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THE ANTIMICROBIAL ACTIVITY OF *ZINGIBER OFFICINALE* EXTRACT (GINGER), COCONUT OIL AND A COMBINATION OF COCONUT OIL AND GINGER EXTRACT FROM DIFFERENT LOCATIONS, AT DIFFERENT CONCENTRATIONS ON WOUND INFECTION BACTERIA

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ABSTRACT

The hexane extracts of ginger root (*Zingiber officinale*) of the Zingiberaceae family and Coconut; *Cocos nucifera* a member of the Arecaceae family, from two locations in Guyana were used for the investigation of their antibacterial properties. To evaluate the scientific basis for the use of the plant and plant product, the antimicrobial activities of the extracts were evaluated against three common bacteria found on wound infections; *Staphylococcus aureus* a gram-positive bacteria, *Klebsiella pneumoniae* a Gram-negative, and *Pseudomonas aeruginosa* a common encapsulated, facultative aerobic and Gram-negative. The disc diffusion method was used to test the antimicrobial activity of the extracts which were evaluated in 20%, 25%, 50%, 75%, and 100% concentrations against each organism in triplicates, and the diameter of the zone of inhibition was measured. There was no significant difference among the plant extracts from the different locations. The results showed that coconut oil in 50% was most effective against *Staphylococcus aureus* forming zones measuring 19-20mm, the 100% ginger concentration was most effective against *Staphylococcus aureus* also and the zones measured 23-15mm, the mixture of ginger and coconut oil was equally effective against *Staphylococcus aureus* and *Pseudomonas aeruginosa* with zones measured 12-15mm. The study also investigated the phytochemical constituents of the plant hexane extracts to test the presence or absence of secondary metabolites. Results of the phytochemical studies revealed the presence of tannins, saponins, alkaloids, flavonoids, glycoside, phenol, and steroids in the extracts were active against the bacteria.

Contribution/Originality: The originality in this study is the unique and organically grown ginger and coconut oil produced and used for testing in its pure form, its combining effects of the organic ginger extract and coconut oil in Guyana, South America in different concentrations against the pathogens a study unique of its kind.

1. INTRODUCTION

Long before the onset of antibiotics and different antimicrobial treatments within the early 20th century, the practice of medicine has been used to treat infections since antiquity. None is however, as reliable as novel antimicrobial medical care; not as safe and effective (Columbus, 2020). However, due to the increased resistance to microbes and the lack of development of new pharmaceutical agents, the possibility of a return to the former antimicrobial era is possible. This research is of major benefits to pharmaceutical companies, health personnel

and the general public because it improves their knowledge on using local plants and making organic medication to heal and prevent the growth of pathogenic organisms, it is an inexpensive method, both plant products are found abundantly in Guyana and has proven to have little to no side effects when the right amount is used.

The method of killing or inhibiting microbes that cause illness is referred to as antimicrobial activity. An antimicrobial is an agent of natural, semi-synthetic, or synthetic origin that destroys or inhibits the growth of microorganisms but does little or no harm to the host. An antibiotic is a low molecular agent formed by a microorganism that destroys or eliminates other microorganisms at low concentrations (Ghosh, Sarkar, Issa, & Haldar, 2019). The greatest accomplishment of recent drugs is presumably the development of antibiotics and alternative antimicrobial therapies. Alexander Fleming the careless lab technician was the first to commercialize antibiotic called penicillin. Since then, there has been the discovery and acknowledgment of resistance besides the invention of the latest antibiotics. Microorganism resistance leaves ancient therapies useless and raises the chance of transmission of infection, but the usage of plant extracts and phytochemicals, possessing each best-known and unknown antimicrobial activities, may be of ample importance in treatments (Ruddaraju, Pammi, Sankar Guntuku, Padavala, & Kolapalli, 2020). Plants possess a wide variety of organic compounds that are biologically active substances and are responsible for the function of defensive mechanisms through interference with molecular targets in herbivores and microbes (Gupta & Birdi, 2017).

Ginger scientifically known as *Zingiber officinale* belongs to the Zingiberaceae family and Coconut; *Cocos nucifera* a member of the Arecaceae family, are both plants of medicinal importance which are both considered nutraceuticals. These plants are capable of providing a variety of phytochemicals that have therapeutic properties and can be used in treating many diseases present today. Ginger has been used since ancient times as a treatment for several sicknesses together with inflammatory diseases. Ginger contains phenolic active ingredients such as gingerol, paradol, and shogoal (Zehsaz, Farhangi, & Mirheidari, 2014). Zingiber officinale has proven to have antimicrobial properties against pathogens such as *Escherichia coli*, *Proteus sp*., etc. Coconut oil was tested to be the source that contains the most lauric acid. Scientists have known since the 1960s about the antiviral, antibacterial, and antifungal effects of medium-chain fatty acids/triglycerides (MCTs) present in coconut oil. Research has shown that the microorganisms coconut oil is capable of inactivating are bacteria, yeast, fungi, and viruses that are enveloped (Sheshala, Ying, Hui, Barua, & Dua, 2013).

2. MATERIALS AND METHODS

2.1. Study Material

Three samples of fresh Ginger (*Zingiber officinale*) and coconut oil was purchased from two locations in Guyana; Berbice (Location 1) and Essequibo (Location 2). For the determination of the antimicrobial activity of these extracts and a mixture of both in different concentrations, the following three bacterial species were used: *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Klebsiella pneumonia*, since they are all associated with wound infections. These bacteria species were isolated from persons who have wound infections and are being treated at the Georgetown Public health Corporation (GPHC) it was then collected from the laboratory at the GPHC and then transported to and preserved in the Department of Biology Laboratory at the University of Guyana.

2.2. Preparation of Ginger and Coconut Oil

4lbs of ginger was collected from each sample (A, B & C) form both location (1 & 2). These samples were collected based on how fresh they were. The ginger was later washed with distilled water thoroughly until all dirt was removed. After washing, the ginger was sliced thinly and placed to air dry for approximately 3 weeks. When the ginger appeared completely dry, a hand grain mill was used to ground it into a fine powder. Powdered ginger was then sieved through a 0.5mm strainer to ensure only powder was set aside to use and all residual materials were removed, it was then weighed, stored in a clean, dry, tightly covered containers, and labeled

specific to location and sample being; Berbice being "Location-1 Sample A/B/C" etc. and Essequibo being "Location-2 Sample A/B/C".

15 coconuts were collected from each location, 5 from each sample area. Coconuts were collected based on the maturity of the coconut and this was determined by the yellowish, brown colour of the husk and once it made a sloshing sound when shaken. Three days before the commencement of the practical aspect of this project, the coconuts were de-husked then cracked into halves and grated using a hand grater where the kernel turned into a very thin grain which made it easy to extract the coconut's milk. After grating, each set of coconuts gave approximately 1143g and each set was then soaked with 1 litre of water for approximately 3 hours. After soaking a thick, oily and creamy layer was formed at the top, that layer was scooped out and added to a clean pot then boiled at 120^0 C for the first hour, it was then reduced to 90^0 C once it started to coagulate and then reduced to 60^0 C one the oil started to separate. Once it started boiling completely, it was stirred for 5 minutes slowly then removed off heat and strained using a muslin cloth. It was then measured and stored in sterile containers in the refrigerator until ready for use.

2.3. Media Preparation

Mueller Hinton Agar was used to prepare the media. 38 g of agar medium was placed in one liter of distilled water. The media was mixed well and boiled for approximately 10 minutes. After that, the media was sterilized at 121^oC for 30 minutes. The agar media was then placed to be cooled at 40 ^oC - 45 ^oC. After cooling, 15ml of media was poured in each plate marking the 4mm line. 66 plates were made using 1ltr of media so, the step was repeated 7 more times and gave a total of 528 plates. The plates were placed into bags and stored safely in the refrigerator and used as necessary (Kamarul, Rowsni, Khan, & Kabir, 2014).

2.4. Samples Preparation

Each sample prepared was prepared in a 10ml dilution at different concentration. The formula used was "$M_1 * V_1 = M_2 * V_2$". The base solvent used to prepare all concentrations was hexane. To make the 100% oil concentrations for all samples from both locations, 10ml of coconut oil was placed into sterile vials and labelled according to concentration, location, sample and type of extract. For the 75% concentrations, 7.5ml of coconut oil was added to each of the vials then 3.5ml of hexane was added to make up the 10ml, the vials were labelled. When making the 50% concentration, 5ml of coconut oil was added to the vials then 5ml of hexane to make up the 10ml, vials were labelled. To make 25% concentration, 2.5ml of coconut oil was added to the vials and 7.5ml of hexane was added to make the 10ml volume, vials were labelled. 2ml of coconut oil was added to vials, followed by 8ml of hexane to make a 20% concentration, vials were labelled and stored in cool, dry place. For each location, 15 vials of coconut oil extract were prepared at 5 different concentrations each for each sample.

