



Antibacterial Activity of Fresh Red and White Onion (*Allium cepa*) Extract against Some Drug Resistant Bacteria

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Authors' contributions

This work was carried out in collaboration among all authors. Author KZA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors LOA and OOW managed the analyses of the study. Authors SC and IOS managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Antibiotics resistance is currently one of the major challenges in the health care system. The antimicrobial properties of some herbs have been used in the treatment of infectious diseases as well as disinfection of surfaces. This in a way helps overcome microbial resistance arising from indiscriminate use of synthetic antimicrobial agents for similar purpose. Some antibiotic resistant

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bacteria- *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Escherichia coli* isolated from cooking wares in homes were investigated using agar well diffusion and agar dilution method to test for the antibacterial activity of fresh *Allium cepa* (onion) extract. All were susceptible to the fresh white and red onion extract except *Staphylococcus aureus* which was susceptible to only the white onion extract. The diameter of zones of inhibition ranged from 2 mm-35 mm. The Minimum Inhibitory Concentration (M. I. C.) and the Minimum Bacterial Concentration (M. B. C.) values of the fresh onion juices against the test bacteria were low ranging from 3.125% v/v – 25.0% v/v. This study indicates that the fresh raw extracts of *Allium cepa* possess significant antibacterial potency against these antibiotic resistant bacteria.

Keywords: Antibiotic resistance; antibacterial activity; onion extract.

1. INTRODUCTION

The development of antibiotic resistance has become a global public health challenge which is causing ineffectiveness of antibacterial agents leading to increase in diseases and death rate. The antimicrobial properties of some herbs have been used in the treatment of infectious diseases as well as disinfection of surfaces. This in a way helps overcome microbial resistance arising from indiscriminate use of synthetic antimicrobial agents for similar purpose [1-3].

Herbal medicines have become more popular in recent years because it is believed that these do not show as much side toxic effects as synthetic medicines. In addition, herbal medicine serves as a cheap and readily available form of medication [5].

A. cepa is the common onion, a biennial garden plant which belongs to the family *Alliaceae* [6]. Its bulbs have been used as food for centuries, and distinctive taste enhance the flavor of meals whilst contributing impressive health benefits. Onion is one of the world's most widely cultivated vegetable [7], with their esteemed culinary and gustatory qualities spanning history and the globe. Equally varied are their health benefits, for they contain a range of phytochemicals with an array of biological effects such as: antioxidative, antimicrobial, antitumor, hypolipidemic, dermatological and cardioprotective properties [8].

Onions are revered to possess antibacterial and antifungal properties; thus the potential use of onion against human pathogenic organisms. It is a rich source of flavonoids, polyphenols, organic sulfur, saponins and many other secondary metabolites, which are mainly responsible for its medicinal activities [9], thus arouse great interest.

Onions are effective against common cold, heart disease, diabetes, osteoporosis, coughs and sore throat [10]. They are also bacteriostatic in action [11]. Certain chemical compounds believed to have anti-inflammatory, anti-cholesterol, anticancer and antioxidant properties such as quercetin are present in onions. They are high in flavonoids which is concentrated on the outer layer of the flesh [11].

Biological scientists have played significant roles in originating meaningful screening and testing models. However there's insufficient data on the comparative antimicrobial properties of the red and white onion as well as the antimicrobial analysis of the fresh juice which is it's most commonly available and used form. Therefore this study investigated the antibacterial action of fresh juice of red and white *A. cepa* (Onion) against some multidrug resistant pathogens isolated from cooking and eating surfaces.

2. MATERIALS AND METHODS

2.1 Sample Collection

The red and white onion bulbs (*A. cepa*) were bought from the vendors in Ogige market, Nsukka. Enugu State, Nigeria.

2.2 Collection of Test Bacteria Samples

Samples were collected with sterile swab sticks moistened with normal saline. Swabbed samples were taken from cooking slabs or tables, kitchen sinks and dining tables.

