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Synergic antibacterial activity of honey-garlic versus honey-ginger on *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* isolated from acute wound infection in Guyana, South America

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Abstract

This study compared the antibacterial activity of honey-garlic and honey-ginger mixtures against two clinically important bacteria frequently implicated in acute wound infections; *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. Individual extracts of honey, ginger, garlic and erythromycin antibiotics (positive control), were also tested. Concentrations of 100%, 75%, 50%, and 25% of the natural extracts and mixtures were prepared by the use of sterilized distilled water as the solvent. The antibacterial activity of each natural extract and the antibiotic was tested against the aforementioned bacterial species at different concentrations using the disc diffusion method. Further, MIC and MBC tests were conducted on each natural mixture using the broth dilution method and spread plate method, respectively. Our results showed garlic exhibited the highest antibacterial activity against both bacteria at 100% concentration, whereas honey and ginger showed the lowest antibacterial activity against *Klebsiella pneumoniae* and *Pseudomonas aeruginosa,* respectively at 25% concentrations. Honey-garlic mixture was the most effective against *Klebsiella pneumoniae*, whereas honey-ginger mixture was the most effective against *Pseudomonas aeruginosa*. The antibiotic was more effective than the natural mixtures against *Pseudomonas aeruginosa*, but not so effective against *Klebsiella pneumoniae*. The MIC of both mixtures against *Pseudomonas aeruginosa* ranged from 25%-50%, while it ranged from 6%-50% for *Klebsiella pneumoniae*. Honey-garlic was the only mixture against *Klebsiella pneumoniae* that exhibited bactericidal effects at 50% (MBC).

Keywords: Antibacterial; Antibiotic resistance; Aqueous extract; *Klebsiella pneumoniae*; *Pseudomonas aeruginosa*

1. Introduction

Based on their etiology, wounds can be divided widely into acute and chronic wounds. Acute wounds are most often caused by conditions such as trauma or burns (Vachhrajani & Khakhkhar, 2019). Acute wounds, if the proper care is required, can usually recover in a short time. In wound healing, quick and full wound healing is often necessary as the resulting scar tissue would be more satisfactory (Vachhrajani & Khakhkhar, 2019). An infected wound is a deformity or excavation of localized skin during which pathogenic organisms have invaded into viable tissue surrounding the laceration (Everts, 2018). In addition to delaying the healing process, infection of the wound induces the body's response, causing inflammation and tissue damage. Additionally, it causes increased pain, irritation and inconvenience, which has deleterious effects on patients and may contribute to life-threatening diseases or even death (Morcandetti & Molnar, 2019). It also delays the healing process, leading to longer hospital visits and higher costs of care in terms of antibiotics, dressings and staff time (Morcandetti & Molnar, 2019).

With advancements in medicine, wound care is continually changing. As wound care practitioners are faced with many obstacles, the quest for the right dressing substance also continues (Daley & Panthaki, 2020). Due to the increase in

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multidrug-resistant infections and the decline of new antibiotics, wound care specialists have turned to traditional and complementary wound treatment therapy to revive historic healing methods (Daley & Panthaki, 2020). The science of pharmacognosy and the application of medicinal plants are known as herbal medicine (also herbalism) (Merrills & Fisher, 2013). Throughout most of human history, plants have formed the basis for medical treatments, and herbal medicine is still frequently used today. Many plant-derived compounds are used in modern medicine as the cornerstone for evidence-based pharmaceutical medicines (Merrills & Fisher, 2013). The financial cost and life-threatening side effects of conventional antibiotics can be greatly minimized with the introduction of complementary and traditional wound treatment drugs (Che & Marobela, 2017). As it is readily accessible in our kitchen, the use of natural remedies is also very convenient to reach and inexpensive (Che & Marobela, 2017).

The use of traditional medicine to treat wound infection in Guyana has won its place in history; there is a snapshot of what nature has provided for a billion years across its 80 percent unspoiled rainforest, extending through wetlands, plains, valleys and mountains of Guyana, there is a biodiversity supply of thousands of native flora and fauna to benefit from, ecosystems to understand and to preserve (Allicock, 2019). With the healing plants used by shamans, the healers of our ancestral people of the rainforest, many mysteries and untold riches await discovery. In a relatively small but diverse country, Guyana offers a botanical wonder amid magnificent scenery (Allicock, 2019). There is no denying that conventional medications typically provide successful antibiotic treatment for bacterial infections, but there is a growing issue of antibiotic resistance, dramatic side effects, a reduced rate of development of new drugs and a persistent need for new solutions (Allicock, 2019). Natural remedies, specifically honey, garlic and ginger, which are readily available in the markets around Guyana and are readily prepared in one's kitchen, can be the solution to this dilemma (Allicock, 2019). Honey is an old remedy that has lately been rediscovered by the medical community, particularly in cases where standard contemporary therapeutic agents have failed to heal infected wounds (Mandal & Mandal, 2011). Honey's antibacterial properties are due to the presence of enzymatically synthesized hydrogen peroxide and acidity created by two processes (glucose and oxygen that chemically react to form gluconic acid) (Mandal & Mandal, 2011). Another antibacterial effect of honey is due to the influence of osmolarity, which inhibits microorganisms. Honey's high sugar content binds water, preventing bacteria from multiplying since they would lack enough water to survive. Honey with an acid pH of 3.2-4.5 is used to inhibit pathogenic bacteria and increase wound healing rates through epithelization (Mandal & Mandal, 2011). Garlic has long been known to have antibacterial, antifungal, anticancer, and antiviral properties (Strika *et al.,* 2016). The oxygenated sulfur molecule thio-2-propene-1-sulfinic acid S-allyl ester, often known as allicin, has long been recognized as garlic's major antibacterial component (Strika *et al.,* 2016). Allicin (diallylthiosulfinate) has a broad antibacterial spectrum against both gram-positive and gram-negative bacteria, including antibiotic resistant species. Allicin reacts with groups of thiols and can inactivate important enzymes (Strika *et al*., 2016). Ginger has a direct antimicrobial effect and is used to treat bacterial infections. Ginger is generally inexpensive and universally acceptable by most individuals due to its convenient availability. It is also "Generally Recognized as Safe" by the US Food and Drug Administration (Rahmani *et al*., 2014).

As a preliminary step in the development of safe and reliable natural remedies against acute wound infections in Guyana, this research identified and compare the antibacterial activity of honey-garlic and honey-ginger mixtures against two clinically important bacteria frequently implicated in acute wound infections; *Pseudomonas aeruginosa* and *Klebsiella pneumoniae.* Individual extracts of honey, ginger, garlic and erythromycin antibiotic (positive control) were tested. This study investigated the lowest concentration of aqueous extract of honey-garlic and honey-ginger mixtures needed to inhibit the growth and survival of those strains.

2. Material and methods

2lb Raw Garlic, 2lb Raw Ginger, 100 mL Pure Honey, Barium Chloride ($BaCl_2$), Distilled Water, Sodium Chloride (NaCl), Sulphuric Acid (H_2SO_4), 500g Mueller Hinton Agar Powder, 500g Nutrient Broth Powder

2.1. Bacterial Strains

Pseudomonas aeruginosa and *Klebsiella pneumonia* were used in this study.

2.2. Maintenance of Bacterial Culture

The pure cultures of each bacterium were sub-cultured on nutrient agar Petri-dishes two days before the commencement of the antibacterial sensitivity testing. The cultures were streaked on sterile Mueller Hinton agar Petri dishes which were subjected to incubation for twenty-four hours at 37˚C in an incubator. The subcultures were stored at 4˚C in the refrigerator.

