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# Phytochemical Analysis and Antibacterial Activity of Ginger (*Zingiber officinale*) Methanolic Extract Against *Escherichia coli* and *Staphylococcus aureus*

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## ABSTRACT

*Escherichia coli* and *Staphylococcus aureus* are among the most prevalent pathogens found in food poisoning. Infections caused by these organisms are associated with high mortality rate in hospitalized patients. This study is aimed at evaluating the antibacterial activities of Ginger (*Zingiberofficinale*), Against *Escherichia coli* and *Staphylococcus aureus* using agar well diffusion method. Based on the phytochemical screening, the extract was tested for the presence of different chemical groups like Alkaloids, Flavonoids, Tanins, Steroids and Saponins. The result of this study revealed that the extract inhibited the growth of the microorganisms to varying proportion with zones of inhibition ranging from 7 to 16mm. The extract produced the highest zone of inhibition on Escherichia coli (16mm) while Staphylococcus aureus(14mm) shows the least. Furthermore, the phytochemical screening of the extract revealed the presence of Alkaloids, Flavonoids, Tanins, Steroids and Saponins in ginger. The presence of these phytochemical constituent in the extract could be responsible for the observed antimicrobial activity. The study confirms the use of Ginger extract in the treatment of wounds infection in the traditional medicine in different part of the world.

**Keywords:** Ginger (*Zingiberofficinale*), *Escherichia coli* and *Staphylococcus aureus.*

## INTRODUCTION

Herbs and spices parts of plants from indigenous or exotic origin are essential parts of human diets as they improve taste, colour and aroma of food (Ramkumar and Karuppusamy *et al*., 2021). They also have antioxidant and antimicrobial properties (Ramkumar and Karuppusamy *et al*., 2021). Antimicrobial agents are essentially important in reducing the global burden of infectious diseases. However, as resistance pathogens develop and spread, the effectiveness of the antibiotic is diminished. This type of bacterial resistance to the antimicrobial agents poses a very serious threat to public health, and for all kinds of antibiotics, including the major last-resort drugs, the frequencies of resistance are increasing worldwide. Therefore, alternatives antimicrobial strategies are urgently needed, and thus this situation has led to evaluation of therapeutic use of ancient remedies, such as plants and plant-based products (Giannenas *et al*., 2020).

Medicinal plant is any plant in which one or more of its parts contain substance (phytochemical) that can be used for therapeutic purpose or which are precursors for the synthesis of useful drugs. About 80 % of the world medicine are originally derived from plants source especially those found in tropical regions (Hawa *et al*., 2010). Phytochemical are bioactive chemicals of plant origin. They are regarded as secondary metabolites because the plants that manufacture them may have little need for them. They are naturally synthesized in all parts of the plant body; bark, leaves, stem, root, flower, fruits, seeds, etc. Phytochemical have been recognized as the basis for traditional herbal medicine practiced in the past and currently in vogue parts of the world (Ganie *et al*., 2022). Most of the knowledge about plants that have useful properties come from people

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occupying a particular locality. These natives pass on the knowledge from generation to generation making the use of techniques available for them to perform plant extractions, such traditional methods of metabolite extraction include, boiling with water, cold infusion, burning into ashes and mixing with oil. During the technological advancement of the last century where contemporary method of extraction utilize principle based on polarity and alteration of pH. This method provides chance to quantify and to study the environmental factors that regulate the synthesis of such chemical compounds (Saidu *et al*., 2022). Furthermore, Medicinal herbs are moving from fringe to mainstream use with greater number of people seeking for remedies and health approaches free from side effects caused by synthetic chemical (Sharma *et al*., 2022). In addition, medicinal plant have been identified and used throughout human history. Plants have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions and to defend against attack from predators such as insect, fungi, and herbivorous mammals. Recently, considerable attention has been paid to eco-friendly and bio-friendly plants, which can prevent and cure different human disease (Sharma *et al*., 2022). According to the World Health Organisation reports, the use of traditional medicine in the first world countries is on the rise due to failure of conventional medicine that can cure chronic disease, emergence of multi-drug resistance pathogens and parasites, adverse effects of chemical drugs, increasing cost and information of herbal medicine (Makhoba *et al*., 2020). Antimicrobial potentiality of different medicinal plants is extensively studied all over the world.

