The Phytochemical Composition and AntibacterialEffects of*Alliumsativum*cloveExtracts onAlbinoRats InfectedwithSomeEnteric BacterialPathogens

ABSTRACT

In this study, the phytochemicalspresent in the cloves of *Allium sativum* were determined and their antibacterial activities against some enteric bacterialpathogens assessed.After the extraction process was completed using water and ethanol as the solvents, the determination of the phytochemicalconstituentswas carried out. Furthermore, the aqueous and ethanolic extractsof *A. sativum* were tested against *Escherichia coli, Salmonella typhi* and *Shigella dysenteriae*isolatedfromfaecesofgastroenteritispatients. The agar diffusiontechnique(punch method) was used for this. Additionally, the minimum inhibitoryconcentration(MIC), andminimumbactericidalconcentration(MBC)oftheextracts were determined.Themean values of the zones of inhibition obtained were statistically analyzed using ANOVA. The leastsignificant difference was determined according to LSD test at $P<0.05$. The results of the phytochemical analysisrevealed the presence of saponin, alkaloids, flavonoids, and tannins. Furthermore, the antibacterial susceptibility test showed that both the aqueous andethanolic extracts possess antibacterial properties against all the test organisms. Theethanolic extract at the concentration of 500mg/ml had zones of inhibition of29.00^{b}against*E. coli*,24.00^{b}against*S.dysentriae*andthelowest20.00^{b}against*S.typhi*.On the other hand, at that same concentration, the aqueous extract had zones of inhibition of 20.00^{b} against *E. coli*,and18.00^{b}against*S.dysentriae*and*S.typhi*.Thisstudysuggests that*A. sativum* extracts possess antibacterial properties. Furthermore, since the ethanolic extract was more effective thanaqueous extract, it could be that the antibacterial potency of *A. sativum* is solvent dependent. In conclusion, the findings from this studysuggest thatfurther purificationoftheconstituentsoftheplantmight lead to the developmentofnovelantibiotics.

Keywords:Phytochemical;antibacterial;gastroenteritis;extracts; Garlic.

1. INTRODUCTION

Plants in general and medicinal plants in particular have been extremely important to humans. A significant advancement in pharmacognosy, the therapeutic use of herbs and shrubs to treat a wide range of physiological and non-physiological disorders has greatly aided the development of contemporary pharmacotherapeutics in Africa [1].

Folklore medicine is a widely practiced field worldwide [2]. According to the consultation committee of the World Health Organisation (WHO), a medicinal plant is any plant that includes compounds that have therapeutic value or that serve as building blocks to produce effective pharmaceuticals [3].

Allium sativum, popularly known as garlic, is a medicinal herb that has been extensively utilised in traditional medicine. One specie within the *Allium* genus of onions is *Allium sativum.* Onion, shallot, leek, chive, and rakkyo are some of its close relatives. Native to central Asia, garlic has been used by humans for over 7,000 years. It is a staple herb in the Mediterranean region and is frequently used as a condiment in Asia, Africa, and Europe. It was utilised for both medical and culinary uses and was known to the Ancient Egyptians [4, 5].

Owing to its several bioactive components, including organic sulphides, saponins, phenolic compounds, and polysaccharides, garlic is a popular spice with numerous health benefits [6]. In China, garlic has long been used as a traditional medicine and is widely consumed there. Numerous research conducted in the last few decades have shown that garlic has amazing biological capabilities, such as antibacterial, anti-diabetic, anti-inflammatory, antioxidant, cardiovascular protective, anticancer, and immunomodulatory properties[7, 8].

In today's medical practice, drug resistance is becoming a significant problem. Furthermore, a lot of synthetic medications are quite hazardous even at optimal dosage levels because side effect issues have plagued a lot of these contemporary therapeutic approaches [9]. There is therefore an urgent need to produce novelbroad-spectrumantibiotics which are not only potent, but also available, accessible, and affordable with minimaltoxicity and resistance. Consequent upon the afore-mentioned properties of this plant, garlic, and its usein traditional medicine, this study was aimed at determining the phytochemicals and assessing theantibacterial property of aqueous and ethanolic extracts of *Allium sativum* against some entericbacterial pathogens.

