

Antimicrobial Activities of Ginger (*Zingiber officinale*) and Garlic (*Allium Sativum*) Against Selected Pathogenic Organisms

Adeyemo I.A Fawa, O.A

Biological Sciences Department, Microbiology Unit, OAUSTECH, Okitipupa, Ondo State

* E-mail of the corresponding author: ia.adeyemo@oaustech.edu.ng.

ORCID No of corresponding author: 0000 – 0001 - 8823 – 5469

Abstract

Garlic and ginger samples were collected and their bio active components were determined using standard methods. Bio active components of ginger were found to include Saponins, Glycosides, Alkaloids, Flavonoids, Tannis, Terpernoides while garlic tested positive to only Cardiac glycosides. *Pseudomonas aueruginosa*, *Klebsiella pneumonia*, *Staphylococcus aureus* were the test organisms. Antimicrobial activities were tested on *Klebsiella pnuemoniae*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* using Antibiotic sensitivity disc and zone of clearance was highest with Ciprofloxacin but resistant with Meropenem and Cefuroxime sodium respectively. Kirby- Bauer method disc diffusion method was used to test the effectiveness of extracts against selected pathogenic bacteria. *Pseudomonas aueruginosa* was susceptible to various dilutions (10^{-1} – 10^{-7}) of extracts of ginger and ethanol (51 – 15mm) but more susceptible to extracts of garlic and ethanol (59 – 22mm) and resistant to some dilutions of extracts of garlic and ginger in combination (18 – 0mm). *Staphylococcus aureus* was susceptible to garlic and ethanolic extracts (44 – 19mm), more susceptible to extracts of ginger and garlic in ethanol (51 – 19mm) but resistant to some dilutions of aqueous and ethanolic ginger extracts. *Klebsiella pneumonia* was resistant to both aqueous and ethanolic extracts at various dilutions singly and in combination. It is however susceptible to the extracts at 10^{-1} dilutions except in ginger warm aqueous extract and garlic cold aqueous extract where it is resistant. The study therefore prove the potency of ginger and garlic in treatment of certain pathogenic bacteria infections.

Keywords: Pathogenic, garlic, ginger, extracts, zone of clearance.

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1. Introduction

The medicinal use of garlic and ginger has a long history. Garlic is probably one of the earliest known medicinal plants (Starek, 2001; Lien *et al.* 2003; Wang and Wang *et al.*, 2005; White, 2007; Yiming *et al.*, 2012; Wichtl, 2004). Over the centuries, garlic has acquired a special position in the folklore of many cultures as a formidable prophylactic and therapeutic medicinal agent. Garlic has been a traditional treatment in many countries, notably the Near East, China, and India. It has attracted particular attention of modern medicine because of its widespread use around the world and the cherished belief that it helps to maintain good health by warding off illnesses and providing more vigor (Mikaili *et al* 2013). Ginger (*Zingiber officinale*, Roscoe Zingiberaceae) is one of the most widely consumed spices worldwide. From its origin in Southeast Asia and its spread to Europe, it has a long history of use as herbal medicine to treat a variety of ailments including vomiting, pain, indigestion, and coldinduced syndromes (More recently, it was reported that ginger also possessed anti-cancer, anticlotting, anti-inflammatory, and analgesic activities (Yiming *et al.*, 2012). Ginger is believed to have originated from China and then spread to India, South East Asia, West Africa and the Caribbean (McGee, 2004; Wang and Wang, 2005; White, 2007). It has been used in Unani, Ayurvedic and Chinese herbal medicines for a wide array of unrelated ailments that include arthritis, rheumatism, sprains, pains, muscular aches, sore throats, indigestion, vomiting, fever, hypertension, cramps, constipation, dementia, helminthiasis and infectious diseases (Ali *et al*, 2008).

Natural products are the most important source for drugs and drug discovery. The WHO estimated that about 65% of the World's populations are mainly relying on natural products derived from plants for their primary health care systems and most of them are from developing countries, the remaining 35% are mostly from developed courtiers who are also used natural products indirectly to maintain a good health [Cragg and Newman 2013]. The use of ginger and garlic as spices is not restricted to food flavoring only, but also used as food preservatives and colorants, extend shelf-life of food, prevent food spoilage, food-borne diseases and frequently prescribed in traditional medicine [Abdallah and Abdalla 2018]. Antibiotics, which have made tremendous successes on bacterial infections at the beginning of the twentieth century, are now becoming less effective, as bacterial cells have developed gradual resistance for decades to common antibiotics while the human host remains unaware that antibiotic resistance catastrophe has emerged [Zaman *et al.* 2017]. Accordingly, medicinal plants could be the new promising alternative to not only reduce antibacterial resistance but to also reduce the intake of antibiotics in tablet and syrup forms to a more naturally edible and acceptable

products. Many natural products being used over the year have been effective antibiotic, ranging from shrubs, fruits, vegetables, and roots.

