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Comparative Bacterial Analysis of Prepared Hibiscus Sabdariffa Leaf Drink Amended With Solutions of Ginger and Garlic Powder

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COMPARATIVE BACTERIAL ANALYSIS OF PREPARED HIBISCUS SABDARIFFA LEAF DRINK AMENDED WITH SOLUTIONS OF GINGER AND GARLIC POWDER.

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ABSTRACT

BACKGROUND

Hibiscus sabdariffa leaf drink, a popular beverage prepared from *Hibiscus sabdariffa*, is widely consumed in Nigeria because of its nutritional and refreshing qualities. However, microbial contamination during processing, storage, and vending poses significant public health concerns. This study evaluated the comparative antibacterial effects of ginger (*Zingiber officinale*) and garlic (*Allium sativum*) powder solutions on the bacterial quality of prepared Hibiscus sabdariffa leaf drink.

METHODOLOGY

Prepared Hibiscus sabdariffa leaf drink samples were obtained from local vendors and divided into four portions: garlic-amended, ginger-amended, garlic-ginger combination, and an untreated control. Two concentrations (0.2 g and 0.5 g in 10 mL sterile distilled water) of each amendment were added to 100 mL portions of Hibiscus sabdariffa leaf drink. Serial dilution, spread plate enumeration, and bacteriological analyses were performed using Nutrient Agar, MacConkey Agar, and Salmonella-Shigella (SS) Agar.

RESULTS

Plates were incubated at 37°C for 24 hours before colony counting and characterization.Results showed that the untreated control recorded the highest microbial loads, with total heterotrophic bacterial counts of 31 and 20 CFU/mL at 10^{-2} and 10^{-6} dilutions, respectively. Samples amended with garlic, ginger, and their combination exhibited marked reductions in bacterial counts, with greater reductions observed at the higher concentration (0.5 g). On MacConkey Agar, coliform counts were similarly reduced in amended samples compared to the control. No detectable growth occurred on SS Agar for any amended sample, whereas the control recorded 2 and 1 colonies, indicating possible contamination with enteric pathogens. Phytochemical analyses revealed the presence of bioactive compounds, including alkaloids, terpenoids, saponins, flavonoids, and tannins, which may contribute to the antimicrobial activities observed.

CONCLUSION

The study demonstrates that ginger and garlic possess significant antibacterial properties capable of reducing the microbial load of Hibiscus sabdariffa leaf drink, with effectiveness increasing as concentration increases. The combined use of these natural additives may enhance the microbiological safety of Hibiscus sabdariffa leaf drink while maintaining its nutritional value. Adoption of proper hygienic practices and aseptic preparation methods is also recommended to minimize contamination and improve consumer safety. This can be due to carelessness on the side of the vendor. The study recommended that complete aseptic techniques and high hygienic practices should be adopted during preparation of Hibiscus sabdariffa leaf drink

Keywords: Hibiscus sabdariffa leaf drink, *Hibiscus sabdariffa*, ginger, garlic, antibacterial activity, bacterial load, food safety, phytochemicals.

Introduction

Hibiscus sabdariffa leaf drink (Roselle juice) which is also regarded as one of the ready-to-eat nutritional drink (RTE) can be defined as food/drink prepared and or sold by vendors on the street and in other public places for immediate consumption, or at a later time without further processing or preparation. Disease that results from this drink are one of the major health problems in developing countries, Razavilar, (2010). The traditional processing methods that are used in preparation of Hibiscus sabdariffa leaf drinks, inappropriate holding temperature and poor personal hygiene of handler are some of the main causes of contamination of street-vended food/drink. Consumers who depend on such food are more interested in its convenience and usually pay little attention to its safety, quality hygiene (Mensah et al., 2002; Barro et al., 2006). Hibiscus sabdariffa leaf drinks contamination with antibiotic resistant bacteria can also be a major threat to public health, since the antibiotic resistance determinants can be transferred to other pathogenic bacteria potentially comprising the treatment of severe bacterial infection (Oladipo and Adejumobi, 2010; Threfall et al., 2000). It is expected that consumption of Hibiscus sabdariffa leaf drink is of more health benefits. However, the recent increase in food infections and poisoning around the world affected the derivatives of these benefits (Balakarami et al., 2016). In spite of nutritional challenges of Hibiscus sabdariffa leaf drinks, it is also often contaminated with diverse group of microorganisms which could be harmful to the individual who consumed it. Drinks such as Hibiscus sabdariffa leaf are frequently linked with gastrointestinal disease such as diarrhea and typhoid fever due to improper handling and serving practice (Nkere et al., 2022; Tambekar et al., 2011). Read to eat food such as Hibiscus sabdariffa leaf drinks are frequently associated with diarrhea disease which occur due to improper use of additive, the presence of pathogenic bacteria, environmental contaminants and disregard of good manufacturing practices (GMPs) and good hygiene practices (GHPs). Mishandling and disregard to hygiene measures on the part of the food vendors have introduce contaminants

