Antibacterial Activity Of Allium cepa (Onions) And Zingiber officinale (Ginger) On Staphylococcus aureus And Pseudomonas aeruginosa Isolated From High Vaginal Swab

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Citation

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Abstract

The antibacterial activity of raw and aqueous extracts of Allium cepa (onions) and Zingiber officinale (ginger) against Staphylococcus aureus and Pseudomonas aeruginosa, (from high vaginal swab) that are common cause of nosocomial (hospital-acquired) and urinary tract infections was investigated using the cup-plate diffusion method. The result showed that ethanolic extract of ginger gave the widest zone of inhibition against the two test organisms at the concentration of 0.8gml-1. However, Pseudomonas aeruginosa was more sensitive to the extract of onion bulbs compared to Staphylococcus aureus. It was also observed that the solvent of extraction and its varying concentrations affected the sensitivity of the two organisms to the plant materials. The minimum inhibitory concentration (MIC) of ginger extracts on the test organisms ranged from 0.1gml-1 - 0.2gml-1, showing that ginger was more effective and produced marked inhibitory effect on the two test organisms compared to the onion extracts. This investigation indicates that, though both plants had antibacterial activity on the two test organisms, ginger had more inhibitory effect thus confirming their use in folk medicine.

INTRODUCTION

Pseudomonas aeruginosa is the epitome of an opportunistic pathogen of humans. The bacterium almost never infects uncompromised tissues, yet there is hardly any tissue that it cannot infect if the tissue defenses are compromised in some manner. Pseudomonas exploits most defenses to initiate an infection. It causes urinary tract infections, respiratory system infections, dermatitis, soft tissue infections, bacteremia, bone and joint infections, gastrointestinal infections and a variety of systemic infections, particular in patients with severe burns and in cancer and AIDS patients who are immuno-suppressed. Other diseases it causes are pneumonia, endocarditis, chronic lung infections and septicemia and the bacterium is the 4th most commonly isolated nosocomial pathogen accounting for 10.1 percent of all nosocomial infections$_1$.

Staphylococcus aureus causes a variety of suppurative (pus-forming) infections and toxinoses in humans. Staphylococcus aureus causes superficial skin lesions such as boils, styes and more serious infections such as osteomyelities and endocarditis. It is the major cause of nosocomial infections of surgical wounds and infections associated with indwelling medical devices. Staphylococcus aureus causes food poison by releasing enterotoxins into food and toxic shock syndrome by the release of super antigens into the blood stream$_2$.

Medicinal plants may be defined as any plant that can be put to culinary or medicinal use and include those we associate with, orthodox drugs such as fox glove and opium poppy, as well as everyday plants, such as garlic$_3$. We shall not forget that all drugs of the past were substances with a particular therapeutic action extracted from plants. More and more researchers find that food and their individual constituents perform similar fashion to modern drugs and sometimes better without the dreaded side effects$_4$. The use of herbs and medicinal plants as the first medicines is a universal phenomenon. Every culture on earth, through written or oral tradition, has relied on the vast variety of natural chemistry found in healing plants for their therapeutic properties$_4$.

The onion 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 Millennia. Onion consists of its herbaceous plant part and its edible bulb part. It is probably a native to southwestern Asia[5]. The leaves are bluish –green and hollow. The bulbs are large, fleshy and firm. There are three main varieties- white, red and purple skinned[6]. The relative pungency of onion has both genetic and environmental components. Sulphur compounds in onions have also been shown to be anti-inflammatory both by inhibiting formation of thromboxanes and by inhibiting the action of platelet-activating factor (PAF). Thiosulfinates condition anti-thrombotic benefits, including antioxidant activity[7,8], reduced serum cholesterol and enhance in vitro platelet activity[9]. This later effect is important for cardiovascular health by reducing the probability that platelets aggregate in the blood, a major cause of heart attacks and strokes[10]. Hence, thiosulphinates found in onion have been shown to inhibit in-vitro platelet aggregation[11,12].

Flavonoids are a second class of health enhancing compound produced by onions, an example is quercetin. Flavonoids are chemical compounds active against microorganisms. They have been found in-vitro to be effective antimicrobial substance against a wide array of microorganisms[13].Ginger, consists of the fresh or dried roots of Zingiber officinale. In humans, ginger is thought to act directly on the gastrointestinal system to reduce nausea[14]. Traditionally, ginger has been used to treat intestinal infections, especially related with digestive problems. Equally, its antibacterial 'power' is effective against preventing numerous intestinal problems that take place as a result of the alteration of the intestinal flora. This is ideal to avoid the formation of ulcers by eliminating the Helicobacter pylori, a bacterium whose secretions of ammonia are responsible for many ulcers, especially those of the duodene, and for other stomach problems like gastritis, since the plant is able to neutralize the excess of gastric acid that is another of the causes that favours the formation of ulcers[7].

