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Antibacterial Activity of Ginger *(Zingiber Officinale* Roscoe and Garlic *(Allium Sativum L.)* Extracts on *Escherichia Coli* and *Salmonella typhi*

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Abstract: The antibacterial activity of the ethanolic extracts of garlic and ginger against *E.coli* and *S. typhi* were positive. Though the growth inhibitory response varied with thetype of bacterial species tested and the type of extract. Ethanolic extract of ginger inhibited *E. coli* showing 9.00 mm diameter while *S. typhi* was inhibited w ith 10.00 mm diameter. The aqueous extract of garlic had no inhibitory effect on the two test organisms, but the aqueous extract of ginger inhibited *S. typhi* showing 8.0 mm diameter. Only the paper disc diffusion method was used and it gave clear zones of inhibition. In determination of minimum inhibitory concentration (MIC), it was observed that the range was between 75 mg mL^{-1} and 250 mg mL^{-1} of the concentrations. At any dilution below 75 mg mL^{-1} concentration, there was no growth inhibition by any extract. The results indicated that the aqueous extract had little or no inhibition while ethanolic extracts had a higher inhibitory effect *in vitro* against specific bacteria, *E. coli* and *S. typhi,* confirming their use in folk medicine.

Key words: Zingiber officinal, Allium sativum L., Salmonella typhi

INTRODUCTION

There has been a great shift from the prescription of antibiotics to the use of medicinal plants. It is estimated that there 250,000 to 500,000 species of plant on earth[1]. A relatively small percentage of these are used as food by both humans and other animal species. It is possible hat even more are used for medicinal purposes[2]. The bible offers description of approximately 30 healing plants. Indeed, frankincense and myrrh probably enjoyed their status of great worth due to medical properties[3].

Many plant extracts have been shown to possess antimicrobial properties active against microorganisms *in vitro*. For instance, water extracts of *Ocimum sanctum* and *Ocimum gratissimum* and alcohol extracts of *Ocimum gratissimum* and *Ocimum sanctum* were highly toxic against fungi after 15 days culture[4]. Furthermore, some extracts of garlic, onion and green peper have been reported to inhibit the growth of *Escherichia coli, Salmonella typhosa, Shigella dysenterae* and *Staphylococcus aureus*[5].

The bactericidal effect of garlic extract was apparent within 1 hour of incubation and 93% killing of *Staphylococcus epidermidis* and *Salmonella typhi* was achieved within 3 h[6].

Flavones, flavonoids and flavonols are chemical compounds active against microorganisms. Flavones are phenolic structures containing one carbonyl group (as opposed to the two carbonyls in quinines.

The addition of a 3-hydroxyl group yields a flavonol[7]. Flavonoids are also hydroxylated phenolic substances but occur as a C6 – C3 unit linked to an aromatic ring, they are synthesized by plants in response to microbial infection[8].

They have been found *in-vitro* to be effective antimicrobial substances against a wide array of microorganisms. More lipophilic flavonoids may also disrupt microbial membranes[9].

It was noticed sometime ago that teas exerted antimicrobial activity[10], and that they contain a mixture of catechin compounds.

These compounds inhibited *in vitro Vibro cholerae, Streptococcus mutans, Shigella* and other bacterial andmicroorganisms[1,11,14].

This work evaluated the antibacterial activity of the spices; ginger *(Zingiber officinale* Roscoe and garlic *(Allium sativum L)* on two enteric microorganisms such as *Escherichia coli* and *Salmonella typhi.* Through ages spices have served humans in many areas such as food, flavours and drugs.

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The work ascertained whether these spices could effect growth inhibition on the test organisms *in vitro*. Furthermore, the research was geared towards finding out if the effects on growth inhibition were dependent on the methods and solvents of extraction of the plant chemicals.

MATERIALS AND METHOD

Collection and Identification of Plant materials: The garlic bulbs and ginger rhizomes were purchased at the Umuahia main market. 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 and classified by Prof. H. O. Edeoga (Taxonomist), of the Department of Biological sciences, Michael Okpara University of Agriculture, Umudike, Nigeria.

Extraction of the plant materials: The plant materials were washed with clean water and allowed to air dry. This was done to reduce the microbial load of the plant material due to handling and transportation. The outer covering of both ginger and garlic were manually peeled off and the materials were sliced into cutlets.

