PRINT ISSN 1119-8362
Electronic ISSN 2659-1502

Full-text Available Online at
https://www.ajol.info/index.php/jasem
http://www.bioline.org.br/ja

J. Appl. Sci. Environ. Manage.
Vol. 27 (6) 1077-1082 June 2023

Antibacterial Effect of *Allium sativum* and *Zingiber officinale* Extracts on Some Clinically Pathogenic Bacteria

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ABSTRACTS: The ability of some pathogenic bacteria to develop antibiotic resistance has become a global health challenge, and there is an increasing rate of bacteria resistance against the available antibiotic drug. This study aimed to determine the inhibitory effect of *Allium sativum* (Garlic) and *Zingiber officinale* (Ginger) extracts on clinical pathogenic bacteria. Four different concentrations (125mg/ml, 250mg/ml, 500mg/ml, and 1000mg/ml) of each extract were tested against the test organisms. The test isolates showed susceptibility to varying concentrations of the crude extracts. For aqueous and ethanolic extracts of Allium sativum, Staphylococcus aureus showed the highest susceptibility (16±0.8mm and 15.7±1.2mm), followed by *Escherichia coli* (13.7±1.2mm and 14±0.8mm), *Pseudomonas aeruginosa* (14±0.8mm and 12±0.8mm) while *Klebsiella pneumonia* showed the least susceptibility of (11±0.8mm and 10±1.3mm). For aqueous and ethanolic extracts of *Zingiber officinale, Staphylococcus aureus also showed the highest susceptibility (16±0.8mm and 15±0.8mm),* followed by Pseudomonas aeruginosa (14±0.8mm and 12±0.8mm), Escherichia coli (13.7±1.2mm and 13±0.8mm) and the least was *Klebsiella pneumonia* at (11±0.8mm and 7±0.8mm). However, all the tested organisms were most susceptible to aqueous extracts of garlic and ginger but showed relatively lower susceptibility to ethanolic extracts of ginger. The minimum inhibitory concentration for garlic and ginger extract was 125mg/ml against *E. coli*, *S. aureus*, and *P. aeruginosa*. The phytochemical screening showed the abundant presence of Tannins, Flavonoids, and carbohydrates in the extracts. This study has revealed that extracts of Garlic and Ginger have antibacterial properties against some pathogenic bacteria isolates. Therefore, this study encourages *Allium sativum* and *Zingiber officinale* to treat diseases caused by these pathogenic bacteria.

DOI: **https://dx.doi.org/10.4314/jasem.v27i6.5**

Cite this paper as: BELLO, A.B; SULEIMAN, A; ATTEH, E.O; OLUTIMAYIN, T.A (2023). Antibacterial Effect of *Allium sativum* and *Zingiber officinale* Extracts on Some Clinically Pathogenic Bacteria. *J. Appl. Sci. Environ. Manage.* 27 (6) 1077-1082

Dates: Received: 19 May 2023; Revised: 04 June 2023; Accepted: 09 June 2023
Published: 30 June 2023

Keywords: Antibiotics; Pathogen; Phytochemicals; *Zingiber officinale*; *Allium sativum*

Commercially available antimicrobial drugs have controlled microbial pathogenicity and other infectious diseases for many decades. The therapeutic method of antibiotics has played an essential role in addressing the issue of human infectious diseases. Still, the overuse of these antibiotics has become the primary factor for the emergence and discrimination of drug-resistant strains (Frieri *et al.*, 2017). The increasing drug resistance is the main hindrance to the successful treatment of infectious diseases and the control of microbial pathogenicity (Fu *et al.*, 2007). Therefore, the continuing spread of multi-drug resistant strains and the increased abuse of antibiotics highlight the need for alternative agents. Traditionally, herbs and spices have been used in many communities to manage several ailments, including antimicrobial substances (Dog, 2006; Leja and Czaczyk, 2016; Dini, 2018). Compared to conventional antibiotics, herbs, and spices are generally considered safe for humans owing to their long history of use in food preparation (Akullo *et al.*, 2022). These plants fall under natural products, a significant source of new natural drugs.

