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The Effects of Allium Sativum and Zingiber Officinale Extracts on Shigella Dysenteriae Isolated from Ready-To-Eat Fried Chicken Sold in Ihiala L.G.A, Anambra State

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Abstract
A number of reports have shown that foods vended on streets including fast foods meant for immediate consumption can have high incidence of pathogenic bacteria which can pose serious public health problems to the consumers and may result to different disease conditions. This study was undertaken to evaluate the effects of *Allium sativum* and *Zingiber officinale* seed extracts on *Shigella dysenteriae* isolated from ready-to-eat fried chicken sold in Ihiala L.G.A., Anambra State. A total of 21 samples were collected from street hawkers (9 samples) and fast foods (12 samples) joints and plated on *Salmonella Shigella* Agar (SSA) using pour plated method at appropriate growth conditions. The bacterial isolate was characterized and identified using colonial descriptions and biochemical reactions. The phytochemical constituents of the extracts of *Allium sativum* and *Zingiber officinale* were determined quantitatively using spectrophotometric method. Tube dilution method was used to determine the Minimal Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) using double-fold serial dilutions at concentrations 25mg/ml to 400mg/ml. The phytochemical analysis of *Allium sativum* and *Zingiber officinale* extracts revealed the presence of alkaloids, saponins, flavonoids, tannins, phenolics, steroids and cardiac glycosides. The ethanolic extracts of both plants showed more activity (9.00 mm, 10.70 mm) than their aqueous extracts (7.30 mm, 7.70 mm) and their activity differed significantly ($p< 0.05$) from that of the ciprofloxacin (21.30 mm). The MICs (200 mg/ml, 400 mg/ml; 200 mg/ml, 400 mg/ml) and MBCs (400 mg/ml, Nil; 400 mg/ml, Nil) values revealed the inhibitory activities of ethanolic and aqueous extracts of *Allium sativum* and *Zingiber officinale* and cidal activities of their ethanolic extracts. The study recommends personal and environmental hygiene as preventive measures against bacterial contamination of foods and suggests that *Allium sativum* and *Zingiber officinale* could be used as alternative therapy for diseases associated with *Shigella dysenteriae.*

INTRODUCTION

Shigella dysenteriae infection has been associated with substantial morbidity and mortality rates in Africa, Southeast Asia and the Indian subcontinent. *S.dysenteriae* causes a significant threat to public health by causing shigellosis. *Shigella* infection is typically by ingestion (faecal oral contamination) and concentrates in areas where the population suffers from malnutrition and do not possess adequate waste management and safe drinking water supplies (Ankita *et al.,* 2012). Reports have shown the contamination of street vended foods including ready-to-eat foods with *Shigella* species, which could be as a result of poor personal hygiene by food handlers (Ankita *et al.,* 2012). Shigellosis is associated with 5-15% of diarrhea and 30-50% of cases of dysentery worldwide. In malnourished children, shigellosis can cause a vicious cycle of "further impaired nutrition absorption, recurrent infection and growth retardation." Without proper care, shigellosis can become life threatening (Ankita *et al.,* 2012).

Since the 1970s, the vigorous use of oral rehydration therapy in developing countries has contributed significantly to reductions in mortality from diarrhoeal dehydration (Ankita *et al.,* 2012). In contrast, this intervention provides little benefit to patients with dysentery caused by invasive bacterial enteropathogens such as *Shigella*. Over the last 50years, *Shigella* has demonstrated extraordinary prowess in acquiring plasmid-encoded resistance to the antimicrobial drugs that previously constituted first-line therapy (Sack 1997). Innovative strategies, including development of vaccines against the most common serotypes, show great promise for the prevention of *Shigella* disease (Coster 1999).

The practice of using of natural sources as a remedy or alternative medicine for the treatment of shigellosis has been proven effective in recent years. *Allium sativum* which belongs to the family *Amaryllidaceae* is native to central Asia and a known home remedy for treating diarrhea. It is known to possess antimicrobial activities which is traced to its phytochemical constituents and have been used both for culinary and medicinal purposes (Bauer, 2012; Debdutta *et al.,* 2012). Zingiber officinale on the other hand, a plant from the family *Zingiberaceae* is indigineous to Southern China. The rhizomes have been used since antiquity as an important

kitchen spice and medicinal values have been attributed to it (Butler, 2012). In view of the clinical burden of drug resistance of *Shigella dysenteriae to* most antibiotics, this research work was undertaken to evaluate the effects of *Allium sativum* and *Zingiber officinale* extracts against *Shigella dysenteriae* isolated from ready-to-eat-fried chicken.

