ISSN 2348-313X (Print)
International Journal of Life Sciences Research ISSN 2348-3148 (online)
Vol. 11, Issue 1, pp: (18-23), Month: January - March 2023, Available at: www.researchpublish.com

The Effects of Aqueous Extract of Garlic on Bacterial Keratitis

[1]Ibeje T., [2]Ikoro N.C., [3]Esenwah E.C., [4]Megwas A.U., [5]Azuamah Y., [6]Eronini Y., [7]Odikemere M.

Department of Optometry, Federal University of Technology, Owerri, Imo State, Nigeria.

DOI: https://doi.org/10.5281/zenodo.7599717

Published Date: 02-February-2023

Abstract: **The use of *Allium Sativum* (garlic) for the treatment of various microbial infections of the human body has been an age-long practice, however many bacterial organisms have developed resistance to most conventional antibiotic drugs. The aim of this study was to demonstrate the effectiveness of aqueous garlic extracts against bacterial keratitis caused by *Staphylococcus aureus* and *Psuedomonas aeruginosa* . The study was carried out both in vitro and in vivo. The in vitro study was carried out to measure zones of inhibition and the minimum inhibitory concentrations while the in vivo study was done to assess the effect of the extracts on the cornea. The data obtained were analysed using the Analysis of Variance at a significance level of P<0.05 and tested against the research hypothesis. The in vivo results showed that the aqueous garlic extracts were effective in treating the keratitis caused by the two microorganisms(p=0.00). 0.3% Ciprofloxacin eye drop was more effective in treating the microbial keratitis than the aqueous garlic extracts. The in vitro results showed that the micro organisms were sensitive to aqueous garlic extract and their sensitivity was concentration dependent. It is therefore safe to conclude that aqueous garlic extract is effective in treating bacterial keratitis.**

***Keywords: Allium Sativum*, Bacterial Keratitis, *Staphylococcus aureus*, *Pseudomonas aeruginosa*.**

1. INTRODUCTION

Garlic (Allium sativuim) has been in use for many years in different parts of the world for both medicinal and culinary purposes. Originating from Asia, it has been seen to grow successfully in almost all parts of the world, spreading its usefulness as a medicinal herb that has curative and preventive properties against a wide range of ailments (Rani *et al*, 2019).

Its medicinal use can be found in its ability to treat various infections caused by different kinds of microorganisms like bacteria, fungi, virus and protozoans. It has also been proved to have antioxidant, anticancer and antihypertensive abilities. Its' role in the enhancement of the reproductive functions in males have also been proved (Harris et al., 2001).

the use of garlic as an antibacterial agent has been the most common, it's effectiveness against a wide range of both gram positive and gram negative bacteria making it a very potent medicinal plant against a wide range of bacterial infections. Even bacterial infections which have been known to be very resistant to conventional antibiotics have been shown to be highly susceptible to garlic(Haris *et al*,2001).

The use of garlic extracts to treat infections of the ocular surface has not been a common practice although some studies have shown the effectiveness of garlic extracts on some infectious diseases of the eyes which are caused by staphylococcus infections on the conjunctiva (Uzodike *et al*., 2005). It has also been proved that garlic extracts are effective agents for the treatment of fungal ocular infections when administered topically. However, the use of garlic as a safe antimicrobial agent for topical use in the treatment of ocular infections is yet to gain general acceptance among eye care professionals.

Many studies have been undertaken to demonstrate the antibiotic effect of garlic on bacteria organisms. Different strains of bacteria organisms have been tested with different extracts of garlic to examine its efficacy against such strains. The emergence of strains of bacteria which are resistant to conventional antibiotics has been one of the major reasons for the desire to access plants such as garlic as effective alternatives in the treatment of such bacterial infections (Epherem *et al*., 2016). Allicin in garlic is found to be the major antimicrobial agent of the herb. It has been shown to effective against a wide range of microbial organisms. Its antibacterial property stems from its ability to inhibit the actions of two particular enzymes which are the most implicated enzymes in the causation of infections. These enzymes are the cysteine proteinases and the alcohol dehydrogenases which serve as the main destructive enzyme in microorganisms and the main enzyme of metabolism in microorganisms respectively. Also the complete inhibition of the RNA synthesis in bacterial organisms by allicin and other thiosulfinate substances of garlic also plays a role in the inhibition of bacterial growth. Allicin in garlic has been known to be effective against both gram positive and gram negative bacteria (Kamal *et al*., 2000).

