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Comparative Study of the Antibacterial Activity of the Underground Stem of Ginger (*Zingiber officinale*) and the Bulb of Garlic (Allium sativum) on Selected Aerobic Bacterial Species

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Research Article

Abstract

Background: In this study, Garlic extract was tested against the extract of Ginger. The rate of action, effect of temperature and pH were measured. Also, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were measured. The zone of inhibition observed in this study confirmed the use of Garlic as a more potent antibacterial agent. The rate of action of Garlic was very high eliminating 65% and 73% of *Pseudomonas aeruginosa* and Antibacterial, Activity, Aerobic, Garlic and Ginger pyogenes respectively while Ginger recorded 47% and 51% action in the first eight hours. The effect of pH and temperature did not alter significantly the activities of the extracts on the test microorganisms. Garlic extract showed a MIC and MBC of 0.0125 and 0.025 concentrations of the stock using Strep pyogenes; and MIC and MBC of 62.5mg and 125 mg concentrations on *Ps. aeruginosa*. Similarly, Ginger showed MIC and MBC of 125 mg and 250 mg concentrations on Strep *pyogenes*; and 250 mg and 500 mg concentrations of Ginger on Ps. *aeruginosa*. The analysis of variance showed that there is no significant difference ($P<0.05$) on the sensitive pattern of both organisms to the extracts. The correlation analysis (spearman's rank model) showed a weak relationship ($r=0.3$) with respect to responses of the microorganisms to pH and temperature but strong relationship with time ($r=0.8$).

Keywords: Antibacterial, Activity, Aerobic, Garlic and Ginger.

1. Introduction

Ginger and Garlic are widely used in Africa as herbal supplements in food and for medical purposes [1,2]. These genera are bulb and rhizoid group of plants traditionally used in Southern Nigeria to add spice to food and to treat some disease [3]. Garlic is a nature to Central Asia and has long been a staple in Mediterranean region as well as seasoning in Asia, Africa and Europe [4]. It is used as fish and meat preservative in Asia and some part of Africa. Medically, its allicin and phytoncide have antibiotic and antifungal activities respectively. Garlic is used traditionally for the treatment of upper respiratory tract infections such as cough and sore throat; and gastrointestinal tract infections [5,6]. Fresh ginger is used as the main spice in making pulse, lentil curries and other vegetable preparations. Traditionally, it is used in preparing food for pregnant women and nursing mothers [1,2].Unlike garlic, not much is known about the antibacterial activities of ginger except that the studies conducted revealed that gingerol found in Ginger is known to have antibacterial properties. However, both fresh and dried roots of ginger have been used in the treatment of cold, colic, asthma, cough and loss of appetite [6-8]. This study is aimed at having a comparative analysis of the extract of both plants in relation to the antibacterial activity of Gentamycin and Ciproxin antibiotics. The minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) of the extracts were determined. Also investigated were the effect of heat and pH; and the kinetics of the extracts.

2. Materials and Methods

The bulb of Garlic and underground stem of Ginger were purchased at Rumuokoro market in Port Harcourt. The aerobic bacteria cells used were *Pseudomonas aeruginosa* and *Streptococcus pyogenes* sampled from isolated culture of five aerobic bacteria species; both isolates were from patients with respiratory, gastrointestinal or urogenital tract infection. Selection of the test microorganisms was done randomly by lottery method out of the numerous bacteria species isolated from upper respiratory samples.

2.1 Alcohol extraction of the active agent

100 g of bulb of Garlic and 100 g of underground stem of Ginger were prepared and put in separate 500 ml Pyrex conical flask. The chips were immersed in 75% ethanol for 72 h. The suspensions were shaken thoroughly and filtered into clean sterile containers, air dried and preserved in the refrigerator for subsequent use.

2.2 Isolation test of the microorganism

a) Aerobic bacteria species were isolated form hospital samples using basic microbiological techniques [9,10]. MacConkey agar, Nutrient agar and Blood agar used were prepared according to manufacturer's instructions. Of the isolated bacteria, *Pseudomonas aeruginosa* and *Streptococcus pyogenes* were selected through lottery method of random sampling.

b) Biochemical tests were done to identify the species of the test microorganisms in line with Bergey's manual of determinative bacteriology [9].

