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Antimicrobial Activity of *Zingiber officinale* Against Multidrug Resistant Microbial Isolates

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Abstract

Aqueous and ethanol extracts of ginger (*Zingiber officinale*) were investigated for their antimicrobial activities against two bacterial isolates: *Pseudomonas aeruginosa*, *Bacillus subtilis* and two fungal isolates: *Aspergillus flavus* and *Candida albicans* which were obtained from UBTH, Benin City. The organisms were identified using standard microbiology procedures. The phytochemical screening of ginger revealed the presence of alkaloids, saponins, tannins, flavonoids, terpenoids, phenols and steroids in both extract. The antimicrobial activity of each extract was evaluated by the agar well diffusion method. The ethanol extract showed considerable activity on the test organisms with zones of inhibition ranging from 7±0.4mm at concentration of 6.25mg/ml to 23.0 ±3.2mm at 100mg/ml and MIC ranging from 6.25mg/ml against *Bacillus subtilis* and *Candida albicans* to 12.5mg/ml in *Bacillus subtilis*. The activity of the aqueous extract was very minimal at low concentration, but at higher concentration (50-100mg/ml), marked activity was observed. The minimum bactericidal concentration of both extracts was also determined and ranged from 25-50mg/ml in both ethanol extract and aqueous extract. The most susceptible organisms were *Bacillus subtilis* and *Candida albicans* while the most resistant was *Aspergillus flavus*. Antibiotics susceptibility test for the selected organisms showed varying degree of resistance to the antibiotics used, with *Bacillus subtilis* and *Pseudomonas aeruginosa* being multi-drug resistant. The most effective drugs were gentamicin, ofloxacin and perfloxacin. The sensitivity of multiple drug resistant organisms to this plant extracts implies an alternative or substitution for existing antibiotics. Therefore, this plant should be further researched into and developed to explore its therapeutic antimicrobial agents and further pharmacological evaluation.

1. Introduction

The increasing reliance on drugs from natural sources has led to the extraction and development of several drugs and chemotherapeutic agent from traditional herbs [1]. Many foods present antibiotics function that are often unknown to the eater and these foods limit the growth of bacteria in their body. Some of these foods are green tea and ginger [2]. Ginger, a common substance found increasingly in the diets of the global population, has known antimicrobial effects and is commonly used together in teas [3].

Ginger has been used in centuries to fight infection. Its components are active against a form of diarrhea which is the leading cause of infant death in developing countries [3].

There has been a shift from the prescription of antibiotics to the use of medicinal plant [4]. Many plant extracts have been shown to possess antimicrobial properties. For example, aqueous and alcohol extracts of *Ocimum sanctum* and *Ocimum gratissimum* were highly toxic against fungi after 15 days of culture [5]. Some extracts of garlic, onion and green pepper have been reported to inhibit the growth of *Escherichia coli*, *Salmonella typhosa*, *Shigella dysenteriae* and *Staphylococcus aureus*. Flavones, flavonoids and flavonols are chemical compounds active against microorganisms and they are synthesized by plant in response to microbial infection [6]. *Zingiber officinale* rhizomes are rich source of phytochemicals, viz; alkaloids, saponins, flavonoids, terpenes and steroids. These drugs are widely used in the treatment of different ailments in the India system of medicine. Ginger mainly contain up to 3% of volatile oil, a mixture of 24 constituents containing monoterpenoids fraction (p-phelladrene, cineol and citral) and sesquiterpenoids (p-sesquiphelladrene, bisabolene and farnasene) with zingiberene [7]. Ginger has been mixed with other plant extracts and synergistic action of the phytochemicals has been observed [8; 9]. Trikatu churna is an equiproportions of powdered fruits of *Piper nigrum*, *Piper longum* and rhizome of *Zingiber officinale* and it has potent antimicrobial activity. In disease of microbial origin, the plants function as a result of antimicrobial activity against the causative agents [10]. Study conducted showed that ginger's constituents acted as strong antioxidant and effective antimicrobial agent that could heal sores and wounds of internal organs such as stomach and liver. In this relation, [11] pointed out that the primary factor associated with gastritis and peptic ulcer diseases was the gram negative bacterium, *Helicobacter pylori*. [12] concluded that the aqueous ginger extract was able to protect the gastric mucosa from stress induced by mucosal lesion and inhibited the growth of *Helicobacter pylori*. Many microorganisms have developed resistance to commonly used antibiotics, therefore there is need to search for new and additional alternative antimicrobial agent such as various plants extract which contain bioactive phytochemical constituents. Hence, this research work was designed to evaluate the antimicrobial activity and phytochemical constituents of *Zingiber officinale*.

