

## **Bactericidal Property of *Zingiber officinale* (Ginger) Extract Against *Staphylococcus aureus*, Methicillin Resistant *Staphylococcus aureus* (MRSA), *Escherichia coli*, and Extended Spectrum Beta Lactamase *Escherichia coli* (ESBLEC)**

**Alfredo V. Corpuz (Project Leader), Mercita Q. Queddeng, Amante P. Cabatu, Jr., and Gerard Gabriel P. Reotutar**  
University of Northern Philippines  
Vigan City, Philippines

### **ABSTRACT**

The researchers proved in this study that *Zingiber officinale* (Ginger) has bactericidal property. An ethanolic extract of ginger was confirmed effective against four test organisms – two (2) drug resistant and two non drug resistant bacteria. Thus, it is remarkable to recognize the potential use of the ginger extract in treating infections caused by *Staphylococcus aureus*, *Escherichia coli*, Methicillin Resistant *Staphylococcus aureus*, and Extended Spectrum Beta Lactamase *Escherichia coli*.

This study determined the efficacy of the ethanolic extract of ginger in inhibiting the growth of four bacteria. There were 72 plates included in the study, of which only 60 were considered valid. Treatments consist of the different concentrations of the ginger extract (150mg/mL, 100mg/mL, 50mg/mL and 25mg/mL). The zone of inhibition is the measure used in determining the growth inhibiting effect of the different treatments.

The results of the study strongly show that ginger extract can inhibit the growth of the four test organisms. Although it is apparent that the ginger extract was not as effective as the positive control of each of the four test organisms, the mean zones of inhibition of the ginger extract (particularly 150mg/mL, 100mg/mL and 50mg/mL concentrations) were statistically significant when compared to that of distilled water. Thus, all three concentrations inhibited the growth of the four test organisms. The 25mg/mL extract did not exhibit antibacterial activity against *S aureus* and MRSA because it may have been very diluted that it lost its inhibitory capacity.

**Keywords:** Bactericidal, *Zingiber officinale*, Methycillin Resistant *Staphylococcus aureus*, Extended Spectrum Beta Lactamase *Escherichia coli*

### **Introduction**

Since the beginning of civilization, plants have been a valuable source of natural products for maintaining human health, especially in the last decade, with more intensive studies for natural therapies. The use of plant compounds for pharmaceutical purposes has gradually increased. According to the World Health Organization (WHO), medicinal plants would be the best source to obtain a variety of drugs. Likewise, Nascimento et al. (2005) said that about 80% of individuals from developing countries use traditional medicine, which has compounds derived from medicinal plants. Therefore, such plants are to be subjected to laboratory experimentation to better understand their properties, safety, and efficiency.

The use of plant extracts and phytochemicals, both with known antimicrobial properties, can be of great significance in healing treatments. In the last few years, several studies have been conducted in different countries to prove such efficiency. Many plants are

utilized in healing because of their antimicrobial traits, which are due to compounds synthesized in the secondary metabolism of the plant (Bidlack et al. 2000).

On the other hand, the bacterial infection causes a high rate of mortality in the human population. Studies on the bacterial infection in the Philippines showed the high prevalence rate of Gram-negative and Gram-positive bacteria, causing community-acquired and healthcare associated infections. In a national prevalence survey of infections in hospitals in the Philippines, most of the diseases were associated with bacterial cases (Philippine Health Advisory, 2012). Together, these reports indicate that bacterial infections are considered to be an emerging health problem in the Philippines, affecting the general population.

Nowadays, the use of antibiotics increased significantly due to many bacterial infections. However, the pathogenic bacteria become resistant to drugs due to indiscriminate use of antibiotics lowering the drug's effectiveness and injurious to the host. Antibiotic-resistant bacteria pose a problem of giving treatment to several infectious diseases, resulting in the use of more expensive treatments over an extended period. Moreover, the cost of the drugs is high, and these drugs may cause adverse effects on the host, which include hypersensitivity and depletion of beneficial microbes in the gut. Other adverse effects of antibiotics include, but not limited to, nephrotoxicity, neuromuscular blockage, and ototoxicity for Aminoglycosides; aplastic anemia for Chloramphenicol; dysglycemia, orthopedic anomalies and retinopathies for Fluoroquinolones; myopathies for Ionophores; hepatitis for Isoniazid; reversible neuropathy and neoplasia for Metronidazole; and vestibular problems for Tetracyclines (Omar et al., 2012).