In Nigeria, spices such as cloves of garlic known as *Allium Sativum* and ginger (*Zingiber officinale*) are not being used as spices only but also as medicinal foods, they are however taken through meat spicing, jollof rice condiment as well as fermented maize (Ogi) being used as food by several millions across the nation. It is believed that they exhibit wide range of antimicrobial activities when they are consumed as food hence their acceptability by all class, age and location notwithstanding. They are readily available and cheap. A number of clinical trials have been undertaken with diverse products containing ginger and garlic products, with various results such as gingerol (S) - (6) and (S) – (8) gingerols. On the other hand garlic contains a number of organosulphur compounds such as S-allyl cysteine, S-allyl mercaptocysteine, allicin, ajoene and diallyl sulphide (Tende, 2015) which are widely believed to be the active agents for several antibiotics.

2. Materials and Methods

Sample Collection

The garlic and ginger was gotten from Bodee market along beree- new garage road in Ibadan. The plant samples collected were taken to the herbarium section in the department of Botany, University of Ibadan for identification. They were identified as *Allium sativum* (Garlic) and *Zingiber officinale* (Ginger) respectively.

Identification of the test organisms

Phenotypic and biochemical tests were used to ascertain the test organisms. The biochemical tests included; Indole test, urease test, catalase test, lactase test, citrate utilization test and coagulase test.

Extraction of bioactive compounds in Ginger and Garlic

The fresh ginger rhizomes and garlic cloves were collected, cleaned, peeled, sliced and dried at room temperature. pieces of *Allium sativum* and *Zingiber officinale* were grinded to fine particles in isolated manner utilizing a suitable grinder (mortar and pestle with blender). 10 grams of the powder *Allium sativum* was weighed and macerated in 100 milliliters of both cold and warm distilled water (D.W) and ethanol. 10 grams of *Zingiber officinale* was also weighed and macerated in 100 milliliters of both cold and warm distilled water and ethanol. The containers were left at 25⁰C for 3 days (72 hours). After 72 hours, the suspensions were filtered and the filtrates (extracts) were delivered into sterile, clean containers with suitable labeling and kept at 4⁰C until used for additional assay.

Production of discs (disks of the extracts)

Whatmann filter paper Discs of 5 mm in diameter were autoclaved in order to sterilize the disks (adjusting the conditions of autoclave to be 121⁰C for 15 min and left to become cold. The discs were allowed to suck up the extract filtrate and maintained for later assay. The produced discs (each one) have the ability to absorb about 0.01ml

Antimicrobial susceptibility test by Kirby-Bauer method

The antibiotics susceptibility was done by Kirby-Bauer method (Kirby and Baucer 1996) using Whatmann filter paper. The organisms under the experiment were evaluated for their susceptibility toward some antimicrobials including: Erythromycin (15µg), Meropenem (10µg), Gentamicin (30µg), Cefuroxime sodium (30µg), Ceftazidime (30µg), Ciprofloxacin (5µg) and Amoxycillin Clavulanic (30 µg) again by disc diffusion procedure. The cultures of test organisms were reactivated by culturing in sterile nutrient agar for 16 hrs at 37⁰C. Turbidity comparison was done against McFarland turbidity standard, sterile inoculating loop were used to transfer the bacterial cultures aseptically and swabbed over nutrient agar petri dishes. A sterilized forceps was used to fix the antibiotic disc aseptically over the cultured petri dishes incubated at 37⁰C for 20-22 hours and subsequently all diameters of inhibition zones were determined.

Phytochemical Analysis

Phytochemical tests were carried out using standard procedures to identify the constituents according (Edeoja *et al.*, 2005).

Test for Tannins

15g of the dried powdered samples was boiled in 20 ml of water in a test tube and then filtered. Two (2) drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black colouration.

Test for Saponin

2g of the powdered sample was boiled in 20 ml of distilled water in a water bath and filtered. 10 ml of the filtrate was mixed with 5 ml of distilled water and swirled vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously, then observed for the formation of emulsion.

Test for Flavonoids

A portion of the powdered plant sample was heated with 10 ml of ethyl acetate over a steam bath for 3 min. The mixture was filtered and 4 ml of the filtrate was shaken with 1 ml of dilute ammonia solution. A yellow colouration was observed, indicating a positive test for flavonoids.