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Their use as an alternative medicine for various diseases has increased in the last few decades (Vuorelaa *et al.,* 2004; Ansari *et al.*, 2006). Compared to formulated drugs, herbs, and spices have fewer side effects. They are inexpensive, show better patient tolerance, and are readily available for low socioeconomic populations (Adeshina *et al.,* 2011; Emmanuel *et al.*, 2021). The antimicrobial activity of spices is due to specific phytochemicals or essential oils (Avato *et al.,* 2000). The main factors that determine the antimicrobial activity are the type and composition of the spice, the amount used, the type of microorganism, the composition of the food, the pH value, and the temperature of the environment (Sagdic, 2003). Several reports have been published that describe the antibacterial and antifungal properties of different herbs and spices. However, still, there is little information about the exact mechanism of their antimicrobial action (Gur *et al.,* 2006; Pattaratanawadee *et al.,* 2006; Yusha'u *et al.,* 2008; Belguith *et al.*, 2010; Poeloengan, 2011; Gull *et al.*, 2012). Ginger (Zingiber officinale) is a member of the family Zingiberaceae. It is widely distributed across the tropics of Asia, Africa, America, and Australia, where it is used as a spice and medicinal plant (Jung Park & Pezzuto, 2002). Several studies have reported on the antimicrobial activity of ginger and Garlic (Akintobi *et al.,* 2013; Indu *et al.*, 2006; Khashan, 2014; Mohammed *et al.,* 2019). There is a broad spectrum of antibacterial activity of ginger rhizome against a range of Gram-negative and Gram-positive bacteria. However, conflicting reports exist about the antibacterial effectiveness of ginger against bacteria from different resources (Abdalla & Abdallah, 2018). Zingiberene in ginger rhizome oils is the most active antibacterial component (El-Baroty *et al.*, 2010), and the therapeutic effectiveness of Garlic is attributed to its oil content and water-soluble organosulfur compounds (Prati *et al.*, 2014). Studies on the diversity of ginger have shown that cultivated ginger exhibits variations in rhizomes and vegetative character, and environmental factors considerably influence the critical bioactive compounds (Kizhakkayil & Sasikumar, 2011; Akullo *et al.,* 2022). Garlic (*Allium sativum*) belongs to the family Alliaceae, which has been found to help prevent many diseases. Numerous modern studies confirm that Garlic has definite antibiotic properties and is effective against a broad spectrum of bacteria, fungi, and viruses (Shuford *et al.*, 2005; Low *et al.,* 2008). In addition, Garlic's antimicrobial activities are linked to some bioactive compounds (Tsao & Yin, 2001). Due to the high morbidity and mortality burden of infectious diseases, many investigations have focused on understanding, controlling, treating, and preventing these diseases (Murugaiyan *et al.,* 2022). Therefore, natural products are a source of medicine used as an alternative medicine for various diseases. This present study aims to study the inhibitory effect of Garlic (*Allium sativum*) and Ginger (*Zingiber officinale)* extract on some clinically pathogenic bacteria.

MATERIALS AND METHODS

Sample collection: Fresh garlic cloves and ginger rhizomes used in this study were obtained from the old market in Bida town. Nigeria.

Test Organisms: The four different clinical isolates used in this study were obtained from the Microbiology Laboratory of the Federal Polytechnic Medical Centre Bida, Niger State. The isolates include *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, and *Staphylococcus aureus*.

*Preparation of Extracts:*The aqueous and ethanolic extracts of ginger and Garlic were prepared separately. The fresh garlic cloves and ginger rhizomes were cut into pieces, air-dried at room temperature, pulverized into powder using a clean mortar and pestle, and then packed into clean bottles for further analysis. Fifty grams (50g) of Garlic and ginger powder was soaked in 120ml of distilled water and ethanol separately in a beaker. The beaker was allowed to stand at room temperature for 72 hours with intermittent agitation and filtered using Whatman paper No.11.2. The ethanol extract was evaporated at 40°C while the aqueous extracts were evaporated at 80°C using a rotary evaporator. The extracts were labelled and kept at 4°C to preserve the antibacterial property.

Culture Preparation. The clinical isolates were inoculated into the nutrient broth, allowed to grow overnight at 37°C, and adjusted to 0.5McFarland standard turbidity before performing the antimicrobial assay.

