

**ANTIBACTERIAL ACTIVITY OF FRESH JUICES OF *ALLIUM CEPA* AND *ZINGIBER OFFICINALE* AGAINST MULTIDRUG RESISTANT BACTERIA****G. O. ADESHINA*, S. JIBO, V.E. AGU, J. O. EHINMIDU**

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ABSTRACT

Antibiotic resistant bacteria are one of the major problems challenging the health care system in general. The antibacterial activity of fresh red and white *Allium cepa* (Onion) and *Zingiber officinale* (Ginger) juices against multidrug resistant bacteria viz: *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli* and *Salmonella typhi* isolated from salad was investigated using agar well diffusion and agar dilution methods. All the bacteria except *Staphylococcus aureus* were susceptible to the fresh red and white onion juices with the diameter of zones of inhibition ranging from 15mm – 35mm while the test bacteria isolates were not sensitive to the fresh ginger juice. The Minimum Inhibitory Concentration (M. I. C.) and the Minimum Bactericidal 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 juices of *Allium cepa* possess significant antibacterial potency against multidrug resistant bacteria.



KEY WORDS

Susceptibility, pathogens, *Allium cepa*, *Zingiber officinale*.

INTRODUCTION

It is believed that the history of herbal medicine began with the earliest man. The first written herbal record was in 2800BC and herbal medicine is practised today in countries around the world¹. Some of the advantages of herbs over the formulated drugs are that they typically have fewer side effects and may be less safe to use over time; they are inexpensive compared to formulated drugs and they are readily available².

Allium cepa L. (Onion) which belongs to the family Alliaceae, is also known as 'garden onion' or 'bulb' onion. It is one of the oldest cultivated vegetables in history. It is thought that bulbs from the onion family have been utilized as a food source for millennia³. Above ground, the onion shows only a single vertical shoot; the bulb grows underground, and is used for energy storage, leading to the possibility of confusion with a tuber which it is not³. The leaves are bluish-green and hollow, the bulbs are large, fleshy and firm⁴. Three main varieties of onion are available viz: red, white and purple skinned⁵. Onions are easily propagated, transported and stored. Onions are effective against common cold, heart disease, diabetes, osteoporosis, coughs and sore throat⁶. They also act as bacteriostatic⁷. Certain chemical compounds believed to have anti-inflammatory, anti-cholesterol, anticancer and antioxidant properties such as quercetin are present in onions⁸. They are high in flavonoids which is concentrated on the outer layer of the flesh^{9, 10}. Onions are also high in polyphenols than other allium vegetables¹⁰.

Ginger is the rhizome of the plant *Zingiber officinale* which belongs to the family Zingiberaceae. Ginger is cultivated in India, China, Japan, Indonesia, Australia (Queensland); Sierra Leone, Nigeria; Jamaica and other West Indies islands. Ginger is thought to act directly on the gastrointestinal system to reduce nausea¹¹. It has been used

to treat intestinal infections, especially related with digestive problems⁴. Ginger is effective in the treatment of cough and cold. Gingerols present in ginger have analgesic, sedative, antipyretic, antibacterial and gastrointestinal tract motility effects⁴.

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. Therefore this study intends to investigate the antibacterial action of fresh juice of red and white *Allium cepa* (Onion) and *Zingiber officinale* (Ginger) against multidrug resistant pathogens isolated from salad.

MATERIALS AND METHODS

(i) Test Organisms:

Multidrug resistant *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli* and *Salmonella typhi* isolated from already prepared salads in some certain restaurants on the campus of Ahmadu Bello University, Zaria, Nigeria and in the Zaria metropolis were characterized by standard microbiology methods. The pure cultures were sub-cultured on Nutrient Agar slants and kept at 4°C until ready for the study.

(ii) Collection of Plant Materials:

Allium cepa (red and white onion bulbs) and *Zingiber officinale* (ginger) were bought from Samaru market, Zaria, Nigeria. They were identified in the herbarium of the Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria.

(iii) Extraction of the Plant Materials:

The onion bulbs 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 re-washed with freshly prepared sterile distilled water. Exactly 25.0 gm each of the red and white onion bulbs were blended separately to form juice. The fresh onion juice was immediately analysed for its antibacterial activity.

Fresh ginger rhizomes were thoroughly washed with freshly prepared sterile distilled water. They were cut into pieces to allow for easy blending. Exactly 20.0 gm of the ginger was blended to form juice. The fresh ginger juice was immediately analysed for its antibacterial activity.

(iv) Preparation of Inoculum ¹²:

Exactly 18 hour broth culture of the test bacteria isolates was suspended into sterile nutrient broth. They were standardized according to National Committee for Clinical Laboratory Standards (NCCLS) by gradually adding normal saline to compare their turbidity to McFarland standard of 0.5 which is approximately 1.0×10^6 cfu/ml.