MATERIALS AND METHODS

Sample Collection: The fresh rhizomes of *Zingiber officinale* and fresh bulbs of *Allium sativum* were obtained from the market in Umuoma village, Uli, Ihiala Local Government Area, Anambra State and authenticated appropriately.

Plate 1: *Allium sativum* bulbs

Plate 2: Rhizome of *Zingiber officinale*

Preparations of Samples for Extraction: The cloves of the fresh bulbs of Allium sativum were separated and the back was peeled alongside with that of the fresh rhizomes. The samples were masticated using mechanical grinder and kept ready for extraction of active ingredients (Nwobu *et al*. 2010).

Extraction Procedure: A 10 g portion of the sample was extracted by maceration in 100 ml of ethanol and water respectively for 3 days. The resulting extracts were subsequently filtered using Whatman No. 1 filter paper. The extracts were evaporated to dryness at room temperature in a steady air current (Nwobu *et al*. 2010).

Preparation of Test Sample: In this study, concentration of 400 mg/ml of the extracts was used to screen for antimicrobial activity. This was done by dissolving 2.0g of the extract in 5 ml of solvent (Iheukwumer and Umedum, 2013).

Isolation and Identification of Test Organism: A total of 21 fried chicken samples were collected aseptically from different street hawkers (9) and fast food joints (12) in Ihiala L.G.A. Anambra State, using sterile aluminum foil. The samples were transported to laboratory within 1 h of collection. A portion was cut from each chicken and one gram (1.0 g) was weighed from each portion. It was ground using sterilized mortar and pestle and then suspended in a test tube, each containing 10 ml of peptone water. The remaining portion of fried chicken was washed using peptone water .One millilitre (1.0 ml) from both grinded and washed chicken samples were pour plated on *Salmonella Shigella* agar and incubated at 37°C for 24 h. The organism obtained was aseptically subcultured on nutrient agar plate and incubated at 37 °C for 24 h. The pure culture of the test organism was identified the colonial description and biochemical reactions (Iheukwumere *et al.,* 2012**).**

Maintenance of Test Organism: The isolated organism was used for the antibacterial sensitivity testing. Prior to the test, the organism was subcultured on nutrient agar plate at 37°C for 24 h. Then the 24 h culture was transferred into nutrient broth and incubated at 37°C for 24 h (Iheukwumere *et al.,* 2012).

Sensitivity Testing Using Agar Well Diffusion Method: This was carried out using the modified method of

Iheukwumere and Umedum (2013). Each labeled plate was uniformly inoculated with the test organism using pour plating method. A sterile cork bore was used to make wells on the mediums. One tenth millilitre (0.1 ml) of various concentrations of the extracts was dropped into each labeled well and incubated at 37°C for 24 h. Antibacterial activity was determined by measuring the diameter of the zones of inhibition (mm) produced after incubation. Ciprofloxacin (500 mg/ml) used as control.

Determination of Minimum Inhibitory Concentration (MIC): This was carried out using the modified method of Iheukwumere and Umedum (2013).Here various concentrations of the test extracts were obtained using double- fold serial dilution. Each dilution was assayed against the test organism using tube dilution method. One milliliter of the test organism was added into each dilution and incubated at 37°C for 24 h. The MIC was defined as the lowest concentration able to inhibit any visible bacterial growth. This was determined and recorded as the MIC.

Determination of Minimum Bacterial Concentration (MBC): This was determined using the modified method of Iheukwumere and Umedum (2013). Here equal volumes of various concentrations of those tubes that did not show any visible growth for MICs were sub cultured on sterile poured plate and incubated at 37°C for 24 h. The lowest concentration of the extracts that showed no visible growth is the MBC.