2. Materials and Methods

2.1. Collection and Preparation of Plant Material

Ginger rhizomes were purchased from Uselu Market. The rhizomes were washed to remove soil and it was then peeled, washed, sliced and dried. The dried materials were powdered. 50g of the powdered ginger was weighed into bottle and 500ml of distilled water was added. The same was done for

ethanol. This was to carryout aqueous and ethanol extract respectively. The plant materials were soaked in the respective solvent for 24hrs and then filtered. The respective filtrate was concentrated to get the crude extract from which different concentrations were prepared. All extracts were stored at 4°C when not in use.

2.2. Phytochemical Screening of Extract

Using the method described by [13], test for alkaloid, saponin, Tannin, Phlobotannin, flavonoid, cardiac glycoside, steroid and Terpenoid, were carried out.

2.2.1. Alkaloid Test

Exactly 5g each of the ginger extract and 5ml of honey was stirred with 5ml of 1% aqueous hydrochloric acid on a steam bath. 1ml of the filtrate was treated with few drops of Draggen doffs reagent. Blue black turbidity serves as preliminary evidence of alkaloids.

2.2.2. Saponin Test

Five gram (5g) each of extract and 5ml of honey was shaken with distilled water in a test tube. Frothing which persist on warming was taken as preliminary evidence for saponin.

2.2.3. Tannins

Five gram (5g) each of extract and 5ml of honey was stirred with 100ml distilled water and filtered. Ferric chloride reagent was added to the filtrate. Formation of blue-black or blue-green precipitate indicated the presence of tannin.

2.2.4. Phlobotannin Test

Disposition of red precipitate when an aqueous extract of the test sample was boiled with 1% hydrochloric acid served as evidence for the presence of phlobotannin.

2.2.5. Flavonoid Test

Five milliliter (5ml) of diluted ammonia solution was added to aqueous filtrate of the samples followed by the addition of concentrated H₂SO₄. A yellow colouration observation was taken as an evidence for the presence of Flavonoids.

2.2.6. Cardiac Glycosides (Keller-Killiani Test)

Five gram (5g) of each of the extract and 5ml of honey was dissolved in 2ml glacial acetic acid containing a drop of ferric chloride solution. 1ml of concentrated H₂SO₄ was added. A browning of the interface indicated the presence of deoxy-sugar characteristic cardenolids. A violet ring may appear below the brown ring, while in the acetic acid layer, a green ring may form, just gradually spread throughout this layer.

2.2.7. Steroids

Two milliliter (2ml) of acetic anhydride was added to 0.5g of extract and 2ml of sulphuric acid was added by the sides of the test tube which was then observed for colour change from violet or blue-green.

2.2.8. Terpenoids (Salkowski Test)

To 0.5g of the extract, 2ml of chloroform was added, concentrated H₂SO₄ (3ml) was carefully added to form a layer. A reddish brown colouration of the interface indicated the presence of terpenoids.

2.3. Test Microorganisms

Pure cultures of *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Aspergillus flavus* and *Candida albicans* were collected from Microbiology laboratory, University of Benin Teaching Hospital (UBTH). Each isolate was subjected to standard morphological and biochemical technique for the identification in microbiology laboratory, UBTH. The morphological and biochemical tests included gram staining, motility test, oxidase test, catalase test, coagulase test and indole test.

2.4. Antimicrobial Susceptibility Testing

2.4.1. Preparation of Different Concentrations

Concentrations of plant extracts were prepared according the method of [14]. Zero point one gram (0.1g) of each extract was weighed into sterile bottle and 1ml of sterile distill water was added, giving a concentration of 100mg/ml. Then concentrations of 50mg/ml, 25mg/ml 12.5mg/ml and 6.25mg/ml were prepared from the stock concentration (100mg/ml) by double dilution procedure.

