



Antimicrobial effects of *Zingiber officinale* Rhizomes extracts on selected pathogenic clinical isolates

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ABSTRACT

The success of microbial chemotherapy lies in the continuous search for novel drugs to counter the challenges posed by resistant strains. The antimicrobial and growth inhibition effect of different *Zingiber officinale* solvent extracts (n-hexane, chloroform, ethyl acetate, petroleum ether, butanol, methanol and water extract) were investigated against five pathogenic bacterial and fungal isolates (*Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas putida*, *Bacillus subtilis* and *Candida albicans*). Agar well diffusion technique was used to determine the antimicrobial activity of the extracts while broth dilution technique was used for Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC). The zones of inhibition on agar wells were observed to vary depending on the microbe and type of extract. The ethyl acetate extract exhibited the most prominent antimicrobial activity as it was effective against all the five pathogenic microbes (*Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas putida* and *Candida albicans*), but was more toxic against *Bacillus subtilis* (14 mm). Butanol extract showed toxicity against *Bacillus subtilis* (10 mm) and *Candida albicans* (12 mm). Chloroform extract showed antimicrobial activity against *Candida albicans* (10.0 mm). However, aqueous, methanol, petroleum and n-hexane extracts did not show any antimicrobial activity against the pathogens studied. Ethyl acetate extract exhibited its antibacterial and antifungal efficacy with an MIC value of 50 mg/ml against *P. putida*, *B. subtilis* and *C. albicans* while chloroform extract was effective at 100 mg/ml against *C. albicans*. Furthermore, butanol and ethyl acetate extracts also showed their antimicrobial efficacy against *C. albicans* and *E. coli* respectively with an MIC of 100 mg/ml. The Minimum Bactericidal Concentration determined for the potent extracts showed that butanol extract had bactericidal efficacy at 25 mg/ml concentration against *Bacillus subtilis*. Ethyl acetate extract also exhibited its antibacterial potency at an MBC of 50 mg/ml against *P. putida*, *B. subtilis* and *S. aureus* but at 100 mg/ml against *E. coli*. For the Minimum Fungicidal Concentration, butanol, ethyl acetate and chloroform extracts were effective as fungicidal agents against *Candida albicans* at MFC of 100 mg/ml, 50 mg/ml and 100 mg/ml respectively. This study has shown the potential of ginger rhizomes as possible antimicrobial agent.

Key words: *Zingiber officinale*, solvent extracts, bacterial, fungal, isolates, antimicrobial.

1.0 INTRODUCTION

The use of medicinal plants and their extracts to treat infections is an age old practice in traditional African medicine. Plant derived products have been used for medicinal purposes for centuries and at present, it is estimated that about 80 % of the world population relies on botanical preparations as medicines to meet their health needs (Sakr and

Al-Amoudi, 2012). Traditional medical practice has been known for centuries in many parts of the world (Parekh and Chanda, 2007). It has, however been, observed that these practices vary from one country to another. Numerous plants and herbs are used all over Nigeria by traditional medical practitioners. The use of herbs is the most ancient approach to healing known. The herbal medicines may

be in form of powders, liquids, or mixtures, which may be raw or boiled, ointments, liniments, and incisions (Onyeagba *et al.*, 2004). Rhizomes, barks, and leaves of various plants are employed in ethnomedicine. Plant extracts are given singly or as concoctions for various ailments. More than 70 % of the people living in Nigeria depend on these various form of concoctions and herbal decoctions for the treatment of some diseases (Kimbi and Fagbenro-Beyioku, 1996).

Within the recent years, infections have increased to a great extent and antibiotics resistance effects have become an ever-increasing therapeutic problem (Mahesh and Satish, 2008). Antimicrobial usage particularly in high-risk cancer patients create selection pressures that lead to the emergence of resistant microorganisms (Rolston, 2009). Researchers have shown that many cancer centres have reported an increase in quinolone resistant bacteria (primarily *Escherichia coli* and *Pseudomonas aeruginosa*) in patients receiving quinolone prophylaxis (Gomez *et al.*, 2003; Cattaneo *et al.*, 2008; Rolston, 2009).