2.3. Preparation of Aqueous Garlic Extract

2 lbs of matured fresh garlic bulbs and ginger rhizomes were purchased from Stabroek Market, Georgetown. The bulbs were peeled, weighed (100 grams) and cleaned. The clean cloves were grated and filtered through sterile cheesecloth to achieve 100% extract. The extract was further diluted to make different concentrations which included 75%, 50% and 25%. Each concentration of the extract was made to have a volume of 10 mL. The dilutions were prepared by diluting the crude extract with corresponding volumes of distilled water using the C1V1=C2V2 formula. The aqueous extracts of each concentration were separately stored in glass vials with secured caps, and labelled. Using a 5mm paper puncher, Whatman discs were punched, after which they were sterilized in the autoclave at 121°C for 15 minutes at 12 Psi, and inserted into each vial. The glass vials were then stored in a refrigerator at 4˚C (Ewnetu *et al*., 2014).

2.4. Preparation of Aqueous Ginger Extract

2 lbs of ginger rhizomes used was purchased from Stabroek Market, Georgetown. The ginger rhizomes were washed with clean sterile distilled water and allowed to air-dry for one hour. The outer covering was manually peeled off, washed again and extracted using the following method: The ginger rhizomes were grated. The grated ginger was then filtered using the sterile cheesecloth. The resulting juice was considered as the 100% concentration of the extract. The extract was further diluted to make different concentrations which included 75%, 50% and 25%. Each concentration of the extract was made to have a volume of 10 ml. The dilutions were prepared by diluting the crude extract with corresponding volumes of distilled water using the C1V1=C2V2 formula. The aqueous extracts of each concentration were separately stored in glass vials with secured caps, and labelled. With the use of a 5mm paper puncher, Whatman discs were punched, after which they were sterilized in the autoclave at 121°C for 15 minutes at 12 Psi, and inserted into each vial. The glass vials were then stored in a refrigerator at 4˚C (Ewnetu *et al*., 2014).

2.5. Filtering and Dilution of Honey

The honey used in this study was obtained from Airy Hall Mahaicony, Region 5, Guyana. The honey sample was first of all filtered with sterile mesh to remove debris to obtain 100% pure honey. The honey was further diluted to make different concentrations which included 75%, 50% and 25%. Each concentration of the extract was made to have a volume of 10 ml. The dilutions were prepared by diluting the honey with corresponding volumes of distilled water using the C1V1=C2V2 formula. The aqueous extracts of each concentration were separately stored in glass vials with secured caps, and labelled. With the use of a 5mm paper puncher, Whatman discs were punched, after which they were sterilized in the autoclave at 121°C for 15 minutes at 12 Psi, and inserted into each vial. The glass vials were then stored in a refrigerator at 4˚C (Ewnetu *et al*., 2014).

2.6. Preparation of Honey-Garlic Mixture

The pure honey and garlic extracts were used in the preparation of the honey-garlic mixture. The mixture was prepared in a 50:50 ratio. A honey-garlic stock was made by adding 10 ml of honey extract with 10 ml of garlic extract in a test tube. The honey and garlic extracts were then stirred with a stirring rod. From the honey-garlic stock, honey-garlic mixtures were made in four (4) dilutions: 100%, 75%, 50% and 25%. The 100% dilution was prepared by adding 10 ml of the stock into a glass vial. The 75% dilution was prepared by mixing 7.5 ml of the stock with 2.5 ml distilled water. The 50% dilution was prepared by mixing 5 ml of stock with 5 ml of distilled water. The 25% dilution was prepared by mixing 2.5 ml of the stock with 7.5 ml distilled water. The aqueous extracts of each concentration of the mixture were separately stored in glass vials with secured caps and labelled. With the use of a 5mm paper puncher, Whatman discs were punched, after which they were sterilized in the autoclave at 121°C for 15 minutes at 12 Psi, and inserted into each vial. The glass vials were then stored in a refrigerator at 4˚C (Ewnetu *et al*., 2014).

2.7. Preparation of Honey-Ginger Mixture

The pure honey and ginger extracts were used in the preparation of the honey-ginger mixture. The mixture was prepared in a 50:50 ratio. A honey-ginger stock was made by adding 10 ml of honey extract with 10 ml of ginger extract in a test tube. The honey and ginger extracts were then stirred with a stirring rod. From the honey-ginger stock, honey-ginger mixtures were made in four (4) dilutions: 100%, 75%, 50% and 25%. The 100% dilution was prepared by adding 10 ml of the stock into a glass vial. The 75% dilution was prepared by mixing 7.5 ml of the stock with 2.5 ml distilled water. The 50% dilution was prepared by mixing 5 ml of stock with 5 ml of distilled water. The 25% dilution was prepared by mixing 2.5 ml of the stock with 7.5 ml distilled water.

2.8. Preparation of Medium

2.8.1. Mueller-Hinton Agar

300 g of Muller-Hinton agar was weighed on the electronic balance, and added into a conical flask, to which thereafter 1000 mL of distilled water was added and the stopper was inserted. The liquid was gently stirred before being placed on a heated plate for roughly 12 hours, allowing the agar to dissolve further. The flask was spun at regular intervals, and the pressure build-up within the flask was reduced by removing the cap. When handling the agar on the hot plate, heat-insulating gloves were employed. When bubbles appeared at the bottom of the flask, the solution was taken from the hot plate and sterilized in an autoclave at 121°C for fifteen minutes at 12 Psi. (Final pH: 7.3+/-0.1 at 25°C). After the solution was autoclaved, it was cooled to 55 ºC by swirling it under running tap water before being deposited into Petri plates (Ewnetu *et al*., 2014)

2.8.2. Nutrient Agar

8 g of nutrient broth powder was weighed on an electronic balance, and added into a conical flask, to which thereafter 600 mL of distilled water was added and the stopper was inserted. The mixture was gently stirred and then placed on a hot plate for about 1/2 hour, thus allowing the nutrient broth to dissolve further. The flask was spun at regular intervals, and the pressure build-up within the flask was reduced by removing the cap. When handling the nutrient broth on the hot plate, heat-insulating gloves were employed. When bubbles appeared at the bottom of the flask, the solution was taken from the hot plate and sterilized in an autoclave at 121°C for fifteen minutes at 12 Psi (Final pH: 6.8 +/-0.2 at 25°). After the solution was autoclaved, it was cooled to 55 ºC by swirling it under running tap water. 2 mL of the broth was then poured into 27 clean test tubes and capped using cotton wool. The capped test tubes were then stored in the refrigerator at 4 ºC for later use in conducting the Minimum Inhibitory Concentration (MIC) tests (Ewnetu *et al*., 2014).

2.9. Preparation of Culture Suspension

A sterile saline solution was used for the preparation of culture suspension. Saline is a 0.9% sodium chloride solution. A total volume of 200 mL was required for the number of test tubes arranged. 200 mL of distilled water was added to 1.8 g of 100% NaCl to prepare 200 mL of 0.9% NaCl. 10 ml of the 0.9% saline solution was poured into each test tube. The test tubes containing the saline solution were autoclaved for approximately 45-60 minutes.

2.10. Preparation of 0.5 MacFarland Standards

To ensure that the appropriate number of microbial organisms were allocated to the agar plates, a broth standard known as the 0.5 McFarland standard (with a Concentration of 1.5 x 10^8 CFU/mL) was used. The preparation of the McFarland Standard was done under the bio-safety cabinet to prevent contamination. In keeping with good aseptic techniques, the biosafety cabinet was swabbed with 70% ethanol with the cotton wool; and to aid in further sterilization, the ultra violet light was turned on for 20 minutes. The spirit lamp was then lit, after which the cabinet was turned on. An electronic balance was used to weigh 85 ml of 1% sulphuric acid, which was then poured into a conical flask. With the use of a volumetric pipette, 0.5 ml of 1.1175% barium chloride was added to the conical flask. Soon after, 100 ml of 1% sulphuric acid was added to the conical flask, and it was stirred with the stirring rod until no clumps were visible. Following that, 10 ml of the barium sulphate precipitate was poured into two test tubes and covered with cotton wool. The test tubes were securely wrapped in aluminum foil and kept at room temperature to minimize evaporation (Ewnetu *et al*., 2014).

