Phytochemical Screening and Antibacterial Evaluation of Conventional Antibiotics, Garlic and Ginger on Isolates from Fish Pond Water Samples in Awka, Anambra State, Nigeria

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Authors’ contributions

This work was carried out in collaboration among all authors. Author ORU designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors ORU, EMI and BOU managed the analyses of the study. Authors ELO and PIE managed the literature searches while Author EIC supervised the work. All authors read and approved the final manuscript.

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ABSTRACT

Medicinal plants are used by almost 80% of the world’s population for their basic health care because of their low cost and ease in availability. In the last few decades, many bacteria have continued to show increasing resistance against current antibiotics.

Aim: In this study, phytochemical screening and antibacterial effects of conventional antibiotics, garlic and ginger on test isolates from fish pond water samples were evaluated between May-November, 2019.

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INTRODUCTION

Medicinal plants are used by almost 80% of the world’s population for their basic health care because of their low cost and ease in availability [1]. From the dawn of civilization, people have developed a great interest in plant-based drugs and pharmaceutical products [1]. In the last few decades, bacteria have continued to show increasing resistance against current antibacterial agents [2]. Herbal drugs made from medicinal plants have been used from ancient times to treat various diseases and their antimicrobial properties make them a rich source of many potent drugs [3]. The use of medicinal plants has always played a positive role in the control and prevention of diseases such as diabetes, heart disorders and various cancers [4]. Some medicinal plants have been used in the production of various drugs, singly or in combination and even as principal raw material for the production of other conventional medicines [5].

Plants have adapted similar strategy in their biochemical battle against diseases. Instead of relying on a single flavonoid to stop pathogens, plants produce families of structurally and functionally diverse antimicrobial compounds. These act together to prevent development of resistance. The results of present study have provided the justification for therapeutic potential use of ginger and garlic. The practice of using ginger and garlic as supplementary or alternative medicine in developing countries like Nigeria will not only reduce the clinical burden of drug resistance development, but also the side effects and cost of the treatment with allopathic medicine. In fact, the findings revealed that the knowledge of the antibacterial activity of the extracts obtained from garlic and ginger can be very useful and can be applied in different areas of research such as the pharmaceutical and food industries. Powdered garlic and ginger can be added as adjuvants to foods as this will help to minimize or prevent infections and diseases. The findings of this research will be useful in managing aquaculture farms, formulating feeds for aquaculture, educating fish farmers and fish feeders and simultaneously prevent epidemic in Anambra State. Unsuccessful treatments (antibiotic resistance by microorganisms) using various antibiotics have led to continuous research on finding new ways of treatments hence, the need for this research; phytochemical screening and antibacterial evaluation of conventional antibiotics, garlic and ginger on isolates from fish pond water samples in Awka, Anambra State, Nigeria. Specific objectives were to: determine the antibiotic susceptibility patterns...
of the bacterial isolates. Determine the qualitative and quantitative screening of the phytochemicals present in pulverized (Allium sativum) garlic cloves and (Zingiber officinale) ginger rhizomes. Extract pulverized garlic cloves and ginger rhizomes using absolute ethanol, absolute methanol and hot water. Determine the susceptibility patterns including minimum inhibitory and bactericidal concentrations of garlic and ginger extracts on the bacterial isolates. Determine the synergistic effect of plants extracts (garlic and ginger) against the bacterial isolates.

2. MATERIALS AND METHODS

2.1 Research Center

The antibacterial analysis was done at Projects Development Institute (PRODA) Enugu and in Applied Microbiology and Brewing laboratory Nnamdi Azikiwe University, Awka Nigeria while molecular analysis was done at Inqaba, South Africa.

Twelve (12) bacteria were isolated from fifteen (15) concrete fish pond water samples in Awka and its environment. Each fish pond water was sampled twice.

Identification of the bacterial isolates from fish pond water samples was done according to [6] and Maldi-Tof DNA spectrometry.

2.2 Antibiotics Susceptibility Assay

The antibiotic susceptibility of the bacterial isolates was determined using Kirby-Bauer disc diffusion technique. The plating medium was Mueller-Hinton agar and antibiotics used were Erythromycin (10µg), Amoxicillin (30µg), Pefloxacin (10µg), Ofloxacin (10µg) and Ciprofloxacin (10µg).

Preparation of 0.5 McFarland Standard and Standardization of Inoculum as described by [6].

Interpretation of the zone sizes was done by referring to the standard tables according to CLSI guidelines and for consideration whether the bacteria is susceptible, intermediate or resistant to that particular antibiotics as described by [7].

2.3 Collection and Preparation of Plant Materials

2.3.1 Garlic cloves and ginger rhizomes sampling

The cloves and rhizomes were purchased from Plateau state, Nigeria. They were placed on a clean table and selected using the following criteria: those with no dead cloves, those with no flowers and rots. The cloves and rhizomes were washed to remove dirt, peeled, weighed, chopped into pieces, dried in an oven at 40°C for 5 days, re-weighed and grounded into powder using an electric blender for ginger while mortar and pestle was used for garlic cloves as described by [8] and as modified in this study.