RESULTS

The results of the quantitative phytochemical analysis of the extracts of *Allium sativum* and *Zingiber officinale* were shown in Table 1.The results revealed the presence of alkaloids, saponins, flavonoids, tannins, phenolics, steroids and cardiac glycosides. The phytochemicals may be responsible for the antibacterial activity of the leaf extracts.Table 2 shows the prevalence of *Shigella dysenteriae* on ready-to-eat fried chicken samples. A total of 21 fried chicken samples were collected from different street hawkers (9) and fast food joints (12) in Ihiala L.G.A. Anambra State. The results showed that 4 (44.44 %) chicken samples out of 9 (42.86 %)) samples collected from street hawkers were positive for *Shigella dysenteriae*. Also 2 (16.67 %) samples out of the total of 12 (57.14 %) chicken samples collected from fast food joints were positive for *Shigella dysenteriae* .Total of 6 (28.57 %) samples out of 21 (100 %) samples analyzed showed presence of *Shigella dysenteriae.* Table 3 shows the characterization and identity of the isolate. *Shigella dysenteriae* was isolated using *Salmonella Shigella* agar (SSA), characterized and identified using Gram reaction, colonial morphology and biochemical tests. Table 4 reveals the diameter zones of inhibition (mm) of the ethanolic and aqueous extracts of *Allium sativum* and *Zingiber officinale against S. dysenteriae.* The ethanolic extracts of both plants inhibited *S. dysenteriae* more than their aqueous extracts and their inhibitory activities differed significantly ($p< 0.05$) from that of the ciprofloxacin. The absolute ethanol (0.1 ml) and distilled water used in this study as extracting solvents had no effect on the tested organism as shown in Table 4.The results of the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) in milligrams per milliliter (mg/ml) of the ethanolic and aqueous extracts of *Allium sativum* and *Zingiber officinale* are shown in table 5. The results revealed that the ethanolic extracts of both plants exhibited more pronounced activity than the aqueous extracts. The ethanolic and aqueous extracts of both plants exhibited similar MIC compared to ciprofloxacin (control) which had more pronounced activity. The ethanolic extracts of *Allium sativum*, *Zingiber officinale* andciprofloxacin (control) showed similar bactericidal activity compared to their aqueous extracts which had no bactericidal activity against *S. dysenteriae.*

Table 1: Quantitative phytochemical constituents of *Allium sativum* and *Zingiber officinale* extracts

Phytochemical constituent	*Allium sativum* (g/100 g)	Zingiber officinale (g/100 g)
Alkaloids	3.16	10.04
Saponins	4.20	0.86
Flavonoids	1.22	5.88
Tannins	1.03	4.34
Phenolics	1.18	1.24
Steroids	0.06	0.02
Cardiac glycosides	0.24	1.01

Table 2: prevalence of *Shigella dysenteriae* on ready-to-eat fried chicken samples

Sampling point	Positive Sample (%)	Negative Sample (%)	Total (%)s
A	2 (16.67)	10 (83.33)	12 (57.14)
B	4 (44.44)	5 (65.56)	9 (42.86)

A= Fast food jointB= Vended foods

Table 3: Characteristics and Identity of the test organism

Parameter	*Shigella dysenteriae*
Appearance on agar plate	Pink on *SalmonellaShigella* agar plate
Gram reaction	-
Morphology	Rod
Catalase	-
Citrate	-
Motility	-
Hydrogen sulfide production	-

Table 4: Diameter zones of inhibition of *Alium sativum* and *Zingiber officinale* extracts against *S. dysenteriae* using 5 mm cork borer

Extract (400mg/ml)	*S. dysenteriae* (x ± SD)
EEA	9.00 ± 0.82
EEZ	10.70 ± 0.47
AEA	7.30 ± 1.25
AEZ	7.70 ± 0.94
CPX	21.30± 1.70
Absolute ethanol (0.1ml)	–
Distilled water (0.1ml)	–

EEA = Ethanolic extract of *Allium sativum,* EEZ = Ethanolic extract of *Zingiber officinale*
AEA = Aqueous extract of *Allium sativum,* AEZ = Aqueous extract of *Zingiber officinale,* CPX = Ciprofloxacin, X = mean, SD = Standard Deviation

