



Phytochemical Profile and Antibacterial Effects of Zingiber Officinale Root Extract on Some Enteric Bacterial Pathogens

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The majority of Africans today depend either totally or partially on medicinal plants for the treatment of various diseases. In some rural communities, ethno-medicine is sometimes the only form of healthcare. Therefore, this work aimed to determine the phytochemical constituents and antibacterial potentials of *Z. officinale*, which is one of the medicinal plants used by some people. The active ingredients of the plant were first extracted using water and ethanol as solvents. This was followed by the phytochemical analysis of the extracts. Furthermore, the antibacterial effects of aqueous and ethanolic extracts of *Z. officinale* on *Escherichia coli*, *Salmonella typhi* and *Shigella dysenteriae* isolated from faeces of gastroenteritis patients were evaluated using the agar diffusion technique (punch method). Additionally, a two-fold tube dilution method was used to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the extracts. The mean values of zones of inhibition obtained were statistically analyzed using ANOVA. The least significant difference was determined according to the LSD test at $P < 0.05$. Phytochemical analysis revealed the presence of saponin, alkaloids, flavonoids, and tannins. The antibacterial results showed that both the aqueous and ethanolic extracts have antibacterial effects against all the test organisms but at varying degrees. At the 500mg/ml concentration, the ethanolic extract of *Zingiber officinale* produced a zone of inhibition of 21.00^b against *S. dysenteriae* and 20.00^b against *E. coli* and *S. typhi*. On the other hand, the 500mg/ml concentration of the aqueous extract had a zone of inhibition of 20.00^b against *S. dysenteriae*, 16.00^b against *E. coli* and 10.00^b against *S. typhi*. The findings from this study lend credence to the claims that *Z. officinale* extracts possess antibacterial potentials. Also, the higher potency in ethanolic than in aqueous extract suggests that the potency might be dose and solvent dependent. Conclusively, owing to the findings from this study, the active ingredients of *Z. officinale* could be harnessed and employed in the development of novel antibacterial therapies.

Keywords: *Escherichia coli*; *Salmonella typhi*; *Shigella dysenteriae*; aqueous; ethanol; antibacterial.

1. INTRODUCTION

In some parts of the world, traditional medicine is still being practised [1]. This often involves the use of a medicinal herb or plant in the treatment of diseases. According to Abayomi et al. [2], a consultative committee of the World Health Organisation (WHO), a medicinal plant is any plant that contains chemicals that have therapeutic value or that serve as precursors to produce effective pharmaceuticals. One of the therapeutic herbs that has been widely used in folk medicine is *Zingiber officinale* (popularly known as ginger), which belongs to the Zingiberaceae family. There are more than 45 genera and 800 species in this family. *Z. officinale* is a perennial plant that grows upright to a height of one to three feet and is a widely used home remedy. The roots of this plant (fresh and dried) have been used in ancient China, India, and other countries to treat various diseases like cold-induced illnesses, nausea, asthma, cough, colic, heart palpitations, swelling, dyspepsia, loss of appetite, and rheumatism [3].

Ginger contains a variety of bioactive substances [4]. Recent research has revealed that the biological properties of ginger, including

anti-inflammatory, antibacterial, antioxidant, and anti-cancer properties, could be attributed to the bioactive compounds present in it. Furthermore, an increasing body of research has shown that ginger may help prevent and treat several diseases, including neurological conditions [5], cardiovascular conditions, obesity, diabetes mellitus, nausea and vomiting and respiratory issues [6].

With the increasing resistance of microorganisms to conventional antibiotics, there is an urgent need to produce novel, more potent, and broad-spectrum antibiotics that are readily available, affordable, and have low levels of toxicity and resistance. Consequent upon the afore-mentioned properties of this plant and its use in traditional medicine, this research aimed at determining the phytochemicals composition and assessing the antibacterial properties of aqueous and ethanolic extracts of *Zingiber officinale* against enteric bacterial pathogens.

2. MATERIALS AND METHODS

2.1 Collection of Plant Samples

Zingiber officinale was purchased from cemetery markets in Aba South Local Government Area of

Abia State, Nigeria. It was identified in the Department of Plant Science and Biotechnology, Imo State University, Owerri. Three hundred grams (300gm) of *Zingiber officinale* was washed, sliced, and sun-dried for seven days. It was later crushed using an electric blender and thereafter packed into a clean polythene bag and labelled accordingly.

