IN-VITRO ANTIBACTERIAL ACTIVITIES AND PRELIMINARY PHYTOCHEMICAL SCREENING OF THE AQUEOUS AND ETHANOLIC EXTRACTS OF *ZINGIBER OFFICINALE*

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ABSTRACT

Studies on the *in-vitro* antibacterial activities and phytochemical screening of the aqueous and ethanolic extracts of *Zingiber officinale* (ginger) against some clinical bacterial isolates (*Escherichia coli, Staphylococcus aureus* and *Pseudomonas aeruginosa*) obtained from ear and urine samples were carried out using standard methods. Phytochemical screening revealed the presence of flavonoids, steroids, tannins and reducing sugars in both extracts while alkaloids and saponins were only present in the ethanolic and aqueous extracts respectively. In addition, a cream, soft and dark-brown ethanolic extract as well as solid, soft and black aqueous extract were recovered. The bioassay studies showed that the ethanolic extract exhibited an activity against *S. aureus* with 8 mm zone of inhibition at a concentration of 1000 μg/disc while no activity was recorded against *E. coli* and *P. aeruginosa* at the same disc potency. On the other hand, the aqueous extract was found to be more active against *E. coli* with 9 mm zone of inhibition at a comparatively higher concentration of 3000 μg/disc. The minimum inhibitory concentration ranged between 250 and 6000 μg/ml. Overall, the results of the study showed that the ethanolic and aqueous extracts of *Z. officinale* have antibacterial potentials against the tested clinical bacterial isolates, thus supporting the plant's ethnomedicinal uses.

Keywords: *Zingiber Officinale,* Extracts, Phytochemistry, Antibacterial Activity, MIC.

INTRODUCTION

Plant-derived products have been used for medicinal purposes for centuries. At present, it is estimated that about 80.0% of the world population relies on botanical preparations as medicines to meet their health care needs. Herbs and spices are generally considered safe and proved to be effective against certain ailments. They are also extensively used particularly in many Asian, African and other countries (Langner *et al*, 1998).

Herbal medicine, sometimes referred to as 'Herbalism' or 'Botanical medicine', is the use of herbs for therapeutic or medicinal value. A herb is a plant or plant part valued for its medicinal, aromatic or savory qualities (Kawo *et al*., 2011). Herbaceous plants produce and contain a variety of chemical substances that act upon the body for the cure of infections of microbial origin. Many drugs commonly used today are of herbal origin. Indeed, about 25% of the prescription drugs dispensed in the United States contain at least one active ingredient derived from plant material. Some are made from plant extracts; others are synthesized to mimic a natural plant compound (Reagor *et al*., 2002).

Among the plants used as herbal medicine is ginger (*Zingiber officinale).* Ginger is a perennial herb which grows from underground rhizomes, and is often mistakenly called the "roots". Botanically, it is the rhizome that provides its slightly hot, citrus-like taste, and wonderful aroma. Its botanical name is *Zingiber officinale*, originating from the plant family called *Zingiberaceae* (Aliyu, 2006). Ginger is one of the most important and widely grown perennial rhizomatous herbs with over 90 species. It is mostly grown in South-Eastern Asia extending to Queensland and Japan. Ginger is mainly used as a spice and to some extent for medicinal purposes (Langner *et al*., 1998). Ancient civilizations in India and China used fresh ginger to treat nausea, asthma, coughs, colic, heart palpitations, loss of appetite and rheumatism, fever, swelling, dysentery, diarrhea and sore (Aliyu, 2006). It has been found to stimulate circulation and improve

blood flow and has been used for centuries to aid in digestion, inhibit vomiting and prevent motion or sea sickness. In India, ginger is used to prevent heart attacks and relieve migraine headaches. It has been found to reduce cholesterol levels and lower blood pressure. Ginger has also been found to have antimicrobial properties as it contains extremely high levels of phytochemicals (plant substances with a healing effect) (Cakir *et al.*, 2004). In addition to its beneficial effects on the heart and its anti-cancer activities, ginger has been reported for its anti-inflammatory effects and has high contents of anti-oxidants properties (Aliyu, 2006). Thus, with respect to historical and cultural records of various medicinal plants and their applications as therapeutics, the present study aimed at determining the *in-vitro* antibacterial effects of the aqueous and ethanolic extracts of *Z. officinale* as well as the phytochemical screening of the extracts to determine their bioactive components with a view to further substantiate its ethno-medicinal potentials.