2.3 Isolation of Test Organisms

MacConkey agar was used for isolation of the bacteria. The swab sticks used to collect the samples were smeared or swabbed on the surface of the dry MacConkey agar. Using a sterile wire loop, the primary inoculum was

streaked across the surface of the agar. The plates were incubated at 37°C for 24-48 hours.

2.4 Sub-culture of Test Organisms

Nutrient agar was used for sub-culture of the test organisms. After 24 hrs, a sterile wire loop was used to pick samples from different colonies from the test organism culture plates. The test sample was then inoculated and streaked on surface of the prepared nutrient agar. The sub-culture was stored in Bijou bottles at 37°C for 18 hours and stored in a refrigerator for further analysis.

2.5 Identification and Characterization of Test Organisms

The following tests were carried out in order to identify and characterize the bacteria colonies isolated from the selective media.

2.6 Gram Staining

Materials: A 24-hr old culture of the test organism, grease free slides, wireloop, crystal violet, lugol's iodine, acetone alcohol, safranin, Bunsen burner, normal saline, clean running water, wash bottle, blotting paper, microscope, immersion oil.

Procedure: The colonies got from the sub-culture media were spread on a clean grease free slide. This was done by placing a drop of normal saline on the slide and a sterile wire loop was used to take a pinch of the organism from the sub-culture media, the pinch was placed in the normal saline and dissolved using the wireloop to make a thin smear. The slide was allowed to air dry and then it was heat fixed by passing over a Bunsen flame.

The slides containing the thin smears of the test organisms were then placed on a staining rack. The smears were first covered with crystal violet (serves as the primary stain) for 60 seconds, and then washed off with running water from a wash bottle. The smears were again covered with lugol's iodine (which is a mordant) for 60 seconds after which it was washed off with running water. The smear was then decolorized with acetone alcohol for few seconds, and then washed off immediately with running water. The smear were then covered with safranin (a counter stain) for another 60 secs after which it was washed off with running water and blotted dry with a blotting paper. The smears were

examined microscopically using oil immersion under x100 objectives lens.

2.7 Catalase Test (Slide Method)

Materials: 3% H₂O₂, 24-hr old culture of the test organism, glass rod, absolute ethanol, slides.

Procedure: A small portion of the test organism culture is transferred to a clean glass slide using a sterile glass rod. The culture was then covered with a drop of 3% H₂O₂ and examined for bubble production within 30 seconds.

2.7.1 Coagulase test (slide method)

Materials: human plasma, grease free slide, 24-h old culture of bacteria, normal saline, wireloop.

Procedure: Normal saline was dropped on two grease free slides; a loopful of the 24-hour old culture was emulsified on the drops of normal saline. A loopful of human plasma was added to one of the slides while the second slide served as control. It was left for 10 seconds and observed for clumping.

2.7.2 Indole test

Materials: A 24-hr old culture of the test organism (in peptone water broth), Kovac's reagent, test tubes.

Procedure: The organism was inoculated in a peptone water broth and incubated for 48 hrs (2 days) at 37°C after which 0.5 ml of kovac's reagent was added to the broth and examined for 10 mins for red coloration on the surface layer.

2.7.3 Lactose fermentation test

Materials: MacConkey agar, a 24-hour old culture of the test organism, petri dishes, incubator, wireloop.

Procedure: Sterilized MacConkey agar was poured into a petri dish and allowed to set. Using a sterile wire loop, the test organism was streaked on the plate and incubated at 37°C for about 24 hours. After 24 hrs, the culture media was observed for color changes. By utilizing the lactose available in the medium, lactose positive bacteria will produce acid, which lowers the pH of the agar below 6.8 and results in the appearance of red/pink colonies. Non- lactose fermenting bacteria cannot utilize lactose and so will use peptone instead. This forms ammonia, which

raises the pH of the agar and leads to the formation of white/colorless colonies.

2.7.4 Citrate utilization test

Materials: Simmon's citrate agar (SCA), a 24-hour old culture of the organism, test tubes.

Procedure: The test organism was inoculated on an SCA in test tube slants and incubated for 24 hours after which it was examined for color change. Color of the agar changes from green to blue is considered to be positive test whereas no color change is a negative test.