and pathogens that survive and multiply in sufficient numbers to cause illness in the consumer (Greig et al., 2007). Symptoms of food borne illness include: diarrhea, vomiting, abdominal cramp and nausea, Nweze, (2010); most of which are caused by *Staphylococcus aureus, Salmonella species, Clostridium perfringens, Clostridium botulinum, Campylobacter sp, Vibrio parahaemolyticus, Bacillus cereus and Entropathogenic Escherichia coli*. Food safefy depends on conditions necessary during the production, processing, storage, and preparation of food to ensure that it is safe, sound, wholesome, and fit for human consumption, FAO/WHO, (1990). The diverse group of pathogenic microorganisms are transmitted by facial oral route, often by the ingestion of contaminated foods, drinks and water. The isolation of unacceptable level of S. aureus , an Enteroxins producer responsible for staphylococcal food poisoning in Hibiscus sabdariffa leaf drink. Food is essential resource needed for the maintenance and functioning of the various system of the body. Foods are classified based on the readiness to consumption including fully, minimally processed and ready-to-eat food. Hibiscus sabdariffa leaf drink is one of the ready-to-eat nutritional drinks consumed by several families in Nigeria irrespective of the socioeconomic status. The impact of Hibiscus sabdariffa leaf drink (Roselle juice) is seen as one of the most vital nutrient drink for the promotion of human health and prevention of disease. Food is regarded as one of human primary needs besides clothing and housing. Due to the important role of food in human existence, it is imperious to maintain high level of food safety in order to ensure that human being is safe from disease or other related health hazard associated with food. Conditions of food safety include efforts to avoid contamination from biological, chemical and other substance that can endanger human health (Adolf and Azis, 2012).

Materials and methods

SAMPLE COLLECTION AND PROCESSING

Already prepared Hibiscus sabdariffa leaf drink was purchased from the women hawking it. It was taken to the laboratory for analysis.

The Hibiscus sabdariffa leaf drink was divided into four portions as follows.

Portion 1: to be amended with garlic (100ml)

Portion 2: to be amended with ginger (100ml)

Portion 3: to be amended with combination of garlic and ginger (100ml)

Portion 4: to serve as control (100ml)

PREPARATION OF GINGER AND GARLIC SOLUTIONS

Each of garlic and ginger was weigh out separately and cut in pieces before sun-drying for 5 days. They were grinded with a laboratory blender which was rinsed with very hot water. Then varying masses of each powder was mixed 10ml of sterilized distilled water. The mixtures were kept to stand for 1 hr to homogenize properly. Little portions of the mixtures were put into the four portions of the Hibiscus sabdariffa leaf drink as shown below.

Hibiscus sabdariffa leaf Amended WithGarlic Solution	**Hibiscus sabdariffa leaf Amended WithGinger Solution**	**Hibiscus sabdariffa leaf Amended WithMixture Of Garlic/Ginger Solution**	**Hibiscus sabdariffa leaf Without Anything**
0.5g of garlic in 10ml of water	**0.5g of ginger in 10ml of water**	**0.5g of mixture of ginger & garlic in 10ml of water**	**Nothing was added**

Then 1ml from the four(4) portions was taken and added to 9ml of sterilized distilled and used to perform 10 fold serial dilution. The second and the sixth dilutions were plated out using spread plate techniques.