2.2 Isolation of Test Organisms

The test organisms used in this research were *Escherichia coli*, *Salmonella typhi* and *Shigella dysenteriae*. These organisms were isolated from stool samples of gastroenteritis patients attending Abia State University Teaching Hospital, Aba. Ethical clearance was obtained from the hospital's ethical committee. The consent of the patients was sought after a brief discussion with the physician. The importance of the research was explained to them. Those who agreed to participate were made to fill out the consent form. The isolation and identification of the bacteria were carried out. Cultural and morphological identification, besides biochemical characterization of isolates, were carried out using the methods described by Cheesbrough. *S. typhi* was serologically differentiated from other *Salmonella* species by the presence of vi antigen [7]. Pure cultures of the bacteria were maintained in a Nutrient agar slant and kept in the refrigerator for future use.

2.3 Extraction

Ninety-eight per cent (98%) of ethanol and distilled water were used for the extraction. The ground root was weighed (150 grams each) and dissolved in 500 ml of the solvent. These were stoppered and kept for ten days with intermittent shaking [8]. Afterwards, the mixtures were filtered with Whatman's number one filter paper. The ethanol extract was concentrated at 40°C under reduced pressure using a Rotary evaporator (R100). A hot air oven was then used to concentrate the aqueous extract overnight at 40°C [9]. The concentrated extract was collected in screw-capped bottles, labelled and stored at 4°C in the refrigerator.

2.4 Phytochemical Screening

The method described by Lajubutu et al. [10] was used for this. The extract was tested for alkaloids, Tannins, Saponins, and flavonoids.

2.5 Antibacterial Assay

The *in vitro* antibacterial assay was carried out using the agar-gel diffusion (punch method) technique, as described by Osadebe and Ukwueze [11]. In this method, the broth culture of the test isolates (0.1ml) was aseptically inoculated by spreading evenly onto the dried surface of Muller-Hinton agar plates using a bent sterile glass rod. Six wells (5.0mm diameter) were then made in the plates using a sterile cork borer. The fifth well served as the negative control, while the sixth well served as the positive control. Sterile distilled water served as the negative control, while ciprofloxacin was used as the positive control. Double dilution of the extracts was made to get the various concentrations as follows: (500mg/ml, 250mg/ml, 125mg/ml and 63mg/ml) that were used for the antibacterial assay. The bottom of wells 1- 4 were sealed with one drop of sterile molten Muller-Hinton agar to prevent diffusion of the extracts under the agar. Fixed volumes (0.1ml) of the four different concentrations of the extracts were transferred into wells 1-4 using a sterile Pasteur pipette. The control wells were filled with 0.1ml of distilled water and 10µg of ciprofloxacin, respectively.

The plates were left on the bench for 40 minutes for pre-diffusion of the extracts to take place [12]. They were then incubated at 37°C for 24 hours. Antibacterial activities of the extracts were determined by measuring the resulting zone diameters of inhibition (mm) against each test organism using a ruler. The experiment was carried out in triplicate and the mean values of the results were taken as antibacterial activity [13,14].

2.6 Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

Inoculum was prepared by making a direct broth of colonies selected from 24-hour agar plates. The suspension was adjusted to match 0.5ml Mac-Farland standard using sterile saline. The MIC and MBC of the potent extracts were determined according to the macro broth dilution technique [15]. Double dilution was also done here to get the four different concentrations of the extracts. Standardized suspensions of the test organisms were inoculated into a series of sterile tubes of peptone water containing dilutions (500, 250, 125, and 63mg/ml) of the

extracts. They were incubated at 37°C for 24 hours. The MIC was read as the lowest concentration, which inhibited visible growth (absence of turbidity) of the test organisms.

For MBC determination, a loopful of the broth from each of the tubes that did not show any visible growth (no turbidity) during MIC determination was subcultured onto extract-free Muller-Hinton agar plates and further incubated for 24 hours at 37°C. The lowest concentration at which no visible growth was observed was noted as the minimum bactericidal concentration (MBC).

