



Antibacterial Activities of the Extracts of *Allium sativum* (Garlic) and *Allium cepa* (Onion) Against Selected Pathogenic Bacteria

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Abstract

Although many antibiotics have been developed against bacterial diseases, the problems of antibiotic resistance and toxicity have made the continuous search for newer drugs an existential necessity. This study determined the phytochemical components and antibacterial activities of *Allium sativum* and *Allium cepa* against *Salmonella typhi*, *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Klebsiella pneumoniae* and *Escherichia coli*. The phytochemical analysis was carried out using standard methods. The antibacterial activities of the aqueous, ethanol and acetone extracts of both plants at different concentrations were determined against all the test organisms using agar well diffusion method. All the extracts were found to contain important phytochemicals such as saponins, flavonoids, alkaloids, phlobatannins and anthraquinones. Ethanol, acetone and aqueous extracts of *Allium sativum* showed maximum activities against *S. aureus* (21.2 ± 0.2 – 28.1 ± 0.2 mm), *S. pneumoniae* (16.8 ± 0.4 – 20.8 ± 0.4 mm) and *S. aureus* (15.5 ± 0.5 – 22.8 ± 1.1 mm) respectively. Ethanol, aqueous, and acetone extract of *Allium cepa* showed maximum activity against *E. coli* (19.1 ± 0.1 – 28.7 ± 0.3 mm), *K. pneumoniae* (16.4 ± 0.2 – 21.2 ± 0.5 mm) and *E. coli* (19.7 ± 0.7 – 26.0 ± 0.0 mm), respectively. All extracts of both plants showed varying minimum inhibitory concentrations which ranged between 10 and 20 mg/mL against the test bacteria. This study has established that the extracts of *Allium* species have antibacterial activities against both Gram positive and Gram negative bacteria.

Keywords: Antibacterial activity, *Allium sativum*, *Allium cepa*, Extract, Phytochemicals.

Introduction

In the Nigerian health care system, the role of plants/herbs as medicines is presently well recognized, and nearly all plants are associated with medicinal uses (Anyawu and Okoye 2017). Garlic (*Allium sativum*) and onion (*Allium cepa*) belong to the genus *Allium*, a monocotyledonous genus of flowering plants informally referred to as the onion genus found

in the family *Alliaceae*. The generic name *Allium* is the Latin word for garlic. Several members of the genus, especially the various edible onions, garlics, cloves and leeks play a vital role in cooking worldwide, as various parts of the plants; either raw or cooked produce large varieties of flavours and textures (Karuppiah and Rajaram 2012). *Allium* species have been used for centuries worldwide to

tackle infectious diseases (Shobana et al. 2009). The use of *Allium* species for medicinal purposes dated as far back as 35 centuries ago as documented in the ancient Egyptian papyrus: Codex Ebers (Gerber 2008). Historically, it was reported that slaves working on the pyramids were usually fed onions and garlic to increase their strength and stamina. It was also reported that in ancient Greece, athletes were normally fed these plants in days preceding the Olympics to serve as energy booster (Rivlin 2001). Louis Pasteur was however, the first scientist to describe the antibacterial effects of onion and garlic juice against both Gram negative and Gram positive bacteria (Whitemore and Naidu 2000).

Staphylococcus aureus, *Escherichia coli*, *Streptococcus pneumoniae*, *Salmonella typhi* and *Klebsiella pneumoniae* have well established history of pathogenicity and have been implicated in various human infections with varying degrees of severity. Although antibiotics have been developed against these pathogenic organisms, the twin problems of drug toxicity and antibiotic resistance have made the continuous search for newer antibiotics against these pathogens a necessity (Price et al. 2017).

All over the world, especially in developing countries of Africa and South-East Asia where the health infrastructures are rudimentary, increased morbidity and mortality are being witnessed due to infections caused by antibiotic resistant bacteria (Friedman et al. 2016). To treat hitherto common and innocuous bacterial infections, prolonged exposure to broad spectrum antibiotics are sometimes required, which in most cases lead to increased toxicity for patients. This has created an ever increasing need for a less toxic, cheap, easily sourced and effective alternative antimicrobial agents that can treat the diseases caused by bacteria and other infectious agents (Wise et al. 2011).