To prepare the ginger extract, 50g of the prepared powdered ginger from each sample was placed into 300ml of hexane in large glass jars. These jars were locked tightly for 24 hours allowing the ginger and hexane to mix. After 24 hours the jars were open and placed in a cool dry area for excessive hexane to vaporize until it was almost to the line of the thick mixture at the bottom. This process took approximately 3 days for the hexane to vaporize to desired result. The liquid was then filtered through a muslin cloth into sterile containers, each measuring approximately 46ml of crude ginger extract after the vaporizing process and filtering. To make the 100% ginger extract concentrations for both locations and each sample, 10ml of crude ginger extract was placed into sterile vials and labelled according to concentration, location and sample. For the 75% concentrations, 7.5ml of crude ginger extract was added to each of the vials then 3.5ml of hexane was added to make up the 10ml, the vials were labelled. When making the 50% concentration, 5ml of crude ginger extract was added to the vials then 5ml of hexane to make up the 10ml, vials were labelled. To make 25% concentration, 2.5ml of crude ginger was added to the vials and 7.5ml of hexane was added to make the 10ml volume, vials were labelled. 2ml of crude

ginger extract was added to vials, followed by 8ml of hexane to make a 20% concentration, vials were labelled and stored in cool, dry place. For each location, 15 vials of crude ginger extract were prepared at 5 different concentrations each for each sample.

To prepare the mixture of ginger and coconut oil, 20ml of crude ginger extract was added to 20ml of coconut oil forming a 1:1 ratio of ginger to coconut oil, this was done of each sample from both locations. This was allowed to mix will for 24 hours and afterwards, the concentrations were prepared. To make the 100% ginger-coconut extract concentrations for both locations and each sample, 10ml of ginger-coconut extract was placed into sterile vials and labelled according to concentration, location and sample. For the 75% concentrations, 7.5ml of ginger-coconut extract was added to each of the vials then 3.5ml of hexane was added to make up the 10ml, the vials were labelled. When making the 50% concentration, 5ml of ginger-coconut extract was added to the vials then 5ml of hexane to make up the 10ml, vials were labelled. To make 25% concentration, 2.5ml of ginger-coconut extract was added to the vials and 7.5ml of hexane was added to make the 10ml volume, vials were labelled. 2ml of ginger-coconut extract was added to vials, followed by 8ml of hexane to make a 20% concentration, vials were labelled and stored in cool, dry place. For each location, 15 vials of crude ginger extract were prepared at 5 different concentrations each for each sample.

2.5. Inoculum Bacteria Preparation & Antibacterial Assay

Filter paper discs (6 mm diameter) were prepared using a puncher to punch Whatman filter paper No.1. The filter paper discs were sterilized in a dry heat sterilizer and kept at room temperature. The discs were the distributed evenly in each vails containing the extracts; the filter paper was left to soak for 24hours in the extracts before using. Mueller Hinton agar plates were placed under the ultraviolet light for 20mins then left under the laminar airflow cabinet to dry for 15mins before the plates were ready to streak and discs were applied. After drying, plates were labelled on the bottom specific to location, sample, organism, concentration, treatment, researcher's name and the date. 2ml of suspension media was then used and a small colony of the bacteria was suspended into the suspension media using a sterile loop until it reached the desired standard of the McFarland Standard. This prepared inoculum was then used to spread onto Mueller Hinton agar, using a sterile cotton swab to make a lawn of bacteria. The lid of the agar plate was slightly lifted and the entire surface of the agar plate was streaked carefully. It was ensured the swab overlapped every time to evenly distribute inoculate. The plate was then turned at a 120 degree and swabbed again repeatedly. The plates were divided into 3 quadrants and one disc of the same concentration was placed in each quadrant. This was done for each sample, each organism, each treatment and both locations. Gentamicin discs were used as a positive control and distilled water was used as the negative control against each bacterium. All plates will be incubated at 37°C for 24 hours. The zones of inhibition were observed and measured in mm on the agar surface and the results were recorded in tables to be tabulated (Kamarul et al., 2014). The zones were compared to the standard reference of the ZOH of antimicrobial test. The interpretation chart follows; resistant: 13mm or less, intermediate: 14-16mm and susceptible 17mm and more (Ewnetu, Lemma, & Birhane, 2014).

2.6. Phytochemical Screening

2.6.1. Alkaliods

2ml of extracts was measured in a test tube to which picric acid solution was added. An orange coloration indicated the presence of alkaloids.

2.6.2. Tannins

To a portion of the extracts diluted with water, 3-4 drops of 10% ferric chloride solution is added. A blue colour is observed for the presence of tannins.

2.6.3. Saponins

0.5ml of extract was measured in a test tube and was shaken with 2ml of water. If foam produced persist for 10mins it indicates the presence of saponins (Prashant, Kumar, Kaur, Kaur, & Kaur, 2011).

2.6.4. Glycosides

25ml of diluted sulphuric acid was added to 5ml extract in a test tube and boiled for 15mins, cooled and neutralized with 10%Sodium Hydroxide (NaOH), then 5ml of Fehling solution added. Glycosides are indicated by a brick red precipitate.

2.6.5. Flavonoids

The extract solution was prepared by adding 1.5ml of 50% methanol to 4ml of water. After the solution was warmed, hydrochloric acid was added. A red color was then observed in the solution due to the presence of flavonoids.

2.6.6. Volatile Oils

A small quantity of hydrochloric acid and 0.1ml Sodium hydroxide was added to the solution. The extract was shaken with a mixture of these two components. A white precipitate formed if the presence of volatile oils is present.

2.6.7. Phenols

A small amount of extract was then treated with ferric chloride solution. The resulting solution exhibited a bluish black color, which indicates that there are phenols in it.

2.6.8. Steroids

For the treatment, 1 ml of extract was mixed with 0.5 ml of acetic anhydride acid and 0.5 ml of chloroform. then gradually added a solution of powerful sulfuric acid. A greenish-blue hue indicates the presence of steroids.

Table 1. Displays the zone of inhibition of coconut oil collected in triplicates from "Location 1" that was tested against 3 bacteria; *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Klebsiella pneumonia in 5 concentrations*. A mean value of each sample, overall mean of samples and standard deviation is displayed.

Location 1- coconut oil							
		Zone of inhibition(mm)					
Organism	**Conc.**	**Sample A**	**Sample B**	**Sample C**	**Mean**	**Standard deviation**	**Mean±S.D**
Staphylococcus aureus	20%	0	0	0	0.00	0.00	0.00±0.00
	25%	9.9	10	9.9	9.93	0.06	9.93±0.06
	50%	19.6	19.4	20	19.67	0.31	19.67±0.31
	75%	10	10	10	10.00	0.00	10.00±0.00
	100%	8	8	8.3	8.10	0.17	8.10±0.17
Klebsiella pneumonia	20%	0	0	0	0.00	0.00	0.00±0.00
	25%	0	0	0	0.00	0.00	0.00±0.00
	50%	5	5	5	5.00	0.00	5.00±0.00
	75%	6.1	6	6	6.03	0.06	6.03±0.06
	100%	6	6	6	6.00	0.00	6.00±0.00
Pseudomonas aeruginosa	20%	5.1	5	5	5.03	0.06	5.03±0.06
	25%	9	9	9	9.00	0.00	9.00±0.00
	50%	10	10	10	10.00	0.00	10.00±0.00
	75%	10	10	10	10.00	0.00	10.00±0.00
	100%	12	12	12	12.00	0.00	12.00±0.00

Note: All samples were collected in triplicated and tested in triplicates (Anzuku, 2021).

3. RESULTS AND DISCUSSION

Present is a table showing the secondary metabolites present in the plant extracts under study and also a comparative analysis of different concentration of ginger, coconut oil and a mixture of ginger and coconut oil from two different locations in Guyana on the different bacteria.

Table 2. Shows the zone of inhibition of coconut oil collected in triplicates from "location 2" that was tested against 3 bacteria; *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Klebsiella pneumonia in 5 concentrations*. A mean value of each sample, overall mean of samples and standard deviation is displayed.