MEDIA PREPARATION

Nutrient agar (NA):

Nutrient Agar was prepared by dissolving 14g of nutrient agar powder in 500 ml of distilled water in a clean flask. The mouth of the flask was plugged with non absorbent cotton wool wrapped with aluminum foil paper that was extended up to the neck of the flask as described by (Syvester et al. (2015). The flask was placed on a Bunsen flame and allows to boil and mix

completely. It was sterilized in an autoclave at 1210C for 15 minutes and allowed to cool to 450C and aseptically dispensed into Petri dishes. Nutrient agar was used for the total bacterial aerobic plate count (Roseline et al, 2006)

MacConkey Agar (MA):

This agar was prepared by dissolving bile salt, then 24g of the powder was dissolved in 500 ml of distilled water. The pH was adjusted to 7.8. It was autoclave at 121^0C for 15 minutes and allowed to cool to a temperature of 45-50^0C before pouring into plates. This was used to determine coliform as described by Cheesebrough (2004). This is a selective and differential media designed to isolate and differentiate organism based on their ability to ferment lactose as described by Sebastia et al., (2012) and for enumeration of total coliform.

Salmonella and Shigella agar

This agar was prepared by dissolving 30g of the powder in 500 ml of distilled water. The pH was adjusted to 7.8. It was autoclave at 121^0C for 15 minutes and allowed to cool to a temperature of 45- 50^0C before pouring into plates. This is a selective media designed to isolate and for enumeration of Salmonella and Shigella as described by Sebastia et al., (2012).

STERILIZATION OF MATERIAL

`All materials that were used in the research such as glassware's were properly washed with detergent and water to remove dirty and contaminations and dried property. The washed glasswares was sterilized in a portable laboratory autoclave at a temperature of 121^0C for 15 minutes as described by Bukar, et al (2015). All media used were also sterilized in the autoclave at a temperature of 1210C for 15 minutes according to Nwachukwu and Osuocha (2014).

BACTERIAL ENUMERATION:

Total bacteria count was determined by Spread plate technique using standard methods. The total bacteria count was obtained by incubation aerobically at 37^0C for 24 hours.

STERILIZATION OF MATERIALS

The glassware and the wire loops were properly washed, air dried, wrapped with kraft paper and sterilized in hot air oven at 180^0C for 2 hours. Alcohol (70%) was used to disinfect working benches in the laboratory and media preparation room.

BACTERIOLOGY ANALYSIS OF SAMPLES

1ml of amended samples was drawn for serial dilution. Tenfold serial was done while second and sixth dilutions were inoculated unto the solidified media. Incubation was done at 37^OC for 24 hrs. Isolation by streaking and biochemical identifications were done on the isolate.

RESULTS

The results obtained after incubation and plate counts were represented as follows,

Table3. 1: Table for analysis and processing of sample

Hibiscus sabdariffa Amended With Garlic Solution	*Hibiscus sabdariffa* Amended With Ginger Solution	*Hibiscus sabdariffa* Amended With Mixture Of Garlic/Ginger Solution	*Hibiscus sabdariffa* Without Anything
0.5g of garlic in 10ml of water	0.5g of ginger in 10ml of water	0.5g of mixture of ginger & garlic in 10ml of water	Nothing was added

Table 4.2: colony count on Nutrient Agar

Hibiscus sabdariffa with Galic (cfu/ml)	*Hibiscus sabdariffa* with Ginger(cfu/ml)	*Hibiscus sabdariffa* with mixture Galic & Ginger (cfu/ml)	Control (cfu/ml)
30	25	*10*	57

Table 4.3: colony count on Maconkey Agar

Hibiscus sabdariffa with Galic (cfu/ml)	*Hibiscus sabdariffa* with Ginger(cfu/ml)	*Hibiscus sabdariffa* with mixture Galic & Ginger (cfu/ml)	Control (cfu/ml)
12	*16*	*2*	35

Table 4.4 : Biochemical Test Results

Organism	Gram	Catalase	Oxidase	Indole	MR	VP	Citrate	Motility
Escherichia coli	-	+	-	+	+	-	-	+
Staphylococcus sp	+	+	-	-	Variable	-	-	-
Klebsiella sp	-	+	-	-	-	+	+	-