(v) Antibacterial Analysis ^{12, 13, 14}:

The susceptibility pattern of the isolates to selected commonly prescribed antibiotics: amoxicillin (25 µg), Augmentin (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 cells/ml and flooded over the prepared Mueller- Hinton 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 hr. Interpretation of the isolates as sensitive or resistance were based on zones of inhibition according to

current NCCLS standards in accordance with WHO requirements.

Cup-plate method was used to test the antibacterial activities of the onion juice and the fresh ginger juice against the test bacteria isolates. Eighteen hour broth cultures were diluted appropriately using McFarland scale (0.5 McFarland which is about 10^6 cfu/ml). The molten sterile nutrient agar (20 ml) 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 (No. 4) was used to bore equidistant cups 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. 0.1ml 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 bored hole. One hour pre-diffusion time was allowed, after which the plates were incubated at 37°C for 18 h. The zones of inhibition were then measured in millimeter. The above method was carried out in triplicates and the mean of the triplicate results were taken. The same method was carried out on the ginger juice.

The M.I.C of the fresh onion and ginger 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. Then 1 ml of standardized 18 h broth culture of test organism (1.0×10^6 cfu/ml) was inoculated into each test tube and thoroughly mixed on a vortex mixer. 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.

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% $\frac{1}{v}$ Tween 80 plates. These plates were then incubated at 37°C for 24 - 48 hours after which they



were examined for presence or absence of growth.

The sensitivity pattern of the bacteria isolated from salad to some commonly prescribed antibiotics is as shown in Table 1.

RESULTS

Table 1
Antibiotic sensitivity pattern of the bacteria isolates from salad.

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

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

The result of the susceptibility test of the bacteria strains to the onion and ginger juice showed that the zone of inhibition of the fresh white onion juice was higher than the other juices (Table 2).

Table 2
Susceptibility of the test bacteria isolates to the plant juices

Test bacteria	Zones of Inhibition (mm)		
	White Onion	Red Onion	Ginger
<i>Ps. aeruginosa</i>	35 ± 0.1	30 ± 0.2	NI
<i>Staph. aureus</i>	NI	NI	NI
<i>E. coli</i>	19 ± 0.5	15 ± 0.2	NI
<i>S. typhi</i>	35 ± 0.2	28 ± 0.1	NI

Key: NI = No Inhibition

The M.I.C. and M.B.C. values are as shown in Table 3. No antibacterial activity was observed in fresh ginger juice against the test bacteria isolates.



Table 3
The Minimum Inhibitory Concentration (M.I.C.) and Minimum Bactericidal Concentration (M.B.C.) of the plant juices.

Test bacteria	White Onion		Red Onion		Ginger	
	M. I. C. (% ^v / _v)	M. B. C. (% ^v / _v)	M. I. C. (% ^v / _v)	M. B. C. (% ^v / _v)	M. I. C. (% ^v / _v)	M. B. C. (% ^v / _v)
<i>Ps. aeruginosa</i>	3.125	6.25	3.125	6.25	NA	NA
<i>Staph. aureus</i>	NA	NA	NA	NA	NA	NA
<i>E. coli</i>	25	50	50	NA	NA	NA
<i>S. typhi</i>	3.125	6.25	6.25	12.5	NA	NA

Key: NA = No Activity

DISCUSSION

The presence of multidrug resistant bacteria in already prepared salad in restaurants is of great concern due to the influx of people who patronize these places in search of fast food. The sources of these bacteria isolates might have been from improper washing of hands, improper treatment of the salad ingredients especially the vegetables which are liable to faecal contamination from either soil or water. Another source of contamination of the salad can be from unclean or untreated water used in washing the salad ingredients.

The 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 activity¹⁵. Polyphenols from plants have been reported to have antibacterial activity¹⁶.

The susceptibility of the test multidrug resistant bacteria especially *Pseudomonas aeruginosa* and *Salmonella typhi* 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 microorganisms¹⁷.

The reason for the ineffectiveness of raw ginger juice against the test bacteria is not known. The result obtained from the antibacterial activity of ginger against the test bacteria strains is contrary to those of Chrubasik *et al.*,¹⁸ and Chandarama *et al.*,¹⁹ who reported that ginger showed antibacterial activity against these bacteria.

CONCLUSION

Allium cepa L. (onion) showed antibacterial activity against multidrug resistant *Pseudomonas aeruginosa*, *Salmonella typhi* and *E. coli*, while *Zingiber officinale* (ginger) did not show antibacterial activity against the organisms. White onion showed more antibacterial activity than red onion against the organisms. Restaurants and individuals can be encouraged to use more of *Allium cepa* L. (onion) especially the white onion in the preparation of salads in order to prevent the proliferation of these bacteria isolates.



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