The materials were placed in a hot air oven fordrying. At the temperature of 65°C, ginger dried within 48 h (2 days) and garlic dried the next day after 72 h (3 days). Using a milling machine from National Root Crops Research Institute, Umudike, Nigeria (NRCRI), the plant cutlets were pulverized into powder. The powder was weighed using Sartorious AG Gottingen electronic weighing balance.

Weights of the total powder

Ginger	67.00g
Garlic	78.25g

Extraction: The two solvents used for extraction were ethanol (80%) and deionized water. The 80% ethanol was prepared by adding 80 mls of 100% ethanol to 20 mL^{-1} distilled water. Five grammes (5g) of the ginger powder were weighed into a 250 mL^{-1} conical flask and 200 mL^{-1} of ethanol was used to dissolve it[15].

Furthermore, 5 g of the ginger was dissolved in the 250 mL^{-1} conical flask using 200 mL^{-1} distilled water. The same was repeated for garlic powder for both the ethanolic and aqueous extraction. The same was repeated for garlic powder for both the ethanolic and aqueous extraction. The mixtures were vigorously stirred with a sterile glass rod. After twenty four 24 h, with interval stirring, the mixture was filtered, using Whatmann No. 1 filter paper[16]. The precipitate was discarded and the supernatant was collected for evaporation. The colour of the filtrates after filtration was;

- Aqueous ginger filtrate light brown
- Aqueous garlic filttrate dark yellow
- Ethanolic ginger filtrate orange yellow
- Ethanolic garlic filtrate yellow

The filtrates were poured into crucibles and placed on a steam bath at 100 0 C for evaporation to dryness. After evaporation the extracts were recovered and weighed[17].

Ginger aqueous extract	3.50g
Ginger ethanolic extract	3.90g
Garlic aqueous extract	2.80g
Garlic ethanolic extract	3.00g

This process of extraction was repeated to recover larger quantity of the extracts and they were stored in the refrigerator at 0 0 C for further use of antimicrobial sensitivity testing. The yields were recovered as percentage of the quantity of the initial plant material (5g) used.

$$\frac{\text{yield} \times 100}{5\text{g}} = \text{yield (\%)}$$

Bacterial species confirmation and test for purity: The bacterial stock cultures *E. coli* a n d *S. typhi* were obtained from the microbiology laboratory of Federal Medical Centre, Umuahia, Abia State, Nigeria. Viability tests for each isolate were carried out by resuscitating the organism in nutrient agar.

They were also confirmed by carrying out Gram staining procedures. *Escherichia coli* is a Gram negative rod, lactose fermenter appearing pink to red in colour on MacConkey agar medium. It was indole positive and motile as observed using hanging drop technique *Salmonella typhi* a gram negative organism appeared black on MacConkey agar medium[18].

The stock on nutrient agar (BL 9-6AU) lab-8, England) was incubated for 24 h at 37°C following refrigeration storage at 4°C until required for sensitivity testing.

Preparation of different concentration of the extracts: The test was conducted using the paper disc diffusion method. The Whatmann No. 1 filter paper was

used. The paper was cut into circular discs of 0.5cm or 5.0 mm in diameter. Each of the discs was found to absorb a maximum volume of 0.052 mL^{-1}. Therefore, 1000 mL^{-1}/2 mL^{-1} solution of each of the extract was prepared by dissolving 1.0g (1000 mg) of the extracts in 2 mls of the suitable solvents.

The alcoholic extracts were reconstituted in Di-Methyl Sulphoxide (DMSO). Then 500 mg mL^{-1}, 250 mg mL^{-1}, 75 mg mL^{-1}, 35.25 mg mL^{-1} solutions of the extracts were prepared from the original 1000 mg mL^{-1} concentration by double dilution procedure.

Sterilization of materials: The dried extracts were exposed to ultra violet light (UV rays for 24 h to sterilize. The sterility was checked by streaking the extracts on nutrient agar plate and incubated at 37° C for 24 h. It was confirmed that there were no artifacts to contaminate the sensitivity testing.

Nutrient agar media and MacConkey agar media were prepared by the manufacturer's specifications. The media, paper discs (which was in aluminium foil) and Petri dishes were autoclaved at 121 0 C for 15 min and 11 atm.

Screening the extracts for antibacterial activity: A serial dilution of the bacterial culture was made using normal saline. A flamed sterile wire loop was used to loop the organisms from the pure culture plate into a test tube containing 10 mL^{-1} of normal saline. From the test tube, serial dilution from 10-1 to 10-5 was made. From the dilution of 10-3 and10-4 a sterile swab stick was used to seed the nutrient media culture plates.