Ginger is used as a herb and also a spice, it is a member of the family *Zinberaceae* and its scientific name is *zingiberofficinale* (Kirby-Bauer *et al.*, 2014). Ginger is a thick scaly rhizomes which are aromatic, thick lobed, branched, have a scaly structure and they posses a spicy lemon like scent (Singh *et al.,* 2011). The syrup from from ancient ginger rhizomes is highly strong and plays role as powerful food maintenance. Ginger (*zingiberofficinale*) is a medicinal plant that have been widely used all over the world, since antiquity, for a wide array of unrelated aliments including arthritis, cramps, rheumatism, sprains, sore throats, muscular aches, pains, constipation, vomiting, hypertension, indigestion, dementia, fever and infectious diseases. Ginger has direct anti-microbial activity and thus can be used in treatment of bacterial infection; ginger is relatively inexpensive due to their easy availability, universally acceptable and well tolerated by most people (Chukwudebe, 2020).

*Zingiber officinale* plays role as powerful food maintenance. In limited studies, ginger was found to has better effectiveness than placebo in relieving disgust produced by sea dizziness, morning dizziness, despite ginger not reported to be preferred on placebo in relieving surgical sickness. The plant is reported to have antibacterial, anti-oxidant, antiprotozoal, anti-insecticidal activity, anti-fungal, anti-emetic, anti-rhinoviral, and anti-inflammatory. Reported pharmacological activities of ginger include antipyretic, analgesic, ant tissue in addition to hypersensitive effect (Saxena, and Kukreti, 2020). Ginger was found to have free radical remover activity (prevent oxidant formation); so prevent lipid oxidation. Moreover, ginger displayed chemopreventive and antineoplastic effect; also ginger shows to have in the future role in cancer inhibition, although additional studies are required to assess this property of ginger (Saxena, and Kukreti, 2020).

Food borne pathogens are widely distributed in the environment and may be a significant cause of mortality and morbidity in the population (Indu *et al*., 2006). Escherichia coli is a significant food borne hazard in

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many countries around the world and infection often causes haemorrhagic diarrhoea and occasionally turns kidney failure and death, also *Staphylococcus aureus* causes food borne illness due to their ability to form heat stable toxins (WHO., 2007). Therefore, keeping in view the important of the plant (ginger) as important medicinal foods,

The presence of these organisms and their growth and multiplication in food do not produce much appreciable changes in foods but leads to food borne infection. In order to prevent the growth of bacterial pathogens in food, various preservative techniques have been used. Consumer concerns on the safety of foods containing synthetic chemicals as preservatives have resulted in a growing need for use of natural antibacterial compounds, having a characteristic flavour, antioxidant and antimicrobial activity. Currently, various natural compounds like spices are preferred and used as food preservatives. Kerala, a south Indian state in tropical Asia is a well-endowed state with numerous varieties of spices. Spices form an integral part of the traditional cuisines in the everyday diet of the common man here. With globalisation and increased recreational travel, intercontinental modification in the local cuisines has resulted in vast changes in traditional cooking methods. The anti-microbial properties of various natural spices against emerging pathogens have to be taken advantage of, with the changing lifestyle. This Research is aimed at evaluating the phytochemical constituents and the antimicrobial activity of ginger extract against some food-borne microorganism.

## MATERIALS AND METHODS

### Colllection of Plant Material

Fresh form of ginger rhizomes were purchase from the local market in Gombe state. The samples were identified by Mal Muhammad chindo of Biological Science Department, Gombe State University. A herbarium specimen with voucher number 110 of the same plant has been deposited. The fresh form will then be clean, slice and dry at room temperature for fourteen days. After drying, pieces of ginger will be grind to fine particle (powder) using clean pestle and mortar.

### Phytochemical Extraction of Ginger Sample

The extraction of phytochemical was conducted using methanol reagent as described by Dhawan, and Gupta *et al*., (2017). Twenty five grams of ginger was weigh and macerated in a container containing 150 millilitres of Methanol. The container was shaken intermittently using rotary shaker for three days at room temperature. The resulting extracts were decanted, filtered using Muslin cloth and stored in sterile bottles, the bottle was labelled and kept at 4 $^{o}$C in refrigerator.

