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Phytochemical Constituents and Antibacterial activity of Ginger (*Zingiber officianale*) Extract on Selected Clinical Isolates Associated with Urinary Tract Infections (UTIs)

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Abstract

Ginger (Zingiber officianale) is a rhizome that is used in traditional medicine for the treatment of cold, fever, stomach disorder, diarrhoea and jaundice and as skin cleanser. To evaluate the scientific basis for the use of the plant, this study investigated the phytochemical constituents of the plant. The phytochemical constituents of the dried powdered ginger plant parts were extracted using aqueous and organic solvents (ethanol and methanol).The antimicrobial activities of extracts of the plant were evaluated against some selected clinical isolates associated with urinary tract infections (UTIs) using the agar well diffusion method. The isolation, characterization and identification of the bacteria isolates were carried out using standard techniques of streak plate and biochemical tests. The results of the phytochemical studies revealed the presence of tannins, saponins, flavonoids, alkaloids and steroids. The bacteria isolates characterized and identified were Pseudomonas aeruginosa, Escherichia coli and Klebsiella sp. The ginger extracts were active (inhibitory) against all the test isolates. The studies on the minimum inhibitory concentration (MIC) of the extracts on the test isolates showed that the lowest MIC were demonstrated against Pseudomonas aeruginosa, Escherichia coli and Klebsiella sp while the highest MIC at 50mg/ml was exhibited against only Escherichia coli. Ginger (Zingiber officianale) showed medicinal efficacy against the test isolates. This justified the ethnobotanical use of Zingiber officianale extract and its medical importance.

***Keywords**:Ginge;, phytochemical; activity; bacteria; diffusion; inhibition*

Introduction

Urinary tract infections (UTI) are bacterial infections that affect any part of the urinary tract. When it affects the lower urinary tract it is known as a simple cystitis (a bladder infection) and when it affects upper urinary tract it is known as pyelonephritis (a kidney infection). The UTIs has become the most common hospital-acquired infection, accounting for as many as 35% of nosocomial infections, and they are the second most common cause of bacteraemia in hospitalized patients (Samm and Norby, 2001). The annual cost to the health care system of the United States attributable to community-acquired UTIs alone is estimated to be approximately $ 1.6 billion (Foxman, 2002). The UTIs are among the most common bacterial infections which are prevalent extra intestinal and affecting people of all ages from neonates to geriatric age group (Kunin, 2005). Worldwide, about 150 million people are diagnosed with UTIs each year. It is estimated that about 35%of healthy women suffer with UTIs at some stage in their lives. About5% of women each year suffer with the problem of painful urination (dysuria) and frequency. The incidence of UTIs is greater in women than men, which may be either due to anatomical predisposition or urothelial mucosal adherence to the muco polysaccharide lining or other host factors (Schaffer *et al*., 2001). The most common cause of UTIs is Gram negative bacteria that belong to the family *Enterobacteriaceae*. Members of this family include *E. coli*, *Klebsiella, Enterobacter* and *Proteus*. Also Gram positive *Staphylococcus* sp. plays a role in the infection (Kunin, 2005).The *E. coli* is one of the most common bacteria capable of causing infection in humans, particularly urinary tract infections (Iroha, 2009). The frequency of *E.coli* in urine samples varies in different studies from 32% (Okada and Usui -Abe, 1994), 40% (Nunezsanchez *et al*., 2003) and 75% (Goldstein, 2000). Many plants belonging to the ginger family, *Zingiberaceae,* have a history of medicinal use in systems of traditional medicine. Best known are ginger (*Zingiber officinale*) and turmeric (*Curcuma longa*), both of which have been the subject of substantial pharmacological and clinical investigations over the last three decades, but many lesser known species are also used, mostly in tropical Asia, where the majority are native. Several species in the family are also important spices (Singhal *et al.*, 2009).