2.11. Antibacterial Assay of Natural Extracts and Mixtures

An inoculating loop was flamed red-hot and then cooled. It was then utilized to gently remove a small amount of bacteria colony from the *Klebsiella pneumoniae* subculture, which was then inserted in one of the test tubes containing 10 ml sterilized distilled water. NB: The tube was flamed after removing and reapplying the stopper. The test tube was gently shaken from side to side, which allowed the bacteria to distribute more uniformly throughout the fluid. After that, it was compared to the McFarland turbidity solution against a Wickerham card. As long as the content of the prepared tube appeared similar to that of the McFarland solution the test tube was labeled *Klebsiella pneumonia* with the use of a marker; this was the bacterial inoculum. The steps above were repeated for *Pseudomonas aeruginosa*. After drying, the plates were sterilized under UV light for 20 minutes. The plates were then labeled with the researcher's initials, date, type of agar, name of bacteria, the specific concentration of the natural extracts (either 100%, 75%, 50% or 25%), and the plate number (either plate 1, 2 or 3 since the experiments was done in triplicates). Using the aseptic technique, a sterile cotton tip applicator was placed into the inoculum of *Klebsiella pneumoniae*, and the excess liquid was carefully removed by gently rotating the cotton tip applicator against the inside of the tube. Using the applicator, the agar plates labeled "*klebsiella pneumoniae"* was streaked to form a bacterial lawn. To obtain uniform growth, the plate was streaked

with the applicator in one direction, then the plate was rotated at 90° and streaked again in that direction. The rotation of the plate was repeated three (3) times. The plate was then allowed to dry for approximately 5 minutes. The vials containing the honey, garlic, ginger, honey-garlic and honey-ginger mixtures at (100%, 75%, 50% and 25% concentrations) were then obtained. A forcep was flamed, allowed to cool, and used to pick up Whatman discs from the 100% concentration of honey, garlic, ginger, honey-ginger and honey-garlic vials, which were placed into the appropriate plates. The discs were gently pressed unto the agar by use of flame-sterilized forceps to ensure that it was attached to them. The same steps were repeated for the 75%, 50% and 25% concentrations of the natural extracts and mixtures. For each concentration, four Whatman discs (inoculum) were administered to a plate, which was labeled as A, B, C and D. The steps above were repeated for *Pseudomonas aeruginosa*. For the negative controls, Whatman discs soaked in sterilized distilled water were used instead of the treatments, where one Whatman disc was inserted into each plate. All the plates were then taped and incubated inverted at 37 °C (98.6 °F) for 24 hours. Inhibition zones were indicated by a clear area around the discs which was measured in millimeters by the use of a ruler to evaluate the degree of susceptibility of the test organisms. NB: These studies were done in triplicates (plates 1, 2 and 3) for each concentration of the natural extracts and mixtures (Ewnetu *et al.,* 2014).

2.12. Antibiotic Sensitivity Testing

NB: The Antibiotic Sensitivity Test was done in duplicates (plates 1 and 2 for each bacteria). The test was done using the Kirby-Bauer disk diffusion method, and a commercially available antibiotic disc- Erythromycin (ER15), was used for this study. After comparing with MacFarland Standard, a sterile cotton tip applicator was placed into the inoculum of the *Pseudomonas aeruginosa* culture, and the excess liquid was carefully removed by gently rotating the swab against the inside of the tube. Using the applicator, the two agar plates were streaked to form a bacterial lawn. To obtain uniform growth, the plate was streaked with the swab in one direction, then the plate was rotated at 90° and streaked again in that direction. The rotation of the plate was repeated three (3) times. The plate was then allowed to dry for approximately 5 minutes. The antibiotic (erythromycin) disc was then dispensed onto the plates by use of flame-sterilized forceps. One disc was inserted into the center of each plate. The forceps were then used to gently press the discs onto the agar to ensure that it was attached to it. The steps above were repeated for *Klebsiella pneumoniae.* The plates were then taped and incubated inverted at an incubation temperature of 37 °C (98.6 °F) for 24 hours. Inhibition zones were indicated by a clear area around the discs which was measured in millimeters by the use of a ruler to evaluate the degree of susceptibility of the test organisms. (Ewnetu *et al*., 2014).

2.13. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the natural mixtures (honey-garlic and honey-ginger)

The Minimum Inhibitory Concentration and Minimum Bactericidal Concentration were done by the broth dilution method. Extracts of honey-garlic and honey-ginger mixtures were diluted to prepare concentrations of 1.5%, 3%, 6%, 12.5%, 25% and 50% with the use of sterilized distilled water. The formula C1xV1=C2xV2 was utilized for the dilutions. The 27 test tubes containing the 2 ml broth were obtained and labeled with the date, name of bacteria, type of treatment, the specific diluted concentrations and the type of broth. The bacteria inoculum was then prepared and compared with MacFarland standard as outlined above. The 27 test tubes containing the nutrient broth were then inserted into the biosafety cabinet and with the use of aseptic techniques, 1 ml of each bacteria inoculum was placed into 26 of the tubes. One of the test tubes containing only the nutrient broth served as the negative control. Two of the 26 tubes containing the nutrient broth and both bacteria inoculums served as the positive controls. 2 ml of the various concentrations of the honey-ginger and honey-garlic mixtures were then added to each of the 24 appropriate test tubes. The tubes were then incubated at 37°C for 24 hours to observe for turbidity (growth) which indicated the MIC of each treatment on the test organisms. The tube that had the lowest concentration of treatment that showed no growth (no turbidity) was considered the MIC value. The minimum bactericidal concentrations (MBCs) were then performed to determine the lowest level of antimicrobial agent that will result in microbial death which was defined as a 99.9% reduction in the initial inoculum. The contents of the nutrient broth used for the MIC tests that showed no growth was subcultured on Mueller Hinton agar media. The plates were then incubated at 37°C for 24 hours. The absence of growth on the plates indicated the Minimum Bactericidal Concentrations (MBC) of the natural mixtures. The inoculated plates were scored as bactericidal if no growth; bacteriostatic if there was light to moderate growth and no antibacterial activity if there was heavy growth (Ewnetu *et al*., 2014).

2.14. Statistical analysis

All statistical analysis was performed using Excel 2013 software and SPSS software version 20. Comparisons of the honey, ginger, garlic, antibiotic and honey-ginger, and honey-garlic mixtures for their overall mean inhibitions against each test organism were analyzed using one-way analysis of variance (ANOVA) and two-way analysis of variance (ANOVA), where values less than 0.05 was considered as significantly different. Where significant values were observed,

Tukey's Post Hoc test was performed to determine where significant differences lie. Appropriate tables and graphs were also generated to represent the mean zone of inhibition and standard deviation, Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) data (Ewnetu *et al*., 2014).

3. Results and discussion

3.1. Negative Control

To create a standard for comparison, a negative control was prepared by inserting Whatman discs soaked in sterilized distilled water into the agar containing both bacteria. The negative control showed bacterial growth, with no zone of inhibition perceived around the discs. These results are in agreement with Malu *et al*. (2009), who found similar results. The distilled water is pure and contains no bioactive compounds or phytochemicals to inhibit bacterial growth, which explains the results.

3.2. Antibacterial effect of honey, ginger and garlic against *P. aeruginosa*

The antibacterial effect of the honey, ginger and garlic extracts at 25%, 50%, 75% and 100% concentrations were evaluated individually against *Pseudomonas aeruginosa* (Table 1).