The gingerols have analgesic, sedative, antipyretic, antibacterial and gastrointestinal tract motility effects. Ginger has the capacity to eliminate harmful bacteria, such as Escherichia coli, responsible for most of the diarrhoea, especially in children[15]. Ginger eases both diarrhoea and constipation; hence it should have impact on the growth of Bacillus cereus, which mainly causes diarrhoea and nausea. It has been shown to reduce the stickiness of blood platelets, hence may help reduce risk of artherosclerosis[15,16].

MATERIALS AND METHODS

TEST ORGANISM CONFIRMATION

The test organisms Staphylococcus aureus and Pseudomonas aeruginosa isolated from high vaginal swab (HVS) samples from patients with urinary tract infection was collected from the Microbiology Laboratory Unit of Ebonyi State University Teaching Hospital Abakaliki (EBSUTH). Similarly, a few tests were carried out to reconfirm the test organisms including gram staining, catalase test, coagulase test, oxidase test and motility test. The pure cultures were subcultured on Nutrient Agar slants and preserved in the refrigerator at 4°C until required for the study.

COLLECTION AND IDENTIFICATION OF PLANT MATERIALS

The plant materials, onion bulbs (Allium cepa) and ginger (Zingiber officinale) were purchased from Abakpa main market Abakaliki. It was confirmed that the cultivation was in the Northern part of Nigeria, from where greater quantities were purchased by the sellers. The plants were identified by Prof. C. U. Okeke (Taxonomist) of Applied Biology Department of Ebonyi State University, Abakaliki, Nigeria.

EXTRACTION OF THE PLANT MATERIALS

ONION EXTRACTION

The onions were washed with clean sterile distilled water and allowed to air dry for one hour. The outer covering of the onion were manually peeled off. The onion bulbs being separated were washed and extracted in the following ways:

1. Exactly 200g of fresh onion bulbs were blended into fine powder and soaked in 100mls of distilled water for 24hrs. The pulp obtained was left in a clean, sterile glass container and shaken vigorously to allow for proper extraction and it was filtered using a sterile muslin cloth after which the extract was obtained, air-dried and stored below ambient temperature until required.

2. Exactly 200g of fresh onion bulbs were blended and soaked in 100mls of hot water for 24hrs; the resultant juice was extracted, air-dried and stored as in (1) above.

3. Exactly 200g of fresh onion bulbs were blended and soaked in 100mls of 95% ethanol for 24hrs and the extract was obtained, air-dried and stored as in (1) above.

4. Exactly 200g of fresh onion bulbs were blended and the raw juice was extracted after standing in a clean glass contained for 24hrs, it was extracted using a sterile muslin cloth and the extract was air-dried and stored as in (1) above.

GINGER EXTRACTION

The ginger rhizomes were washed with clean sterile distilled water and allowed to air-dry for one hour. Then the outer covering of the ginger were manually peeled off and the ginger was washed again and extracted using the following procedures:

1. Exactly 200g of fresh ginger were blended into fine powder and soaked in 100mls of distilled water for 24hrs. The pulp obtained was left in a clean, sterile glass container and shaken vigorously to allow for proper extraction and it was filtered using a sterile muslin cloth after which the extract was obtained, air-dried and stored below ambient temperature until required.
2. Exactly 200g of fresh ginger were blended and soaked in 100mls of hot water for 24hrs; the resultant juice was extracted, air-dried and stored as in (1) above.
3. Exactly 200g of fresh ginger were blended and soaked in 100mls of 95% ethanol for 24hrs and the extract was obtained, air-dried and stored as in (1) above.
4. Exactly 200g of fresh ginger were blended and the raw juice was extracted after standing in a clean glass contained for 24hrs, it was extracted using a sterile muslin cloth and the extract was air-dried and stored as in (1) above.