Nowadays, drug resistance is a huge growing problem in treating infectious diseases like malaria, tuberculosis, diarrheal diseases, urinary tract infections, etc. According to Goldman and Huskins (2010) the improper and uncontrolled use of many antibiotics resulted in the occurrence of antimicrobial resistance, which became a major health problem worldwide. In the last 3 decades, there have been a lot of reports in the scientific literature on the inappropriate use of antimicrobial agents and the spread of bacterial resistance among microorganisms causing UTIs (Tenver and McGowan Jr, 2004; Kurutepe *et al*., 2005). A number of phyto-therapy manuals have mentioned various medicinal plants for treating infectious diseases due to their fewer side effects and reduced toxicity (Lee *et al*., 2007). There are several reports on the antimicrobial activity of different herbal extracts (Islam *et al*., 2008; de Boer *et al*., 2005). Many plants have been found to cure

gastrointestinal disorders, respiratory diseases and cutaneous infections (Somchit *et al*., 2003; Santos *et al*., 2005). According to WHO, medicinal plants would be the best source for obtaining a variety of drugs (Santos *et al*., 2005). Several studies have evaluated ginger's effectiveness in preventing motion sickness or sea sickness and the potential mechanisms for this effect (Islam *et al*., 2009). In a randomized cross-over trial of eight healthy volunteers, ginger supplements were significantly more effective than placebo in alleviating vertigo associated with motion sickness. In a randomized controlled trial of naval cadets, ginger was significantly more effective than placebo in preventing sea sickness, both vomiting and vertigo (Lee *et al*., 2008). This research is aimed at determining phytochemical analysis and antibacterial activity of ginger *(Zingiber officianle)* extract on selected clinical isolates associated with urinary tract infections (UTTs).

Plate 1: Rhizome (underground stem) of Ginger (*Zingiber officinale)*

Materials and Methods

Collection and Preparation of Plant Material

Rhizome was collected from *Zingiber officianale* in Hunkuyi, Kaduna State and was deposited in a sterile polythene bag. The rhizome was taken to the department of Biological science, Faculty of science Kaduna state University, for identification and authentication. The rhizome was dried and ground with mortar and pestle and was deposited in a sterile rubber and kept in a dry place until when needed.

Collection of Samples

Urine sample was collected from Diamond Hospital Kaduna, in a sterilized a bottle and was transported to the microbiology laboratory where it was kept in a refrigerator at a temperature of 4 ^{o}C, until needed.

Preparation of Plant Extract

Fifty (50) g of the dry leaf was weighed and extracted with 95% ethanol and methanol and distilled water in the ratio of 1:6 (50 g of leaf to 500 mL each of distilled water and ethanol).Each was blended using electric blender. The use of water for extraction was to justify the condition in which the traditional practitioner generally uses the herbs, and ethanol was used because of its broad spectrum and relative non-selective property of extraction (Iyamabo, 2008).The water and ethanol extraction was for 24 hours at 40 ^{o}C with occasional shaking, each mixture was filtered and evaporating dish on a steam both at a temperature of 70 ^{o}C to obtain a brown semi-solid substance. This extract was stored in a screw-capped bottle and kept in the laboratory refrigerator for further research (Parija, 2006 and Sofowora, 2008).

Phytochemical Screening of the Plant Extract

The phytochemical constituent/screening of the plant extract for presence of bioactive components was performed following the method of Sofowora (2008).

Detection of Alkaloids

The extract was stirred with aqueous hydrochloric acid on a steam bath and the filter was treated separately with a few drops of Wagner`s reagent (lugols iodine). The formation of brown precipitate indicates the presence of alkaloids.

Detection of Saponins

About 20 mL of distilled water was added to 0.5 g of each extract and was mixed in a graduated cylinder for 15 minutes. The formation of 1cm layer of form indicates the presence of saponins.

Detection of Flavonoids

The extract was treated with few drops of lead acetate solution. The formation of yellow colour precipitate indicates the presence of flavonoids.

Detection of Steroids

Five (5) drops of concentrated H_2SO_4 was added to 1ml of the extract. The formation of reddish brown colour indicates the presence of steroids.

Isolation and Identification of Test Organisms from Urinary Tract Infections (UTIs)

Preparation of Media

MacConkey agar was prepared according to manufacturer directions. About 4.7 g of MacConkey powder was weighed and dissolved in 100 ml of distilled water. The mixture was placed on hot plate to obtain a homogenous solution. The mixture was sterilized by autoclave at 121 ºC for 15 minutes and allowed to cool to 45 ºC before dispensing 20 ml into sterile petri dish and was allowed to solidify (Cheesbrough, 2002).