3. RESULTS

3.1 Phytochemical Components of *Zingiber officinale*

Table 1 shows the phytochemicals or bioactive compounds present in the extracts of Ginger (*Zingiber officinale*). The phytochemical analysis showed the presence of alkaloids, saponins, flavonoids, and tannins.

3.2 Antibacterial Effects of *Zingiber officinale* Root Extracts on Some Enteric Pathogens

Table 2a below shows the mean diameter of the zones of inhibition produced by the different concentrations of *Zingiber officinale* root ethanolic extract and the control treatments on the test organisms. There is a concentration-dependent inhibition of the growth of the

pathogens. That is to say, the highest zones of inhibition (20.00mm, 20.00mm, and 21.00mm for *E. coli*, *S. typhi*, and *S. dysenteriae*, respectively) were seen in the highest concentration (500mg/mL). However, when compared with the zones of inhibition produced by the positive control (ciprofloxacin), those produced by the latter were higher (24.00mm, 25.00mm, and 27.00mm for *E. coli*, *S. typhi*, and *S. dysenteriae*, respectively). On the other hand, at the lowest concentration (63mg/ml), no zone of inhibition (0.00) was seen. This corresponds with the no zone of inhibition (0.00mm) observed in the negative control (distilled water) for all the pathogens.

Table 2b below shows the mean diameter of the zones of inhibition produced by the different concentrations of *Zingiber officinale* root aqueous extract and the control treatments on the test organisms. There is a concentration-dependent inhibition of the growth of the pathogens. That is to say, the highest zones of inhibition (16.00mm, 10.00mm, and 20.00mm for *E. coli*, *S. typhi*, and *S. dysenteriae*, respectively) were seen in the highest concentration (500mg/mL). However, when compared with the zones of inhibition produced by the positive control (ciprofloxacin), those produced by the latter were higher (22.00mm, 20.00mm, and 26.00mm for *E. coli*, *S. typhi*, and *S. dysenteriae*, respectively). On the other hand, at the lowest concentration (63mg/ml), no zone of inhibition (0.00) was seen. This corresponds with the no zone of inhibition (0.00mm) observed in the negative control (distilled water) for all the pathogens.

Table 1. Phytochemical components of *Zingiber officinale*

Extract	Alkaloids	Saponin	Flavonoids	Tannins
<i>Zingiber officinale</i>	+	+	+	+

Key: Present = (+), Absent = (-)

Table 2a. Mean* diameter of zone of inhibition (in millimetres) of different concentrations of *Zingiber officinale* root ethanolic extract and the control treatments on the test organisms.

Treatments	Dose	Zones of Inhibition [in millimetre (mm)]		
		<i>E. coli</i>	<i>S. typhi</i>	<i>S. dysenteriae</i>
<i>Z. officinale</i>	500mg/ml	20.00 ^b	20.00 ^b	21.00 ^b
<i>Z. officinale</i>	250mg/ml	16.00 ^c	6.00 ^c	15.00 ^c
<i>Z. officinale</i>	125mg/ml	6.00 ^d	0.00 ^d	0.00 ^d
<i>Z. officinale</i>	63mg/ml	0.00 ^e	0.00 ^d	0.00 ^d
Ethanol	0.1ml	0.00 ^e	0.00 ^d	0.00 ^d
Ciprofloxacin	10µg/ml	24.00 ^a	25.00 ^a	27.00 ^a
LSD		1.485	1.369	1.369

Key: Means on the same column with different letter superscripts are significantly different ($P < 0.05$), according to the LSD test; Ethanol = Negative Control; and Ciprofloxacin = Positive Control

Table 2b. Mean* diameter of zone of inhibition (in millimetres) of different concentrations of *Zingiber officinale* root aqueous extract and the control treatments on the test organisms

Treatments	Dose	Zones of Inhibition [in millimetre (mm)]		
		<i>E. coli</i>	<i>S. typhi</i>	<i>S. dysenteriae</i>
<i>Z. officinale</i>	500mg/ml	16.00 ^b	10.00 ^b	20.00 ^b
<i>Z. officinale</i>	250mg/ml	10.00 ^c	6.00 ^c	12.00 ^c
<i>Z. officinale</i>	125mg/ml	6.00 ^d	0.00 ^d	4.00 ^d
<i>Z. officinale</i>	63mg/ml	0.00 ^e	0.00 ^d	0.00 ^e
Distilled water	0.1ml	0.00 ^e	0.00 ^d	0.00 ^e
Ciprofloxacin	10µg/ml	22.00 ^a	20.00 ^a	26.00 ^a
LSD		1.627	1.369	0.939

Key: Means on the same column with different letter superscripts are significantly different ($P < 0.05$), according to the LSD test; Distilled water = Negative Control; and Ciprofloxacin = Positive Control

Table 3a. The minimum inhibitory concentration of different concentrations of *Zingiber officinale* root ethanolic extract and the control treatments on the test organisms.