2.4 Detection of Phytochemicals

Phytochemical analysis for the qualitative detection of biologically important compounds including alkaloids, saponins, tannins, flavonoids, phenols, terpenoids, glycosides in garlic and ginger were examined according to the method of [8] and as modified in this study.

Ten (10) grams of the powdered samples were weighed into a 250 ml labelled conical flask each. 100mls of the solvents (absolute ethanol, absolute methanol and hot water) were added, capped and placed in a laboratory shaker for 24 hours. They were filtered using Whatman no. 1 filter paper and the filtrates were used for qualitative screening of active compounds.

Tannin, alkaloid, cardiac glycoside, saponin, flavonoid, terpenoid and phenol tests were carried out as described by [9-13] respectively.

2.4.1 Quantitative screening of garlic and ginger powder

Determination of alkaloid, saponin, flavonoid, terpenoid, phenol, glycoside and tannin were carried out according to the methods of [14-19] respectively.

2.4.2 Preparation of plant extract for sensitivity test

The method described by [20] and as modified in this study was used for the extraction. The solvents; absolute ethanol, methanol and boiled water were used for the extraction of crude extract. Water was chosen as a solvent so as to mimic the traditional style, since some of these plant parts were administered as either infusions or decoctions. One hundred grams of powdered plant material (garlic and ginger separately) was macerated in 500 ml of the boiled water in five different extraction pots such that the level of the water was above that of the plant material. The macerated mixtures were then left on the shaker for 48 hours at room temperature. The extracts were filtered out from the macerated mixture.
using a Whatman no. 1 filter paper, transferred into a brown coloured beaker and concentrated in a freeze dryer away from sunlight at Projects Development Institute (PRODA) before being used for antibacterial efficacy test.

Soxhlet apparatus was used for 95% ethanol and methanol extraction. One hundred grams of the powdered garlic and ginger were extracted separately in 500 ml of the Analar grade (AR) solvents contained in a 500 ml round bottom flask in five different soxhlet apparatus. The extracts were later combined in a brown colored 1000 ml beaker and concentrated in a water bath at 40°C for 3 days before being used for antibacterial efficacy test.

2.4.3 Percentage yield

\[ \frac{W_E}{W_p} \times 100 \]

\( W_E \): Weight of extract or yield.
\( W_p \): Weight of ginger or garlic powder used for extraction.

2.4.4 Preparation of susceptibility test discs

Discs of 5 milliliters were prepared from Whatman no.1 filter paper. This was done by punching the filter papers using a paper punch. The discs prepared filled four McCartney bottles. The discs were then sterilized by autoclaving at 121°C for 15 minutes after which the autoclave was left to cool before removing the McCartney bottles containing the discs. The discs were then dried in hot air oven at 50°C for 24 hours to remove moisture according to [21] and as modified in this study.

2.5 In vitro antibacterial screening of the test isolates

2.5.1 Neat Examination

The antibacterial study was carried out by disc diffusion technique as described by [22] and as modified in this study. The discs used for the antibacterial activity were impregnated into the stock solution (neat) of the plant extracts using sterile forceps to check whether they are effective or not before dilution. This was done beside a Bunsen flame to maintain asepsis. The forceps were sterilized after every pick. The discs were then left to stay in the plant extracts stock solution for one hour. The discs were then removed and placed in sterile Petri dish and left to dry at room temperature for 30 minutes as described by [23]. Muller-Hinton agar was prepared according to manufacturer’s instruction and allowed to cool to about 45°C before dispensing into sterile Petri plates. The standardized inocula (0.5 McFarland) were spread on the gelled medium using swab stick and the discs impregnated into the extracts were picked using sterilized forceps and seeded onto the gelled medium. They were allowed for 20 minutes before incubating upside down at 37°C for 24 hours. The inhibition zone diameters were measured in mm using a meter rule. Each extract and isolate was done in duplicates. This was done to check the efficacy of the extracts.

2.6 Serial Dilution

This was carried out by disc diffusion technique as described by [22] and as modified in this study. Two fold serial dilution of the extracts ranging from 500 mg/ml or 1000mg/2ml to 15.6 mg/ml was done. 4% Dimethylsulfoxide (DMSO) was used as the diluent. One gram or 1000 milligram of the extract was weighed into the first test tube containing 2 ml of 4% DMSO and serially diluted. A known number of the sterile discs were impregnated into the labelled test tubes using a sterile forcep and allowed to absorb the extracts for an hour. The discs were then removed using a sterile wire loop into a sterile labelled Petri-plates to air-dry for 30 minutes at room temperature beside a Bunsen burner as described by [23]. Muller-Hinton agar was prepared according to manufacturer’s instruction and allowed to cool to about 45°C before dispensing into sterile Petri plates. The standardized inocula (0.5 McFarland) were spread atop the gelled medium using swab stick and the discs impregnated into the extracts were then picked by sterilized wire loop and seeded onto the gelled medium. They were allowed for 20 minutes before incubating upside down at 37°C for 24 hours. The inhibition zone diameters were measured in mm using a meter rule. Each extract and isolate were done in duplicates and their average determined. Sterility test was done on the agar and extracts. Positive controls were commercially bought antibiotic discs containing Amoxacillin (30 µg), Pefloxacin (10µg), Ofloxacin (10µg), Erythromycin (10µg) and Ciprofloxacin (10ug). Negative controls were 4% DMSO, absolute ethanol, absolute methanol (AR). Sterility test was carried out on the extract, agar and 4% DMSO.
Interpretation of the zone sizes for antibiotics was done by referring to the standard tables according to NCCLS guidelines and for consideration whether the organism is susceptible, intermediate or resistant to that particular antibiotics as described by [24].

