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Antibacterial activity of *Zingiber officinale* extract on clinical isolates; *Salmonella typhi, Staphylococcus aureus* and *Escherichia coli*

S.S*. Alingo, M. Yaji, E.O. Agada

Department of Microbiology, Faculty of Biological Science, Joseph Sarwuan Tarka University, Makurdi

*Correspondence E-mail: eagada02@gmail.com

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Abstract

This study investigated the antibacterial activity of *Zingiber officinale* (ginger) extracts against selected pathogenic bacteria. Fresh rhizomes of *Z. officinale* were collected from Wadata market in Makurdi, Benue State, air-dried, and pulverized into powder. Aqueous and ethanolic extracts were prepared at varying concentrations (500, 250, 125, 62.5, and 31.25 mg/mL). Phytochemical screening revealed the presence of bioactive compounds including phenols, quinones, terpenoids, tannins, alkaloids, saponins, and steroids. Test organisms used were *Escherichia coli*, *Staphylococcus aureus*, and *Salmonella* sp., which were obtained and confirmed through cultural, morphological, and biochemical methods in the Microbiology Laboratory of Joseph Sarwuan Tarka University, Makurdi. Ciprofloxacin (500 mg/mL) served as the control drug. Antibacterial susceptibility testing was carried out, and zones of inhibition were measured in millimetres. At 500 mg/mL, ethanolic extract showed the highest activity, with *S. aureus* exhibiting the largest inhibition zone (16.33±3.19 mm), followed by *E. coli* (12.33±6.17 mm), while *Salmonella* sp. showed the lowest inhibition (3.33±3.33 mm). Interestingly, both aqueous and ethanolic extracts inhibited *S. aureus* and *E. coli*, whereas *Salmonella* sp. responded only to aqueous extract. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) were determined in triplicates, and results were analysed using Analysis of Variance (ANOVA) in SPSS version 21. The findings demonstrate that *Z. officinale* extracts possess significant antibacterial activity against *S. aureus*, *E. coli*, and *Salmonella* sp., suggesting their potential as effective, affordable alternatives to conventional antibiotics for managing bacterial infections.

Keywords: Quinones, Inhibitory, Alkaloids, Antibacterial, *Zingiber officinale*

Introduction

Abuse of antibiotics have been a leading cause of the emergence of antibiotics resistance mechanism amongst pathogenic bacteria (1,3). Antibiotic resistance to antimicrobial agents can be natural, acquired genetically, phenotypically or biologically [2-4]. Furthermore, resistance may be developed due to spontaneous mutation in genes, acquisition of plasmid or transposon, the physiological change in the state of a bacteria cell or decrease in the permeability of cell [8,9]. Bacteria develop resistance in various ways such as enzymatic drug inactivation, drug target alteration and drug permeability reduction [5,7].

Herbs and spices are parts of plants from indigenous or exotic origin and they are essential part of human diet as they improve taste, colour and aroma of food [11,13]. In addition, they are preservatives in many foods, they also have antioxidants [8,9] and antimicrobial properties [12]. Herbs have also been utilized in human and veterinary medicine [2,10].

Zingiber officinale is also used as herb and spice, especially in the east. *Zingiber officinale* is a thick, scaly rhizoid and aromatic, thick lobed, branched, have scaly structures and possess a spicy lemon like scent. The rhizomes contain both aromatic and pungent components [8,13]. *Zingiber officinale* is widely used in culinary as medicine [12,17]. It has a pungent hot flavour but mellows and improves with cooking. It has also been utilized to fight infections such as, cold, cough, asthma, diarrhoea, flu, headache, sore throat, abdominal discomfort and respiratory tract infections [9,15].

