## *Original Research Article*

# *In-vitro* Antibacterial Activity of *Allium sativum* on Common Food Poisoning Bacteria


**ABSTRACT**

***Background:*** The use of herbal medicines for food poisoning in inadequate resource countries has greatly increased with the limited establishment of antibacterial activity. This study aimed at establishing the antibacterial activity of the aqueous *Allium sativum* extract against *Staphylococcus aureus*, *Escherichia coli* and *Salmonella typhimurium*.

***Methods:*** *Allium sativum* L. (Amaryllidaceae) was purchased from a farmer in Kasese District and was authenticated. The material was then extracted using distilled water, filtered and lyophilized. Evaluation of the phytochemicals in the extract was done using standard procedures. High-Performance Liquid Chromatography (HPLC) fingerprint of the extract was conducted to monitor the reproducibility of the extract. The antibacterial activity of the aqueous garlic extract against the organisms above was evaluated and expressed as Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC).

***Results:*** Phytochemical screening of the garlic extract showed the presence of amino acids, alkaloids, glycosides and saponins, flavonoids, steroids and terpenoids. HPLC fingerprints showed 39 and 98 peaks at 220nm and 254nm wavelengths respectively and the presence of various phytochemical compounds in *A. sativum*. The extract showed antibacterial activity against *S. aureus*, *E. coli* and *S. typhi*.

***Conclusion:*** The aqueous garlic extract exhibited antibacterial activity against tested common food poisoning bacteria, with a high potency against gram-positive bacteria.



*Keywords: Allium sativum, antibacterial, food poisoning, Staphylococcus aureus, Escherichia coli, Salmonella typhimurium*


## 1. INTRODUCTION

*Allium sativum* L. (garlic) is a small, ubiquitous species in the family Amaryllidaceae, Genus *Allium* and commonly used food spice for food processing [1]. In addition to being an aromatic herbaceous plant that is consumed worldwide as food, *A. sativum*

is also used as a traditional remedy for various diseases such as food poisoning, bronchitis, intestinal worms, etc. [2]. Among these, food poisoning has increasingly become a global health challenge, which requires interventions, stemming up right from our local communities [3]. According to the World Health Organization, an estimated 600 million; almost 1 in 10 people in the world fall ill after eating contaminated food and 420,000 die every year, with the WHO African and South-East Asia Regions having the highest morbidity and mortality rates, including children under the age of 5 years [4]. Food poisoning is a group of illnesses acquired by consumption of foods contaminated with infective organisms and/or their toxins, chemical contaminants, whether metallic or organic [5]. Food contamination occurs from food processing, preparation, and storage leading to sickness; hospitalizations or eventually death otherwise called foodborne diseases [6]. The infective organisms that contaminate food include bacteria, viruses and parasites [6], which cause more than 200 diseases like diarrhea, cancers, etc. [4]. Antimicrobials such as antibiotics are usually recommended for infections caused by bacteria such as food poisoning bacteria. However, their prolonged administration and abuse have been linked to the emergence and spread of resistant bacteria, rendering the treatment of infectious diseases ineffective [7]. This increasing microbial resistance to conventional drugs has revived the interest in plants with antibacterial activities. Available literature shows that garlic has been widely known for its potency of dietary and medicinal use to cure various diseases, including infectious diseases for centuries [8]. Garlic has also been used as a home-based prophylactic and symptom management agent in the recent COVID-19 emergence, it was reported to have therapeutic efficacy against multiple symptoms observed in advanced COVID-19 patients [9]. The antibacterial activity of *A. sativum* has been mainly attributed to three phytochemical families, fructans (mainly inulin), phenolic compounds (flavonoids), and organosulfur compounds (OSCs) [10]. It is also known that the most sensitive bacterium to garlic is the deadly *Bacillus anthracis* which causes the disease anthrax [11]. Some studies have also shown garlic to have antibacterial activity against bacteria [12, 13] . However, the antibacterial activity of garlic on common food poisoning bacteria has not been extensively studied and this was investigated in this study.



## 2. METHODOLOGY

### 2.1 Study Design

It was a laboratory-based study in which *A. sativum* active ingredients were extracted using distilled water, then evaluation of the phytochemicals in the extract was done. The aqueous garlic extract obtained was evaluated for antibacterial activity against *Staphylococcus aureus* (Cat. 25923, ATCC$^{TM}$), *Escherichia coli* (Cat. 25922, ATCC$^{TM}$) and *Salmonella typhimurium* (Cat. 14028, ATCC$^{TM}$).

