



# The Phytochemical Composition and Antibacterial Effects of *Allium sativum* Clove Extracts 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

In this study, the phytochemicals present in the cloves of *Allium sativum* were determined, and their antibacterial activities against some enteric bacterial pathogens were assessed. The phytochemical constituents were determined after the extraction process was completed using water and ethanol as the solvents. Furthermore, the aqueous and ethanolic extracts of *A. sativum* were tested against *Escherichia coli*, *Salmonella typhi* and *Shigella dysenteriae* isolated from faeces of gastroenteritis patients. The agar diffusion technique (punch method) was used for this. Additionally, the extracts' minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined. The mean values of the zones of inhibition obtained were statistically analyzed using ANOVA. The least significant difference was determined according to the LSD test at  $P < 0.05$ . The results of the phytochemical analysis revealed the presence of saponin, alkaloids, flavonoids, and tannins. Furthermore, the antibacterial susceptibility test showed that the aqueous and ethanolic extracts possess antibacterial properties against all the test organisms. The ethanolic extract at the concentration of 500mg/ml had zones of inhibition of 29.00<sup>b</sup> against *E. coli*, 24.00<sup>b</sup> against *S. dysenteriae* and the lowest 20.00<sup>b</sup> against *S. typhi*. On the other hand, at that same concentration, the aqueous extract had zones of inhibition of 20.00<sup>b</sup> against *E. coli* and 18.00<sup>b</sup> against *S. dysenteriae* and *S. typhi*. This study suggests that *A. sativum* extracts possess antibacterial properties. Furthermore, since the ethanolic extract was more effective than the aqueous extract, it could be that the antibacterial potency of *A. sativum* is solvent-dependent. In conclusion, the findings from this study suggest that further purification of the constituents of the plant might lead to the development of novel antibiotics.

**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 practised field worldwide [2]. According to the World Health Organization (WHO) consultation committee, 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 utilized in traditional medicine. One species within the *Allium* genus of onions is *Allium sativum*. Some of its close relatives are onion, shallot, leek, chive, and rakkyo. 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 used for medical and culinary purposes 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 recent research studies 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]. Therefore, there is an urgent need to produce novel broad-spectrum antibiotics that are potent but also available, accessible, and affordable with minimal toxicity and resistance. Consequent upon the afore-mentioned properties of this plant, garlic, and its use in traditional medicine, this study was aimed at determining the phytochemicals and assessing the antibacterial properties of aqueous and ethanolic extracts of *Allium sativum* against some enteric bacterial pathogens.

## 2. MATERIALS AND METHODS

### 2.1 Collection of Plant Samples

Fresh cloves of *Allium sativum* were purchased from cemetery markets in Aba South Local Government Area of Abia State, Nigeria. This was identified in the Department of Plant Science and Biotechnology, Imo State University, Owerri. Three hundred grams (300 grams) of *A. sativum* clove was washed, sliced, and dried at room temperature for seven days. It was later crushed using an electric blender. It was thereafter packed into a clean polythene bag and labelled accordingly.

### 2.2 Isolation of the Test Organisms

*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 *Salmonella* species. In Nutrient Agar Slant, pure cultures of the bacteria were preserved for later use and refrigerated.

### 2.3 Extraction

Ninety-eight percent (98%) of ethanol and distilled water were used for the extraction. The ground clove was weighed (150 grams each) and soaked in 500 ml of the solvent. These were stoppered and kept for ten days with intermittent shaking [11]. 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 [12]. The concentrated extract was collected in screw-capped bottles, labelled and stored at 4°C in the refrigerator.

### 2.4 Phytochemical Screening

This was done using the method described by Lajubutu et al. [13]. 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 [14]. 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 made in the plates using a sterile cork borer. The fifth well was the negative control, while the sixth was the positive control. Sterile distilled water was the negative control, while ciprofloxacin was the positive control. Double dilution of the extracts was made to get the various concentrations: 500mg/ml, 250mg/ml, 125mg/ml and 63mg/ml 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 the pre-diffusion of the extracts [15] and then incubated at 37°C for 24 hours. The extracts' antibacterial activities 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 [16,17].

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

Inoculum was prepared using 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 [18]. 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 the extracts' dilutions (500, 250, 125, and 63mg/ml). 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 tube 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 MBC.

### 3. RESULTS

#### 3.1 Phytochemical Components of *Allium sativum*

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

#### 3.2 Antibacterial Effects of *Allium sativum* clove Extracts on Some Enteric Pathogens

Table 2a shows the mean diameter of the zones of inhibition produced by the different concentrations of *Allium sativum* clove 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 (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) was observed in the negative control (ethanol) for all the pathogens.

Table 2b shows the mean diameter of the zones of inhibition produced by the different

concentrations of *Allium sativum* clove 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 (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.

#### 3.3 Minimum Inhibitory Concentration (MIC)

Table 3a shows the MIC of the different concentrations of *Allium sativum* clove ethanolic extract and the control treatments on the test organisms. The minimum inhibitory concentration of *E. coli* was 125mg/ml, while that of *S. typhi* and *S. dysenteriae* was 250mg/ml.