Bacterial keratitis is the bacterial infection of the cornea , the most common form of microbial keratitis which is prevalent among various population groups around the globe. Its effect on the cornea can go from the cornea epithelium through to the stroma of the cornea. Bacterial keratitis is commonly caused by three bacterial organisms: *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Streptococcus pneumonoia*. These organisms have the capacity to invade through the cornea epithelium down to the stroma. Of these organisms, *Pseudomonas aeruginosa* is the most common, having the capacity to cause corneal epithelial perforation just within three days of infecting the cornea(Al Mujaini,2009).

2. MATERIALS AND METHODS

This research work was carried out in Owerri, Imo State, Nigeria. A randomized controlled study was done in which the effectiveness of aqueous garlic extract on bacterial infections of the cornea was compared to that of 0.3% Ciprofloxacin eye drop using forty healthy albino rats and two different strains of bacterial organisms; *Staphylococcus aureus* and *Pseudomonas aeruginosa* as the keratitis inducing organisms.

3. METHOD: PREPARING THE ANIMAL MODELS

Forty albino wistar rats were purchased from the Animal Friends Farm located at Royce Road, Owerri, Imo State from where they were transported in cages to the laboratory after being certified free of any systemic disease or infection by a veterinary doctor. They were between the ages of 4 to 6 weeks old, weighing between 80 to 100g.

Preparation of the Microorganisms

Two kinds of bacterial microorganisms were used for this study. They are clinical isolates of Pseudomonas aeuriginosa and *Staphylococcus aureus* species. Strains of these microorganisms were procured from the Microbiology Laboratory of Federal Medical Centre Owerri and transported in nutrient agar plates to the research laboratory. They were grown on nutrient broth for a period of twenty-four hours and under standard temperature of 37 . This was then followed by decantation of the media and the washing of the microorganisms in sterile distilled water using the centrifuge at a speed of 3000 rotations per minute for a period of ten minutes. The microorganisms were then suspended in sterile distilled water and standardized to an optical density of about 0.50 at a wavelength of 540um using a spectrophotometer.

Preparation of the Garlic Extract: Fresh garlic bulbs were first purchased from the local market, their cloves washed with sterile distilled water and allowed to air dry. 150g portion of the raw garlic was weighed using a weighing balance (kalan weighing balance).About 50g of the raw garlic was then homogenized in an electric blender with about 700ml of sterile distilled water. The homogenate was then filtered using sterile musclin cloth, thereafter, the resultant filtrate was filtered the second time using a whatman number 1 filter paper to obtain the fresh aqueous garlic extract. The aqueous extract was then weighed using the kalan weighing balance and its weight was found to be 37.54g.The volume of the aqueous extract was also measured and found to be 650ml.The aqueous garlic extract was then heated over a water bath at a temperature of about 55 C until a paste form of it was obtained. This paste form of the raw garlic extract was put in a sterile container and weighed using the kalan weighing balance and it's weight was found to 22.86g.This was then stored in the refrigerator at a temperature of about 4 C and kept for use throughout the study.

ISSN 2348-313X (Print)
International Journal of Life Sciences Research ISSN 2348-3148 (online)
Vol. 11, Issue 1, pp: (18-23), Month: January - March 2023, Available at: www.researchpublish.com

The Experiment:

The experiment was done both in vivo and in vitro in the laboratory. The in vitro test was done in order to determine the sensitivity of the garlic extract on the microorganisms. This was achieved by first determining the zones of inhibition of the various concentrations of the garlic extracts to each of the organisms, the minimum inhibition concentration and finally the minimum bactericidal concentrations of the extracts to the microorganisms. For the study, about 11.3g of the garlic paste was dissolved in 100ml of sterile distilled water in order to obtain a stalk concentration of 113mg/ml. This was then diluted using sterile distilled water to 50%, 25% and 12.5% to obtain lower concentrations of 56.5mg/ml, 28mg/ml and 14mg/ml. These three concentrations were used for the in vitro study to determine the zones of inhibition and the minimum inhibitory concentration of the garlic to the two microorganisms. For the determination of the zone of inhibition, the agar well diffusion method was used while the broth dilution method was used in the determination of the minimum inhibitory concentration. For the minimum bactericidal concentration, the pour plate method was used to determine it.