The rising incidence of antibiotic resistance among bacteria is alarming worldwide. Methicillin-Resistant *Staphylococcus aureus* (MRSA) has become a major problem in the hospital as well as in the community setting. MRSA is one of the most focal threats to public health due to the fast dissemination and diversification of MRSA strains with more virulence and anti-microbial resistance (Omar et al. 2012; Enright et al. 2002).

Moreover, another study (Nagba et al. 2012) on the antibiotic sensitivity profile of *Escherichia coli* in water samples from water hand pumps in Iligan City, Philippines showed the presence of antibiotic-resistant *E. coli* and 51% of the tested bacteria demonstrated multiple drug non-susceptible strains. The study further showed that children drinking water with greater than 1000 *E. coli* per 100 mL had significantly higher rates of diarrheal disease than those drinking less contaminated water. The sudden outbreak in multidrug resistant *Pseudomonas aeruginosa* in the neonatal intensive care unit of the Philippine General Hospital was also studied, and it showed that the use of antibiotics was associated with the risk of colonization and infection with multi-resistant bacteria. In another study on the antimicrobial resistance of pathogens from tracheal and endotracheal aspirates of patients with clinical manifestations of pneumonia in Bacolod City, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* were found to be the most frequent bacterial isolates (Juayang et al. 2015).

New classes of antibiotics are needed to combat this trend (Freile-Pelegrin & Morales, 2004). There is an increasing demand for therapeutic drugs from the vastly bio-diverse natural resources, because approximately 25% of prescribed pharmaceuticals are from plant sources. Plants are the main sources of biologically active compounds. Plants are always in contact with many groups of microorganisms, and they have evolved with chemical defense strategies

by synthesizing an array of secondary metabolites to defend themselves against the microbial threats. Plants have a broad range of biological activities such as antioxidant, antibacterial, antifungal, anti-inflammatory, anti-tumoral, and anti-viral. With plants having diverse biologically active compounds, examining the pharmacological and antimicrobial potentials of the metabolite compounds derived from plants can lead to the production of an alternative to antibiotics.

Ginger (*Zingiber officinale*) is a plant that belongs to the Zingiberaceae family. It is a perennial herb that develops a rhizome with a characteristic flavor and odor due to its volatile oil content. Zingerole, shogaols, and gingerol are the components of this volatile oil and the latter gives the plant its major pungent element (Girhepunje, 2006).

Studies have shown that Ginger has antibacterial activity against non fastidious bacteria (Auta et al. 2011; Hassan et al. 2012; Yahaya et al. 2012; Yassen et al. 2016). However, the researchers did not find previous studies investigating the antibacterial activity of Ginger extract against drug-resistant gram-negative bacteria, specifically Extended Spectrum Beta Lactamase gram negative bacilli.

With this background, this study determined the bactericidal property of Ginger extract against selected species of bacteria. Specifically, the researchers determined the antibacterial effect of the different dosage concentrations (25mg/mL, 50mg/mL, 100mg/mL, 150mg/mL) of Ginger extract using 95% ethyl alcohol as extracting solvent against *Staphylococcus aureus*, Methicillin-Resistant *Staphylococcus aureus*, *Escherichia coli*, and Extended Spectrum Beta-Lactamase *Escherichia coli*. Establishment of the antibacterial potential of this plant may lead to the pharmaceutical attraction on the development of Ginger as alternative agent against pathogens.

## Objectives of the Study

The study determined the bactericidal property of the ethanolic extract of *Zingiber officinale* against *Staphylococcus aureus*, Methicillin Resistant *Staphylococcus aureus* (MRSA), *Escherichia coli*, and Extended Spectrum Beta Lactamase *Escherichia coli* (ESBLEC).

Specifically, it determined: (a) the inhibitory activities of the different concentrations of the ginger extract against the four test organisms by measuring their zones of growth inhibition; (b) the significant difference between and among the mean zones of inhibition of the different concentrations of the ginger extract against *S. aureus*, MRSA, *E. coli* and ESBLEC, and (c) the Minimum Inhibitory Concentration (MIC) and the Minimum Bactericidal Concentration (MBC) of the ginger extract against the test organisms.

## Materials and Methods

### Preparation of the Ginger Rhizome

The plant materials were the organically grown, native variety of ginger, harvested from the upland municipality of Cervantes, Ilocos Sur, Philippines. The Bureau of Plant Industry of the Department of Agriculture, Manila identified and authenticated the samples.

The researchers washed the ginger rhizomes with tap water to remove all materials that adhered to it and rinsed with distilled water. Finally, they air-dried the rhizomes for 48 hours at room temperature.