Food borne pathogens are widely distributed in the environment and may be a significant cause of mortality and morbidity in the population [6,16,20]. *Escherichia coli* is a significant food borne pathogen in many countries around the world. Infections which often cause haemorrhagic diarrhoea occasionally leads to kidney failure and death. *Salmonella typhi* is another bacterium that is a cause of food borne illness, mainly from food of animal origin throughout

the world. *Staphylococcus aureus* causes food borne illnesses due to their ability to form heat stable toxins [7, 21,22,30]

Several attempts have been made to come up with a plant-based medicine, because of the cost implications of manufactured drugs, toxicity and resistance of pathogenic microorganisms to most of the industrially produced drugs (antibiotics), hence the need for cheap, alternative and readily available method. This research therefore intends to discover the phytochemical constituents responsible for the efficacy of *Zingiber officinale* against certain infections caused by bacteria.

Materials and Methods

Study Area

The study area is Makurdi, Makurdi Local Government Area of Benue State. Makurdi is located at latitude 7.44°N and

8.31°E on the map, the city is located in North Central Nigeria [2].

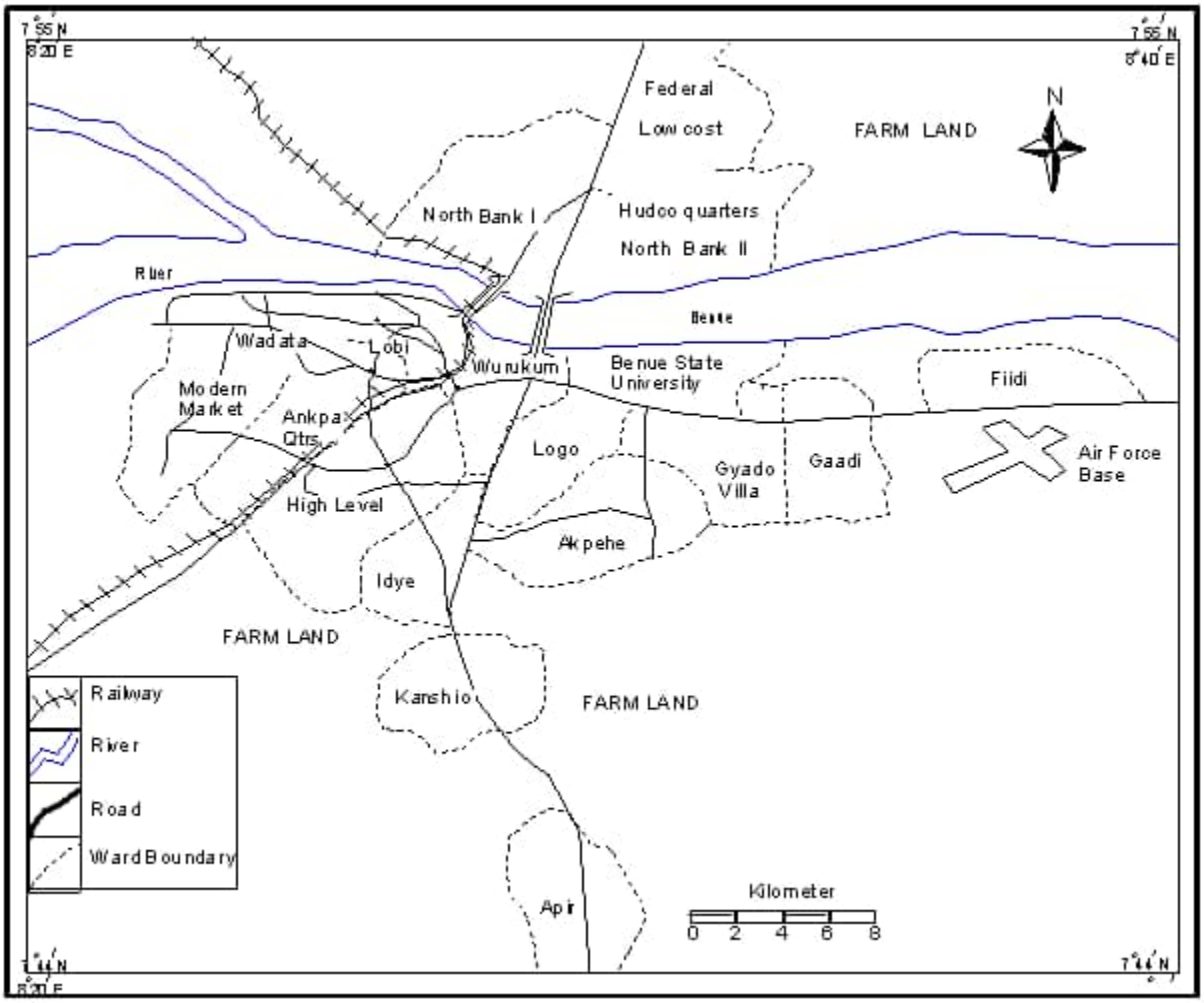

Fig: 1. Map of Makurdi

Collection and Preparation of Plant Material

The *Zingiber officinale* plant was collected from Wadata market in Makurdi Local Government Area of Benue State and transported to the Department of Botany, Joseph Sarwuan Tarka University Makurdi, for authentication by renowned taxonomists. The outer covering of *Zingiber officinale* (ginger) was washed with clean water, peeled, air dried and then pulverized using mortar and pestle [18].

Drying and Storage of Zingiber officinale

The clean *Zingiber officinale* was shade dried at room temperature (32-35 °C) to constant weight over a period of 5 days and grounded with the aid of mortar and pestle into coarse powder. The coarse powder obtained from the rhizome/corm bark of *Zingiber officinale* was sieved using dry test sieve, packaged in polythene bags and stored for further analysis [29].

Preparation of Crude Extracts

Aqueous Extraction

One hundred (100 g) gram of the powdered rhizome/corm bark of *Zingiber officinale* was weighed and soaked in 500 mL of distilled water for three days (72 hours) and was constantly agitated [9]. The extract was filtered using a Whatman No. 1 filter paper to remove the residue. The filtrate was evaporated at 50 °C in a water bath to get the crude extract [2].