Table 1 Mean zone of inhibition and standard deviation of honey, ginger and garlic at 25%, 50%, 75% and 100% concentrations against *Pseudomonas aeruginosa*

Pseudomonas aeruginosa			
Concentrations (%)	**Mean zone of inhibition ± SD (mm) Treatments**		
	Honey	**Ginger**	**Garlic**
25	4.0±6.9	2.0±3.4	4.3±3.7
50	6.2±6.0	2.6±2.3	4.6±4.1
75	4.2±3.6	5.0±1.9	5.7±1.9
100	2.7±4.7	7.2±1.5	7.6±0.5
Total	4.3±4.8	4.2±2.9	5.6±2.8

Garlic displayed the highest zone of inhibition at 25%, 75%, and 100% concentrations against *P. aeruginosa,* thus making it the most effective natural treatment against this organism. This is in line with the report of Mauti *et al.* (2015). Garlic's antimicrobial properties are due to the presence of allicin, which disrupts the normal process of RNA production and lipid synthesis, affecting the synthesis of protein and cell wall of the microorganism, as well as other compounds present in garlic such as adjoene, enzymes (peroxidase and miracynase), different amino acids such as cysteine, glutamine, and methionine, and vitamins B and C. (Borlinghaus *et al*., 2014), which may also be responsible for its antimicrobial activity (Borlinghaus *et al*., 2014). The garlic was the most effective at 100% concentration and least effective at 25% concentration against *P. aeruginosa*. These results agree with Hovana *et al*. (2011), who proved that garlic when used in its raw form (100% concentration) has better antibacterial activity. Pure garlic extract would be more concentrated and thus may contain a higher percentage of phytochemicals. On the other hand, ginger showed the least zone of inhibition at 25% concentration against *P. aeruginosa*, thus making it the least effective natural treatment against this bacterium. These findings align with the results of Gull *et al.* (2012), who found that several multi-resistant bacteria, including *P. aeruginosa* displayed poor susceptibility to aqueous ginger extract, compared to garlic, thus indicating that most of the bioactive agents in the ginger may not be water soluble. Abdalla and Abdallah (2018), reported that the bioactive property of ginger is mainly attributed to the presence of volatile oils, the output of which typically ranges between 1% and 3%. In this research, ginger displayed higher zones of inhibition at 100% concentration. Just as with garlic, this may be explained by the decreased percentage of photochemical upon dilution of ginger (Abdalla & Abdallah, 2018). Honey was more effective against *P. aeruginosa* at 50% concentration, and the least effective at 100% concentration. This may be due to the viscosity of the honey at 100% concentration, which made it harder to penetrate the bacterial cells. With the dilution of the honey, it was more likely to pass through the cells and caused an effect. This is in line with studies by Grace *et al, (*2017). Honey's antibacterial properties are ascribed to its high osmolarity, acidity (low pH), and presence of hydrogen peroxide (H2O2) and non-peroxide components (Mandal & Mandal, 2011).

The two-way ANOVA tests showed that there was no significant difference in the antibacterial effect of the treatments within each concentration against *P. aeruginosa*, as shown by the p-value of 0.51. This means that at each concentration, the respective treatments had the same effect. Also, there were no significant differences in the antibacterial effect among honey, ginger and garlic against *P. aeruginosa*, as indicated by the P-value of 0.55. This means that the natural treatments displayed similar antibacterial effects against this bacterium.

3.3. Antibacterial effect of honey, ginger and garlic against *K. pneumoniae*

The antibacterial effect of the honey, ginger and garlic extracts at 25%, 50%, 75%, and 100% concentrations were evaluated individually against *Klebsiella pneumoniae* (Table 2).

Table 2 Mean zone of inhibition and standard deviation of honey, ginger and garlic at 25%, 50%, 75% and 100% concentrations against *Klebsiella pneumoniae*

Klebsiella pneumoniae			
Concentrations (%)	**Mean zone of in hibition ± SD (mm) Treatments**		
	Honey	**Ginger**	**Garlic**
25	2.8±3.0	8.4±0.5	19.5±3.2
50	7.3±0.7	9.3±0.3	26.7±4.6
75	10.5±5.8	7.0±0.6	28.5±0.8
100	6.5±1.6	7.5±0.0	32.5±1.4
Total	6.7±4.0	8.0±0.2	26.8±5.5

Garlic displayed the highest zone of inhibition at all concentrations; thus, making it the most effective natural treatment against this organism. The results are similar to research conducted by Alemseged *et al, (*2018), where various nosocomial organisms implicated in acute wound infections were treated with aqueous garlic extract and the results showed *K. pneumoniae* to be the most susceptible organism. The factors responsible for the high susceptibility observed can be attributed to the secondary metabolites of garlic, including γ glutamyl peptides, scordinins, steroids, ketones, carbohydrates, lipids, alkaloids, terpenoids, flavonoids and other phenols (Njue *et al.,* 2014). Also, in this research, the highest zone observed for garlic was at 100% concentration, and the lowest zone observed was at 25% concentration. These findings are similar to studies done by Yadav *et al., (*2015) At a higher concentration, garlic extract is more concentrated, and thus may contain a higher percentage of phytochemicals. Unlike *P. aeruginosa*, honey displayed the highest mean zone of inhibition at 75% and the lowest zone of inhibition at 25% against *K. pneumoniae*. These results are in contrast to studies done by Molan & Rhodes (2015), who found that the antibacterial agent in honey is generated by glucose oxidase, an enzyme that is inactive under the low level of free water present in honey, but becomes active if the honey becomes diluted. However, in this study, higher concentrations were more potent against *K. pneumoniae,* which suggests that the results obtained may be because of the reaction of this specific organism to the honey. Moreover, honey has a low pH, high osmolarity, and viscous properties that hinder microbial proliferation (Mandal & Mandal, 2011). Despite these properties, honey was the least effective natural treatment against this organism. This may be due to the decreased penetrance of honey into the cells of the organism as a result of the test organism being gram-negative and having an outer protective membrane (Molan & Rhodes, 2015). Ginger showed a higher zone of inhibition at 50% concentration and a lower zone at 75% concentration against *K. pneumoniae*.

The two-way ANOVA tests also showed that there was no significant difference in the antibacterial effect of the treatments within each concentration against *K. pneumoniae*, as shown by the p-value 0.27. This means that at each concentration, the respective treatments had the same effect. However, there were overall significant differences in the antibacterial effect among honey, ginger and garlic against *K. pneumoniae*, as indicated by the P-value of 0.00 (Table 4). This means that the natural treatments did not have the same effect on this bacterium. Tukey's Post Hoc test was therefore conducted to check for individual significant differences between honey, ginger, and garlic against *K. pneumoniae*. The results showed a significant difference in the antibacterial effect of honey and garlic (p-value=0.00) and ginger and garlic (p-value=0.00). However, no significant difference was found between honey and ginger (p-value=0.87).

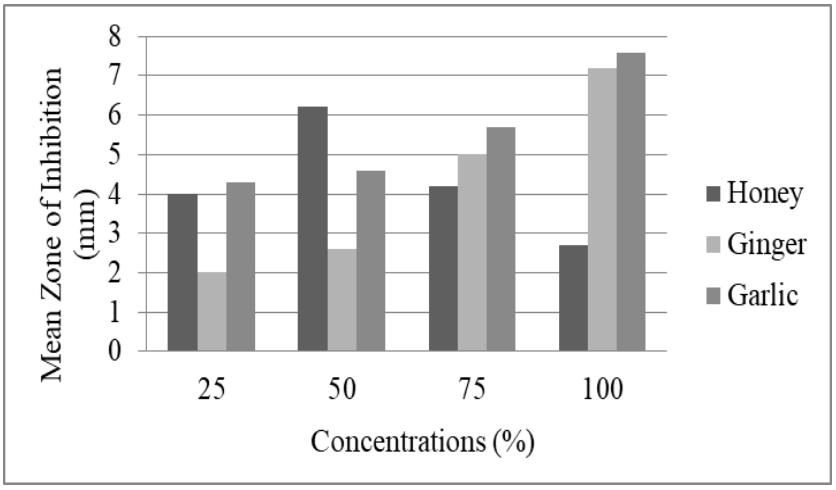

Figure 1 Mean zone of inhibition of honey, ginger and garlic at 25%, 50%, 75% and 100% concentrations against *Pseudomonas aeruginosa*