Treatments	Dose	Bacterial Pathogens		
		<i>E. coli</i>	<i>S. typhi</i>	<i>S. dysenteriae</i>
<i>Z. officinale</i>	500mg/ml	-	-	-
<i>Z. officinale</i>	250mg/ml	-	-	-
<i>Z. officinale</i>	125mg/ml	+	+	+
<i>Z. officinale</i>	63mg/ml	+	+	+
Ethanol	0.1ml	+	+	+
Ciprofloxacin	10µg/ml	-	-	-

Key: (-) = inhibition (no growth); (+) = no inhibition (growth); Ethanol = negative control; Ciprofloxacin = positive control

Table 3b. The minimum inhibitory concentration of different concentrations of *Zingiber officinale* root aqueous extract and the control treatments on the test organisms

Treatments	Dose	Bacterial Pathogens		
		<i>E. coli</i>	<i>S. typhi</i>	<i>S. dysenteriae</i>
<i>Z. officinale</i>	500mg/ml	-	-	-
<i>Z. officinale</i>	250mg/ml	-	+	+
<i>Z. officinale</i>	125mg/ml	+	+	+
<i>Z. officinale</i>	63mg/ml	+	+	+
Distilled water	0.1ml	+	+	+
ciprofloxacin	10µg/ml	-	-	-

Key: (-) = inhibition (no growth); (+) = no inhibition (growth); Distilled water = negative control; Ciprofloxacin = positive control

3.3 Minimum Inhibitory Concentration (MIC)

Table 3a below shows the minimum inhibitory concentration of different concentrations of *Zingiber officinale* root ethanolic extract and the control treatments on the test organisms. The minimum inhibitory concentration of all the test organisms was (250mg/mL) which is the least concentration that inhibited the growth of all the test organisms.

Table 3b below shows the minimum inhibitory concentration (MIC) of different concentrations of

Zingiber officinale root aqueous extract and the control treatments on the test organisms. The MIC of *E. coli* was 250mg/ml, while that of *S. typhi* and *S. dysenteriae* was 500mg/ml.

3.4 Minimum Bactericidal Concentration (MBC)

Table 4a below shows the minimum bactericidal concentration (MBC) of different concentrations of *Zingiber officinale* root ethanolic extract and the control treatments on the test organisms. The MBC of all the test organisms (*E. coli*, *S. typhi* and *S. dysenteriae*) was 500mg/ml.

Table 4a. The minimum bactericidal concentration of different concentrations of *Zingiber officinale* root ethanolic extract and the control treatments on the test organisms

Treatments	Dose	Bacterial Pathogens		
		<i>E. coli</i>	<i>S. typhi</i>	<i>S. dysenteriae</i>
<i>Z. officinale</i>	500mg/ml	-	-	-
<i>Z. officinale</i>	250mg/ml	+	+	+
<i>Z. officinale</i>	125mg/ml	+	+	+
<i>Z. officinale</i>	63mg/ml	+	+	+
Ethanol	0.1ml	+	+	+
Ciprofloxacin	10µg/ml	-	-	-

Key: (-) = inhibition (no growth); (+) = no inhibition (growth); Ethanol = negative control; Ciprofloxacin = positive control

Table 4b. The minimum bactericidal concentration of different concentrations of *Zingiber officinale* root aqueous extract and the control treatments on the test organisms

Treatments	Dose	Bacterial Pathogens		
		<i>E. coli</i>	<i>S. typhi</i>	<i>S. dysenteriae</i>
<i>Z. officinale</i>	500mg/ml	-	+	+
<i>Z. officinale</i>	250mg/ml	+	+	+
<i>Z. officinale</i>	125mg/ml	+	+	+
<i>Z. officinale</i>	63mg/ml	+	+	+
Distilled water	0.1ml	+	+	+
Ciprofloxacin	10µg/ml	-	-	-

