

Research Article

Antibacterial Activity of *Allium Cepa* (Onion) On Some Pathogenic Bacteria Associated With Ocular Infections

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Abstract: Research was carried out on antibacterial activity of *Allium cepa*. Two different extracts were made from *Allium cepa* (onion) using distilled water. The extracts include fresh onion extract and cold water extract. The antibacterial activity of the onion was tested on four bacteria isolates associated with various ocular infections. The bacterial isolates include: *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus pyogenes*, and *Streptococcus pneumoniae*. Among the two extracts, fresh onion extracts exhibited antagonistic effects on the test organisms ranging from observed zone of inhibition (average value) of 15mm for *Escherichia coli*, 17mm for *Staphylococcus aureus*, 20mm for *Streptococcus pyogenes*, and 8mm for *Streptococcus pneumoniae*. The antibiotic used as control was ciprofloxacin with zone of inhibition of 17mm for *Streptococcus pneumoniae*, 17mm for *Escherichia coli*, 19mm for *Staphylococcus aureus* and 20mm for *Streptococcus pyogenes* respectively. The minimum inhibitory concentration of the potent onion extracts shows positive inhibitory effect when an undiluted extracts was used. Also at the lowest concentration (0.015625 and 0.0068) of fresh onion extracts there was growth of all the organisms. From the research conducted it was concluded that there was no antibacterial activity on the test isolates observed with cold water extracts. Further research is encouraged on the pharmacokinetics of the active components; invitro study of the effectiveness of the purified active component, measuring dosage and some other parameters, on various implicated organisms from ocular infections.

Keywords: Antibacterial activity, *Allium cepa*, Pathogenic bacteria and Ocular infection.

INTRODUCTION

Traditional healers have long used plants to prevent or cure infectious conditions. Western medicine is trying to duplicate their successes [10]. Plants are rich in a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids and flavonoids, which have been found in vitro to have antimicrobial properties [5]. However, since many of these compounds got from plants material are currently available as unregulated botanical preparations and their use by the public is increasing rapidly, it is therefore necessary to consider the consequences (positive or negative) of patients' self medication with these preparations and to confirm the potency of such preparations. Onion is a good example of such plants, which has been found out to be used locally against infectious, particularly in some cases of eye infections [5].

Onion bulbs contain a good number of phytochemicals, most of which are hydrocarbons and their derivatives. These include: Dipropyl disulphide (which is used as a flavour compound), Allicin (which has antidiabetic, antihypertensive, antibiotic and antithrombotic activities), diethyl sulphide (which is of insecticidal property), Dimethyl disulphide (which is used as a gas odorant and in chemical synthesis), Mercaptopropane or propylmercaptan (which is used as flavour compound) [7]. Moreover, in addition to all previously stated uses of onion as food and food condiments. Several parts of the plant have traditional place in folk medicine in

many countries and that the extracts from onion and garlic, which is another species of *Allium*, have antimicrobial properties [10].

In a research works also discovered that crude juices of onion and garlic bulbs exert inhibition on the growth of *E. coli*, *Pseudomonas pyocyaneus*, *Salmonella typhi*, *Bacillus subtilis*, in vitro [1]. Also, [9] confirmed the sensitivity of certain food-borne bacterial pathogens to Allicin, which is the major component of garlic extracts, including onion [7]. [9] Used chemically synthesized and purified Allicin and discovered the inhibitory property of Allicin on *Salmonella typhimurium*, *Shigella dysenteriae*. All these food-borne pathogens were inhibited by Allicin in a dose-dependent manner. Thus, various medicinal properties have been ascribed to natural herbs. Therefore, since it had been confirmed that the extracts from onion have some inhibitory effects on some food-borne pathogens generally have antimicrobial properties [10], it is therefore necessary to confirm the scope of the inhibitory properties of onion extracts, most especially fresh onion extracts of onion on some microorganisms implicated in various eye infections such as blepharitis (inflammation of the eyelid), mostly caused by *Staphylococci* i.e. *Staphylococcus blepharitis*, impetigo contagiosa often caused by *Streptococcus pyogenes*[2].