Antibacterial Activity Testing Using Agar Well Diffusion: The bacterial cultures were swabbed onto the surface of solidified Muller Hinton agar using a sterile cotton swab. The agar wells were prepared using a sterile 10mm diameter cork-borer. The wells were filled with different concentrations (125mg/ml, 250mg/ml, 500mg/ml, and 1000mg/ml) of the extracts. The plates were incubated uprightly at 37℃ for 18 hours. The diameters of growth inhibition were measured in millimeter (mm) and recorded. The inhibition zones with a diameter of less than 10 mm are considered to have no antibacterial activity (Dulger & Gonuz, 2004).

Determination of Minimum Inhibitory Concentration (MIC): The tube dilution method was used to determine the minimum inhibitory concentration. The inoculums were prepared using 24-hour old cultures broth and were adjusted to 0.5McFarland standard turbidity. In the test tubes, 9ml of sterile nutrient broth was dispensed, 1ml of the extract of varying concentrations (125mg/ml, 250mg/ml, 500mg/ml, and 1000mg/ml) was added into different test tubes and 0.1ml of standardized organism was inoculated and incubated for 24 hours at 37oC. The test tube with the least concentration of extract that showed no turbidity was taken as the minimum inhibitory concentration (Mounyr *et al.,* 2016).

RESULT AND DISCUSSION

The Inhibitory activity of Garlic (cloves) and Ginger extracts was investigated against four clinical isolates. Among the clinical isolates, *Staphylococcus aureus, Escherichia coli, and Pseudomonas aeruginosa* were more susceptible than *Klebsiella*. The inhibitory activity of garlic and ginger extracts revealed that *Staphylococcus aureus* (16mm) and *Escherichia coli* (15mm) were more susceptible to the aqueous extract of Garlic than the ethanol extract of Garlic (Tables 1 and 2). While in Ginger extract, *Staphylococcus aureus* (16mm) and *Pseudomonas aeruginosa* (14mm) were more susceptible to aqueous extract than the ethanol extract of ginger (Tables 3 and 4). The result of activity in this study is similar to previous reports by Gull *et al.,* (2012), Riaz *et al.,* (2015), and Anyamaobi *et al*. (2020); however, it is at variance with the result of Adeshina e*t al*., (2011), which showed high susceptibility of *Escherichia coli* and *Pseudomonas aeruginos*a but no activity against *Staphylococcus aureus*. Furthermore, a study by Akintobi *et al*., (2013) reported that Ginger extract was ineffective in *Pseudomonas aeruginosa*, *contrary* to this study. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the extract on the isolates are shown in Table 5. The minimum inhibitory concentration for garlic extract was 125mg/ml against *E. coli*, *S. aureus*, and *P. aeruginosa*. In the case of the minimum inhibitory concentration, ginger recorded 125mg/ml against *P. aeruginosa.*

Table 1: Result of Antibacterial Effect of Aqueous Extract of *Allium sativum* on the test organisms

Conc. mg/ml	***Escherichia coli***	***Pseudomonas aeruginosa***	***Klebsiella pneumonia***	***Staphylococcus aureus***
1000	15±1.3 mm	13.3±1.7mm	12±0.8mm	16±0.8mm
500	11±1.3mm	9.7±1.3mm	10±0.8mm	14±0.8mm
250	11±0.8mm	5.7±1.3mm	6±0.8mm	10.3±1.3mm
125	6.7±1.3mm	3.7±0.5mm	NA	4.7±1.3mm

Key = each value is the mean ± SD of the triplicate determination, Non Active (NA)

Table 2: Result of Antibacterial Effect of Ethanol Extract of *Allium sativum* on the test organisms

Conc. mg/ml	***Escherichia coli***	***Pseudomonas aeruginosa***	***Klebsiella pneumonia***	***Staphylococcus aureus***
1000	14±0.8mm	12±0.8mm	10±1.3mm	15.7±1.2mm
500	10±0.8mm	10±0.8mm	7±0.8mm	10.7±2.2mm
250	8.7±1.3mm	7.7±1.2mm	4±0.8mm	9.3±1.2mm
125	8±0.8mm	6.7±1.2mm	3±0.8mm	5.7±1.2mm

