

In Vitro Evaluation on the Antibacterial Potential of *Zingiber officinale* (Ginger) and *Allium sativum* (Garlic) against Clinically Relevant Bacterial Strains

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Abstract

Cases of fake/weak drugs are abundant everywhere in the country today, especially in the use of antibiotics, which leads to an increase level of drug resistance among microorganisms which were known to be susceptible to particular antibiotics. Thus, the need to test for the antibacterial activity of ginger and garlic against some pathogenic microorganisms should be employed to support its use as an ingredient for herbal medicines. This study explores the potential of ginger ethanol extracts, ginger chloroform extract, garlic ethanol extract and garlic chloroform extract tested against some clinically relevant bacterial strains. These include; *Citrobacter* sp, *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas auregenosa*, using agar well diffusion technique. Ciprofloxacin (1 mg/ml) was used as standard control. The test concentration of each extract was set as 100 mg/ml, 50 mg/ml, 25 mg/ml and 12.5 mg/ml. The phytochemical screening revealed the presence of tanins, flavonoids, saponins, cardiac glycosides, steroid, alkaloid, volatile oils, balsams and terpenoids. The results showed a higher zone of inhibition of 28.45 mm at 100 mg/ml concentration of ginger ethanol extract against *S. aureus* and garlic chloroform extract with the least activity against *Citrobacter* spp. with 6.30 mm at 100 mg/ml. The MIC of ginger ethanol extract against the test organisms was determined, which show values between 12.5 and 95 mg/ml. In comparison, the ginger chloroform extract had MIC values between 12.5 and 50 mg/ml. The garlic ethanol extract had MIC value of 50 mg/ml while the garlic chloroform extract had MIC value of 100 mg/ml. The MBC of ginger ethanol and chloroform extracts was between 12.5 and 100 mg/ml. The garlic ethanol and chloroform extracts had MBC values only at 100 mg/ml. The result of this study show that the extracts had activity against the test organisms and as such could be a potential therapeutic against the tested organisms.

Keywords: *Zingiber officinale* (Ginger); *Allium sativum* (Garlic) Phytochemicals; Antibacterial

Introduction

Antibiotic resistance has become a serious problem and affects almost every bacterial specie. Resistance to multiple antibiotics has developed among many common pathogens, such as *Staphylococcus*, *Pneumococci*, *Pseudomonas* organisms and this problem is steadily increasing worldwide (Olofsson and Cars, 2007). Around 90-95% of *Staphylococcus aureus* strains is penicillin resistance worldwide. In Asian countries, 70-80% of the same strains are methicillin resistance (Hemaiswarya *et al.*, 2008). Sometimes antibiotics are associated with adverse effects on host, which include depletion of beneficial gut, mucosal microorganisms, immune suppression, hypersensitivity and allergic reaction. Some drug-resistant bacteria have complicated the treatment of infectious diseases in immune compromised AIDS and cancer patients (McGaw *et al.*, 2000). One way to beat this downside of drug resistance is by getting new molecules from natural resources.

Plants are known to produce a variety of compounds and medicinal properties to prevent infections from a wide range of microorganisms including plant pathogens and environmental organisms (Bentley, 1997; Savithramma *et al.*, 2011; Chung *et al.*, 2011). Therefore, alternative antimicrobials are used from botanical sources which provide flexibility and diversity. In many developing countries large portion of population depends on the traditional system of medicine to treat variety of disease (McGaw *et al.*, 2000). The World Health Organization (WHO) reported that 80 % of world population relies chiefly on traditional medicine, which involve the medicinal plant extracts or their active constituents (Ahmad *et al.*, 1998). Subsequently advancement of common antimicrobial agents from plant source would serve as a promising approach.