### Phytochemical Analysis

The phytochemical constituents such as alkaloids, saponins, tannins, flavonoids and steroid were measured according to the method described by Iram *et al*., (2018). Specifically, alkaloids were extracted using dilute Hydrochloric acid, Tannins, and flavonoids were extracted using sodium chloride, and steroids using acetic anhydride $H_2SO_4$. The phytochemical analysis was conducted on the plants crude extracts to identify the constituents as described by;

#### *Detection of alkaloids*

Extracts were dissolved in dilute Hydrochloric acid and filtered. Filtrates were treated with Wagners reagent; Presence of alkaloids was confirmed by the formation of yellow coloured precipitate.

#### *Detection of saponins*

About 0.5g of extract was shaken with 2 ml of water. Foam was produced and persists

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for ten minutes which confirmed the presence of saponins.

***Detection of tannins***

To a small portion of extract, 2 mL of 1% gelatin solution containing sodium chloride was added to the mixture, after sometime, the production of white precipitate indicated the present of tannins.

***Detection of flavonoids***

Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids.

***Detection of steroids***

Two milliliters of acetic anhydride were added to 0.5 mL of the extracts and then followed with 2 mL $H_2SO_4$. There was no change of colour from violet to blue or green which indicates the absent of steroids.

**Collection and Maintenance of Organisms**

*Staphylococcus aureus* and *Escherichia coli* were obtained and confirmed at the microbiology laboratory of the Gombe State University. They were maintained on slanted Nutrient Agar medium inside a bijou bottle and kept at 4$^{o}$C. Twenty-four hour old pure cultures were prepared for use each time.

**Identification of the Test Isolates**

*Escherichia coli* were subjected to gram staining and biochemical tests for confirmation. Indole test, citrate utilization test, and catalase test were carried out to identify and confirmed that the isolate were *E. coli*. *Staphylococcus aureus* was also subjected to gram staining and biochemical tests. It was first sub-cultured onto Mannitol Salt Agar (MSA), followed by catalase test and coagulase test were carried out to identify and confirmed the isolate was *S. aureus*.

**Preparation of Stock Solution of the Extract**

In the study of the anti-bacterial activities of this plant, concentrations of 400 mg/mL of the extract were used for the screening. This was done by dissolving 0.4 g of the extracts in 1 mL of DMSO.

**Standardization of the Inocula**

Turbidity standard equivalent to McFarland 0.5; is a barium sulphate standard against which the turbidity of the test inocula were compared. When matched with the standard, the inocula gave confluent growth of $10^8$CFU (Cheesbrough, 2006). For the preparation of the MacFarland standard, 1% v/v solution of sulphuric acid was prepared by adding 1 ml of concentrated sulphuric acid to 99 ml of water. Then 1% w/v solution of barium chloride was prepared by dissolving 0.5 g of dihydrate barium chloride ($BaCl_2.2H_2O$) in 50 ml of distilled water. 0.6 ml of the barium chloride solution was added to 99.4 ml of the sulphuric acid solution, and mixed. A small volume of the turbid solution was transferred to a screw-cap bottle of the same type as used for preparing the test and control inocula (Cheesbrough, 2006).

**Sensitivity Test**

Mueller-Hinton Agar was prepared according to the manufacturers instruction, autoclaved and dispensed at 20 mL per plate in 12 x 12 cm Petri dishes. Set plates were incubated overnight to ensure sterility before use. Suspension of microorganisms was made in sterile normal saline and adjusted to 0.5 Macfarlandstandard ($10^8$ Cfu/mL) (NCCLS, 2000). From the stock of 100 mg/mL extract, serial dilutions were made to 50, 25, 12.5, 6.25, mg/mL (NCCLS, 2000). Each labeled medium plate was uniformly inoculated with a test organism by using a sterile cotton swab rolled in the suspension to streak the plate surface in a form that lawn growth can be observed.

A sterile cork borer of 6mm diameter was used to make wells on the medium. 0.1 mL

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of the various extract concentration were dropped into each, appropriate labeled well (Shahidi, 2004). The inoculated plates were kept aside for 1 hour to allow the extracts to diffuse into the agar (Atata *et al*., 2003). The Mueller Hinton Agar plates were incubated at 37$^{o}$C for 24 h. Antibacterial activity was determined by measuring the diameter of zones of inhibition (mm) produced after incubation. Ciprofloxacin and Agumentin were the positive control used.