2.0. MATERIALSANDMETHODS

2.1. CollectionofPlantSamples.

The fresh cloves *of Allium sativum* was purchased from cemetery markets in Aba South LocalGovernment Area of Abia State, Nigeria. This was identified in the Department of Plant Scienceand Biotechnology, Imo State University, Owerri. Three hundred grams (300gm) *of A. sativum*clove was washed, sliced and dried at room temperature for seven days. This was later crushedusinganelectricblender.Itwasthereafterpackedintocleanpolythenebagandlabeledaccordingly.

2.2. Isolation of the TestOrganisms

Salmonella typhi, Shigella dysenteriae, and *Escherichia coli* were the test organisms used in this study. Stool samples from gastroenteritis patients at Abia State University Teaching Hospital Aba were used to isolate these microbes. It received ethical clearance from the hospital's ethical committee. After consulting with the doctor, the patients' consent was requested. They were informed of the significance of the study. The consent form has to be filled out by those who accepted to participate. It was necessary to isolate and identify the bacterium. The protocols outlined by Cheesbrough were used to identify isolates morphologically and culturally in addition to characterising them biochemically. According to Cheesbrough [10], the presence of the vi-antigen allowed *S. typhi* to be serologically distinguished from other salmonellae. In Nutrient Agar Slant, pure cultures of the bacteria were preserved for later use and refrigerated.

2.3. Extraction

Ninety- eight percent (98%) ethanol and distilled water were used for the extraction. The grindedleaf was weighed (150 grams each) and dissolved in 500ml of the solvent. These were stopperedand kept for ten days with intermittent shaking [11]. 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 overnight at 40^{o}C [12].The

concentratedextractwerecollectedinscrewcappedbottles,labeledand storedat4^{o}Cintherefrigerator.

2.4. PhytochemicalScreening

The method described by Lajubutu*et al.*[13] was used for this. Alkaloids, Tannins, Saponinsandflavonoids weretestedforinthe extract.

2.5. AntibacterialAssay

In-vitroantimicrobialassaywascarriedoutusingagar-geldiffusion(punchmethod)techniqueas described by Osadebe and Ukwueze [14]. 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. Six wells (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 dilutionof the extracts was made 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 [15] 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 theresultweretakenas antimicrobialactivity[16, 17].

2.6. DeterminationofMinimumInhibitoryConcentration(MIC)andMinimumBactericidal Concentration(MBC)

Innoculum was prepared by making a direct broth of colonies selected from 24-hour agar plates.Suspensionwasadjustedto match0.5mlMac-Farlandstandardusing sterile saline.The MIC and MBC of the potent extracts were determined according to the macro broth dilutiontechnique[18].Doubledilution wasalsodoneheretogetthefourdifferent concentrations of the extracts. Standardized suspensions of the test organisms wereinoculatedintoaseriesof steriletubesof peptonewatercontainingdilutions(500,250,125,and63mg/ml) of the extracts and were incubated at 37°C for 24 hours. The MIC was read as the 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).

3.0. RESULTS

3.1. Phytochemical components of *Allium sativum*.

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

Table 1:Phytochemicalcomponentsof*Alliumsativum.*

Extract	Alkaloids	Saponin	Flavonoids	Tannins
Alliumsativum	+	+	+	+

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

3.2. Antibacterial Effects of *Allium sativum*cloveextracts on Some enteric Pathogens.

Table 2a below shows the mean diameter of the zones of inhibition produced by the differentconcentrationsof*Allium sativum*cloveethanolic 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 (29.00mm, 20.00mm, and 24.00mm 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 (40.00mm, 24.00mm, and 28.00mm for *E. coli, S. typhi,* and *S. dysenteriae*, respectively). On the other hand, at the lowest concentration (63mg/ml), *E. coli* had a zone of inhibition of 12.00mm while *S. typhi,* and *S. dysenteriae* had no zones of inhibition (0.00mm). Furthermore, no zone of inhibition (0.00mm) observed in the negative control (ethanol) for all the pathogens.

Table2a:Mean* diameterofzoneofinhibitionofdifferentconcentrationsof*Allium sativum*clove ethanolic extract and the control treatments on the testorganisms.