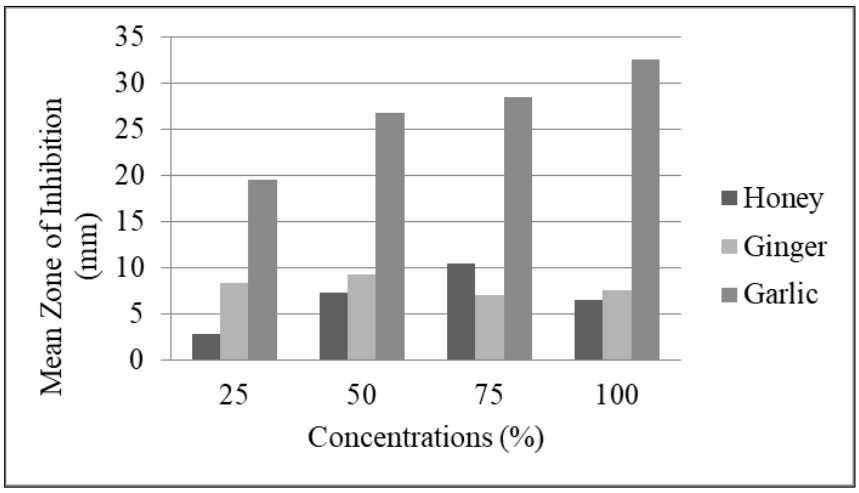

Figure 2 Mean zone of inhibition of honey, ginger and garlic at 25%, 50%, 75% and 100% concentrations against *Klebsiella pneumoniae*

3.4. Antibacterial effect of honey-ginger and honey-garlic mixtures against *P. aeruginosa*

The antibacterial effect of honey-garlic and honey-ginger mixtures at 25%, 50%, 75%, and 100% concentrations were evaluated against *Pseudomonas aeruginosa* (Table 3).

Honey-ginger showed the highest mean zone of inhibition at 75% and 100% concentrations against P*. aeruginosa*, thus making it the most effective natural mixture against this organism. These results are exceptionally similar to that of Omoya & Akharaiyi (2011), who recorded high zones of inhibition of honey and ginger mixtures against *P. aeruginosa*. Honey in its saturated solution of sugar may have caused an osmotic effect on the bacteria and ginger in its spicy nature may have reduced the formation of free radicals and performed other toxic factors which may have caused the antibacterial effect observed in this study (Omoya & Akharaiyi, 2011). The lowest zones observed for honey-ginger were at 25% and 50% concentrations, which may be explained by the loss of phytochemicals upon dilution of the mixture. Honey-garlic on the other hand displayed the lowest mean zone of inhibition at all concentrations as compared to honey-ginger, thus making it the least effective natural mixture against *P. aeruginosa*. These findings are in contrast to Agbagwa et al. (2021), who found honey-garlic to be very effective against *P. aeruginosa*. The differences in the potency of honey-garlic and honey-ginger mixtures against *P.aeruginosa*, may be due to structural and/or metabolic differences of the organism which caused it to respond differently to each mixture (Agbagwa et al., 2021). The lowest zone observed for honey-garlic was at 50% concentration, and the highest zone observed was at 100% concentration.

Table 3 Mean zone of inhibition and standard deviation of honey-garlic and honey-ginger mixtures at 25%, 50%, 75% and 100% concentrations against *Pseudomonas aeruginosa*

Pseudomonas aeruginosa		
Concentrations (%)	**Mean Zone of Inhibition ± SD (mm) Treatments**	
	Honey-garlic	**Honey-ginger**
25	1.7±1.5	0.0±0.0
50	0.0±0.0	0.0±0.0
75	4.5±1.2	7.8±1.3
100	5.5±1.1	7.1±1.2
Total	2.9±2.4	3.7±4.0

The two-way ANOVA tests showed that there was a significant difference in the antibacterial effect of the treatments within each concentration against this bacterium, as shown by the p-value 0.04. This means that at each concentration, the respective mixtures did not have the same effect. Since a significant difference was found among the concentrations of each mixture, Tukey's Post Hoc test was further conducted to check for individual significant differences between each concentration (25%, 50%, 75% and 100%) against *P. aeruginosa*. The results showed significant differences between 25% and 75% (0.01), 25% and 100% (0.00), 50% and 75% (0.00) and 50% and 100% (0.00). However, there were no significant differences between 25% and 50% (0.09) and 75% and 100% (0.35). There were no significant differences in the antibacterial effect between honey-ginger and honey-garlic mixtures against *P. aeruginosa*, as indicated by the P-value of 0.50. This means that the natural mixtures displayed almost the same effect on this bacterium.

3.5. Antibacterial effect of honey, ginger and garlic against *K. pneumoniae*

The antibacterial effect of the honey-garlic and honey-ginger mixtures at 25%, 50%, 75% and 100% concentrations were evaluated against *Klebsiella pneumoniae* (Table 4).

Table 4 Mean zone of inhibition and standard deviation of honey-garlic and honey-ginger mixtures at 25%, 50%, 75% and 100% concentrations against *Klebsiella pneumoniae*

Klebsiella pneumoniae		
Concentrations (%)	**Mean Zone of Inhibition ± SD (mm) Treatments**	
	Honey-garlic	Honey-ginger
25	12.8±1.6	8.0±0.1
50	18.3±1.6	6.5±2.3
75	20.1±0.6	6.1±2.1
100	22.4±0.3	6.8±0.6
Total	18.4±3.8	6.8±1.5

Honey-garlic displayed the highest mean zone of inhibition at all concentrations, thus making it the most effective mixture against this organism. These results are similar to those of Alemseged *et al. (*2018), who conducted studies on the potency of the aqueous honey-garlic extract on various gram-negative bacteria, and *K. pneumoniae* was found to be the most susceptible. The highest zone observed was at 100% concentration, and the lowest zone observed was at 25% concentration. As the mixture becomes more diluted, the percentage of the photochemical present in the extract may have decreased, and as such, the potency of the mixture exhibited decreased antibacterial activity (Alemseged *et al.* 2018). On the other hand, honey-ginger showed the lowest zone of inhibition at all concentrations against *K.*

pneumoniae, thus making it the least effective mixture. This result was in contrast to research done by (Ewnetu *et al.,* 2014), who found through rigorous research that the honey-ginger mixture was very effective against *K. pneumoniae. K. pneumoniae* responded differently to the mixtures which may be due to genetic and metabolic variations of the organism that enabled it to absorb one mixture over the other (Ewnetu *et al.,* 2014), The highest zone observed was at 25% concentration and the lowest zone was at 75% concentration.

Further, two-way ANOVA tests showed that there was no significant difference in the antibacterial effect of the treatments within each concentration against *K. pneumoniae*, as shown by the p-value 0.68. This means that at each concentration, the respective mixture had the same effect. However, there were overall significant differences in the antibacterial effect between honey-ginger and honey-garlic against *K. pneumoniae*, as indicated by the P-value of 0.01. This means that the natural treatments did not have the same effect on this bacterium.