Nutrient agar was prepared by weighing 5.6 g of powder in 200 ml of distilled water and was allowed to soak for 10 minutes; it was swirled to mix and then sterilized by autoclaving at 121 ºC for 15 minutes. It was cooled to 47 ºC. then poured into bijou bottles. The bijou bottles were kept in slanted position (Parija, 2006).

Nutrient agar (NA), nutrient broth (NB) were used for preparation of inoculums. Mueller Hinton Agar (MHA) and nutrient broth (NB) were used for screening the plant extract for antibacterial activities. The media were prepared according to the instruction of the manufacturer and sterilized 121 ºC for 15 minutes (Akerele *et al.,* 2008).

Isolation of Escherichia coli

A sterile wire loop was dipped into the urine sample and was streaked on the MacConkey agar media. Plates were incubated for 24 hours at 37 ºC.The observed microbial growth was sub-cultured on Eosin methylene blue (EMB) agar and was incubated at 37 ºC for 24 hours. The appearance of a greenish metallic sheen colour indicates preliminary presence of *E. coli*. The isolates were sub – cultured on slants. The slant bottles were stored in a refrigerator at 4 ºC for further studies (Cheesbrough, 2002).

Isolation of Pseudomonas aeruginosa and Klebsiella species

A sterilized wire loop was dipped into the urine sample and was streaked on the CLED agar media. Plates were incubated for 24 hours at 37°C.The isolates were sub – cultured on agar slants. The slant bottles were stored in a refrigerator at 4°C for further studies (Cheesbrough, 2002).

Characterization and Identification of Bacterial Isolates

The procedures used Oyeleke and Manga (2008) were employed for the biochemical tests. The biochemical tests performed include: Gram's staining, catalase test, indole test, citrate test, hydrogen sulfide test, Methyl-Red Vogues Proskauer test (MR-VP), Oxidase test for bacteria characterization and identification.

Preparation of McFarland Turbidity Standard

One percent (1%) solution of sulphuric acid was prepared by adding 1ml of concentrated H_2SO_4 into 99ml of water. One percent (1%) solution of barium chloride ($BaCl_2$) was also prepared by dissolving 0.5 g of dehydrated barium chloride in 50 mL distilled water. Exactly 0.5 mL of Barium chloride solution was added to 99.5 mL of sulphuric acid

solution to yield 1.0% barium sulphate suspension. The turbid solution formed was transferred into a test tube as the standard for comparison.

Preparation of Standard Inoculum of Test Organism

The test bacteria were first inoculated into tubes of nutrient broth separately and incubated at 37 ^{o}C for 18 hours. Using sterilized cotton swab, enough material from an over-night broth culture of the test organisms was transferred into a tube containing 2.0 mL normal saline separately and turbidity were adjusted to match 0.5 McFarland turbidity standard.

Preparation of Different Concentration of the Ginger Extracts

A stock solution was prepared by dissolving 1.6 g of the extracts in 4 mL of distilled water for each extract, serial dilution was then carried out in four test tubes containing 2 mL of distilled (sterile) water each for both extracts to obtain the concentration. Exactly 400 mg/ml, 200 mg/mL, 100 mg/mL, 50 mg/mL, respectively. Exactly 2 mL of the stock solution was transferred into 2 mL of distilled water then in to the next test tubes until the final concentration was reached. The procedure was carried out for all extracts used (Parija, 2006).

