PhytochemicalProfile and AntibacterialEffectsof*Zingiberofficinale*Root Extract onAlbinoRatsInfectedwithSomeEnteric BacterialPathogens

ABSTRACT

Majority of Africans today depend either totally or partially on medicinal plants for the treatment of various diseases. In some rural communities, ethno-medicineissometimestheonlyform of healthcare. This work, therefore, was aimed at determining thephytochemicalconstituentsandantibacterialpotentialsof *Z.officinale*which is one of the medicinal plants used by some persons. 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 andethanolic extracts of *Z. officinale* on*Eschrichia coli, Salmonella typhi* and*Shigelladysentriae*isolatedfromfaecesofgastroenteritispatientswas evaluated usingagar diffusiontechnique(punch method). Additionally, two-fold tube dilution method was used to determine the minimuminhibitory concentration (MIC), and minimum bactericidal concentration (MBC) of the extracts.The mean values of zones of inhibition obtained were statistically analyzed using ANOVA. Theleast significant difference was determined according to LSD test at $P<0.05$. Phytochemicalanalysis revealed the presence of saponin, alkaloids, flavonoids, and tannins. The antibacterial results showed thatboth the aqueous andethanolic extracts have antibacterial effects against all the test organisms but at varying degrees. At the concentration of 500mg/ml, the ethanolic extract of *Zingiber officinale* produced zone of inhibitionof21.00^{b}against*S.dysentriae*and20.00^{b}against*E.coli* and*S.typhi*.On the other hand, the 500mg/ml concentration of the aqueous extract had zone of inhibition of 20.00^{b} against *S.dysentriae*,16.00^{b}against*E.coli*and10.00^{b}againstand *S.typhi*.The findings from this study lend credence to the claims that*Z. officinale* extracts possess antibacterial potentials. Also, the higher potency seen in ethanolic than in aqueous extract suggests that the potency might be doseand 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 emerging antibacterial therapies.

Keywords:Ginger, *Escherichia coli, Salmonella typhi, Shigella dysenteriae*, Aqueous, Ethanol.

1. INTRODUCTION

In some parts of the world, traditional medicine is still being practiced [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). It 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 likecold-induced illnesses, nausea, asthma, cough, colic, heart palpitations, swelling, dyspepsia, loss of appetite, and rheumatism [3].

Ginger contains a variety of bioactive substances, including phenolic and terpene chemicals. The primary phenolic chemicals that give ginger its different bioactivities are gingerols, shogaols, and paradols[4]. Recent research has revealed 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 severaldiseases, including neurological conditions [5], cardiovascular conditions, obesity, diabetes mellitus, nausea and vomiting and respiratory issues [6].

With increasing resistance of microorganisms to conventional antibiotics, there is an urgent need to produce novel, more potent, andbroad-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 andassessing the antibacterial properties of aqueous and ethanolic extracts of *Zingiber officinale* againstentericbacterialpathogens.

2. MATERIALSANDMETHODS

2.1 CollectionofPlantSamples.

Zingiber officinale was purchased from cemetery markets in Aba South Local Government AreaofAbiaState,Nigeria.ThiswasidentifiedintheDepartmentofPlantScienceandBiotechnology,ImoSta teUniversity,Owerri.Threehundredgrams(300gm)*ofZingiberofficinale* was washed, sliced and sun dried for seven days. This was later crushed using anelectricblender. Itwasthereafter packedintocleanpolythenebagandlabeledaccordingly.

2.2 Isolation of TestOrganisms

The test organisms used in this research were *Escherichia coli, Salmonella typhi* and *Shigelladysenteriae.*Theseorganismswereisolatedfromstoolsamplesofpatientssufferingfromgastroenter itis attending Abia State University Teaching Hospital Aba. Ethical clearance wasobtained from the ethical committee of the hospital. The consent of the patients was sought afterdiscussing with the physician. The importance of the research was explained to them. Those thatagreed to participate were made to fill the consent form. The isolation and identification of

thebacteriawascarriedout.Culturalandmorphologicalidentificationbesidesbiochemicalcharacterization of isolates were carried out using the methods described by Cheesbrough. *S.typhi*was serologically differentiatedfrom othersalmonellaebythepresenceof vi antigen[7]. Pure culture of the bacteria were maintained inNutrient agar slant andkeptintherefrigeratorforfuture use.