Location 2- coconut oil							
		Zone of inhibition(mm)					
Organism	**Conc**	**Sample A**	**Sample B**	**Sample C**	**Mean**	**Standard deviation**	**Mean±S.D**
Staphylococcus aureus	20%	0	0	0	0.00	0.00	0.00±0.00
	25%	9.8	10	10	9.93	0.12	9.93±0.12
	50%	19.4	19.8	17.7	18.97	1.12	18.97±1.12
	75%	10	10	10	10.00	0.00	10.00±0.00
	100%	8	8	8.1	8.03	0.06	8.03±0.06
Klebsiella pneumonia	20%	0	0	0	0.00	0.00	0.00±0.00
	25%	0	0	0	0.00	0.00	0.00±0.00
	50%	5	5	5	5.00	0.00	5.00±0.00
	75%	6	5.8	6	5.93	0.12	5.93±0.12
	100%	6	6	5.8	5.93	0.12	5.93±0.12
Pseudomonas aeruginosa	20%	5.1	5	5	5.03	0.06	5.03±0.06
	25%	9	9	9	9.00	0.00	9.00±0.00
	50%	10	10	10	10.00	0.00	10.00±0.00
	75%	9.8	10	10	9.93	0.12	9.93±0.12
	100%	11.5	12	12	11.83	0.29	11.83±0.29

Note: All samples were collected in triplicated and tested in triplicates (Anzuku, 2021).

Table 3. Displays the zone of inhibition of ginger collected in triplicates from "location 1" that was tested against 3 bacteria; *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Klebsiella pneumonia in 5 concentrations*. A mean value of each sample, overall mean of samples and standard deviation is displayed.

Location 1- ginger							
		Zone of inhibition(mm)					
Organism	**Conc**	**Sample A**	**Sample B**	**Sample C**	**Mean**	**Standard deviation**	**Mean±S.D**
Staphylococcus aureus	20%	0	0	0	0.00	0.00	0.00±0.00
	25%	0	0	0	0.00	0.00	0.00±0.00
	50%	15.7	15.6	15.4	15.57	0.15	15.57±0.15
	75%	19.8	19.7	19.7	19.73	0.06	19.73±0.06
	100%	24.8	25	24.7	24.83	0.15	24.83±0.15
Klebsiella pneumonia	20%	0	0	0	0.00	0.00	0.00±0.00
	25%	0	0	0	0.00	0.00	0.00±0.00
	50%	14.1	14	14	14.03	0.06	14.03±0.06
	75%	15.7	15.8	15.8	15.77	0.06	15.77±0.06
	100%	0	0	0	0.00	0.00	0.00±0.00
Pseudomonas aeruginosa	20%	0	0	0	0.00	0.00	0.00±0.00
	25%	0	0	0	0.00	0.00	0.00±0.00
	50%	6.5	6.6	6.8	6.63	0.15	6.63±0.15
	75%	5.3	5.4	6.1	5.60	0.44	5.60±0.44
	100%	0	0	0	0.00	0.00	0.00±0.00

Note: All samples were collected in triplicated and tested in triplicates (Abdalla & Abdallah, 2018).

Table 4 presents the zone of inhibition of ginger collected in triplicates from "Location 2" that was tested against Staphylococcus aureus, Pseudomonas aeruginosa, and Klebsiella pneumonia in 5 concentrations. The table shows the mean value of each sample, overall mean of samples and the standard deviation.

Table 4. The zone of inhibition of ginger collected in triplicates from "location 2" that was tested against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Klebsiella pneumonia in 5 concentrations*. The table shows the mean value of each sample, overall mean of samples and the standard deviation.

Location 2- ginger							
		Zone of inhibition					
Organism	**Conc**	**Sample A**	**Sample B**	**Sample C**	**Mean**	**Standard deviation**	**Mean±S.D**
Staphylococcus aureus	20%	0	0	0	0.00	0.00	0.00±0.00
	25%	0	0	0	0.00	0.00	0.00±0.00
	50%	15.5	15.6	15.7	15.60	0.10	15.60±10.0
	75%	19.5	19.7	19.7	19.63	0.12	19.63±12.0
	100%	24.5	24.4	24.7	24.53	0.15	24.53±15.0
Klebsiella pneumonia	20%	0	0	0	0.00	0.00	0.00±00.0
	25%	0	0	0	0.00	0.00	0.00±00.0
	50%	14.1	13.5	13.3	13.63	0.42	13.63±42.0
	75%	15.4	15.7	15.4	15.50	0.17	15.50±17.0
	100%	0	0	0	0.00	0.00	0.00±00.0
Pseudomonas aeruginosa	20%	0	0	0	0.00	0.00	0.00±00.0
	25%	0	0	0	0.00	0.00	0.00±00.0
	50%	6.7	6.4	6.6	6.57	0.15	6.57±15.0
	75%	5.4	5.5	5.4	5.43	0.06	5.43±06.0
	100%	0	0	0	0.00	0.00	0.00±00.0

Note: All samples were collected in triplicated and tested in triplicates (Abdalla & Abdallah, 2018).

Table 5. Portrays the zone of inhibition of coconut oil + ginger collected in triplicates from "Location 1" that was tested against 3 bacteria; *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Klebsiella pneumonia in 5 concentrations*. A mean value of each sample, overall mean of samples and standard deviation is displayed.

Location -1 coconut-ginger							
	Zone of inhibition(mm)						
Organism	**Conc**	**Sample A**	**Sample B**	**Sample C**	**Mean**	**Standard deviation**	**Mean±S.D**
Staphylococcus aureus	20%	0	0	0	0.00	0.00	0.00±0.00
	25%	4.6	5.4	5.2	5.07	0.42	5.07±0.42
	50%	8.1	7.4	7.1	7.53	0.51	7.53±0.51
	75%	9.5	9.3	9.1	9.30	0.20	9.30±0.20
	100%	13.6	13.6	13.8	13.67	0.12	13.67±0.12
Klebsiella pneumonia	20%	0	0	0	0.00	0.00	0.00±0.00
	25%	0	0	0	0.00	0.00	0.00±0.00
	50%	0	0	0	0.00	0.00	0.00±0.00
	75%	6.2	6.6	6.6	6.47	0.23	6.47±0.23
	100%	9.2	9.6	9.3	9.37	0.21	9.37±0.21
Pseudomonas aeruginosa	20%	0	0	0	0.00	0.00	0.00±0.00
	25%	0	0	0	0.00	0.00	0.00±0.00
	50%	0	0	0	0.00	0.00	0.00±0.00
	75%	9.2	9.3	9.2	9.23	0.06	9.23±0.06
	100%	14.4	14.2	14.3	14.30	0.10	14.30±0.10

Note: All samples were collected in triplicated and tested in triplicates.

Table 6 presents the zone of inhibition of coconut oil + ginger collected in triplicates from "Location 1" that was tested against 3 bacteria; Staphylococcus aureus, Pseudomonas aeruginosa, and Klebsiella pneumonia in 5 concentrations. A mean value of each sample, overall mean of samples and the standard deviation.

Table 6. The zone of inhibition of coconut oil + ginger collected in triplicates from "Location 1" that was tested against 3 bacteria; *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Klebsiella pneumonia in 5 concentrations.* A mean value of each sample, overall mean of samples and the standard deviation.

Location -2 coconut-ginger							
		Zone of inhibition(mm)					
Organism	**Conc**	**Sample A**	**Sample B**	**Sample C**	**Mean**	**Standard deviation**	**Mean±S.D**
Staphylococcus aureus	20%	0	0	0	0.00	0.00	0.00±0.00
	25%	5.3	3.4	4.6	4.43	0.96	4.43±0.96
	50%	7.4	7.8	7.2	7.47	0.31	7.47±0.31
	75%	9.4	9.8	9.6	9.60	0.20	9.60±0.20
	100%	14.4	14.5	13	13.97	0.84	13.97±0.84
Klebsiella pneumonia	20%	0	0	0	0.00	0.00	0.00±0.00
	25%	0	0	0	0.00	0.00	0.00±0.00
	50%	0	0	0	0.00	0.00	0.00±0.00
	75%	6.5	6.5	6.1	6.37	0.23	6.37±0.23
	100%	9.3	9.5	9.4	9.40	0.10	9.40±0.10
Pseudomonas aeruginosa	20%	0	0	0	0.00	0.00	0.00±0.00
	25%	0	0	0	0.00	0.00	0.00±0.00
	50%	0	0	0	0.00	0.00	0.00±0.00
	75%	8.5	9.1	9.4	9.00	0.46	9.00±0.46
	100%	14.4	14	14.6	14.33	0.31	14.33±0.31

Note: All samples were collected in triplicated and tested in triplicates.