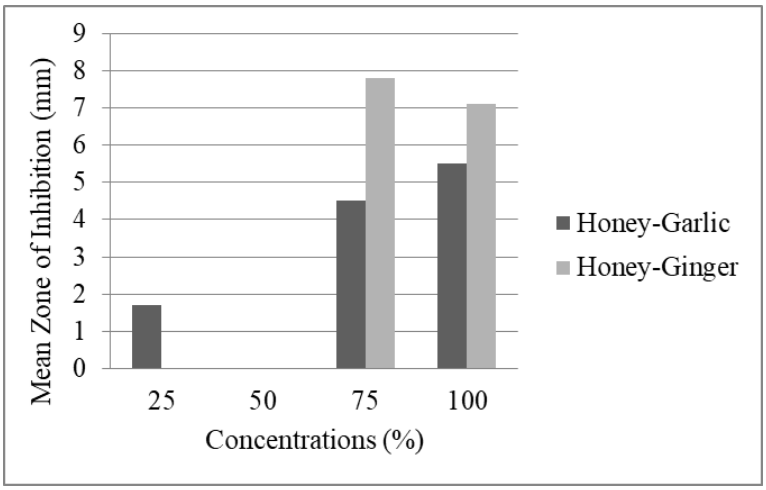

Figure 3 Mean zone of inhibition of honey-garlic mixture versus honey-ginger mixture at 25%, 50%, 75% and 100% concentrations against *Pseudomonas aeruginosa*

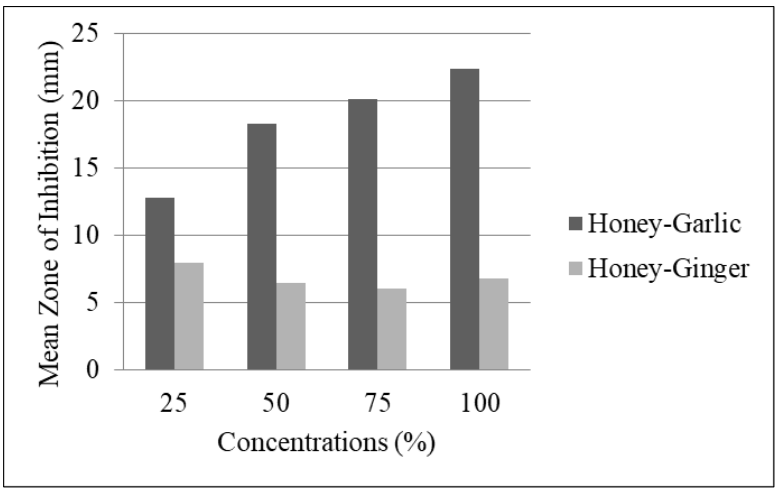

Figure 4 Mean zone of inhibition of honey-garlic mixture versus honey-ginger at 25%, 50%, 75% and 100% concentrations against *Klebsiella pneumoniae*

3.6. Antibacterial effect of honey-ginger and honey-garlic versus erythromycin against *P. aeruginosa*

The antibacterial effect of the honey-garlic and honey-ginger mixtures at 25%, 50%, 75% and 100% concentrations versus the erythromycin antibiotic was evaluated against *Klebsiella pneumonia* (Table 5).

Table 5 Overall mean zone of inhibition and standard deviation of honey-garlic mixture, honey-ginger mixture and erythromycin (antibiotic) against *Pseudomonas aeruginosa*

Pseudomonas aeruginosa	
Treatments	**Mean zone of inhibition ± SD (mm)**
Honey-Garlic	2.9±2.4
Honey-Ginger	3.7±4.0
Antibiotic (Erthryomcin)	5.5±0.7

When the honey-ginger and honey-garlic mixtures versus the erythromycin antibiotic (positive control) were compared against *P. aeruginosa*, the erythromycin antibiotic displayed higher zones of inhibition compared to both honey-garlic and honey-ginger mixtures against *P. aeruginosa*. The results are in contrast with Alemseged *et al, (*2018), who found *P. aeruginosa* to be more susceptible to honey-ginger compared to erythromycin antibiotic, but also in agreement with findings from Muley *et al., (*2018), who observed erythromycin antibiotics as more effective compared to honey-garlic. This may be attributed to the fact that erythromycin, as a conventional antibiotic, is prepared using reproducible manufacturing processes and procedures, whereas extracts of herbal medicines are subject to degradation and decomposition on storage (Muley *et al*., 2018). This coupled with *P. aeruginosa* is known for its high resistance to antimicrobial agents, which may explain the results observed.

The mean zone of inhibition of honey-garlic mixture and erythromycin (synthetic antibiotic) against *Pseudomonas aeruginosa* was compared by use of ANOVA: single-factor test. The p-value obtained from the analysis was 0.42 which means that there is no significant difference in the antibacterial effect of honey-garlic mixture and erythromycin (synthetic antibiotic) against *Pseudomonas aeruginosa.*

The mean zone of inhibition of honey-ginger mixture and erythromycin (synthetic antibiotic) against *Pseudomonas aeruginosa* was compared by use of ANOVA: single-factor test. The p-value obtained from the analysis was 0.40 which means that there is also no significant difference in the antibacterial effect of honey-ginger mixture and erythromycin (synthetic antibiotic) against *Pseudomonas aeruginosa.*

3.7. Antibacterial effect of honey-ginger and honey-garlic versus erythromycin against *K. pneumoniae*

The antibacterial effect of the honey-garlic mixture and honey-ginger mixture versus erythromycin (synthetic antibiotic) was evaluated against *Klebsiella pneumoniae*. Honey-garlic showed the highest overall mean zone of inhibition (18.4 mm) compared to erythromycin (8.5 mm) against *Klebsiella pneumonia* (Table 6).

Table 6 Overall mean zone of inhibition and standard deviation of honey-garlic mixture and erythromycin (antibiotic) against *Pseudomonas aeruginosa*

Klebsiella pneumonia	
Treatments	**Mean zone of inhibition ± SD**
Honey-Garlic	18.4±3.8
Honey-Ginger	6.8±1.5
Antibiotic (Erythromycin)	8.5±0.7

Honey-garlic mixture showed a larger zone of inhibition compared to erythromycin (antibiotic), thus making it an effective alternative to the use of synthetic antibiotics against this organism. These results are in agreement with those of Alemseged *et al, (*2018), who conducted sensitivity tests of various antibiotics including erythromycin against several bacteria, and found that *K. pneumoniae* was the most resistant to erythromycin antibiotic. These results may be due to the chemical makeup of this organism, as well as the bioactive constituents of honey and garlic. Another reason may be due to the prolonged use and misuse of erythromycin antibiotics against *K. pneumoniae*, which enabled the bacteria to develop resistance (Alemseged *et al.,* 2018).

Further, the mean zone of inhibition of honey-garlic mixture and erythromycin (synthetic antibiotic) against *Klebsiella pneumoniae* was compared by use of ANOVA: single-factor test which is shown in table 15. The p-value obtained from the analysis was 0.00 which means that there is a significant difference in the antibacterial effect of honey-garlic mixture and erythromycin (synthetic antibiotic) against *Klebsiella pneumoniae.*

In addition, the mean zone of inhibition of honey-ginger mixture and erythromycin (synthetic antibiotic) against *Klebsiella pneumoniae* was compared by use of ANOVA: single-factor test. The p-value obtained from the analysis was 0.06 which means that there is no significant difference in the antibacterial effect of honey-ginger mixture and erythromycin (synthetic antibiotic) against *Klebsiella pneumoniae.* This is in line with the results of Ewnetu *et al.* (2014), who found erythromycin to be slightly more effective than honey-ginger against *K. pneumoniae.*

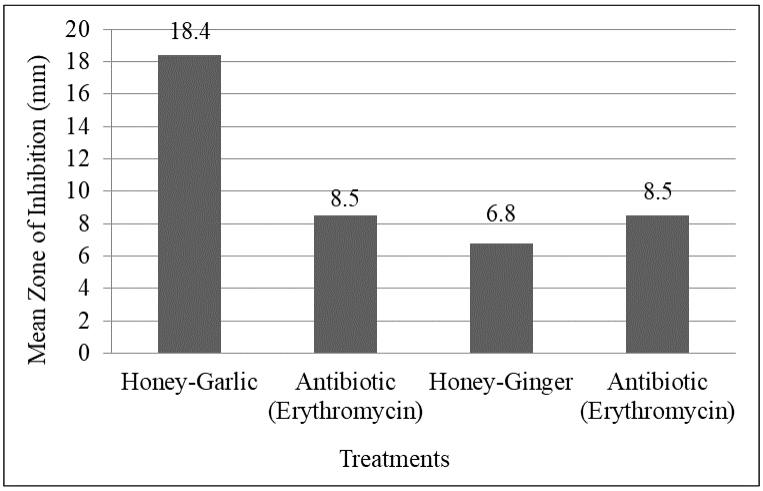

Figure 5 Mean zone of inhibition of honey-garlic and honey-ginger mixtures versus erythromycin (antibiotic) against *Klebsiella pneumoniae*

