

Research Article

Antimicrobial Activities of Ginger extract on Selected Pathogenic Microorganisms

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Abstract:

Antimicrobial activities of ginger (*Zingiber officinale*) were investigated for its microbial activity against clinical isolates of *Salmonella*, *Staphylococcus aureus*, *Escherichia coli*, and *Bacillus* using agar well diffusion method. Freshly harvested rhizomes of both local and agric ginger from the Clifford University Farm were employed for this study. The rhizomes were washed to remove adhering soil particles, sliced, dried and milled to powder, extraction was done using ethanol and water. Tests carried out on both ethanol and water extracts of local and agric ginger were phytochemicals, antimicrobial activities, Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC). The phytochemical test result on both local and Agric ginger typically showed the presence of bioactive compounds such as tannins, alkaloids, saponins, cardiac glycosides, flavonoids, anthraquinone derivatives with traces of steroids. The study showed that ethanol extracts of ginger exhibited significant antimicrobial activity, producing larger zones of inhibition compared to water extracts which could be as a result of better extraction with alcohol solvents.

Keywords: Ginger, Antimicrobial properties, Clinical isolates, ethanol and water extracts.

1. Introduction

Antibiotics are antimicrobial substances with the ability to either kill or inhibit the growth of microorganisms. They are commonly used in non-medical applications as well as the treatment of bacterial infections in humans and animals [1].

One of the antibiotics of interest is ginger which can be regarded as natural antibiotic or antimicrobials, which is a member of the family Zingiberaceae (*Zingiber officinale*). This is a flowering plant whose roots, or rhizome, are commonly used as a spice and medicinal drug. Ginger plants are said to have some genetic variation in Southern Asia, where they are said to have originated from the tropical rainforest of the Indian subcontinent. Many nations, including Bangladesh, Taiwan, India, Jamaica, and Nigeria, cultivate ginger extensively. Due to their shared family tree, the ginger plant and other medicinal plants like cardamom (*Elettaria cardamomum*) and turmeric (*Curcuma longa*) are closely related. Carbohydrates (60–70%), water (9–12%), proteins (9%), lipids (3–6%), ash (8%), crude fiber (3–8%), and essential oil (2–3%) are the components of ginger. In addition to these macronutrients, minerals like sodium, potassium, calcium, magnesium, and phosphorus are also present in ginger, along with a variety of bioactive compounds [2].

Ginger is used to treat conditions like rheumatism and bleeding, also, it is claimed to be a treatment for nausea, hair loss, snakebite, toothaches, and respiratory conditions were among the conditions for which it was allegedly used [3]. Some Africans believe that regularly

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consuming ginger will help repel mosquitoes, while in Arabian medicine ginger is considered an aphrodisiac. Worldwide, ginger is used as a culinary spice, condiment, and herbal medicine. For nausea, stomach pain, and other fitness-related problems, it is a well-liked home remedy. It is thought to have been among the first spices to be exported from Asia and was utilized by the ancient Greeks and Romans [4]. For many years, people all over the world have used the rhizome of the ginger plant as a spice. Ginger's fleshy, upright, elongated pseudo stems, which are attached to an underground multibranched rhizome, give rise to the leaves.

Ginger leaves taste mild, grassy, peppery, and vegetal, similar to rhizomes but with a more sophisticated flavor. They can be fed raw or cooked. The leaves' fiber content can help regulate the digestive tract, while the potassium balances fluid levels, the magnesium helps maintain optimal nerve function, and the calcium helps build strong bones and teeth. Ginger leaves are used in herbal medicines throughout Asia, particularly in China, where they have antibacterial and antifungal qualities. Bioactive substances like phenolic compounds, flavonoids, terpene, and certain volatile compounds found in ginger, the essential oils are primarily responsible for the health benefits of ginger for humans.

Numerous illnesses have been linked to ginger's treatment or prevention, including post-chemotherapy nausea and vomiting [5], blood pressure lowering (anti-hypertension) [6], anti-diabetic activity by lowering blood glucose levels [7], anti-hyperlipidemia [8], and other conditions. Furthermore, due to its ability to suppress reactive oxygen species and function as an antioxidant, ginger is widely used to prevent a number of chronic diseases [9].