2.6.1 Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

These were determined by adopting the techniques specified by Clinical and Laboratory Standards Institute [7] and as modified in this study.

Minimum inhibitory concentration was examined using the dilution method. Two fold serial dilution of the extracts ranging from 500 mg/ml or 1000mg/2ml to 15.6 mg/ml was done. 4% Dimethylsulfoxide (DMSO) was used as the diluent. One gram or 1000 milligram of the extract was weighed into the first test tube containing 2 ml of 4% DMSO and serially diluted. 0.1 ml of the standardized inocula were aseptically inoculated into the test tubes, capped and incubated at 37°C for 24 hours. The tube containing the least concentration of extract with no growth (turbidity) was confirmed as the minimum inhibitory concentration.

Minimum bactericidal concentration was performed by plating out the minimum inhibitory concentration tube upwards on Muller-Hinton agar using a swab stick, incubated at 37°C for 24 hours. The least concentration of the extract with no growth after incubation was confirmed as the minimum bactericidal concentration.

2.6.2 Synergistic effects of ginger and garlic extracts on the bacteria

The method described by [22] and as modified in this study was used for the synergistic test.

The different extracts were combined in the ratio of 0.5g/ml: 0.5g/ml which gave a concentration of 500 mg/ml or 1000mg/2ml. 4% Dimethylsulfoxide (DMSO) was used as the diluent. The combinations were; Garlic ethanol: Ginger ethanol, Garlic methanol: Ginger methanol, Garlic water: Ginger water. One gram or 1000 milligram of the extract was weighed into a test tube containing 2 ml of 4% DMSO. A known number of the sterile discs were impregnated into the labelled test tubes using a sterile forcep and
allowed to absorb the extracts for an hour. The discs were removed using a sterile wire loop into a sterile labelled Petri-plates to air-dry for 30 minutes at room temperature beside a Bunsen burner as described by [23]. Muller-Hinton agar was prepared according to manufacturer’s instruction and allowed to cool to about 45°C before dispensing into sterile Petri plates. The standardized inocula (0.5 McFarland) were spread atop the gelled medium using swab stick and the discs impregnated into the extracts were then picked by sterilized wire loop and seeded onto the gelled medium. They were allowed for 20 minutes before incubating upside down at 37°C for 24 hours. The inhibition zone diameters were measured in mm using a meter rule. Each extract and isolate were done in duplicates and their average determined. The average zones of inhibition formed when the extracts were used in combinations against the test isolates were compared with the zones of inhibition when they were used singly.

2.7 Statistical Analysis

The results were analyzed statistically using two-way analysis of variance (ANOVA).

3. RESULTS

The outcome of the phytochemical analysis using antibiotics, garlic, and ginger are present in the tables beneath.

4. DISCUSSION

The sequencing of 16s rRNA gene showed the presence of *Shigella flexneri* strain RPK33, *Pseudomonas aeruginosa* strain ARa, *Pseudomonas fluorescens* strain 3 and *Acinetobacter calcoaceticus* strain A3. The bacterial isolates showed the sequence, 99% identities, single number of matches, 100% query cover, sequence length and 1% and 0% gaps.

According to [24] standard, *Pseudomonas aeruginosa* was resistant to pefloxacin (10µg), ofloxacin (10µg) and amoxicillin (30µg) and this may be attributed to the synthesis of biofilm by this bacterium. *Bacillus subtilis* was resistant to erythromycin (10µg) and amoxicillin (30µg). All the isolates except *Staphylococcus aureus*, *Vibrio cholerae*, *Vibrio parahaemolyticus* and *Salmonella typhi* were resistant to amoxicillin (30µg) (Table 1).

A medicinal plant is any plant in which one or more of its organs, contains substances that can be used for therapeutic purposes of which are precursors for the synthesis of useful drugs. The medicinal value of these plants lies in bioactive phytochemical constituents that produce definite physiological action in fishes and huanan [25]. It is no longer news that herbal plants possess in them great healing effects, which is why they are often resorted to for treatment due to the emergence of resistant strains of bacterial pathogens which has rendered most available synthetic drugs inactive. The use of natural products from herbal plants for various curative measures has continued to play significant roles as alternative sources of medication in many parts of the world (Nigeria inclusive); and these compounds originating from natural products including medicinal plants have also served and are being considered as leading agents in the development of novel and potent antimicrobial agents to solve some of the mysteries of drug resistance which is prevalent in our health institutions across the world.

In this study, the phytochemical screening and *in vitro* antibacterial activity of garlic and ginger extracts were evaluated. The phytochemical investigation (qualitative and quantitative) indicates the presence of alkaloids (11.32% and 15.38%), saponin (2.84% and 2.92%), tannins (0.36% and 2.46%), flavonoids (6.10% and 13.62%), terpenoids (1.08% and 1.75%) and cardiac glycosides (0.04% and 0.18%) (Table 2). This is in agreement with the work done by [26]. These classes of compounds especially alkaloids, saponins, tannins and flavonoids are known to have curative activity against several pathogens [25].

