat 0.5 g, carbohydrate 33 g, fiber 2.1 g, Ca 181 mg, P 34 mg, Fe 1.7 mg, and ascorbic acid 31 mg.[11] This composition aligns with other vegetables in its class. Some of these compounds have notable health benefits. For instance, allicin exhibits significant antimicrobial and anti-inflammatory activities[12] while sulfides have shown anticancer properties.[13] Allicin has also demonstrated broad-spectrum activity against a variety of pathogens, including bacteria, viruses, and fungi.[14] Garlic's flavonoids and phenolic compounds contribute to its antioxidant activity.[15] Thus, garlic is widely recognized for its diverse medicinal applications. The antimicrobial effects of garlic are utilized in both clinical and natural medicine to combat infections and support immune function.

Bacterial conjunctivitis is a contagious eye infection which presents with redness, discharge, swelling, tearing, and irritation.[16] The discharge can be mucopurulent or just purulent, consisting of cellular (leukocytes, bacteria, epithelial cells) and non-cellular (fibrin, protein, mucus) material.[17] Common pathogenic organisms causing bacterial conjunctivitis include *Staphylococcus aureus, Haemophilus influenza, Streptococcus viridans, Neisseria gonorrhea, Neisseria meningitides, Escherichia coli*, *Pseudomonas aeruginosa* and *Corynebacterium diphtheria*.[18] It is usually treated with antibiotic eye drops or ointment. The problem of antibiotic resistance is a concern in global healthcare, significantly impacting the management of bacterial infections. This problem is also present in the field of eye care, where antibiotic-resistant strains complicate the treatment of ocular infections such as conjunctivitis.[19] In light of the growing resistance to antibiotics and the limitations of existing treatments, there is an interest in exploring herbal and plant-based remedies. Traditional medicinal plants have been utilized across various cultures for their therapeutic properties, including antimicrobial effects.[20] Investigating the antimicrobial properties of these plant-based remedies could provide valuable insights into their potential as alternative treatments. This study aimed to assess the antibacterial activity of ethanolic and aqueous extracts of *Allium sativum* (garlic) against selected ocular bacterial isolates.

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MATERIALS AND METHODS

Collection of Plant Material: Fresh cloves of garlic were purchased from a local market and were identified and authenticated by Prof. Mrs. C.P. Anyanwu, a botanist at the Department of Crop Science and Technology, Federal University of Technology, Owerri. A voucher number (FUTO/001/597/2024) was assigned to it. The fresh seeds of the garlic were taken to the Department of Microbiology Laboratory, Federal University of Technology Owerri, for processing. The seeds were washed using distilled water, weighed, sliced and oven dried at 50 °C. The dried sample was then blended to powder under laboratory conditions using a sterile electric blender, and it was sieved to uniform particle size using 1 mm mesh sieve, and was weighed and stored in an air tight sterilized container to avoid contamination until time of extraction.[21]

Ethanol Extraction of Allium sativum: The ethanolic extraction of *Allium sativum* was performed using the soxhlet extraction method.[22] To obtain the ethanolic extract, three hundred milliliters (300 ml) of 95% ethanol was poured into a soxhlet flask and 100 grams of the Garlic (*Allium sativum*) was packed into the extractor. The soxhlet apparatus was mounted on a heating mantle set at the boiling point of the solvent (78 °C). When the solvent was boiling, the vapor evaporated through the extractor arm into the extraction chamber, while the condenser at the top condensed the vapor. The liquid condensate dripped into the center which contained the *Allium sativum* sample to be extracted. The extract filled the siphon tube, where it flowed back down into the soxhlet flask. This was allowed to continue to circulate until the extraction was completed. It was then removed from the tube, and the ethanol was evaporated to remain only the *Allium sativum* extract in the soxhlet flask.

Aqueous Extraction of Allium sativum: The aqueous extraction of *Allium sativum* was performed using the cold maceration process.[23] To obtain the aqueous extract, thirty (30) grams of *Allium sativum* was weighed and added into a sterile 250 ml conical flask and 100 ml of distilled water was poured into the garlic, mixed thoroughly, corked, and allowed to soak for 24 hours at a temperature of 4 °C. The mixture was filtered after 24 hours using sterile filter cloth and a hot air oven was used for evaporation at 60 °C, leaving only the *Allium sativum* extract.

Collection and Transportation of Bacterial Swabs: Twenty-Four bacterial swab samples were collected from 24 patients presenting with signs and symptoms of bacterial conjunctivitis at the Department of Optometry Eye Clinic, Federal University of Technology, Owerri, Nigeria. Ethical approval for the study was obtained from the Ethics Committee, School of Health Technology, Federal University of Technology, Owerri, Nigeria, with reference number FUT/SOHT/C1/065 dated October 17, 2024. All the participants provided informed consent to be part of the study and participation in the study was voluntary. Upon ocular examination of the external ocular tissues using a penlight, swab samples were gently obtained from the inferior conjunctival sac with a sterile swab stick from patients who presented with red eyes and purulent conjunctival discharges. The swab samples were transported to the Microbiology Department Laboratory at Federal University of Technology, Owerri for culturing and identification of microorganisms using the Amies transport medium.[24]

Identification of Bacteria Isolates: The media preparation was done according to the manufacturer's specification[21] prior to collection of swabs. The swab samples underwent inoculation on Nutrient agar, Blood agar, Mac-Conkey agar, and Chocolate agar plates to isolate and grow individual bacterial colonies from the mixed microbial population. The bottom of the agar plate was labelled with relevant information, including sample identification number, specimen source, and date. Starting at one edge of the agar plate, the swab was streaked over the surface of the agar in a zigzag motion while rotating the plate slightly. After completing the first streak, without touching the initial streak, a second quadrant of the plate was streaked by spreading the bacteria from the first streak into the second quadrant. The streaking process was repeated for the third and fourth quadrants. The agar plate lid was closed immediately after streaking to prevent contamination. The inoculated agar plate was then placed in an incubator for 24 hours at 37°C. Bacterial isolates were identified based on standard biochemical characteristics, employing both microscopic and macroscopic analyses.[25] Gram staining and motility tests were conducted. For gram-negative identification, biochemical tests such as indole, citrate, oxidase, catalase, H_2S production, lysine decarboxylase, lactose fermentation, urea hydrolysis, and gas production were employed. Gram-positive bacteria were identified using catalase, coagulase tests, and observing haemolysis patterns on blood agar. The sterility of culture media was verified by incubating 3–5% of the batch at 37 °C

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overnight and observing for bacterial growth. The media that produced growth were examined for color, shape, elevation and pattern of growth.