PREPARATION OF MCFARLAND STANDARD

Exactly 0.5 McFarland equivalent turbidity standard was prepared by adding 0.6ml of 1% barium chloride solution (BaCl2. 2H2O) to 99.4ml of 1% sulphuric acid solution (H2SO4) and mixed thoroughly. A small volume of the turbid solution was transferred to capped tube of the same type that was used to prepare the test and control innocula. This was then stored in the dark at room temperature (25°C). Exactly 0.5 McFarland gives an equivalent approximate density of bacteria $1x10^{-8}$ cfu_{17}.

INNOCULUM PREPARATION BY DIRECT COLONY SUSPENSION METHOD

A small volume of sterile water was poured inside a test tube to which general colonies of the test organisms, taken directly from the plate were emulsified and the suspension was adjusted to match the 0.5 McFarland's standard which has a similar appearance of an overnight broth culture by adding distilled $water_{18}$.

ANTIMICROBIAL SCREENING TEST

The sensitivity of the test organisms, Staphylococcus aureus and Pseudomonas aeruginosa to the extracts of Allium cepa (onions) and Zingiber officinale (ginger) were carried out using the cup-plate diffusion method as described by_{19}. A glass dropper was used to add 0.02mls of the suspension to an already prepared medium. A sterile cotton swab was used to spread by streaking the organisms all over the surface of the medium and allowed to dry for about 5 minutes. Cups of 6mm in diameter were made in the agar using sterile cork borer.

Different dilutions of the plant extracts prepared in the order of $0.1gmI^{-1}$, $0.2gmI^{-1}$, $0.4gmI^{-1}$, $0.6gml^{-1}$ and $0.8gml^{-1}$ respectively were prepared in five different test tubes and placed in a test tube rack. About $0.3gml^{-1}$ of erythromycin was also prepared along side, which served as a positive control. Exactly 0.02ml of each concentration was introduced into each hole on the medium and was allowed to stand on the bench for about one hour for proper diffusion. It was thereafter incubated at 37°C for 24hrs. The sensitive bacteria grew everywhere except in areas around the holes in the medium. Then, the resulting inhibition zones obtained were measured in millimeters and recorded against the corresponding concentrations.

RESULTS

The results of the biochemical tests and the antibacterial activities of the extracts on the test organisms are shown in tables below:

Figure 1

Table 1: Biochemical characteristics of the test organisms

G S	Cat	Coa	Oxi	Mot	Probable isolate
+	+	+	–	–	*Staphylococcus aureus*
–	–	–	–	+	*Psuedomonas aeruginosa*

Key: + = Positive reaction; - = Negative reaction; G S = Gram staining; Cat = catalase; Coa = coagulase; Oxi = oxidase; Mot = motility

Figure 2

Table 2: Sensitivity pattern of and to raw onion juice

Concentration (gml^{-1})	Zone of inhibition diameter (mm)	
	S. aureus	*P. aeruginosa*
0.1	N.I	N.I
0.2	N.I	9
0.4	N.I	13
0.6	N.I	15
0.8	N.I	17
0.3 (Erythromycin control)	26	30

N.B: Values are means of duplicate readings; N.I = No inhibition

Figure 3

Table 3: Sensitivity pattern of and to cold-water extract of onion.

Concentration (gml^{-1})	Zone of inhibition diameter (mm)	
	S. aureus	*P. aeruginosa*
0.1	N.I	N.I
0.2	N.I	N.I
0.4	N.I	N.I
0.6	N.I	N.I
0.8	N.I	19
0.3 (Erythromycin control)	28	29

N.B: Values are means of duplicate readings; N.I = No inhibition

Figure 4

Table 4: Sensitivity pattern of and to hot-water extract of onion.

Concentration (gml^{-1})	Zone of inhibition diameter (mm)	
	S. aureus	*P. aeruginosa*
0.1	N.I	N.I
0.2	N.I	N.I
0.4	N.I	N.I
0.6	9	N.I
0.8	11	N.I
0.3 (Erythromycin control)	28	30

N.B: Values are means of duplicate readings; N.I = No inhibition

Figure 5

Table 5: Sensitivity pattern of and to ethanolic- extract of onion.

Concentration (gml^{-1})	Zone of inhibition diameter (mm)	
	S. aureus	*P. aeruginosa*
0.1	N.I	N.I
0.2	N.I	N.I
0.4	N.I	N.I
0.6	N.I	N.I
0.8	N.I	11
0.3 (Erythromycin control)	28	29

N.B: Values are means of duplicate readings; N.I = No inhibition

Figure 6

Table 6: Sensitivity pattern of and to raw ginger extract.