The antimicrobial activity of extracts is due to specific phytochemicals or essential oils [27]. The main factors that determine the antimicrobial activity are the type and composition of the plant, amount used, type of microorganism, solvent and method used for extraction, temperature of the environment. The *in vitro* antibacterial activity of ethanol, methanol and hot aqueous preparation of garlic and ginger extracts against all the bacteria isolated from the fish pond water samples were determined. All the bacterial isolates used for the study were very sensitive to ethanol and methanol garlic and ginger extracts except for hot water extracts which recorded lesser zones of inhibition. This may be attributed to higher extractive capacity of alcohols and therefore extracted more of the phytochemical
constituents in garlic and ginger responsible for inhibition (Table 3). Garlic extracts inhibition activity were in this order: Methanol > ethanol > hot water. Ginger extracts inhibition activity were in this order: Ethanol > methanol > hot water. This is in agreement with the report of [28] who asserted that alcohol is a better extracting solvent. The neat was tested against the isolates to determine the level of efficacy of the extracts before dilution (Table 4). All the isolates were resistant (0 mm) to these extracts at 15.6 mg/ml. *Staphylococcus aureus* and *Bacillus subtilis* were resistant (0 mm) to hot water and ethanol extracts of garlic at 31.25 mg/ml. *Vibrio parahaemolyticus* recorded 0 mm zone of inhibition for hot water extract of garlic. Methanol garlic extract at 500 mg/ml had the highest zone of inhibition (22 mm) on *Proteus mirabilis* and least (18 mm) on *Staphylococcus aureus*, *Escherichia coli* and *Vibrio parahaemolyticus* (Table 5). *Staphylococcus aureus* and *Bacillus subtilis* were also resistant (0 mm) to hot water extracts of ginger at 31.25 mg/ml and this may be attributed to the nature of their peptidoglycan (Gram positive bacteria) and is similar to the report of [29]. Ethanol ginger extract at 500 mg/ml had the highest zone of inhibition (24 mm) on *Proteus mirabilis* and least (20 mm) on *Staphylococcus aureus*, *Escherichia coli* and *Vibrio parahaemolyticus* (Table 6) which is same with the report of [29] who stated that ethanol extract of *Zingiber officinale* produced the highest zone of inhibition on *Proteus mirabilis* (17 mm) and a slight inhibitory effect on other bacteria. Garlic minimum inhibitory concentration for all the isolates ranged from 31.25 mg/ml to 62.5 mg/ml (only hot water extracts of *Staphylococcus aureus* and *Bacillus subtilis* had 62.5 mg/ml) (Table 7). Garlic minimum bactericidal concentration for all the isolates ranged from 62.5 mg/ml to 125 mg/ml while ginger minimum bactericidal concentration for all the isolates ranged from 62.5 mg/ml to 125 mg/ml (Table 7). The garlic MIC and MBC is similar to the values recorded by [30]. Better zones of inhibition were observed during synergistic analysis (garlic + ginger) at 500 mg/ml. Ethanol extracts of garlic + ginger had inhibition zones ranging from 24 mm to 29 mm on all the isolates, methanol garlic and ginger combination had inhibition zones ranging from 22 mm to 28 mm and hot aqueous combination of garlic and ginger had inhibition zones ranging from 18 mm to 25 mm. (Table 8). This is similar to the report of [31] who recorded inhibitory values ranging from 9 mm to 20 mm against Gram-positive and Gram-negative bacteria except *Streptococcus pyogenes*. In general, ginger extracts had more inhibitory effects on most of the isolates than garlic extracts and this may be attributed to higher amounts of phytochemical constituents present in ginger. (Table 3 and fig.2). The antibacterial activity of garlic is widely attributed to allicin which is an important component of garlic extract. The antimicrobial activity of ginger may be attributed to the fact that it contains antimicrobial substances such as zingiberol, zingerine and bisabolene [32]. Allicin affects the growth of bacteria by inhibiting there DNA and proteins synthesis partially and also by inhibiting RNA synthesis as primary target [33]. The results of antibacterial effect of ginger in this study are in accordance with most of the reports published by [27] regarding ginger antibacterial activity.

![Fig. 2. Synergistic effect of ginger and garlic extract. GIAE: Ginger, garlic, ethanol. GIAM: Ginger, garlic, methanol, GIAW: Ginger, garlic, water](image-url)
Table 1. Susceptibility test of the test isolates against antibacterial agents and negative controls in milliliter (mm)