Preparation and Standardization of Test Microorganisms: The test organisms that were employed in this study were bacterial isolates of *Staphylococcus aureus, Streptococcus viridans, Escherichia coli* and *Pseudomonas aeruginosa*. The micro-organisms obtained from the conjunctival swabs were identified and cultured at the laboratory. They were inoculated in the petri-dishes using sterile wire loop and the spreading method to enable them grow in the nutrient agar at 37 °C for 24 hours. The test organisms were then picked up by a sterile loop from the culture and transferred and suspended into a tube containing sterile normal saline. This was then placed in an incubator for 5-10 minutes until it achieved turbidity. Turbidity was reached when the test organism reached McFarland 0.5 turbidity standard. A McFarland standard is taken as a reference to adjust the turbidity of bacterial suspensions as bacterial suspensions could cause potential bias in the result if they vary in turbidity. Turbidity implies the presence of the organism.

Test for Antibacterial Activity of Allium sativum Extracts: The modified agar well diffusion method[26] was performed to determine the antimicrobial activities for ethanolic and aqueous leaves extracts. A stock solution of extract was prepared by dissolving 10 grams of the extract in 100 ml of their respective solvents (distilled water and ethanol) to produce a concentration of 100 mg/ml. The stock solution was then prepared at concentrations of 50 mg/ml, 25 mg/ml, 12.5 mg/ml and 6.25mg/ml by dissolving 10 grams of the extract in 200 ml, 400 ml, 800 ml and 1600 ml of the solvent respectively. Sterile paper discs were impregnated with *Allium sativum* extracts (ethanol and aqueous) at varying concentrations and placed onto the agar plates inoculated with bacterial cultures using sterile forceps. The set up was incubated aerobically at 37 °C for 24 hours. At the end of the incubation period, inhibition zones formed on the agar were measured in millimeter (mm) using a ruler and the results were recorded.

Antibiotic Sensitivity Testing: Ciprofloxacin 500 mg tablets served as a control in this study and were purchased from a reputable pharmacy. The samples were diluted in distilled water to prepare different concentrations. The 500 mg sample was dissolved in 5 ml distilled water to produce 100 mg/ml. To produce other concentrations of 50, 25, 12.5, and 6.25 mg/ml, the 500 mg was dissolved in 10, 20, 40, and 80 ml of distilled water, respectively. Antibiotic sensitivity testing was performed using the disc-diffusion method.[26] By plating out, the test organism was seeded onto Mueller-Hinton agar. The sterile paper discs were impregnated with different concentrations of ciprofloxacin. The antibiotic sensitivity disc was then placed on agar plates inoculated with bacterial cultures using sterile forceps. The setup was incubated under aerobic conditions at 37 °C for 24 hours. The zones of inhibition diameters were measured in millimetres (mm) using a meter rule after 24 hours of incubation, and the results were recorded.

***Minimum Inhibitory Concentration (MIC) Determination*:** The Minimum Inhibitory Concentration (MIC) is the lowest concentration of an antimicrobial agent required to inhibit visible growth of a microorganism. This was assessed using broth dilution, with clear endpoints indicating microbial inhibition.[25] To determine the MIC, increasing concentrations (5% - 100% with 5% intervals) of each extract were prepared in 9 ml tubes of sterile nutrient broth. Exactly 100 µl of each standardized test organism was then introduced into each tube of extract. A tube containing only nutrient broth and bacteria without extract served as negative control while another tube containing just the extract and broth without bacteria served as positive control. Each tube was incubated for 18 hours and then examined for visible growth or turbidity. The concentration of the extract in the tube in which no visible growth was observed when compared with the controls was taken as the MIC.

Minimum Bactericidal Concentration (MBC) Determination: The Minimum Bactericidal Concentration **(**MBC) refers to the lowest concentration of an antimicrobial that kills 99.9% of the initial bacterial population. MBC was determined by sub culturing samples from tubes showing no visible growth in the MIC test onto a growth medium without the antimicrobial agent. The absence of bacterial colonies confirms the bactericidal endpoint.[26] To determine the MBC for each extract, samples from the test tubes used in MIC test that showed no visible growth after the period of incubation were inoculated on sterile nutrient agar plates (which had no antimicrobial incorporated) in them using sterile swab sticks. The plates were incubated at 37 ^{o}C for 18-24 hours and were then observed for growth. The concentration at which absence of growth was observed (bactericidal activity) was taken as the MBC.

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Statistical Analysis: The data collected from this study were uploaded to the Statistical Package for Social Sciences (SPSS) version 23 software for analysis. Analysis of Variance (ANOVA) was used to compare the antibacterial activities of the ethanolic and aqueous extracts of *Allium sativum* and ciprofloxacin, at a significant level of 0.05.