Key = each value is the mean ± SD of the triplicate determination

Table 3: Result of Antibacterial Effect of Aqueous Extract of *Zingiber officinale* on the test organisms

Conc. mg/ml	***Escherichia coli***	***Pseudomonas aeruginosa***	***Klebsiella pneumonia***	***Staphylococcus aureus***
1000	13.7±1.2mm	14±0.8mm	11±0.8mm	16±0.8mm
500	11.6±1.2mm	12±1.6mm	8±0.8mm	12±1.6mm
250	8.7±1.2mm	7±0.8mm	5±0.8mm	8±0.8mm
125	4.6±1.2mm	3.3±0.9mm	NA	NA

Key = each value is the mean ± SD of the triplicate determination, Non Active (NA)

Table 4: Result of Antibacterial Effect of Ethanol Extract of *Zingiber officinale* on the test organisms

Conc. mg/ml	***Escherichia coli***	***Pseudomonas aeruginosa***	***Klebsiella pneumonia***	***Staphylococcus aureus***
1000	13±0.8mm	12±0.8mm	7±0.8mm	15±0.8mm
500	9±0.8mm	9.0±0.8mm	5.7±1.3mm	9.7±1.3mm
250	5.7±1.3mm	8±0.8mm	4.0±1.6mm	5±0.8mm
125	4±1.6mm	7±0.8mm	NA	3.7±1.3mm

Key = each value is the mean ± SD of the triplicate determination, Non Active (NA)

This indicates that the crude extracts of Garlic may yield potential molecules in treating infections caused by pathogenic bacteria. The extract's minimum bactericidal concentration (MBC) showed effectiveness at 1000mg/ml for *E. coli*, *S. aureus* and *P. aeruginosa*, and *K. pneumoniae* for both garlic and ginger extracts. The result is similar to the report by Adeshina *et al*., (2011), Gull *et al.*, (2012), and Riaz *et al*., (2015), which stated that the higher the concentration of the extract, the greater the inhibitory effect of extracts. The phytochemical characteristics of garlic and ginger extracts are presented in Table 6. The result revealed the presence of alkaloids, flavonoids, tannins, saponins, and phlobatannins.

Table 5: Comparison of the susceptibility of the test isolate to the extract minimum inhibiting and the minimum bacterial concentration

Organism	Conc. mg/ml	Garlic Extract A	Garlic Extract E	Ginger Extract A	Ginger Extract E
E. coli	125	+	+	+	+
	1000	$+^x$	$+^x$	$+^x$	$+^x$
P. aeruginosa	125	-	+	-	+
	1000	$+^x$	$+^x$	$+^x$	$+^x$
K. pneumonia	125	-	-	-	-
	1000	$+^x$	$+^x$	$+^x$	$+^x$
S. aureus	125	+	+	_	_
	1000	$+^x$	$+^x$	$+^x$	$+^x$

Key = - = No activity; $+^x$ = MBC, + = MIC; A = Aqueous; E = Ethanol

Table 6: Phytochemical Screening of Plant Extracts

Phytochemical	Garlic Aqueous	Ethanol	Ginger Aqueous	Ethanol
Saponins	+	+	++	+
Tannins	++	++	+	+
Flavonoids	++	++	+	+
Alkaloids	++	+	+	++
Carbohydrate	++	++	++	++
Phlobatannins	+	+	+	+

Key = + moderate present, ++ abundantly present

The presence of these components may be responsible for the antibacterial activity of the extracts. A study by Kumar *et al*., (2011), Kamppiah and Rajaram (2012), and Riaz *et al.*, (2015) also reported similar phytochemical compounds from garlic and ginger extract identified in this study.

Conclusion: This study revealed the potential antibacterial activity of Garlic and Ginger extracts against some clinically pathogenic bacteria isolates. Findings from this study have shown that aqueous extract of *Allium sativum* (Garlic) and *Zingiber officinale* (Ginger) showed the highest antibacterial activity, especially against *Staphylococcus aureus.* However, for the full potential of the extracts, more studies should be carried out to discover and validate active components responsible for antibacterial activity to serve as an alternative to antibiotics.

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