

ISSN 3027-2513 (ONLINE)

ISSN 3027-169X (PRINT)

Volume 2 Number 2, March 2024 https://journals.journalsplace.org/index.php/Ekete

ANTIBACTERIAL ACTIVITY OF ZINGIBER OFFICINALE (GINGER) AGAINST SELECTED DRUG RESISTANT ORGANISMS (STAPHYLOCOCCUS AUREUS AND ESCHERICHIA COLI)

Onyekachi Fidelis Igwe^{1*}, Cynthia Ugochi Egbule¹, Chijioke Ugbala Ekpono²; Lilian Eze¹;

Julius Okemini Ikporo² Aja Monique Iheoma³ and Aniokete Ugonna Cassandra²

¹Department of Science Laboratory Technology, Akanu Ibiam Federal Polytechnic, Unwana; Ebonyi State.

²David Umahi Federal University of Health Sciences Uburu, Ebonyi State, Nigeria.

³Institute of Maternal and child health, University of Nigeria, Ituku-Ozalla Campus, Enugu state.

*Corresponding Author: igwe2612@gmail.com

Abstract

The aim of this research was to determine the antibacterial effects of the aqueous and ethanol extracts of Zingiber officinale (Ginger) against selected drug resistant organisms of Staphylococcus spp and Escherichia coli. We carried out antibacterial tests on the selected drug resistant organisms using paper disc diffusion by kirby-bauer disc diffusion method. Whiteman filter paper was cut into pieces of 9mm, sterilized with an autoclave and dipped into the different concentrations of the extracts that were serially diluted for 30minutes and placed on the pre-inoculated plates of the test organisms, incubated for 24hours after which zone of inhibition diameter (ZID) were measured in MM. The results indicated that 50mg/ml concentration and the control antibiotics (AUG) were effective against staphylococcus spp in the ethanol extract with ZID of 10 and 20.while 400mg/ml and control drug were effective against Escherichia coli for ethanol ginger extract with ZID of 11 and 10MM respectively. In aqueous ginger extract, 400mg/ml, 50mg/ml and control antibiotics showed zones of inhibition of 13, 10 and 11 respectively; 400mg/ml and control drug (AUG-30Mg) were 10 and 13 against Escherichia coli. The MIC was 50mg/ml for Staphylococcus aureus on both aqueous and ethanol extracts while 400mg/ml is both the MIC and MBC for Escherichia coli on both aqueous and ethanol extracts. Both the control and different concentrations of the extracts were active against the selected resistant organisms while some strains were still resistant to the extracts. The result showed that Ginger could be very effective as an antibacterial agent against drug resistant organisms of Staphylococcus aureus and Escherichia coli mostly at high concentration dosages and dependent on the type of drug resistant organism.

Keywords: Zingiber officinale, extracts, resistance, Staphylococcus aureus, Escherichia coli

Igwe et al. Antibacterial activity of *Zingiber officinale* (ginger) against selected drug resistant organisms (*Staphylococcus aureus and Escherichia coli*)

Introduction

Background to the study

Both misuse and overuse of antibiotics have been a leading cause of the evolution of antibiotics resistance mechanism amongst pathogenic bacteria. Bacteria have developed different methods to inhibit the effect of antibiotics (WHO, 2014). The resistance to antimicrobial agents can be natural, acquired, genetic, phenotypic or biological (WHO, 2015). Furthermore, resistance may be developed due to spontaneous mutation in genes, acquisition of plasmid or transposon, the physiological change in the state of bacterial cell or decreasing of the permeability of cell. Bacteria develop resistance in various ways such as enzymatic drug inactivation, drug target alteration and drug permeability reduction (Griffith *et al.*, 2012). Thus bacteria continue to grow in the presence of a given antimicrobial agent.

Recently, the list of various non-antibiotics approaches was done based on their antimicrobial activities in treatment and prevention of infectious diseases such as probiotic, bacteriophage and phytomedicine (Yu, 2011). Moreover, plants are not in introductory phase in the history of medicine, they have been used as medicines for long and are known to be the good cure of various infectious and non-infectious diseases worldwide (Goossens, 2009). According to the report of WHO, nearly 80 percent of the world population depend primarily on traditional medicine which influence the high involvement of the use of both plants extracts and their active substances. Ginger extracts is one of those medicinal plants that played a huge role in treatment of different diseases. Ginger has strong properties that kill or inhibit the growth of pathogenic bacteria; and previously it was seen that active components of ginger inhibit multiplication of digestive bacteria (Pakyz *et al.*, 2008).