Table 5: Minimum inhibitory concentration (MIC) and minimum bactericidal concentration of the extracts against *S. dysenteriae*

Extract	MIC (mg/ml)	MBC (mg/ml)
EEA	200	400
EEZ	200	400
AEA	400	–
AEZ	400	–
CPX	50	100

DISCUSSION

Natural products are a major source of new natural drugs and their use as an alternative medicine for treatment of various diseases has been increased in the last few decades (Gull *et al*. 2013). *Shigella dysenteriae,* the causative agent of human shigellosis is associated with epidemics of dysentery. Studies have shown the occurrence of *Shigella* species in ready-to-eat chicken and other meats (Ankita *et al.* 2012).The microbial pathogenecity have been controlled by the use of commercially available antimicrobial drugs. Tremendous use of antibiotics has developed multiple drug resistance (MDR) in the bacteria and is the main hindrance in successful treatment of the infectious disease (Fu *et al.* 2007). In this study, the effects of *Allium sativum* and *Zingiber officinale* extracts on *Shigella dysenteriae* isolated from ready- to- eat fried chicken was evaluated. The data clearly revealed a pronounced activity of the extracts against the tested bacterium.

The presence of the phytochemicals seen in the studied extracts may be responsible for the antibacterial activity exhibited by the extracts of *Allium sativum* and *Zingiber officinale*. Similar findings were made, as described by many researchers (Iheukwumere *et al*. 2012; Gazuwa *et al.* 2013; Iheukwumere and Umedum 2013). Some phytochemicals work by intercalating with DNA of the organism (alkaloid), interferes with protein synthesis and disrupt cell membrane (e.g. saponins) while others interfere signal transduction pathway, metabolic processes, damage metabolic and cellular enzymes, disrupt proton motive force, electron flow, coagulation of cell component and modulation of gene expression (Fu *et al*. 2007).

The antibacterial activity shown by *Allium sativum* and *Zingiber officinale* extracts in this study agrees with similar findings of different researchers (Patil and Shethgar, 2010; Gull *et al*. 2012). It is reported that sesquiterpenoids are the main component of *Zingiber officinale* which attributes its antibacterial activity (Fu *et al.* 2007) while the antibacterial properties of *Allium sativum* is traced to its chemical compound, allicin (Gazuwa *et al*.2013).

Allium sativum and *Zingiber officinale* ethanolic extracts showed more activity against *S.dysenteriae* than their aqueous extracts. This showed that the active phytochemical constituents of the extracts had more ability to dissolve in ethanol (organic solvent) than in water (inorganic solvent). Similar conclusion was drawn by

different researchers (Sebioma *et al*. 2011; Gull *et al*. 2012; Iheukwumere *et al*. 2012; Iheukwumere and Umedum 2013). Though aqueous extract produced higher amount of extract but exhibited relatively lower activity than the ethanolic extract which was obtained in lower quantity. This indicates that the amount of yield did not always influence the inhibition of microbial growth but the active ingredients found in the extract play the major role. Similar observation was made by Iheukwumere *et al.* (2012). The study further highlighted that ethanol was able to extract more of the phytochemical constituents because ethanol is an organic and polar solvent. And most of the phytochemical constituents are organic in nature. This observation suggested that the organic solvent extraction is suitable to verify the antibacterial properties of medicinal plants. (Ali *et al.* 2001; Iheukwumere *et al.* 2012).

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the extracts of *Allium sativum* and *Zingiber officinale* showed that both their ethanolic and aqueous extracts exhibited similar activity compared to ciprofloxacin (control) which had more pronounced activity. Also the ethanolic extracts of both plants exhibited similar antibactericidal activity with ciprofloxacin (control). This means that infections caused by *S. dysenteriae* could be managed effectively using the single dose of this seed extract. Also, further research involving *In vivo* assays will be needed to establish the relationship between MICs and MBCs obtained in this study and the effective dosage that should be administered in ethnomedical practice.

CONCLUSION

The results of the present study have provided the justification for therapeutic potential of spices. The use of garlic and ginger as a natural supplement is considered healthy choice and alternative medication for the treatment of shigellosis. It will not only reduce the clinical burden of drug resistance development, but also the side effects and cost of treatment with modern medicine

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