2.8 Preparation of Onion Extract

The onions were washed with freshly prepared sterile distilled water. The outer covering of the bulbs was manually peeled off and the fleshy part of the onion was rewashed with freshly prepared sterile distilled water and sterilized with alcohol. Exactly 25.0 gm each of the red and white onion bulbs were blended separately to form juice. The fresh onion juice was used immediately for analysis.

2.9 Standardization of Test Organism

Overnight broth culture of the test bacteria isolates was suspended into sterile nutrient broth and was standardized. The standardization of the test organism was carried out by standing six test tubes each containing 2 mls of normal saline. Serial dilution was done by taking 1ml of the test organism from an overnight broth culture of the test organism and inoculating into the first test tube, making it 3mls, then 1ml was taken from the first test tube and inoculated into the second test tube, making it 3mls, this was serially repeated for the rest of the test tubes. After dilution, their turbidity was compared to McFarland standard of 0.5 which is approximately 1.0×10^6 cfu/mL.

2.10 Antibacterial Analysis of Some Commonly Prescribed Antibiotics

The susceptibility pattern of the isolates to selected commonly prescribed antibiotics: amoxicillin (25 µg), augumentin (30 µg), co-trimoxazole (25 µg), gentamicin (10 µg), nalidixic acid (30 µg), nitrofurantoin (300 µg), ofloxacin (30 µg), and tetracycline (30 µg) was determined using Kirby Bauer disc diffusion method.

Overnight cultures of the isolated bacteria species in nutrient broth were standardized to 1.0×10^6 cfu/mL and flooded over the prepared agar

plates. Excesses were drained off and allowed to dry in a warm incubator for about 15–20 min. Antibiotics impregnated discs were placed on the dried inoculated agar plates aseptically. The plates were left at room temperature for 25 min to allow for the diffusion of the antibiotics into the agar medium and then incubated at 37°C for 24 hrs.

2.11 Antibacterial Analysis of onions

Cup-plate method was used to test the antibacterial activities of the onion juice against the test bacteria isolates. Overnight broth culture was diluted appropriately using McFarland scale (0.5 McFarland which is about 10^6 cfu/mL). The molten sterile nutrient agar (20 mLs) was poured into sterile petri dish and allowed to set. The sterile nutrient agar plates were flooded with 1.0 mL of the standardized inoculum and the excess was drained off. A sterile cork borer was used to bore equidistant holes into the agar plate. One drop of the molten agar was used to seal the bottom of the bored hole, so that the extract will not sip beneath the agar. 0.1 ml of the juice was added to fill the bored holes. A control was prepared by putting 0.1 ml of freshly prepared sterile distilled water in one of the bored hole. One hour pre-diffusion time was allowed, after which the plates were incubated at 37°C for 24 hrs. The zones of inhibition were then measured in millimeter (mm). The above method was carried out in quadruplicates and the mean of the quadruplicate results were calculated.

2.12 Minimum Inhibition Concentration (M.I.C)

The M.I.C of the fresh onion juice against the test bacteria was determined using the broth dilution method. One (1.0) mL of the juice was added to 1 mL of nutrient broth and subsequently transferred. One (1.0) mL from the first test tube to the next, for up to the seventh test tube in serial dilution. Then 1 mL of standardized overnight broth culture of test organism (1.0×10^6 cfu/mL) was inoculated into each test tube and thoroughly mixed. The test tubes were then incubated at 37°C for 24 h. The tube with the lowest dilution with no detectable growth was considered as the M.I.C.

2.13 Minimum Bactericidal Concentration (M.B.C)

Minimum Bactericidal Concentrations were determined by taking 0.1 mL of the content of last tubes showing no visible growth and

inoculated into sterile nutrient agar containing inactivating agents 3% v/v Tween 80 plates. These plates were then incubated at 37°C for 24 - 48 hours after which they were examined for the presence or absence of growth.