Ginger (Zingiber officinale), because of its antimicrobial ability against different microbial pathogens, it has been used as naturopathy (Tacconelli, 2009). Ginger has been against number of pathogenic microorganisms in its natural state, without transforming it which is a proof of using it as a strong medicine in treatment of infectious diseases (WHO, 2014). Ginger belongs to Zingiberaceae family, the Zingiberaceous plants are naturally with strong aromatic and medicinal properties and are characterized by their tuberous or non-tuberous rhizomes. Ginger is available and accessible at low cost for everyone to use it, it is universally acceptable and well tolerated by most people (Griffith et al., 2012). Ginger has both gingerols and shogaols that are rich in antimicrobial effects, helps in resolving stomach infection and other health outcomes. It has strong antimicrobial properties and active constituents of ginger inhibit the replication of colon bacteria. It inhibits the growth of Escherichia coli, Staphylococcus spp., and more (WHO, 2015). Otit mentioned that the antimicrobial activity of ginger may be due to phenolic compounds content (Yu, 2011). Traditionally, Ginger was used in treatment of intestinal infections, especially those related to digestive health outcomes. It is against this background that this study assessed the antibacterial activity of ginger against selected resistant bacteria strains of Staphylococcus aureus and Escherichia coli. Antibiotic resistance is one of the biggest epidemic to global health especially in low and middle income world. Drugs resistance is a leading cause of higher costs, prolonged hospital stays and increase mortality (Griffith et al., 2012). Number of studies have been carried out on plant medicines aimed to show the ability of plants to fight against bacterial infections but few studies has been done on the effect of ginger on drug resistant organisms. Even though number of studies have been done, their findings and recommendations were not taking into consideration by policy makers to facilitate easy accessibility of health care in low and middle income countries

Resistance: A Growing Global Concern

The discovery of antibiotics once heralded victory in the battle against infectious diseases. However, the emergence of antimicrobial resistance has escalated the war in favor of bacteria. Infectious diseases remain significant contributors to morbidity and mortality worldwide, with lower respiratory infections, diarrheal diseases, HIV/AIDS, and malaria among the top contributors (WHO, 2014). The misuse and overuse of antimicrobial agents have exacerbated the problem, leading to resistance shortly after the approval of new drugs (WHO, 2015).

Antimicrobial agents target various bacterial mechanisms, including cell wall synthesis, cell membrane depolarization, protein synthesis, nucleic acid synthesis, and metabolic pathways. However, improper stewardship, increased consumption, and improper prescription have contributed to widespread resistance. Overuse of antibiotics, driven by factors like low cost and low toxicity, has led to the emergence of resistant organisms (Griffith *et al.*, 2012; Yu, 2011).

The use of antibiotics in raising food animals has also contributed to the development of antimicrobial-resistant organisms. Evidence suggests that resistant organisms can be transferred to humans through consumption or direct contact with animals (Landers *et al.*, 2012). This escalating resistance has led to fewer treatment options, increased morbidity and mortality rates, prolonged illnesses, and heightened healthcare costs (CDC, 2013; Maragakis *et al.*, 2008). To combat resistance, various antimicrobial stewardship strategies have been proposed, including the use of diverse antimicrobial agents to minimize resistance selection (Pakyz *et al.*, 2008).

Persistence Versus Resistance

It is essential to differentiate between resistance and persistence in bacteria. While resistant bacteria possess resistance genes, persistent bacteria exhibit tolerance to antimicrobial agents without possessing resistance genes. This tolerance arises from the stationary growth phase of some bacterial cells, rendering them unaffected by antimicrobial agents (Wood *et al.*, 2013).

Origins of Resistance

Bacteria exhibit varying levels of susceptibility and resistance to antimicrobial agents. Natural resistance may be intrinsic or induced, with mechanisms such as reduced permeability of cell membranes and efflux pumps playing crucial roles (Martinez, 2014). Acquired resistance, facilitated by horizontal gene transfer and mutations, further complicates the issue (Coculescu, 2009).

Mechanisms of Antimicrobial Resistance

Antimicrobial resistance mechanisms include limiting drug uptake, modifying drug targets, inactivating drugs, and active drug efflux. Gram-negative bacteria predominantly utilize all four mechanisms, while gram-positive bacteria exhibit variations due to structural differences (Mahon, 2014).

Limiting drug uptake involves structural barriers such as the outer membrane in gram-negative bacteria, preventing the entry of certain antimicrobial agents. Biofilm formation further protects bacteria from antimicrobial agents and facilitates horizontal gene transfer (Mah, 2012).

Igwe et al. Antibacterial activity of *Zingiber officinale* (ginger) against selected drug resistant organisms (*Staphylococcus aureus and Escherichia coli*)

Modification of drug targets, such as alterations in penicillin-binding proteins, reduces drug efficacy. Bacteria may also inactivate drugs through hydrolysis or chemical group transfer, primarily using enzymes like β -lactamases (Blair et al., 2015).

Beta-lactamases and Drug Efflux Mechanisms in Bacterial Resistance:

 β -lactam drugs constitute a vital class of antimicrobial agents, whose efficacy is increasingly compromised due to bacterial resistance mechanisms, notably β -lactamases and drug efflux pumps. Understanding these mechanisms is crucial for combating antimicrobial resistance.

B-lactamases:

 β -lactamases, including penicillinases and cephalosporinases, mediate resistance by hydrolyzing the β -lactam ring of antibiotics, rendering them ineffective. These enzymes are widespread and represent the primary mechanism of resistance in gram-negative bacteria, posing significant challenges in treating infections.

Classification of β-lactamases:

Structurally, β -lactamases are categorized into four groups (A, B, C, D), while functionally, they are grouped based on substrate specificity into cephalosporinases, serine β -lactamases, and metallo β -lactamases. These enzymes, often carried on plasmids, contribute to multidrug resistance in various bacterial species.

Carbapenemases:

Emergence of carbapenemases, such as Klebsiella pneumoniae carbapenemases (KPCs) and Carbapenem-Resistant Enterobacteriaceae (CRE) enzymes, poses a grave threat. While KPCs are serine β -lactamases, CRE enzymes are metallo- β -lactamases, resistant to β -lactam drugs and challenging to inhibit.