2.4.2. Bacteria Inoculum Preparation

The inocula were prepared by inoculating the test organisms in nutrient broth and incubating them for 24 hours at 37°C. After incubation, a milliliter of the cultures was inoculated onto solidify nutrient agar at 45°C using a Pasteur pipette.

2.4.3. Agar Well Diffusion Techniques

The ability of the various extracts to inhibit the growth of the clinical test organisms was determined using the agar well techniques. The inoculated Nutrients agars and potato dextrose agar plates were allowed to dry. After which, wells were bored on the surface of inoculated agar plates using 4mm cork borer. 0.2 ml of the different concentration of each

extract were transferred into the well using Pasteur pipette. Sterile distilled water was used as a negative control. The wells were sufficiently spaced to prevent the resulting zones of inhibition from overlapping. The plates were incubated at 37°C for 24hours and 28°C for 48 hours. The experiment was performed in triplicate and the resulting zones of inhibition were recorded as mean ± standard error.

2.4.4. Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The minimum inhibitory concentration (MIC) of the methanol extracts was determined for each of the test organisms in triplicates at varying concentrations of 100, 50, 25, 12.5, 6.25 and 3.125 mg/ml). 1 ml of nutrient broth was added and then a loopful of the test organism previously diluted to 0.5 McFarland turbidity standard was introduced to the tubes. A tube containing nutrient broth only was seeded with the test organism to serve as control. All the tubes were then incubated at 37°C for 24 hours and then examined for growth by observing for turbidity. The minimum bactericidal concentration (MBC) of the plant extract on the clinical bacterial isolates was carried out according to [15]. Briefly, 1 ml bacterial culture was pipetted from the mixture obtained in the determination of MIC tubes which did not show any growth and were sub-cultured onto nutrient agar and incubated at 37°C for 24 hours. After incubation the concentration at which there was no single colony of bacteria was taken as MBC

2.5. Antibiotics Susceptibility Pattern

Antimicrobial disc tests of the isolates were performed according to the recommendations of [16] using the following antibiotic discs: tetracycline (20ug), ampiclox (30ug), zinnacef (20ug), amoxicillin (30ug), rocephin (25ug), ciprofloxacin (10ug), Nitrofurantoin (20ug), streptomycin (30ug), erythromycin (10ug), gentamycin (10ug), septrin (30ug), chloramphenicol (25ug), perfloxacin (10ug), and ofloxacin (30ug) and antibiotics resistance was interpreted by diameter of inhibition zones around the antibiotic discs.

3. Results

Table 1. Phytochemical screening of ethanolic and aqueous extracts of *Zingiber officinale*.

Phytochemicals	Solvent	
	Ethanol	Aqueous
Flavonoids	+	+
Tannins	-	-
Phenols	+	+
Terpenoids	+	+
Saponins	+	+
Alkaloids	+	+
Steroids	+	-

Key:

+ = Present

- = Absent

Table 2. Antimicrobial activity of ethanolic extract of *Zingiber officinale*.

Test organisms	Concentrations				
	100mg/ml	50mg/ml	25mg/ml	12.5mg/ml	6.25mg/ml
Bacteria					
<i>Bacillus subtilis</i>	23.0 ±3.20	19.0 ±0.05	15.3±1.20	10.0±0.60	7±0.40
<i>P. aeruginosa</i>	14.0 ±0.60	11.0 ±0.01	7.0±1.1	5.0±0.07	0±0.00
Fungi					
<i>Aspergillus flavus</i>	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00
<i>Candida albican</i>	19±1.80	16.0±0.20	11.0±0.30	8.0±0.20	5.0±0.01

Bacillus subtilis was sensitive to all concentrations with zones of inhibition ranging from 7±0.40mm at 6.25mg/ml to 23.0 ±3.20mm at 100mg/ml. *Pseudomonasaeruginosa* was resistant to ginger ethanol extract at 6.25mg/ml while inhibition zone of 14.0 ±0.60mm was observed at 100mg/ml. *Aspergillus flavus* was found to be resistant to all concentrations of the plant extract while *Candida albican* was sensitive, with inhibition zones of 5.0±0.01mm at 6.25mg/ml and 19±1.80mm at 100mg/ml

Table 3. Antimicrobial activity of Aqueous extract of *Zingiber officinale*.