RESULTS

Four bacterial isolates including *Staphylococcus aureus*, *Streptococcus viridans*, *Escherichia coli*, and *Pseudomonas aeruginosa* were selected for this study. Table 1 shows the distribution of the mean zones of inhibition of the ethanolic and aqueous extracts of *Allium sativum*, and Ciprofloxacin on the bacterial isolates. Ciprofloxacin was used as the control. The zones of inhibition of ethanolic and aqueous extracts of *Allium sativum*, and Ciprofloxacin on the selected bacterial isolates were measured at concentrations of 100 mg/ml, 50 mg/ml, 25 mg/ml, 12.5 mg/ml and 6.25mg/ml. The ethanolic extract of *Allium sativum* produced zones of inhibition against all the bacteria isolated at 100 mg/ml, 50 mg/ml and 25 mg/ml concentrations. Against *S. aureus*, there was a mean (± standard error mean) zone of inhibition of 19.45 ± 0.42 mm with 100 mg/ml concentration, 11.85 ± 0.60 mm with 50 mg/ml, and 3.85 ± 0.57 mm with 25 mg/ml. There was no zone of inhibition with 12.5 mg/ml and 6.25 mg/ml concentrations. Against *S. viridans*, the mean (± standard error mean) zone of inhibition was 18.13 ± 0.85 mm with 100 mg/ml concentration, 10.50 ± 0.25 mm with 50 mg/ml, and 2.58 ± 0.43 mm with 25 mg/ml. There was no zone of inhibition with 12.5 mg/ml and 6.25 mg/ml concentrations. Against *E. coli*, the mean (± standard error mean) zone of inhibition was 17.56 ± 0.52 mm with 100 mg/ml concentration, 9.33 ± 0.64 mm with 50 mg/ml, and 0.78 ± 0.13 mm with 25 mg/ml. There was no zone of inhibition with 12.5 mg/ml and 6.25 mg/ml concentrations. Against *P. aeruginosa*, the mean (± standard error mean) zone of inhibition was 16.66 ± 0.30 mm with 100 mg/ml concentration, 8.63 ± 0.26 mm with 50 mg/ml, and 0.83 ± 0.13 mm with 25 mg/ml. There was no zone of inhibition with 12.5 mg/ml and 6.25 mg/ml concentrations. The aqueous extract of *Allium sativum* produced zones of inhibition at higher concentrations of 100 mg/ml and 50 mg/ml with all the bacteria isolated. They were however lower in diameter when compared to the ethanolic extract of *Allium sativum*. Against *S. aureus*, there was a mean zone of inhibition of 8.35 ± 0.79 mm with 100 mg/ml concentration, and 1.48 ± 0.43 mm with 50 mg/ml. There was no zone of inhibition with 25 mg/ml, 12.5 mg/ml and 6.25 mg/ml concentrations. Against *S. viridans*, the mean zone of inhibition was 7.60 ± 0.49 mm with 100 mg/ml concentration, and 1.28 ± 0.25 mm with 50 mg/ml. There was no zone of inhibition with 25 mg/ml, 12.5 mg/ml and 6.25 mg/ml concentrations. Against *E. coli*, the mean zone of inhibition was 6.88 ± 0.32 mm with 100 mg/ml concentration, and 1.13 ± 0.10 mm with 50 mg/ml. There was no zone of inhibition with 25 mg/ml, 12.5 mg/ml and 6.25 mg/ml concentrations. Against *P. aeruginosa*, the mean zone of inhibition was 4.76 ± 0.29 mm with 100 mg/ml concentration, and 0.79 ± 0.29 mm with 50 mg/ml. There was no zone of inhibition with 25 mg/ml, 12.5 mg/ml and 6.25 mg/ml concentrations. With Ciprofloxacin which served as a control, the mean zone of inhibition against *S. aureus* was 28.75 ± 0.96 mm with 100 mg/ml concentration, 18.03 ± 0.68 mm with 50 mg/ml, 9.55 ± 0.44 mm with 25 mg/ml, 2.10 ± 0.12 mm with 12.5 mg/ml, and 0.83 ± 0.21 mm with 6.25 mg/ml. Against *S. viridans*, the mean zone of inhibition was 24.15 ± 0.19 mm with 100 mg/ml concentration, 16.63 ± 0.43 mm with 50 mg/ml, 7.63 ± 0.43 mm with 25 mg/ml, 1.70 ± 0.24 mm with 12.5 mg/ml, and 0.60 ± 0.08 mm with 6.25 mg/ml. Against *E. coli*, the mean zone of inhibition was 27.33 ± 0.22 mm with 100 mg/ml concentration, 15.33 ± 0.38 mm with 50 mg/ml, 8.63 ± 0.17 mm with 25 mg/ml, 2.23 ± 0.21 mm with 12.5 mg/ml, and 0.33 ± 0.17 mm with 6.25 mg/ml. Against *P. aeruginosa*, the mean zone of inhibition was 25.71 ± 0.47 mm with 100 mg/ml concentration, 17.53 ± 0.41 mm with 50 mg/ml, 8.45 ± 0.31 mm with 25 mg/ml, 1.94 ± 0.14 mm with 12.5 mg/ml, and 0.32 ± 0.09 mm with 6.25 mg/ml. Figures 1 to 4 show a comparison of the mean zones of inhibition of ethanol and aqueous extracts of *Allium sativum* and ciprofloxacin on the bacterial isolates. The standard errors of the mean values are indicated by the error bars. Statistical comparison of the mean zones of inhibition of ethanol and aqueous extracts of *Allium sativum* and ciprofloxacin on the selected bacterial isolates was performed using one-way ANOVA at a significance level of 0.05. Among all the bacterial isolates, ciprofloxacin showed a significantly higher mean zone of inhibition [p (0.00) <0.05] than the ethanolic and aqueous extracts of *Allium sativum*. Post-hoc analyses with Tukey's test showed that the ethanolic extract of *Allium sativum* showed significantly higher

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mean zones of inhibition [p (0.00) <0.05] than the aqueous extracts of *Allium sativum* at 100, 50, and 25 mg/ml. At lower concentrations of 12.5 mg/ml and 6.25 mg/ml, there was no difference [p(1.00)>0.05] between the ethanolic and aqueous extracts of *Allium sativum*.