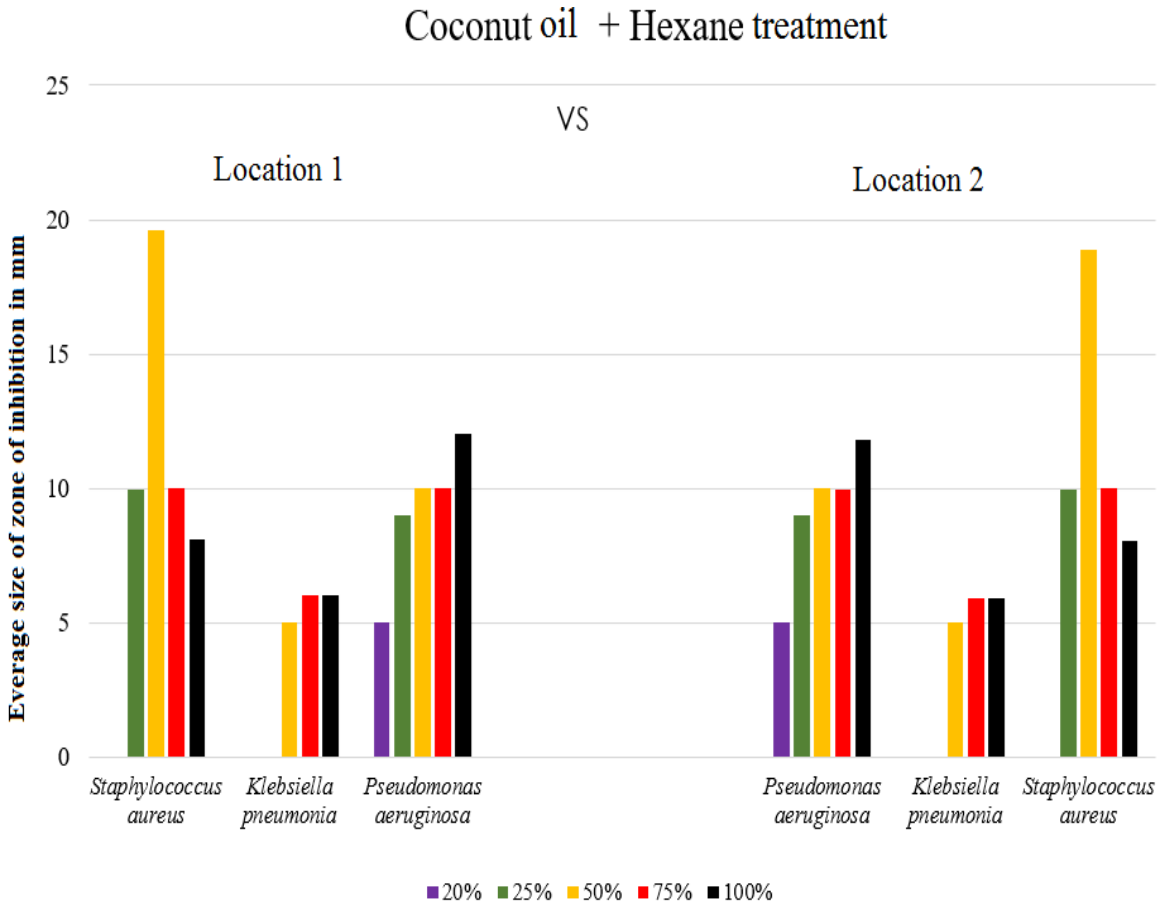

Figure 1. The comparison of the average zone of inhibition formed using coconut oil and hexane in different concentrations from "location 1" and "location 2" against *staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Klebsiella pneumonia.*

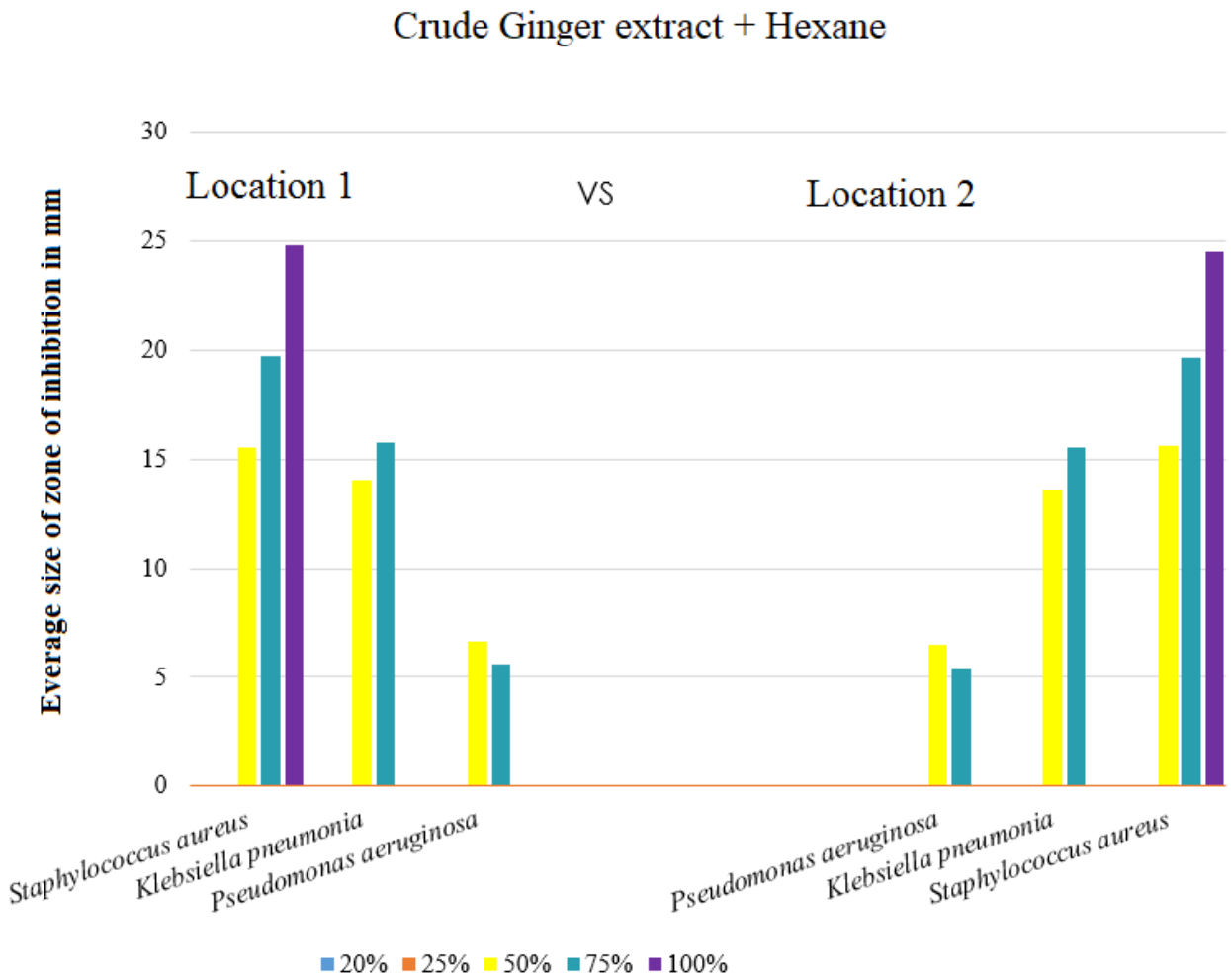

Figure 2. The comparison of the average zone of inhibition formed using ginger extract and hexane in different concentrations from "location 1" and "location 2" against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Klebsiella pneumonia*.
Note: No zones of inhibition were present when using the 20% concentration.