The in vivo test was carried out by first dividing the animals into two broad groups, A&B and then counting the bacterial colonies in the eyes of all the animals prior to infecting them with the microorganisms. For the animals in group A, their right eyes were swapped for the colony counting while for those in group B their left eyes were swapped. This was done by using sterile swab sticks that were dipped into normal saline and swabbing them on the surface of the cornea of the eyes of all the animals. From the swabs, the organisms were cultured and grown on nutrient agar using the agar pour plate method for a period of 24 hours at a temperature of about 37 degrees Celsius. After the 24 hour period, the bacterial colonies on the agar plates were counted using the direct visualization method and the colonies were recorded as the bacterial bioload before infection for each of the animal models. Before inoculation with the microorganisms, each of the two groups was further divided into four subgroups of (A1, A2, A3, A4 & B1, B2, B3, B4) containing five animals in each of the subgroups. The animals in group A (A1, A2, A3 & A4) all had their right eyes inoculated topically with *Staphylococcus aureus* strains using the swab stick while those in group B (B1, B2, B3 & B4) were inoculated topically on the left eyes with *Pseudomonas aeruginosa* using sterile swab sticks also .The animals first had their corneal surfaces scrapped with sterile syringes on the eyes that were to be inoculated in order to initiate a wound /aberration on the surface of the cornea before the inoculations were done according to the method proposed by Kathleen *et al*,(2001). The inoculations with the microorganisms took place simultaneously and at room temperature and continued for about two days consecutively until infections were visibly noticed on the cornea. The parameter for ascertaining the presence of infections on the cornea was the appearance of signs of bacterial corneal infections. Once the infections were visibly noticed, a colony count of the microbial flora under the state of infection was taken for all the infected animals.

The first group which served as the control did not receive any treatment either with the garlic extract nor the ciprofloxacin. The second group was treated with the 56.5mg/ml garlic extract while the third group was treated with the 113mg/ml garlic extract .The fourth group was treated with 0.3%ciprofloxacin eye drop. The treatment regimen was three drops of the garlic extract and 0.3% Ciprofloxacin twice daily until all the signs of infection had completely resolved.

Colony count of the bacterial bioload was done every 24hours during the course of the treatment until the infections were all resolved. At the final resolution of the clinical signs, swabs were also taken from the eyes of the rats and colony counting was done for the various groups.

The data obtained were analysed using the one way analysis of variance(ANOVA) at a confidence level of 0.05%.

4. RESULTS

Table 1: Antimicrobial activity of *Allium Sativum* aqueous extract on *Staphyllococcus aureus* isolates

	Zone of Inhibition (mm)	
Allium Sativum (mg/ml)	Mean Zone of Inhibition (mm)	% Inhibition
0	37.50 ± 3.54	0.00 ± 0.00
28.25	17.50 ± 3.54	53.33 ± 13.89
56.5	6.50 ± 0.71	82.67 ± 3.54
113	5.50 ± 0.71	85.33 ± 0.51
Ciprofloxacin (0.3%)	0.00 ± 0.00	100.00 ± 0.00

Results are mean ± standard deviation of three (3) determinations

ISSN 2348-313X (Print)
International Journal of Life Sciences Research ISSN 2348-3148 (online)
Vol. 11, Issue 1, pp: (18-23), Month: January - March 2023, Available at: www.researchpublish.com

Table 2: Antimicrobial activity of *Allium Sativum* aqueous extract on *Pseudomonas aeruginosa* isolates

Zone of Inhibition (mm)		
Allium Sativum (mg/ml)	Mean Zone of Inhibition (mm)	% Inhibition
14.13	15.75 ± 0.006	0.00 ± 0.00
28.25	11.00 ± 1.41	30.16 ± 13.71
56.5	8.50 ± 0.71	46.03 ± 8.14
113	4.50 ± 0.71	71.43 ± 6.43
Ciprofloxacin (0.3%)	0.00 ± 0.00	100.00 ± 0.00

.3%).