Vibrio sp	-	+	+	-	+	-	+	+
Bacillus sp	+	+	+	-	N/A	N/A	Variable	+
Organism	Gram	Catalase	Oxidase	Indole	MR	VP	Citrate	Motility
Escherichia coli	-	+	-	+	+	-	-	+
Staphylococcus sp	+	+	-	-	Variable	-	-	-
Klebsiella sp	-	+	-	-	-	+	+	-
Vibrio sp	-	+	+	-	+	-	+	+
Bacillus sp	+	+	+	-	N/A	N/A	Variable	+

Table 6: Results of phytochemicals present in the garlic.

Phytochemical Test	**Results**
Alkaloid test	+
Test for reducing sugar	-
Flavonoids test	-
Terpenoids test	+
Glycosides test	-
Tannins test	-
Saponins test	+
Anthraquinones test	-
Carbohydrate test	-

Table 7: Phytochemical Screening of Ginger

Phytochemical Test	Results

Tannin	+++
Saponin	+++
Alkaloid	++
Flavonoid	++
Terpenoid	+
Steroid	+
Glycoside	+

Table 4.5: Results of phytochemicals present in the garlic.

Phytochemical Test	**Results**
Alkaloid test	**+**
Test for reducing sugar	**-**
Flavonoids test	**-**
Terpenoids test	**+**
Glycosides test	**-**
Tannins test	**-**
Saponins test	**+**
Anthraquinones test	**-**
Carbohydrate test	**-**



Figure 4.1: Soaked Hibiscus sabdariffa leaf and Distilled water for serial dillution