Ginger, *Zingiber officinale* (commonly called 'jinja' in Igbo, 'citta' in Hausa and 'Atale' in Yoruba). Ginger is a member of the family *Zingiberaceae* a small family with more than 45 genera and 800 species, its scientific name is *Zingiber officinale* (Foster, 2011). Ginger is an erect, slim herbaceous perennial plant growing from one to three feet in height. Its stem is surrounded by the sheathing bases of the two ranked leaves. A club like spike of yellowish, purple lipped flowers, it has a greenish yellow bracts with rarely flowers in cultivation (Tyler, 2002). It possesses a fleshy and thick underground rhizome and having one or more aerial leafy stems that grows up to 1.25m tall. Ginger is grown in the tropical weathers of Australia, West Africa, India, Jamaica, Brazil, China, and some parts of the United States (Suruchi *et al.*, 2016). In the first year of growth, it produces a green straight stalk like stem about 60 cm high growing from the rhizome. Its leaves grow and measures about 12-30 cm long which dies off each year. The crop grows preferably in warm, sunny conditions, and may profit from shade during hot days, especially when young. Shading is however generally considered redundant. The optimum rainfall is 2500-3000 mm, well distributed over the year. (Shubha, 2015). Ginger plays an important medicinal roles due to the presence of certain constituents such as gingerol, paradol, shogaol, zingerone, zerumbone, terpenoids and ginger flavonoids (Arshad *et al.*, 2014).

Garlic, *Allium sativum* (commonly called 'aayu' in Yoruba, 'ayo-ishi' in Igbo and 'tafarnua' in Hausa), is a perennial bulbous plant that initially came from middle Asia and is at present grown globally. It belongs to the family *Alliaceae*. Garlic can grow up to 2 feet in height or more. The

bulb is the main part of the plant which is used for medicine (Steven, 2015). Each garlic bulb is made up of 4 to 20 cloves. Each garlic clove may weigh about 1 gram in weight. Fresh, aged, or dried garlic can be used as garlic supplements. Each of the supplements may have different effects to the body (Sethi *et al.*, 2014). It is commonly used as seasoning. Its close relatives include the onion, shallot and leek. The head of garlic (the most commonly used plant part) comprises numerous discrete cloves whereas the leaves and stems are sometimes eaten, particularly while immature and tender. The medicinal potency of garlic is due to glycoside, vitamin B, C, and D allisatin II and I. It also contains volatile sulphur oil, which has a vermifugal action (Arshad *et al.*, 2014). It has been used throughout recorded history for both culinary and medicinal purposes. It has a characteristic pungent, hot, flavour that mellows and sweetens considerably with cooking. Its typical pungent odour antibacterial activity depend on allicin, which is produced by enzymatic (alliin-lyase) hydrolysis of alliin after cutting and crushing of the cloves (Onyeagba *et al.*, 2004)

METHODS

Sample Collection and Processing

The plants materials (Garlic and Ginger) were obtained from Kabuga market, Kano, Kano State, Nigeria. They were placed in separate polythene bags and transported immediately to the laboratory of the Department of Microbiology, Bayero University, Kano. The samples were identified and authenticated by comparing them with known samples. the plant materials were washed with clean water to get rid of sand particles. They were chopped and partially allowed to air-dry in the shade at room temperature for five days in order to remove excess moisture. The garlic bulbs were separated into cloves. The cloves skins were peeled off and the cloves were sliced and also air dried at ambient temperature for about four weeks. After drying, pieces of *Allium sativum* and *Zingiber officinale* were grinded to fine particles each, utilizing a suitable sterile electric blender to obtain a homogenous sample.

Preparations of Extract

25 g of the powdered samples each was extracted with 250 mls of ethanol and chloroform by cold maceration method as described by Handa *et al.* (2008); Jolly *et al.* (2022), with some slight modifications. The containers were left at 25 °C for 4 days (96 hours). The suspensions were filtered using Whatman no.1 filter paper. The filtrates were concentrated at 90 °C using water bath and delivered into sterile clean containers with suitable labeling and were kept at 4°C in a refrigerator until further use. The percentage yield of each extract obtained was calculated using the formula.

$$\frac{We}{Wp} \times 100$$

Where; We = weight of the extract,

Wp = weight of the powdered material used for the extraction.