Table 1: Distribution of Mean Zones of Inhibition (mm) of *Allium sativum* and Ciprofloxacin on selected bacterial isolates

Bacteria isolates	***Allium sativum* Ethanolic extract**									
	100 mg/ml		**50 mg/ml**		**25 mg/ml**		**12.5 mg/ml**		**6.25 mg/ml**	
	n	**Mean ± SE (mm)**	**n**	**Mean ± SE (mm)**	**n**	**Mean ± SE (mm)**	**n**	**Mean ± SE (mm)**	**n**	**Mean ± SE (mm)**
Staphylococcus aureus	23	19.45 ± 0.42[a]	23	11.85 ± 0.60[a]	23	3.85 ± 0.57[a]	23	0.00 ± 0.00[b]	23	0.00 ± 0.00[b]
Streptococcus viridans	22	18.13 ± 0.85[a]	22	10.50 ± 0.25[a]	22	2.58 ± 0.43[a]	22	0.00 ± 0.00[b]	22	0.00 ± 0.00[b]
Escherichia coli	21	17.56 ± 0.52[a]	21	9.33 ± 0.64[a]	21	0.78 ± 0.13[a]	21	0.00 ± 0.00[b]	21	0.00 ± 0.00[b]
Pseudomonas aeruginosa	21	16.66 ± 0.30[a]	21	8.63 ± 0.26[a]	21	0.83 ± 0.13[a]	21	0.00 ± 0.00[b]	21	0.00 ± 0.00[b]
	***Allium sativum* Aqueous extract**									
	100 mg/ml		**50 mg/ml**		**25 mg/ml**		**12.5 mg/ml**		**6.25 mg/ml**	
	n	**Mean ± SE (mm)**	**n**	**Mean ± SE (mm)**	**n**	**Mean ± SE (mm)**	**n**	**Mean ± SE (mm)**	**n**	**Mean ± SE (mm)**
Staphylococcus aureus	23	8.35 ± 0.79[a]	23	1.48 ± 0.43[a]	23	0.00 ± 0.00[b]	23	0.00 ± 0.00[b]	23	0.00 ± 0.00[b]
Streptococcus viridans	22	7.60 ± 0.49[a]	22	1.28 ± 0.25[a]	22	0.00 ± 0.00[b]	22	0.00 ± 0.00[b]	22	0.00 ± 0.00[b]
Escherichia coli	21	6.88 ± 0.32[a]	21	1.13 ± 0.10[a]	21	0.00 ± 0.00[b]	21	0.00 ± 0.00[b]	21	0.00 ± 0.00[b]
Pseudomonas aeruginosa	21	4.76 ± 0.29[a]	21	0.79 ± 0.29[a]	21	0.00 ± 0.00[b]	21	0.00 ± 0.00[b]	21	0.00 ± 0.00[b]
	Ciprofloxacin (Control)									
	100 mg/ml		**50 mg/ml**		**25 mg/ml**		**12.5 mg/ml**		**6.25 mg/ml**	
	n	**Mean ± SE (mm)**	**n**	**Mean ± SE (mm)**	**n**	**Mean ± SE (mm)**	**n**	**Mean ± SE (mm)**	**n**	**Mean ± SE (mm)**
Staphylococcus aureus	23	28.75 ± 0.96[a]	23	18.03 ± 0.68[a]	23	9.55 ± 0.44[a]	23	2.10 ± 0.12[a]	23	0.83 ± 0.21[a]
Streptococcus viridans	22	24.15 ± 0.19[a]	22	16.63 ± 0.43[a]	22	7.63 ± 0.43[a]	22	1.70 ± 0.24[a]	22	0.60 ± 0.08[a]
Escherichia coli	21	27.33 ± 0.22[a]	21	15.33 ± 0.38[a]	21	8.63 ± 0.17[a]	21	2.23 ± 0.21[a]	21	0.33 ± 0.17[a]
Pseudomonas aeruginosa	21	25.71 ± 0.47[a]	21	17.53 ± 0.41[a]	21	8.45 ± 0.31[a]	21	1.94 ± 0.14[a]	21	0.32 ± 0.09[a]

*SE: Standard Error of Mean; a = Values for corresponding rows in indicate a significant difference (p < 0.05); b = Values for corresponding rows indicate no significant difference (p > 0.05)

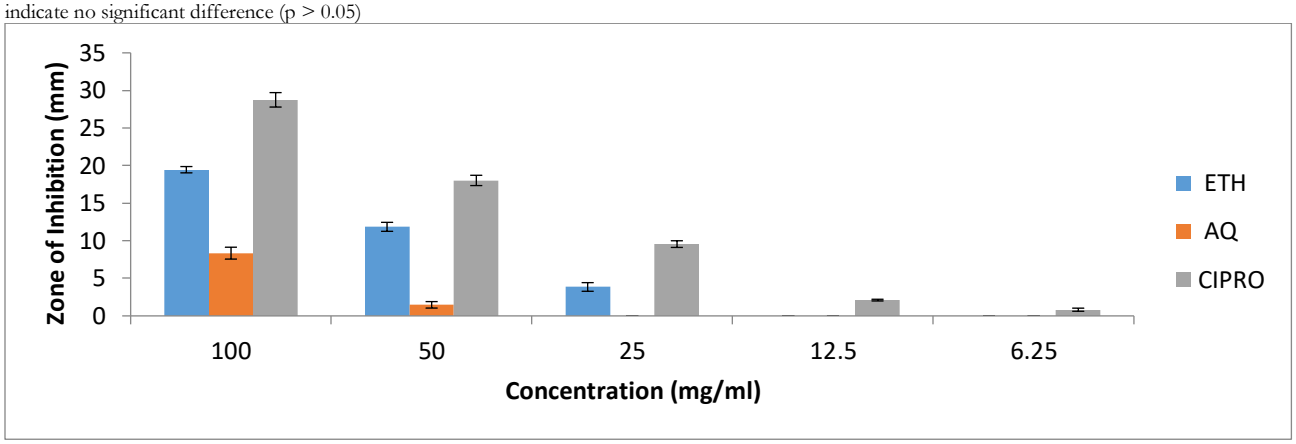

Figure 1: Comparison of mean zones of inhibition of Ethanol and Aqueous extracts of *Allium sativum* and Ciprofloxacin on *Staphylococcus aureus*