MATERIALS AND METHODS

Collection, Identification and Preparation of the Plant Materials

Fresh ginger plant rhizome was purchased from a local market in the metropolitan Kano, northern Nigeria. Its botanical identity was first authenticated at the field using standard keys and descriptions as reported by Sofowora (1993) and Aliyu (2006). A further confirmation and authentication of the plant at the herbarium section of the Department of Plant Science, Bayero University Kano, Nigeria was carried out, where voucher specimens were preserved and stored for future reference. The rhizome was thoroughly washed with sterile distilled water and then allowed to air-dry for few days. It was then ground into powder form using pestle and mortar, sieved through 250µm mesh to obtain fine powder which was stored at room temperature in sealed containers until required for use.

Extraction Protocols

The extraction protocols described by Fatope *et al* (1993) were adopted. Two different solvents were used in the extraction process: 95% ethanol and water. Here, 100 grams of the fine grounded powder of the rhizome was taken and percolated with a liter (1000 ml) of ethanol to give a dilution ratio of 1:10. This was allowed to stand for a period of two weeks with regular shaking using magnetic shaker. The content was then filtered using Whatman No. 1 filter paper and the filtrate obtained was transferred into a pre-weighed beaker and then evaporated to dryness in a water bath at 30°C until the solvent escaped completely leaving behind the ethanolic extract. This was then weighed to find the percentage yield of the extract. Similarly, another 100 grams of the powder was percolated with a litre of sterile distilled water in Kilner jar. This was also placed in the magnetic shaker to ensure regular shaking for a period of one week. The aqueous percolation was allowed for only one week as leaving it to percolate for more than this period may lead to fungal growth on the surface of the solution. The aqueous solution was filtered using Whatman No. 1 filter paper, poured into a pre-weighed beaker and evaporated to dryness in a water bath at 30°C until the solvent escaped completely leaving behind the aqueous extract only. The percentage yield of the extracts was also determined.

Phytochemical Screening of the Extracts

Test for Alkaloids

This was carried out qualitatively according to Cuili (1994). Using a pipette, 1.0 ml of the aqueous or ethanolic extract was placed in two separate test tubes. Using a dropper, three drops of Dragendoff's and Meyer's reagents were separately added. An orange-red precipitate or turbidity with Dragendoff's reagent or white precipitate with Meyer's reagent was indicative of the presence of alkaloids.

Test for Saponins

This was carried out according to the method reported by Brain and Turner (1975). To 0.5 g of the aqueous or ethanolic extract in a test tube, 5.0 ml of sterile distilled water was added and shaken vigorously. A froth that persisted for 15 minutes was indicative of the presence of saponins.

Test for Flavonoids

This was carried out in accordance with the

method as reported by Sofowora (1993). A 4 mg weight of the aqueous or ethanolic extract and a piece of magnesium ribbon were added followed by concentrated HCl drop-wise. A color change from crimson to magenta indicated the presence of flavonoids in the extract.

Test for Steroids

This was carried out according to Cuili (1994). A 2 grams weight of the aqueous extract was taken in a test tube and evaporated to dryness. The extract was then dissolved in acetic anhydride followed by the addition of chloroform and then concentrated sulphuric acid was added by the side of the test tube. Appearance of a brown ring at the interface of the two liquids and the appearance of violet colour in the supernatant layer indicated the presence of steroids in the extract. The same procedure was followed for the ethanolic extract.

Test for Tannins

This was carried out according to Cuili (1994). A 2 grams weight of the aqueous extract was diluted in sterile distilled water in a test tube. Then, 2-3 drops of 5% ferric chloride ($FeCl_3$) solution were added. A green-black or blue-black colouration was indicative of the presence of tannins in the extract. Same procedure was adopted for the ethanolic extract but using dimethylsulphoxide (DMSO) as the diluent.

Test for Reducing Sugars

This was carried out according to the method of Brain and Turner (1975). Here, one gram each of the extracts was weighed and introduced into separate test tubes. The ethanolic and aqueous extracts were diluted with 2.0 ml each of DMSO and sterile distilled water respectively. To the solution thus obtained were added Fehling's solution and the mixture was then warmed. A brick-red precipitate at the bottom of the test tubes was indicative of the presence of reducing sugars in the extracts.