| Bacterial isolates          | Ciprofloxacin (10µg) | Perflo xacin (10µg) | Oflo xacin (10µg) | Amoxicillin (30 µg) | Erythromycin (10µg) | 4% DMSO | 95% Ethanol | 95% Methanol |
|-----------------------------|----------------------|---------------------|-------------------|---------------------|---------------------|---------|-------------|--------------|
| Klebsiella pneumonia        | 25                   | 24                  | 23                | 15                  | ND                  | 0       | 0           | 0            |
| Acinetobacter calcoaceticus | 27                   | 29                  | 26                | 15                  | ND                  | 0       | 0           | 0            |
| Escherichia coli            | 29                   | 28                  | 23                | 10                  | ND                  | 0       | 0           | 0            |
| Staphylococcus aureus       | 29                   | 24                  | ND                | 22                  | 18                  | 0       | 0           | 0            |
| Vibrio cholera              | 28                   | 28                  | 26                | 20                  | ND                  | 0       | 0           | 0            |
| Pseudomonas fluorescens     | 26                   | 25                  | 17                | 12                  | ND                  | 0       | 0           | 0            |
| Pseudomonas aeruginosa      | 17                   | 10                  | 10                | 7                   | ND                  | 0       | 0           | 0            |
| Proteus mirabilis           | 23                   | 21                  | 20                | 3                   | ND                  | 0       | 0           | 0            |
| Vibrio parahaemolyticus     | 25                   | 25                  | 20                | 18                  | ND                  | 0       | 0           | 0            |
| Bacillus subtilis           | 20                   | 18                  | ND                | 2                   | 2                   | 0       | 0           | 0            |
| Shigella flexneri           | 23                   | 24                  | 18                | 10                  | ND                  | 0       | 0           | 0            |
| Salmonella typhi             | 29                   | 25                  | 21                | 19                  | ND                  | 0       | 0           | 0            |

Key: R: Resistant, I: Intermediate, S: Sensitive, ND: Not done; < 16: Resistant, 16-21: Intermediate, > 21: Sensitive (CLSI, 2002)

Table 2. Qualitative and quantitative analyses of ginger and garlic

| Phytochemical Constituents | Garlic Ethanol | Garlic Methanol | Garlic Hot water | Ginger Ethanol | Ginger Methanol | Ginger Hot water | Garlic Weight (%) | Ginger Weight (%) |
|---------------------------|----------------|-----------------|------------------|----------------|-----------------|------------------|-------------------|-------------------|
| Alkaloids                 | +              | +               | +                | +              | +               | +                | 11.32             | 15.38             |
| Saponins                  | +              | +               | -                | +              | +               | -                | 2.84              | 2.92              |
| Tannins                   | +              | +               | -                | +              | +               | +                | 0.36              | 2.46              |
| Flavonoids                | +              | +               | +                | +              | +               | +                | 6.10              | 13.62             |
| Phenols                   | -              | -               | -                | -              | -               | -                | -                 | -                 |
| Terpenoids                | +              | +               | +                | +              | +               | +                | 1.08              | 1.25              |
| Glycosides                | +              | +               | +                | +              | +               | +                | 0.04              | 0.18              |
Table 3. Quantity of ginger and garlic powder, volume of solvent used, weight (gram) of crude extract, yield percentage and colour of the crude extract

| Garlic and Ginger (gram) | Volume (ml) and type of solvent | Weight (gram) crude extract yield | Yield (%) | Colour of extract |
|-------------------------|---------------------------------|---------------------------------|-----------|------------------|
| 500g of garlic powder   | 2500ml ethanol                  | 71                              | 14.2      | Orange yellow    |
| 500g of garlic powder   | 2500ml methanol                 | 80                              | 16        | Orange yellow    |
| 500g of garlic powder   | 2500ml hot water                | 54                              | 10.8      | Orange yellow    |
| 500g of garlic powder   | 2500ml ethanol                  | 85                              | 17        | Brown            |
| 500g of garlic powder   | 2500ml methanol                 | 73                              | 14.6      | Brown            |
| 500g of garlic powder   | 2500ml hot water                | 51                              | 10.2      | Brown            |

Table 4. *In vitro* antibacterial screening using neat ginger and garlic crude extracts in millimeters (mm)

| Bacterial isolates                  | Ginger ethanol | Ginger methanol | Ginger hot water | Garlic ethanol | Garlic methanol | Garlic hot water |
|-------------------------------------|----------------|-----------------|------------------|----------------|-----------------|-----------------|
| Klebsiella pneumoniae               | 29             | 27              | 24               | 24             | 26              | 21              |
| Acinetobacter calcoaceticus        | 23             | 22              | 20               | 20             | 21              | 18              |
| Escherichia coli                   | 23             | 22              | 19               | 19             | 21              | 18              |
| Staphylococcus aureus              | 23             | 21              | 20               | 19             | 21              | 18              |
| Vibrio cholerae                    | 22             | 21              | 20               | 19             | 21              | 18              |
| Pseudomonas fluorescens            | 23             | 22              | 18               | 18             | 20              | 17              |
| Pseudomonas aeruginosa             | 22             | 22              | 20               | 20             | 21              | 19              |
| Proteus mirabilis                  | 33             | 29              | 23               | 24             | 28              | 22              |
| Vibrio parahaemolyticus            | 23             | 22              | 20               | 20             | 21              | 18              |
| Bacillus subtilis                  | 22             | 21              | 20               | 20             | 21              | 19              |
| Shigella flexneri                  | 23             | 23              | 21               | 21             | 22              | 21              |
| Salmonella typhi                   | 25             | 25              | 22               | 21             | 23              | 19              |
Table 5. *In vitro* antibacterial screening (mm) using garlic extracts with respect to various concentrations in milligram per milliliter (mg/ml)