Because ginger extract has the potential to offer novel therapeutic options, support sustainable healthcare practices, inspire the development of novel pharmaceuticals derived from its compounds which could lead to the synthesis of more potent and targeted antimicrobial agents and aid in the global fight against antimicrobial resistance, this study was therefore initiated to investigate its antimicrobial activities against specific pathogens and to determine the phytochemical components of ginger.

2. Materials and Methods

2.1 Study Site/ Sample Collection

Fresh ginger rhizomes (local and agric ginger) were uprooted from Clifford university farm. Only healthy-looking rhizomes without any signs of spoilage or damage were selected for further processing.

2.2 Preparation of Media

Media used for the work were Nutrient agar and Muller Hinton agar for isolation and for growth of bacteria respectively which were prepared according to the manufacturer's instruction.

2.3 Ginger Extract procedure

Mature ginger rhizomes were uprooted; the uprooted ginger rhizomes were washed thoroughly to remove adhering sand particles, debris and impurities. The outer skin of the ginger rhizomes was peeled off using clean knife, the peeled ginger rhizomes were dried under shield at room temperature to reduce moisture content and improve shelf life. The peeled and dried ginger rhizomes were grounded into a fine powder with the use of grinder to increase the surface area and facilitate the extraction process.

Using the method of Akintobi *et al.*, [10] with modifications, the active ingredients were extracted by cold maceration method, using ethanol and water as the solvents. The extract was filtered through Whatman filter paper to remove any solid particles. The filtered extract was concentrated by evaporating the solvent under reduced pressure using a water bath at 45 °C. The dried active ingredients gotten after the evaporation was weighed with digital weighing balance and was later transferred into a separate clean sample bottles for storage with proper labelling for documentation purposes and kept inside fridge until needed.

2.4 Determination of Percentage Yield

The percentage yield of the extract was calculated using the following formula:

$$\% \text{Yield} = \text{mass of extract} / \text{mass of ginger powder} \times 100 / 1$$

2.5 Sterilization and Inoculation/ Incubation

The media used for isolation and the Dimethyl Sulfoxide (DMSO) used for the dilution were sterilized with the use of autoclave at 121°C for 15 minutes at 15 psi. 0.5g of ginger extract was weighed into 5ml of the sterile DMSO and a 6-fold serial dilution was prepared.

Bacterial strains *E. coli*, *Staphylococcus*, *Salmonella*, and *Bacillus* were obtained from clinical isolates from the Microbiology laboratory Clifford University. Pure cultures were maintained on agar slants and sub-cultured prior to the experiments. Agar diffusion method was used, 6 wells were bored with a sterile well borer on the Muller Hinton agar plates which have been inoculated with test microorganisms and the wells were impregnated with various concentrations of ginger extracts (250 mg/ml, 125 mg/ml, 62.5 mg/ml, 31.3 mg/ml, 15.6 mg/ml, and 7.8 mg/ml) in duplicates. The plates were then incubated at 37°C for 24 hours. The sensitivity of the test organisms to ethanol and water extract of ginger was indicated by a clear zone of inhibition around the wells. The diameter of the clear zone (Zone of inhibition) was measured to the nearest mm using a transparent ruler. The zone of inhibition was taken as an index of the degree of sensitivity of the test organisms to ethanol and aqueous extracts.

2.6 Phytochemical Test

Phytochemical tests involve several qualitative tests to identify the presence of bioactive compounds present in ginger. The phytochemical tests include; Alkaloids, Flavonoids, Tannins, Saponins, Steroids, Anthraquinone Derivatives, and Cardiac Glycosides.

2.6.1 Alkaloids

0.5 gram of ginger extract was weighed. It was dissolved in 5 ml of methanol for 20 minutes in a water bath and was allowed to cool. 2 ml of each filtrate was dispensed into four different test tubes. 1 % hydrochloric acid was added into the four test tubes and was steamed, after which 6 drops of Mayer's solution was added to each tube. The presence or absence of colours of precipitates in each tube was noted.