The degree of antibacterial activity expressed by many plants has been found to correlate very well with the extraction solvents used (Altemimi et al. 2017). Water, ethyl acetate and ethanol are more frequently used

compared to other solvents such as acetone, butanol and chloroform (Gupta et al. 2010, Gangadhar et al. 2012, Lekshmi et al. 2015a). This study is focused on the antibacterial activities of the extracts of *Allium sativum* and *Allium cepa* against selected pathogenic bacteria using water, acetone and ethanol.

Materials and Methods

Sample collection

Garlic (*Allium sativum*) and onions (*Allium cepa*) used in the present study were purchased from Kure Ultra-Modern Market, Minna, Niger State, Nigeria.

Microorganisms

The test microorganisms were obtained from the Department of Microbiology, School of Life Sciences Federal University of Technology Minna, Niger State, Nigeria. Conventional biochemical tests were used to identify and confirm the test organisms as *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Streptococcus pneumoniae*, *Escherichia coli* and *Salmonella typhi*.

Preparation of plant materials and extraction

Cold maceration method of extraction as described by Sankeshwari et al. (2018) was used in this study. Garlic and onions were skinned, sliced and washed using sterile distilled water and were dried at room temperature for four weeks. After four weeks of drying, the samples were pounded separately into powder forms. 100 g of the garlic and onion powders were weighed separately and dissolved in different conical flasks containing 500 mL of ethanol (95%). Another 100 g of the powder of each of garlic and onion was weighed and dissolved in 500 mL of acetone in two different conical flasks. The flasks were corked and kept under agitation for 72 hours using rotary shaker. This same procedure was repeated using distilled water as solvent. After 72 hours of agitation, the mixture comprising the solvent and the plant materials in each conical flask was filtered using muslin cloth of

200 µm pore size and whatman no. 1 filter paper, the filtrates were collected in separate beakers and labelled accordingly. The filtrates were then evaporated to dryness using water bath at 40 °C. Each dried extract was packed in a well labeled sterile sample container and stored in the refrigerator at 4 °C until further use.

Phytochemical screening of plant extracts

The extracts of *Allium sativum* and *Allium cepa* were screened for the presence or otherwise of phytochemical compounds such as saponins, tannins, flavonoids, alkaloids, phlobatannins, and anthraquinones according to the method described by Khan et al. (2016), Auwal et al. (2014) and Gul et al. (2017).

Test for tannins: Bromine water (10 mL) was added to 0.5 g of extracts. Presence of tannins was indicated by decolouration of bromine water.

Test for saponins: Distilled water (5 mL) was added to 0.5 g of plant extract in a test tube and shaken vigorously. Formation of persistent frothing showed the presence of saponins.

Test for flavonoids: Two millilitres (2 mL) of 2.0% sodium hydroxide was added to 0.5 g of plant extract in a test tube. The mixture produced yellow colouration, which became colourless on addition of 2 drops of 10.0% hydrogen chloride to indicate presence of flavonoids.

Test for anthraquinones: The extract (0.5 g) was mixed with 2 mL of 10.0% hydrochloric acid in a test tube. The mixture was filtered and cooled at room temperature before the addition of equal volume of chloroform to the filtrate. Few drops of 10.0% ammonia were added to the mixture before heating. Presence of anthraquinones was indicated by formation of pink colouration

Test for phlobatannins: The extract (0.5 g) was dissolved in 2 mL of dilute hydrochloric acid in a test tube. Formation of red precipitate indicated the presence of phlobatannins.

Test for alkaloids: The extracts (0.5 g) was dissolved in 2 mL of 2.0% sulphuric acid, the mixture was warmed for 2 minutes. The mixture was filter before the addition of 3 drops of Dragendorff's reagent to the filtrate. Formation of orange red precipitate indicated the presence of alkaloids.