Furthermore, there has been tremendous increase in the resistance of diverse bacterial pathogens; this has greatly affected the ability to successfully treat patients (Kaye *et al.*, 2004; Finch and Hunter, 2005). There is therefore need for continual search for new safe and consistent ways of tackling this challenge. Interest has always been focused on exploration of plant materials to solve diverse health issues.

Many investigators have demonstrated the antimicrobial activity of the constituents of some plants (Onyeagba *et al.*, 2004; Ogundare *et al.*, 2006; Oloyede *et al.*, 2012). A number of chemical compounds of plant origin have been shown to possess antimicrobial activities (Ijah and Oyebanji, 2003; Khanom *et al.*, 2003). Herbs and spices are generally considered safe and proved to be effective against certain ailments (Indu *et al.*, 2006; Maharjan *et al.*, 2011; Uzama *et al.*, 2012).

Zingiber officinale (Zingiberaceae) also known as ginger is one of the medicinal plants that have been used as a stimulant and carminative, dyspepsia and colic (Ali *et al.*, 2008). Ginger may also decrease joint pain from arthritis, may have blood thinning and cholesterol lowering properties and may be useful for the treatment of heart diseases and lungs diseases

(Ghayur *et al.*, 2005; Altman. and Marcussenck, 2011). The chemical constituents of this rhizome that might be responsible for its pharmacological activities have been evaluated. Eleazu *et al.*, (2012) reported the presence of saponins, alkaloids, flavonoids, tannins, and cyanogenic glycosides in the plant. Therefore, due to the growing interest in the use of medicinal plants in the food and the pharmaceutical industries, a systematic study on *Zingiber officinale* extracts have become very important.

Moreover, it is also known that the chemical constituent of a plant material may vary regionally which may influence its medical and economic uses. Thus, the investigation of the medical potential of a common plant material from different localities cannot be over emphasized. In this study, the antibacterial and antifungal activities of different solvent extracts of the rhizomes of *Zingiber officinale* on selected pathogenic microbes was investigated.

2.0 MATERIALS AND METHODS

2.1 Plant collection and identification

The ginger rhizomes used in this study were purchased at the popular mile 12 vegetable market in Ketu-Agboyi local council development area of Lagos state, Nigeria and was certified with herbarium number 3560 at the herbarium unit of the Department of Botany, Faculty of Science, University of Lagos, Nigeria. The ginger rhizomes were properly washed with clean water, dried at room temperature and then oven dried at 35-40°C. The dried ginger rhizomes were thoroughly grinded with an electric blender before it was subjected to exhaustive soxhlet extraction process using distilled water and 6 most frequently used solvent of extraction. They are volatile organic solvents with different polarities; n-hexane, chloroform, ethyl acetate, petroleum ether, butanol, methanol.

2.2 Test microorganism

The test microorganisms (pathogenic clinical isolates) used in this study was obtained at the microbiology laboratory of the Department of Pharmaceutical Microbiology, Faculty of Pharmacy, University of Lagos, Nigeria. The pathogenic clinical isolates include; *Pseudomonas putida*, *Escherichia*

coli, *Bacillus subtilis*, *Staphylococcus aureus* and *Candida albicans*.

2.3 Extraction and concentration of extracts

Aqueous extract from ground ginger rhizomes was obtained by maceration of 200 g ground ginger rhizomes in distilled water (1 L) for 48 hr followed by sieving with muslin cloth. The aqueous extract obtained was concentrated on water bath at 40°C and then freeze dried (8 g). The freeze-dried extract was then kept in the fridge at 4°C. This process was followed by an exhaustive extraction of 200 g ground ginger rhizomes in a soxhlet extractor using six different volatile organic solvents (1 L) which are; n-hexane, chloroform, ethyl acetate, petroleum ether, butanol, and methanol. The various extracts obtained were respectively concentrated in a rotary evaporator (40° C) to obtain a semi-solid which was further freeze dried (1.5, 2.5, 0.8, 3, 5, 3) g and then kept in the refrigerator at 4°C.