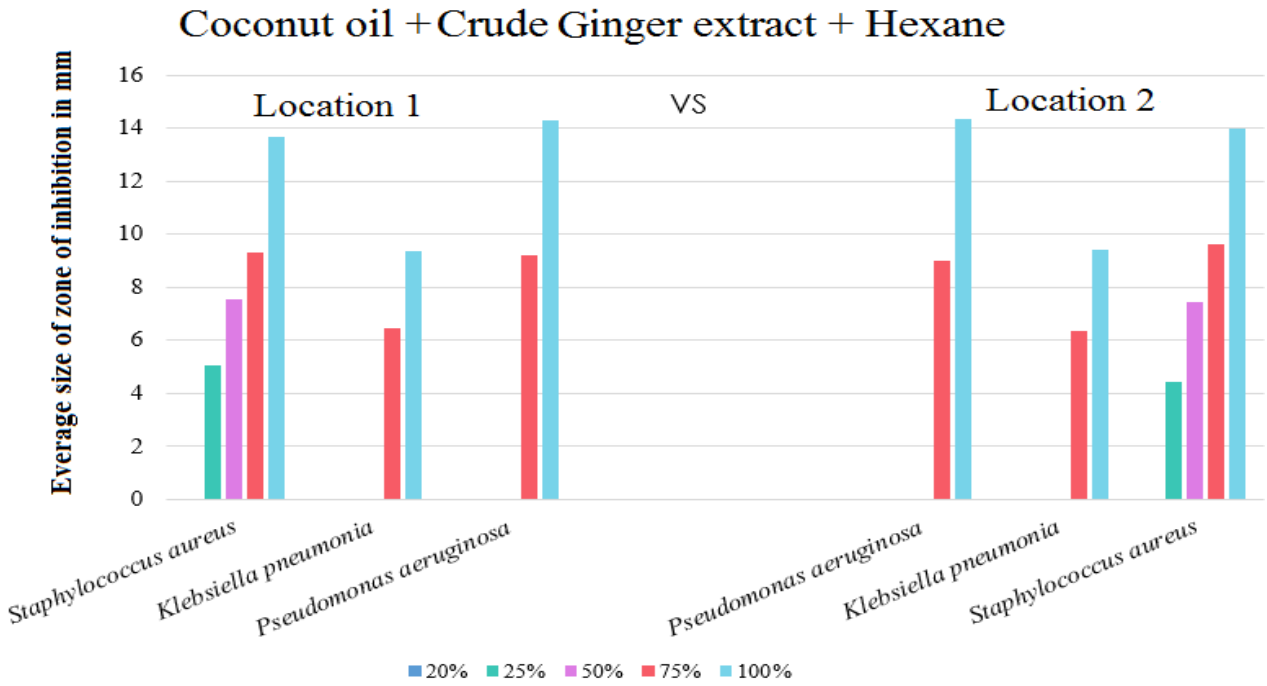

Figure 3. The comparison of the average zone of inhibition formed using coconut oil + crude ginger extract and hexane in different concentrations from "location 1" and "location 2" against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Klebsiella pneumonia*.
Note: No zone of inhibition was present when using the 20% concentration.

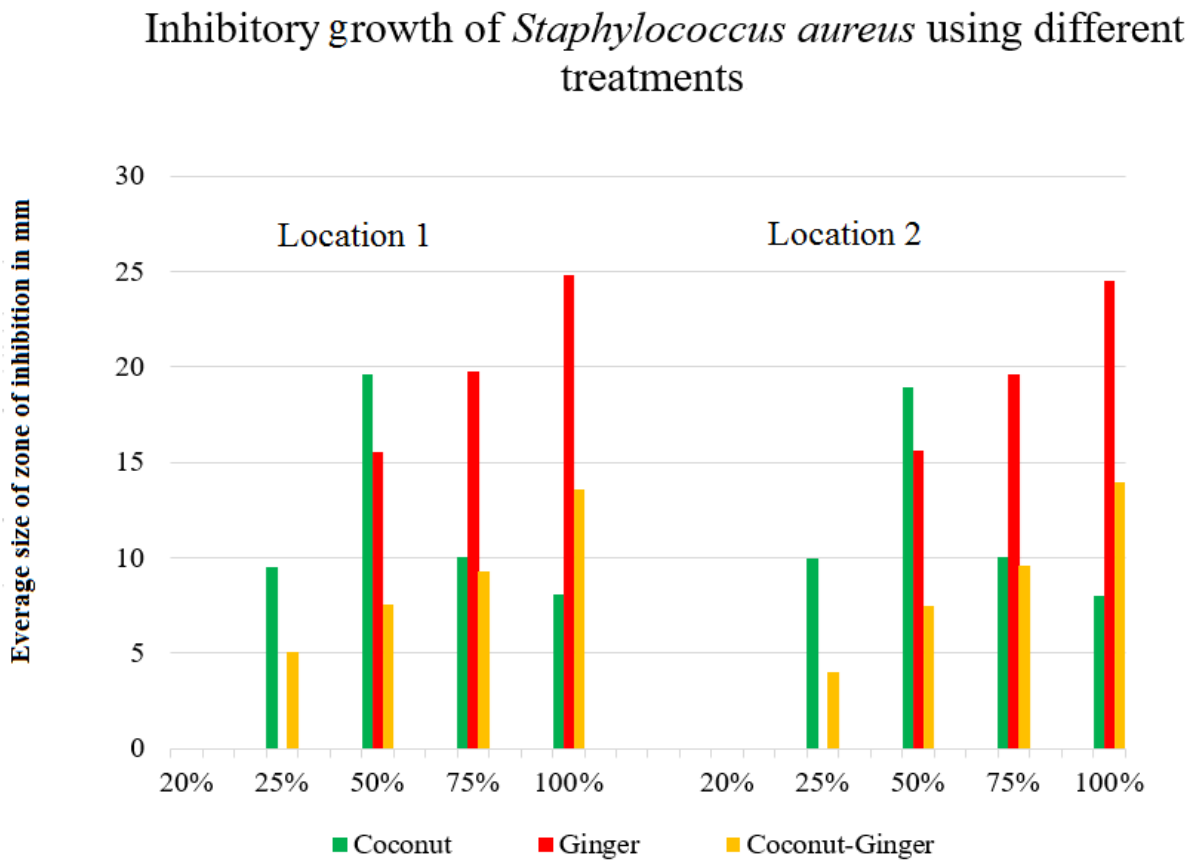

Figure 4. The comparison of the average zone of inhibition formed using ginger extract and hexane, coconut oil and hexane, and coconut + ginger extract and hexane in different concentrations from "location 1" and "location 2" against *Staphylococcus aureus.*
Note: No zone of inhibition was present when using the 20% & 25% concentrations.

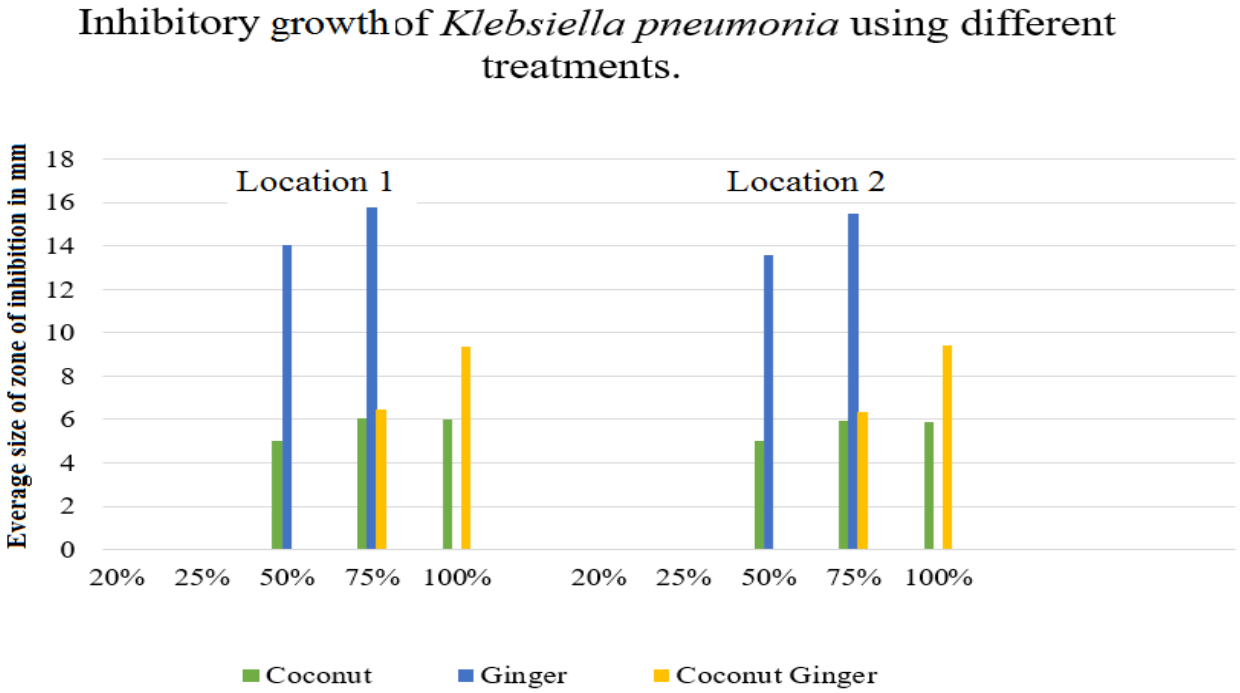

Figure 5. The comparison of the average zone of inhibition formed using ginger extract and hexane, coconut oil and hexane, and coconut + ginger extract and hexane in different concentrations from "location 1" and "location 2" against *Klebsiella pneumonia.*
Note: No zone of inhibition was present when using the 20% & 25% concentrations.