There are various ophthalmic drugs and antibiotics that have been found in extensive application in the treatment of eye infections, which may be classified into two groups: therapeutic and diagnostic examples which include: atropine, hematropine, pilocarpine, etc for treating ocular injury and diseases[6]. Antibiotics such as chloramphenicol, tetracycline, streptomycin (mostly in the form of drops and ointments) are also very useful as therapeutic agents of eye infections. However, the increasing resistance of microorganism and other pathogenic microbes to conventional antibiotics resulted in a strong effort to develop antimicrobial compounds with new mechanisms of action. Hence, this research work of confirming the microbial properties of onion (*Allium cepa*) on some microorganisms implicated in ocular infections is of great relevance [6]. Traditional healers have long used plant to prevent or cure infectious diseases; western medicine is trying to duplicate their success. Plants are rich in a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids and flavonoids which have been found invitro to have antimicrobial properties. This research was designed to find out the antibacterial activity of onion (*Allium cepa*) on bacteria associated with ocular infections [6].

MATERIALS AND METHODS

Sample Collection

The onion bulbs used were bought from the vendors in Sokoto Markets. The onions were obtained in a newly clean polythene bags. Eye swab samples were collected from different patients at Specialist Hospital and Usmanu Danfodiyo Teaching Hospital Sokoto. The samples were collected using sterile swab sticks. All the samples were transported to Microbiology Laboratory of Usmanu Danfodiyo University Sokoto for analysis.

Isolation and Identification of the Organisms

Using the collected swab sample sticks, streaking method was used for the process. Isolation was made from each swab sample on plates containing Nutrient agar. The agar plates were incubated aerobically at 37°C for 24 hours. In cases of mixed cultures, the isolates were sub cultured to get pure isolates.

Subculture

Pure culture of bacteria was isolated by streak plate method. Separate colony on agar plate was picked with sterilized wire loop and was used to inoculate several portions and subsequently to three other areas on the plate. The plates were incubated at 37°C for 24hrs [3].

Gram staining

A drop of distilled water was placed on a grease free glass slide and a colony in the nutrient Agar plate was picked with a heated wire loop after allowing it to cool and emulsified to make a desirable smear. The

glass was passed over a burning flame for three times to heat fix. The smear was flooded with crystal violet and allowed for 60sec, it was then rinsed with distilled water. Lugol's iodine was added to the smear for 60sec and rinsed with distilled water. It was then decolorized with acetone and rinsed immediately with distilled water. The smear was counterstained with safranin for 1-2 minutes and rinsed with distilled water. The smear was then allowed to air dry after which oil immersion was added and viewed under a microscope using x100 objectives lens [3].

Biochemical Tests

Catalase Test

This demonstrates the presence of an enzyme catalase that catalyses the enzymatic breakdown of hydrogen peroxide to water with subsequent release of oxygen gas seen as bubbles. The container containing the hydrogen peroxide solution was shaken to expel the dissolved oxygen. One drop of the solution was dropped on a loop full of 24hr-old inoculums of the slide indicate a positive reaction whereas in negative reaction no gas bubbles was observed [4].

Coagulase Test

A drop of physiological saline was put on a clean glass slide, followed by making a smear of 24hr-old isolate of the test organism. Then a drop of human plasma was added unto it to makes a suspension. Clumping indicates positive result which implies the ability of the test organism to produce coagulase, an enzyme that coagulates blood plasma. While in a negative result no clumping was observed [4].

Citrate Utilization Test

The test aids in identification and differentiation of enterobacteria. It is based on the ability of an organism to utilize citrate as the sole source of carbon and energy and ammonium salt as the sole source of nitrogen for growth. Simmon's citrate agar slant was inoculated with large inoculums of the isolate and incubated at 37°C for 24hrs. Presence of growth leads to the increase in turbidity and pH of the medium resulting in the change of colour from green to blue for positive reaction. Presence of the original green colour indicates that citrate was not utilized and this gives a negative reaction [4].

Indole Test

This test demonstrates the ability of certain bacteria to decompose the amino acid (tryptophan) to indole, a volatile substance that rises and accumulates at the surface of the medium. The isolates were grown for 24-48hrs on peptone water at 37°C then 0.5ml of the Kovac's reagent was added to the culture inside the test-tube which was shaken gently. Production of indole was confirmed by the formation of red ring colouration on the surface of the medium. An indication of the reaction between the produced indole and Kovac's

reagent is a positive test. In a negative test, red coloration at the surface was not produced [8].

MR (Methyl Red)

The methyl red test was employed to detect the production of sufficient acid during the fermentation of glucose and the maintenance of condition, the pH of a 24hr-old culture was sustained below a value of about 4.5 as shown by a change in colour of the incubation. The Voges-Proskauer test was ran to test the ability of the organism to produce acetylmethylcarbinol. The tests were ran using the commercially prepared MR medium.