Evaluation of in-vitro antibacterial activity of plant extracts

Evaluation of antibacterial activity was done using agar well diffusion method as described by Balouiri et al. (2016). Mueller Hinton agar was prepared according to the manufacturer's instruction and dispensed aseptically into petri dishes after which they were allowed to solidify. The solidified medium was streaked entirely on the surface with 0.1 mL of overnight culture of test organism which was adjusted to 0.5 McFarland turbidity, using sterile swab stick. Four adequately spaced out wells were bored in the culture medium using a sterile 4 mm diameter cork borer after in which 0.1 mL each of the three different concentrations of extracts (100 mg/mL, 150 mg/mL and 200 mg/mL) were added to different wells using sterile syringe and needle. The remaining well which served as a negative control had distilled water. The plates were allowed to stand for one hour for proper diffusion of extracts before incubation at 37 °C for 24 hours. The diameters of zones of inhibition observed were measured and recorded in millimetres. This was done in triplicates.

Determination of Minimum Inhibitory Concentration (MIC)

The MIC was determined by checking for the minimum concentration of the extract that inhibited the growth of the test organisms according to the method described by Doughari et al. (2007). Nutrient broth (2 mL) was added to 0.5 mL of varying concentrations of extracts (50 mg/mL, 100 mg/mL, 150 mg/mL and 200 mg/mL) in a test tube, followed by addition of loopful of the test microorganisms previously adjusted to 0.5 McFarland turbidimetric

standard. The same procedure was used for all the test organisms at varying concentrations and a test tube containing nutrient broth and loopful of organisms was used as a control. All the test tubes were inoculated at 37 °C for 24 hours after which the test tubes were observed for turbidity. The lowest concentration of the extract that showed no observed turbidity was recorded as MIC for the extract.

Results

Phytochemicals constituents of plant extracts

The phytochemical screening of both garlic and onion extracts indicated the presence important phytochemicals such as saponins, tannins,

flavonoids, alkaloids, phlobatannins, and anthraquinones (Tables 1 and 2). All the extracts of *Allium sativum* contained flavonoids, saponins and anthraquinones. Tannins were present in aqueous and acetone extracts but not found in ethanol extract. Alkaloids were absent in both aqueous and acetone extracts but present in ethanol extract, while only acetone extract of *Allium sativum* lacked phlobatannins. Similarly, all the extracts of *Allium cepa* contained flavonoids, saponins, alkaloids and anthraquinones. Phlobatannins and tannins were present in aqueous and ethanol extracts but absent in acetone extract, while anthraquinones and alkaloids were absent in aqueous extracts of *Allium cepa*.

Table 1: Phytochemical constituents of crude extracts of *Allium sativum*

Test	Inferences		
	Aqueous extract	Acetone extract	Ethanol extract
Tannins	+	+	-
Flavonoids	+	+	+
Saponins	+	+	+
Alkaloids	-	-	+
Phlobatannins	+	-	+
Anthraquinones	+	+	+

Keys: + = present; - = absent

Table 2: Phytochemical constituents of crude extracts of *Allium cepa*

Test	Inferences		
	Aqueous extract	Acetone extract	Ethanol extract
Tannins	+	-	+
Flavonoids	+	+	+
Saponins	+	+	+
Alkaloids	-	+	+
Phlobatannins	+	-	+
Anthraquinones	-	+	+

Keys: + = present; - = absent

Antibacterial activities of extracts of *Allium sativum* and *Allium cepa* against test bacteria

Extracts of *Allium sativum* showed varying antibacterial activities against the test bacteria at concentrations of 100 mg/mL, 150 mg/mL and 200 mg/mL (Table 3). Aqueous extract of garlic showed maximum activity against *S. aureus* (15.5 ± 0.5 – 22.8 ± 1.1 mm), moderate activity against *K. pneumoniae* (11.8 ± 0.2 –

17.8 ± 0.3 mm), *S. pneumoniae* (10.0 ± 0.0 – 16.3 ± 0.2 mm) and *E. coli* (10.0 ± 0.1 – 16.0 ± 0.1 mm), while the minimum activity was shown against *S. typhi* (4.0 ± 0.0 – 11.1 ± 0.4 mm). Ethanol extract of garlic showed maximum activity against *S. aureus* (21.2 ± 0.2 – 28.1 ± 0.2 mm) and *S. pneumoniae* (20.0 ± 0.0 – 22.4 ± 0.7 mm), while it showed moderate activity against *E. coli* (9.8 ± 0.4 – 20.0 ± 0.1