ETH: Ethanol extracts; AQ: Aqueous extracts; CIPRO: Ciprofloxacin

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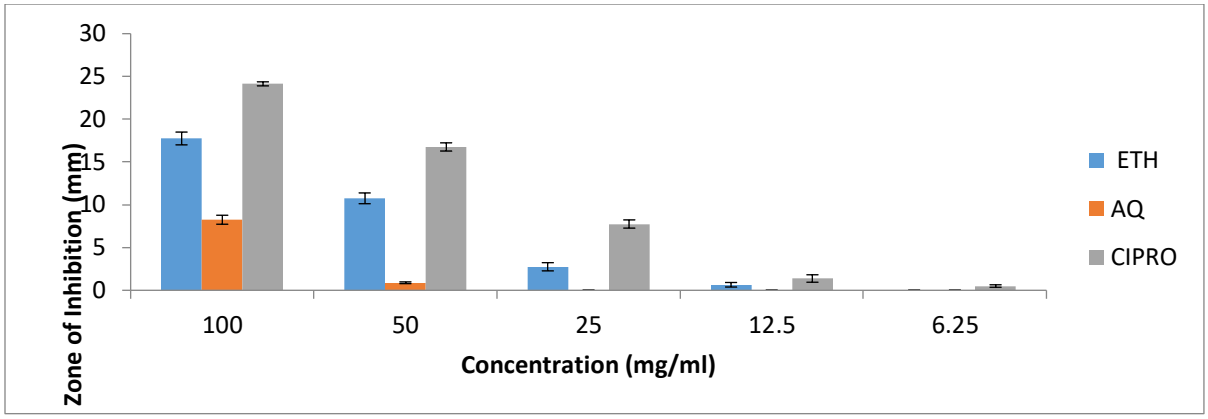

Figure 2: Comparison of mean zones of inhibition of Ethanol and Aqueous extracts of *Allium sativum* and Ciprofloxacin on *Streptococcus viridans*
ETH: Ethanol extracts; AQ: Aqueous extracts; CIPRO: Ciprofloxacin

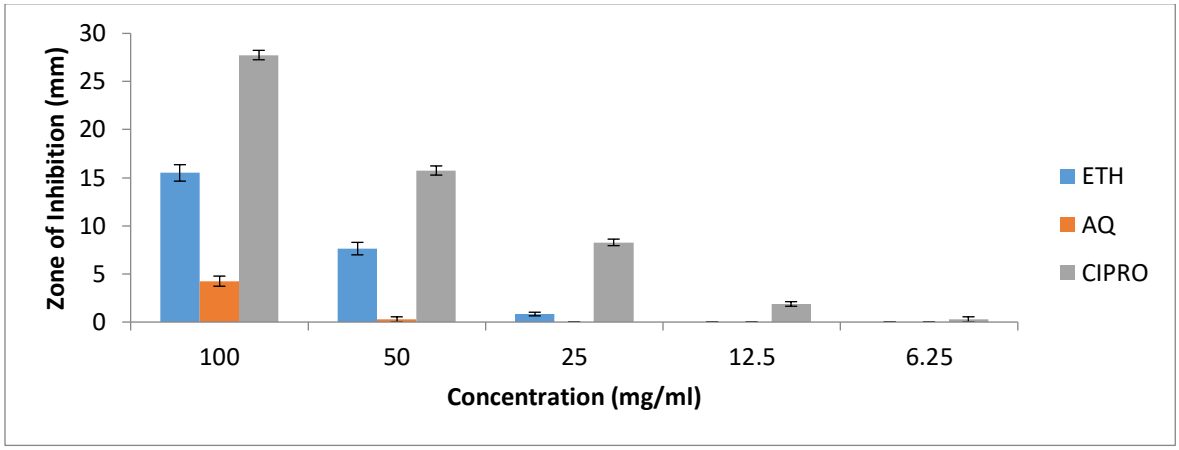

Figure 3: Comparison of mean zones of inhibition of Ethanol and Aqueous extracts of *Allium sativum* and Ciprofloxacin on *Escherichia coli*
ETH: Ethanol extracts; AQ: Aqueous extracts; CIPRO: Ciprofloxacin

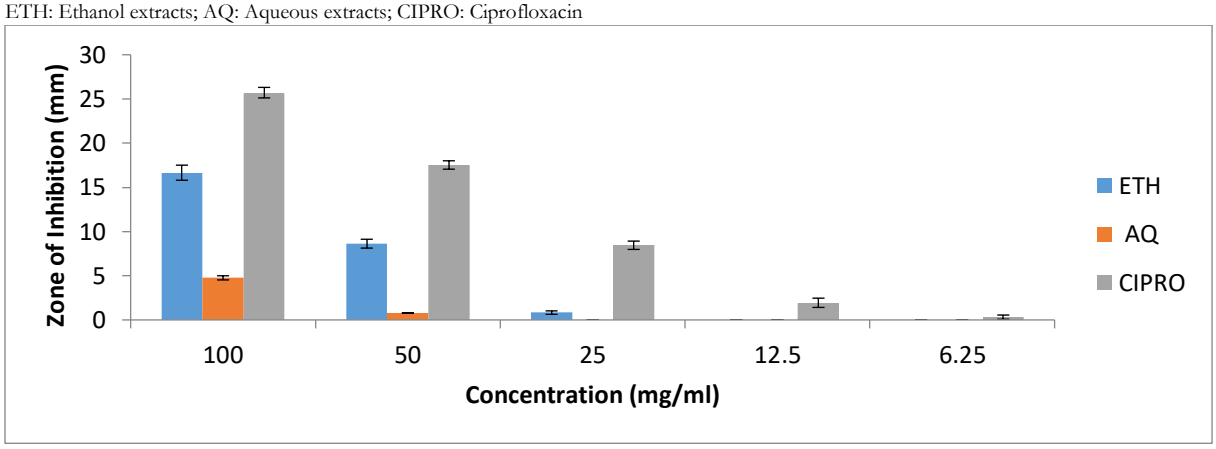

Figure 4: Comparison of mean zones of inhibition of Ethanol and Aqueous extracts of *Allium sativum* and Ciprofloxacin on *Pseudomonas aeruginosa*
ETH: Ethanol extracts; AQ: Aqueous extracts; CIPRO: Ciprofloxacin