The crude aqueous extract was stored in a sample bottle and used for further analysis.

Ethanolic Extraction

One hundred (100 g) gram of the powdered root bark of *Zingiber officinale* was weighed and soaked in 500 ml of ethanol for three days (72 hours) and was constantly agitated [9]. The extract was filtered using a Whatman No. 1 filter paper to remove the residue. The filtrate was evaporated at 50 °C in a water bath to get the crude extract. The crude aqueous extract was stored in a sample bottle and used for further analysis.

Phytochemical Screening Extracts.

Test for Tannins

One (1) mL of the aqueous and ethanolic extracts was diluted in 4 mL of water in a ratio of 1:4 and few drops of 10 % ferric chloride was added and the mixture was observed for precipitation or colouration which indicated the presence of tannins.

Test for Saponins

Exactly 1 mL of aqueous and ethanolic extracts was added to 4 mL of distilled water in a test tube was stoppered, shaken vigorously for about 30 seconds and was then allowed to stand for half an hour. A honey comb-forth formation was an indication of presence of saponins.

Test for Terpenoids

A few drops of chloroform were reacted with 2 mL of the extract and a few drops of concentrated sulphuric acid (H_2SO_4) were added to form a layer. A reddish-brown precipitate produced immediately indicated the presence of terpenoids.

Test for Steroids

Two (2) mL of the aqueous and ethanolic extracts was mixed with 1ml of acetic acid anhydride followed by the addition of concentrated sulphuric acid carefully down the side of the test tube while observing for the formation of interphase layer of reddish-brown colour which shows the presence of steroids.

Test for Phenols

A few drops of Ferric Chloride solution were added to 2 mL of the extract in a test tube. The presence of a green colour indicated the presence of phenol.

Test for Quinones

Exactly 2ml of the extract was mixed with concentrated sulphuric acid, blue-green or red colour indicated the presence of quinones.

Alkaloids (Mayer's Test)

Exactly 2 mL of the extracts was added to a few drops of 2HCl, an aqueous layer formed which was decanted and one or two drops of Mayer's reagent was added. Formation of white turbidity or precipitate indicated the presence of alkaloids.