Concentration (gml^{-1})	Zone of inhibition diameter (mm)	
	S. aureus	*P. aeruginosa*
0.1	N.I	N.I
0.2	N.I	9
0.4	N.I	10
0.6	N.I	11
0.8	N.I	15
0.3 (Erythromycin control)	28	30

N.B: Values are means of duplicate readings; N.I = No inhibition

Figure 7

Table 7: Sensitivity pattern of and to cold-water extract of ginger.

Concentration (gml^{-1})	Zone of inhibition diameter (mm)	
	S. aureus	*P. aeruginosa*
0.1	13	12
0.2	14	13
0.4	15	14
0.6	16	15
0.8	19	17
0.3 (Erythromycin control)	28	30

N.B: Values are means of duplicate readings; N.I = No inhibition

Figure 8

Table 8: Sensitivity pattern of and to hot-water extract of ginger.

Concentration (gml^{-1})	Zone of inhibition diameter (mm)	
	S. aureus	*P. aeruginosa*
0.1	N.I	N.I
0.2	N.I	13
0.4	N.I	14
0.6	N.I	15
0.8	N.I	16
0.3 (Erythromycin control)	28	30

N.B: Values are means of duplicate readings; N.I = No inhibition

Figure 9

Table 9: Sensitivity pattern of and to ethanolic extract of ginger.

Concentration (gml^{-1})	Zone of inhibition diameter (mm)	
	S. aureus	*P. aeruginosa*
0.1	N.I	9
0.2	N.I	11
0.4	10	14
0.6	17	18
0.8	20	22
0.3 (Erythromycin control)	28	30

N.B: Values are means of duplicate readings; N.I = No inhibition

DISCUSSION

The result of this work indicates that the water-soluble extracts of onions and ginger have antibacterial properties.

When the extracts were tested on Staphylococcus aureus and Pseudomonas aeruginosa, the widest zones of inhibition were obtained with P. aeruginosa. These differences in the zones of inhibition may be directly related to the susceptibility of each test organisms to the onions and ginger extracts. The factors responsible for this high susceptibility of P. aeruginosa to the extracts are not exactly known but may be attributed to the presence of secondary plant metabolites[20].

It was clear from this work that the solvent of extraction affected the degree of antibacterial activity of the extracts. It was observed that the ethanolic extract of ginger gave the widest zone of inhibition (22mm) using the concentration of $0.8gml^{-1}$ while the ethanolic extract of onion gave 11mm with $0.8mgl^{-1}$ each against P. aeruginosa. This credit to ethanol extraction was supposed to ethanol being an organic solvent and will dissolve organic compounds better, hence liberate the active component required for antimicrobial activity[13].

It was also observed that raw onion and ginger extracts had activity only on P.aeruginosa and no effect on S.aureus. The reason for this is not clear because the raw juice is thought to be more concentrated than the other extracts since the growth of S.aureus was not inhibited at all. The hot water extracts of onions did not inhibit the growth of Pseudomonas aeruginosa. This may be explained by the fact that the antimicrobial substance in the onion extracts, which are mainly phenolic compounds are destroyed by heat from the hot-water which might have raised the temperature of the extracts inactivating them[21]. In general, antibacterial components in the spice plants are heat-labile; hence all the spices lost their antibacterial activities within 20minutes at $100°C$[21]. Since, the hot- water extract of ginger showed activity on P.aeruginosa, the reason for this variation is not very clear. The cold-water extract of onion did not inhibit at all the growth of S.aureus but inhibited P. aeruginosa at $0.8gml^{-1}$ while the cold-water extract of ginger inhibited both organisms at all concentrations. It could be said that cold-water as an extractant could liberate the active constituents of ginger better compared to onions.

It is note worthy that the antibacterial activities of these plants extracts were dependent on the concentration of the extracts as reported by[13]. Also, if the extract has high molecular weight, the rate of diffusion is always slow, reduced and also takes longer time, whereas an extract of low molecular weight diffuses faster and at a quicker rate.

The result obtained is evidence that Zingiber officinale (ginger) produced marked inhibitory effect on the two test organisms compared to Allium cepa (onions). Hence, it has been reported that ginger extract and its pungent compounds demonstrated greater antibacterial activity against a variety of bacterial species including Helicobacter pylori, Staphylococcus aureus, Pseudomonas aeruginosa and Escherichia coli, although mixed result is attributed to different ginger preparations and varying strength[22].

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