2.3 Determination of the antibacterial activity of the extracts

500 mg per ml of the extracts was screened for antibacterial activity using Well-in-Agar gel diffusion method of well size 3 mm in diameter [11]. Sensitivity of the tests microorganisms to the extracts against synthetic broad spectrum antibiotics (Gentamycin and Ciproxin) was measured in millimeter (mm) after 18-24 h aerobic incubation at 35°C.

I. **Total bacterial cell count:**

A 1/10 serial dilution of stock culture of the test bacteria was made in normal saline. 1 ml of each dilution seeded into each molten agar incorporated with 500 mg/ml of the extracts, mixed properly and poured into sterile petri dishes. The seeded media were allowed to solidify and then incubated at 35°C for 18-24 h. Growth as seen on different concentration plates were enumerated [11].

II. **Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)** of the extracts were determined using the methods described in medical laboratory manual for tropical countries [11].

A 1 in 2 dilution of the stock extracts (500 mg/ml) was made using physiological saline into 5 test tubes. A 0.5 ml of the test microorganisms inoculated into the various dilutions and incubated overnight at 35°C. The overnight culture were inoculated into freshly prepared nutrient agar plate and incubated aerobically for 18-24 h at 35°C.

2.4 Kinetics of the extracts

0.1 ml of the test microorganisms was inoculated into a test tube containing 5 ml of Nutrient broth and O.1 ml of the broth culture was taken from the test tube for bacterial cell count at time zero and subsequently, two hours interval for a maximum of ten hours. Enumeration of bacterial cells was done using the pour plate method [9,11,12].

2.5 Effect of heat on extracts

The extracts were heated to boiling temperature. The precipitated white deposits were re-suspended in 1ml of distilled water. Both the supernatant and the re-suspended deposits were tested for antibacterial activity using well-in-agar gel diffusion method [11,12].

2.6 Effect of relatively weak acid medium

The effect of pH was tested by adding to the extracts 0.1ml of 0.1N Hydrochloric acid to 5 ml of 500 mg extracts and the antibacterial activity of the compound tested using well-in-agar gel diffusion method [11].

3. Results

This study compared the antibacterial activity of the extracts of Garlic and Ginger with Ciproxin and Gentamycin antibiotics using cultures of *Pseudomonas aeruginosa*, *Streptococcus* pyogenes, *Streptococcus pneumonia*, *Klebsiella pneumoniae* and *Staphylococcus aureus*. The zones of inhibition recorded by test microorganisms on the antibacterial agents are shown in Table 1.

In Table 2, Garlic treated culture recorded a reduction to 3.2 x 10^3 cfu/ml of *P. aeruginosa* from the stock of 5.0 x 10^6 cfu/ml. Ginger showed a slight reduction to 5.0 x 10^5 cfu/ml of *P. aeruginosa*. Also, *S. pyogenes* recorded reduction of cell count of 5.5 x 10^3 cfu/ml and 4.0 x 10^4 cfu/ml on extracts of Garlic and Ginger respectively. There is significant difference in the antibacterial activity of Garlic extract than Ginger ($p<0.05$) as shown in Table 2.

Duration of incubation affected (r=0.8) the activity of the extracts on the test microorganism as shown in Table 3 above.

Boiling or freezing the extract did not significantly affect (r=0.3) the activities of the extract as shown in Table 4 below. From Table 5, Garlic extract showed an MIC and MBC of 62.5 mg/ml and 125 mg/ml concentrations using *S. pyogenes* while *P. aeruginosa* showed MIC and MBC of 125 mg/ml and 250 mg/ml

Table 1. Antibacterial Screening Test.

Species	Garlic (mm)	Ginger (mm)	Ciproxin (mm)	Gentamycin (mm)	Normal Saline Controle (mm)
P. aeruginosa	4	2.6	3.6	3	NA
Strep. pyogenes	4.5	2	4.1	2.4	NA
Strep pneumonia	4	3	4.6	3.4	NA
K. pneumonia	3.8	3.8	4	4.2	NA
Staph. aereus	3.8	NA	4.2	3.5	NA

KEY: NA= No activity

Table 2. Total Bacterial Count.