#### Extraction of the Ginger

The researchers subjected three kilograms of the finely chopped ginger to the fruit juice extractor and soaked the ginger juice and the pulp in 95% ethanol at a 1:2 proportion (ginger: alcohol) for 72 hours with constant agitation. They filtered the extract and collected the filtrate into flasks and then concentrated it by evaporating the ethanol in a rotary evaporator. The water bath controlled at 50°C further dehydrated the syrupy extract. The researchers collected, dried and weighed the residue produced after evaporation.

Mueller Hinton Agar (MHA) - Difco & BBL Manual, 2<sup>nd</sup> Ed.

The Mueller Hinton Agar determined the antimicrobial activity of the different concentrations of the *Zingiber officinale* extract against the four test organisms. The MHA is prepared by mixing 19 grams of the powdered culture medium with 500mL of distilled water and heating the mixture until the powder is fully dissolved. An autoclave set at 121°C and 15 pounds per square inch (psi) pressure for 15 minutes sterilized the culture medium. Then, the medium was cooled at 45°C and dispensed in sterile petridishes.

Nutrient Agar (NA) - Difco & BBL Manual, 2<sup>nd</sup> Edition

The researchers utilized the colonies grown on Nutrient Agar medium to prepare the microbial suspension used in the bioassay. The NA is prepared by suspending fourteen (14) grams of the NA powder in 500 mL distilled water and heating the mixture until the powder is fully dissolved. The preparation was sterilized in an autoclave at 121°C for 15 minutes at 15 psi pressure. The culture medium was allowed to cool at 45°C and dispensed in sterile petridishes.

Tryptic Soy Broth (TSB) – Becton Dickinson (BD)

The inoculum suspension for the MIC and MBC determination utilized the use of TSB. In the preparation of the TSB, the researchers suspended three (3) grams of the TSB powder in 100 mL distilled water, heated on a hot plate with continuous stirring to dissolve the medium completely, and transferred five (5) mL of the liquid medium to glass test tubes. The researchers sealed the tubes with cotton plugs, and autoclaved at 121°C, 15 psi for 15 minutes, cooled at room temperature and stored inside the refrigerator until use.

Preparation and Standardization of Bacterial Suspension

A tertiary government hospital in Northern Philippines provided all the test organisms utilized in this study. It was also the venue of the bioassay. *Staphylococcus aureus* and *Escherichia coli* have identification numbers ATCC 25923 and ATCC 25922, respectively, while the MRSA and ESBL<sup>EC</sup> were clinical isolates from the hospital patients and confirmed by a reference laboratory in Manila, Philippines. The stock bacterial cultures were re-grown in Nutrient Agar before use to ensure that the bacteria were young and viable.

The researchers prepared the suspension of the four test organisms by transferring a colony of each bacterium on sterile plastic tubes containing 5mL normal saline solution (NSS). The McFarland calibrator standardized each test tube containing the bacterial suspension to ensure uniformity of the number of bacteria inoculated.

#### Preparation of the Test Solutions

The researchers prepared the test solutions (25, 50, 100, and 150 mg/mL ginger extract) by mixing the specified weight of the powdered extract with an appropriate volume of sterile distilled water. The researchers prepared the test solutions just before the conduct of the experiment.

#### Preparation of the Negative Control and Test Discs

The positive control discs were the commercially available antibiotic discs - Ampicillin 0µg was the positive control antibiotic for *Staphylococcus aureus*, Gentamicin 10µg for *E. coli*, Linezolid 30µg for MRSA, and Imipenem 10µg for ESBL *E. coli*. The researchers utilized sterile distilled water as negative control for all bacteria.

#### Bioassay Proper Using Kirby Bauer Disc Diffusion Test - as proposed by the Clinical Laboratory Standards Institute (CLSI).

The Kirby Bauer Disc Diffusion test determined the inhibitory activity of the different concentrations of the ginger extract against the test organisms.

The researchers employed twenty-four (24) sterile petridishes containing an equal amount of Mueller Hinton Agar (MHA) in every replication. They inoculated each of the test organisms from the previously prepared bacterial suspension in six petridishes utilizing the multiple streaking technique using sterile cotton swabs.

All in all, there were 144 samples (consisting of six discs on each of the 24 plates). On each plate contained the positive control disc, the negative control disc, and the four concentrations of the ginger extract under study. The discs were distributed on the surface of the Mueller Hinton Agar at equal distances using a sterile forcep. The researchers incubated the plates in an inverted position at 37°C and observed the result after 18-24 hours of incubation period.

#### Macro tube dilution test – as recommended by Delost (2004).

The researchers performed the macro tube dilution test to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the ginger extract.