Test Organisms

Cold stored Agar slant cultures of already identified *Escherichia coli, Stapylococcus aureus* and *Salmonela* spp. were obtained from the Microbiology Laboratory of the Joseph Tarka Sarwuan University, Makurdi, Benue State.

Viability test of each organism was carried out by resuscitating the sourced isolates in buffered peptone broth and thereafter sub cultured unto nutrient agar medium and incubated at 37 °C for 24 hours. The probable identity of the isolates was further confirmed by exposing the cultures to an array of biochemical test which include coagulase production, catalase, indole and citrate utilization [4,9]. The results of the biochemical reactions elicited by the test isolates were compared with standard identification keys [4].

Catalase Test

Two (2) mL of Hydrogen peroxide (H_2O_2) was poured into Petri dishes and a sterile applicator stick was used to immerse several colonies into it and carefully observed for immediate bubbling. The presence of bubbles indicated a positive result [4].

Citrate Test

Simmons citrate agar was used to prepare a slant in a test tube. Using a sterile wire loop; the slant was streaked with the inoculum and then stabbed. It was allowed for 24 hours. A colour change from green to blue indicated a positive result [13].

Indole Test

Peptone water (5 mL) was introduced I nto a test tube with the test organism inoculated into it with a sterile wire loop and it was allowed to stand for 48 hrs. Positive result is seen by a colour change in the test tube upon addition of Kovac's reagent while negative result is seen by no colour change in the test tube [13].

Preparation of Concentrations of the Zingiber officinale root bark Extracts

One (1g) gram each of the aqueous and ethanolic extracts was added to two millilitres (2 mL) of distilled water to give a concentration of 500 mg/mL, and other concentrations of

250 mg/mL, 125 mg/mL, 62.5 mg/mL and 31.25 mg/mL were prepared by double broth dilution method [15].

Determination of the Antibacterial Properties of Zingiber officinale the Extracts

Susceptibility testing was carried out using agar well diffusion method according to the recommendation of Nutritional Committee for Clinical Laboratory Standard, 2000. Mueller-Hinton Agar was prepared according to the manufacturer's direction. It was sterilized by autoclaving for 15 minutes at 120 ℃ [24,29].

In this method, 0.5 MacFarland turbidity standard of 24 hours *Escherichia coli*, *Staphylococcus aureus* and *Salmonella* spp. were prepared in a normal saline broth. Exactly 0.5 mL each of the organism from the 24 hours' normal saline broth was pipetted onto a petri dish after which the prepared Muller-Hinton agar was pour plated [2,28], it was swirled evenly and then allowed to solidify.

After the cultured plates have gelled, a sterile cork borer (8 mm) was used to bore wells on the surface of the agar plate and then labelled. Exactly 0.2 mL of the different concentrations of each extract was transferred into the wells using a Pasteur pipette. The wells were sufficiently spaced to prevent the resulting zones of inhibition from overlapping. Ciprofloxacin (500 mg/mL) was used as control. The experiment was performed in triplicates and the resulting zones of inhibition were measured by the diameter of the well using a ruler calibrated in millimeters. The average of the reading was taken to be the zone of inhibition of the isolates in question to that particular concentration [22,31,32].

Determination of Minimum Inhibitory Concentration (MIC)

The MIC of the extracts was determined according to the macro broth dilution technique [26]. Standardized suspension of test organisms was inoculated into a double fold serial dilution of the extract in a normal saline broth in a series of four sterile test tubes. The mixtures in sterile test tubes were incubated at 37℃ for 24 hours and observed for turbidity (signifying growth) or absence of it (signifying inhibition). The minimum inhibitory concentration was recorded as the least concentration of the extract solution that inhibited microbial growth [2,27].

Minimum Bactericidal Concentration (MBC)

The MBC of the respective extracts was determined by the procedure in [23,25 32]. An aliquot of the test mixture was taken from the MIC tube that showed no visible growth and sub cultured onto a freshly prepared Nutrient agar plate which was prepared according to manufacturer's direction and was incubated at 37 ℃ for 24 hours. The minimum bactericidal concentration was recorded as the least concentration of extracts that showed no bacterial growth [25].