Large inoculum of the test organism was picked and inoculated into the prepared and sterilized test-tubes. Three [3] drops of methyl red indicator was added into the culture to the test-tube and the test-tube was shaken. A red colour indicates a positive reaction while a yellow colour, indicates a negative result [4].

Triple Sugar iron Agar (TSI Agar)

The medium contains three sugars namely: glucose, sucrose, and lactose as well as amino acid. Gas production is also detectable on this medium, likewise the production of hydrogen-sulphide (H₂S). Some enterobacteria and other urinary tract pathogens ferment all the three sugar present and produce sufficient acid which change the colour of the indicator to yellow.

Other microorganisms ferment only one of these sugars, producing little amount of acid indicated by yellow colour at the bottom. The sugars and protein were attached oxidatively to release ammonia, which turn the indicator to pink-red in the medium and the production of media along the stabbed line. Gas production can also be detected by the production of bubbles cracks or by separation of the medium.

The freshly obtained 24hr-old culture from the plate was picked and streaked on the surface of the slope and stabbed in the bottom by using straight line wire-loop and incubate at 37⁰C for 24 hrs. It was then observed for the above reaction [4].

Urease Test

The production of ammonium gas leads to the change in the pale yellow colour of the urease medium incorporated with about 0.3ml of urea bright pink colour.

Heavy inoculum was obtained from the test isolates and inoculated on a slope of the medium in universal bottle and was incubated for 24hrs at 37⁰C. A pink-red colour indicates a positive test while a negative result retains the colour of the urease medium [4].

Carbohydrate Fermentation Test

The Fermentation medium (containing 0.5% of the chosen carbohydrate) was inoculated with two loops full of the isolate suspension. The tubes containing

inverted Durham tubes were then incubated at 37⁰C and observed 24 hourly for two days. Growth, acid and or gas production were observed and recorded. The various carbohydrates tested were mannitol and Raffinose.

Preparation of the Extract

Two different fresh onion extracts were made from onion bulbs using sterilized distilled water. The extracts were cold water extract and fresh onion extracts.

For the preparation of cold water extract, 5 onion bulbs were peeled, rinsed with distilled water and cut into small pieces. The moisture of the onion was removed by drying inside oven at constant temperature of 60⁰C. The resulted onion crimps was grinded using mortar and pestle. 11.9g of the onion powder was weighed and was dissolved into a conical flask containing 100mls of distilled water. The mixture was sterilized using autoclave at 121⁰C for 15 minutes and was left to soak for 2 days after which it was filter aseptically and was transferred in to sterile bottle.

The fresh onion extract was prepared by blending five bulbs of onion that was surface sterilized by using 70% alcohol and rinsed-off with sterile distilled water. The liquid content was sieved aseptically and collected with sterile bottle. All extracts were properly stored at 4⁰C throughout the experimental period.

Determination of antagonistic activities of *Allium cepa*

Disc diffusion method was used. The discs were prepared with Whatman paper which is around 6mm in diameter. The paper was sterilized using hot air oven at 160⁰C for 2 hours. The prepared paper was aseptically soaked into the extract. Sterile nutrient agar was aseptically poured into sterile Petri dishes and allowed to solidify. Test organisms were taken from overnight culture to inoculate the dried agar plates by streaking. Sterile forceps was used and discs were aseptically place on the surface of Agar plates. Ciprofloxacin was used in the nutrient agar plates as a control and the plates were incubated at 37⁰C for 24 hours. The plates were examined for zones of inhibitions.

Minimum Inhibitory Concentration

One in one dilution method was used with slight modification. 10mls of fresh water extract was made from 10mls each of sterile distilled water inside series of sterile distilled water inside series of sterile 25mls bottles (a set of 8 plates) and war allowed to solidify. The plates were then seeded with the isolates by after which they were incubated at 37⁰C for 24 hours.