Efforts in Combating Resistance:

Efforts to combat resistance include developing novel β -lactamase inhibitor combinations, like ceftolozane/tazobactam. However, metallo- β -lactamases present a formidable challenge due to their structural diversity and resistance to inhibitors.

Drug Efflux Mechanisms:

Bacteria employ efflux pumps to extrude antibiotics, limiting their intracellular accumulation. Five main families of efflux pumps, like RND pumps, are implicated in multidrug resistance. These pumps, found in both gram-negative and gram-positive bacteria, contribute to the broad-spectrum resistance observed. Antibiotic resistance mediated by β -lactamases and efflux pumps poses a significant threat to public health. Addressing this challenge requires a multifaceted approach, including the development of novel inhibitors and alternative therapeutic strategies. Understanding these mechanisms is paramount in mitigating the spread of resistant bacteria and preserving the efficacy of existing antibiotics.

Medical Importance of Ginger:

Ginger, a commonly used spice worldwide, has garnered significant attention for its diverse medicinal properties. This paper aims to provide a succinct overview of the multifaceted health benefits of ginger, supported by scientific evidence **Anti-diabetic Activity:** With diabetes emerging as a global health concern, ginger offers potential as a therapeutic agent in glycemic control. Research suggests that ginger may help lower fasting blood glucose levels and mitigate diabetic complications through its anti-hyperglycemic effects (Maiti *et al.*, 2004).

Neuroprotective Effect: Ginger demonstrates neuroprotective properties attributed to its phenolic and flavonoid compounds. Studies indicate its potential in safeguarding against neurodegenerative conditions and ischemic injuries by modulating antioxidant defense mechanisms (Shanmugam *et al.*, 2011; Sharma *et al.*, 2011).

Effect on Osteoarthritis: In the realm of musculoskeletal health, ginger emerges as a promising adjunctive therapy for osteoarthritis. Clinical trials have shown significant symptom relief and comparable efficacy to conventional anti-inflammatory drugs, with fewer adverse effects (Haghighi *et al.*, 2006).

Gastroprotective Effect: Ginger's gastroprotective properties are attributed to its ability to increase mucin secretion and suppress gastric contractions. These mechanisms contribute to its efficacy in preventing peptic ulcers, offering a natural alternative to conventional treatments (Bhattarai *et al.*, 2001).

Anti-emetic Effect: Ginger's antiemetic effects make it a valuable remedy for nausea and vomiting, particularly in postoperative settings. Its constituents exhibit serotonin receptor antagonism, providing relief from these distressing symptoms (Bhattarai *et al.*, 2001).

Hepato-protective Effect: Studies highlight ginger's protective effects against hepatotoxicity induced by various toxins, including chemicals and drugs. Its antioxidant properties contribute to liver health and may mitigate liver damage in diverse pathological conditions (Patrick *et al.*, 2007; Li *et al.*, 2012).

Effect on Migraine and Eye Health: Ginger shows promise in alleviating migraine symptoms and managing complications of diabetes, such as retinopathy. Experimental evidence suggests its potential in reducing the severity and frequency of migraine attacks, as well as inhibiting the formation of advanced glycation end products in the eyes (Saraswat *et al.*, 2009).

Safety, Efficacy, and Toxicity: Extensive research supports the safety and efficacy of ginger, with recommended dosages exhibiting minimal adverse effects. Animal studies have established tolerable dose ranges, ensuring its suitability for long-term use in health management (Weidner *et al.*, 2001; Rong *et al.*, 2009).

Anti-microbial Activity: Ginger and its constituents exhibit potent antimicrobial properties against various pathogens, including *Escherichia coli*, *Salmonella typhi*, *Bacillus subtilis*, and *Candida albicans*. Studies have highlighted the efficacy of ginger extracts in inhibiting microbial growth, making it a promising natural alternative to antibiotics (Atai *et al.*, 2009; Ficker *et al.*, 2003). With the discovery of antibiotics, the healthcare community thought that the battle with infectious diseases was won. However, now that so many bacteria have become resistant to multiple antimicrobial agents, the war has seemingly escalated in favor of the bacteria.

Aim of the Study

The purpose of this study was to determine the antibacterial effects of the ethanolic and aqueous extracts of ginger against selected drug resistant strains of *staphylococcus spp* and *Escherichia coli*.

Objectives of the study

The specific objectives are to:

- **Igwe et al.** Antibacterial activity of *Zingiber officinale* (ginger) against selected drug resistant organisms (*Staphylococcus aureus and Escherichia coli*)
 - A. Collect and identify the selected drug resistant strains of *Staphylococcus spp* and *Escherichia coli*.
 - B. Carry out the aqueous and ethanol extraction of Ginger
 - C. Determine the antibacterial effects of the aqueous and ethanol extracts of Ginger on the selected drug resistant strains.
 - D. Determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the extracts on the selected drug resistant strains of *Staphylococcus spp and Escherichia coli*

Scope of the Study

This study involved the collection of selected drug resistant strains of *Staphylococcus spp* and *Escherichia coli* from Romic hospital Afikpo, aqueous and ethanol extraction of Ginger, determination of the antibacterial effects of the extracts on the selected drug resistant strains of *Staphylococcus aureus* and *Escherichia coli*, determination of their MIC and MBC.