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DISCUSSION

Allium sativum, commonly known as "garlic", is a widely cultivated plant with a rich history of use in traditional medicine and culinary practices across the globe. It has been the subject of numerous investigations due to its potential antimicrobial and therapeutic properties.[27-29] In this study, *Allium sativum* ethanolic and aqueous extracts demonstrated significant antibacterial activity. The ethanolic extracts however, had greater antibacterial activity than the aqueous extracts. Similar studies[30,31] have reported greater antibacterial activity of the ethanolic extract over the aqueous extract of *Allium* sativum. This could be because the ethanolic extracts tend to contain higher amounts of bioactive compounds, such as allicin, diallyl, disulfide, and diallyl trisulfide, which are responsible for the antibacterial activity.[11] Also, ethanol is a more effective solvent than water for extracting these bioactive compounds, leading to a higher concentration of antibacterial agents. Ethanolic extracts may also be more stable, as ethanol helps to preserve the bioactive compounds and prevent degradation. The results obtained from this study also revealed that both ethanolic and aqueous extracts of *Allium sativum* have inhibitory effect against both gram-positive (*Staphylococcus aureus* and *Streptococcus viridians*) and gram-negative (*Pseudomonas aeruginosa* and *Escherichia coli*) bacteria. The inhibitory effect was greater at higher concentrations than in lower concentrations. Hence, the antibacterial activities of the extracts are concentration-dependent, with higher concentrations of the extract exhibiting greater antibacterial activity. This finding is consistent with the concentration-dependent effect that is frequently found in pharmacological research.[32] According to this relationship, the effectiveness of a chemical has been shown to rise with increasing concentrations. In a study[33] to determine the antibacterial efficacy of crude garlic extract against Methicillin Resistant *Staphylococcus aureus* (MRSA), all MRSA strains assessed were significantly sensitive to the ethanolic extracts at higher concentrations. This concentration-dependent trend suggests that higher concentrations may be required for therapeutic applications, particularly for pathogens with greater resistance.

The increasing prevalence of antibiotic resistance highlights the need for alternative antimicrobials. *Allium sativum* extracts, with their diverse phytochemicals, offer a multifaceted approach that reduces the likelihood of resistance development. Studies[34,35] support the use of garlic essential oil and extracts in combating multidrug-resistant strains. Ciprofloxacin, a conventional drug for treating bacterial infections which served as a control in this study showed significant inhibition of bacterial growth, with higher concentrations exhibiting greater effectiveness. Overall, Ciprofloxacin displayed stronger anti-bacterial activity against the bacteria when compared to both the ethanolic and aqueous extracts of *Allium sativum*. This could be because ciprofloxacin kills the bacteria by disrupting their ability to create and repair their DNA. It specifically targets bacterial DNA gyrase and topoisomerase IV, which are essential enzymes for bacterial DNA replication leading to a bactericidal effect, directly killing the bacterial cells. While a study[36] highlighted the effectiveness of ciprofloxacin as a powerful topical and systemic antibacterial drug for treating infections, another study[37] indicated growing resistance of bacteria like *Staphylococcus aureus* to ciprofloxacin. Just like other antibacterial medications, chronic usage of ciprofloxacin may result in the problem of antibiotic resistance.

The ability of *Allium sativum* extracts to inhibit both gram-positive and gram-negative bacteria suggests the plant possesses broad-spectrum antibacterial activity, potentially making it a valuable source for developing new drugs with a broad spectrum of activities. The garlic phytochemicals exert their antibacterial effects through different mechanisms, including disruption of bacterial cell membranes and inhibition of vital enzymes.[38] The observed variations in the antibacterial activity of *Allium sativum* extract between the ethanol and aqueous extracts highlight the importance of extraction methods in determining the bioactivity of plant-derived compounds. The findings of this study emphasize the need for investigating traditional medicinal plants for the purpose of determining their potential therapeutic applications, particularly in view of the growing prevalence of antibiotic resistance. This observation highlights the importance of solvent choice in determining the antibacterial potential of plant extracts, as solvents like ethanol are more effective at extracting a broader range of bioactive compounds. The antibacterial activity of garlic extracts against multidrug-resistant bacteria, as reported by Noman *et al.*[39] further underscores its potential role in combating antibiotic resistance. Further research should focus on the isolation and characterization of specific bioactive compounds responsible for garlic's antibacterial effects. Studies should be conducted through in vivo investigations in order to evaluate the therapeutic relevance and safety profile of the substance. Other areas for further research

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include extraction techniques such as supercritical fluid extraction, hydro-distillation, ultrasound-assisted extraction, enzyme-assisted extraction, and microwave-assisted extraction. In addition, exploring the synergistic effects of *Allium sativum* extracts with antibiotics could pave the way for innovative combination therapies to enhance the efficacy of existing drugs while mitigating resistance development.

With the rising concerns of antibiotic resistance, natural remedies are potential alternatives to conventional antibiotics in the treatment of bacterial infections including ocular infections such as bacterial conjunctivitis. The results from this study indicate that *Allium sativum* can be used to make herbal remedies for ocular bacterial infections. However, this was an in-vitro laboratory study from which results of the antibacterial activity of *Allium sativum* was derived. In-vivo studies are recommended in future studies to fully appreciate its clinical significance in the treatment of bacterial conjunctivitis and other ocular infections. Garlic is widely available and relatively affordable in local markets in Nigeria, and with the rising problem of antibiotic resistance, this makes it an attractive plant for promoting healthcare due to its potential antibacterial health benefits.