2.3 Extraction

Ninety eight percent (98%) ethanol and distilled water were used for the extraction. The grindedleafwasweighed(150gramseach)anddissolvedin500mlofthesolvent.Theseweretopperedand kept for ten days with intermittent shaking [8]. Afterwards, the mixtureswere filtered with Whatman's number one filter paper.The ethanol extract was concentrated at40°C under reduced pressure using Rotary evaporator (R100).Hot air oven was then used in theconcentration of aqueous extract overnightat40^{o}C[9]. Theconcentratedextractwerecollectedinscrewcappedbottles,labeledand storedat4^{o}Cintherefrigerator.

2.4 PhytochemicalScreening

The method described by Lajubutu*et al*., [10] was used for this. Alkaloids, Tannins, Saponinsandflavonoids weretestedforinthe extract.

2.5 AntibacterialAssay

In-vitroantimicrobialassaywascarriedoutusingagar-geldiffusion(punchmethod)techniqueas described by Osadebe and Ukwueze[11]. In this method, broth culture of the test isolates(O.1ml) was aseptically inoculated by spreading evenly onto the dried surface of Muller-Hintonagar plates using a bentsterile glass rod. Sixwells (5.0mm diameter) were then madein theplates using a sterile cork borer. The fifth well served as the negative control, while the sixthwells served as the positive controls. Sterile distilled water served as the negative control, whileciprofloxacin was used as the positive control. Double dilution of the extracts wasmade togetthe various concentrations as follows: (500mg/ml, 250mg/ml, 125mg/ml and 63mg/ml) that wereused for the antimicrobial assay.The bottom of the wells 1-4 were sealed with one drop ofsterile molten Muller-Hinton agar to prevent diffusion of the extracts under the agar. Fixedvolumes (O.lml)of thefourdifferentconcentrations of the extracts were transferredinto thewells 1-4 using asterile pastuerpipette. The control wellswerefilled with O.lml of distilledwaterandl0µgofciprofloxacinrespectively.

The plates were left on the bench for 40 minutes for pre-diffusion of the extracts [12], and then incubated at 37°C for 24hours. Antimicrobial activities of the extracts weredetermined by measuring the resulting zone diameters of inhibition (mm)againsteach testorganism using a ruler. The experiment was carried out in triplicate and the mean values of theresultweretakenasantimicrobialactivity[13, 14].

2.6. DeterminationofMinimumInhibitoryConcentration(MIC)andMinimumBactericidal Concentration(MBC)

Innoculum was prepared by making a direct broth of colonies selected from 24-hour agar plates.Suspensionwasadjustedtomatch0.5mlMac-Farlandstandard usingsterile saline.The MIC and MBC of the potent extracts were determined according to the macro broth dilutiontechnique[15].Doubledilution wasalsodoneheretogetthefourdifferent concentrations of the extracts. Standardized suspensions of the test organisms wereinoculated into a series of sterile tubes of peptone water containing dilutions (500, 250, 125, and63mg/ml) of the extracts and were

incubated at 37°C for 24 hours. The MIC was read as leastconcentrationthatinhibitedvisiblegrowth(absenceofturbidity)ofthe test organisms.

For MBC determination, a loopful of the broth from each of the tubes that did not show anyvisiblegrowth(noturbidity)duringMICdeterminationwassub-culturedontoextractfreeMuller-Hinton agar plates, and further incubated for 24 hours at 37°C. The least concentration, atwhich no visible growth was observed, was noted as the MBC, whereas the least concentration atwhichvisiblegrowthoccuredwasregardedastheMinimumBacteriostaticConcentration(MBS).

3. RESULTS

3.1. Phytochemical components of *Zingiberofficinale.*

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

Table1:Phytochemicalcomponentsof*Zingiberofficinale.*

Extract	Alkaloids	Saponin	Flavonoids	Tannins
Zingiber officinale	+	+	+	+

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

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 differentconcentrationsof*Zingiber officinale* root ethanolic extract and the control treatments on the testorganisms. 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 (Ciproxin) 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.

Table2a:Mean* diameterofzoneofinhibition(in millimeter) ofdifferentconcentrationsof*Zingiber officinale* root ethanolic extract and the control treatments on the testorganisms.

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

Key: Meansonthesamecolumnwithdifferentlettersuperscriptsaresignificantlydifferent ($P < 0.05$),accordingtoLSDtest; Ethanol = Negative Control; and Ciproxin = Positive Control.

Table 2b below shows the mean diameter of the zones of inhibition produced by the differentconcentrationsof*Zingiber officinale* root aqueous extract and the control treatments on the testorganisms. 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 (Ciproxin) 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.

Table2b: Mean*diameterofzoneofinhibition (in millimeter) of differentconcentrationsof*Zingiber officinale* root aqueous extractand the control treatments on the testorganisms.