Table 3: Minimum inhibitory concentration of *Allium Sativum* aqueous extract on *Pseudomonas aeruginosa* and *Staphyllococcus aureus* isolates

	Minimum Inhibitory Concentration (MIC)			
Isolates	*Allium sativum* extract (mg/ml)			
	0	14.13	28.25	56.50
Pseudomonas aeruginosa	+	-	-	-
Staphyllococcus aureus	+	-	-	-

– = No visible growth

+ = Growth

Table 4: Minimum bactericidal concentration of *Allium Sativum* aqueous extract on *Pseudomonas aeruginosa* and *Staphyllococcus aureus* isolates

	Minimum Bactericidal Concentration (MBC)			
Isolates	*Allium Sativum* extract (mg/ml)			
	0	14.13	28.25	56.50
Pseudomonas aeruginosa	+	+	+	-
Staphyllococcus aureus	+	+	+	-

– = No visible growth

+ = Growth

Table 5: Effect of *Allium Sativum* aqueous extract on ocular total bacterial count of rats in *Staphyllococcus aureus* induced keratitis

	Total bacteria count in colony forming unit (CFU)/ml x 10^3										
Groups	Pre-Inoculation	Post-Inoculation	Post-treatment (Days)								
			1	2	3	4	5	6	7	8	9	10
Group A1 (Control)	79.60 ± 9.33	318.40 ± 37.32^a	382.08 ± 44.78^a	458.50 ± 53.74^a	446.44 ± 51.25^a	432.94 ± 49.45^a	386.16 ± 15.27^a	420.29 ± 49.26^a	424.45 ± 48.48^a	445.60 ± 42.50^a	415.25 ± 46.48^a	374.91 ± 14.83^a
Group A2 (*A. sativum* 56.5mg/ml)	74.05 ± 1.98	296.20 ± 7.92^a	246.83 ± 6.60^b	222.93 ± 23.65^b	194.16 ± 3.61^b	167.70 ± 4.70^b	123.42 ± 3.30^b	107.32 ± 2.87^b	84.05 ± 1.98^b	70.05 ± 2.23^b	54.05 ± 1.88^b	40.71 ± 1.65^b
Group A3 (*A. sativum* 113 mg/ml)	73.10 ± 12.72	292.40 ± 50.89^a	243.67 ± 42.41^b	203.06 ± 7.83^{b,c}	182.60 ± 4.92^b	154.12 ± 2.38^{b,c}	114.40 ± 4.72^{b,c}	96.93 ± 7.32^b	66.06 ± 3.13^{b,c}	51.06 ± 4.16^{b,c}	59.85 ± 3.13^{b,c}	38.20 ± 2.36^b
Group A4 (Ciprofloxacin 0.3%)	68.45 ± 2.33	273.80 ± 9.31^a	182.53 ± 6.21^c	171.13 ± 5.82^c	142.52 ± 2.09^c	126.77 ± 7.35^c	107.37 ± 3.65^c	91.27 ± 3.10^b	44.71 ± 3.37^c	37.85 ± 3.41^c	30.80± 2.62^c	26.84 ± 0.91^c

Results are mean ± standard deviation of five (5) determinations. Mean values bearing different superscript letters across columns are significantly different ($p<0.05$).