Test for Steroids

Two ml of acetic anhydride was added to 0.5 g ethanolic extract of each sample with 2 ml H₂SO₄. The colour changed from violet to blue, indicating the presence of steroids.

Test for Terpenoids (Salkowski test)

4ml of the extract was mixed in 2 ml of chloroform, and concentrated H₂SO₄ (3 ml) was carefully added to form a layer. A reddish brown colouration of the inter face was formed to show positive results for the presence of terpenoids.

Test for Cardiac Glycosides (Keller – Killiani test)

5 ml of the extracts was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underlayered with 1 ml of concentrated sulphuric acid. A brown ring at the interface indicates a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer.

Test for Anthraquinones

0.5 g of the extract was boiled with 10 ml H₂SO₄ and filtered while hot. The filtrate was shaken with 5 ml of chloroform. The chloroform layer was pipette into another test tube and 1 ml of dilute ammonia was added. The resulting solution was observed for colour change.

Test for Alkaloids

0.5 g of the powdered extracts was stirred in 5 ml of 1% HCl solution on a steam bath for 5 mins. The mixture was then filtered using Whatman’s No1 filter paper. To the filtrate, 2-4 drops of Dragendoff’s reagent was added to 1 ml of the filtrate. An orange colour was observed indicating the presence of alkaloids.

3. Results

Table 1: Biochemical Test result

Isolates	Gram staining	Urease	Indole	Oxidase test	Lactose fermentation	Catalase
<i>Pseudomonas aueruginosa</i>	-	-	-	+	AG	+
<i>Klebsiella pneumonia</i>	-	-	-	-	AG	+
<i>Staphylococcus aureus</i>	+	+	-	-	AG	+

KEY: AG; Acid and Gas production; - negative; + positive

Table 2: Phytochemical screening test for garlic

COMPOUND	TEST
Anthroquinone	-ve
Glycosides	-ve
Cardiac glycosides	+ve
Steriods	-ve
Flavonoids	-ve
Tannis	-ve
Terpernoides	-ve

KEY: + Present - Absent

Table 3: Phytochemical screening test for Ginger

COMPOUND	TEST
Saponins	+ve
Glycosides	+ve
Akaloids	+ve
Steriods	-ve
Flavonoids	+ve
Tannis	+ve
Terpernoides	+ve

KEY: + Present - Absent

Table 4: Antibiotic susceptibility test using antibiotic disc

	Erythromycin (mm)	Meropenem (mm)	Cefuroxime sodium (mm)	Gentamicin (mm)	Ceftazidime (mm)	Ciprofloxacin (mm)	Amoxicillin Clavulanic (mm)
<i>Klebsiella pneumoniae</i>	- (R)	-(R)	-(R)	18(S)	-(R)	35(S)	-(R)
<i>Staphylococcus aureus</i>	18(S)	-(R)	-(R)	18(S)	20(S)	22(S)	18(S)
<i>Pseudomona aeruginosa</i>	-(R)	-(R)	-(R)	24(S)	-(R)	28(S)	-(R)

KEY : <15mm = Resistance(R); >15= Sensitive (S)

Table 5: Antimicrobial activity of the plant extracts against *Pseudomonas aeruginosa*

Plant	10 ⁻¹	10 ⁻²	10 ⁻³	10 ⁻⁴	10 ⁻⁵	10 ⁻⁶	10 ⁻⁷
Extracts							
A	18(S)	17(S)	13(S)	8(S)	3(R)	(R)	(R)
B	17(R)	13(R)	10R	2R	R	R	R
C	51(S)	49(S)	45(S)	34(S)	27(S)	21(S)	15(S)
D	22(S)	18(S)	12(R)	10(R)	7(R)	5(R)	(R)
E	20(S)	18(S)	14(R)	10(S)	7(R)	5(R)	1(R)
F	59(S)	54(S)	50(S)	44(S)	39(S)	30(S)	22(S)
G	33(S)	27(S)	22(S)	19(S)	12(R)	10(R)	6(R)
H	18(S)	15(S)	10(R)	7(R)	4(R)	2(R)	R

Key points: A- Ginger + aqueous (Cold) extract, B- Ginger aqueous (warm) water, C- Ginger + ethanol, D- Garlic + aqueous (cold) water, E- Garlic + aqueous (warm) water, F- Garlic + ethanol G- Ginger + Garlic (cold) water, H- Ginger + Garlic ethanol

Key : <15 = Resistance(R); >15= Sensitive (S)

Table 6: Antimicrobial activities of plants extracts against *Staphylococcus aureus*