CONCLUSION

Allium sativum extracts showed antibacterial activity against the ocular bacterial isolates selected in this study, especially with the ethanolic extract and at higher concentrations. Thus, it has potential as an alternative herbal ophthalmic medication for the treatment of bacterial infections. Due to its affordability and accessibility in Nigeria, herbal medications from garlic are a suitable option especially in rural areas where people do not have access to adequate healthcare.

Declarations

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Authors Contributions: YCA and MPO conceptualized and designed the study. MPO and WB worked on data collection and laboratory work. YCA performed data analysis and interpreted the data. All the authors contributed to the development of the final manuscript and approved its submission.

Ethics Approval and Informed Consent: Ethical approval for the study was obtained from the Ethics Committee, School of Health Technology, Federal University of Technology, Owerri, Nigeria, with reference number FUT/SOHT/C1/065 dated October 17, 2024. All the participants provided informed consent. Participation in the study was voluntary.

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REFERENCES

1. Gao L, Zhang M, Li Y. Nutritional composition and health benefits of garlic. Food Chem 2016; 200: 240-250. doi: 10.1016/j.foodchem.2015.11.045
2. Salehi B, Zucca P, Sharifi-Rad M, Pezzani R, Rajabi S, Setzer WN. et al. Phytotherapeutics in cancer invasion and metastasis: from plant extracts to mechanistic insights. Phyto Res 2019; 33(10): 2791-2811. doi:10.1002/ptr.6464
3. Faridi K, Amini N, Mozaffari S, Keshavarz S, Rostami S. Anti-inflammatory effects of garlic and its constituents. J Inflamm Res 2022; 15: 233-245. doi: 10.2147/JIR.S358974
4. Magryś A, Olender A, Tchórzewska D. Antibacterial properties of *Allium sativum* L. Against the most emerging multidrug-resistant bacteria and its synergy with antibiotics. Arch Microbiol 2021; 203(5): 2257-2268. doi:10.1007/s00203-021-02248-z.
5. Shang A, Cao SY, Xu XY, Gan RY, Tang GY, Corke H. et al. Bioactive compounds and biological functions of garlic (*Allium sativum*). Foods 2019; 8(7): 246. doi: 10.3390/foods8070246
6. Zhang Y, Liu X, Ruan J, Zhuang X, Zhang X, Li Z. Phytochemicals of garlic: Promising candidates for cancer therapy. Biomed Pharmaco 2020; 2020: 109730. doi: 10.1016/j.biopha.2020.109730
7. Batiha GE, Beshbishy AM, Wasef LG, Elewa YHA, Sagan AA, El-Hack MEA. Chemical Constituents and Pharmacological Activities of Garlic (*Allium sativum* L.): A Review. Nutrients 2020; 12(3): 872. doi: 10.3390/nu12030872
8. Sahidur MR, Islam S, Jahurul MHA. Garlic (*Allium sativum*) as a natural antidote or a protective agent against diseases and toxicities: A critical review. Food Chem Adv 2023; 3: 100353. doi: 10.1016/j.focha.2023.100353
9. Yaniv BZ. Garlic as a medicine throughout the ages (Review). World Acad Sci J 2025; 7: 49. doi: 10.3892/wasj.2025.337