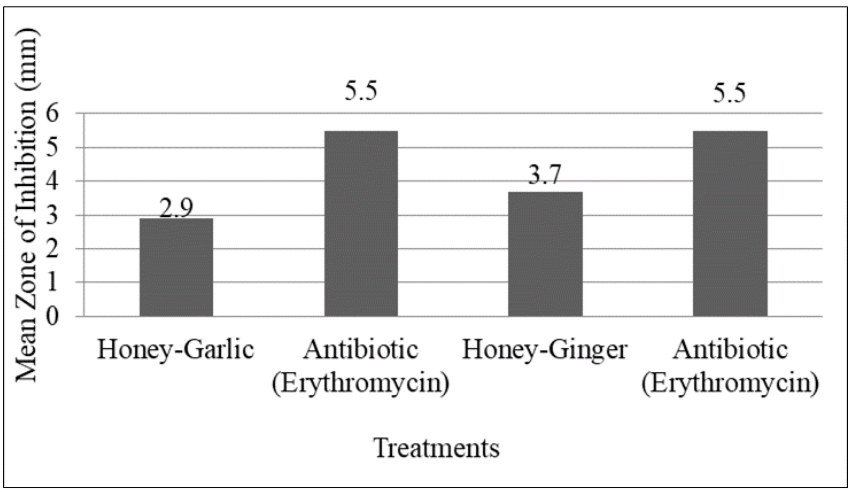

Figure 6 Mean zone of inhibition of honey-garlic and honey-ginger mixtures versus erythromycin (antibiotic) against *Pseudomonas aeruginosa*

3.8. Antibacterial effect of honey, ginger, garlic (individually) honey-ginger and honey-garlic mixtures against *P. aeruginosa*

The antibacterial effect of the honey, ginger and garlic (individually) and honey-garlic and honey-ginger mixtures was evaluated against *Pseudomonas aeruginosa* (Table 7).

Table 7 Overall mean zone of inhibition and standard deviation of honey, ginger and garlic (individually) and honey-garlic and honey-ginger mixtures against *Pseudomonas aeruginosa*

Pseudomonas aeruginosa	
Natural extracts and mixtures	**Overall mean zone of inhibition ± SD (mm)**
Honey	4.3±4.8
Ginger	4.2±2.9
Garlic	5.6±2.8
Honey-Garlic	2.9±2.4
Honey-Ginger	3.7±4.0

Garlic (used individually) displayed the largest zone of inhibition among all the treatments, and the honey-garlic mixture showed the least zone of inhibition among all the treatments against *P*. *aeruginosa*, thus rendering them the most effective and least effective natural treatments respectively against this organism. These results are in agreement with Palaksha *et al*. (2010), who found garlic to be highly effective against *P. aeruginosa.* Allicin was the most important compound in garlic thought to be responsible for the antimicrobial effect seen. The action of Allicin includes immediate and total inhibition of RNA synthesis, inhibition of cell wall synthesis due to enzymes involved in the cross-linking of the polysaccharide chains of the bacterial cell wall, and activation of lytic enzymes (Palaksha *et al.,* 2010). However, the results are also in contrast with those Al- Masaudi & Al-Bureikan (2012), who found that the mixture of honey and garlic exhibited very high inhibition zones against *P. aeruginosa,* unlike what was observed in this study against this bacterium. Honey (used individually) displayed a higher zone of inhibition compared to honey-garlic and honey-ginger mixtures. Also, ginger and garlic (used individually) displayed higher zones of inhibition compared to honey-ginger and honey-garlic mixtures respectively against *P. aeruginosa*. These findings do not align with the results of Andualem (2013) and Ewnetu *et al.* (2014) who found that the synergy of honey-ginger and honey-garlic produced exceptionally higher zones when compared with the extracts being used individually against *P. aeruginosa*. The result observed in this study may be due to the low inhibition capacity of honey when used in synergy. The results may also be explained by the dilution factor of the mixtures. The mixtures used in this study were divided into 50:50 ratios. Mixtures of 100:100 ratios may have shown higher inhibition capacity.

The mean zone of inhibition of honey, ginger, and garlic (individually) and honey-garlic and honey-ginger mixtures against *Pseudomonas aeruginosa* was compared by use of ANOVA: single-factor test. The p-value obtained from the analysis was 0.71 which means that there is no significant difference in the antibacterial effect of honey, ginger and garlic (individually) and honey-garlic and honey-ginger mixtures against *Pseudomonas aeruginosa.*

3.9. Antibacterial effect of honey, ginger, garlic (individually) honey-ginger and honey-garlic mixtures against *K. pneumoniae*

The antibacterial effect of the honey, ginger and garlic (individually) and honey-garlic and honey-ginger mixtures was evaluated against *Klebsiella pneumoniae* (Table 8).

Table 8 Overall mean zone of inhibition and standard deviation of honey, ginger and garlic (individually) and honey-garlic and honey-ginger mixtures against *Klebsiella pneumoniae*

Klebsiella pneumonia	
Natural extracts and mixtures	**Overall mean zone of inhibition ± SD (mm)**
Honey	6.7±4.0
Ginger	8.0±1.0
Garlic	26.8±5.5
Honey-Garlic	18.4±3.8
Honey-Ginger	6.8±1.5

Garlic (used individually) displayed the largest zone of inhibition among all the treatments, and honey (used individually) showed the least zone of inhibition among all the treatments, thus rendering them the most effective and least effective natural treatments respectively against this organism. These results are similar to that of Muley *et al.* (2018), who found garlic to be more effective against *K. pneumoniae.* Compared to that honey. Garlic is known universally as a very potent natural treatment against a wide variety of bacteria, which it owes to the antibacterial activity of allicin that carries out its reactions with the thiol groups of various enzymes, e.g., alcohol dehydrogenase, thioredoxin reductase, and RNA polymerase (Muley *et al*., 2018). The low inhibition effect observed for honey against *K. pneumoniae,* maybe a result of multiple factors such as viscosity, type of honey, and the chemical makeup of the bacteria as discussed above. Honey (used individually) displayed a lower zone of inhibition compared to honey-garlic and honey-ginger mixtures. This aligns with the findings of Omoya & Akharaiyi (2011) and Agbagwa *et al.,* (2021), who found honey-ginger and honey-garlic mixtures to be more effective against *K. pneumoniae* compared to the use of honey alone. With the addition of ginger and garlic to honey, more phytochemicals are added and the potency is expected to be greater (Omoya & Akharaiyi, 2011). These findings also related to the chemical and metabolic makeup of the organism, since these results were not observed in *P. aeruginosa,* but shown against *K. pneumoniae.* On the other hand, ginger and garlic (used individually) displayed higher zones of inhibition compared to honey-ginger and honey-garlic mixtures respectively against *K. pneumoniae.* These results are in contrast to those of Andualem (2013) and Ewnetu *et al. (*2014), who found the mixtures to be more effective against *Klebsiella pneumoniae* compared to ginger and garlic alone. In this study, the results may be due to the dilution factor of the mixtures. The mixtures used in this study were divided into 50:50 ratios. Mixtures of 100:100 ratios may have shown higher inhibition capacity.