2.4 Assay of antimicrobial activity of *Zingiber officinale* Rhizomes extracts

The antimicrobial assays included sensitivity test, Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC) and Minimum Fungicidal Concentration (MFC). Analyses were carried out in triplicates of each set up.

2.4.1 Preparation of starter cultures

The bacterial isolates were sub-cultured from nutrient agar slant using sterile swab, then inoculated into sterile nutrient broth and incubated at 37°C for 24 hr to obtain fresh colonies of bacteria species; *Pseudomonas putida*, *Escherichia coli*, *Bacillus subtilis* and *Staphylococcus aureus*. The fungi isolate; *Candida albicans* was sub-cultured from Sabouraud Dextrose Agar (SDA; Oxoid, UK) and then cultured on SDA plate at 37 °C for 24 hr.

2.4.2 Sensitivity test

Antimicrobial activity of the different solvent extracts of the ginger rhizomes was evaluated by agar well diffusion method on Mueller Hinton Agar (MHA) medium (Forbes *et al.*, 1990). The antimicrobial activity was assessed based on the inhibitory effect of the extract on bacterial growth on agar plate. The zone of inhibition (mm) of microbial growth

was compared to that of known antibacterial (ceftriaxone) and antifungal (ketonazole) agents. The sterile Mueller Hinton Agar (MHA) medium (20 ml) in petri dishes were uniformly smeared using sterile cotton swabs moistened with pure cultures suspension (0.5 macfarland) of the respective human pathogenic bacteria; *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas putida*. Eight wells in equidistant were bored round the plate using a sterile cork borer and 2 ml (100 mg/ml) of each of the seven solvent extracts were put into seven of the wells while ceftriaxone (30 µg/ml) and ketonazole (30 µg/ml) which were used as positive controls were put into the eighth well for bacterial species and the fungi (*Candida albicans*) respectively. The plates were left for 1 hr for proper diffusion after which they were incubated at 37°C for 24 hr. The plates were examined for zone of inhibition which was measured with vernier calipers and the value recorded.

2.4.3 Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) of the extracts that showed potent antimicrobial activity during the sensitivity test were determined by serial dilution in nutrient broth with concentrations ranging from 6.25, 12.5, 25, 50, and 100 mg/ml. Double strength nutrient broth (2 ml) was placed in 5 test tubes, labelled 1-5, then 2 ml of the prepared stock extract (200 mg/ml) was placed in 2 ml of the double strength nutrient broth to obtain an extract concentration of 100 mg/ml in test tube 1. Thereafter, 2 ml of the mixture in test tube 1 was taken and placed in test tube 2 to obtain an extract concentration of 50 mg/ml in the second test tube. The same procedure was repeated for test tube 3, 4 and 5 to obtain an extract concentration of 25, 12.5 and 6.25 mg/ml respectively. The test tubes were inoculated with inoculums prepared from fresh overnight broth culture in nutrient broth, then incubated at 37°C for 24 hr. The series of dilution test tubes were observed for microbial growth, which was indicated by turbidity and/or pellet of microorganisms at the bottom of the test tubes. Minimum inhibitory concentration (MIC) was recorded as the lowest extract concentration demonstrating no visible growth in the broth (Prescot *et al.*, 1996).

2.4.4 Minimum Bactericidal Concentration (MBC)

The MBC was determined using the content from the broth dilution of MIC test. The surface of sterile Mueller Hinton Agar (MHA) medium (20 ml) in petri dishes were uniformly smeared using sterile cotton swabs moistened with the contents of the test tubes from MIC test. The plates were then incubated at 37°C for 24 hr. The minimum bactericidal concentration (MBC) was recorded as lowest concentration of the extract that showed no growth on the agar.