Justification of the Study

The rate of Multi-drug resistance of bacteria against most antibiotics used for treatment of diseases is quite alarming. Antibiotics resistance has become a global challenge putting serious pressure on the available antibiotics and increasing the cost of health care and most time leads to serious morbidity and mortality. This is caused by over use of drugs, abuse, indiscriminate use of and prescription of antibiotics by clinicians, quacks and patients. This has given room to exploit opportunities to tackle this menace including the determination of the antibacterial effects of most herbal extracts against some drug resistant organisms.

Materials and Method

Materials

Chemicals

The following chemicals were used: hydrogen peroxide (Damazo Nig.), crystal violet, (Afis Biochemicals Nig), Ethanol (Mark, England) Ginger, and Distilled water.

Media

The culture media used includes: Mannitol salt agar, MacConkey agar and Muller-Hinton agar (Oxoid Ltd, Basingstoke, Hampshire, England), Nutrient agar (Oxide Ltd., UK).

Equipment

The following equipment were used: refrigerator, (Harier thermocool Nig), Incubator, (Kankoid Germany), autoclave, hot air oven (Culfexmed, England), laminar flow chamber (Kankoid Germany) syringes, foil spatula, weighing balance, petri-dishes, masking tape, hand gloves, Bunsen burner, tripod stand, wire guaze, wire loop, gas cylinder, pressure pot, measuring cylinder, test tube, forceps, swab stick, microscope, slide, meter ruler (Graduated in mm).

Antibiotics Discs: This antibiotics disc was used Augmentin (30mg).

Methods

Collection and Preparation of the Aqueous and Ethanol Ginger extracts

The ginger rhizomes were bought at the local Eke market, Afikpo, transported directly to microbiology laboratory unit, Akanu Ibiam Federal Polytechnic Unwana; Peeled, washed, Chopped into pieces and pounded with sterilized mortar, weighed 50g and dissolved into 200ml of both distilled water and 95% ethanol respectively, stored for 72 hours, shake intermittently for proper dissolution, was filtered using cheese cloth, weighed with a yield of 2g ie 4% yield and

reconstituted with 5ml of DMSO to get 0.4g/ml multiplied by 1000 to get 400mg/ml concentration which was further diluted serially in two folds to get 200mg/ml,100mg/ml and 50mg/ml concentrations, respectively.

Collection, Identification and standardization of the test organisms

The resistant strains of both *Staphylococcus spp* and *E. coli* were collected from the laboratory unit of Romic hospital Afikpo, transported in a nutrient broth media directly to the microbiology laboratory of Akanu Ibiam Federal Polytechnic Unwana for identification and characterization through cultural and biochemical methods and were standardized to 0.5 mcfarland standard for antibacterial assays.

Media Preparation and Material Sterilization

The media such as macConky and mannitol salt agar were prepared according to manufacturer's instructions, sterilized with an autoclave at 121°C for 15minutes, was aseptically poured into petridishes and allowed to cool before streaking to obtain pure culture of the isolates.

Preparation of Mueller-Hinton Agar: 3.8g of Mueller-Hinton agar was weighed and dissolved in 100ml of distilled water, autoclaved for 15mins, 121c and was allowed to cool to the temperature of 40°C, 20ml of the agar was aseptically poured into different petri-dishes for antibacterial assay.

Determination of the Antibacterial Activity of the Extract

The extracts were serially diluted to obtain 1.0%, 0.5%, 0.25%, and 0.125% solutions in sterile test tubes. Sterilized 9mm filter paper disc soaked in the diluted extracts were placed on the plates and incubated for 24hours at room temperature. The plates were examined for clear zones of inhibition. Presence of zones of inhibition indicated activity. The zones were measured in mm using a meter rule while the MIC and MBC were also determined accordingly.

Determination of the Minimum Inhibitory Concentrations (MIC)

The MIC was determined using tube dilution technique according to (Cheesebrough, 200).

The MIC was determined by taking 2ml of the concentrations of the extracts that inhibited the growth of the resistant strains and mixing it with 2ml of the nutrient broth containing 0.1ml of standardized inoculum of 0.5 Mcfarland standard, the tubes were incubated for 24hrs at 37°c.it has a tube containing broth and inoculum without the extract as the control. The lowest concentration that showed no visible turbidity (inhibited microbial growth) is regarded as the MIC.

Determination of the MBC

Sterile Mueller hinton agar plates were incubated with samples from each of the test tubes that showed no visible growth from the MIC test. The plates were then incubated at 37°c for 24hrs. The lowest concentration of the extract yielding no growth was recorded as the minimum bactericidal concentration (MBC)

Results

The observations from the antibacterial studies of both aqueous and ethanol extracts of ginger on resistant strains of *Staphylococcus aureus* and *Escherichia coli* were summarized in the following tables:

Table 4.0: Zone of Inhibition Diameter (ZID) in mm of different concentrations of ethanol and aqueous ginger extracts.

Ginger ethanol

Igwe et al. Antibacterial activity of *Zingiber officinale* (ginger) against selected drug resistant organisms (*Staphylococcus aureus and Escherichia coli*)

Organisms/co	400mg/ml	200mg/ml	100mg/ml	50mg/ml	Control
ncentrations					
S. aureus	0	0	0	10	20
E. coli	11	0	0	0	10

Aqueous Ginger

Organisms/co	400mg/ml	200mg/ml	100mg/ml	50mg/ml	Control
ncentrations					
Staph	13	0	0	10	11
E.coli	10	0	0	0	13

Table 4.1: Resistance and susceptibility of the selected resistant strains of the organisms against different concentrations of the ginger extracts.