Plant	Zone of inhibition (mm)						
Extracts	10 ⁻¹	10 ⁻²	10 ⁻³	10 ⁻⁴	10 ⁻⁵	10 ⁻⁶	10 ⁻⁷
A	31(S)	26(S)	22(S)	19(S)	11(R)	5(R)	1(R)
B	R	R	R	R	R	R	R
C	44(S)	40(S)	35(S)	31(S)	28(S)	24(S)	19(S)
D	22(S)	18(S)	12	9(R)	6(R)	3(R)	1(S)
E	27(S)	21(S)	15(R)	10(S)	8(R)	5(R)	2(R)
F	32(S)	27(S)	22(S)	17(S)	14(R)	9(R)	5.5(R)
G	R	R	R	R	R	R	R
H	51(S)	48(S)	42(S)	37(S)	32(S)	24(S)	19(S)

Key points: A- Ginger + aqueous (Cold) water, B- Ginger aqueous (warm) water, C- Ginger + ethanol, D- Garlic + aqueous (cold) water, E- Garlic + aqueous (warm) water, F- Garlic + ethanol, G- Ginger + Garlic (cold) water, H- Ginger + Garlic ethanol

Key : <15 = Resistance(R); >15= Sensitive (S)

Table 7: Antimicrobial activities of plants extracts against *Klebsiella pneumoniae*

Plant Extracts	<i>Klebsiella pneumoniae</i> Zone of inhibition (mm)						
	10 ⁻¹	10 ⁻²	10 ⁻³	10 ⁻⁴	10 ⁻⁵	10 ⁻⁶	10 ⁻⁷
A	15(S)	11(R)	8(R)	6(R)	4(R)	(R)	(R)
B	13(R)	10(R)	6(R)	3(R)	R	R	R
C	19(S)	17(R)	11(R)	6(R)	2(R)	(R)	(R)
D	13(R)	9(R)	3(R)	(R)	(R)	(R)	(R)
E	18(S)	14(R)	8(R)	5(R)	(R)	(R)	(R)
F	26(S)	21(S)	18(S)	14(R)	10(R)	8(R)	6(R)
G	16(S)	14(R)	11(R)	5(R)	1(R)	R	R
H	19(S)	12(R)	10(R)	6(R)	R	R	R

Key points : A- Ginger + aqueous (Cold) water, B- Ginger aqueous (warm) water C- Ginger + ethanol extract, D- Garlic + aqueous (cold) water E- Garlic + aqueous (warm) water, F- Garlic + ethanol extract, G- Ginger + Garlic (cold) water, H- Ginger + Garlic ethanol

Key : <15 = Resistance(R); >15= Sensitive (S)

4. Discussion

Table 1 shows that *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, and *Staphylococcus aureus* are lactose fermenter, positive to catalase test and all negative to indole test. *Staphylococcus aureus* is the only gram-positive stained organism and the only organism that is urease positive while *Pseudomonas aeruginosa* is the only oxidase positive.

Table 2 reveals the Phytochemical screening test for Garlic. It showed that garlic is only positive to Cardiac glycosides while it was negative to Anthroquinone, Glycosides, Steriods, Flavonoids, Tannis and Terpernoides. However, ginger tested positive to all the phytochemicals of Anthroquinone, Glycosides, Steroids, Flavonoids, Tannis and Terpernoides which makes ginger to be more bio actively fortified, hence a reason it exhibits more antimicrobial potency. Table 3 is the result of phytochemical screening teste for ginger revealing the presence of Saponins, Glycosides, Akaloids, Flavonoids, Tannis and Terpernoides and the absence of steroids.

Tables 4 showed the antibiotics sensitivity test result with all the test organisms being susceptible to Gentamicin (18mm, 18mm and 24mm) for *Klebsiella pneumonia*, *Staphylococcus aureus* and *Pseudomona aeruginosa* respectively and Ciprofloxacin (35mm, 22mm and 28mm) for *Klebsiella pneumonia*, *Staphylococcus aureus* and *Pseudomona aeruginosa* accordingly hence Ciprofloxacin is the most potent of the synthetic antibiotics in the antibiotic sensitivity disc test with a zone of clearance (35 mm) against *Klebsiella pneumonia* followed by Gentamycin with highest zone of clearance (24 mm) against *Pseudomona aeruginosa*. The test organisms are resistant against Meropenem, Cefuroxime sodium and Ceftazidime with Erythromycin and Amoxycillin Clavulanic having weak resistance of 18mm against *Staphylococcus aureus*. Tables 5, 6 and 7 showed that ethanolic extracts of ginger and garlic are more potent than their cold and warm water extracts against tested organisms.