2.6.2 Flavonoids

0.5 gram of ginger extract was weighed. It was treated with 5 ml of dilute Sodium hydroxide (NaOH) and filtered. Dilute HCL and magnesium was added to 2 ml filtrate. Solubility and colour was noted.

2.6.3 Tannins

0.5 gram of ginger extract was weighed. 5 ml of distilled water was added, dissolved and filtered. 2 ml of the filtrate was dispensed into a test tube then 2 ml of 15% ferric chloride (FeCl₃) was added. The resultant color was noted.

2.6.4 Saponins

0.5 gram of ginger extract was weighed. 5 ml of distilled water was added, dissolved and filtered. 0.5 ml of filtrate was measured out into a test tube. 5 ml of distilled water was added and was shaking vigorously and left for 10 minutes.

2.6.5 Steroids

0.5 gram of ginger extracts was weighed. 5 ml of chloroform was added and filtered. 2 ml of filtrate was measured into a test tube. This was followed by the addition of 2 ml acetic anhydride. Concentrated H₂SO₄ was finally added to the mixture.

2.6.6 Anthraquinones Derivatives

0.5 gram of ginger extract was weighed. 10 ml of chloroform was added, dissolved and filtered. 1 ml of dilute (10 %) ammonia was added to 2 ml of filtrate. The colour change was recorded.

2.6.7 Cardiac Glycosides

0.5 gram of ginger extract was weighed. 5 ml of distilled water was added, dissolved and filtered. 2 ml of filtrate was measured out into a test tube. 1 ml of glacial acid was added followed by FeCl_3 and concentrated H_2SO_4 .

2.7 Minimum Inhibitory concentration (MIC) and Minimum Bactericidal Concentration (MBC) of Ginger extracts

The tube dilution method was employed to establish the MIC of the *Zingiber officinale* extracts. Various concentration of the extracts (500, 250, 125, 62.5, 31.25, and 15.62 mg/ml) were prepared and placed into a separate test tube with DMSO (Dimethyl sulfoxide) and Nutrient broth. Standard inoculum containing 1.5×10^8 was added to the tubes, followed by incubation period at 37°C for 24 hours to check the turbidity or growth. Then, the concentration with no turbidity of the medium, was considered as the MIC. The MBC was determined by transferring the contents of the test tubes onto sterile Muller Hinton agar plates using a wire loop, and then the plates was incubated at 37°C . The presence and absence of growth was observed to determine MBC.

3. Results

Ginger has demonstrated effectiveness against a range of microbes, including *E. coli*, *Staphylococcus*, *Salmonella*, and *Bacillus* by showing a clear zone. These findings suggest that ginger extract could be used as a natural alternative to synthetic antimicrobial agents. The findings of the present study revealed that *Zingiber officinale* combine potent antimicrobial property against tested microbes. The results of qualitative phytochemical analysis of both agric and local extract are presented in Table 1 and Table 2 respectively.

Table 1: Qualitative Phytochemical Analysis of Agric Ginger Extract (ginger root).

Bioactive compounds	Ethanol extract	Water extract
Tannins	+++	++
Alkaloids	++	+
Saponins	+++	++
Cardiac Glycosides	++	+
Steroids	+	-
Flavonoids	+++	++
Anthraquinone Derivatives	+	+

Table 2: Qualitative Phytochemical Analysis of Local Ginger Extract (ginger root).

Bioactive compounds	Ethanol extract	Water extract
Tannins	+++	+
Alkaloids	++	+
Saponins	+++	+
Cardiac Glycosides	++	+
Steroids	++	+
Flavonoids	+++	++
Anthraquinone Derivative	++	+

Bioactivity key: - absent

+++ = abundantly present

++ = moderately present

+ = fairly present.

The results of antimicrobial activities of both ethanol and water extract of local ginger on some test organisms are presented in Table 3 and Table 4 respectively

Table 3: Inhibition Zone Diameters of Local Ginger Ethanol Extract on Some Test Organisms Against Different Concentrations.