They set-up thirteen sterile test tubes with caps and labelled them 1 through 13. Tube 11 was the inoculum control, tube 12 was the broth control, and tube 13 was the extract control. They transferred one (1) mL of the Tryptic Soy Broth (TSB) to tubes 2 through 10, tube 11 and tube 13 while two mL into tube 12. To the working extract solution, the researchers transferred 1 mL to tubes 1, 2, and 13. Then they made two-fold dilutions in 1 mL amounts in tubes 2 through 10 by mixing tube two (2) and transferring 1 mL of its contents to tube three (3). The process continued to tube ten (10) then discarded one (1) mL from tube ten (10) to have a uniform volume for all test tubes.

The McFarland portable machine (VITEC) standardized the bacterial inocula to a final dilution of 1:200 and a final concentration of  $1 \times 10^5$  to  $1 \times 10^6$  cfu/mL. The researchers incubated the tubes at  $37^\circ\text{C}$  for 18 to 24 hours, then, they determined the MIC of each sample by measuring the Optical density in a spectrophotometer at 620 nanometers (nm), comparing the sample reading with the non-inoculated TSB.

The researchers confirmed the MIC result by preparing a 1:100 dilution of the inocula and plating 0.01 mL of the dilution onto a blood agar plate. They incubated the plates at  $37^\circ\text{C}$  for 18 to 24 hours. The tube with the lowest ginger extract concentration showing colony growth is considered to be the MIC while the MBC is the lowest extract concentration permitting no survival of the organism.

#### Measurement of the Zones of Inhibition

The researchers enforced strict aseptic technique during the measurement of the zones of inhibition on all the samples. They expressed all measurements in millimeters.

The table below presents the manner how the zones of inhibition were interpreted.

Table 1  
Descriptive Interpretation of the Zones of Inhibition

<b>Zones of Inhibition (mm)</b>	<b>Interpretation</b>
6	No zone of inhibition (actual size of the paper discs)
7-10	Ineffective
11-14	Partially Effective
15-18	Effective
19 and above	Very effective

Adopted from Diagnostic Microbiology by Maria Delost, 2004.

The researchers initially processed the data gathered from the experiment by manual computation then confirmed using the software SPSS. Coefficient of Variation values determined the reliability of the experiment.

Descriptive statistics (mean and standard deviation) were computed and later used for making statistical inferences. One way Analysis of Variance (ANOVA) indicated the presence of a true difference between mean zones of growth inhibition of the experimental and control groups. Post hoc tests were employed only when F-tests are significant. The post hoc tests were Tukey Honesty Significant Difference (HSD) and Games-Howell Test, to compare the different extract concentrations and determine which among the concentrations are significantly different from one another. The Tukey HSD was used for the data set that satisfied the assumption of the equality of variances.

## RESULTS AND DISCUSSION

The researchers determined the antibacterial property of the *Zingiber officinale* (ginger) extract through its inhibitory activity and MIC/MBC against *Staphylococcus aureus*, MRSA, *Escherichia coli*, and ESBL *Escherichia coli*. The former utilized the Kirby-Bauer disk diffusion method, while the latter made use of the Macro tube dilution test. To establish the validity and reliability of the test performed, positive and negative control groups were included in each set of tests.

Initially, there were a total of 24 plates for a total of 144 samples per replication. After the 24-hour incubation period, only 20 plates, for a total of 120 samples qualified as the final study sample. The remainder had either contaminants, overlapping zones of inhibition or that the readings are outside the +/- 2SD.

Table 2 presents the result of the Kirby Bauer disc diffusion test of the ginger extract against the four test organisms. It shows that the ginger extract exhibited varying degrees of inhibitory activity against the four test organisms. As expected, higher extract concentrations produced wider zones of inhibition.

Table 2  
 Inhibitory Activity of the Ginger Extract Against the Test Organisms

Ginger Extract	Mean Zones of Inhibition (in mm)			
	<i>S. aureus</i>	MRSA	<i>E. coli</i>	ESBLEC
25 mg/mL	6.00	6.00	10.67	8.47
50 mg/mL	8.27	7.80	17.53	14.40
100 mg/mL	14.73	10.73	20.13	18.67
150 mg/mL	19.33	13.60	23.87	21.80
Control Antibiotic	32.80 (Ampicillin 10 µg)	29.73 (Linezolid 30 µg)	30.60 (Gentamicin 10 µg)	24.73 (Imipenen 10 µg)

Legend: 6.00 – No zone of Inhibition

Revised 05 March 2021; Accepted 01 April 2021

- 7.0 – 10.0 – Ineffective  
 11.0 – 14.0 – Partially Effective  
 15.0- 18.0 – Effective  
 19.0 and above – Very Effective