mm), *S. typhi* (11.5 ± 0.0 – 16.0 ± 0.0 mm) and *K. pneumoniae* (9.1 ± 0.3 – 14.0 ± 0.0 mm). Acetone extract of garlic showed maximum activity against *S. pneumoniae* (16.8 ± 0.4 – 20.8 ± 0.4 mm), moderate against *S. aureus* (15.4 ± 0.2 – 16.9 ± 0.6 mm), *S. typhi* (10.6 ± 0.5 – 15.7 ± 0.8 mm) and *E. coli* (5.7 ± 0.8 – 13.4 ± 0.7 mm), while it showed no activity against *K. pneumoniae*. All the extracts of *Allium cepa* showed activity against the test organisms at 100 mg/mL, 150 mg/mL and 200 mg/mL concentrations as shown in Table 4. Aqueous extract showed maximum activity against *K. pneumoniae* (16.4 ± 0.2 – 21.2 ± 0.5 mm), moderate activity against *E. coli* (14.0 ± 0.0 – 18.7 ± 0.3 mm) and *S. aureus* (16.9 ± 0.5 – 19.5 ± 0.6 mm) and it showed minimum activity against *S. typhi* (7.6 ± 0.2 – 14.3 ± 0.1 mm) and *S. pneumoniae* (6.6 ± 0.7 – 10.1 ± 0.4 mm). Ethanol extract showed maximum activity against *E. coli* (19.1 ± 0.1 – 28.7 ± 0.3 mm) and *S. aureus* (16.7 ± 0.2 – 25.5 ± 0.2 mm) and moderate activity against *K. pneumoniae* (14.0 ± 0.0 – 20.1 ± 0.1 mm), *S. typhi* (11.5 ± 0.2 – 16.7 ± 0.3 mm) and *S. pneumoniae* (10.5 ± 0.5 – 16.6 ± 0.2 mm). Acetone extract showed maximum activity against *E. coli* (19.7 ± 0.7 – 26.0 ± 0.0 mm), moderate activity against *S. aureus* (14.3

± 0.3 – 16.7 ± 1.1 mm), *S. pneumoniae* (14.7 ± 0.3 – 20.1 ± 0.1 mm), *K. pneumoniae* (15.2 ± 0.6 – 17.4 ± 0.6 mm) and *S. typhi* (14.4 ± 0.4 – 15.7 ± 1.1 mm).

Evaluation of MIC of extracts of *Allium sativum* and *Allium cepa*

The minimum inhibitory concentrations of the extracts of *Allium cepa* and *Allium sativum* shown in Table 5, ranged from 10 to 20 mg/mL. Aqueous extract of *Allium sativum* showed MIC at 10 mg/mL for *S. aureus* and *E. coli* and 10 mg/mL for *S. typhi*, *S. pneumoniae* and *K. pneumoniae*. Ethanol extracts showed MIC at 10 mg/mL for all the tested bacteria except *S. typhi* (20 mg/mL), while acetone extracts also showed MIC at 20 mg/mL for all the test bacteria but had no inhibitory effect on *K. pneumoniae*. Aqueous extract of *Allium cepa* showed MIC at 20 mg/mL for *S. typhi* and *S. pneumoniae* and 10 mg/mL for *E. coli*, *S. aureus* and *K. pneumoniae*, ethanol extract showed MIC at 10 mg/mL for all test organisms, while acetone extract showed MIC at 20 mg/mL for *S. typhi* and *S. pneumoniae* and 10 mg/mL for *E. coli*, *S. aureus* and *K. pneumoniae*.

Table 3: Diameters of zones of inhibition (mm) of extracts of *Allium sativum* at 100 mg/mL, 150 mg/mL and 200 mg/mL concentrations