Conc. (Mg/ml)	<i>Staph aureus</i>	<i>E. coli</i>	<i>Salmonella</i>	<i>Bacillus</i>
500	25 ± 0.00	22.5 ± 2.5	27.5 ± 2.5	20 ± 5
250	17.5 ± 7.5	20 ± 5	12.5 ± 2.5	18.5 ± 3.5
125	17.5 ± 7.5	16.5 ± 3.5	0.00 ± 0.00	17.5 ± 2.5
62.5	0.00 ± 0.00	12.5 ± 2.5	0.00 ± 00	0.00 ± 0.00
31.25	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
15.63	0.00 ± 0.00	10 ± 5	0.00 ± 0.00	0.00 ± 0.00

All values are expressed as mean zones of inhibition (mm) ± standard deviation.

Table 4: Inhibition Zone Diameters of Local Ginger Water Extract on Some Test Organisms Against Different Concentration.

Conc. (Mg/ml)	<i>Staph aureus</i>	<i>E. coli</i>	<i>Salmonella</i>	<i>Bacillus</i>
500	17.5 ± 2.5	23 ± 0.00	27.5 ± 2.5	17.5 ± 2.5
250	15 ± 5	22.5 ± 2.5	27.5 ± 2.5	12.5 ± 2.5
125	12.5 ± 2.5	20 ± 0.00	17.5 ± 2.5	12.5 ± 2.5
62.5	12.5 ± 2.5	19 ± 1	14 ± 5.1	10 ± 5
31.25	0.00 ± 0.00	10 ± 0.00	0.00 ± 0.00	5 ± 0.00
15.63	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

All values are expressed as mean zones of inhibition (mm) ± standard deviation.

The results of antimicrobial activities of both ethanol and water extract of agric ginger on some test organisms are presented in Table 5 and Table 6 respectively

Table 5: Inhibition Zone Diameters of Agric Ginger Ethanol Extract on Some Test Organisms Against Different Concentration.

Conc. (Mg/ml)	<i>Staph aureus</i>	<i>E. coli</i>	<i>Salmonella</i>	<i>Bacillus</i>
500	19 ± 1.00	20 ± 0.00	0.00 ± 0.00	20 ± 0.00
250	14 ± 1.00	20 ± 5	0.00 ± 0.00	12.5 ± 0.5
125	11.5 ± 1.5	17.5 ± 2.5	0.00 ± 0.00	15 ± 0.00
62.5	12.5 ± 2.5	12.5 ± 2.5	0.00 ± 0.00	0.00 ± 0.00
31.25	12.5 ± 2.5	10 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
15.63	7.5 ± 2.55	7.5 ± 7.55	0.00 ± 0.00	0.00 ± 0.00

All values are expressed as mean zones of inhibition (mm) ± standard deviation.

Table 6: Inhibition Zone Diameters of Agric Ginger Water Extract on Some Test Organisms Against Different Concentration.

Conc. (Mg/ml)	<i>Staph aureus</i>	<i>E. coli</i>	<i>Salmonella</i>	<i>Bacillus</i>
500	16 ± 4	20 ± 5	19 ± 6	0.00 ± 0.00
250	15 ± 3	17.5 ± 2.5	17.5 ± 2.5	0.00 ± 0.00
125	± 2.4	15 ± 5	15 ± 0.00	0.00 ± 0.00
62.5	11.5 ± 1.5	11 ± 1	12.5 ± 2.5	0.00 ± 0.00
31.25	10 ± 0.00	9 ± 1.00	7.5 ± 2.55	0.00 ± 0.00
15.63	0.00 ± 0.00	10.00 ± 0.00	5 ± 0.00	0.00 ± 0.00

All values are expressed as mean zones of inhibition (mm) ± standard deviation.

Table 7 is the Minimum Inhibitory Concentration values of the test organisms against local ginger extracts.

Table 7: Minimum Inhibitory Concentration (MIC) values of Test Organisms against Local ginger extracts.

Extract	MIC values in (mg/ml)			
	<i>Staphylococcus</i>	<i>Salmonella</i>	<i>Bacillus</i>	<i>E. coli</i>
Ethanol	250	250	125	250
Water	250	250	250	125

Table 8 is the Minimum Inhibitory Concentration values of the organisms against agric ginger extracts.