Having set a sterile working bench in the inoculating chamber, the already prepared discs were impregnated on the seeded culture plates using sterile forceps. Three discs of the 1000 mg mL^{-1} concentration were used as well as the discs from 500 mg mL^{-1},250 mg mL^{-1}, 75 mg mL^{-1} and 35.25 mg mL^{-1}. Appropriate labeling and date were made.

The plates were incubated for 24 h and the zone diameter of inhibition were measured and recorded. The same procedure was repeated for the four different exracts on the two test organisms; *E. coli* and *S. typhi.*

Determination of minimum inhibitory concentration (MIC): The MIC is the concentration giving the least inhibitory activity and below which there is no further inhibition.

It was regarded as the concentration giving the lowest possible zones of inhibition using a sterile forceps the paper discs of different concentration (from 500 mg mL^{-1}, 250 mg mL^{-1} 75.0 mg mL^{-1}, 35.25 mg mL^{-1} and 1000 mg mL^{-1}) were placed at different portions of the seeded plates.

They were labeled as 10 -1 , 10 -2 , 10 -3 , 10 -4 , 10 -5 respectively. The plates were incubated at 37 0 C for 24 h. The zones of inhibition in each case were measured as the diameter of the clearing zones and recorded.

Mean zones of Inhibition: The various concentrations of 1000 mg mL^{-1}, 500 mg mL^{-1}, 250 mg mL^{-1}, 75 mg mL^{-1} and 35.25 mg mL^{-1} were tested on the test organisms using the disc diffusion method.

Control experiment using antibiotics: The control experiment was carried out to compare the diameter zone of clearing from the extracts and already standardized antibiotics.

This will enable further prescription of either antibiotics or plants with antimicrobial activities. The antibiotics used were erythromycin, tetracycline and chloramphenicol.

Erythromycin: One tablet of erythromycin (500 mg) was crushed manually and dissolved in 10 mL^{-1} of water. This was the working solution; dilute stock 1/10 (1 mL^{-1} + 9 mls) of distilled water. Then the concentration was 5 mg mL^{-1}, 0.1 mL^{-1} was added to 25 discs.

Tetracycline: One capsule (250 mg) was dissolved in 25 mL^{-1} water, concentration 10 mL^{-1}/ mL^{-1} working solution: dilute 3/5 (3 mL^{-1} + 2 mL^{-1}) distilled water: to make a concentration of 6 mg mL^{-1}. 0.1 mL^{-1} was added to 25 discs

Chloramphenicol: One capsule (250 mg) was dissolved in 25 mL^{-1} of water, to make a 10 mL^{-1} / mL^{-1} working solution. 3/5 dilution was made to get 6 mg mL^{-1} concentration. 0.1 mL^{-1} was added to 25 discs. The discs were placed on plates containing *E. coli* and *S. typhi* inocula using sterile forceps.

Incubation was at 37 0 C for 24 h. The zones of inhibition were measured and recorded.

Statistical analysis Analysis Of Variance (ANOVA) was the statistical analysis used throughout in this work[19].

RESULTS

Effects of extraction methods on percentage yields: The yields of the extracts of *Zingiber officinale* Roscoe and *Allium sativum* L. with respect to solvents are shown in Table 1.

The percentage yield of ethanolic extract of *Zingiber officianale* (Ginger was highest with (78%). The next was the aqueous extract of ginger with (70%).

Table 1: Yields of extracts of plants with respect to solvents

Plants	Solvents	Yield(g)	Yield (%)
Ginger	Aqueous	3.50	70.00
	Ethanol	3.90	78.00
Garlic	Aqueous	2.80	56.00
	Ethanol	3.00	60.00

Thepercentage yields were calculated against the 5.0g powder of the plant material subjected to each extraction method

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Table 2: Antimicrobial activity of the various extracts *Ginger*

	Ginger		Garlic	
Bacterial spp	Ethanol	Aqueous	Ethanol	Aqueous
E. coli	++	-	++	-
S. typhi	++	++	++	-

Key: ++ = Inhibition > 6.00mm diameter
- = No inhibition

Table 3: Diameter of Inhibition (mm)

	Ginger		Garlic	
Bacterial spp.	Ethanol	Aqueous	Ethanol	Aqueous
E. coli	9.00	0.00	8.00	0.00
S. typhi	10.00	8.00	8.00	0.00

Figures above are zones of clearing in millimeter of the original working solution

The percentage yield of *Allium sativum* (garlic) was (60% and 56%) for ethanol and aqueous extracts, respectively.