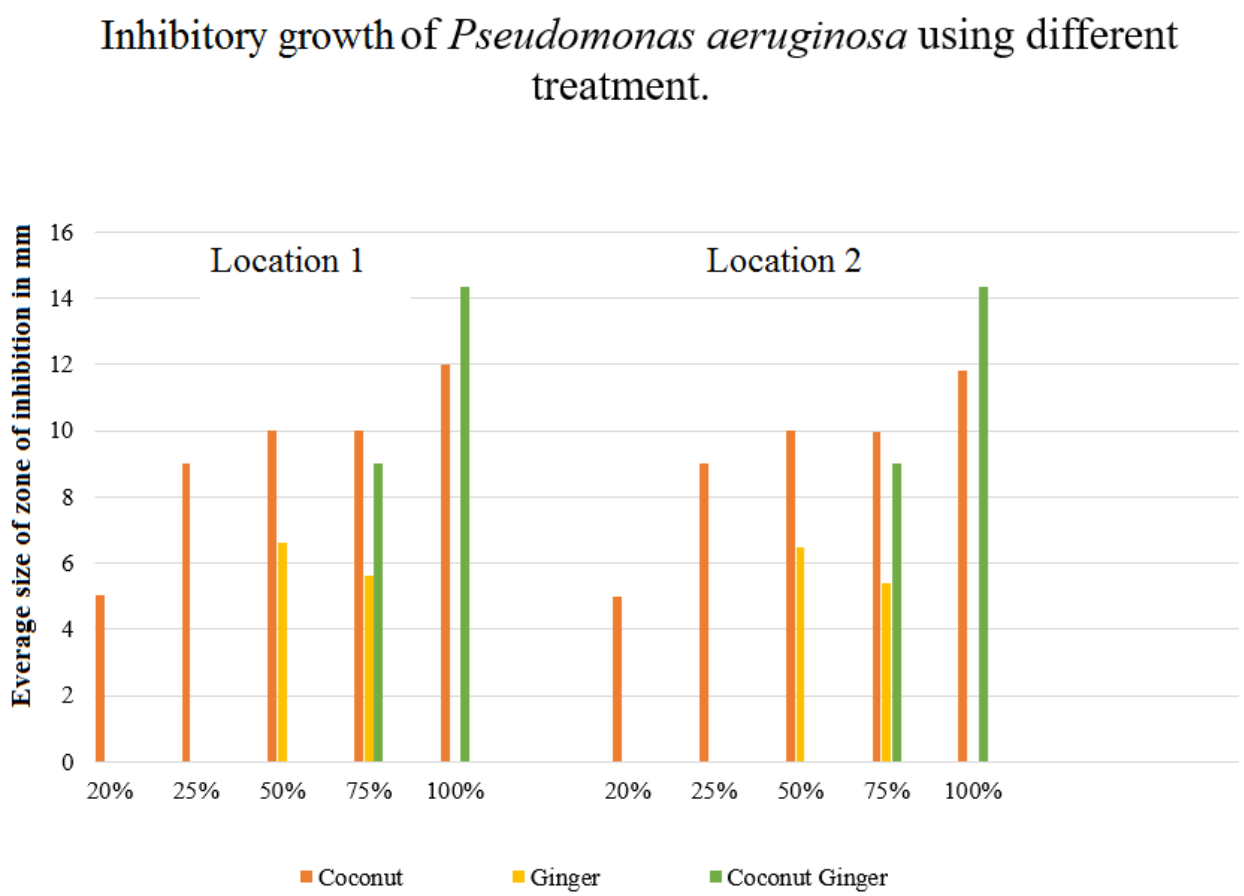

Figure 6. The comparison of the average zone of inhibition formed using ginger extract and hexane, coconut oil and hexane, and coconut + ginger extract and hexane in different concentrations from "location 1" and "location 2" against *Pseudomonas aeruginosa*.

Table 7. The secondary metabolites present or absent in crude ginger extract, coconut oil and a mixture of coconut oil and ginger. (L1- Location 1, L2- Location 2).

Phytochemical screening						
Secondary metabolites	**Crude ginger extract L1**	**Crude ginger extract L2**	**Coconut oil extract L1**	**Coconut oil extract L2**	**Coconut oil + ginger extract L1**	**Coconut oil + ginger extract L2**
Alkaloids	+	+	-	-	-	-
Saponins	+	+	+	+	-	-
Glycosides	-	-	+	+	+	+
Flavonoids	+	+	-	-	-	-
Volatile oils	-	-	+	+	+	+
Phenol	+	+	-	-	-	-
Steroids	-	-	-	-	-	-
Tannins	+	+	-	-	-	-

Note: (+) Presence of constituent , (-) Absence of constituent.

Table 7 shows the secondary metabolites present or absent in Crude ginger extract, coconut oil and a mixture of coconut oil and ginger. (L1- Location 1, L2- Location 2).

The plant extracts of ginger, coconut oil and a mixture of coconut oil and ginger were collected from two locations in triplicates. These plant extracts were investigated at five different concentrations of 100%, 75%, 50%, 25% and 20% using hexane as the solvent. The Area of the Zone of Inhibitions (AZOI) were used as indicators of the plant extracts antimicrobial activity against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Klebsiella pneumonia*. The data presented on Table 1 shows the zone of inhibition using the average zone of each of the three samples collected from location 1 when tested against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Klebsiella pneumonia* in different concentrations. The table displays that the Area of the zone of inhibition

when using coconut oil to test against *Staphylococcus aureus* was largest at the 50% concentration measuring 19.6mm, 75% concentration of coconut oil created average zone measuring 10mm, 25% concentration showed an average of zone measuring 9.93mm while 100% concentration of coconut oil showed zone measuring an average of 8.1mm and 20% concentration of coconut oil showed no zone of inhibition. 50% concentration of coconut oil worked best because the plant extract worked by dehydrating the proteins slowly and managed to diffuse in and under the cell wall of the gram positive organism then all the way through the organism which prevented the bacteria to grow in the area the treatment inhibited. With the organism being gram positive, it has a thick peptidoglycan cell wall but it is easily penetrable (Navarre & Schneewind, 1999). The organism was inhibited and formed zone considered susceptible because it measured >17mm. The same principle worked for 75% and 25% concentrations, however if the treatment was presented in higher dose than 25% and lower dose than 75% it would have worked better, 100% had a smaller zone than 50% , 75% and 25% because of the viscosity of the coconut oil , the oil was too thick to diffuse through the cell wall of the organism to denature it's proteins as it did in 25%, 75% and 50% concentrations, resulting in a smaller zone of inhibition (Sungpud, Panpipat, Chaijan, & Sae Yoon, 2020). However 20% concentration of coconut oil showed no zone of inhibition since the treatment was unable to penetrate the walls of the organism to prevent any growth. This is due likely to the possibility that the majority of hexane in the treatment may have caused the treatment to evaporate easily. Based on the standard reference of antimicrobial activity with resistant being 13mm or less, intermediate being 14-16mm and susceptible being 17mm or more, it is evident that *Staphylococcus aureus* is susceptible to 50% concentration of coconut oil and resistant to 100%, 75%, 25% and 20% concentrations. Coconut Oil collected from location 1 when tested against *Klebsiella pneumonia* as shown in Table 1 displays that there were no zones of inhibition when using 20% and 25% concentrations of coconut oil while zones measured an average of 5mm when using 50%, 6.03mm when using 75% and 6mm when using 100% concentration of coconut oil. It showed that the larger zones were present at 75% and 100% while 50% concentration of coconut oil has the smallest zone. All the zone of inhibition displayed with coconut oil from location 1 was used against *Klebsiella pneumonia* shows that the organism is resistant to the treatment since no zone measured >13mm. *Klebsiella pneumonia* is a gram-negative bacteria meaning it has three protective layers; outer membrane, peptidoglycan cell wall and inner membrane. *Klebsiella pneumonia* also possesses a capsules composed of complex acidic polysaccharides and these capsules are associated with the virulence of the organism and protects the bacteria from phagocytosis or when it is exposed to bactericidal serum factors. Due to the presence of the protective layers and the complex acidic capsule, the treatment was unable to severely denature the proteins present in the walls. This therefore caused the organism to created average inhibition of 5-6mm even in highest concentrations and organism is deemed resistant to the treatment. Table 1 shows, the antimicrobial activity of coconut oil collected from location 1 and tested against *Pseudomonas aeruginosa* in different concentrations. The table shows the area of inhibition of 20% concentration being 5.03mm, 25% concentration of coconut oil measuring 9mm, 50% and 75% of coconut oil concentration inhibited 10mm and 100% concentration produced zones that measured 12mm. As the concentration increased, the zone of inhibition were larger, with 12mm being the largest and 5.03mm being the smallest zone of inhibition. Like *Klebsiella pneumonia*, *Pseudomonas aeruginosa* is a gram negative bacteria and has three protective layers. The treatment of coconut oil surprisingly showed antimicrobial activity at all concentrations even though this organism is characterized by biofilm-mediated resistant. This treatment may have inhibited growth at all concentrations because it is postulated that the mechanism of lauric acid and monolaurin both present in coconut oil may inactivate the bacteria dissolving the lipid component present in bacterial cell membrane (Yang et al., 2018). However based on the antimicrobial activity zone of inhibition reference, even though antimicrobial activity was present, *Pseudomonas aeruginosa* is resistant to the treatment since no zone measured >13mm , this treatment may have great potential to be a possible treatment if tested in higher doses. Table 2 shows, the zone of inhibition using the average zone of each of the three samples collected