Based on the result, the 25mg/mL ginger extract has no inhibitory activity against *S. aureus* and MRSA (6mm) but has weak to moderate inhibitory activity against *E. coli* and ESBLEC (10.67mm and 8.47mm respectively). The 150mg/mL extract exhibited the widest zone of inhibition against the test organisms. Using the interpretative description provided by Delost (2004) in her book, Diagnostic Microbiology, the 150mg/mL concentration was “very effective” against *S. aureus*, *E. coli*, and ESBLEC while partially effective only against MRSA. The inhibitory activity of the ginger may be due to the presence of potential active chemical constituents in the rhizome. These include but not limited to sesquiterpene compounds like bisapolene, zingiberene, zingiberol, sesquiphellandrene, curcurnene, phenolic compounds like shogaols and gingerols, and other compounds like 6-dihydrogingerdione, galanolactone, gingesulfonic acid, zingerone, geraniol, neral, monoacyldigalactosylglycerols and gingerglycolipis (Singh et al. 2003; O’Hara et al., 1998; Yassen, 2016). These substances act individually or in synergy with one another.

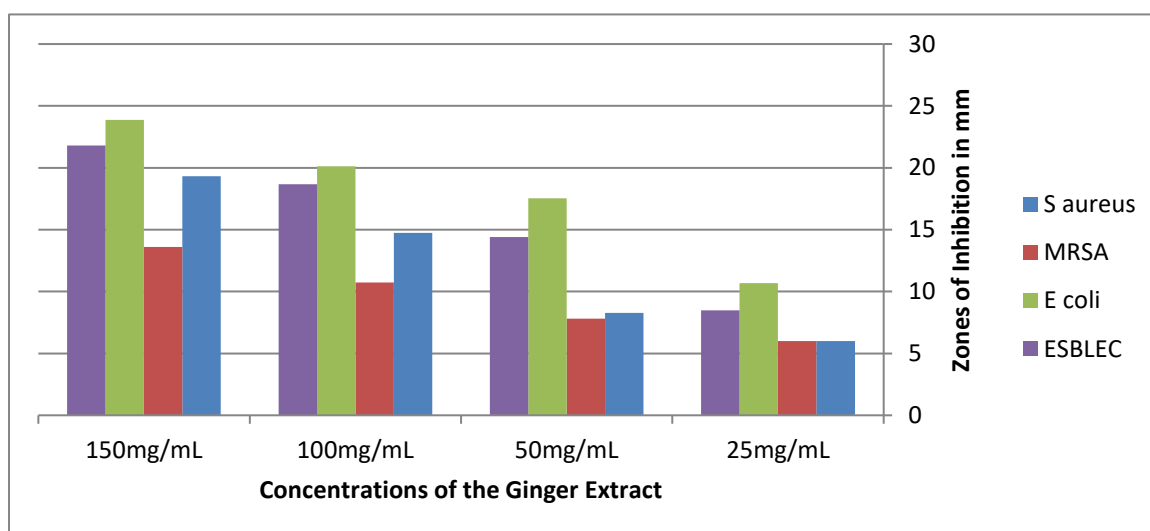


Figure 1. Inhibitory Activity of the Different Concentrations of the Ginger Extract Against the Test Organism

Figure 1 compares the inhibitory activity of the four test organisms used in the study. It shows that the ginger extract is more effective against *E. coli* and ESBLEC than against *S. aureus* and MRSA. The former two bacteria are gram negative while the latter are gram positive. The extract may have caused irreparable damage to the outer membrane of the gram negative bacteria causing their eventual death. Gram negative bacteria have hydrophilic outer membrane owing to the presence of lipopolysaccharide molecules permitting only lipophilic compounds and macromolecules. If these molecules have antibacterial activity, they can penetrate the middle layer of gram negative bacteria (which comprises a very thin

peptidoglycan layer) and disturb cellular function, metabolism, and loss of cellular constituents, leading to bacterial death. Rajeshwar et al. (2005) and Kuete et al. (2007) also reported a similar explanation in their previous studies.

Similarly, the ginger extract targeted the cell walls of *S. aureus* and MRSA, causing irreversible damage. However, the specific mechanism of action of the extract against the test organisms is beyond the scope of this study.