Table 8: Minimum Inhibitory Concentration (MIC) values of Test Organisms against Agric ginger extracts.

Extract	MIC values in (mg/ml)			
	<i>Staphylococcus</i>	<i>Salmonella</i>	<i>Bacillus</i>	<i>E. coli</i>
Ethanol	250	250	250	250
Water	250	250	125	250

Table 9 is the Minimum Bactericidal Concentration values of test organism against Local ginger extract.

Table 9: Minimum Bactericidal Concentration (MBC) of Test Organism against Local ginger extract.

Test organisms	MBC (mg/ml)	
	500	250
<i>Staphylococcus</i>	Presence of growth	Presence of growth
<i>Salmonella</i>	Presence of growth	Presence of growth
<i>E. coli</i>	Presence of growth	Presence of growth
<i>Bacillus</i>	Presence of growth	Presence of growth

Table 10 is the Minimum Bactericidal Concentration values of test organism against Agric ginger extract.

Table 10: Minimum Bactericidal Concentration (MBC) of Test Organism against Agric ginger extract.

Test organisms	MBC (mg/ml)	
	500	250
<i>Staphylococcus</i>	Presence of growth	Presence of growth
<i>Salmonella</i>	Presence of growth	Presence of growth
<i>E. coli</i>	Presence of growth	Presence of growth
<i>Bacillus</i>	Presence of growth	Presence of growth
Presence of growth (No MBC)		

4. Discussion

The phytochemical activities of both Agric and local ginger (Tables 1- 2 and Appendix - Figure 1) typically showed the presence of bioactive compounds such as tannins, alkaloids, saponins, cardiac glycosides, flavonoids, anthraquinone derivatives with traces of steroids. It was reported that flavonoids and gingerol are the main component of ginger which attributes to its antibacterial activity [11]. These phytochemicals are responsible for ginger medicinal properties, such as anti-inflammatory, antioxidant, and antimicrobial activities [12].

Our study found that ethanol extracts of ginger exhibited significant antimicrobial activity, producing larger zones of inhibition compared to water extracts which could be as a result of better extraction with alcohol solvents (Tables 3 – 6 and Appendix – Figure 2). This observation aligns with the findings of Saheed and Tariq, [13], Akintobi *et al.*, [10] and Dharmapala and Amarakoon [14] who reported that ethanol extracts of ginger showed substantial antibacterial activity against both Gram-positive and Gram-negative bacteria, with ethanol proving to be a more efficient solvent for extracting bioactive compounds. Consistent with our results, Singh *et al.*, [15] demonstrated that ginger extract had more pronounced effects on Gram-positive bacteria such as *Staphylococcus aureus* and *Bacillus subtilis*, while showing moderate activity against Gram-negative bacteria like *E. coli* and *Salmonella*, also, a study conducted by Melvin *et al.* [16], found that the ginger extract exhibited maximum inhibitory effect against *P. aeruginosa* while the antimicrobial activity against *E. coli* was found to be moderate. Similarly, Mascolo *et al.*, [17] found that gingerol, a primary active component in ginger, exhibited strong antibacterial activity against both Gram-positive and Gram-negative bacteria. A study in 2022 as reported by Morales-Brown [18] suggested that ginger could effectively inhibit a variety of bacteria, including *Streptococcus mutans*, *Enterococcus faecalis*, *Staphylococcus* species, and *Lactobacillus* species.