Table 3b shows the minimum inhibitory concentration of the different concentrations of *Allium sativum* clove aqueous extract and the control treatments on the test organisms. The minimum inhibitory concentration of *E. coli* and *S. dysenteriae* was 250mg/mL, while that of *S. typhi* was 500mg/ml.

#### 3.4 Minimum Bactericidal Concentration (MBC)

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

Table 4b shows the minimum bactericidal concentration (MBC) of the different concentrations of *Allium sativum* clove aqueous extract and the control treatments on the test organisms. The MBC of *E. coli* and *S. dysenteriae* was 500mg/ml, while *S. typhi* did not exhibit any bactericidal effect.

**Table 1. Phytochemical components of *Allium sativum***

Extract	Alkaloids	Saponin	Flavonoids	Tannins
<i>Allium sativum</i>	+	+	+	+

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

**Table 2a. Mean<sup>\*</sup> diameter of zone of inhibition of different concentrations of *Allium sativum* clove ethanolic extract and the control treatments on the test organisms**

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

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 Ciproxin = Positive Control

**Table 2b. Mean<sup>\*</sup> diameter of zone of inhibition of different concentrations of *Allium sativum* clove aqueous extract and the control treatments on the test organisms**

Treatments	Dose	Zones of Inhibition [in millimeters (mm)]		
		<i>E. coli</i>	<i>S. typhi</i>	<i>S. dysenteriae</i>
<i>A. sativum</i>	500mg/ml	20.00 <sup>b</sup>	18.00 <sup>b</sup>	18.00 <sup>b</sup>
<i>A. sativum</i>	250mg/ml	16.00 <sup>c</sup>	8.00 <sup>c</sup>	14.00 <sup>c</sup>
<i>A. sativum</i>	125mg/ml	14.00 <sup>d</sup>	0.00 <sup>d</sup>	0.00 <sup>d</sup>
<i>A. sativum</i>	63mg/ml	0.00 <sup>e</sup>	0.00 <sup>d</sup>	0.00 <sup>e</sup>
Distilled water	0.1ml	0.00 <sup>e</sup>	0.00 <sup>d</sup>	0.00 <sup>e</sup>
Ciproxin	10µg/ml	24.00 <sup>a</sup>	20.00 <sup>a</sup>	22.00 <sup>a</sup>
LSD		0.939	0.996	1.485

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 Ciproxin = Positive Control

**Table 3a. The minimum inhibitory concentration (MIC) of the different concentrations of *Allium sativum* clove 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>A. sativum</i>	500mg/ml	-	-	-
<i>A. sativum</i>	250mg/ml	-	-	-
<i>A. sativum</i>	125mg/ml	-	+	+
<i>A. sativum</i>	63mg/ml	+	+	+
Ethanol	0.1ml	+	+	+
Ciproxin	10µg/ml	-	-	-

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

**Table 3b. The minimum inhibitory concentration of the different concentrations of *Allium sativum* clove 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>A. sativum</i>	500mg/ml	-	-	-
<i>A. sativum</i>	250mg/ml	-	+	-
<i>A. sativum</i>	125mg/ml	+	+	+
<i>A. sativum</i>	63mg/ml	+	+	+
Distilled water	0.1ml	+	+	+
Ciproxin	10µg/ml	-	-	-

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

**Table 4a. The minimum bactericidal concentration (MBC) of the different concentrations of *Allium sativum* clove 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>A. sativum</i>	500mg/ml	-	-	-
<i>A. sativum</i>	250mg/ml	-	+	+
<i>A. sativum</i>	125mg/ml	+	+	+
<i>A. sativum</i>	63mg/ml	+	+	+
Ethanol	0.1ml	+	+	+
Ciproxin	10µg/ml	-	-	-

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

**Table 4b. The minimum bactericidal concentration of the different concentrations of *Allium sativum* clove 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>A. sativum</i>	500mg/ml	-	+	-
<i>A. sativum</i>	250mg/ml	+	+	+
<i>A. sativum</i>	125mg/ml	+	+	+
<i>A. sativum</i>	63mg/ml	+	+	+
Distilled water	0.1ml	+	+	+
Ciproxin	10µg/ml	-	-	-

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

#### 4. DISCUSSION

The increasing resistance of bacterial organisms to conventional antibiotics has fueled the development of alternative antimicrobials that are sustainable, more effective, and less toxic. With advances in ethnomedicine, there is a need to explore the antibacterial potentials of some medicinal plants. Hence, this research aimed to determine the phytochemical composition and evaluate the antibacterial effects of garlic (*Allium sativum*) on selected 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]. However, they have been reported to possess other properties, including the 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.

This study's determination of the presence of alkaloids, saponins, flavonoids, and tannins

following phytochemical analysis of the ethanolic extract of *Allium sativum* clove 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 *Allium sativum* exhibited antibacterial effects against all the test organisms, although with different levels 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], who reported that *Allium sativum* clove possesses antibacterial properties. 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 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 *Gongronema latifolium*.

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], Ezeifeke 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 *Gongronema latifolium*. 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.

## 5. 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 they can serve as a source of

antibiotics, which justifies the traditional use of this plant for therapeutic purposes.

## CONSENT

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

## ETHICAL APPROVAL

As per international standards or university standards written ethical approval has been collected and preserved by the author(s).

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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