RESULTS

A total number of Eighteen (18) samples were collected from patient suffering from ocular infections. Four (4) bacteria species were identified, they includes *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus pyogenes*, *Streptococcus pneumonia*. Table 1 indicated the percentage frequency of occurrence. From the result obtained, *Escherichia coli* has the highest percentage frequency of (33.3) followed by *Staphylococcus aureus* and *Streptococcus pneumonia* with (26.7%) while *Streptococcus pyogenes* had the lowest with (13.3) percentage frequency. Table 2 showed the antibiotic sensitivity test of bacterial isolates. From the result obtained, all the organisms were sensitive to ciprofloxacin, *Streptococcus pyogenes* was highly sensitive with 20mm followed by *Staphylococcus aureus* with 19 mm then *Escherichia coli* and *Streptococcus pneumonia* were less sensitive with 17mm. Table 3 showed the antagonistic action of the onion extracts. The cold water onion extract exerted no inhibitory effects on the bacterial isolates. However, *Streptococcus pyogenes* and *Staphylococcus aureus* were sensitive to the fresh onion extracts with the zone of inhibition ranging from 17mm in *Staphylococcus aureus* to 20mm in *Streptococcus pyogenes*. Moreover, *Escherichia coli* was sensitive to fresh onion extracts with the zone of inhibition of 15mm in diameter and *Streptococcus pneumonia* had a zone of inhibition of 8mm on fresh onion extracts. Table 4 showed the minimum inhibitory concentration of the fresh onion

extracts for bacterial isolates from ocular infection. Fresh onion extracts inhibited the growth of the organism at 100% concentration and all the organisms grow at the lowest concentration of the fresh onion extracts.

Table 1: Percentage Frequency of Occurrence of Bacteria Isolates (%)

Bacteria species	Frequency of occurrence (%)
<i>Staphylococcus aureus</i>	26.7
<i>Escherichia coli</i>	33.3
<i>Streptococcus pyogenes</i>	13.3
<i>Streptococcus pneumonia</i>	26.7

Table 2: Antibiotic sensitivity test on the bacterial isolate

Isolates	Sensitivity test (mm)
<i>Escherichia coli</i>	17
<i>Staphylococcus aureus</i>	19
<i>Streptococcus pneumonia</i>	17
<i>Streptococcus pyogenes</i>	20

Table 3: Antagonistic effect of the onion extracts on bacterial isolates

Isolates	Cold water onion extract	Fresh onion extract
<i>E. coli</i>	–	15mm
<i>S. aureus</i>	–	17mm
<i>S. pyogens</i>	–	20mm
<i>S. pneumonia</i>	–	8mm

Key: – Indicate no zone of inhibition

Table 4: Minimum inhibitory concentration of fresh onion extract on bacterial isolates

Isolates	Concentrated extract dilution/ml						
	0.50	0.25	0.125	0.0625	0.03125	0.015625	0.0068
<i>S.pyogenes</i>	–	–	–	–	–	+	+
<i>S. aureus</i>	–	–	–	–	–	+	+
<i>S.pneumoia</i>	–	–	–	–	–	+	+
<i>E. coli</i>	–	–	–	+	+	+	+

Key: – indicates no growth
+ indicates growth

DISCUSSION

Among the two different extracts obtained from onion, cold water extract and fresh onion extracts,it was discovered that cold water extract could

not exert any inhibitory effect on all the tested organisms isolated from ocular infection. This indicated the absence of the antibiotic property in cold water onion extracts, this might be as a result of high

temperature 121⁰C used during autoclaving, which may denature the active component [7]. However, the fresh onion extract was observed to inhibit the growth of the bacteria isolated from ocular infection. This finding is in agreement with the documentation of [10], that extract from onion and garlic has antibacterial properties. Hence, comparing the antibacterial activity of the onion extracts with commercially available antibiotic (ciprofloxacin) used in this research; it was observed that both have antibacterial effect on ocular infections. *Streptococcus pyogenes* and *Staphylococcus aureus* were sensitive to the fresh onion extracts with the zone inhibition ranging from 17mm in *Staphylococcus aureus* to 20mm in *Streptococcus pyogenes*. Moreover, *Escherichia coli* was sensitive to fresh onion extracts with the zone of inhibition of 15mm in diameter and *Streptococcus pneumoniae* has a zone of inhibition of 8mm on fresh onion extracts.

However, since it had earlier been confirmed by [7], that allicin has an antibiotic property, and also [10] affirmed the antibacterial property of onion, it could then be inferred that some varying quantities of allicin was present in the onion extract and largest quantity of it that was present in fresh onion extract conferred its profound inhibitory effect on all the bacterial isolates as it could be observed from the results of minimum inhibitory concentrations obtained in the research.

CONCLUSION

From the research conducted it was observed that there was no antibacterial activity on the test isolates observed with cold water extracts. Further research is encouraged on the pharmacokinetics of the active components; invitro study of the effectiveness of the purified active component, measuring dosage and some other parameters, on the various implicated organisms from ocular infections.

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