Sterility of the Extracts

One (1) ml of the extracts was added separately in two test tubes containing 5 ml of sterile nutrient broth. They were incubated at 37 °C for 24 hours. The extracts were cleared after incubation indicating the absence of contaminant which could have caused a turbid appearance in the tube (Iotsor *et al.*, 2019).

Qualitative Phytochemical Screening

Phytochemical screening of the extracts were conducted qualitatively as stated by Harborne (1973); Yusha'u *et al.* (2009); Tafinta *et al.* (2020). Tanins, flavonoids, saponins, cardiac glycosides, reducing sugars, steroid, alkaloid, volatile oils, balsams and terpenoids were carried out accordingly.

Test Microorganisms

The test organisms were collected from Microbiology Department Bayero University Kano. The isolates were subjected to Gram's staining and other biochemical tests according to standard procedures and identified as *Staphylococcus aureus* (MRSA), *Escherichia coli*, *Pseudomonas auregenos* and *Citrobacter* sp. (Clarke and Cowan, 1952; Daniel, 2000). The pure isolates were then stored on Nutrient agar slant bottles at a temperature of 4°C until further use.

Preparation of extract concentration

The concentration was prepared by dissolving 0.1 g of crude extract into one milliliter of Dimethylsulphuroxide (DMSO) in a clean grease free vial bottle, to obtain a stock of 100mg/ml concentration. Using double serial dilution methods the following concentration of 50mg/ml, 25mg/ml and 12.5 mg/ml were made. 1mg/ml of ciprofloxacin was used as positive control (Afolabi *et al.*, 2020).

Antibacterial Assay

The agar well diffusion method was used to investigate the antimicrobial properties of the extracts as described by NCCLS (2000); Schumacher *et al.* (2018). All media were prepared and sterilized according to manufacturer's instructions. Within 15 minutes of adjusting the turbidity of the inocula suspension, a sterile swab stick was used to inoculate the inocula onto dried surface of sterile prepared Mueller Hinton agar plates. In each case, streaking was repeated two more times, rotating the plate approximately 60° each time to ensure an even distribution of the inoculums. The inoculated plates were allowed to stay for about 3-5 minutes for the surface of the agar to air-dry. While the plates were drying, four various concentrations of the extracts were prepared. A sterilized cork borer of an internal diameter of 4mm was then used to punch five holes in the inoculated medium, the bottom of the wells were then closed using 1 ml of sterile Mueller Hinton agar and the various concentrations of the Prepared extracts were dispensed into the respective labeled holes (100 mg/ml, 50 mg/ml, 25 mg/ml and 12.5 mg/ml). 1 mg/ml of Ciprofloxacin was used as positive control. Four plates were made for each test organism and the procedure was repeated for the other organisms. The plates were kept in refrigerator for about 4 - 5 hours for complete diffusion of the extracts and incubated at 37°C for 24 - 48 hours. After the incubation

period, the diameter of each zone of inhibition was measured with a handheld vernier caliper in mm and the results were recorded.

Determination of Minimum inhibitory concentration (MIC)

The results of antibacterial activity were used for the determination of MIC. Using single serial dilution method using dimethylsulphuroxide (DMSO) to obtain a stock of 200mg/ml. the following concentrations of 100 mg/ml, 95 mg/ml, 90 mg/ml and 85 mg/ml were prepared but depending on the least concentration that had reasonable activity against the test organisms. If reasonable activity starts from 50mg/ml then the MIC concentrations starts from 50, 45, 40 and 35 mg/ml. The test organism was standardized using 0.5 McFarland standards. Six test tubes were used, each test tube was leveled according to concentration while the last two test tubes serves as positive and negative control. 2ml of nutrient broth was dispensed into sterile test tubes and autoclaved at 121°C for 15 min. the following volume (1 ml, 0.95 ml, 0.9 ml, and 0.85 ml) were removed from the autoclaved nutrient broth and replace with appropriate concentration from the stock solution. One hundred micro liter (0.1 ml) of standardized organisms were added into five test tubes respectively. Tubes containing broth and extracts serves as positive control while tubes containing broth and inocula serves as negative control. This procedure was repeated for the remaining extracts and test organisms. The tubes were observed after 24 hours of incubation to determine minimum inhibitory concentration, i.e the lowest concentration that showed no evidence of growth or turbidity (Yusha'u *et al.*, 2009).