Organisms/concentration	400mg/ml	200mg/ml	100mg/ml	50mg/ml	Control	Ethanol ginger
Staphylococcus spp	+	+	+	-	-	
E.coli	-	+	+	+	-	
						Aqueous
						Ginger
Staphylococcus spp	-	+	+	-	-	
E.coli	_	+	+	+	-	

Keys: - = inhibition or susceptibility

+= no inhibition or resistance

MIC=50mg/ml for *Staphylococcus spp in* both ethanol and aqueous ginger extracts.

MBC=50mg/ml for same organisms

MIC=400mg/ml for *E.coli for both ethanol and aqueous extracts of Ginger*

Table 4.2

Descriptive analysis for the zone of inhibition for different doses of ethanolic extract of Zingiber officinale (EEZO)

		N	Mean	Std. Deviation	Std. Error
	Control	2	10.0000	.02828	.02000
	50mg/ml	2	.0000	.00000	.00000
E. coli	100mg/ml	2	.0000	.00000	.00000
	200mg/ml	2	.0000	.00000	.00000
	400mg/ml	2	11.0000	.01414	.01000

	Total	10	4.2000	5.43242	1.71788
	Control	2	20.0000	1.41421	1.00000
	50mg/ml	2	10.0000	.14142	.10000
C4 1-	100mg/ml	2	.0000	.00000	.00000
Staph	200mg/ml	2	.0000	.00000	.00000
	400mg/ml	2	.0000	.00000	.00000
	Total	10	6.0000	8.44604	2.67087

Table4.3 **ANOVA table (Post Hoc Tests)**

Multiple Comparisons

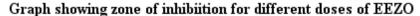
Dunnett t (2-sided)^a

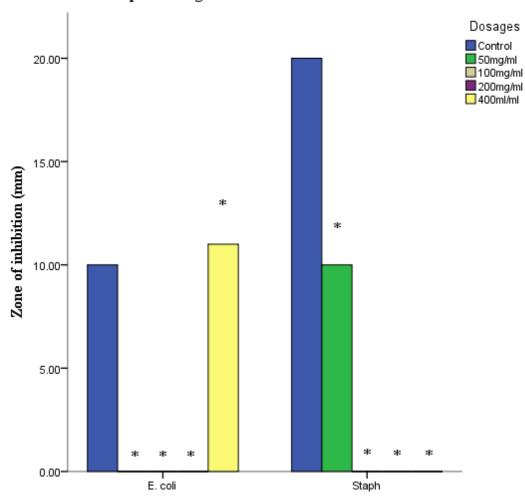
Dependent Var	riable(I) Dosages	(J) Dosages	Mean Difference (I-	Std. Error	Sig.
			J)		
	50mg/ml	Control	-10.00000*	.01414	.000
E soli	100 mg/ml	Control	-10.00000*	.01414	.000
E. coli	200 mg/ml	Control	-10.00000*	.01414	.000
	400 mg/ml	Control	1.00000^*	.01414	.000
	50mg/ml	Control	-10.00000*	.63561	.000
Stoph	100 mg/ml	Control	-20.00000*	.63561	.000
Staph	200 mg/ml	Control	-20.00000*	.63561	.000
	400mg/ml	Control	-20.00000*	.63561	.000

^{*.} The mean difference is significant at the 0.05 level.

a. Dunnett t-tests treat one group as a control, and compare all other groups against it.

Igwe et al. Antibacterial activity of *Zingiber officinale* (ginger) against selected drug resistant organisms (*Staphylococcus aureus and Escherichia coli*)





Values are expressed as mean \pm SEM, n = 2.

Table 4.4

Descriptive analysis for the zone of inhibition for different doses of aqueous extract of Zingiber officinale (AEZO)

		N	Mean	Std. Deviation	Std. Error
	Control	2	13.0000	.05657	.04000
	50mg/ml	2	.0000	.00000	.00000
E. coli	100mg/ml	2	.0000	.00000	.00000
E. Con	200mg/ml	2	.0000	.00000	.00000
	400mg/ml	2	10.0000	.07071	.05000
	Total	10	4.6000	6.02226	1.90440
Staph	Control	2	11.0000	1.41421	1.00000

^{* =} significantly different from control at P < .05

50mg/ml	2	10.0000	.02828	.02000
100mg/ml	2	.0000	.00000	.00000
200mg/ml	2	.0000	.00000	.00000
400mg/ml	2	13.0000	.70711	.50000
Total	10	6.8000	5.96379	1.88592

Table 4.5 **ANOVA table (Post Hoc Tests) Multiple Comparisons**Dunnett t (2-sided)^a