The antimicrobial properties of ginger are largely attributed to its bioactive compounds, such as gingerol and shogaol, which can disrupt bacterial cell membranes and inhibit essential enzymes. Mao *et al.*, [9] confirmed these mechanisms, supporting our findings that these compounds exhibit antimicrobial, antiviral, and antifungal effects. Our study showed that aqueous extracts of ginger had lower antimicrobial activity compared to ethanol extracts as presented in Tables 3 and 4 for local ginger and Tables 5 and 6 for Agric ginger. This is consistent with Gull *et al.*, [19], who reported poor susceptibility of bacterial strains of *Zingiber officianle* aqueous extracts. The higher efficacy of ethanol extracts is attributed to their ability to more effectively extract compounds like gingerol, shogaols, and paradols. Tables 7 - 8 and Appendix – Figure 3 showed the Minimum Inhibitory Concentration (MIC) while Minimum Bactericidal Concentration (MBC) were presented in Tables 9 and 10 Appendix – Figure 4 for all four test organisms (*Salmonella*, *Staphylococcus*, *E. coli*, and *Bacillus*). The concentration with no visible growth (indicating no turbidity of the medium) was considered the MIC. As reported by Mounyr *et al.*, [20], this method helps to ascertain the potency of the ginger extract against specific microorganisms, signifying the lowest amount of extract needed to inhibit bacterial growth. The MBC is the lowest concentration that not only inhibits growth but also kills the bacteria. The presence of growth on sub-cultures plated from test tubes with no visible growth (for all tested bacteria) indicated the lack of MBC in the extracts.

The results of this study, supported by existing literature, indicate that ginger extract exhibits antimicrobial activity against a range of pathogens, with ethanol extracts showing greater efficacy. This confirms gingers' potential as a medicinal plant for treating various

infections. Further research into the characterization and standardization of ginger extracts could enhance its application in developing therapeutic formulations for human and veterinary medicine.

5. Conclusions

It is concluded that ginger rhizome, a natural compound with long history of traditional use, has been found to possess significant antimicrobial activities against a range of pathogens, including bacterial (*Staphylococcus aureus*, *Salmonella*, *E. coli*, *Bacillus*) making it a promising remedy for the prevention and treatment of various infections. The bioactive compounds present in ginger such as gingerols and shagaols, have been shown to inhibit the growth of microorganisms, disrupt biofilm formation, and induce apoptosis in microbial cells. The antimicrobial properties of ginger extract making it a potential natural remedy for the prevention and treatment of various infections, including respiratory, gastrointestinal, and skin infections, immune support, digestion, immunity booster, and have shown potential in managing certain health conditions. However, Ginger can be used as potential source of novel antibiotic. The use of these medicinal plant materials will aid the promotion of human health system.

Appendix



Figure 1: Phytochemical screening

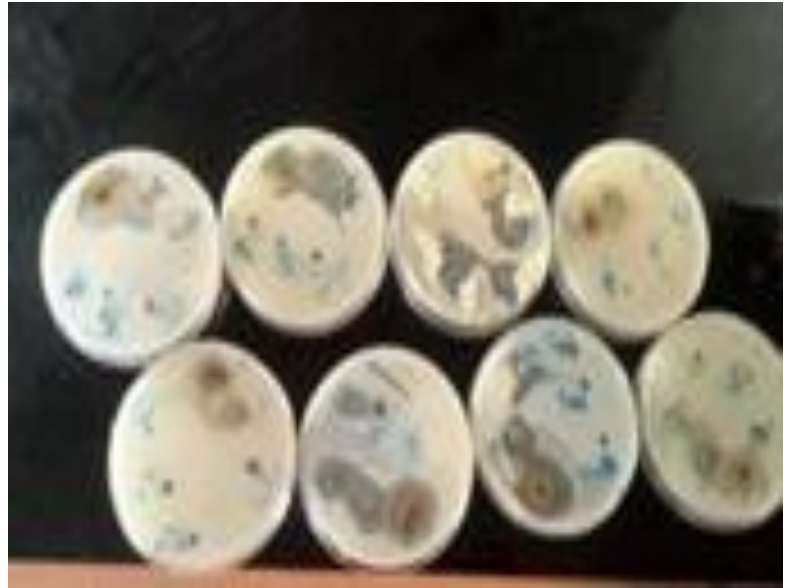


Figure 2: Zone of Inhibition on Nutrient agar.



Figure 3: Minimum Inhibitory Concentration (MIC) Screening



Figure 4: Minimum Bactericidal (MBC) plates streaked with test organisms

Conflict of Interest

The Authors declare that there is no conflict of interest.

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