Table 3  
Statistical Analysis Using One-Way ANOVA

Measured Variable	Source of Variation	Sum of Squares	Df	Mean Square	F	Sig.
Zone of Growth Inhibition Against <i>Staphylococcus aureus</i>	Between Groups	1363.509	4	340.877	926.297	.000
	Within Groups	3.680	10	.368		
	Total	1367.189	14			
Zone of Growth Inhibition Against Methicillin Resistant <i>Staphylococcus aureus</i>	Between Groups	1079.696	4	269.924	973.284	.000
	Within Groups	2.773	10	.277		
	Total	1082.469	14			
Zone of Growth Inhibition Against Extended Spectrum Beta Lactamase <i>Escherichia coli</i>	Between Groups	489.957	4	122.489	665.703	.000
	Within Groups	1.840	10	.184		
	Total	491.797	14			

Table 4  
Statistical Analysis Using Welch Test

	Statistic <sup>a</sup>	df1	df2	Sig.
Zone of Growth Inhibition Against <i>Escherichia coli</i>	7424.424	4	4.477	.000

The three sets of data in the previous tables satisfy the assumption of equality of variances. Thus, tests for significant difference between and among mean zones of inhibition can proceed using one way analysis of variance (ANOVA) while the last set of data (Table 4) was subjected to Welch's test.

All sets of data above resulted in significant F values, meaning that at least one pair of zones of growth inhibition among the measured variables are significantly different from one another. Tukey HSD (for the data set that satisfies the assumption of the equality of variances) and Games-Howell (for the last data set) were applied to determine which pairs were significantly different from one another.

Table 5

Multiple Comparisons Between and Among the Zones of Inhibition Caused by the Different Concentrations of the Ginger Extract

Post Hoc Tests	Dependent variable	(I) Treatment	(J) Treatment	(I-J) Mean Difference	Sig.
Tukey HSD	Zone of Growth Inhibition Against <i>S. aureus</i>	25mg/ml	50mg/ml	-2.26667*	.007
			100mg/ml	-8.73333*	.000
			150mg/ml	-13.33333*	.000
		50mg/ml	Ampicillin 10µg	-26.80000*	.000
			100mg/ml	-6.46667*	.000
			150mg/ml	-11.06667*	.000
		100mg/ml	Ampicillin 10µg	-24.53333*	.000
			150mg/ml	-4.60000*	.000
			Ampicillin 10µg	-18.06667*	.000
	150mg/ml	Ampicillin 10µg	-13.46667*	.000	
	Zone of Growth Inhibition Against Methicillin Resistant <i>S. aureus</i>	25mg/ml	50mg/ml	-1.80000*	.013
			100mg/ml	-4.73333*	.000
			150mg/ml	-7.60000*	.000
		50mg/ml	Linezolid 30µg	-23.73333*	.000
			100mg/ml	-2.93333*	.000
			150mg/ml	-5.80000*	.000
		100mg/ml	Linezolid 30µg	-21.93333*	.000
			150mg/ml	-2.86667*	.000
			Linezolid 30µg	-18.06667*	.000
	150mg/ml	Linezolid 30µg	-16.13333*	.000	
	Zone of Growth Inhibition Against Extended Spectrum Beta Lactamase <i>E. coli</i>	25mg/ml	50mg/ml	-5.93333*	.000
			100mg/ml	-10.20000*	.000
			150mg/ml	-13.33333*	.000
		50mg/ml	Imipenem 10µg	-16.26667*	.000
100mg/ml			-4.26667*	.000	
150mg/ml			-7.40000*	.000	
100mg/ml		Imipenem 10µg	-10.33333*	.000	
		150mg/ml	-3.13333*	.000	
		Imipenem 10µg	-6.06667*	.000	
150mg/ml	Imipenem 10µg	-2.93333*	.000		
Games-Howell	Zone of Growth Inhibition Against <i>Escherichia coli</i>	25mg/ml	50mg/ml	-6.86667*	.001
			100mg/ml	-9.46667*	.000
			150mg/ml	-13.20000*	.000
			Gentamicin 10µg	-19.90000*	.000

	50mg/ml	100mg/ml	-2.60000*	.002
		150mg/ml	-6.33333*	.003
		Gentamicin 10µg	-13.03333*	.000
	100mg/ml	150mg/ml	-3.73333*	.005
		Gentamicin 10µg	-10.43333*	.000
	150mg/ml	Gentamicin 10µg	-6.70000*	.004

\*The mean difference is significant at the 0.05 level.

The multiple comparisons show that all paired mean zones of inhibition are significantly different from one another. The implication of this is that the different treatments (concentrations of the ginger extract) have significantly different effects on the test organisms. Specifically, as the concentration of the ginger extract increases, the zone of inhibition also increases.