Microorganisms	Extracts	Zone of inhibition (mm)		
		100 mg/mL	150 mg/mL	200 mg/mL
<i>Salmonella typhi</i>	Aqueous	4.0 ± 0.0	8.3 ± 0.1	11.1 ± 0.4
	Ethanol	11.5 ± 0.0	12.6 ± 0.8	16.0 ± 0.0
	Acetone	10.6 ± 0.5	12.3 ± 0.2	15.7 ± 0.8
<i>Staphylococcus aureus</i>	Aqueous	15.5 ± 0.5	22.6 ± 0.6	22.8 ± 1.1
	Ethanol	21.2 ± 0.2	24.7 ± 0.5	28.1 ± 0.2
	Acetone	15.4 ± 0.2	17.3 ± 0.2	16.9 ± 0.6
<i>Streptococcus pneumoniae</i>	Aqueous	10.0 ± 0.0	10.7 ± 1.2	16.3 ± 0.2
	Ethanol	20.0 ± 0.0	22.0 ± 0.2	22.4 ± 0.7
	Acetone	16.8 ± 0.4	20.4 ± 0.4	20.8 ± 0.9
<i>Klebsiella pneumoniae</i>	Aqueous	11.8 ± 0.2	14.0 ± 0.4	17.8 ± 0.3
	Ethanol	9.1 ± 0.3	9.4 ± 0.3	14.0 ± 0.0
	acetone	0.0	0.0	0.0
<i>Escherichia coli</i>	Aqueous	10.0 ± 0.1	12.0 ± 0.0	16.0 ± 0.1
	Ethanol	9.8 ± 0.4	17.4 ± 0.8	20.0 ± 0.1
	Acetone	5.7 ± 0.8	10.0 ± 0.9	13.4 ± 0.7

Table 4: Diameters of zones of inhibition (mm) of extracts of *Allium cepa* at 100 mg/mL, 150 mg/mL and 200 mg/mL concentrations

Microorganisms	Extracts	Zone of inhibition (mm)		
		100 mg/mL	150 mg/mL	200 mg/mL
<i>Salmonella typhi</i>	Aqueous	7.6 ± 0.2	10.0 ± 0.0	14.3 ± 0.1
	Ethanol	11.5 ± 0.2	14.0 ± 0.5	16.7 ± 0.3
	Acetone	14.4 ± 0.8	14.7 ± 0.5	15.7 ± 1.1
<i>Staphylococcus aureus</i>	Aqueous	16.9 ± 0.5	18.0 ± 0.6	19.5 ± 0.6
	Ethanol	16.7 ± 0.2	19.7 ± 0.5	25.5 ± 0.2
	Acetone	14.3 ± 0.3	14.3 ± 0.7	16.7 ± 1.1
<i>Streptococcus pneumoniae</i>	Aqueous	6.6 ± 0.7	10.7 ± 0.5	10.1 ± 0.4
	Ethanol	10.0 ± 0.5	14.6 ± 0.2	16.6 ± 0.2
	Acetone	14.7 ± 0.3	15.9 ± 0.4	20.1 ± 0.1
<i>Klebsiella pneumoniae</i>	Aqueous	16.4 ± 0.2	20.2 ± 0.4	21.2 ± 0.5
	Ethanol	14.0 ± 0.0	16.2 ± 0.5	20.1 ± 0.1
	acetone	15.2 ± 0.6	16.1 ± 0.3	17.4 ± 0.6
<i>Escherichia coli</i>	Aqueous	14.0 ± 0.0	16.2 ± 0.1	18.7 ± 0.3
	Ethanol	19.1 ± 0.1	24.5 ± 0.3	28.7 ± 0.3
	Acetone	19.7 ± 0.7	24.4 ± 0.3	26.0 ± 0.0

Table 5: Minimum inhibitory concentrations of extracts against test bacteria

Microorganisms	Minimum inhibitory concentrations (mg/mL)					
	<i>Allium sativum</i>			<i>Allium cepa</i>		
	Aqueous	Ethanol	Acetone	Aqueous	Ethanol	Acetone
<i>S. typhi</i>	20	10	10	20	10	20
<i>S. aureus</i>	10	20	10	10	10	10
<i>S. pneumoniae</i>	20	10	10	20	10	20
<i>K. pneumoniae</i>	20	10	NI	10	10	10
<i>E. coli</i>	10	10	10	10	10	10

NI: No inhibition

Discussion

The qualitative phytochemical analysis of the extracts indicated the presence of the important phytochemical compounds in *Allium sativum* and *Allium cepa* such as tannins, saponins, flavonoids, alkaloids and anthraquinones which were present in all the extracts of both plants. This was in agreement with the work done by Jadon and Dixit (2014), Lekshmi et al. (2015a) and Ahmad and Beg (2001) who reported similar findings that these compounds were found in *Allium* species and have been found to have significant therapeutic applications against human pathogens including bacteria, fungi and virus. The phytochemical constituents present are known

to possess antimicrobial activities. Tannins from *Allium* species have been reported to inhibit growth of pathogenic microorganism, while alkaloids indicate that the plants can be used as antibacterial agents (Dalhat et al. 2018). The presence of saponins and flavonoids in *Allium* species have been reported to possess both bacteriostatic and bactericidal effects on some strains of bacteria (Gazuwa et al. 2013).