Bioassay Studies

Collection, Identification and Authentication of Clinical Bacterial Isolates

The test organisms (clinical bacterial isolates) included one Gram-positive bacterium (*Staphylococcus aureus)* and two Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*). They were collected from the Pathology Department of Murtala Mohammed Specialist Hospital (MMSH), Kano, Nigeria. Two isolates for each species were collected: one isolate from urine sample and the other from ear swab. Identification and confirmation of the isolates was carried out using the Gram's reaction, cultural, morphological and biochemical tests (Chesebrough, 2000).

Preparation of Extract-impregnated Paper Discs

This was carried out as described by Chesebrough (2000). Here, discs of 6mm diameter were punched out from Whatman No.1 filter paper with the aid of paper puncher and placed into Bijou bottles in batches of 100 discs. The discs were then sterilized by autoclaving at 121°C for 15 minutes and then allowed to cool.

Preparation of Extract Treatment Concentrations

Two grams of ethanolic and aqueous extracts were each dissolved in 2 mL of DMSO and water in separate Bijou bottles to yield 1,000,000 µg/ml (1 g/ml) solutions of ethanolic and aqueous extracts respectively. These were labeled as ethanolic and aqueous stock solutions respectively. From the ethanolic extract stock solution, 0.5 ml was measured and introduced into a clean bottle containing 0.5 ml DMSO. This gave a concentration of 500,000 µg/ml. Then, 100 discs were added such that after even distribution (with the help of shaking at equilibrium) each disc absorbed 0.01 ml of the extract, which is equivalent to 5,000 µg/disc. From the same stock solution, 0.4, 0.3, 0.2 and 0.1 ml was each pipetted into separate sterile bottles containing 0.6, 0.7, 0.8 and 0.9 ml of DMSO respectively. This was followed by the addition of 100 discs into each bottle such that each disc absorbed 0.01 ml of the solution to arrive at concentrations of 4000, 3000, 2000 and 1000 µg/disc respectively. These were stored in a refrigerator at 4°C until required for use. The same procedure was repeated for the

aqueous extract using sterile distilled water (Chesebrough, 2000).

Preparation of Turbidity Standard and Standardization of Inoculum

The standard turbidity solution (barium sulphate standard turbidity solution) was prepared as described by Chesebrough (2000). To 99 ml of sterile distilled water, 1 ml of concentrated sulphuric acid (H_2SO_4) was added to arrive at 1% (v/v) solution of the acid. A quantity (0.5 g) of barium chloride ($BaCl_2.2H_2O$: BDH) was dissolved in water until the final volume of the solution was 50 ml. Then, 0.6 ml of the barium chloride solution was added to 99.4 ml of the 1% H_2SO_4 solution. This resulted in turbid solution. A small quantity of this turbid solution was taken in a test tube, which served for comparison during standardization of the inocula. For standardizing the inoculum, the test organisms were sub-cultured onto nutrient agar (oxoid) plates and incubated at 37°C overnight after which period an overnight colony was taken, transferred into a tube containing 2.0 ml of normal saline until the turbidity of the suspension matched with the turbidity of the standard barium sulphate solution. This was confirmed by comparing the turbidity of the diluted inoculum with that of the standard turbidity solution against the background of a printed white paper (where the inoculum was more turbid, a little more normal saline was added until its turbidity matched with 0.5 Macfarland standard of 3.30×10^6 cfu/ml).

Susceptibility Testing of the Clinical Bacterial Isolates

This was carried out using agar diffusion technique as described by Kirby-Bauer *et al* (1996). Here, nutrient agar (oxoid) plates were prepared and the surface of the agar was dried in a hot-air oven. Using sterile swab sticks, the nutrient agar plates were aseptically inoculated with the test organism. With the aid of sterile forceps and syringe, discs containing different concentrations of the extract (5000, 4000, 3000, 2000 and 1000 μg/disc) were impregnated firmly on to the surface of inoculated plates. Control discs (30 μg/g chloramphenicol as a positive control while DMSO was used as a negative control) were also incorporated onto the inoculated plates. The discs were sufficiently spaced out to prevent overlapping of the zones. The plates were then allowed for the pre-diffusion time of 15 minutes after which they were incubated at 37±1°C for 24 hours. Diameters of zones of inhibition were measured using millimeter rule and the results expressed in mm.