Table 6  
MIC and MBC of the Ethanolic extract in mg/mL

Test Organism	Spectrophotometric Readings			MIC (mg/mL)	MBC (mg/mL)
	With growth	No Growth	TSB (Standard)		
<i>Staphylococcus aureus</i>	0.010	0.015	0.02	50	75
MRSA	0.010	0.015	0.02	50	75
<i>Escherichia coli</i>	0.0025	0.010	0.02	12.5	25
ESBLEC	0.0025	0.010	0.02	12.5	25

MIC and MBC are computed by dividing the sample by the standard readings multiplied by 100.

The researchers determined the minimum inhibitory and bactericidal concentrations of the ginger extract against the test organisms through spectrophotometric measurements using the method proposed by Delost (2004).

The results showed that the MIC values varied from 12.5mg/mL to 50mg/mL while the MBC values ranged from 25mg/mL to 75mg/mL. These results harmonized with the findings in Table 1, where the ginger extract is more effective against the gram (-) bacteria than against gram (+) bacteria. The common mechanisms of action of plant extracts with antibacterial property are either inhibition of cell wall synthesis for gram positive (Cowan, 1999; Marcucci et al., 2001), accumulate in bacterial membranes causing energy depletion (Conner, 1993), or interfere in the permeability causing disruption of the cell membrane function for gram negative bacteria (Kim et al., 1995).

## CONCLUSIONS AND RECOMMENDATIONS

This study established that *Zingiber officinale* (ginger) extract significantly inhibits the growth of *Staphylococcus aureus*, Methicillin Resistant *Staphylococcus aureus*, *Escherichia*

*coli*, and Extended Spectrum Beta Lactamase *Escherichia coli*. This study supported the use of ginger as an antimicrobial in the traditional system of medicine. Furthermore, this pre-clinical study, which successfully utilized the Kirby Bauer Disk Diffusion technique, hopefully will pave the way for future researches on ginger. Efforts must continue in the research field to find cures for diseases caused by the bacteria using medicinal plants.

Therefore, it is suggested that the bioactive constituents found in ginger be subjected to further laboratory assays that would determine the specific mechanisms of action of each, synergistic or antagonistic properties, and the main active marker for its active ingredient.

### ACKNOWLEDGMENT

The Department of Science and Technology (DOST), Philippine Council for Health Research and Development (PCHRD), and the Region 1 Health Research and Development Consortium funded this study.

### LITERATURE CITED

1. Auta, K.I., Galadima, A.A., Bassey, J.U., Olowoniyi, O.D., Moses, O.O., & Yako, A.B. (2011). Antimicrobial Properties of the Ethanolic Extracts of *Zingiber officinale* (Ginger) on *Escherichia coli* and *Pseudomonas aeruginosa*. *Research Journal of Biological Sciences*, 6, 37-39. Retrieved from <http://medwelljournals.com/fulltext/?doi=rjbsci.2011.37.39>
2. Bidlack, W.R., Omaye, S.T., Meskin, M.S., & Topham, D.K. (2000). *Phytochemicals as*
3. *Bioactive Agents*. Lancaster, PA: Technomic Publishers. Retrieved from <https://books.google.com.ph/books/>
4. Conner, D.E., Davidson, P.T., & Branen, A.L. (1993). *Naturally Occuring Compounds in Antimicrobials in Foods*. Mareel Dekker, New York. Retrieved from [https:// books.google.com.ph/](https://books.google.com.ph/)
5. Cowan, M. M. (1999). *Plant Products as Antimicrobial Agents*. *Clinical Microbiology*.12: 564-582. (Pubmed). Retrieved from <https://books.google.com.ph/>.
6. Delost, M.D. (2004). *Introduction to Diagnostic Microbiology: A Text and Workbook*. Philippines: Elsevier (Singapore) Pte Ltd. Retrieved from <https://www.google.com.ph/>
7. Enright, M.C., Robinson, D.A., Randle, G., Feil, E.J., Grundmann, H., & Spratt, B.G. (2002). The evolutionary history of methicillin-resistant *Staphylococcus aureus* (MRSA). *Proceedings of the National Academy of Sciences of the United States of America*. Retrieved from <http://www.pnas.org/content/99/11/7687.full>
8. Freile-Pelegrin, Y., & Morales, J.L. (2004). Antibacterial activity in marine algae from the coast of Yucatan, Mexico. *Botanica Marina* 47 (2). Retrieved from <https://www.Researchgate.net/publication/249927030>