Print ISSN: 0189-9287 Online ISSN: 2992-345X

10. Indira M, Bhuvaneshwari G, Premkumar L, Neelusree P. Antibacterial Activity of the *Allium sativum* Crude Extract against Methicillin-resistant *Staphylococcus aureus*. J Pure Appl Microbiol 2024; 18(2): 1297-1304. doi: 10.22207/JPAM.18.2.50
11. Ried K, Sullivan T, Parker J. Garlic and cardiovascular disease: A critical review. J Clin Hypertens 2016; 18(7): 688-694. doi: 10.1111/jch.12756
12. Friedman M. Garlic and onions: Their bioactivity and potential health benefits. Food Chem 2019; 311: 126085. doi: 10.1016/j.foodchem.2019.126085
13. El-Saadony MT, Saad AM, Korma SA, Salem HM. Garlic bioactive substances and their therapeutic applications for improving human health: a comprehensive review. Front Immunol 2024; 15: 1277074. doi: 10.3389/fimmu.2024.1277074
14. Furdak P, Pienkowska N, Kapusta I, Bartosz G. Comparison of Antioxidant and Antiproliferative Effects of various forms of Garlic and Ramsons. Molecules 2023; 28(18): 6512. doi: 10.3390/molecules28186512
15. Li J, Zhang J, Yang S. Saponins in garlic and their health benefits. J Food Sci Technol 2017; 54(12): 4337-4344. doi: 10.1007/s11483-017-9996-6
16. Salmon JF. Kanski's Clinical Ophthalmology. 10th ed. Oxford: Butterworth-Heinemann. 2019; 109-120.
17. Trojan R, Razdan L, Singh N. Antibiotic Susceptibility Patterns of Bacterial Isolates from Pus Samples in a Tertiary Care Hospital of Punjab, India. Int J Microbiol 2016; 2016: 9302692. doi: 10.1155/2016/9302692
18. Woldemariam M, Aklilu A, Manilal A, Mengistu M, Tadesse D, Siraj M. Microbial profile and associated factors of external ocular bacterial and fungal infections in Arch Minch General Hospital: a cross sectional study. Sci Rep 2024; 14: 28744. doi: 10.1038/s41598-024-77723-w
19. Morehead MS, Scarbrough C. Emergence of Global Antibiotic Resistance. Prim Care 2018; 45(3): 467-487. doi: 10.1016/j.pop.2018.05.006
20. Karami S, Shamshiri S, Abdollahi M, Rahimi R. An Evidence-based Review of Medicinal Plants used in Traditional Persian Medicine for Treatment of Osteoarthritis. Curr Drug Discov Technol 2021; 18(2): 244-271. doi: 10.2174/1570163817666200316105658
21. Madigan MT, Bender KS, Buckley DH, Sattley WM, Stahl D. Brock Biology of Microorganisms 16th Ed. San Fransisco: Pearson. 2020; 144-183.
22. Zhang M, Zhao J, Dai X, Li X. Extraction and Analysis of Chemical Compositions of Natural Products and Plants. Separations 2023; 10(12): 598. doi: 10.3390/separations10120598
23. Abdullahi A, Haque M. Preparation of Medicinal Plants: Basic Extraction and Fractionation Procedures for Experimental Purposes. J Pharm Bio Sci 2020; 12(1): 1–10. doi: 10.4103/jpbs.JPBS_175_19
24. Boiko I, Krynytska I. Comparative performance of commercial Amies transport media with and without charcoal for *Neisseria gonorrhoeae* culture for gonococcal isolation and antimicrobial resistance monitoring in Ukraine. Germs 2021; 11(2): 246-254. doi: 10.18683/germs.2021.1261
25. Franco-Duarte R, Cernakova L, Kadam S, Kaushik KS, Martins N, Rodrigues CF. Advances in Chemical and Biological Methods to Identify Microorganisms from Past to Present. Microorg 2019; 7(5): 130. doi: 10.3390/microorganisms7050130
26. Balouiri M, Sadiki M, Ibnsouda SK. Methods for in vitro evaluating antimicrobial activity: A review. J Pharma Anal 2016; 6(2): 71–79. doi: 10.1016/j.jpha.2015.11.005
27. Bello AB, Suleiman A, Atteh EO, Olutimayin TA. Antibacterial Effect of *Allium sativum* and *Zingiber officinale* Extracts on Some Clinically Pathogenic Bacteria. J Appl Sci Environ Man 2023; 27 (6): 1077-1082. doi: 10.4314/jasem. v27i6.5
28. Gabriel T, Vestine A, Kim KD, Kwon SJ, Sivanesan I, Chun SC. Antibacterial Activity of Nanoparticles of Garlic (*Allium sativum*) Extract against Different Bacteria Such as *Streptococcus mutans* and *Poryphormonas gingivalis*. Appl Sci 2022; 12: 3491. doi: 10.3390/app12073491
29. Chung SK, Choi HY, Kwon DY, Kim HJ. Allicin in garlic: a review of its antimicrobial and anticancer effects. Food Chem 2016; 211: 166-175.
30. Oyawoye OM, Olotu TM, Nzekwe SC, Idowu JA, Abdullahi TA, Babatunde SO. Antioxidant potential and antibacterial activities of Allium cepa (onion) and Allium sativum (garlic) against the multidrug resistance bacteria. Bull Nat Res Cen 2022; 46:214. doi: 10.1186/s42269-022-00908-8
31. Muchtaromah B, Ahmad M, Romaidi R, Nazilah LA, Naja NA. Antibacterial activity of water and ethanol extract of Allium sativum, Curcuma manga, and Acorus calamus combination. Berk Pen Hay 2018; 24(1): 8-15. doi: 10.23869/30
32. Tilanus A, Drusano G. Inoculum-Based Dosing: A Novel Concept for combining time with Concentration-Dependent Antibiotics to Optimize Clinical and Microbiological Outcomes in Severe

Print ISSN: 0189-9287 Online ISSN: 2992-345X

Gram-Negative Sepsis. Antibiotics 2023; 12(11): 1581. doi: 10.3390/antibiotics12111581
33. Mohammed SON, Abuzeid NMK, Abdelghani S, Eltayeb LB. In-Vitro Antibacterial Activity of crude Garlic (Allium sativum) Extract Against Clinical Isolates of Methicillin Resistant *Staphylococcus aureus*. Biomed Pharmacol J 2021; 2021:14(3). doi: 10.13005/bpj/2247
34. Bernaldez JL, Manuela CG. Antibacterial Activity of Soap Formulated from Garlic (*Allium sativum*) Extract. J Adv Microbiol 2021; 21(1): 63-67. doi: 10.9734/jamb/2021/v21i130320
35. Yasin G, Jasim SA, Mahmudiono T, Al-Shawi SG, Shichiyakh RA, Shoukat S. Investigating the effect of garlic (Allium sativum) essential oil on foodborne pathogenic microorganisms. Food Sci. Technol 2022; 42: e03822. doi: 10.1590/fst.03822.
36. Talwar D, Thulasidas M. Ciprofloxacin: Rationale for Use in Intraocular Infections. J Pharmacol Clin Toxicol 2023; 11(1): 1171. doi: 10.4172/2333-7079.1001171
37. Chatterjee S, Agrawal D, Gomase SN, Parchand SM, Gangwe AB, Mishra M. Fluoroquinolone resistance in bacterial isolates from ocular infections: Trend in antibiotic susceptibility patterns between 2005-2020. Ind J Ophthalmol 2022; 70(12): 4391–4398. doi: 10.4103/ijo.IJO_469_22
38. Lionel OO, Adegboyega IP, Ezekiel AO, Olufunke BC. Antimicrobial activity of garlic (Allium sativum) on selected uropathogens from cases of urinary tract infection. Ann Trop Pathol 2020; 11(2): 133-138. doi: 10.4103/atp.atp_9_20
39. Noman ZA, Anika, TT, Sachi S, Ferdous J, Sarker YA, Sabur MA. Evaluation of antibacterial efficacy of garlic (Allium sativum) and ginger (Zingiber officinale) crude extract against multidrug-resistant (MDR) poultry pathogen. J Adv Vet Ani Res 2023; 10(2): 151-156. doi: 10.5455/javar. 2023.j664

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