		Zones of Inhibition [in millimetre (mm)]		
Treatments	**Dose**	***E. coli***	***S. typhi***	***S. dysenteriae***
A.sativum	500mg/ml	29.00^{b}	20.00^{b}	24.00^{b}
A.sativum	250mg/ml	20.00^{c}	6.00^{c}	16.00^{c}
A.sativum	125mg/ml	18.00^{d}	0.00^{d}	0.00^{d}
A.sativum	63mg/ml	12.00^{e}	0.00^{d}	0.00^{d}
Ethanol	0.1ml	0.00^{f}	0.00^{d}	0.00^{d}
Ciproxin	10µg/ml	40.00^{a}	24.00^{a}	28.00^{a}
LSD		1.788	1.369	0.996

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

Table 2b below shows the mean diameter of the zones of inhibition produced by the differentconcentrationsof*Allium sativum*clove 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 (20.00mm, 18.00mm, and 18.00mm 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, 20.00mm, and 22.00mm for *E. coli, S. typhi,* and *S. dysenteriae*, respectively). On the other hand, at the lowest concentration (63mg/ml), no zone of inhibition (0.00mm) 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* diameterofzoneofinhibitionofdifferentconcentrationsof *Allium sativum*clove aqueous extract and the control treatments on the testorganisms.

		Zones of Inhibition [in millimetre (mm)]		
Treatments	**Dose**	***E. coli***	***S. typhi***	***S. dysenteriae***
A.sativum	500mg/ml	20.00^{b}	18.00^{b}	18.00^{b}
A.sativum	250mg/ml	16.00^{c}	8.00^{c}	14.00^{c}
A.sativum	125mg/ml	14.00^{d}	0.00^{d}	0.00^{d}
A.sativum	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	24.00^{a}	20.00^{a}	22.00^{a}
LSD		0.939	0.996	1.485

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*Allium sativum* clove ethanolic extract and the control treatments on the testorganisms. The minimum inhibitory concentration of *E. coli* was 63 mg/mL while that of *S. typhi* and *S. dysenteriae* was 125mg/ml.

Table 3a: The minimum inhibitory concentration (MIC) of different concentrationsof *Allium sativum* clove ethanolic extract and the control treatments on the testorganisms.

		Bacterial Pathogens		
Treatments	**Dose**	***E. coli***	***S. typhi***	***S. dysenteriae***
A.sativum	500mg/ml	-	-	-
A.sativum	250mg/ml	-	-	-
A.sativum	125mg/ml	-	+	+
A.sativum	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 of different concentrations of*Allium sativum* clove aqueous extract and the control treatments on the testorganisms. The minimum inhibitory concentration of *E. coli* was 125 mg/mL while that of *S. typhi* and *S. dysenteriae* was 250 mg/ml.

Table 3b: Theminimum inhibitory concentration of different concentrations of*Alliumsativum*cloveaqueousextractandthecontroltreatmentsonthetestorganisms.

		Bacterial Pathogens		
Treatments	**Dose**	***E. coli***	***S. typhi***	***S. dysenteriae***
A.sativum	500mg/ml	-	-	-
A.sativum	250mg/ml	-	+	-
A.sativum	125mg/ml	+	+	+
A.sativum	63mg/ml	+	+	+
Distilled water	0.1ml	+	+	+
Ciproxin	10µg/ml	-	-	-

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

4.4. Minimum Bactericidal Concentration (MBC)

Table 4a below shows the minimum bactericidal concentration (MBC) of different concentrations of*Allium sativum* clove ethanolic extract and the control treatments on the testorganisms. The MBC of *E. coli* was 250mg/ml while that of *S. typhi* and *S. dysenteriae* was 500mg/ml.

Table4a: Theminimumbactericidalconcentration(MBC)ofdifferentconcentrations of *Allium sativum* clove ethanolic extract and the control treatmentsonthetestorganisms.

		Bacterial Pathogens		
Treatments	**Dose**	***E. coli***	***S. typhi***	***S. dysenteriae***
A.sativum	500mg/ml	-	-	-
A.sativum	250mg/ml	-	+	+
A.sativum	125mg/ml	+	+	+
A.sativum	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*Allium sativum* clove aqueous extract and the control treatments on the testorganisms. The MBC of *E. coli* and *S. dysenteriae* was 500mg/ml while that of *S. typhi* could not be determined.