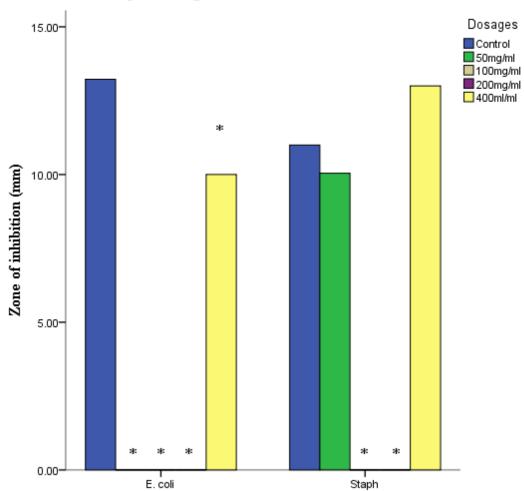
Dependent Variabl	e(I) Dosages	(J) Dosages		Std. Error	Sig.
			Difference (I- J)		
	50mg/ml	Control	-13.00000*	.04050	.000
E sali	100 mg/ml	Control	-13.00000*	.04050	.000
E. coli	200 mg/ml	Control	-13.00000*	.04050	.000
	400mg/ml 50mg/ml	Control Control	-3.00000* -1.00000	.04050 .70722	.000 .494
C41-	100mg/ml	Control	-11.00000*	.70722	.000
Staph	200mg/ml	Control	-11.00000*	.70722	.000
	400mg/ml	Control	2.00000	.70722	.100

^{*.} The mean difference is significant at the 0.05 level.

a. Dunnett t-tests treat one group as a control, and compare all other groups against it.

Igwe et al. Antibacterial activity of *Zingiber officinale* (ginger) against selected drug resistant organisms (*Staphylococcus aureus and Escherichia coli*)





Values are expressed as mean \pm SEM, n = 2. * = significantly different from control at P < .05

Discussion, Conclusion and Recommendation

The results indicated that 50mg/ml concentration and the control antibiotics were effective against *staphylococcus* spp and 400mg/ml and control drug were effective against *Escherichia coli* for ethanol ginger extract. In aqueous ginger extract, 400mg/ml, 50mg/ml and control antibiotics showed zones of inhibition of 13, 10 and 11 respectively; 400mg/ml and control drug (AUG-30Mg) were 10 and 13 against *E.coli*. *The* MIC was 50mg/ml for *Staphylococcus aureus* on both aqueous and ethanol extracts while 400mg/ml is both the MIC and MBC for *E. coli* on both aqueous and ethanol extracts respectively. Both the control and different concentrations of the extracts were active against the selected resistant organisms while some strains were still resistant to the extracts. The result showed that Ginger could be very effective as an antibacterial agent against drug resistant organisms of *Staphylococcus aureus* and *Escherichia coli* mostly at a high concentration dosages and dependent on the type of drug resistant organisms.

Our results were in agreement with the work of Emmanuel *et al.*, 2021 in which the ethanol, methanol and aqueous extracts of zingiber officinale and allium sativum inhibited the growth of clinical isolates

Of Staphylococcus aureus, Escherichia coli, pseudomonas aeruginosa, Salmonella typhi. it indicated that fresh ethanol and aqueous extracts of Ginger are broad spectrum antibacterial agents which is also in tandem to the work of (Gull et al., 2012) where their fresh extracts of ginger and garlic also had broad spectrum activity on the clinical isolates of S. aureus, E.coli, Pseudomonas spp.

Different concentrations of the aqueous extracts inhibited the resistant Staphylococcus strains which also agreed with the work of Gull *et al.*, 2012 that have all the concentrations inhibiting the growth of their isolates.

Table 1.0 revealed that the highest concentration of the aqueous extracts that inhibits the growth of the resistant strains of staphylococcus is 50mg/ml while 400mg/ml is the highest concentration that inhibited the growth of *Escherichia coli* which proved that ethanol extracts of ginger inhibited the drug resistant strain of *Staphylococcus aureus* only at that concentration of 50mg/ml which differs with the work of Emmanuel *et al.*,2021 where different concentrations of the ginger had inhibitory effects on *Staphylococcus aureus*.

We compared the inhibition zones of both extracts and discovered that aqueous extract of ginger exhibited a better activity against the test organisms than the ethanol extract as observed from table 1.0. which implies that distilled water is a better extraction agent than ethanol for the bioactive compounds in ginger. it was also observed that all the resistant strains were susceptible to control antibiotics of AUG, which suggests that *Augumentin* is better antibiotics for resistant strains of *Staphylococcus aureus* and *Escherichia coli*. From table 4.2, 4.3, 4.4 it was observed that resistant strain of *Staphylococcus aureus* was more susceptible to the extracts compare to the Escherichia coli., and all the concentrations of the extracts were significantly different in their activity to the control antibiotics as seen from the descriptive analysis figures in the results.

Conclusion

Antimicrobial resistance has become a global phenomenon and posed a serious health challenge to the world. The development of resistance to antibiotics by most resistant bacteria strains like *staphylococcus aureus* and *Escherichia coli* could be largely attributed to misuse and overuse of antibiotics by clinicians, quacks, patients, farmers, etc. This has reached to a zenith level of a possibility of causing crisis in the health sector, increase in cost of health care, and increase of morbidity and mortality hence the need to intensify effort in research to screen for phytomedicine or alternative medicine with less side effect but huge potential activity and effect against drug resistant organisms. Fresh ethanol and aqueous ginger extracts from our study has shown sufficient evidence to be effective against drug resistance strains of Staphylococcus aureus and *Escherichia coli* hence the need for more research on the antibacterial potentials of various products of ginger against drug resistant organisms.

Recommendation

From the findings of this research we will recommend the use of various products of ginger extracts in combating the menace of drug resistance organisms and will also suggest further studies on its phytochemical contents, further antibacterial studies with dry or powered ginger extracts against several other multidrug resistant organisms.