ISSN 2348-313X (Print)
International Journal of Life Sciences Research ISSN 2348-3148 (online)
Vol. 11, Issue 1, pp: (18-23), Month: January - March 2023, Available at: www.researchpublish.com

Table 6: Effect of *Allium Sativum* aqueous extract on ocular total bacterial count of rats in *Pseudomonas aeruginosa induced keratitis*

Total bacteria count in colony forming unit (CFU)/ml x 10^3										
Groups	Pre-Innoculation	Post-Innoculation	Post-treatment (Days)							
			1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Group B1 (Control)	69.10 ± 4.20	276.40 ± 16.79^a	365.10 ± 52.06^a	438.12 ± 62.48^a	426.80 ± 61.62^a	415.81 ± 60.95^a	405.12 ± 60.44^a	401.61 ± 57.27^a	436.60 ± 64.00^a	427.74 ± 65.00^a	408.81 ± 64.00^a	417.27 ± 62.26^a
Group B2 (*A. sativum* 56.5mg/ml)	58.70 ± 4.78	283.80 ± 14.29^a	278.16 ± 14.64^b	271.97 ± 13.06^b	184.62 ± 12.00^b	167.71 ± 13.66^b	156.53 ± 12.75^b	116.60 ± 5.18^b	83.86 ± 6.83^b	70.50 ± 6.75^b	62.28 ± 6.44^b	48.27 ± 6.37^b
Group B3 (*A. sativum* 113 mg/ml)	71.25 ± 8.63	285.00 ± 34.51^a	256.03 ± 18.02^b	242.07 ± 5.50^b	156.33 ± 16.88^{b,c}	144.50 ± 14.15^{b,c}	128.02 ± 9.01^{b,c}	94.76 ± 10.03^b	70.15 ± 3.23^b	58.30 ± 7.00^c	42.60 ± 5.80^{ab}	31.01 ± 4.51^b
Group B4 (Ciprofloxacin 0.3%)	69.55 ± 6.80	278.20 ± 27.19^a	209.17 ± 8.08^c	159.88 ± 6.47^c	132.62 ± 15.12^c	101.62 ± 9.93^c	109.58 ± 8.94^c	78.54 ± 6.81^b	55.71 ± 2.08^c	29.60 ± 3.40^c	22.00 ± 2.20^c	21.40 ± 2.07^c

Results are mean ± standard deviation of five (5) determinations. Mean values bearing different superscript letters across columns are significantly different ($p<0.05$).

5. DISCUSSION

The in vivo results of this study showed that the aqueous garlic extracts were effective in the treatment of the bacterial keratitis caused by the *Pseudomonas aeruginosa* and *Staphylococcus aureus* organisms. The in vitro results showed a higher zone of inhibition for 0.3% ciprofloxacin eye drop than the aqueous garlic extracts for all the concentrations for both micro organisms with the least percentage inhibition shown by the aqueous extract at a concentration of 14.13mg/ml. The in vivo study which was carried out to demonstrate the effectiveness of the aqueous garlic extract on treating the bacterial infections caused by the two different organisms showed a slight discrepancy being more pronounced on the *Staphylococcus aureus* infection than the *Pseudomonas aeruginosa* infections. This observation was also seen in the in vitro study where the percentage of inhibition was higher for the S.aureus than for the *P.aeruginosa*. This could be as a result of the bioactive compounds(allicin and other thiosulfinates) contained in garlic which has been shown to have more effective on S.aureus than the *P.aeruginosa*. The second reason could be due to the mode of extraction as aqueous garlic extracts have been shown to be more effective against S.aureus than it's *Pseudomonas aeruginosa* counterpart because of the presence of the cell wall of *S aureus* which contains no polysaccharides and very little amount of lipids making it possible for allicin to penetrate most readily through it's cell wall. The in vivo study also showed a good comparison between the 0.3% Ciprofloxacin and the aqueous garlic extracts in their effectiveness in treating bacterial keratitis caused by both micro organisms. The results showed that the ciprofloxacin had a greater antibacterial effect on the infected cornea than the two concentrations of the aqueous garlic extracts, being able to resolve the bacterial infections caused by both organisms at a shorter time interval than the aqueous garlic extracts. This is similar to the work carried out by Uzodike et al (2005) on comparing the effectiveness of aqueous garlic extract on *S.aureus* infections of the conjunctiva.This effect could be attributed to the concentration of garlic used as higher concentrations of the aqueous extract could prove to have a shorter time interval in the treatment of the bacterial keratitis than the 0.3% Ciprofloxacin. Moreover, the mode of preparation of the garlic extract which involved the heating of the aqueous extract over a water bath at a temperature of 58 C could have reduced it's antibacterial efficacy.