Statistical Analysis of Data

Data were analysed for mean and standard deviation. Difference in parameter was tested for statistical difference at $P<0.05$ using student ANOVA. All the analysis was done using a statistical package service solution (SPSS) version 21, 2010.

Results

Table 1: Shows the Cultural, Morphological and Biochemical Characteristics of clinical isolates, confirming *Staphylococcus aureus*, *Salmonella* sp. and *Escherichia coli.*

Table 2: Shows the presence of phenols, quinones, terpenoids, tannins, alkaloids, saponins and steroids in the aqueous ethanolic extracts of *Zingiber officinale*.

Table 3: Shows the zones of inhibition (in millimetres) of *Zingiber officinale* on *Staphylococcus aureus*. It was noticed that the ethanolic extract has the highest zones of inhibition than the aqueous extract across the different concentrations. Statistical analysis shows that there is a significant difference across the different concentrations, where P-value = 0.580 for aqueous extract and P-value = 0.003 for ethanolic extract, and the degree of freedom is given at 2.

Table 4: Shows the zones of inhibition (in millimetres) of *Zingiber officinale* on *Escherichia coli*. It was noticed that the ethanolic extract has the highest zones of inhibition than the aqueous extract across the different concentrations. Statistical analysis shows that there is a significant difference across the different concentrations where P-value = 0 for aqueous extract and P-value = 0.165 for ethanolic extract, and the degree of freedom is given at 2.

Table 5: Shows the zones of inhibition (in millimetres) of *Zingiber officinale* on *Salmonella Spp*. It was noticed that the ethanolic extract has the highest zones of inhibition than the aqueous extract across the different concentrations. Statistical analysis shows that there is a significant difference across the different concentrations where P-value = 0.452 for aqueous extract and P-value = 0 for ethanolic extract, and the degree of freedom is given at 2.

Table 1: Cultural, Morphological and Biochemical Characteristics of clinical isolates

Colony Colour	Colony Shape	Elev	Morph	Gram Rxn	Cat	Cit	Ur	Ind	Mot	H_2S	Coa	Bacteria Spp.
Yellow	Circular	Raised	Cocci	+	+	+	-	-	-	-	+	*Stapylococcus aureus*
Pink	Circular	Raised	Rod	-	+	-	-	+	+	-	NA	*Escherichia coli*
Pale	Circular	Raised	Rod	-	+	+	-	-	+	+	NA	*Salmonella* Sp.

Key; Positive = (+)**,** Negative = (-), NA – Not Applicable, Coa – Coagulase, H_2S – Hydrogen Sulphide, Ind – Indole, Cit – Citrate, Cat – Catalase, Ur – Urease, Rxn – Reaction, Morph – Morphology, Elev – Elevation

Table 2: Phytochemical constituents of the Aqueous and Ethanolic Extracts of *Zingiber officinale*

Phytochemical constituent	Aqueous Extract	Ethanolic Extract
Phenols	+	+
Quinones	+	+
Terpenoids	+	+
Tannins	+	+
Alkaloids	+	+
Saponins	+	+

Key; Present = (+)**,** Absent = (-)

Table 3: Zones of Inhibition (mm) of *Zingiber officinale* on *Stapylococcus aureus*

Concentration (mg/mL)	Aqueous Extract	Ethanolic Extract
500	4.67 ± 4.67	16.33 ± 3.18[9]
250	4.33 ± 4.33	16.00 ± 2.00[9]
125	0.00	10.00 ± 5.00[9]
62.5	0.00	0.00 ± 0.00[6]
31.25	0.00	0.00 ± 0.00[6]
P – value	0.580	0.003
df = 2		p < 0.003

Table 4: Zones of Inhibition (mm) of *Zingiber officinale* on *Salmonella* Sp.