| Bacterial isolates          | Solvents used for extraction | 500 garlic | 250 garlic | 125 garlic | 62.5 garlic | 31.25 garlic | 15.6 garlic |
|-----------------------------|------------------------------|------------|------------|------------|-------------|--------------|-------------|
| *Klebsiella pneumoniae*     | Ethanol                      | 19         | 18         | 17         | 11          | 6            | 0           |
|                             | Methanol                     | 20         | 19         | 18         | 11          | 9            | 0           |
|                             | Hot water                    | 18         | 17         | 16         | 10          | 5            | 0           |
| *Acinetobacter calcoaceticus* | Ethanol                    | 18         | 17         | 16         | 11          | 8            | 0           |
|                             | Methanol                     | 19         | 18         | 17         | 11          | 9            | 0           |
|                             | Hot water                    | 17         | 16         | 15         | 9           | 8            | 0           |
| *Escherichia coli*          | Ethanol                      | 17         | 16         | 15         | 12          | 9            | 0           |
|                             | Methanol                     | 18         | 17         | 16         | 13          | 10           | 0           |
|                             | Hot water                    | 16         | 15         | 14         | 10          | 9            | 0           |
| *Staphylococcus aureus*     | Ethanol                      | 17         | 16         | 15         | 9           | 0            | 0           |
|                             | Methanol                     | 18         | 17         | 16         | 9           | 8            | 0           |
|                             | Hot water                    | 16         | 15         | 14         | 7           | 0            | 0           |
| *Vibrio cholerae*           | Ethanol                      | 18         | 17         | 16         | 11          | 9            | 0           |
|                             | Methanol                     | 18         | 17         | 16         | 12          | 10           | 0           |
|                             | Hot water                    | 16         | 15         | 14         | 10          | 8            | 0           |
| *Pseudomonas fluorescens*   | Ethanol                      | 18         | 17         | 16         | 11          | 9            | 0           |
|                             | Methanol                     | 19         | 18         | 17         | 12          | 9            | 0           |
|                             | Hot water                    | 17         | 16         | 15         | 10          | 8            | 0           |
| *Pseudomonas aeruginosa*    | Ethanol                      | 18         | 17         | 16         | 11          | 6            | 0           |
|                             | Methanol                     | 19         | 18         | 17         | 12          | 10           | 0           |
|                             | Hot water                    | 17         | 16         | 15         | 10          | 0            | 0           |
| *Proteus mirabilis*         | Ethanol                      | 20         | 19         | 18         | 11          | 8            | 0           |
|                             | Methanol                     | 22         | 21         | 20         | 12          | 9            | 0           |
|                             | Hot water                    | 19         | 18         | 17         | 9           | 7            | 0           |
| *Vibrio para- haemolyticus* | Ethanol                      | 17         | 16         | 15         | 9           | 6            | 0           |
|                             | Methanol                     | 18         | 17         | 16         | 10          | 9            | 0           |
|                             | Hot water                    | 17         | 16         | 15         | 9           | 0            | 0           |
| *Bacillus subtilis*         | Ethanol                      | 17         | 16         | 15         | 9           | 0            | 0           |
|                             | Methanol                     | 19         | 18         | 17         | 11          | 8            | 0           |
|                             | Hot water                    | 16         | 15         | 14         | 7           | 0            | 0           |
| *Shigella flexineri*        | Ethanol                      | 18         | 17         | 16         | 11          | 9            | 0           |
|                             | Methanol                     | 19         | 18         | 17         | 11          | 9            | 0           |
|                             | Hot water                    | 17         | 16         | 15         | 9           | 8            | 0           |
| *Salmonella typhi*          | Ethanol                      | 18         | 17         | 16         | 10          | 8            | 0           |
|                             | Methanol                     | 20         | 19         | 18         | 11          | 9            | 0           |
|                             | Hot water                    | 17         | 16         | 15         | 9           | 7            | 0           |
Table 6. *In vitro* antibacterial screening (mm) using ginger extracts with respect to various concentrations in milligram per milliliter (mg/ml)