2.4.5 Minimum Fungicidal Concentration (MFC)

The MFC was determined using the content from the broth dilution of MIC test. The surface of sterile Sabouraud Dextrose Agar (SDA) medium (20 ml) in petri dishes was uniformly smeared with sterile cotton swabs moistened with the contents of the test tubes from MIC test. The plates were then incubated at 37°C for 24 hr. The minimum fungicidal concentration (MFC) was recorded as lowest concentration of the extract that showed no growth on the Sabouraud Dextrose Agar (SDA).

3.0 RESULTS AND DISCUSSION

The exploration of medical plants, herbs and spices for development of new therapeutic agents has been known over the years. Likewise the mutation and resistance of disease causing agents to therapeutic medicines thus leading to the continual search for better ways to treat, cure, manage and prevent health problems. In this study, the agar well diffusion method was used to evaluate whether the extracts possessed antimicrobial potential by measuring the zone of inhibition against the test microorganisms. The ethyl acetate extract exhibited the most prominent antimicrobial activity as it was effective against all the five pathogenic microbes (*Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas putida* and *Candida albicans*) used in this study, but was more toxic against *Bacillus subtilis* (14 mm) whereas chloroform extract only showed antimicrobial activity against *Candida albicans* (10.0 mm). Butanol extract also showed toxicity against *Bacillus subtilis* (10 mm) and *Candida albicans* (12 mm) but

showed no antibacterial activity against other bacteria species used in this study (Table 1). The aqueous extract, methanol extract, petroleum extract and n-hexane extract did not exhibit any antimicrobial activity against the pathogens studied. Similar results have been obtained with aqueous and ethanolic extracts of ginger (Onyeagba *et al.*, 2004). However, the observation that ginger rhizomes exhibits antimicrobial properties corroborate previous studies on spices possessing antimicrobial activity (Indu *et al.*, 2006; Maharjan *et al.*, 2011). The antimicrobial properties of ginger have been attributed to its chemical constituents such as tannin, saponin, alkanoids and flavonoids (Ali *et al.*, 2008; Bhargava *et al.*, 2012). Gingerone and gingerol which are the major pungent components of ginger have been suggested to have strong inhibitory activity against pathogenic bacteria (Ali *et al.*, 2008). The observation that some of the extraction media did not exhibit antimicrobial activity highlights the need to use appropriate solvents in the preparation of medicinal plant concoctions.

Table 1: Dimension of zones of inhibition of *Zingiber officinale* rhizomes extracts against some selected pathogenic microbes

Isolates	zone of inhibition of extracts (mm)								
	Aq.	Pet.	Met.	But.	Eth.	Chl.	Hex.	Cef.	Ket.
<i>P. putida</i>	0.0	0.0	0.0	0.0	8.0	0.0	0.0	30.0	-
<i>E. coli</i>	0.0	0.0	0.0	0.0	11.0	0.0	0.0	25.0	-
<i>S. aureus</i>	0.0	0.0	0.0	0.0	11.0	0.0	0.0	27.0	-
<i>B. subtilis</i>	0.0	0.0	0.0	10.0	14.0	0.0	0.0	30.0	-
<i>C. albican</i>	0.0	0.0	0.0	12.0	12.0	10.0	0.0	-	16

Aq. = Aqueous; Pet. = Petroleum ether;
Met. = Methanol; But. = Butanol;
Eth. = Ethyl acetate; Chl. = Chloroform;
Hex. = n-hexane; Cef. = ceftriaxone;
Ket. = ketonazole

The various extracts of *Zingiber officinale* rhizomes which exhibited antimicrobial potential and subsequently subjected to Minimum Inhibitory Concentration (MIC) assay showed that ethyl acetate extract had MIC of 100, 100, 25, 50 and 50 (mg/ ml) when tested against *P. putida*, *E. coli*, *S. aureus*, *B. subtilis* and *C. albicans* respectively (Table 2). Butanol exhibited MIC value of 25 and 100 (mg/ ml) against *B. subtilis* and *C. albicans* respectively while chloroform had MIC of 100 mg/ ml against *C. albicans*. The possession of potent

antimicrobial activity by the ethyl acetate extract of the ginger rhizomes against both Gram positive and Gram negative bacterial isolates suggest that the ethyl acetate extract may contain components that may have disrupted the membrane and/or genetic make up of the isolates. The anticandidal effect of the extracts of *Zingiber officinale* is in agreement with previous research findings, as such ginger extract could be used in the treatment of oral candidiasis (Atai *et al.*, 2009).