Determination of the Minimum Inhibitory Concentration

This was carried out in accordance with the methods described by Morello *et al.* (2003) and Barnett (1992). Extract concentrations of 2000, 1000, 500 and 250 μg/ml were prepared. A quantity (0.1 ml) of the suspension of the test bacterium was inoculated onto fresh nutrient agar (oxoid) plates at the different extract concentrations. The plates were incubated at 37±1°C for 18-24 hours. The lowest concentration of the extract that inhibited the growth of the test bacterium was noted and recorded as the minimum inhibitory concentration (MIC).

RESULTS

Physical and Phytochemical Characteristics of the Extracts

The results of the physical characteristics of the extracts showed that yields of 9.2 and 8.6 grams of the aqueous and ethanolic extracts were obtained respectively. The aqueous extraction was black, pungent, soft and solid while the ethanolic was dark-brown, pungent, soft and creamy (Table 1).

Table 1: Physical Characteristics of Ethanolic and Aqueous Extracts of *Zingiber officinale*

Extract	Initial weight (g)	Final weight (g)	Colour	Odour	Texture	Appearance
Ethanolic	100.0	8.6	Dark-brown	Pungent	Soft	Creamy
Aqueous	100.0	9.2	Black	Pungent	Oily	Solid

Results of the phytochemical screening of the extracts showed the presence of flavonoids, steroids, tannins and reducing sugars in both the aqueous and ethanolic extracts while alkaloids and saponins were only present in the ethanolic and aqueous extracts respectively (Table 2).

Table 2: Phytochemical Characteristics of Aqueous and Ethanolic Extracts of *Z. Officinale*

Extract	Alkaloids	Saponins	Flavonoids	Steroids	Tannins	Reducing sugar
Ethanolic	+	-	+	+	+	+
Aqueous	-	+	+	+	+	+

KEY: + = present
- = absent

The results of the bioassay studies showed that the ethanolic extract was active only at concentration of 1000 µg/disc and the activity was pronounced on *S. aureus* II while for *S. aureus* I, the activity started at 4000 µg/disc. The ethanolic extract was found to be active against *P. aeruginosa* II only at 2000 µg/disc and at 3000 µg/disc against *P. aeruginosa* I while it was found to be active at concentration of 4000 µg/disc against *E. coli* II and inactive at all at all concentrations against *E. coli* I (Table 3).

Table 3: Antibacterial Activities of the Ethanolic Extract of *Zingiber Officinale*

	Concentration of discs (µg/disc)						
Isolates	1000	2000	3000	4000	5000	Positive control	Negative control
	Diameter of zone of inhibition (mm)						
E. coli I 00	00	00	00	00	00	00	00
E. coli II	00	00	00	08	09	14	00
S. aureus I	00	07	07	08	09	12	00
S. aureus II	08	08	09	10	11	12	00
P. aeruginosa I	00	07	08	09	10	11	00
P. aeruginosa II	07	08	08	09	10	13	00

KEY: *E. coli* I, *P. aeruginosa* I and *S. aureus* 1 are isolates obtained from urine samples.

E. coli II, *P. aeruginosa* II and *S. aureus* II are isolates obtained from ear swabs.

The aqueous extract had its lowest activity at 3000 µg/disc, but it was more pronounced against *E. coli* II with 9 mm zone of inhibition while on *E. coli* I, there was no activity at all concentrations. On *S. aureus* I and II, the extract was active at 4000 µg/disc with 8 mm zone of inhibition each while on both *P. aeruginosa* I and II, 3000 µg/disc was active with 8 mm zone of inhibition each (Table 4).

Table 4: Antibacterial Activities of the Aqueous Extract of *Zingiber Officinale*

	Concentration of discs (µg/disc)						
Isolates	1000	2000	3000	4000	5000	Positive control	Negative control
	Diameter of zone of inhibition (mm)						
E. coli I 00	00	00	00	00	10	00	00
E. coli II	00	00	09	09	10	12	00
S. aureus I	00	00	00	08	09	13	00
S. aureus II	00	07	07	08	08	15	00
P. aeruginosa I	00	00	08	08	09	10	00
P. aeruginosa II	00	07	08	08	11	13	00

KEY: *E. coli* I, *P. aeruginosa* I and *S. aureus* I are isolates obtained from urine samples.