The study also showed a discrepancy in the time interval required for the resolution of the bacterial infections caused by the two organisms. The 0.3% ciprofloxacin eye drop was able to completely resolve the bacterial infections caused by the two organisms within the first week of the treatment regimen(day 6 of the treatment).The 113mg/ml of garlic extract resolved the infection caused by the S.aureus on the 6th day while it resolved that caused by the *P.aeruginosa* on the 8th day.For the 56.5mg/ml, no resolution of the infections caused by the each of the micro organisms was observed until ten days post treatment.

6. CONCLUSION & RECOMMENDATION

From the results of this study, it is safe to conclude that aqueous extract of garlic is an effective antibacterial agent which can effectively treat bacterial infections of the cornea. Its antibacterial effect can be attributed to the presence of its active component allicin which is responsible for not only its antibacterial effect but also other pharmacological effects. Aqueous

garlic extract although effective against bacterial infections of the cornea is not more efficient than ciprofloxacin eye drops in the treatment of bacterial keratits that are caused by Staphylococcus aureus and Pseudomonas aeruginosa as the later is able to treat bacterial corneal infection at a shorter time interval. This however is based upon the concentration of the garlic extract that was used for this study.

Future research studies should be done using higher concentrations in order to ascertain if there will be any changes in effectiveness of aqueous garlic extracts on bacterial keratitis in comparison to ciprofloxacin and other more recent brands of fluroquinolones. I will also recommend aqueous extracts of garlic to be used as an adjunct medication in the treatment of bacterial infections of the cornea.

REFERENCES

[1] Al-Mujaini, A., Nadia, A., Archana,T. and Upender K. (2009). Bacterial Keratitis: Perspective on Epidemiology, Clinico-Pathogenesis, Diagnosis and Treatment. *SQU Med J*, 9(2) :184-195.

[2] Eja,M.,Bassey,E. and Clement,A.(2007).A comparative assessment of the antimicrobial effects of garlic and antibiotics on diarrheagenic organisms. *Southeast Asian Journal Of Tropical Medicine And Public Health*,38(2):343-347.

[3] Ephrem, A. and Asefaw, B. (2016). Antibacterial effects of garlic against clinical isolates of S. aureus and E.coli from hospital patients in Ethiopia. *Journal Of Infectious Diseases And Treatment*,2(2):18.

[4] Hadis,M.,Maliye,T.,Farznch,A.,Marayam,H.,Fardin,A.&Abbasa,M.(2018).Effects of garlic on male fertility; a systematic review. *Journal Of Herbmed Pharmacology*.7(4):306-312.

[5] Kamael, A. and Saleh,M. (2000). Recent studies on the chemistry and biological activities of the organo sulpfur compounds of garlic. *Studies in Natural Products Chemistry*, 23:455-485.

[6] Kathleen H. , Yanwen L & Swapan K. (2001). Pseudomonas aeruginosa corneal infection affects cholinergic enzymes in rat lacrimal gland. *Archives Of Microbiology*,177 (1) : 47–53.

[7] Rani,V. and Kumkum,S.(2021). Anticancerous and antimicrobial properties of garlic. *Journal Of Natural Products And Plant Resources*,11(2):1-5.

[8] Sun,C., Jiru,X., Gudiang,L., Xudong,M., Lisha,L., Zhao,X., Yuejiao,W., Gao,Z. and Jing,J. (2014). Fresh garlic extracts enhances the antimicrobial activity of antibiotics on resistant strains in vitro. *Journal Of Microbiology*, 8(5):1-6.

[9] Tsao,M. and MeiChin,Y.(2001). Invitro activity of garlic oil and four disallyl sulphides against resistant P. aeruginosa and K. pneumonia. *Journal Of Antimicrobial Chemotherapy*, 47(6): 665-670.

[10] Uzodike, E.B. and Igwe, I.C. (2005). Effects of Garlic extract on bacterial conjunctivitis. *Journal of Nigerian Optometry Association*, 12: 18-22.

[11] Zago,V. and Ana,L.(2013). Laboratory in the diagnosis of bacterial and fungal keratitis. *Article On Common Eye Infections*,4(4):61-84.