3. RESULTS

Result of Gram Staining: The gram-stained smears showed Gram-negative rods in singles, Gram-positive cocci in clusters and Gram negative coccobacilli. This suggests they could be *E. coli*, *S. aureus* and *P. aeruginosa* respectively. Further biochemical tests were carried out to confirm these organisms.

Table 1 show that *S. aureus* is a gram-positive, catalase positive and coagulase positive cocci. *E. coli* is a gram-negative, lactose fermenting bacilli, which can split tryptophan into indole, pyruvic acid and ammonia. While *P. aeruginosa* is a Gram negative, catalase positive, citrate positive rods, and can split tryptophan into indole, pyruvic acid and ammonia.

Table 2 shows that among the eight most prescribed antibiotics used in this experiment, *S. Aureus* is sensitive to Cotrimoxazole (30 Kg), Gentamicin (10 Kg), Nitrofuratoin (30 Kg), Ofloxacin (30 Kg). *E. coli* is sensitive to Cotrimoxazole (30 Kg), Nalidixic acid (30 Kg),

Nitrofuratoin (30 Kg), Ofloxacin (30 Kg). While *P. aeruginosa* is sensitive to Cotrimoxazole (30 Kg), Gentamicin (10 Kg), Ofloxacin (30 Kg).

Table 3 shows the result of the susceptibility test of the bacteria strains to the onion juice showed that the zone of inhibition of the fresh white onion juice was wider than that of the fresh red onion juice. The susceptibility pattern of the test bacteria isolates to the fresh raw extract of the red and white onion juice. The zone of inhibition was measured in millimeter (mm) with an error limit of ± 0.5 . The experiment was carried out at room temperature and the sensitivity culture was incubated at 37°C for 24hours.

4. DISCUSSION

The presence of multidrug resistant bacteria on kitchen cooking surfaces and dining tables in homes is of great concern due to the fact that these are where most things that have to do with food preparation, dishing and eating in homes are centered. The sources of these bacteria isolates might have been from improper washing cleaning of the surfaces, leftover food crumbs from previous meals, spilling of drinks. Another source of contamination of this cooking surfaces and dining tables can be from unclean or untreated cleaning towels used in cleaning these surfaces.

Table 1. Results of confirmatory tests for *E. coli*, *S. aureus* and *P. aeruginosa*

Test characterization	<i>S. aureus</i>	<i>E. coli</i>	<i>P. aeruginosa</i>
Catalase	+	-	+
Coagulase	+	-	-
Indole	-	+	+
Lactose fermentation	-	+	-
Citrate utilization	-	-	+

KEY: - = Negative, + = Positive

Table 2. Antibiotic sensitivity pattern of the bacteria isolates from cooking surfaces and dining tables

Antibiotics	Test bacteria isolates		
	<i>S. aureus</i>	<i>E. coli</i>	<i>P. aeruginosa</i>
AMO (25 Kg)	R	R	R
AUG (30 Kg)	R	R	R
COT (30 Kg)	S	S	S
GTC (10 Kg)	S	R	S
NAL (30 Kg)	R	S	R
NFT (300 Kg)	S	S	R
OFL (30 Kg)	S	S	S
TET (30 Kg)	R	R	R

Key: AML – Amoxycilin, AUG – Augmentin, COT – Cotrimoxazole, GTC – Gentamicin, NAL– Nalidixic acid, NFT – Nitrofuratoin, OFL – Ofloxacin, TET – Tetracycline, S – sensitive, R – resistant

Table 3. Susceptibility of the test bacteria isolates to the plant juices

Test bacteria	Zones of inhibition (mm) ± 0.5							
	Red onion				White onion			
	A	B	C	D	A	B	C	D
<i>S. aureus</i>	NIL	NIL	NIL	NIL	NIL	2	3	NIL
<i>E. coli</i>	12	8	14	15	29	15	14	16
<i>P. aeruginosa</i>	24	16	20	26	28	27	35	26

Key: NIL= no zone of inhibition

Table 4. The minimum inhibitory concentration (m.i.c) and minimum bacteriocidal concentration (m.b.c) of the plant juices