Antimicrobial Susceptibility testing of the Ginger Extracts

The antimicrobial screening was carried out using the agar diffusion techniques of Kirby-Bauer (well method) as described by Muhammad *et al*. (2013). Broth cultures of the test organisms (*Pseudomonas aeruginosa, Klebsiella* sp *and Escherichia coli*) were made and spread on the surface of the solidified plate of Muller Hinton agar using swab stick for each respectively. A sterilized 8 mm cork borer was used to make 4 wells for different concentrations of the extracts on each plate containing culture (excluding the control well) of the test organisms and were labelled A-D. Five (5) drops of each concentration of the extract were then introduced into the four wells (excluding the well for control) using a sterilized micro-pipette. This preparation was for all the extracts. A standard antibiotic (Ciprofloxacin) was used as a positive control while the distilled water served as negative control. There were plates prepared for media sterility control (MSC) and extract sterility control (ESC). All the cultures, including plates of MSC and ESC were incubated at 37 ^{o}C for 24 hours but allowed to set for 3 hours before incubation, for the organisms to set on the media. After 24 hours antimicrobial activity it was determined by measuring the diameter zones of inhibition (mm) against the test organisms around each of the wells containing the extracts, water and the antibiotic (Parija, 2006).

Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory (MIC) of the plant extracts was determined using the broth dilution method as described by Lar *et al*. (2001). The least concentration showing inhibition (within visible growth sign) using turbidity as a criterion was determined by

pipetting 5 mL of sterile nutrient broth into 5 set of test tubes for each organism that was inhibited by the extract. Serial dilutions were carried out using 5 ml of the concentration for four tubes to give corresponding dilution for each test organisms and were inoculated into the labelled tube and incubated aerobically at 37 ºC for 24 hours. The fifth test tube served as control. These were carried out for each extract for antimicrobial sensitivity test.

Determination of Minimum Bactericidal Concentration (MBC)

The Minimum Bactericidal Concentration (MBC) was determined by first selecting the tubes that showed no growth during the MIC determination. One loopful from each of these tubes was sub-cultured onto the surface of extract free nutrient agar and incubated for 24 hours at 37 °C. The lowest concentration at which no growth was observed on the agar was noted as the MBC (Clinical Laboratory Standard Institute, 2002).

Results

The phytochemical constituents of the *Zingiber officianale* extract included alkaloids, saponins, tannins, steroids, flavonoids. The ethanoic and methanolic extract had more phytochemical compounds than the aqueous extracts (Table 1). The inhibitory activity of the ginger extract tested on the organisms showed that the ethanol extract had higher zone of inhibition compared to the aqueous extract and the methanolic extract. The ethanolic extract at 400 mg/mL had inhibitory effects on *Escherichia coli*, *Klebsiella* sp and *Pseudomonas aeruginosa* with zones of inhibition of 23 mm, 22 mm and 12 mm respectively compared to 11 mm, 10 mm, and 8 mm recorded zones of inhibition respectively from the effect of the aqueous extract of the ginger on the test organisms (Tables 2, 3 and 4). The result of the minimum inhibitory concentration of *Zingiber officianale* extract on the test organisms is shown on table 4. The MIC was interpreted as the lowest concentration that showed no turbidity. In the ethanolic extract, the MIC was observed in the concentration 50mg/ml, 100mg/ml from the aqueous extract and 100mg/ml from the methanolic extract (Table 5). Table 6 showed the minimum bactericidal concentrations of *Zingiber officianle* extract on the test organisms. The minimum bactericidal concentration (MBC) was determined by first selecting the tubes that showed no growth during the MIC determination. It was interpreted as the lowest concentration at which no growth was observed on the agar. The MBC was noted at concentrations of 25 mg/mL for both the ethanolic, methanolic and aqueous extracts, respectively (Table 6).

Table 1: Phytochemical Screening of *Zingiber officianale* Extracts

Phytochemicals	Ethanol	Aqueous	Methanol
Alkaloids	+	+	+
Flavonoids	+	-	+
Steroids	+	-	+

Tannins	+	-	+
Saponins	+	+	+

Key+: Present, -: Absent

Table 2: Antibacterial activity of *Zingiber officianale* against *Klebsiella* sp

	Zones of Inhibition (mm)		
Concentration mg/ml	**Ethanolic extract**	**Aqueous extract**	**Methanolic**
400	15.0	-	11.5
200	20.0	-	21.5
100	20.0	-	2.3
50	20.5	-	2.2

Keys - : No zone of inhibition

Table 3: Antibacterial activity of *Zingiber officianale* against *Eschericia coli*

	Zones of Inhibition (mm)		
Concentration mg/ml	**Ethanolic extract**	**Aqueous extract**	**Methanolic**
400	-	-	-
200	-	-	-
100	-	-	-
50	-	-	-