## 2.2 Study area

*Allium sativum* L. (family Amaryllidaceae) was obtained from a local garden in Mubuku village, Kasese district, Uganda and the study was conducted in Pharmaceutical Chemistry/Analysis Laboratory of Mbarara University of Science and Technology, Mbarara (MUST), where garlic extraction and evaluation of the phytochemicals in the extract was done, and the Microbiology Laboratory of MUST, where the determination of the antibacterial activity of the garlic extract against standard food poisoning bacteria was done.

## 2.3 Study population

*Allium sativum* L. was used in this study. The purposive sampling method was used to obtain the garlic from the local farmer's garden in Mubuku village, Kasese district, before it was authenticated by Dr. Olet Eunice, a botanist in the Biology Department of the Faculty of Science, MUST.

## 2.4 Preparation of garlic extract

The sterile aqueous extract of garlic was obtained using a method by Benmeziane *et al*. [14], where the cloves on fresh bulbs garlic obtained were peeled using a kitchen knife and washed thoroughly under running tap water to obtain the edible portion. The garlic extract was prepared immediately from 1 kg to avoid degradation of allicin [15], by cutting into pieces [16] and crushed to release allinase under aseptic conditions before it was mixed with distilled water to allow alliin to be converted to allicin by enzyme allinase [17]. The homogenate was recovered by filtration through a double-layered sterile fine mesh cloth into an Eppendorf tube [18]. An extract sample of 800 mL was then lyophilized using a freeze-dryer for 3 days. The obtained garlic powder was stored at 4 °C till use [19].

## 2.5 Phytochemical Screening of garlic extract

Following methods were used to determine presence of phytochemicals: quinones (Borntrager's test) [20], alkaloids (Dragendorff's test) [21], glycosides (Benedict's test) [22], phenols ($FeCl_3$) [23], tannins (Folin-Ciocalteu method) [24], flavonoids (Ammonia test) [25], saponins (Frothing test) [26], steroids (Chloroform and concentrated $H_2SO_4$ meth) [27], terpenoids (Chloroform and concentrated $H_2SO_4$ meth) [23] and thiosulphates ethyl (p-hydroxybenzoate) [28], amino acids (ninhydrin test) [29].

## 2.6 High Performance Liquid Chromatography Apparatus for Fingerprint

The high-performance liquid chromatography (HPLC) fingerprint was performed on a *UFLC* Shimadzu Prominence Model HPLC system (Tokyo, Japan) at the Analytical and Pharmaceutical Laboratory, Mbarara University of Science and Technology, Uganda, comprising an LC-20AD pump, a Phenomenex Luna $C_{18}$ column (250 × 4.6 mm. 5 µm), temperature-controlled sample trays, an online degasser DGU-20A5R and an ultraviolet (UV) detector. A reversed-phase HPLC assay was carried out using a binary isocratic elution with a flow rate of 1.0 mL/min at a column temperature of 25 °C, a mobile phase of methanol/acetonitrile /0.01% trifluoroacetic acid (6:1:3). The injection volume was 10 µL at detection wavelengths of 220 and 254 nm while the acquisition time was 40 min.

## 2.7 Evaluation of antibacterial activity

Antibacterial activities of the sterile aqueous garlic extracts were evaluated using agar well diffusion method on Mueller Hinton Agar (MHA) (Cat. R454084, Thermofisher). The Zones of inhibition were measured using a ruler. Single colonies on agar plates were used to prepare the bacterial suspension with a turbidity of 0.5 McFarland (equal to 1.5×108 colony-forming units (CFU)/mL) [14].

## 2.8 Determination of MIC and MBC

The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of aqueous garlic extract were determined using following the Alirezaei's method [30] and Shetty's method [31] respectively.

### 2.8.1 Determination of Minimum Inhibitory Concentration (MIC)

The MHA plates were inoculated with standard bacterial strain under aseptic conditions and wells of 8 mm diameter were made using a sterile cork borer. *A. Sativum* extracts were prepared in a series of decreasing concentrations. One hundred (100) µL of extracted garlic were introduced into the wells using automatic microliter pipettes, and all plates were incubated at 37 °C for 24 hours. The sensitivity of *S. typhimurium, E. coli* and *S. aureus* was determined by measuring the diameter of the zone of inhibition.