| Bacterial isolates          | Solvents used for extraction | 500 ginger | 250 ginger | 125 ginger | 62.5 ginger | 31.25 ginger | 15.6 ginger |
|-----------------------------|------------------------------|------------|------------|------------|-------------|--------------|-------------|
| *Klebsiella pneumoniae*     | Ethanol                      | 23         | 22         | 21         | 13          | 11           | 0           |
|                             | Methanol                     | 21         | 20         | 19         | 12          | 10           | 0           |
|                             | Hot water                    | 19         | 18         | 17         | 10          | 6            | 0           |
| *Acinetobacter calcoaceticus* | Ethanol                     | 21         | 20         | 19         | 13          | 10           | 0           |
|                             | Methanol                     | 20         | 19         | 18         | 12          | 10           | 0           |
|                             | Hot water                    | 18         | 17         | 16         | 10          | 8            | 0           |
| *Escherichia coli*          | Ethanol                      | 21         | 20         | 19         | 15          | 12           | 0           |
|                             | Methanol                     | 21         | 19         | 18         | 14          | 11           | 0           |
|                             | Hot water                    | 17         | 16         | 15         | 12          | 9            | 0           |
| *Staphylococcus aureus*     | Ethanol                      | 20         | 19         | 18         | 12          | 10           | 0           |
|                             | Methanol                     | 19         | 18         | 17         | 11          | 9            | 0           |
|                             | Hot water                    | 16         | 15         | 14         | 8           | 0            | 0           |
| *Vibrio cholerae*           | Ethanol                      | 20         | 19         | 18         | 14          | 11           | 0           |
|                             | Methanol                     | 19         | 18         | 17         | 13          | 11           | 0           |
|                             | Hot water                    | 17         | 16         | 15         | 11          | 9            | 0           |
| *Pseudomonas fluorescens*   | Ethanol                      | 21         | 20         | 19         | 13          | 11           | 0           |
|                             | Methanol                     | 20         | 19         | 18         | 13          | 10           | 0           |
|                             | Hot water                    | 17         | 16         | 15         | 11          | 9            | 0           |
| *Pseudomonas aeruginosa*    | Ethanol                      | 21         | 20         | 19         | 14          | 11           | 0           |
|                             | Methanol                     | 20         | 19         | 18         | 13          | 10           | 0           |
|                             | Hot water                    | 18         | 17         | 16         | 11          | 6            | 0           |
| *Proteus mirabilis*         | Ethanol                      | 24         | 23         | 21         | 14          | 11           | 0           |
|                             | Methanol                     | 23         | 22         | 21         | 13          | 10           | 0           |
|                             | Hot water                    | 21         | 20         | 19         | 11          | 8            | 0           |
| *Vibrio para-haemolyticus*  | Ethanol                      | 20         | 19         | 18         | 12          | 10           | 0           |
|                             | Methanol                     | 19         | 18         | 17         | 11          | 9            | 0           |
|                             | Hot water                    | 17         | 16         | 15         | 9           | 6            | 0           |
| *Bacillus subtilis*         | Ethanol                      | 21         | 20         | 19         | 13          | 10           | 0           |
|                             | Methanol                     | 20         | 19         | 18         | 12          | 9            | 0           |
|                             | Hot water                    | 18         | 17         | 16         | 9           | 0            | 0           |
| *Shigella flexineri*        | Ethanol                      | 21         | 20         | 19         | 13          | 10           | 0           |
|                             | Methanol                     | 20         | 19         | 18         | 12          | 10           | 0           |
|                             | Hot water                    | 18         | 17         | 16         | 10          | 9            | 0           |
| *Salmonella typhi*          | Ethanol                      | 22         | 21         | 20         | 13          | 10           | 0           |
|                             | Methanol                     | 21         | 20         | 19         | 12          | 9            | 0           |
|                             | Hot water                    | 19         | 18         | 17         | 10          | 8            | 0           |
Table 7. Minimum inhibitory and bactericidal concentration (MIC/MBC) of garlic and ginger extracts (mg/ml) with respect to various bacterial isolates