Figure 4.2: Biochemical Analysis

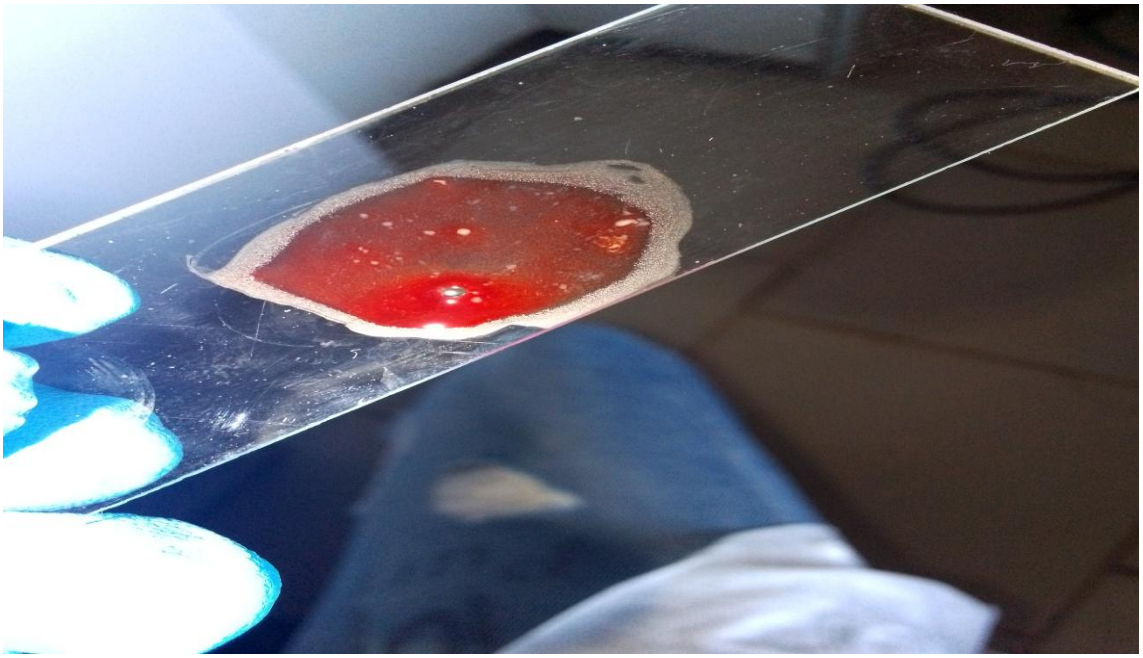


Figure 4.3: Coagulase test results

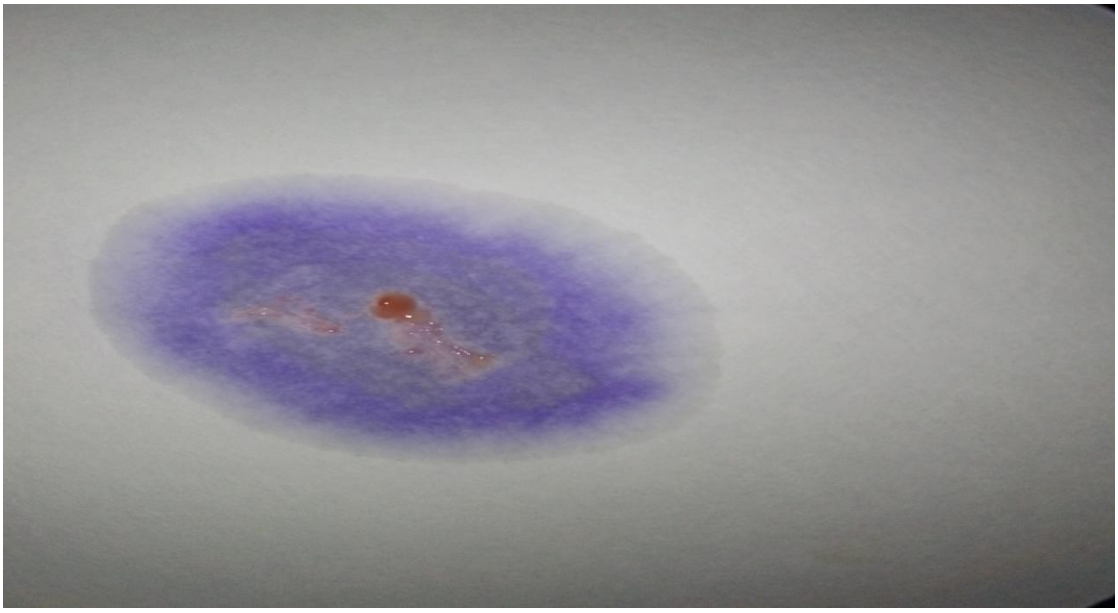


Figure 4.4: Oxidase test result



Figure 4.5: Raw garlic and Ginger powder



Figure: Hibiscus sabdariffa leaf leaf



Plate 4.2 : Plate of Maconkey Agar showing growth



Plate 4.3 : Some plates of SS agar and MaconKey Agar

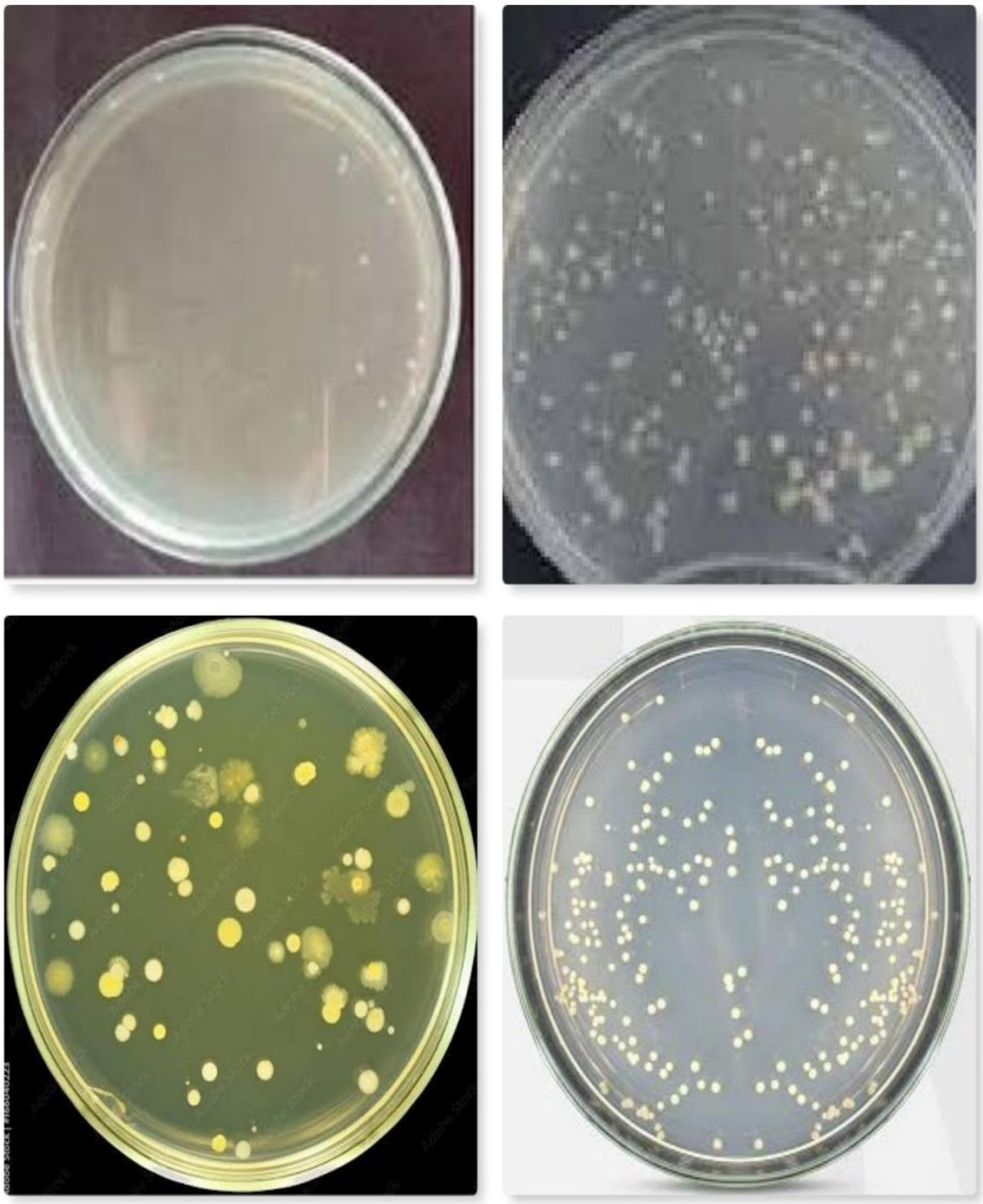


Plate 4.4 ： Plate of Nutrient Agar showing growth

DISCUSSION

This study evaluated the bacteriological quality of *Hibiscus sabdariffa* drink amended with garlic (*Allium sativum*) and ginger (*Zingiber officinale*) powders. The results demonstrated that the addition of garlic, ginger, and their combination significantly reduced the bacterial load of the drink when compared with the untreated control sample. These findings suggest that both garlic and ginger possess considerable antimicrobial properties that can improve the microbiological quality and safety of *Hibiscus sabdariffa* beverages (Nwachukwu and Ezejiaku, 2025).

The total heterotrophic bacterial counts obtained on Nutrient Agar showed a clear variation among the different treatments. The control sample recorded the highest bacterial count (57 CFU/ml), while the samples amended with garlic, ginger, and the garlic-ginger mixture recorded counts of 30 CFU/ml, 25 CFU/ml,

and 10 CFU/ml respectively. The lowest bacterial count observed in the garlic-ginger mixture indicates that the combined treatment was the most effective in suppressing bacterial growth. This finding is consistent with reports by Nwachukwu and Ezejiaku (2025), who observed that garlic and ginger contain bioactive compounds capable of inhibiting spoilage and pathogenic microorganisms in locally produced beverages.

The coliform counts obtained on MacConkey Agar further confirmed the antimicrobial effectiveness of the treatments. The untreated control sample recorded the highest coliform count (35 CFU/ml), while garlic-treated, ginger-treated, and garlic-ginger-treated samples recorded 12 CFU/ml, 16 CFU/ml, and 2 CFU/ml respectively. Coliform bacteria are generally regarded as indicators of poor sanitary conditions and possible fecal contamination of foods and beverages (FAO/WHO, 2020). Therefore, the lower coliform counts recorded in the amended samples indicate improved microbiological quality and enhanced safety of the beverage.