Test bacteria	Red onion		White onion	
	M. I. C. (%v/v)	M. B. C.(%v/v)	M. I. C.(%v/v)	M. B. C.(%v/v)
<i>S. aureus</i>	NA	NA	NA	NA
<i>E. coli</i>	50	NA	25	50
<i>P. aeruginosa</i>	3.125	6.25	3.125	6.25

KEY: NA= no activity

A. cepa (onion) showed antibacterial activity against multidrug resistant *P. aeruginosa*, and *E. coli*, with the white onion showing more antibacterial activity than red onion against the organisms. This antibacterial activity of onion juice can be attributed to the presence of flavonoids and polyphenols which has been reported to have broad spectrum of antibacterial activity [12-14]. Volatile oil of onion has been shown to be highly effective against some gram positive bacteria [14]. The inhibitory effect of onion oil was demonstrated against the growth of various isolates of bacteria representing Gram-positive (four isolates), Gram-negative (four isolates) species and nine different species of dermatophytic fungi. The results showed that onion oil was highly active against all Gram-positive bacteria tested as well as *Klebsiella pneumoniae*, while all the fungi tested were inhibited at different concentrations [15]. The antibacterial activity of onion extracts on *Streptococcus mutans* and *Streptococcus sobrinus*, showed that the onion extracts possess bacteriocidal effect on all test bacterial strains [16]. Volatile oil of onion has also been shown to be highly effective against the growth and aflatoxin production of some fungi genera, including *Aspergillus niger*, *Brettanomyces anomalus*, *Candida albicans*, *Cladosporium werneckii*, *Fusarium oxysporium*, *Geotrichum candidum* and *Saccharomyces cerevisiae* [17]. In different studies, onion showed protective effect against pathogenic bacteria (*Escherichia coli*, *Pseudomonas spp*) and fungi (*Candida sp*, *Cryptococcus sp* and *Malassezia sp*) in dose dependent manner [18].

Aqueous onions extract (AOE) showed dose-dependent protective effect against pathogenic yeasts and dermatophytes [19]. AOE inhibits the growth of *Trichophyton rubrum* and *T. mentagrophytes* by affecting their morphology at cellular and sub cellular level as it disrupts the cell membrane and other membrane-bound structures [20]. Welsh onion extracts have been reported to exert more inhibitory activity towards aflatoxin production by some fungi genera (like *Aspergillus* and *Candida*) than the preservatives sorbate and propionate at pH values near 6.5, even at concentrations 3-10 folds higher than maximum level used in foods [21]. Benkeblia [22] reported that the essential oil of onion possessed significant antimicrobial activity. In another study, it was found that onion inhibit the pro-inflammatory messenger and bacterial growth due to the presence of various organic sulfur compounds [23]. The ethyl acetate subfraction of onion showed the prevention against microbes. This may have been due to the presence of flavonoids in these extracts [24]. Organosulphur compounds in onions have also been reported to be responsible for antibacterial effects of onion extract against oral pathogenic bacteria causing dental caries [25]. When grated and kept at 37 degrees for more than two days, extract from onions lose their potency and the enzymatic destabilization of thiosulfates was proposed for the change in effectiveness. This antimicrobial activity of onion is variety dependent [26].

The susceptibility of the test multidrug resistant bacteria especially *P. aeruginosa* and *E. coli* to the onion juice is encouraging because of the

health crisis caused by these organisms all over the world. Some of the advantages that herbal preparations have over the synthetic ones are that they do not act directly on bacteria but create an adverse environment for them, thus threatening their survival and they have also been found to deter the development of resistant strains of microorganisms [27,28].

5. CONCLUSION

Most people especially in the developing countries are suffering from many infectious diseases due to poor hygiene and unavailability or high cost of disinfectants. Therefore there is a need for cheaper, effective and available natural alternatives like onion which exhibits antimicrobial activities. Restaurants and individuals can be encouraged to use more of *A. cepa* (onion) especially the white onion in food preparation to prevent the proliferation of these bacteria isolates. Incorporation of onion especially in its raw form in daily meals will have beneficial effect on human life and health especially for the common man.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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