### 2.8.2 Determination of Minimum Bactericidal Concentration (MBC)

Brain Heart Infusion (BHI) broth (Cat.17580547, Oxoid, Thermofisher, China) of 900 µL was poured into sterile tubes. Then, each tube was inoculated with 100 µL of the standardized microbial suspension (0.5 Mc-Farland). After mixing, the inoculated

tubes were incubated. The negative control used was distilled water while the tubes were sub-cultured on MHA and incubated for 24 hours at 37 °C.

### 2.9 Data Analysis

All quantitative data were expressed as mean ± standard error of mean (SEM) while the variation in a set of data was analyzed through the one-way Analysis of Variance. The difference among the means was considered at 95% confidence level using the post-hoc method of Tukey's Multiple Comparison Test through GraphPad Prism9.

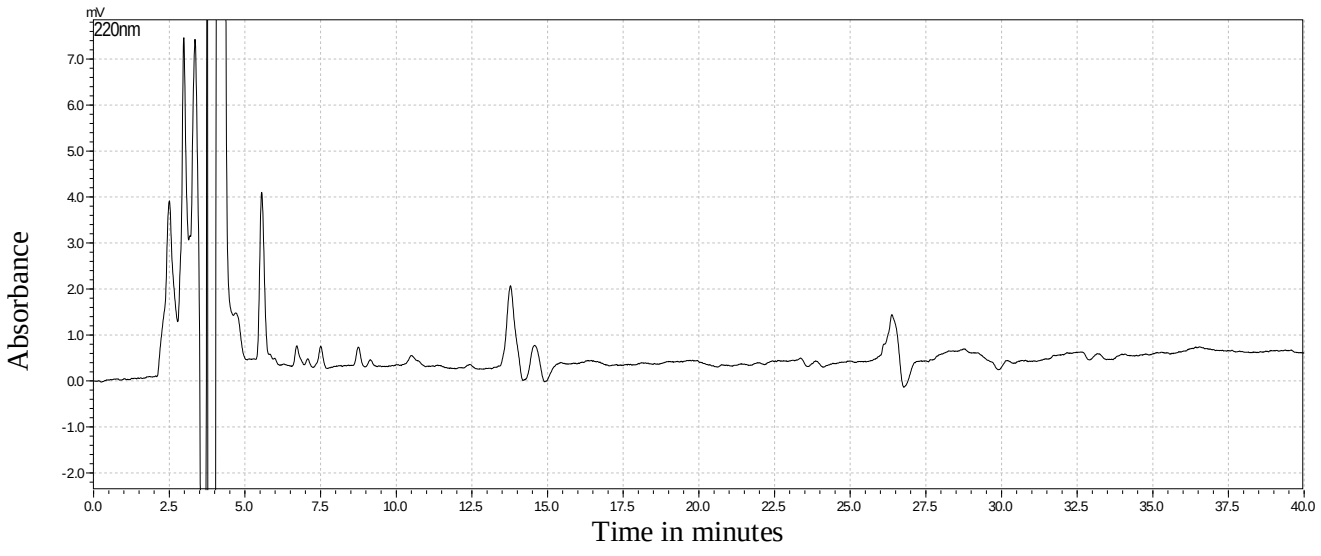
## 3.0 RESULTS

### 3.1 Phytochemical screening & antibacterial activity of the extract

The phytochemical analysis of the extract of *Allium sativum* showed presence of amino acids, alkaloids, glycosides, saponins, flavonoids, steroids and terpenoids. The extract showed antibacterial activity against all the tested microorganisms that is *Staphylococcus aureus*, *Escherichia coli* and *Salmonella typhimurium*. In order to ensure the reproducibility of the extract for quality control purposes, the HPLC fingerprint conducted using both 220 and 254nm showed 39 and 98 peaks, respectively.