Determination of Minimum Bactericidal Concentration (MBC)

Mueller-Hinton agar was prepared and the petri plates were separately inoculated with sample from each of the test tubes that showed no evidence of growth. The plates were further incubated at 35 °C for 24 hours and observed. The least concentration at which the organism did not grow was taken as the minimum bactericidal concentration. (Iotsor *et al.*, 2019; Yusha'u *et al.*, 2009).

RESULTS

Table 1.0 shows the physical properties of Ginger and Garlic Extracts, ginger extract was recovered as a dark brown, loose substance that smelled spicy-sweet while the garlic extract was a golden-yellow, sticky residue with a harsh disagreeable scent. The percentage yield of extract show that ginger ethanol extract yielded the highest amount of 10 %, followed by ginger chloroform extract with 8.4 %, followed by garlic ethanol extract with 3.6 % and garlic chloroform extract with 2.4 %.

Table 1: Physical Properties of the Plant Extracts

Characteristics	ETH. ginger	CCl4 ginger	ETH. garlic	CCl4 garlic
Weigh	25g	25g	25g	25g
Odour	Spicy sweet	Spicy sweet	Harsh scent	Hash scent

Volume of solvent	250ml	250ml	250ml	250ml
Weight of extract yield	2.5g	2.1g	0.9g	0.6g
Percentage yield	10%	8.4%	3.6%	2.4%

Key: ETH.ginger = Gnger ethanol extract, ETH.garlic= Garlic ethanol extract, CCl4.ginger = Ginger chloroform extract, CCl4.garlic = Garlic chloroform extract

Table 2: Phytochemical Constituents Found in Ginger and Garlic Extracts

Phytochemical	ETH. Ginger	CCl4 ginger	ETH. garlic	CCl4 garlic
Tanins	-	-	+	-
Flavonoids	+	+	-	-
Saponins	+	+	+	-
Cardiac glycosides	+	+	+	+
Reducing sugas	-	-	-	-
Steroids	+	+	-	-
Alkaloids	+	-	+	-
Volatile oil	+	+	+	+
Balsams	+	+	-	-
Terpenoids	+	+	-	-

Key: ETH.ginger = Gnger ethanol extract, ETH.garlic= Garlic ethanol extract, CCl4.ginger = Ginger chloroform extract, CCl4.garlic = Garlic chloroform extract, + = present and - = absent.

Table 3: Zone of inhibition for the ginger ethanol extract, measured in millimeters

Test Organism	Concentration/zone inhibition				
	100mg/ml	50mg/ml	25mg/ml	12.5mg/ml	Control
<i>Citrobacter spp.</i>	17.15	7.97	4.00	4.00	33.18
<i>E. coli</i>	4.00	4.00	4.00	4.00	4.00
<i>S. aureus</i>	28.45	28.00	27.12	26.51	4.00
<i>P. aeruginosa</i>	14.03	12.84	7.27	6.99	10.21

Key: Diameter of cork borer is 4.00 mm

Table 4: Zone of inhibition for the ginger chloroform extract, measured in millimeters

Test Organism	Concentration/zone inhibition				
	100mg/ml	50mg/ml	25mg/ml	12.5mg/ml	Control
<i>Citrobacter spp.</i>	20.87	15.28	10.77	6.62	30.60
<i>E. coli</i>	4.00	4.00	4.00	4.00	4.00
<i>S. aureus</i>	25.98	22.78	21.58	15.79	4.00
<i>P. aeruginosa</i>	7.55	7.11	4.00	4.00	6.65