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

Key: Meansonthesamecolumnwithdifferentlettersuperscriptsaresignificantlydifferent (P <0.05),accordingtoLSDtest; Distilled water = Negative Control; and Ciproxin = 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 testorganisms. The minimum inhibitory concentration of the all the test organisms was (250mg/mL) which is the least concentration that inhibited the growth of all the test organisms.

Table 3a:Theminimum inhibitory concentration of different concentrations of*Zingiber officinale* root ethanolic extract and the control treatments on the testorganisms.

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

Key: (-) =inhibition(nogrowth); (+) = no inhibition (growth); Ethanol =negativecontrol; Ciproxin= positivecontrol.

Table 3b below shows the minimum inhibitory concentration (MIC) of different concentrations of*Zingiber officinale* root aqueous extract and the control treatments on the testorganisms. The MIC of *E. coli* was 125mg/ml while that of *S. typhi* was 250mg/ml and that of and *S. dysenteriae* 125mg/ml.

Table 3b: Theminimum inhibitory concentration of different concentrations of*Zingiber officinale* root aqueous extractand the control treatments on the testorganisms.

		Bacterial Pathogens		
Treatments	**Dose**	***E. coli***	***S. typhi***	***S. dysenteriae***
Z. officinale	500mg/ml	-	-	-
Z. officinale	250mg/ml	-	+	-
Z. officinale	125mg/ml	+	+	+
Z. officinale	63mg/ml	+	+	+
Distilledwater	0.1ml	+	+	+
Ciproxin	10µg/ml	-	-	-

Key: (-) =inhibition(nogrowth); (+) = no inhibition (growth); Distilledwater=negativecontrol; Ciproxin= positivecontrol.

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 testorganisms. The MBC of all the test organisms was(*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 testorganisms.

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

Key: (-) =inhibition(nogrowth); (+) = no inhibition (growth); Ethanol=negativecontrol; Ciproxin= positivecontrol.

Table 4b below shows the minimum bactericidal concentration (MBC) of different concentrations of*Zingiber officinale* root aqueous extract and the control treatments on the testorganisms. The MBC of *E. coli* was 500mg/ml while that of S. typhi and S. dysenteriae could not be determined.

Table 4b: The minimum bactericidal concentration of different concentrations of*Zingiber officinale* root aqueous extractand the control treatments on the testorganisms.

		Bacterial Pathogens		
Treatments	**Dose**	***E. coli***	***S. typhi***	***S. dysenteriae***
Z. officinale	500mg/ml	-	+	+
Z. officinale	250mg/ml	+	+	+
Z. officinale	125mg/ml	+	+	+
Z. officinale	63mg/ml	+	+	+
Distilledwater	0.1ml	+	+	+
Ciproxin	10µg/ml	-	-	-

Key: (-) =inhibition(nogrowth); (+) = no inhibition (growth); Distilledwater=negativecontrol; Ciproxin= positivecontrol.

4. DISCUSSION

This research was carried out to determine the phytochemicals present in *Z. officinale* and evaluate the antibacterial effects of the plant (ginger clove) extract on selected enteric pathogens. The phytochemicals that were identified in the garlic (*Z. officinale*) clove used in this study are alkaloids, saponins, flavanoids and tannins. These phytochemicals have long been reported to bepresentin higher plants by Kaufman *et al.* [16] and were said

toconferantibacterialpotentialstoanyplantinwhichtheyare found. Aside from the antibacterial properties, these compounds are widely believed to be responsible for other medicinal properties the plants in which they are contained possess[16, 17].

Furthermore, the phytochemical result ofthe ethanolic extract of *Zingiber officinale* in this study corroborates the earlier findings byWahab *et al.* [18] and Osabor*et al*. [19]. Although, tannin, which was found in this study, was not reported by Osabor et al.[18]. 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 theirconcentrations 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 andNowacki[22] disagreed with that proposition and suggested that the production of phytochemicals could result from plants' responsestothreats. 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.

Theresultsobtainedinthisresearchindicatedthatbothaqueous andethanolicextracts of*Zingiber officinale* exhibited antibacterial action against all the test organisms, although withdifferent 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 possessesantibacterialproperties. 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 both 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*Gongronemalatifolium.*

Generally,andin thisstudy,the ethanolicextracts of *Zingiber officinale*were more effectivethan the aqueous extract, indicating that ethanol is a better solvent than water. This agrees with the reports of Ogunjobi andNnadozie[25],Ezeifeka*et al.*, [26], andAnyanwu *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 *Gongronemalatifolium*. 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.

Conclusion

Thesuccessful 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.

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