Antibacterial activity of different extracts: The sensitivity of different organisms with the concentrated extracts using the paper disc method are shown in Table 2.

E. coli and *S. typhi* were sensitive with ethanolic extracts of ginger, while only *S. typhi* was sensitive with the aqueous extract of ginger. *E. coli* was not inhibited.

Similarly, the garlic ethanolic extract, inhibited both *Salmonella typhi* and *E. coli.* The aqueous extract of garlic could not inhibit both *S. typhi* and *E. coli.* The zones of inhibition in (mm) diameter are shown in Table 3. The ethanolic extract of ginger gave the widest inhibition zone of 10.0 mm.

The second widest zone of inhibition was with ethanolic extract of ginger on *E. coli* showing 9.0 mm. The garlic ethanolic extract inhibited *S. typhi* and *E. coli* showing the same diameter of inhibition (8.0 mm). There was no inhibition with the aqueous extract of garlic on both *S. typi* and *E. coli.*

Mean Zones of Inhibition: All the ethanolic extracts showed inhibition of varying diameter with the different test organisms. The mean zone (x-) of inhibition of ginger ethanolic extract was 5.2 mm on *E. coli.* The highest mean zone (x -) of inhibition of ginger ethanolic extract of ginger on *S. typhi* was 6.2 mm. The garlic ethanolic extract had a mean zone of 2.8 mm with *E. coli* and 3.4 mm with *S. typhi* as shown in Table 4.

Table 4: Mean zone diameter of inhibition (mm) of ethanolic extract of different plants with respect to various concentrations in mg mL^{-1}

	Ginger						Garlic					
	1000	500	250	75	35.25	x⁻	1000	500	250	75	35.25	x
E. coli	9.0	7.0	6.0	4.0	0.0	5.2	8.0	4.0	2.0	0.0	0.0	2.8
S. typhi	10.0	8.0	7.0	6.0	0.0	6.2	7.0	5.0	3.0	2.0	0.0	3.4

The mean zone was deduced from the summation of various zones of inhibition

DISCUSSION

The yield of garlic was less than the yield of ginger. Garlic powder seemed hard in dissolving and forming solution. However, the ethanolic extract of all the plant materials exhibited inhibition on the test microorganisms. This credit to ethanol extraction was supposed to be because ethanol is an organic solvent and will dissolve organic compounds better, hence liberate the active component required for antimicrobial activity.

It was clear from this work that the solvent of extraction and method affected the degree of antimicrobial activity. During the preliminary testing, it was observed that ethanolic extract of ginger gave the widest diameter zone of inhibition ((10.00 mm) using the concentration of 1000 mg mL^{-1}. No detectable growth of *E. coli* was found around the discs containing 1000 mg mL^{-1} of ethanolic extracts of ginger and garlic.

The antibacterial activities of various plant extracts as found in this work were dependent on the concentration of the extracts. For instance, 1000 mg mL^{-1} of ethanolic ginger extract inhibited *E. coli* with 9.00 mm, while the same concentration inhibited *Salmonella typhi* with 10.00 mm. On the other hand, garlic ethanolic extract inhibited *E. coli* with 8.00 mm while *S. typhi* while *S. typhi* inhibited with 7.00 mm of the 1000 mg mL^{-1} concentration.

Consequently, upon these results, it became expedient to detect the Minimum Inhibitory Concentration (MIC) of different extracts. Ginger ethanolic extract had the over all widest diameter zone of inhibition of 10.00 mm with 1000 mg mL^{-1} concentration.

Next was the ethanolic extract of ginger on *E. coli* which gave 9.00 mm with the 1000 mg mL^{-1}. This was the highest zone of inhibition of ginger on *E. coli* as compared with the other dilutions.