Concentration (mg/mL)	Aqueous Extract	Ethanolic Extract
500	3.33 ± 3.33	0.00
250	0.00	0.00
125	0.00	0.00
62.5	0.00	0.00
31.25	0.00	0.00
P – value	0.452	0.00

Table 5: Zones of Inhibition (mm) of *Zingiber officinale* on *Escherichia coli*

Concentration (mg/mL)	Aqueous Extract	Ethanolic Extract
500	0.00	12.33 ± 6.17
250	0.00	11.00 ± 5.51
125	0.00	9.33 ± 4.67
62.5	0.00	0.00
31.25	0.00	0.00
P – value	0.00	0.165
df = 2		p > 0.05

Discussion

The result of the phytochemical analysis of the aqueous and ethanolic extracts showed that *Zingiber officinale* extracts has some bioactive agents such as phenols, quinones, terpenoids, tannins, alkaloids, saponins and steroids. The antibacterial activity of *Zingiber officinale* is due to the

different Phytochemical constituents of the plants. This agrees with the findings of [15,18]

Zingiber officinale contains a good number of phytochemicals, most of which are hydrocarbons and their derivatives. These includes phenols, quinones, terpenoids, tannins, alkaloids, saponins and steroids. The phenolic compounds are mainly gingerols, shogaols and paradols. Gingerols are the major component present in the rhizome of *Zingiber officinale* and are renowned for their antimicrobial, anti-inflammatory and antioxidant activities [18]. Against Bacteria (Gram-positive bacteria), gingerols (particularly [6]-gingerol) disrupt bacterial cell walls and membranes, leading to leakage of intracellular components. They show inhibitory activity against *Staphylococcus aureus* (including MRSA), *Streptococcus mutans*, and *Bacillus subtilis.* Against Gram-negative bacteria, it is effective against *Escherichia coli, Salmonella typhi, Pseudomonas aeruginosa,* and *Klebsiella pneumoniae*. Their lipophilic side chains enhance penetration into bacterial membranes, impairing growth. Gingerols mechanisms of action include; disruption of cell membranes, Inhibition of bacterial quorum sensing (reducing virulence factor production), Interference with enzymes involved in energy metabolism [18]. Shogoals impacts the characteristic pungent taste to dried *Zingiber officinale.* Antimicrobial activity includes, active against bacteria (*E. coli, Staphylococcus aureus*), fungi, and some viruses. [18]. Like gingerols and shogaols, paradols are another class of pungent phenolic compounds found in ginger (*Zingiber officinale*), particularly in dried and thermally processed ginger. They are formed by the hydrogenation of shogaol. Activities of Paradols in Ginger are antioxidant activity which include; strong free radical scavenging ability. Protects cells from oxidative damage. Its anti-inflammatory activity include suppresses pro-inflammatory enzymes and cytokines. Useful in reducing chronic inflammation [18]

In the course of this research, it was discovered that crude extratcts of *Zingiber officinale* inhibits the growth of *Escherichia coli, Salmonella typhi, Staphylococcus aureus in vitro*. The inhibitory effect of *Zingiber officinale* on bacterial pathogens is due to the presence of gingerols, shagoals and paradols of the phenolic group [18].

This research showed that *Zingiber officinale* had antibacterial activity on *Staphylococcus aureus*, *Salmonella* sp. and *Escherichia coli* with a little form of resistance observed. This also agreed with the findings of [25] where he reported that zones of inhibition are formed depending on the concentration of the extracts used.

Conclusion

From the results of this study, it could be concluded that *Zingiber officinale* extracts had antibacterial activity on *Staphylococcus aureus*, *Salmonella* sp. and *Escherichia coli*. This suggests that *Zingiber officinale* could be used for the treatment of infections caused by these bacteria, and as well could serve as cheaper alternative to expensive antibiotics.

Recommendations

1. *Zingiber officinale* has antibacterial activity against bacteria pathogens and should be used in the treatment of bacterial infections.
2. In situations whereby, some bacteria pathogens are resistant to antibiotics and the cost of more effective antibiotics is high, *Zingiber officinale* extract can be used as it is effective in the treatment of bacterial infections as well as very cheap and affordable.

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