Key: Diameter of cork borer is 4.00 mm

Table 5: Zone of inhibition for the garlic ethanol extract, measured in millimeters

Test Organism	Concentration/zone inhibition				
	100mg/ml	50mg/ml	25mg/ml	12.5mg/ml	Control
<i>Citrobacter spp.</i>	4.00	4.00	4.00	4.00	30.60

<i>E. coli</i>	4.00	4.00	4.00	4.00	4.00
<i>S. aureus</i>	16.60	13.59	4.00	4.00	4.00
<i>P. aeruginosa</i>	4.00	4.00	4.00	4.00	4.19

Key: Diameter of cork borer is 4.00 mm

Table 6: Zone of inhibition for garlic chloroform extract, measured in millimeters

Test Organism	Concentration/zone inhibition				
	100mg/ml	50mg/ml	25mg/ml	12.5mg/ml	Control
<i>Citrobacter spp.</i>	6.30	4.00	4.00	4.00	25.19
<i>E. coli</i>	4.00	4.00	4.00	4.00	4.00
<i>S. aureus</i>	4.00	4.00	4.00	4.00	4.00
<i>P. aeruginosa</i>	5.63	4.00	4.00	4.00	15.21

Key: Diameter of cork borer is 4.00 mm

Table 7: Minimum Inhibitory Concentration (MIC) of ethanol and chloroform Extracts of ginger and garlic on test organisms.

Test Organism	Concentration (mg/ml)			
	ETH. Ginger	CCl4 ginger	ETH. garlic	CCl4 garlic
<i>Citrobacter spp.</i>	95	25	ND	100
<i>E. coli</i>	ND	ND	ND	ND
<i>S. aureus</i>	12.5	12.5	50	ND
<i>P. aeruginosa</i>	45	50	ND	100

Key: ETH.ginger = Ginger ethanol extract, ETH.garlic= Garlic ethanol extract, CCl4.ginger = Ginger chloroform extract, CCl4.garlic = Garlic chloroform extract and ND = Not detected.

Table 8: Minimum bactericidal Concentration (MBC) of ethanol and chloroform extract of ginger and garlic extracts.

Test Organism	Concentration (mg/ml)			
	ETH. Ginger	CCl4 ginger	ETH. garlic	CCl4 garlic
<i>Citrobacter spp.</i>	95	50	ND	100
<i>E. coli</i>	ND	ND	ND	ND
<i>S. aureus</i>	12.5	12.5	100	ND
<i>P. aeruginosa</i>	90	100	ND	100

Key: ETH.ginger = Ginger ethanol extract, ETH. garlic= Garlic ethanol extract, CCl4.ginger = Ginger chloroform extract, CCl4.garlic = Garlic chloroform extract and ND = Not detected.

DISCUSSION

The findings of this study show that solvents with high polarity yielded higher bioactive compounds. This work correlate with the findings of Wolde *et al.* (2018) who reveals that the higher the polarity of a solvent, the more amount of bioactive compound to be obtained.

The outcomes of the phytochemical screening of ethanol and chloroform extracts of ginger and garlic indicated that majority of the phytochemicals were slightly present only in the ethanol and chloroform extract of garlic, but they were abundantly found in the ethanol and chloroform extract of ginger. Only garlic ethanol extracts contain tannins. The study findings show that the ginger ethanol and chloroform extracts contain flavonoid, which is known to act as a potent barrier against bacterial infection (Namadina *et al.*, 2021). Both the ginger ethanol and chloroform extracts contain saponin, while only the garlic ethanol extract did. High levels of saponin was shown to have structure-dependent therapeutic effects (Ayoola *et al.*, 2008). The ginger and garlic ethanol and chloroform extracts contain cardiac glycosides and volatile oil content. There was no reducing sugar in any of the extracts. Both the ginger ethanol and chloroform extracts contain steroid. It is well known that plant-derived steroids have cardiogenic effects in addition to having antibacterial and insecticidal capabilities (Alexei *et al.*, 2001). The ethanol extract of ginger and garlic contain alkaloid. Many alkaloids derived from medicinal plants exhibit biological activities such as antimicrobial, cytotoxic and pharmacological effects (Dua *et al.*, 2013; Benbott *et al.*, 2012). Terpenoids were detected in both the ethanol and chloroform extracts of ginger, whereas balsams were also discovered in both.