Though the inhibitory effect of garlic extract to *E. coli* has been established by this method; 1000 mg mL^{-1} of ethanolic garlic extract inhibited *E. coli* and

Table 5: Minimum inhibitory concentration (mg mL^{-1}) of ethanolic and aqueous extracts of ginger and garlic (MIC)

	Ginger Ethanol	Aqueous	Garlic Ethanol	Aqueous
	1000 500 250 75	MIC 1000 500	MIC 1000 500	MIC 1000 500
	35.25 MIC	250 75 35.25	250 75 35.25	250 75 35.25
E. coli	9.00 7.00 6.00	75 mg mL^{-1} very weak 0.00	1000 mg mL^{-1} 8.00 4.00 2.00	250 mg mL^{-1} 0.00 0.00 0.00
	4.00 0.00	0.00 0.00 0.00	0.00 0.00	0.00 0.00NIL
S. typhi	10.00 8.00 7.00	75 mg mL^{-1} 6.00 4.00	250 mg mL^{-1} 7.00 5.00	75 mg mL^{-1} 0.00 0.00
	6.00 0.00	3.00 0.00 0.00	3.00 2.00 0.00	0.00 0.00 0.00NIL

Table 6: Antimicrobial sensitivity of different extracts on the test microorganism concentration (mg mL^{-1})

	Ginger Ethanol	Aqueous	Garlic Ethanol	Aqueous
	1000 500 250	1000 500 250 75	1000 500 250 75	1000 500 250
	75 35.25 MIC	35.25 MIC	35.25 MIC	75 35.25MIC
E. coli	++ ++ + + -	75 mg mL^{-1} * - - - -	++ + + - -	250 mg mL^{-1} - - - - - NIL*S. typhi*
	++ ++ ++ + -	75 mg mL^{-1} + + + - -	++ ++ + + + -	75 mg mL^{-1} - - - - - NIL

Key: ++ = Inhibition > 6.00mm diameter, + = Inhibition < 6.00mm diameter, * = Weak inhibition, - = Noinhibition

Table 7: Diameter zone of inhibition (mm) control experiment using standard antibiotics

	Bacterial *spp*	
Antibiotics	*E. coli*	*S. typhi*
Erythromycin	15.00	9.00
Tetracycline	9.00	4.00
Chloramphenicol	16.00	5.00

Zones of clearing of standard antibiotics against *E. coli and S. typhi.*

S. typhi with 8.00 mm and 7.00 mm respectively. The variation in percentage of garlic extracts required to inhibit the growth of various microorganisms could be due to the presence of more lipoprotein type SH enzymes.

The MIC results of different extracts were gotten from the extrapolation of zone diameter of inhibition of the concentration. For instance, at the dilution of 75 mg mL^{-1} for ethanolic extract of ginger, the MIC was observed for E. coli and S. typhi, which gave 4.00 mm and 6.00 mm diameter zone of inhibition, respectively.

The aqueous extract of ginger had the MIC at the 1000 mg mL^{-1} concentration with E. coli, though the inhibition of *E. coli* at this concentration was regarded as very weak. It was at this dilution that after which no sign of inhibsition was observed, while S. typhi was inhibited at the same concentration with 6.00 mm diameter of inhibition.

As reported by Kyung *et al.*,[19] varying concentration of garlic extract exhibited varying zones of inhibition. The widest zone of inhibition of ethanolic garlicextract was 1000 mg mL^{-1}.

The MIC of the extract occurred at 250 mg mL^{-1} of E. coli (with 2.0 mm diameter zone of inhibition), 75 mg mL^{-1} for S. typhi with (2.00 mm diameter zones of inhibition).

Any concentration below these was not inhibitory to the test organisms. There was no MIC recorded for the aqueous extract of garlic because there was no inhibition with the highest concentration of 1000 mg mL^{-1} as shown in Tables 5

For the control experiment, chloramphenicol had the widest zone of inhibition against E. coli showing 16.00 mm diameter zone of inhibition. The next was 15.00 mm of erythromycin against E. coli as shown in Table 7. The MIC fell in the range of 75 mg mL^{-1} and 250 mg mL^{-1} concentration.

Lowest inhibition occurred with tetracycline against S. typhi with 4.00 mm diameter, showing that it is not the right antibiotic for treating S. typhi – caused illness.

Although the MIC of the different plant extracts varied yet they fell in the range of 75 mg mL^{-1} and 250 mg mL^{-1} concentration.

Plates 1 shows the zone of inhibition of ethanolic extract of garlic on S. typhi.

Plates 2 shows the zone of inhibition of ethanolic extract of ginger on E. coli while plate 3 shows the zone of inhibition of standard antibiotics on E. coli.

In conclusion, Zingiber officinale Roscoe (ginger) and Allium Sativum L. (garlic) produced marked inhibitory effect on S. typhi and E. coli as representative of enteric microorganisms. The results indicated that the plants have growth inhibitory effect *in vitro* against pathogenic bacteria.

Finally, the dosage to completely inhibit *in vitro* these pathogens should be the next line of research.

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