No growth was observed on Salmonella-Shigella Agar in all the samples analyzed. The absence of *Salmonella* and *Shigella* species is noteworthy because these organisms are important enteric pathogens associated with food-borne infections and gastroenteritis (Amusa *et al*., 2025). Their absence suggests that the processing conditions and the antimicrobial constituents present in the treatment solutions may have contributed to inhibiting their growth.

The biochemical characterization identified the bacterial isolates as *Escherichia coli*, *Staphylococcus* species, *Klebsiella* species, *Vibrio* species and *Bacillus* species. The occurrence of these organisms indicates contamination from environmental, human, and water sources during processing and handling of the beverage. The isolation of *Escherichia coli* suggests possible contamination from water used during preparation or poor sanitary practices among handlers. According to Nweze (2020), the presence of *E. coli* in ready-to-consume foods and drinks is an indication of fecal contamination and poor hygienic conditions.

Similarly, the isolation of *Staphylococcus* species suggests contamination resulting from food handlers. *Staphylococcus aureus* is commonly found on human skin, hands, nostrils, and clothing and can gain access to food through improper handling (Omemu and Aderoju, 2024). The presence of this organism is of public health concern because some strains produce enterotoxins capable of causing food poisoning characterized by vomiting, diarrhea, abdominal pain, and nausea (WHO, 2017).

The detection of *Klebsiella* species may be attributed to contamination from water, processing equipment, dust, and environmental surfaces. Members of this genus are recognized opportunistic pathogens associated with respiratory tract infections, urinary tract infections, and septicemia. Their presence in the beverage suggests inadequate environmental sanitation during processing (Nkere *et al*., 2022).

The occurrence of *Vibrio* species is particularly significant because some species within this genus are known food-borne pathogens capable of causing gastroenteritis and cholera-like diseases. Their presence may be associated with the use of contaminated water during beverage preparation (Tambekar *et al*., 2021). This finding underscores the necessity of using potable water during the production of *Hibiscus sabdariffa* beverages.

The isolation of *Bacillus* species may be attributed to contamination from soil, air, plant materials, utensils, and processing environments. Members of the genus *Bacillus* are capable of producing spores that survive harsh environmental conditions, making them common contaminants of food products (Ibitoye *et al*., 2017). Their presence in the samples suggests inadequate sanitary controls during production and storage.

The antimicrobial activity observed in the amended samples can be attributed to the phytochemical constituents detected in garlic and ginger. Garlic was found to contain alkaloids, terpenoids, and saponins, while ginger contained tannins, saponins, alkaloids, flavonoids, terpenoids, steroids, and glycosides. These phytochemicals have been widely reported to possess antimicrobial properties

through mechanisms such as disruption of microbial cell membranes, inhibition of microbial enzymes, and interference with nucleic acid synthesis (Osuji *et al*., 2025; Aziz *et al*., 2025).

The phytochemical screening results also support previous studies that reported strong antimicrobial activities associated with flavonoids, tannins, terpenoids, and saponins present in medicinal plants. Tannins and flavonoids found in ginger are known to inhibit microbial growth by interfering with bacterial metabolism and membrane permeability, while the allicin compound present in garlic exerts broad-spectrum antibacterial effects against both Gram-positive and Gram-negative bacteria (Afolabi *et al*., 2018).

The superior performance observed in the garlic-ginger combination treatment may be due to synergistic interactions among the phytochemical compounds present in both plants. This synergistic effect likely enhanced bacterial inhibition beyond that achieved by either garlic or ginger alone. Similar observations have been reported by Nwachukwu and Ezejiaku (2025), who demonstrated improved antimicrobial effectiveness when natural plant preservatives were used in combination rather than individually.

Furthermore, the proximate composition of ginger and garlic revealed appreciable levels of crude protein, crude fibre, ash, ether extract, and nitrogen-free extract, indicating their nutritional value. Besides contributing to the microbial safety of the beverage, the addition of these plant materials may also improve its nutritional and medicinal properties (Arvind *et al*., 2021; Bamishaiye *et al*., 2021).