**Minimum Inhibitory Concentration (MIC) Determinations**

The minimum inhibitory concentration was determined using the Broth dilution method. Nutrient broth was prepared by dissolving 10 mL media by dispensing into test-tubes, sterilized by autoclaving and allowed to cool. McFarland's turbidity standard scale number 0.5 was prepared to give a turbid solution. Dilution of the test microbes was in normal saline until turbidity matched that of the McFarlands scale and the concentration of the test microbes was taken to be 1.5×10 cfu/mL. The stock extract was diluted serially to obtain 400 mg/mL, 200 mg/mL, 100 mg/mL, and 50 mg/mL concentrations. The test microbes were then inoculated into the different concentrations (Shahidi, 2004) and incubated at 37°C for 24 hr. There after the test tubes were observed for turbidity or growth. The lowest concentration of extracts in the sterile broth which showed no turbidity was recorded as the minimum inhibition concentration (MIC). Two control tubes were maintained. These include tube-containing extract without inoculum and the tube containing the growth medium and inoculum.

**Minimum Bactericidal Concentration (MBC) Determinations**

This was carried out to determine the concentration of extracts with high bactericidal effect. Mueller Hinton Agar was prepared, sterilized at 121°C for 15 min, poured into sterile Petri dishes and allowed to cool. The contents of the test tube with the determined MIC was then sub-cultured onto prepared media, incubated at 37°C for 24 hr. after which the plates were observed for any colony growth. MBC plates with lowest concentration of extract without a colony growth were considered as MBC (Shahidi, 2004).

## RESULTS AND DISCUSSION

Freshly prepared extract of the Ginger (Figure 1) was subjected to preliminary phytochemical screening for various constituents and the results of the methanolic extracts were summarized in Table 1.

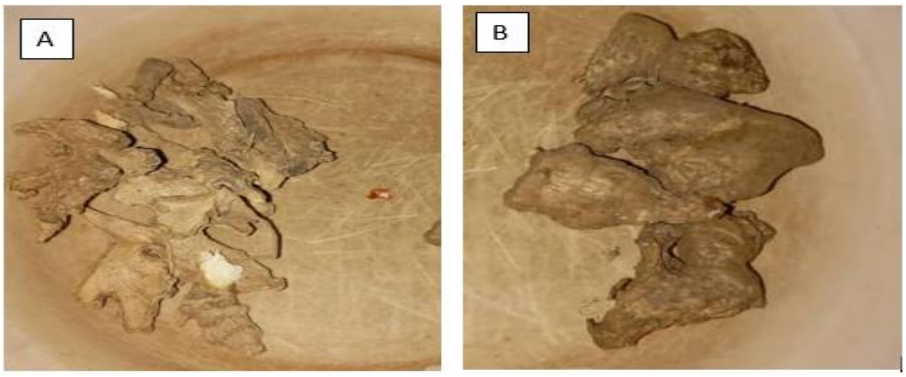


**Figure 1:** Pictures of Ginger Tuber. (a) Dried (b) Fresh.

After the extract of the plant was extracted, it was then characterized based on its physical properties such as color, texture, and consistency. Based on that, the plant extract was found to be pale yellow, smooth texture and liniments in consistency. Table 1 shows the results of phytochemical screening of ginger extract. It can be seen from the result

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that alkaloids, saponins, tannins, flavonoids, and steroids were all present. This corresponds to the work of (Fakhri, *et al*., 2023) who worked on ginger and found out that steroids are absent. The reason may be due to the aqueous solvent used which does not extract the extract from ginger like methanol does. The above table shows the result of phytochemical screening of ginger extract. It can be seen from the result that Flavonoids, Tannins, Saponins, Steroids, and Alkaloids were found to be present.

**Table 1:** Results of the Phytochemical screening of ginger.

| Test of Active Principles | Flavonoids | Tannins | Saponins | Steroids | Alkaloids |
|---|---|---|---|---|---|
| Ginger Extract | + | + | + | + | + |

Key: + Present.

The potential antibacterial effect of methanolic Ginger extract of Ginger at different concentrations was tested against some clinical isolates *E. coli* and *S. aureus*. The justification for varying the concentrations of the plant extract is due to the following reasons; (1) to determine the optimum level of the extract to inhibit the growth of the bacteria, (2) to determine the tolerance level of the bacteria against the increase in the concentrations of the extract 50% extract of ginger was found to produce the least zone of inhibition of 10 and 7 in *E.coli* and *S. aureus*. As the concentration was increased to 100%, the zone of inhibitions was increased to 12 and 13%. The zone of inhibition decreases from 13 to 9 in *E. coli* and 14 to 8 in *S. aureus*. Moreover, the control experiment produces the zone of inhibition of 35 and 12 in E. coli and S. aureus respectively (Table 2).