In the present investigation, the ginger extracts exhibited high degree of inhibitory activity against most of the 4 isolates with the exception of *E. coli* followed by the garlic extracts which showed less activity at only higher concentrations. Among the test organisms, *Staphylococcus aureus* and

Citrobacter sp. were most susceptible then followed by *P. aeruginosa* which show the least susceptibility to garlic chloroform extract. *E. coli* was found to be resistant to all the four extracts and this could be related to the fact that the lipopolysaccharide (LPS) layer of gram-negative bacteria in outer membrane have a high hydrophobicity which acts proly as a strong barrier against the bioactive molecules. Certain molecules can pass through cell wall of Gram-positive bacteria easier than the gram-negative bacteria because cell wall of the gram-positive bacteria contained only peptidoglycan (Ababutain, 2011). Similar finding was obtained from other researchers (Akrayi, 2014; Ababutain, 2011; Keskin & Toroglu, 2011; Nanasombat & Lohasupthawee, 2011), where they found that so many extracts of spices and herbs did not have antibacterial activity against *E. coli* tested in their studies.

The least activity of garlic observed in this study was in disagreement with earlier reports that garlic is highly effective against microorganisms (Belguith *et al.*, 2010; Yin *et al.*, 2002; Bakht *et al.*, 2011; Iwalokun *et al.*, 2004; O’Gara *et al.*, 2000). The resistivity might be as a result of the heat applied during the evaporating phase of the extracts filtrates in a water bath which may have caused the denaturing of some bioactive compounds in the garlic used. According to Gupta and Ravishankar (2005), commercial garlic showed antimicrobial activity only at 4 °C and 8 °C, indicating that antimicrobial activity of garlic is temperature dependent. Apart from temperature it is also believed that geographical location of a plant, temperature, and seasonal variation of an area may have influence over the yield of medicinal plants (Akrayi, 2014). Hence, the low or no inhibition zones observed.

However, the study findings demonstrated that compared with all the extracts and ciprofloxacin as the control drug had higher zone of inhibition producing to 30 mm against all the test isolates with the exception of *Staphylococcus aureus* that showed resistance to Ciprofloxacin. Ginger ethanol and chloroform extracts performed better than the Ciprofloxacin on *Staphylococcus aureus*.

Finally, The susceptibility screening of *Citrobacter spp*, *Escherichia coli*, *Staphylococcus aureus* and *pseudomonas auregenosa*, on the extracts were further evaluated in order to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) all the result obtained are within the range of 12.5mg/ml to 100mg/ml.

Conclusion

This study found that the ethanol and chloroform extract of ginger (*Zingiber officinale*) displayed strong inhibitory action against *Staphylococcus aureus* than ethanol and chloroform extract of garlic. The ginger could be regarded as a potential antibacterial agent with therapeutic potential in the treatment of bacterial infection especially those caused by *Staphylococcus aureus*.

Authors’ Contributions

Yero I.H. conceived the idea and wrote the original draft of manuscript and was also involved particularly in Protocol development, Collection of Ginger and Garlic sample and scientific identification, Aminu A.I. involved in review of the manuscript and experimental design, Ishaq S.A. assisted in result collation and data analysis. All author’s reviewed and edited the final version of the manuscript.

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Conflict of Interest

The authors declare no conflict of interest exist

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