Table 2: Minimum inhibitory concentration (MIC) of *Zingiber officinale* rhizomes extracts against some selected pathogenic microbes

Extract + Isolate	Concentration of extracts (mg/ml)				
	6.25	12.50	25.00	50.00	100.00
Butanol extract + <i>B. subtilis</i>	+	+	-	-	-
Butanol extract + <i>C. albicans</i>	+	+	+	+	-
Ethyl acetate extract + <i>P. putida</i>	+	+	+	+	-
Ethyl acetate extract + <i>E. Coli</i>	+	+	+	+	-
Ethyl acetate extract + <i>S. aureus</i>	+	+	-	-	-
Ethyl acetate extract + <i>B. subtilis</i>	+	+	+	-	-
Ethyl acetate extract + <i>C. albicans</i>	+	+	+	-	-
Chloroform extract + <i>C. albicans</i>	+	+	+	+	-

+ = Turbid (Growth),

- = NoTurbidity (No Growth)

Furthermore, the extracts of *Zingiber officinale* rhizomes which showed antimicrobial potential was evaluated for their Minimum Bactericidal Concentration (MBC) and showed that ethyl acetate extract exhibited bactericidal efficacy with an MBC value of 50, 100, 100 and 50 mg/ml against *P. putida*, *E. coli*, *S. aureus* and *B. subtilis* respectively (Table 3) while its MFC potential against *C. albicans* was at 50 mg/ml. Similarly, butanol extract had its bactericidal efficacy at 25 mg/ml against *B. subtilis* while its fungicidal potency was at 100 mg/ml likewise; chloroform exhibited an MFC at 100 mg/ml against *C. albicans*. MBC values which revealed the concentration of the extract at which the organisms are completely killed suggest that ethyl acetate, butanol and chloroform could be exploited as potent solvents in extracting bactericidal and fungicidal active constituents of ginger rhizomes.

Table 3: Minimum bactericidal concentration (MBC) of *Zingiber officinale* rhizomes extracts against some selected pathogenic microbes

Extract + Isolate	MBC of extracts (mg/ml)
Butanol extract + <i>B. subtilis</i>	25
Ethyl acetate extract + <i>P. putida</i>	50
Ethyl acetate extract + <i>E. coli</i>	100
Ethyl acetate extract + <i>S. aureus</i>	50
Ethyl acetate extract + <i>B. subtilis</i>	50

Table 4: Minimum fungicidal concentration (MFC) of *Zingiber officinale* rhizomes extracts against some selected pathogenic microbes

Extract + Isolate	MFC of extracts (mg/ml)
Butanol extract + <i>C. albicans</i>	100
Ethyl acetate extract + <i>C. albicans</i>	50
Chloroform extract + <i>C. albicans</i>	100

CONCLUSION

The present study showed the antibacterial and antifungal potential of different solvent extracts of *Zingiber officinale* rhizomes. The data obtained suggest that ethyl acetate, butanol and chloroform extracts have antimicrobial activity while aqueous, petroleum ether, methanol and n-hexane extracts may not have antimicrobial effects under the conditions of extraction. Ethyl acetate extract was a more potent antimicrobial agent than butanol and chloroform extracts as it showed its antimicrobial efficacy against all five (*S. aureus*, *B. subtilis*, *E. coli*, *P. putida* and *C. albicans*) pathogenic microbes used in this study. Chloroform extract showed its antimicrobial potential against *Candida albicans* while butanol extract was effective against *Bacillus subtilis* and *Candida albicans*.

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