9. Girhepunje, N.S. (2016). Standardization of Some Bioactives in Ginger Extract. *Journal of Harmonized Research in Pharmacy* 5 (2). Retrieved from <https://www.academia.edu/31768059/>
10. Hasan, H.A., Mohammed, R.R.A., Abd Razik, B.M., & Hassan, A.R.B. (2012). *Chemical Composition and Antimicrobial Activity of the Crude Extracts Isolated from Zingiber Officinale by Different Solvents*. Retrieved from <http://omicsonline.org/2153-35.1000184.pdf>
11. Juayang, A., Maestral, D., & Gallega, C. (2015). Review on the Antimicrobial Resistance of
12. Pathogens from Tracheal and Endotracheal Aspirates of Patients with Clinical
13. Manifestations of Pneumonia in Bacolod City in 2013. *International Journal of*
14. *Bacteriology*. Retrieved from <https://www.ncbi.nlm.nih.gov>.
15. Kim, J., Marshal, M., & Wei, C. (1995). Antibacterial Activity of Some Essential Oil Components Against Five Food Borne Pathogens. *Journal of Agriculture, Food & Chemicals* 4. Retrieved from <https://www.semanticscholar.org/paper/f7786e152fd912f259d15febda8e4779683ca399>.
16. Kuete V., Nguemeving, J.R., Beng, V.P., Azebaze, A.G., Etoa, F.X., Meyer, M., Bodo, B., & Nkengfack, A.E. (2007). Antimicrobial Activity of the Methanolic Extracts and Compounds from *Vismia laurentii* De wild (Guttiferae). *Journal of Pharmacology* 109 (3). Retrieved from <https://www.ncbi.nlm.nih.gov/pubmed/16971076>
17. Marcucci M. C., Ferreres, F., Garcia-Viguera C., Bankova, V.S., De Castro, S.L., Dantas, A.P., Valente, P.H. & Paulino, N. (2001). Phenolic Compounds from Brazilian propolis with Pharmacological Activities. *Journal of Pharmacology* 74 (2). Retrieved from <https://www.ncbi.nlm.nih.gov/pubmed/11167028>.
18. Nacimiento, G., Locatelli, J., Freitas, P.C., & Silva, G.L. (2005). Antibacterial Activity of
19. Plant Extracts and Phytochemicals on Antibiotic Resistant Bacteria. *Brazilian*
20. *Journal of Microbiology* 31 (4). Retrieved from <http://www.scielo.br/scielo.php>.
21. Nagba, M., Palangan, N., Yu, I., Opena, E.L., & Baguio, M. (2012). Presence of Pathogenic
22. Bacteria in Drinking Waters of Selected Public Elementary Schools of Iligan City,
23. Philippines. *Mindanao Journal of Science and Technology* 10 (1). Retrieved from
24. <https://ejournals.ph/article.php?id=6725>.
25. O'Hara, M., Keifer, D., Farrel, K., & Kemper, K. (1998). A Review of 12 Commonly Used Medicinal Herbs. *Archives of Family Medicine* 7 (6). Retrieved from <https://www.ncbi.nlm.nih.gov/pubmed/9821826>.

26. Omar, H.H., Gungumjee, N.M., Shiekh, H.M., & El-Kazan, M.M. (2012).  
Antibacterial
27. Activity of extracts of marine algae from the Red Sea of Jeddah, Saudi Arabia.
28. *African Journal of Biotechnology* 11. Retrieved from <https://www.researchgate.net/publication/268336689>.
29. Philippine Health Advisories 2012. Department of Health. Retrieved from <https://www.doh.gov.ph/sites/default/files/publications/PhilippineHealthAdvisories2012.compressed.pdf>.
30. Rajeshwar Y., Gupta, M., & Mazumder, U. (2005). In Vitro Lipid Peroxidation and Antimicrobial Activity of *Mucana pruriens* Seeds. *Iranian Journal of Pharmacology* 4. Retrieved from <https://www.academia.edu/5930794/>
31. Singh, A. P., & Malhotra, S. (2003). Medicinal Properties of Ginger. *Indian Journal of Natural Products and Resources* 2 (4). Retrieved from <http://nopr.niscair.res.in/handle/123456789/12292>.
32. Yahaya, O., Yabefa, J.A., Umar, I.O., Datshen, M.M., Egbunu, Z.K., & Ameh, J. (2012). Combine Antimicrobial Effect of Ginger and Honey on Some Human Pathogens. *British Journal of Pharmacology and Toxicology*. Retrieved from <http://maxwellsci.Com/print/bjpt/v3-237-239.pdf>
33. Yassen, Doaa, et a., (2016). Antibacterial Activity of Crude Extracts of Ginger (*Zingiber officinale* Rosque) on *Escherichia coli* and *Staphylococcus aureus*: A Study In Vitro. *Indo American Journal of Pharmaceutical Research*. Retrieved from [www.iajpr.com](http://www.iajpr.com).