Key: (-) = inhibition (no growth); (+) = no inhibition (growth); Distilled water = negative control; Ciprofloxacin = positive control

Table 4b below shows the minimum bactericidal concentration (MBC) of different concentrations of *Zingiber officinale* root aqueous extract and the control treatments on the test organisms. The MBC of *E. coli* was 500mg/ml. However, the extract did not exhibit any bactericidal activity against *S. typhi* and *S. dysenteriae*.

4. DISCUSSION

This research was carried out to determine the phytochemicals in *Z. officinale* and evaluate the antibacterial effects of the plant extract on selected enteric pathogens. The phytochemicals that were identified in ginger (*Z. officinale*) roots used in this study are alkaloids, saponins, flavonoids and tannins. These phytochemicals have long been reported to be present in higher plants by Kaufman et al. [16] and were said to confer antibacterial potentials to any plant in which they are found. Aside from the antibacterial properties, these compounds are widely believed to be responsible for other medicinal properties in plants in which they are contained [16,17].

Furthermore, the phytochemical result of the ethanolic extract of *Zingiber officinale* in this study corroborates the earlier findings by Wahab

et al. [18] and Osabor et al. [19]. Although, tannin, which was found in this study, was not reported by Osabor et al. [19]. This additional component (tannin) found in this study might have contributed significantly to the higher rate of inhibition that was recorded. This variation in phytochemicals and their concentrations could be due to the variation in habitats, as opined by Farooq et al. [20]. This might be true as the phytochemical analysis of *Datura stramonium* (Jimson weed) by Dike-Ndudim et al. [21] showed the presence of Tannin, Phenol, flavonoid, alkaloid, phytate, and hydrogen cyanide. However, Walter and Nowacki [22] disagreed with that proposition and suggested that the production of phytochemicals could result from plants' responses to threats. Nevertheless, it could be that the combination of these factors plays a key role in the variation in the composition and concentration of phytochemicals in medicinal plants. Therefore, this area of research requires further studies.

The results obtained in this research indicated that both aqueous and ethanolic extracts of *Zingiber officinale* exhibited antibacterial action against all the test organisms, although with different levels of sensitivities to the extracts. As revealed in this research, the antibacterial

properties of *Z. officinale* extract agree with the report by Nassan and Mohamed [23], who reported that *Zingiber officinale* possesses antibacterial properties. The antibacterial properties observed with both aqueous and ethanolic extracts suggest that water and ethanol could be used as solvents in the extraction process. Reports from previous studies have validated this proposition. The study by Dike-Ndudim et al. [21] reported that the aqueous and ethanolic extracts of Jimsonweed (*Datura stramonium*) possess antibacterial properties. A similar report was obtained from Ndubueze et al. [24] with aqueous and ethanolic leaf extracts of *Gongronema latifolium*.

Generally, and in this study, the ethanolic extracts of *Zingiber officinale* were more effective than the aqueous extract, indicating that ethanol is a better solvent than water. This agrees with the reports of Ogunjobi and Nnadozie [25], Ezeifeke et al. [26-27], and Anyanwu et al. [8]. However, it contradicts the report by Ndubueze et al. [24], in which no statistically significant difference was reported in both aqueous and ethanolic leaf extracts of *Gongronema latifolium*. Furthermore, since both extracts proved to possess antibacterial properties, future research with medicinal plants should continue exploring these solvents and other possible methods of extraction. Modifications and enhancement of the extraction methods would ensure that the findings from studies of this nature are indisputable.

5. CONCLUSION

The successful inhibition of enteric organisms by *Zingiber officinale*'s extracts offers hope for mitigating the diseases caused by pathogens. Considering the level of multi-resistance these bacteria have developed against conventional antibiotics over the years, harnessing the active ingredients of this medicinal plant might go a long way in treating the infections caused by the organisms.

CONSENT

As per international standards or university standards, patient(s) written consent has been collected and preserved by the author(s).

ETHICAL APPROVAL

Ethical clearance was obtained from the hospital's ethical committee.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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