Table 4b: The minimum bactericidal concentration of different concentrations of*Alliumsativum*cloveaqueousextractandthecontroltreatmentsonthetestorganisms.

		Bacterial Pathogens		
Treatments	**Dose**	***E. coli***	***S. typhi***	***S. dysenteriae***
A.sativum	500mg/ml	-	+	-
A.sativum	250mg/ml	+	+	+
A.sativum	125mg/ml	+	+	+
A.sativum	63mg/ml	+	+	+
Distilled water	0.1ml	+	+	+
Ciproxin	10µg/ml	-	-	-

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

DISCUSSION

The increasing resistance of bacterial organisms to conventional antibiotics, has fueled the development of alternative antimicrobials that are sustainable, more effective, and with less toxicity. With advances in ethnomedicine, there is need to explore the antibacterial potentials of some medicinal plants. Hence, this research was aimed at determining the phytochemical

composition and evaluating the antibacterial effects of garlic (*Allium sativum*) onselected enteric pathogens.

The phytochemical analysis of garlic (*Allium sativum*) in this study showed the presence of alkaloids, saponins, flavonoids and tannins. These four phytochemicals have been reported to be present in higher plants and are said to be responsible for the antibacterial properties of the plants containing them [19, 20]. Although, they have been reported to possess other properties, including antiparasitic (*Plasmodium* sp) properties of *Allium sativum*[21]and *Carica papaya* [22], as well as the antifungal properties of *Datura stramonium*[23]. The estimation of the concentrations of these phytochemicals was not covered in this study. Future research on this should do so as it might go a long way in establishing which phytochemicals are in higher concentration and might be mainly responsible for the antibacterial properties of garlic.

The determination of the presence of alkaloids, saponins, flavonoids, and tannins following phytochemical analysis ofthe ethanolic extract of *Allium sativum* clove in this study corroborates the report of Divya et al. [24] in which the same phytochemicals were observed. However, it disagrees with the reports of Dike-Ndudim and Ndubueze [21], in which, in addition to these four phytochemical components, anthraquinones, triterpenoid, glycosides, steroids, and phytates were also observed.

The results obtained in this research indicated that both aqueous and ethanolic extracts of *Alliumsativum* exhibited antibacterial effects against all the test organisms, although with differentlevels of sensitivities to the extracts. The antibacterial properties of *Allium sativum* clove extract, as revealed in this research, agree with the report by Utami et al. [25] and Tuyishime et al. [26],whoreportedthat*Alliumsativum*clove possesses antibacterialproperties. The antibacterial activities of both aqueous and ethanolic extracts indicate that water and ethanol could be utilised as solvents during the extraction procedure. Previous study findings have verified this concept. Dike-Ndudim et al. [23] found that both the aqueous and ethanolic extracts of Jimsonweed (*Datura stramonium*) have antibacterial activities. Ndubueze et al. [27] reported a similar result using aqueous and ethanolic leaf extracts of *Gongronemalatifolium.*

In general, and in this study, the ethanolic extracts of *A. sativum* clove were more effective than the aqueous extracts, indicating that ethanol is a better solvent than water, as reported by Ogunjobi and Nnadozie[28], Ezeifeka et al. [29], and Anyanwu et al. [11]. However, it contradicts a result by Ndubueze et al. [27], who found no statistically significant difference between aqueous and ethanolic leaf extracts of *Gongronemalatifolium.* Furthermore, because both extracts demonstrated antibacterial capabilities, future research with medicinal plants should focus on these solvents and alternative extraction procedures. Modifications and enhancements to the extraction procedures would ensure that the conclusions of such investigations are consistent.

Conclusion

This research has demonstrated that *A. sativum* clove extracts have potential antibacterial action on enteric bacteria pathogens. Inhibition of Gram-negative organisms by these plant extracts indicates that theycan serve as a source of antibiotics, which justifies the traditionaluse ofthis plantfortherapeuticpurposes.

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