Igwe et al. Antibacterial activity of *Zingiber officinale* (ginger) against selected drug resistant organisms (*Staphylococcus aureus and Escherichia coli*)

References

- Ali B.H, Blunden G, Tanira M.O, Nemmar A. (2008). Some phytochemical, pharmacological and toxicological properties of ginger (Zingiber officinale Roscoe): a review of recent research. *Food Chem Toxicol*. 46:409–420.
- Al-Tahtawy R.H.M, El-Bastawesy A.M, Abdel Monem M.G, Zekry Z.K, Al-Mehdar H.A, El-Merzabani M.M. (2011). Antioxidant activity of the volatile oils of Zingiber officinale (ginger) Spatula DD. 1:1–8.
- Atai Z, Atapour M, Mohseni M. (2009). Inhibitory effect of Ginger Extract on Candida Albicans. *Am J Applied Sci.* 6:1067–1069.
- Beceiro A, Tomás M, Bou G. (2013). Antimicrobial resistance and virulence: a successful or deleterious association in the bacterial world? *Clin Microbiol Rev.* 26:185–230.
- Bellik Y. (2014). Total antioxidant activity and antimicrobial potency of the essential oil and oleoresin of Zingiber officinale Roscoe. *Asian Pac J Trop Dis.* 4:40–44.
- Bevan E.R, Jones A.M, Hawkey P.M. (2017). Global epidemiology of CTX-M β-lactamases: temporal and geographical shifts in genotype. *J Antimicrob Chemoth*. 72:2145–2155.
- Bhattarai S, Tran V.H, Duke C.C. (2001). The stability of gingerol and shogaol in aqueous solutions. *J Pharm Sci.* 90:1658–1664.
- Blair J.M, Richmond G.E, Piddock L.J. (2014). Multidrug efflux pumps in Gram-negative bacteria and their role in antibiotic resistance. *Future Microbiol*. 9:1165–1177.
- Chancey S.T, Zähner D, Stephens D.S. (2012). Acquired inducible antimicrobial resistance in Grampositive bacteria. *Future Microbiol*. 7:959–978.
- Choi Y.Y, Kim M.H, Hong J, Kim S, Yang W.M. (2013). Dried Ginger (Zingiber officinalis) Inhibits Inflammation in a Lipopolysaccharide-Induced Mouse Model. *Evid Based Complement Alternat Med.* 914563.
- Coculescu B.I. (2009). Antimicrobial resistance induced by genetic changes. J Med Life. 2:114–123.
- Costa S.S, Viveiros M, Amaral L. (2013). Multidrug efflux pumps in Staphylococcus aureus: an update. *Open Microbiol J.* 7:59–71.
- Emmanuel, S.E., Ehinmitan, E.O., Bodunde, R.S and Joseph, J.C.(2021). Antimicrobial Activity of Zingiber Officinale and Alium sativum on some Drug resistant bacterial isolates. *J.appl.Sci. Environ*.Manage.25:6, 1053-1058.
- Ficker C, Smith M.L, Akpagana K. (2003). Bioassay-guided isolation and identification of antifungal compounds from ginger. *J Phytother Res.* 17:897–903.
- Filice G.A, Nyman J.A, Lexau C. (2010). Excess costs and utilization associated with methicillin resistance for patients with Staphylococcus aureus infection. *Infect Cont Hosp Ep.* 31:365–373.
- Friedman N.D, Tomkin E, Carmeli Y. (2016). The negative impact of antibiotic resistance. *Clin Microbiol Infect*. 22:416–422.

- Goossens H. (2009). Antibiotic consumption and link to resistance. *Clin Microbiol Infec*. 15 3:12–15.
- Griffith M, Postelnick M, Scheetz M. (2012). Antimicrobial stewardship programs: methods of operation and suggested outcomes. *Expert Rev Anti-Infe*. 10:63–73.
- Gull, I., Saeed, M., Shaukat, H., Anslam, S.M., Samra, Z. Q and Atha, A. M(2012). Inhibitory effect of Allium sativum and Zingiber officinale extracts on clinically important drug resistant pathogenic bacteria. Annals of Clinically Microbio. *Antimicrobials*, 11:8:1-6.
- Habib S.H, Makpol S, Abdul Hamid N.A, Das S, Ngah W.Z, Yusof Y.A. (2008). Ginger Extract (Zingiber Officinale) has Anti-Cancer and Anti-Inflammatory Effects on Ethionine-Induced Hepatoma Rats. *Clinics*. 63:807–813.
- Haghighi A, Tavalaei N, Owlia M.B. (2006). Effects of ginger on primary knee osteoarthritis. *Indian J Rheumatol*. 1:3–7.
- Karna P, Chagani S, Gundala S.R, Rida P.C, Asif G, Sharma V, Gupta M.V, Aneja R. (2012). Benefits of whole ginger extract in prostate cancer. *Br J Nutr*. 107:473–84.
- Kourtesi C, Ball A.R, Huang Y.Y. (2013). Microbial efflux systems and inhibitors: approaches to drug discovery and the challenge of clinical implementation. *Open Microbiol J.* 7:34–52.
- Landers T.F, Cohen B, Wittum T.E. (2012). A review of antibiotic use in food animals: perspective, policy, and potential. *Public Health Rep.* 127:4–22.
- Li Y, Tran V.H, Duke C.C, Roufogalis B.D. (2012). Preventive and Protective Properties of Zingiber officinale (Ginger) in Diabetes Mellitus, Diabetic Complications, and Associated Lipid and Other Metabolic Disorders: A Brief Review. *Evid Based Complement Alternat Med*:516870.
- Ling H, Yang H, Tan S.H, Chui W.K, Chew E.H. (2010) 6-Shogaol, an active constituent of ginger, inhibits breast cancer cell invasion by reducing matrix metalloproteinase-9 expression via blockade of nuclear factor-κB activation. *Br J Pharm.* 161:1763–77.
- Liu Y, Whelan R.J, Pattnaik B.R, Ludwig K, Subudhi E, Rowland H, Claussen N, Zucker N, Uppal S, Kushner D.M, Felder M, Patankar M.S, Kapur A. (2012). Terpenoids from Zingiber officinale (Ginger) induce apoptosis in endometrial cancer cells through the activation of p53. *PLoS One*. 7:e53178.
- Mah T.F. (2012). Biofilm-specific antibiotic resistance. Future Microbiol. 7:1061–1072.
- Maiti R, Jana D, Das U.K, Ghosh D. (2004). Antidiabetic effect of aqueous extract of seed of Tamarindus indica in streptozotocin-induced diabetic rats. *J Ethnopharmacol*. 92:85–91.
- Maragakis L.L, Perencevich E.N, Cosgrove S.E. (2008). Clinical and economic burden of antimicrobial resistance. *Expert Rev Anti-Infe*. 6:751–763.
- Martinez J.L. (2014). General principles of antibiotic resistance in bacteria. *Drug Discov Today*. 11:33–39.
- Miller W.R, Munita J.M, Arias C.A. (2014). Mechanisms of antibiotic resistance in enterococci. *Expert Rev Anti-Infe*. 12:1221–1236.