from location 2 when tested against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Klebsiella pneumonia* in different concentrations. Observations made during the experiment and also from looking at the table displayed at Table 2 and Figure 1, shows that despite the coconut collected to prepare the oil was collected from a different location where they were grown in a possible different soil type, weather patterns, temperature, etc. they showed the same antimicrobial activity after undergoing the same preparations. Phytochemical screening was done on the oils from both locations and they presented the same secondary metabolites where alkaloids, flavonoids, steroids and tannins were absent and saponins, glycoside, volatile oils and phenol were present.

Ginger was collected in triplicates from two locations and with each sample, crude ginger extract was prepared in concentrations of 100%, 75%, 50%, 25% and 20% using hexane as the base solvent. The crude ginger extract in different concentrations was tested against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Klebsiella pneumonia* in triplicates. The zone of inhibitions was measured and the Area of the Zone of Inhibition was calculated and placed into Table 3. The table displays an ascending activity with increase concentrations. *Staphylococcus aureus* showed an average zone measuring 24.83mm when 100% concentration was used, 75% ginger extract concentration showed the mean value of zones being 19.73mm, 50% concentration formed zones with an average of 15.5mm while 25% and 20% concentrations showed no antimicrobial activity. With the size of the zones achieved at 50%, 75% and 100% concentrations, it shows that *Staphylococcus aureus* is sensitive to the treatment of crude ginger extract. 50% - 100% of crude ginger plant extract concentration worked by dehydrating the proteins slowly and managed to diffuse in and under the cell wall of the organism then all the way through the organism which prevented the organisms to grow in the area the treatment inhibited, the organism is also gram positive and even though it has a thick peptidoglycan cell wall, it is penetrable (Navarre & Schneewind, 1999). 50% extract formed an intermediate zone of inhibition measuring <17 but >13mm, while 75% and 100% concentrations formed susceptible zones when tested against *Staphylococcus aureus*. Crude ginger extract in 100%, 75%, 50%, 25% and 20% concentrations were tested against *Klebsiella pneumonia*, the trends observed in Table 3 shows, that there was no zone of inhibition present at 20%, 25% and 100% concentrations while the zone was largest at 75% measuring 15.76mm and showed a measurement of 14.03mm at 50%. The 100%, 25% and 20% concentrations formed no zones due to the 100% concentration's viscosity, the 100% concentration was too thick and was unable to pass through the three protective layers of the organism to break down the protein, while the 20% and 25% did not work due to the hexane present in the extract and may have caused the treatment to evaporate before it can begin to denature the bacteria protein. Based on the zones present when compared to the antimicrobial activity standard reference it showed that *Klebsiella pneumonia* shows intermediate sensitivity at 50% and 75% concentrations while it is resistant to 100%,25%,20% crude ginger extract. When the treatment of crude ginger extract was tested against *Pseudomonas aeruginosa*, antimicrobial activity was present at 75% measuring 5.6mm and 6.63mm at 50%. Although there were antimicrobial activity present, the size of the zones did not qualify to the organism being sensitive to the treatment, at all concentrations the organism was resistant to crude ginger extract. This is possible since *Pseudomonas aeruginosa* is characterized by biofilm-mediated resistant and the organism also has three protective layers. However, the components associated with the susceptibility of the test of any organism to ginger are not fully determined but may be related to phytochemicals or secondary metabolites that may be present in ginger. Ginger collected in triplicates from location 2 was also tested in triplicates against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Klebsiella pneumonia* in different concentrations. It was observed and recorded that when 20% and 25% concentration was tested against *Staphylococcus aureus*, no zone of inhibition was present , hence the organism was resistant to treatment at the concentrations , however sensitivity was shown when using 50% , 75% and 100% concentrations which gave zone measuring 15.6mm, 19.63mm and 12.53mm respectively. The gram negative bacteria with three protective layers; *Klebsiella pneumonia* was resistant to treatment at concentrations 100%, 25% and 20% showing no zone of inhibition, while the organism showed

intermediate sensitivity against 50% measuring 13.65 mm and 75% measuring 15.5mm. Despite *Pseudomonas aeruginosa* being resistant to all concentrations, antimicrobial activity was present at concentrations 50% measuring 6.5mm while 75% measured 5.4mm. When comparing the size of the zones present when using the treatments from different locations, it is safe to say that there are no difference based on the size of the zones based on location. However, the components associated with the susceptibility of the test organisms to ginger are not fully determined but may be related to phytochemicals or secondary metabolites that may be present in ginger (Mao et al., 2019). When phytochemical screening was done for ginger from location 1 and 2, alkaloids, saponins, flavonoids, phenol and tannins were present in the crude ginger extract while glycosides, volatile oils and steroids were absent.

Coconut and ginger collected from location 1 was mixed at a 1:1 ratio then concentrations of 100%, 75%, 50%, 25% and 20% were formed. These different concentrations were tested in triplicates against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Klebsiella pneumonia*. Even though antimicrobial activity was present against *Staphylococcus aureus* at concentrations 25% measuring an average zone of 5.06mm, 50% forming a zone of 7.53mm and 75% creating zones measuring 9.3mm, the organism was resistant to the treatment in those concentrations according to the antimicrobial activity reference standard. 20% concentration of coconut and ginger showed no antimicrobial activity while *Staphylococcus aureus* deemed intermediately sensitive to the treatment after 100% concentration formed inhibition zone measuring 13.96mm. At all concentrations, coconut and ginger mixture was able to penetrate the cell wall of the organism, denaturation the protein and forming the zone of inhibition, however if treatment is tested in higher doses or other concentrations , it may work better as a multi-plant extract. When this mixture was tested at all five concentrations in triplicates against *Klebsiella pneumonia*, results obtained that are displayed in Table 5 shows, that there was no antimicrobial activity at 20%, 25% and 50% concentrations while 75% produced zones measuring 6.6mm and 100% concentration formed zones measuring 9.3mm. Although antimicrobial activity was present, when measurements were compared to reference standard, the organism was deemed resistant to the treatment. *Pseudomonas aeruginosa* was intermediately sensitive to the 100% concentration of coconut ginger treatment producing zone measuring 14.3mm while 75% concentration produced average zones of 9.23mm and 20%, 25% and 50% displayed no antimicrobial activity. Three sample of a mixture of coconut and ginger were collect from location 2 and tested against three organism in triplicates at five concentrations. The zones produced were very similar of those collected from locations 1. The treatment produced zone of 13.96mm, 9.6mm, 7.46mm, 4.43mm and 0mm to 100%, 75%, 50%, 25% and 20% respectively against *Staphylococcus aureus*. The growth of *Klebsiella pneumonia* was inhibited by zone measuring 6.36mm and 9.4mm at 75% and 100% respectively, while there were no antimicrobial activity at 20%, 25% and 50%. Coconut ginger mixture at 100% produced zone measuring 14.33mm and deemed *Pseudomonas aeruginosa* intermediately sensitive while 75% concentration produced 9mm average zone measurement and 20%, 25% and 50% showed no antimicrobial activity. *Staphylococcus aureus* is a gram positive bacterial while *Klebsiella pneumonia* and *Pseudomonas aeruginosa* are gram negative bacteria with *Pseudomonas aeruginosa* being biofilm mediated. The mixture of coconut and ginger worked best on the *Staphylococcus aureus* since the treatment was easily diffused through the cell wall while it was more difficult to diffuse through the three protective layers of the *Klebsiella pneumonia* and *Pseudomonas aeruginosa*. This mixture worked better against the *Pseudomonas aeruginosa*, than *Klebsiella pneumonia* since there was a ratio of oil present in the mixture and coconut oil work against *Pseudomonas aeruginosa* since lauric acid and monolaurin both present in coconut oil may inactivate bacteria dissolving the lipid component present in bacterial cell membrane (Nasir, Abllah, Azura Jalaludin, Azura Shahdan, & Hayati Wan Abd Manan, 2018). Significant inhibitory effect was not shown when using a mixture of the two plant extract. This could be due to the fact that the plants did not contain enough secondary compounds with active antibacterial activity when combined and tested against these pathogens. This antimicrobial activity was moderately low, which may possibly be related to the fact that