| Bacterial isolates        | Solvents used for extraction | Minimum inhibitory concentration (Garlic) | Minimum bactericidal concentration (Garlic) | Minimum inhibitory concentration (Ginger) | Minimum bactericidal concentration (Ginger) |
|---------------------------|------------------------------|------------------------------------------|---------------------------------------------|------------------------------------------|--------------------------------------------|
| *Klebsiella pneumoniae*   | Ethanol                      | 31.25                                    | 125                                         | 31.25                                    | 62.5                                       |
|                           | Methanol                     | 31.25                                    | 62.5                                        | 31.25                                    | 62.5                                       |
|                           | Hot water                    | 31.25                                    | 125                                         | 31.25                                    | 125                                        |
| *Acinetobacter calcoaceticus* | Ethanol                    | 31.25                                    | 62.5                                        | 31.25                                    | 62.5                                       |
|                           | Methanol                     | 31.25                                    | 62.5                                        | 31.25                                    | 62.5                                       |
|                           | Hot water                    | 31.25                                    | 62.5                                        | 31.25                                    | 62.5                                       |
| *Escherichia coli*        | Ethanol                      | 31.25                                    | 62.5                                        | 31.25                                    | 62.5                                       |
|                           | Methanol                     | 31.25                                    | 62.5                                        | 31.25                                    | 62.5                                       |
|                           | Hot water                    | 31.25                                    | 62.5                                        | 31.25                                    | 62.5                                       |
| *Staphylococcus aureus*   | Ethanol                      | 62.5                                     | 125                                         | 31.25                                    | 62.5                                       |
|                           | Methanol                     | 31.25                                    | 125                                         | 31.25                                    | 62.5                                       |
|                           | Hot water                    | 62.5                                     | 125                                         | 62.5                                     | 125                                        |
| *Vibrio cholerae*         | Ethanol                      | 31.25                                    | 62.5                                        | 31.25                                    | 62.5                                       |
|                           | Methanol                     | 31.25                                    | 62.5                                        | 31.25                                    | 62.5                                       |
|                           | Hot water                    | 31.25                                    | 62.5                                        | 31.25                                    | 62.5                                       |
| *Pseudomonas fluorescens* | Ethanol                      | 31.25                                    | 62.5                                        | 31.25                                    | 62.5                                       |
|                           | Methanol                     | 31.25                                    | 62.5                                        | 31.25                                    | 62.5                                       |
|                           | Hot water                    | 31.25                                    | 62.5                                        | 31.25                                    | 62.5                                       |
| *Pseudomonas aeruginosa*  | Ethanol                      | 31.25                                    | 125                                         | 31.25                                    | 62.5                                       |
|                           | Methanol                     | 31.25                                    | 62.5                                        | 31.25                                    | 62.5                                       |
|                           | Hot water                    | 62.5                                     | 125                                         | 31.25                                    | 125                                        |
| *Proteus mirabilis*       | Ethanol                      | 31.25                                    | 62.5                                        | 31.25                                    | 62.5                                       |
|                           | Methanol                     | 31.25                                    | 62.5                                        | 31.25                                    | 62.5                                       |
|                           | Hot water                    | 31.25                                    | 125                                         | 31.25                                    | 62.5                                       |
| *Vibrio para-haemolyticus* | Ethanol                    | 31.25                                    | 125                                         | 31.25                                    | 62.5                                       |
|                           | Methanol                     | 31.25                                    | 62.5                                        | 31.25                                    | 62.5                                       |
|                           | Hot water                    | 62.5                                     | 125                                         | 31.25                                    | 125                                        |
| *Bacillus subtilis*       | Ethanol                      | 62.5                                     | 125                                         | 31.25                                    | 62.5                                       |
|                           | Methanol                     | 31.25                                    | 125                                         | 31.25                                    | 62.5                                       |
|                           | Hot water                    | 62.5                                     | 125                                         | 62.5                                     | 125                                        |
| *Shigella flexineri*      | Ethanol                      | 31.25                                    | 62.5                                        | 31.25                                    | 62.5                                       |
|                           | Methanol                     | 31.25                                    | 62.5                                        | 31.25                                    | 62.5                                       |
|                           | Hot water                    | 31.25                                    | 62.5                                        | 31.25                                    | 62.5                                       |
| *Salmonella typhi*        | Ethanol                      | 31.25                                    | 62.5                                        | 31.25                                    | 62.5                                       |
| Bacterial isolates         | Solvents used for extraction | Minimum inhibitory concentration (Garlic) | Minimum bactericidal concentration (Garlic) | Minimum inhibitory concentration (Ginger) | Minimum bactericidal concentration (Ginger) |
|---------------------------|-----------------------------|------------------------------------------|--------------------------------------------|------------------------------------------|--------------------------------------------|
|                           | Methanol                    | 31.25                                    | 62.5                                      | 31.25                                    | 62.5                                      |
|                           | Hot water                   | 31.25                                    | 125                                       | 31.25                                    | 62.5                                      |

Table 8. Synergistic effects (mm) of ginger and garlic extracts (mg/ml) on the bacteria

| Bacterial isolates         | Ginger + Garlic ethanol (500mg/ml) | Ginger + Garlic methanol (500mg/ml) | Ginger + Garlic hot water (500mg/ml) |
|---------------------------|-----------------------------------|-------------------------------------|--------------------------------------|
| Klebsiella pneumoniae     | 29                                | 28                                  | 25                                   |
| Acinetobacter calcoaceticus | 24                              | 22                                  | 18                                   |
| Escherichia coli          | 25                                | 24                                  | 20                                   |
| Staphylococcus aureus     | 25                                | 24                                  | 22                                   |
| Vibrio cholerae           | 25                                | 23                                  | 20                                   |
| Pseudomonas fluorescens   | 25                                | 24                                  | 21                                   |
| Pseudomonas aeruginosa    | 25                                | 22                                  | 20                                   |
| Proteus mirabilis         | 29                                | 27                                  | 22                                   |
| Vibrio parahaemolyticus   | 24                                | 24                                  | 18                                   |
| Bacillus subtilis         | 25                                | 23                                  | 19                                   |
| Shigella flexineri        | 25                                | 23                                  | 21                                   |
| Salmonella typhi          | 24                                | 24                                  | 20                                   |
Furthermore, comparison of the inhibitory activity of garlic and ginger extracts with both Gram-positive and Gram-negative antibiotics revealed that ciprofloxacin (10µg) and pefloxacin (10µg) had the highest zones of inhibition against most bacterial isolates than the extracts when used singly. Inhibitory zones resulting from the combination of garlic and ginger were similar to that of ciprofloxacin (10µg) and pefloxacin (10µg). Alcohol garlic and ginger extracts performed better than ofloxacin (10µg), amoxicillin (30µg) and erythromycin (10µg).

5. CONCLUSION

Some of the bacterial isolates were resistant to the antibiotics used in this study. Alcoholic ginger extracts had more inhibitory effects on most of the isolates than garlic extracts and conventional antibiotics except ciprofloxacin (10µg) and pefloxacin (10µg), synergistic analysis (garlic + ginger) at 500 mg/ml had better zones of inhibition than commercial antibiotics. The antibacterial study using garlic and ginger has justified the antibacterial properties of garlic and ginger extracts. In fact, the findings revealed that the knowledge of the antibacterial activity of the extracts obtained from garlic and ginger can be very useful and can be applied in different areas of research such as the pharmaceutical and food industries. Powdered garlic and ginger can be added as adjuvants to fish feeds during milling as this will help to minimize or prevent diseases in fishes.

CONSENT

It is not applicable

ETHICAL APPROVAL

It is not applicable

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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