E. coli II, *P. aeruginosa* II and *S. aureus* II are isolates obtained from ear swabs.

Results of the minimum inhibitory concentrations (MICs) of the ethanolic extract on the test organisms were 4000, 8000, 250, 6000 and 4000 µg/ml against *E. coli* II, *S. aureus* I, *S. aureus* II, *P. aeruginosa* I and *P. aeruginosa* II respectively. For the aqueous extract, the MICs for *E. coli* II, *S. aureus* I, *S. aureus* II, *P. aeruginosa* I and *P. aeruginosa* II were 3000, 4000, 3000, 6000 and 3000 µg/ml respectively (Table 5).

Table 5: MICs of the Aqueous and Ethanolic Extracts of *Zingiber Officinale*

Isolates	Aqueous extract (µg/ml)	Ethanolic extract (µg/ml)
E. coli II	3000	4000
S. aureus I	4000	8000
S. aureus II	3000	250
P. aeruginosa I	6000	6000
P. aeruginosa II	3000	4000

KEY: *E. coli* I, *P. aeruginosa* I and *S. aureus* I are isolates obtained from urine samples.
E. coli II, *P. aeruginosa* II and *S. aureus* II are isolates obtained from ear swab.

DISCUSSION

The activities of the aqueous and ethanolic extracts of *Z. officinale* against the bacterial isolates in this study could be attributed to the presence of the bioactive ingredients such as alkaloids, tannins and saponins in the extracts. These compounds have been reported to have exhibited antibacterial potentials (Fatope *et al.,* 1993; Cakir *et al.*, 2004; Kawo *et al.*, 2011). According to the present study, preparing an extract with organic solvent such as alcohol has been shown to have exhibited a better antibacterial activity. This is in accordance with the results earlier reported by Kwa *et al* (2012) who reported ethanol as the best solvent that dissolves multivariable type of compounds. The effectiveness of the organic solvent (ethanol) might be attributed to two reasons: firstly, the nature of biologically-active components; their activities could be enhanced in the presence of organic solvents. Secondly, the strength of the extraction capacity of the ethanol could have allowed more of the bioactive constituents responsible for the antibacterial activities. On the other hand, the ethanolic extract was found to be more active against the Gram-positive isolates (*S. aureus)* while the aqueous extract was found to be more active against the Gram-negative isolates (*P. aeruginosa* and *E. Coli*).

This variability could be related to the ability of the different solvents to dissolve some of the chemical constituents in the extracts. However, the resistance of *E. coli* I to both extracts (Tables 3 and 4) could be attributed to the presence of phytochemicals that have poor solubility in the gut as reported in Table 2 or the antibiotics taken by the patient(s) from which it was isolated; as such it could have developed resistance to such antibiotics and become a resistant strain (Kubmarawa *et al.*, 2008; Kumurya *et al.*, 2010). It could also be as a result of cross resistance that might have been developed by the bacterium.

The findings of the present study have shown that the isolates obtained from the ear swabs exhibited a greater susceptibility to the extracts than those obtained from the urine samples. This observation could be attributed to the fact that the organisms from urine (*E. coli* I, *S. aureus* I and *P. aeruginosa* I) might have been subjected to various drugs and antibiotics than those from the ear swabs (*E. coli* II, *S. aureus* II and *P. aeruginosa* II). In addition, the kidney, which acts as an important organ for excreting the urine, is also involved in metabolizing drugs and antibiotics taken up by a patient. Thus, resistance could be developed by such organisms. On the other hand, the organisms from the ear swabs could be less subjected to antibiotics unless in rare cases, hence might not develop such a resistance.

CONCLUSIONS AND RECOMMENDATIONS

The results obtained in this study justify the popularity enjoyed by this plant and it also

rationalizes the use of the plant for the treatment of cutaneous and gastro-intestinal infections. The range of bacteria inhibited suggests that the plant could be used in the treatment of respiratory and urinary tract as well as wound infections. The high vulnerability of *P. aeruginosa* to the extracts tested in this study is worthy of note as this bacterium is resistant to most of the available disinfectants and antibiotics (Neu, 1983; Timasz, 1994). It is therefore recommended that further research needs to be carried out using other solvents to ascertain the antibacterial potentials of the extracts. In addition, there is need to isolate, purify and characterize the~~se~~ phytochemical constituents responsible for the observed bioactivities with a view to supplementing conventional drug development and/or probably serve as lead compounds for various pharmaceuticals especially in developing countries like Nigeria. Finally, it would be worthwhile to establish the toxicological properties of ginger by determining its minimum lethal dose (LC_{50}).

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