when phytochemical screening was carried out, the mixture only had the presence of glycosides and volatile oils while there were no presence of alkaloids, flavonoids, saponins, phenol, steroids and tannins.

Figure 1 shows that coconut oil + hexane treatment produced the largest zones against *Staphylococcus aureus*, it however did not work in all concentrations. This treatment showed antimicrobial activity against *Pseudomonas aeruginosa* in all concentrations and very little antimicrobial activity against *Klebsiella pneumonia*. Based on overall antimicrobial activity, *Staphylococcus aureus* is susceptible to the treatment while *Pseudomonas aeruginosa* and *Klebsiella pneumonia* are resistant. The graph also displays the comparison of the zones of inhibition produced with coconut oil collect from the different locations. Based on the graph and with the p-value being 0.250 which is greater than 0.05 we fail to reject the null hypothesis and conclude there is no significant difference no the antimicrobial effects of coconut oil collected from locations 1 and 2 when tested against organisms. Figure 2 shows that crude ginger extract + hexane treatment produced the largest zones against *Staphylococcus aureus*, it however did not work at all concentrations. Crude ginger extract at different concentrations showed the second largest zones when tested against *Klebsiella pneumonia* and very little antimicrobial activity was displayed against *Pseudomonas aeruginosa*. *Staphylococcus aureus* deemed susceptible to the treatment, *Klebsiella pneumonia* is intermediately sensitive to this treatment and *Pseudomonas aeruginosa* is resistant to the treatment. The graph displays the comparison of ginger extract obtained for location 1 and 2 and the size of the area inhibited by each at a specific concentration. According to the graph and with the p-value being 0.250 which is greater than 0.05 we fail to reject the null hypothesis and conclude there is no significant difference no the antimicrobial effects of crude ginger extract collected from locations 1 and 2 when tested against organisms. Figure 3 shows that a mixture of coconut and ginger + hexane treatment produced the largest zones against *Pseudomonas aeruginosa*, it however only worked at a concentration of 100%. Coconut ginger + hexane at different concentrations showed the second largest zones when tested against *Staphylococcus aureus* and very little antimicrobial activity was displayed against *Klebsiella pneumonia*. *Pseudomonas aeruginosa* and Staphylococcus *aureus* deemed intermediately sensitive to the treatment, while *Klebsiella pneumonia* is resistant to the treatment. The graph displays the comparison of coconut ginger extract obtained for location 1 and 2 and the size of the area inhibited by each at a specific concentration. According to the graph and with the p-value being 0.250 which is greater than 0.05 we fail to reject the null hypothesis and conclude there is no significant difference no the antimicrobial effects of coconut ginger extract collected from locations 1 and 2 when tested against organisms.

Figure 4 shows the comparison of zone of inhibition of each treatment at a different concentration level (100%, 75%, 50%, 25% or 20%). It shows that at different concentration of each of the treatment (coconut oil, ginger extract or coconut-ginger) against *Staphylococcus aureus*, the zone of inhibition differs. According to statistical analysis the concentration levels were found to be statistically significant with a significant level of 0.00 (p-value). This implies that the antimicrobial activity varied significantly across the different concentration levels. Figure 5 shows the comparison of zone of inhibition of each treatment at a different concentration level (100%, 75%, 50%, 25% or 20%). It shows that at different concentration of each of the treatment (coconut oil, ginger extract or coconut-ginger) against *Klebsiella pneumonia*, the zone of inhibition differs. According to statistical analysis the concentration levels were found to be statistically significant with a significant level of 0.00 (p-value). This implies that the antimicrobial activity varied significantly across the different concentration levels. Figure 6 shows the comparison of zone of inhibition of each treatment at a different concentration level (100%, 75%, 50%, 25% or 20%). It shows that at different concentration of each of the treatment (coconut oil, ginger extract or coconut-ginger) against *Pseudomonas aeruginosa*, the zone of inhibition differs. According to statistical analysis the concentration levels were found to be statistically significant with a significant level of 0.00 (p-value). This implies that the antimicrobial activity varied significantly across the different concentration levels.

Figure 4, 5 and 6 is also showing the size of the zones of inhibition formed when the treatments were used at different concentration for each location. It is evident from the graphs with the use of different concentration at each location that the zone of inhibition did not vary. Statistically, the interaction of concentration and location was found to be insignificant with a p-value of 0.431. The antimicrobial activity did not vary with the interaction of these two variable. All the tables and graphs above displays the measure of the zone of the inhibition formed when either ginger extract, coconut oil or coconut ginger mixture was used. The presence of a value for the Zone of Inhibition (ZOI) indicates that these treatments have antimicrobial effects. It was shown that each treatment reacted differently with each organism and that the antimicrobial effects varied based on the organism the treatments were used on. Statistically, the p-value of 0.000 indicates significance and implies that the antimicrobial activity varies based on the treatment and organism used. The interaction of concentration and treatment and concentration an organism were both found to be statistically significant with recorded p-values of 0.000 and 0.000 respectively, so the antimicrobial activity varied across levels of the interaction of concentration and treatment and concentration an organism. The interaction of Location and treatment and location and organism was found to be statistically insignificant, p-values for those interactions were recorded as 0.085 and 0.135 respectively, meaning that the antimicrobial effects does not vary with the interaction of location and treatment and the interaction of location and organism. The interaction of treatment and organism and concentration, treatment and organism were all found to be statistically significant, both interactions had recorded p-values of 0.000 , this indicated that the antimicrobial effect varies based on the interactions of treatment and organism and organism and concentration, treatment and organism. All other interactions containing location were found to be statistically insignificant.

4. CONCLUSION

According to the results obtained from this research the researcher observed the following findings; the crude ginger extract was tested to be most effective against *Staphylococcus aureus* and least effective against *Pseudomonas aeruginosa*. The coconut oil extract was tested to be most effective against *Staphylococcus aureus* and least effective against *Klebsiella pneumonia*. The combination of coconut oil and crude ginger extract was tested to be most effective against *Staphylococcus aureus* and least effective against *Klebsiella pneumonia*. When using 100% crude ginger extract against *Staphylococcus aureus*, the largest zone (23mm) was formed when using this treatment. When using 50% of the coconut oil extract against *Staphylococcus aureus*, the largest zone (18mm) was formed using this treatment. When using 100% crude ginger extract mixed with coconut oil against *Pseudomonas aeruginosa*, the largest zone (15mm) was formed when using this treatment. It concludes that the treatments tested against the organism showed antimicrobial activity and that they possess great potential as antimicrobial agents. The researcher however suggests for the best results to be obtained, a wider range of solvent should be used plant extracts should be tested against fungus in order to do a comparison against bacteria and the use of pure ginger extracts.

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Authors' Contributions: All authors contributed equally to the conception and design of the study.

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