Allium species have been known to exhibit antibacterial activities against both Gram negative and Gram positive bacterial pathogens (Lekshmi et al. 2015b). In the present study, all the extracts of *Allium sativum* and *Allium cepa* showed varying antibacterial activities against both Gram negative and Gram positive

pathogens. *Allium sativum* extracts showed maximum antibacterial activities against *S. aureus* and *S. pneumoniae*, while moderate activities were observed against *E. coli*, *S. typhi* and *K. pneumoniae* except acetone extract which showed no antibacterial activity against *K. pneumoniae* at all the concentrations used. The antimicrobial activities of *Allium sativum* extracts obtained in this study agree with those of Zakaria and Astal (2003), they studied the antibacterial effects of garlic extracts on certain Gram positive and Gram negative bacteria. A study by Shobana et al. (2009) revealed that alcohol extract of *Allium sativum* has the highest inhibitory activity against all test bacteria, while Zakari and Astal (2003) also reported the inhibition of the growth of *S. aureus*, *S. typhi* and *E. coli* by fresh garlic extract.

In the present study, all the extracts of *Allium cepa* showed antibacterial activities against the test bacteria (Table 4), the zones of inhibition ranging from 7.6 ± 0.2 to 21.2 ± 0.5 mm for the aqueous extract, 11.5 ± 0.2 to 28.7 ± 0.3 mm for the ethanol extract and 14.3 ± 0.3 to 26.0 ± 0.0 mm for the acetone extracts. The maximum antibacterial activity was observed against *E. coli* (28.7 ± 0.3 mm) at 200 mg/mL, and this is similar to the observation of the study of Induja and Geetha (2018) in which the extracts of *Allium cepa* were most effective against *E. coli* with zone of inhibition of 28 mm at 200 µg concentration. Moderate antibacterial activities were observed against *K. pneumoniae*, *S. pneumoniae* and *S. aureus*, while the minimum activity was observed against *S. typhi* (7.6 ± 0.2 mm). This study shows that *Allium cepa* has a broad spectrum of activities against both Gram positive and Gram negative bacteria which is in agreement with the study of Bakht et al. (2013), who reported that *Allium cepa* showed antibacterial activities against both Gram negative and Gram positive bacteria.

Out of the three solvents used for extraction, ethanol exhibited maximum antibacterial activity against all the test bacteria; this could be as a result of better

solubility of active compounds in alcohol. *E. coli* was the most susceptible to *Allium cepa* extracts, while *S. aureus* was the most susceptible to *Allium sativum* extracts. Factors responsible for high susceptibility of these organisms were not confirmed but presumed to be attributed to the presence of phytochemical constituents such as saponins, alkaloids, tannins, flavonoids, anthraquinone and phlobatannins in the plant extracts. The results of the minimum inhibitory concentrations of the extracts obtained in this research confirmed the profound inhibitory effects of *Allium* species against pathogenic bacteria. Minimum inhibition concentrations of both *Allium sativum* and *Allium cepa* were low, which is suggestive of the best antibacterial potentials of the bioactive principles of the extracts of the plants.

Conclusion

This study has shown that the extracts of *Allium sativum* and *Allium cepa* have strong activities against all the test bacteria which are known pathogens to humans. The phytochemical screening of these extracts revealed that they contain phytochemical constituents such as saponins, anthraquinones, tannins, phlobatannins, alkaloids and flavonoids which are invariably responsible for the antibacterial activities observed. The minimum inhibition concentrations of extracts observed in this study showed that the plants have inhibitory effect, thereby having a potential for use as medicines. Further clinical evaluation of the effectiveness of *Allium species* in *in vivo* experiment is recommended.

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