**Table 2:** Antibacterial activity of ginger extracts.

| Test Organisms | 50% | 100% | 200% | 400% | C |
|---|---|---|---|---|---|
| **Zones of Inhibition (MM)** | | | | | |
| *E.coli* | 10 | 12 | 13 | 9 | 35 |
| *S. aureus* | 7 | 13 | 14 | 8 | 12 |

Key: C= Control. The above shows the result of antibacterial testing indicating zones of inhibition measured in millimetres. The positive control used was Ciprofloxacin and Augmentin an Antibiotic.

This confirmed that as the concentration of the plant extract increases, the activity of the extract against the bacteria also increases, and vise vasa. This might be due to the presence of phytochemicals such as cardiac glycosides, flavonoids, alkaloids, Carbohydrate, tannins, terpens, and saponins which were all confirmed in this research and were presented in Table 1 of this study. This result was found to agree with the research of Hamza *et al*., (2024) which found the same secondary metabolite of the plant extract when screening out the extract obtained from other plant such as *A. digitata* leaves, *A. mannii* leaves, and *A. Alboviolaceum* fruit, *A. Polyanthum*fruit and *O. Gratissimum* twigs.

The zone of inhibition observed on *S. aureus* was almost the same as that of *E. coli*. This correlates with the work of (Islam *et al*., 2021) which showed the strong activities of the extract of Ginger against some Gram-positive and Gram-negative organisms, and the work of (Dalia *et al*., 2014) which showed the strong activities of the ginger extract against some pathogenic bacteria Isolated from Patients with otitis media. The antibacterial effect of methanol extract against these organisms may be due to the ability of the methanol to extract some of the active properties of these plants like phenolic

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compounds, saponin, bryophyllin and other secondary metabolites which are reported to be antimicrobial (HumayunRiaz *et al*., 2015).

**Minimum Inhibitory Concentration (MIC) of the Extract**

The result of the Minimum Inhibitory Concentration (Table 3) showed that the methanol extract was not most effective with MIC at a higher concentration of 100mg/mL and the Minimum Bactericidal Concentration (Table 4) showed the MBC at 200mg/ml. This is contradictory to the work of (Zanariah *et al*., 2015) that showed the most effective extract of ginger with MIC at 0.05mg/ml and MBC at 1.0mg/ml. This contradiction may be due to the variation in the concentrations applied in the well and the solvents used, since in the work of (Zanariah *et al*., 2015) ethanol-water and methanol were the solvents of choice.

The results of the MIC test of ginger extract shown in Table 3 below showed that the 200mg/ml and 100mg/ml concentrations of the extract inhibited the growth of the tested isolates. Still, they were able to grow in 50 mg/ml concentration of the extracts after 24 hours. This implies that the MIC of each extract on the *S. aureus and E. coli* is 100 mg/ml. There was also no growth on the control tube containing Augmentin and Ciprofloxacin.

**Table 3:** Minimum Inhibitory Concentration of the extract.

| Concentration (mg/ml) | Change in Broth Turbidity |
|---|---|
| 200 | No |
| 100 | No |
| 50 | Yes |
| Control (Augmentin and Ciprofloxacin) | No |

MIC = 100mg/ml

**Minimum Bactericidal (MBC) of the Extract**

The results of the MBC test of ginger extract are shown in Table 4 below which shows that total cell death from the MIC test occurred in the 200 mg/ml concentration. Therefore MBC of the extract on the tested isolates was taken as 200 mg/mL.

**Table 4:** Minimum Bactericidal Concentration of the extract.

| Concentrations (mg/ml) | Appearance of Growth |
|---|---|
| 200 | Negative |
| 100 | Positive |

MBC = 200 mg/mL

## CONCLUSION

This study shows that ginger extract has antibacterial properties, and it exerts its effect on both gram-positive and gram-negative bacteria. Therefore this result supports the traditional medicine use of ginger traditional medicine. Based on this research, it is recommended that further research should be carried out on the activity of the plant-separate phyto-compounds to discover potentially active compounds that can serve as sources and templates for the synthesis of new antimicrobial drugs. Based on this research, it is recommended that further research should be carried out on the activity of the plant separate phyto-compounds to discover potentially active compounds that can serve as source and template for the synthesis of new antimicrobial drugs.

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