Species	Untreated Stock Culture (cfu/ml)	Garlic Treated Culture (cfu/ml)	Ginger Treated Culture (cfu/ml)
P. aeruginosa	5.0 x 10^6	3.2 x 10^3	5.0 x 10^5
Strep pyogenes	3.8 x 10^6	5.5 x 10^3	4.0 x 10^4

Table 3. Kinetics of the Extracts

Time (hours)	Extracts	*P.Aeruginosa* (cfu/ml)	*Strep pyogenes* (cfu/ml)
0	A	1.0 x 10^7	1.0 x 10^7
	B	1.0 x 10^7	1.0 x 10^7
2	A	8.5 x 10^6	6.6 x 10^6
	B	9.3 x 10^6	9.1 x 10^6
4	A	5.2 x 10^5	5.0 x 10^5
	B	6.9 x 10^6	6.2 x 10^6
6	A	3.5 x 10^5	2.3 x 10^4
	B	4.1 x 10^6	4.1 x 10^6
8	A	2.1 x 10^5	1.7 x 10^5
	B	3.8 x 10^6	2.6 x 10^6

Key: A= Garlic Extract; B= Ginger Extract

Table 4. Effect of Temperature.

Temperature	Extracts	*P. aeruginosa*	*Strep pyogenes*
2-8oC	A	4.0mm	5.0mm
	B	2.2mm	2.0mm
35oC	A	4.2mm	4,8mm
	B	2.1mm	2.0mm
Boiling temperature	A	3.9mm	4.9mm
	B	2.0mm	1.9mm

Key: A= Garlic Extract; B= Ginger Extract

Table 5. Minimum Inhibition and Bactericidal Concentrations (MIC and MBC)

Dilutions	Garlic Extract		Ginger Extract	
	P.aeruginosa	S.pyogenes	P.aeruginosa	S.pyogenes (cfu/ml)
Stock (500mg/ml)	NG	NG	NG	NG
250mg/ml	NG	NG	3.0x10^3	NG
125mg/ml	5.0x10^3	NG	2.1x10^6	1.2x10^2
62.5mg/ml	1.2x10^4	2.0x10^2	1.25x10^7	1.2x10^8

Key: NG= No Growth

concentrations respectively. Similarly, Ginger extract showed MIC and MBC of 125 mg/ml and 250 mg/ml concentration respectively for *S. pyogenes* while MIC and MBC for *P. aeruginosa* on Ginger extract are 250 mg/ml and 500 mg/ml respectively.

4. Discussion

The study compared the antibacterial activity of the extracts on selected aerobic bacteria species isolated from patients queried for upper respiratory tract infection, gastrointestinal tract and urogenital tract infections. The result of the study showed the potential of Garlic for the treatment of ailments due to *Pseudomonas aeruginosa* and *Streptococcus pyogenes* infection if fully exploited. The zone of inhibition observed in Table 1 confirmed the work of [5,6] on the use of Garlic as an antibacterial agent. The first two hours witnessed the elimination of 35% *Pseudomonas aeruginosa* and 46% *Streptococcus* pyogenes by Garlic extract. The change in pH and temperature did not alter significantly the activities of the extracts on test microorganisms. The fact that Ginger showed lesser activity than Garlic is supported by the work of [7,8] that little is known about the antibacterial activity of Ginger. Also, synergistic effect was not established in this work as the mixture of both extracts did not alter the potency of the extracts on the tested microorganisms. The study compared the antibacterial activity of the extracts on selected aerobic bacteria species isolated from patients queried for upper respiratory tract infection, gastrointestinal tract and urogenital tract infections. The result of the study showed the potential of Garlic for the treatment of ailments due to *Pseudomonas aeruginosa* and *Streptococcus pyogenes* infection if fully exploited. The zone of inhibition observed in

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5. Conclusion

This work has proved the antibacterial activity of both plant extract. Though more activity was shown by Garlic on Ps. aeruginosa and Str. pyogenes, Ginger showed promising antibacterial activity. Their stability under varying environmental conditions and encouraging MIC and MBC are advantages to be considered for their usage. It is, therefore, recommended that more work be done especially on Garlic to ascertain its bioavailability and biosafety on human cells.

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