Keys - : No zone of inhibition

Table 4: Antibacterial activity of *Zingiber officianle* Against *Pseudomonas aeruginosa*

	Zones of Inhibition (mm)		
Concentration mg/ml	**Ethanolic extract**	**Aqueous extract**	**Methanolic**
400	17.0	18.5	13.5
200	21.5	20.5	19.0
100	23.0	24.5	23.5
50	24.0	27.5	25.5

Keys
- : No zone of inhibition

Table 5: Minimum Inhibitory Concentration (MIC) of *Zingiber officianle* against the test Isolates

	Extract		
Concentration (mg/ml)	**Ethanolic**	**Aqueous**	**Methanolic**
200	-	-	-

100	-	*	*
50	*	+	+
25	+	+	+

Keys:
- : No growth
+: Turbidity
*: MIC

Table 6: Minimum Bactericidal Concentration (MBC) of *Zingiber officianale* the test Isolates

	Extract		
Concentration (mg/ml)	**Ethanolic**	**Aqueous**	**Methanolic**
200	-	-	-
100	-	-	-
50	-	-	-
25	-	-	-

Keys: -: No growth

Discussion

The phytochemical screening of *Zingiber officianale* showed that ethanolic extract had higher concentration of bioactive components, followed by methanolic extract, then aqueous. This could be as a result of the differences in solvent polarity. The presence of the phytochemical constituents in the extract may be responsible for the antimicrobial activity of the plant as reported by Marorie (2004). The observed activities of the various extracts may be due to varying degree of solubility of the bioactive constituents in the three solvents used, and since the ethanol extract had more bioactive component it may be the reason behind high antibacterial activity of the ethanolic and methanolic extract and it is in agreement with the findings of Marorie (2004). The associated bacteria with UTIs were identified as *Escherichia coli, Klebsiella* sp, and *Pseudomonas aeruginosa.* Their presence could be attributed to weak immunity, personal hygiene or long time exposure of the individual. The antibacterial sensitivity test of the ethanolic, methanolic and aqueous extracts showed inhibitory activity of the extracts on the organisms tested at different concentrations. *Escherichia coli* was most sensitive, followed by *Klebsiella* sp, and *Pseudomonas aeruginosa* had the least sensitive to all extracts at different concentration. This agrees with the report of Chah *et al.* (2006). In literature, it has been indicated that medicinal plants are the backbone of traditional medicine (Worth, 2008). And antibacterial activity of plant extract is due to different chemical agents in the extracts which were classified as active antimicrobial compounds as reported by Rojas *et al.* (2010). The minimum inhibitory concentration (MIC) was determined at 100 mg/ml concentration for

all the organisms tested except for *Escherichia coli* (ethanol extract) which had MIC at 50 mg/mL. This lends support from the findings of Rojas *et al.* (2010).Therefore, the observed low MIC values against the bacteria, could be as a result of the plant having the potential to treat any ailment associated with the bacteria pathogen effectively. The results of this study showed that *Zingiber officianale* has promising medicinal property. The plant could be exploited in the development of phytomedicines for the control or management of resistant bacteria. The results of this study are in agreement with previous reports and have validated the folkloric use of *Zingiber officianle* in the treatment of diarrhoea, gastrointestinal disorders, dysentery and wounds as reported by Chah *et al.* (2006). In conclusion, the presence of important phytochemical constituents, antimicrobial activities against the test organisms (Gram negative bacteria), as observed in this study and low MIC values, justified the ethnobotanical use of *Zingiber officianale* extract and its medical importance.

Recommendations

1. Due to increasing menace of typhoid fever, diarrhea, food poisoning, secondary bacterial infection, gastroentitis in Nigeria, further work on *Zingiber officianle* should be carried out since it inhibited the causative agents of these ailment *in-vitro.*
2. *Zingiber officianale* extract should be subjected to further *in-vivo* and clinical trials since it showed antibacterial activity *in-vitro.*
3. The antifungal, viral, molluscidal, antimalarial activity of *Zingiber officianle* plant should be carried out.

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Conflict of Interest

The Authors of this research declared no conflict of interest.

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