- **Igwe et al.** Antibacterial activity of *Zingiber officinale* (ginger) against selected drug resistant organisms (*Staphylococcus aureus and Escherichia coli*)
- Pakyz A, Powell J.P, Harpe S.E. (2008). Diversity of antimicrobial use and resistance in 42 hospitals in the United States. *Pharmacotherapy*. 28:906–912.
- Putman M, van Veen H.W, Konings W.N. (2000). Molecular properties of bacterial multidrug transporters. *Microbiol Mol Biol Rev*. 64:672–693.
- Rahmani A.H, Albutti A.S, Aly S.M. (2014). Therapeutics role of olive fruits/oil in the prevention of diseases via modulation of anti-oxidant, anti-tumour and genetic activity. *Int J Clin Exp Med*. 7:799–808.
- Redgrave L.S, Sutton S.B, Webber M.A. (2014). Fluoroquinolone resistance: mechanisms, impact on bacteria, and role in evolutionary success. *Trends Microbiol*. 22:438–445.
- Rong X, Peng G, Suzuki T, Yang Q, Yamahara J, Li Y. (2009). A 35-day gavage safety assessment of ginger in rats. *Regul Toxicol Pharmacol*. 54:118–23.
- Saraswat M, Reddy P.Y, Muthenna P, Reddy G.B. (2009). Prevention of non-enzymic glycation of proteins by dietary agents: prospects for alleviating diabetic complications. *Brit J Nutr*. 1011714–1721.
- Shyur L.F., Tsung J.H, Chen J.H, Chiu C.Y, Lo C.P. (2005). Antioxidant Properties of Extracts from Medicinal Plants Popularly Used in Taiwan Oxidatative stress play a significant effect in the pathogenesis of various types of disease. *Int J Appl Sci Eng.* 3:195–202.
- Tacconelli E. (2009). Antimicrobial use: risk driver of multidrug resistant microorganisms in healthcare settings. *Curr Opin Infect Dis*. 22:352–358.
- Tjendraputra E, Tran V.H, Biu-Brennan D, Roufogalis B.D, Duke C.C. (2001). Effect of ginger constituents and synthetic analogues on cyclooxygenase-2 enzyme in intact cells. *Bioorg Chem*. 29:156–163.
- Toth M, Antunes N.T, Stewart N.K. (2016). Class D β -lactamases do exist in Gram-positive bacteria. Nat *Chem Biol*. 12:9–14.
- Wegener H.C. (2012). Improving food safety through a One Health approach. Washington: National Academy of Sciences. Antibiotic resistance—linking human and animal health; pp. 331–349.
- Wood T.K, Knabel S.J, Kwan B.W. (2013). Bacterial persister cell formation and dormancy. *Appl Environ Microbiol*. 79:7116–7121.
- World Health Organization (2015). Global action plan on antimicrobial resistance. (2015).
- World Health Organization (2014). World Health Statistics
- Yu V.L. (2011) Guidelines for hospital-acquired pneumonia and health-care-associated pneumonia: a vulnerability, a pitfall, and a fatal flaw. *Lancet Infect Dis.* 11:248–252.
- Zhanel G.G., Lawson C.D., Adam H. (2013) Ceftazidime-Avibactam: a novel cephalosporin/β-lactamase inhibitor combination. Drugs. 73:159–177.
- Zick, S.M., Djuric, Z., Ruffin M.T, Litzinger A.J., Normolle D.P., Alrawi S, Feng M.R, Brenner D.E. (2008). Pharmacokinetics of 6-gingerol, 8-gingerol, 10-gingerol, and 6-shogaol and conjugate metabolites in healthy human subjects. *Cancer Epidemiol Biomarkers Prev.* 17:1930–1936.