Further, the mean zone of inhibition of honey, ginger, and garlic (individually) and honey-garlic and honey-ginger mixtures against *Klebsiella pneumoniae* was compared by use of ANOVA: single-factor test. The p-value obtained from the analysis was 0.00 which means that there is a significant difference in the antibacterial effect of honey, ginger, and garlic (individually) and honey-garlic and honey-ginger mixtures against *Klebsiella pneumoniae.* Because a significant difference was observed among the treatments, a Tukey's Post Hoc test was performed to determine where the differences lie for each treatment. The results showed that no significant difference was found in the antibacterial effect of honey and ginger (p-value=0.98), honey and honey-ginger (p-value=1.00) and ginger and honey-ginger mixture (p-value=0.98). However, there was a significant difference in the antibacterial effect of honey and garlic (p-value=0.00), honey and honey-garlic mixture (p-value=0.00), ginger and garlic (p-value=0.00), ginger and honey-garlic mixture (p-value=0.00), garlic and honey-garlic mixture (p-value=0.02), garlic and honey-ginger mixture (p-value=0.00) and honey-garlic and honey-ginger mixture (p-value=0.02).

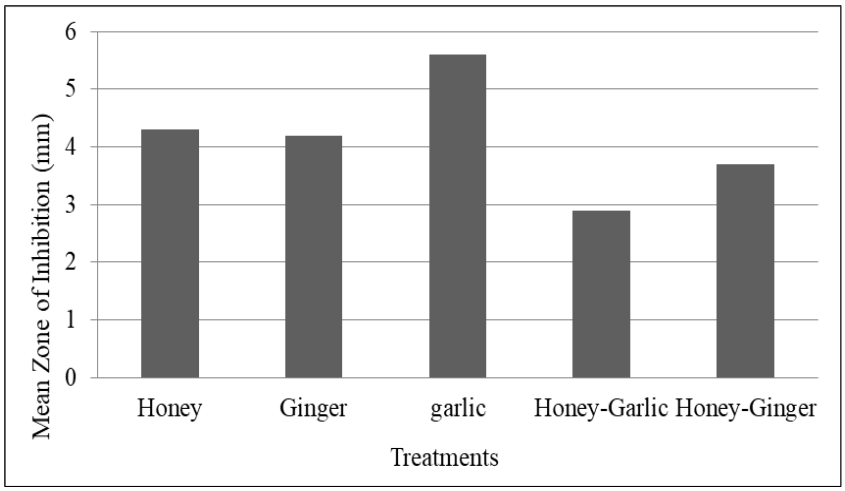

Figure 7 Mean zone of inhibition of honey, ginger and garlic (individually) and honey-garlic and honey-ginger mixtures against *Pseudomonas aeruginosa*

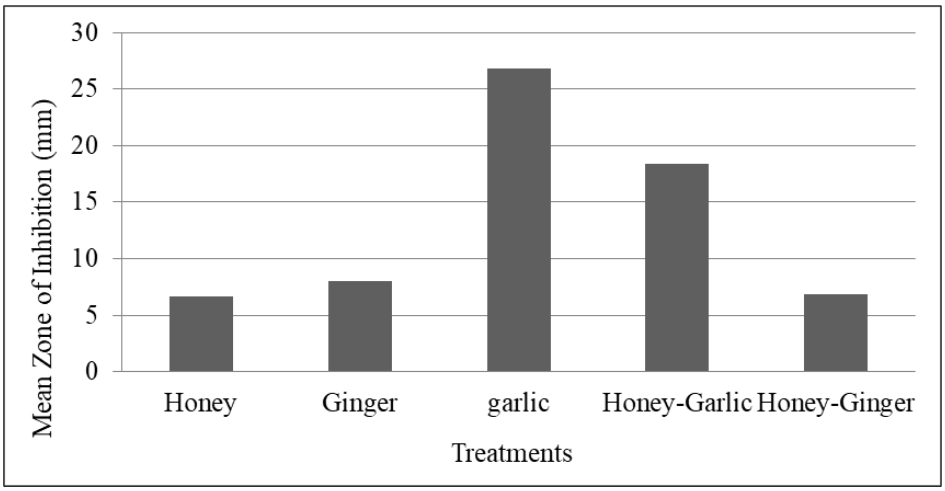

Figure 8 Mean zone of inhibition of honey, ginger and garlic (individually) and honey-garlic and honey-ginger mixtures against *Klebsiella pneumoniae*

3.10. Comparison of the Minimum Inhibitory Concentration (MIC) of honey-ginger and honey-garlic against *Pseudomonas aeruginosa* and *Klebsiella pneumonia*

Honey-garlic displayed lower MIC values compared to honey-ginger against both bacteria. These findings align with those of Saxena *et al. (*2020). Honey-garlic was more effective than honey-ginger against both organisms; since lower concentrations were shown to inhibit their growth. This suggests that garlic may exhibit a higher percentage of more potent compounds and photochemical than those present in ginger. Therefore, when combined with honey that has high osmolarity, acidity, and a high content of hydrogen peroxide, the effects were magnified (Saxena *et al*., 2020). The effectiveness of honey-garlic mixture as an antimicrobial agent at such low concentrations makes this mixture a novel source of an effective drug for resistant bacteria strains. Further, honey-garlic showed a lower MIC value against *K. pneumoniae* compared to *P. aeruginosa*. Suggesting that it was more effective in inhibiting the growth of *K. pneumoniae* compared to *P. aeruginosa*. These findings lead back to the difference in the chemical and metabolic makeup as well as the specific resistance mechanism each bacterium possesses, which causes them to respond differently to various treatments (Saxena *et al*., 2020).

3.11. Comparison of the Minimum Bactericidal Concentration (MIC) of honey-ginger and honey-garlic against *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*

Honey-garlic at 50% was the only mixture that showed bactericidal activity against *K. pneumoniae*. This result is similar to those of Andualem (2013). No bacterial growth was shown at 25% concentration, however, bacteriostatic activity was shown at 12.5% and 6% concentrations, as indicated by moderate and light bacterial growth respectively. Honey-ginger showed no antibacterial activity at both 50% and 25% concentrations against *K. pneumoniae*. In terms of *Pseudomonas aeruginosa*, honey-ginger and honey-garlic showed no antibacterial activity at both 50% and 25% concentrations, as indicated by heavy bacterial growth. The mixtures at the concentrations tested therefore mostly showed bacteriostatic activity compared to bactericidal activity, thus suggesting that maybe higher concentrations of the mixtures were required to cause the death of the organisms. These findings are in agreement with Agbagwa *et al, (*2021), who found predominantly bacteriostatic activities of honey-ginger and honey-garlic mixtures at concentrations similar to those used in this study against *P. aeruginosa* and *K. pneumoniae*.

4. Conclusion

Our results showed that garlic was the most effective individual treatment against both *P. aeruginosa* and *K. pneumoniae,* while ginger was the least effective against *P. aeruginosa* and honey was the least effective against *K. pneumoniae.* Further, honey-ginger mixture was more effective against *P. aeruginosa,* while honey-garlic mixture was more effective against *K. pneumoniae.* Honey-garlic mixture was also an effective bacteriostatic and bactericidal agent in this study compared to honey-ginger. This study has provided a natural alternative to frequently used medications in the treatment of wounds and bacterial infections. Aside from their accessibility and the small amount of each substance employed, the results provide the benefit of enhanced antibacterial and wound healing efficiency with no side effects and at a cheap cost. Combining natural products might lead to a novel therapeutic range, potentially preventing microbial drug resistance. However, further research is needed to identify the underlying mechanism of synergistic

activity and how it interferes with wound healing. In the future, more extensive work can be done with a wide array of solvents rather than solely utilizing one solvent as done in this research. Solvents such as ethanol, methanol, acetone among others, as well as a wider array of bacteria.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors declare that there are no conflicts of interest.

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