Overall, the findings of this study indicate that locally prepared *Hibiscus sabdariffa* drinks are susceptible to contamination by potentially pathogenic microorganisms due to poor handling practices, environmental contamination, and inadequate sanitation. However, the incorporation of garlic and ginger, particularly in combination, significantly reduced microbial contamination and improved the bacteriological quality of the beverage.

5.2 Conclusion

This study successfully investigated the comparative bacteriological quality of *Hibiscus sabdariffa* drink amended with garlic and ginger powders. The findings revealed that untreated *Hibiscus sabdariffa* drinks contained higher bacterial and coliform loads than treated samples, confirming the susceptibility of locally prepared beverages to microbial contamination arising from poor handling practices, contaminated water sources, and unhygienic processing environments (Omemu and Aderoju, 2024).

The study identified the presence of *Escherichia coli*, *Staphylococcus* species, *Klebsiella* species, *Vibrio* species, and *Bacillus* species in the beverage samples. The occurrence of these microorganisms indicates public health risks associated with consumption of improperly prepared *Hibiscus sabdariffa* drinks and highlights the importance of adequate sanitation during production (Nweze, 2020; WHO, 2017).

Garlic- and ginger-amended samples recorded significantly lower microbial loads compared with the untreated control. Among all treatments, the mixture of garlic and ginger produced the greatest reduction in both total heterotrophic bacterial counts and coliform counts. This demonstrates the synergistic antibacterial activity of the phytochemical compounds present in both plants. Phytochemical screening confirmed the presence of alkaloids, tannins, flavonoids, terpenoids, glycosides, steroids, and saponins, which are known for their antimicrobial effects. These bioactive compounds were responsible for the inhibition of bacterial growth observed in the treated samples (Osuji *et al.*, 2025).

Based on the findings, it can be concluded that garlic and ginger are effective natural antimicrobial agents capable of improving the microbiological quality and safety of *Hibiscus sabdariffa* beverages. Their combined application offers a cost-effective and natural preservative strategy that may help reduce food-borne illnesses associated with locally produced drinks.

5.3 Recommendations

Based on the findings of this study, the following recommendations are made:

1. Producers of *Hibiscus sabdariffa* beverages should incorporate appropriate concentrations of garlic and ginger extracts as natural preservatives to reduce microbial contamination and improve product safety (Nwachukwu and Ezejiaku, 2025).
2. The combined use of garlic and ginger should be encouraged because it demonstrated superior antibacterial activity compared with their individual applications.
3. Potable and microbiologically safe water should always be used in the washing, soaking, extraction, and preparation of *Hibiscus sabdariffa* beverages to prevent contamination by enteric pathogens (FAO/WHO, 2020).
4. Food handlers should maintain high standards of personal hygiene, including regular hand washing, proper use of protective clothing, and avoidance of direct hand contact with processed products (WHO, 2017).
5. Processing equipment, storage containers, bottles, and packaging materials should be adequately cleaned and sterilized before use to minimize contamination from environmental microorganisms.
6. Production environments should be kept clean and protected from dust, insects, rodents, and other contamination sources that can introduce pathogenic microorganisms into beverages (Nkere *et al*., 2022).
7. Regulatory agencies such as NAFDAC and local health authorities should intensify routine inspection and monitoring of beverage production facilities to ensure compliance with food safety standards.
8. Regular training programs on Good Manufacturing Practices (GMP) and Good Hygiene Practices (GHP) should be organized for beverage producers and street vendors to improve food safety awareness (FAO/WHO, 2020).
9. Further studies should investigate different concentrations of garlic and ginger extracts to determine the optimum levels necessary to achieve maximum antimicrobial effects without affecting consumer acceptability.

10. Additional research should evaluate the effect of garlic and ginger treatments on the shelf life, sensory characteristics, physicochemical properties, and consumer preference of *Hibiscus sabdariffa* beverages.
11. Routine microbiological quality assessment of locally prepared beverages should be encouraged as a strategy for protecting public health and reducing the incidence of food-borne diseases (Wada *et al*., 2025).
12. Public enlightenment campaigns should be conducted to educate consumers and producers on the importance of food safety practices and the risks associated with contaminated beverages (WHO, 2017).

COMPETING INTEREST

The authors are saying there is no competing interest in this research work.

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