### 3.2 High Performance Liquid Chromatography Fingerprint

The HPLC fingerprints obtained at wavelengths of 220 and 254nm as shown in figure 1 and 2 below showed chromatograms with 39 peaks (at 220nm) and 98 peaks (at 254nm). The peaks seen in the fingerprints correspond to the phytochemical compounds in the *Allium sativum***.**



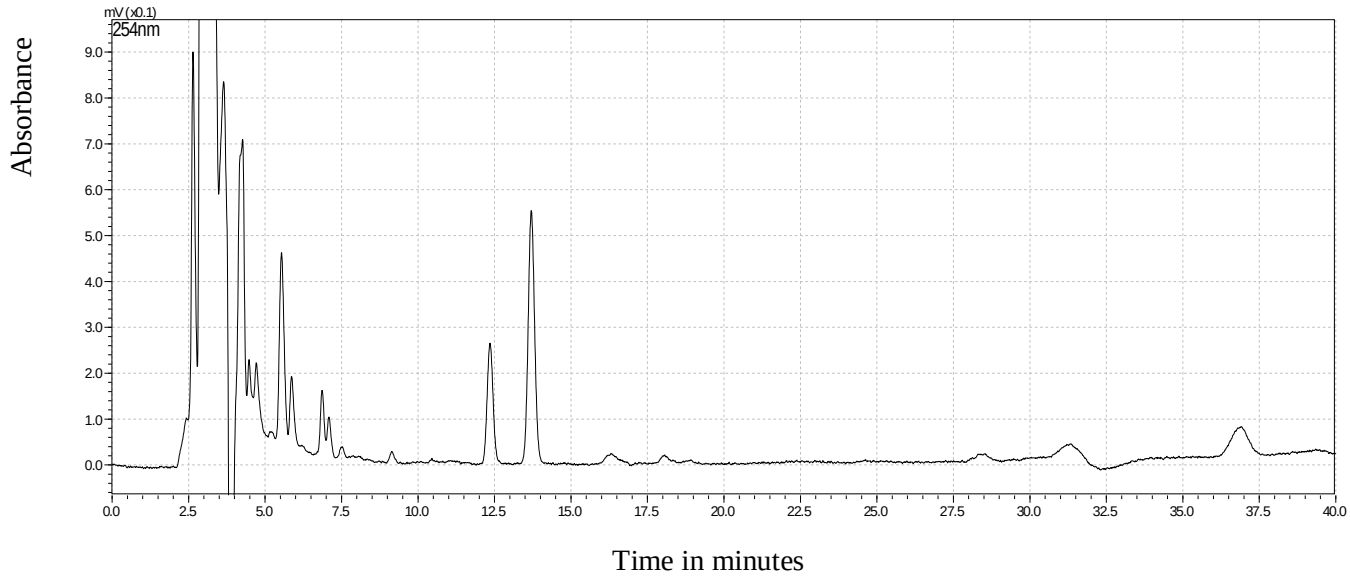



**Figure 2:** HPLC fingerprint of *A. sativum* extract at 254 nm wavelength

**Antibacterial activity of *A. sativum* extract**

The *Allium sativum* extract showed a profound antibacterial activity against all the tested organisms with lowest Minimum Inhibitory Concentration (MIC) value of 31.25 mg/mL against *Staphylococcus aureus*, and highest (62.5 mg/mL) against both *Escherichia coli* and *Salmonella typhimurium* (Table 1).

**Table 1:** MIC values of the aqueous Allium sativum extract

| **Organisms** | **Average MIC (mg/mL)** |
|---|---|
| ***Staphylococcus aureus* (ATCC 25923)** | 31.25 |
| ***Escherichia coli* (ATCC 25922)** | 62.5 |
| ***Salmonella typhimurium* (ATCC 14028)** | 62.5 |

Also, the Minimum Bactericidal Concentration of the aqueous *Allium sativum* extract on *S. aureus* and *S. typhimurium* had the highest MBC of 125 mg/mL while on *E. coli,* it had the least MBC value of 250 mg/mL (Table 2).

**Table 2:** MBC values of the aqueous Allium sativum extract

| **Organisms** | **Mean MBC (mg/mL)** |
|---|---|
| ***Staphylococcus aureus* (ATCC 25923)** | 125 |
| ***Escherichia coli* (ATCC 25922)** | 250 |
| ***Salmonella typhimurium* (ATCC 14028)** | 125 |



## 1. DISCUSSION

In previous study, it was reported that the antibacterial activity of garlic is attributed to its phytochemical constituents such as fructans (mainly inulin), phenolic compounds (flavonoids), and organosulfur compounds (OSCs) [32]. Likewise in this study, phytochemical screening showed presence of amino acids, alkaloids, glycosides, saponins, flavonoids, steroids and terpenoids and these could be responsible for the observed antibacterial activity of the *A. sativum* extract against *Staphylococcus aureus*, *Escherichia coli* and *Salmonella typhimurium*, which is in agreement with a study by Njue, et al 2014 (Njue, Kanja [33].