Table 5 is the test result for potency of ginger against tested *Pseudomonas aeruginosa* ranging from 18 mm zone of clearance in aqeous cold extract against 51mm in ethanolic extract but more susceptible to extracts of garlic in ethanol, this is in accordance with the studied carried out by Ravi *et al.* (2018) who posited that aqueous garlic extract is more potent than that of ginger extract when tested against certain pathogenic bacteria. Garlic and ginger extracts when used in combination also showed some level of potency against *Pseudomonas aeruginosa* although Tende (2015) posited that the consumption of the combination of the two plant pose a dangerous health effects on the kidney and liver of the consumers. On the other hand, the heated extracts of *Z.officinale* had no antimicrobial activity against the test organisms except for its ethanolic extract which is highly effective against all the test organisms. Table 6 showed the potency of the various extracts of ginger against *Staphylococcus aureus* with ethanolic ginger extract showing potency ranging from 44 mm in to 19mm zone of clearance across various dilutions while a combination of ginger and garlic extracts gave a higher potency with zones of clearance ranging from 55mm to 19 mm across the increasing dilution factors, this result is very much in accordance with the findings of Ankri, and Mirelman (1999); Ali *et.al*; 2008 and Al-Qattan *et.al.*, 2006 who also showed in their researches that ginger and garlic have some phytochemical and pharmacological anti- toxicological properties against pathogenic bacteria including *Staphylococcus aureus*. Table 7 also showed the potency of garlic and ginger extracts against *Klebsiella pneumonia* although none of the extracts (aqueous and ethanolic) showed any serious potency against *Klebsiella pneumonia* with the highest zone of clearance from the ethanolic garlic extract (26mm). This result has proven to be in accordance with the works of Uchida *et al.* (1975), Yusha'u *et al.* (2008), Mukhtar and Ghori (2012), Omoya (2012), and Akintobi *et al.* (2013) who have also reported that garlic and ginger preparations have been shown to exhibit a wide spectrum of

antibacterial activity against Gram-negative and Gram-positive bacteria including species of *Escherichia*, *Salmonella*, *Staphylococcus*, *Streptococcus*, *Klebsiella*, *Proteus*, *Bacillus*, and *Clostridium*. Acid-fast bacteria such as *Mycobacterium tuberculosis* are also reported to be sensitive to garlic. Sivam *et al.* (1997), Ankri and Mirelman (1999), Whitmore and Naidu (2000) while Ross *et al.* (2001) have also confirmed that they are not only effective against bacteria but also possess antiviral and antifungal activity. The result of this study has also proved that application of heat may have caused the denaturing of some bioactive compounds in the spices used, it is possible that the compounds in the extracts which could have given higher anti-microbial activity may have been evaporated during the evaporation and boiling processes of the extraction. This may have interfered with the efficacy of the plant extracts and possibly the outcome of the study with aqueous hot extracts, it can therefore be inferred that bioactive components of garlic and ginger are sensitive to heat and therefore should not be cooked when using them for medicinal purposes. According to Madkor *et al.*, 2010, the paste of commercial garlic showed antimicrobial activity only at 4 °C and 8 °C (about 1 log CFU/g reduction), while fresh ginger paste showed antimicrobial activity only at 8 °C indicating that antimicrobial activity of garlic and ginger are temperature dependent as also revealed by this study. Apart from temperature, it is also believed that, geographical location of a plant and seasonal variation of an area may have influence over the yield of medicinal plants. The antimicrobial activity of a plant is due to specific photochemical or essential oils present in it (Avato *et al.*, 2000). Also it is concluded that this spice are very useful in the body because of the active ingredients present in them, therefore it is recommended to be taken in homes and used as an alternative means of medicine, although in amount reasonable for the body this will help combat antibacterial resistances which is on the increase.

5. Conclusion

Pathogenic and opportunistic organisms abound around man, but nature has also provided natural herbs and spices in the same environment with man that can help control these harmful or potentially harmful organisms while still maintaining a healthy balance in the ecosystem. Treatment of infectious organisms in hospitals is mainly based on the application of synthetic antibiotics. The adverse effects of these synthetic antibiotics on human health increases the demand to search for potentially effective, healthy safer and natural control against pathogenic organisms. The plant extracts which proved to be potentially effective as (ginger (*Zingiber officinale*) and garlic (*Allium sativum*) can be used as natural alternative preventives to control and cure bacterial infections thereby avoiding health hazards of chemically antimicrobial agent applications.

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