The peaks observed from the HPLC fingerprints in Figures 1 and 2 correspond to the phytochemical compounds in the *Allium sativum* extract which is comparable to a study by Zhao, Li [34]. Zhao, Li and colleagues standardized the HPLC fingerprint by running standard solutions with their extracts. Therefore, they corresponded the peaks obtained from the extracts to those obtained from the standards and were able to specifically identify the phytochemical compounds, unlike in this study where standards were not used. The peak areas are equal to the peak height times width at half height. The larger the area, the more the amount of the phytochemical present in the extract. Peaks obtained at the same retention time at the two wavelengths represent the same compound. Some peaks are observed at one wavelength and not the other as shown in Figures 2 and 3. Presence of a peak at retention times between 25 and 27.5 minutes only under a wavelength of 220nm

indicated presence of a compound that is visualized at 220nm wavelength and not at 254nm. It is therefore always necessary to run the HPLC of the garlic extract at two different wavelengths in order to identify all the phytochemical compounds in the extract.

The antibacterial activity of the aqueous *Allium sativum* extract on three standard organisms studied varies with the type and composition of the extract, concentration used, types of microorganism, pH value and temperature of the environment [35]. The results of this study indicated that the aqueous extract of garlic had an antibacterial activity on the tested organisms. The extract was more active at high concentration and reduced at very low concentrations which is in accordance with a study by De Moura Oliveira, Santos-Mendonça [36]. Thus, the inhibition of bacterial growth by the aqueous garlic extract showed a dose dependent activity as reported by Shams *et al*. [37]. The susceptibility of the tested organisms to the garlic extract was expressed as MIC and MBC.

The MIC value of the garlic extract was lowest (31.25 mg/mL) against *Staphylococcus aureus*, a gram-positive bacterium and highest (62.5 mg/ml) against both *Escherichia coli* and *Salmonella typhimurium,* gram negative bacteria. The extract in our study is thus more potent against gram positive bacteria than gram negative bacteria, as earlier reported by Nakamoto and Abubakar [38, 39].

Additionally, the MIC values obtained in our study indicate that the extract is bacteriostatic against *Staphylococcus aureus* at an MIC of 31.25mg/mL, which slightly differs from a study by Simeon and Falilat [40] where a bacteriostatic activity of the garlic extract against Staphylococcus aureus was obtained at an MIC of 25mg/mL.

On the other hand, a bacteriostatic activity of the extract against *Escherichia coli* obtained at the MIC of 62.5 mg/mL varied from the report of Simeon and Falilat [40] which was obtained at a slightly higher MIC value of 100 mg/mL while in *Salmonella typhimurium,* the bacteriostatic activity of the extract obtained at the MIC of 62.5mg/mL was slightly higher than that reported by Simeon and Falilat [40] which was obtained at the MIC of 25 mg/mL. The variation in the results obtained could be attributed to the difference in the garlic source, which directly affects the quantity of phytochemical composition responsible for the antibacterial activity of garlic [32].

The MBC values obtained in this study were 125 mg/mL for *S. aureus* and 250 mg/mL for *E. coli*, which indicates that the extract was bactericidal against the organisms at those concentrations. This varied from a study by *Mohammad et al. (2020)* [41] who obtained the bactericidal activity at an MBC of 100mg/mL for *S. aureus* and an MBC of 6.25 mg/mL for *E. coli* whereas for *S. typhimurium,* the bactericidal activity obtained at the MBC of 125 mg/mL was slightly higher than that in a study by Simeon and Falilat [40] which was obtained at the MBC of 50 mg/mL.

The quantification of active components using HPLC could not be done, since a standard was not used. Further studies to quantify the active compounds of *Allium sativum* and *in-vivo* studies to ascertain their bioavailability in body fluids, which is key to the efficacy of drugs, need to be conducted.

## 4. CONCLUSION

The study showed that the aqueous garlic extract from Mubuku in Kasese District exhibited antibacterial activity against tested organisms, with a high potency against gram positive bacteria. The extract contained bioactive phytochemicals which could be responsible for its antibacterial activity.

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