Test organisms	Concentrations				
	100mg/ml	50mg/ml	25mg/ml	12.5mg/ml	6.25mg/ml
Bacteria					
<i>Bacillus subtilis</i>	19.0 ±1.20	17.3 ±0.05	13.0±1.20	7.0±0.60	0.0±0.00
<i>P. aeruginosa</i>	10.0 ±0.10	8.0 ±0.01	5.0±0.30	0.0±0.00	0±0.00
Fungi					
<i>Aspergillus flavus</i>	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00
<i>Candida albican</i>	18.0 ±1.30	14.0±0.60	11.0±0.90	6.0±0.10	4.0±1.10

In the aqueous extract, *B. subtilis* was resistant at 6.25mg/ml but sensitive at 12.5mg/ml (7.0±0.60mm) while *P. aeruginosa* was resistant at 6.25mg/ml and 12.5mg/ml but sensitive at 25mg/ml (5.0±0.30mm). *Candida albican* was sensitive to all concentrations of aqueous extract with inhibition zones of 4.0±1.10mm at 6.25mg/ml and 18.0 ±1.30mm at 100mg/ml, while *A. flavus* was resistant to all concentrations.

Table 4. Minimum inhibitory concentration and minimum bactericidal concentration of *Zingiber officinale* extracts.

Test organisms	MIC (mg/ml)		MBC (mg/ml)	
	Ethanol	Aqueous	Ethanol	Aqueous
Bacteria				
<i>Bacillus subtilis</i>	6.25	12.50	25.00	50.00
<i>P. aeruginosa</i>	12.50	25.00	50.00	50.00
Fungi				
<i>Aspergillus flavus</i>	ND	ND	ND	ND
<i>Candida albicans</i>	6.25	6.25	25.00	25.00

Lowest MIC was observed against *B. subtilis* at 6.25mg/ml in the ethanol extract and 12.50mg/ml in the aqueous extract. Similarly, lowest MBC of 25mg/ml was observed against *B. subtilis* in the ethanol extract and 50mg/ml in the aqueous extract. In both extracts, *Candida albican* had 6.25mg/ml as MIC and 25mg/ml as MBC.

Table 5. Antibiotics sensitivity pattern of bacteria isolates.

Organisms	CPX	S	SXT	E	PEF	CN	APX	Z	AM	R
Gram positive										
<i>Bacillus subtilis</i>	R	R	R	R	S	R	R	R	R	R
Gram -ve										
<i>P. aeruginosa</i>	TE	NB	AX	OF	C	CF	AM	N	CN	CPX
	R	R	R	S	R	R	R	R	S	R

Bacillus subtilis was resistant to all antibiotics except pefloxacin while *P. aeruginosa* was sensitive to both ofloxacin and gentamicin and resistant to others. Key: CPX-Ciprofloxacin, R-Rocephin, S-Streptomycin, TE-tetracycline, SXT-Septrin, NB-Nitrofurantoin, E-Erythromycin, C-Chloramphenicol, PEF-Pefloxacin, CF- ciprofloxacin, CN-Gentamicin, N-Nalidixic, APX-Ampiclox, AM-Amoxicillin, Z-Zinnacef, AX- Azithromycin and OF- Ofloxacin

4. Discussion

Many plants, due to their phytochemical constituents exhibit various properties and have often been used by different people around the world for different purposes. Some of these plants do not only serve as food for man but also help to boost the consumer's health [17]. Many researchers have reported on the medicinal, antimicrobial, phytochemical, anti-inflammatory and antioxidant properties of various plants in the last decades and have found these plant as not just supportive to antibiotics but as an alternative, should microorganisms develop total resistant to antibiotics [18; 19; 17]. This research work was undergone to evaluate the

phytochemical and antimicrobial properties of the ethanolic and aqueous extracts of *Zingiber officinale* (Ginger). The phytochemical screening revealed the presence of alkaloids, flavonoids, phenols, terpenoids and saponins in both ethanol and aqueous extract. Steroids was however only present in ethanolic extract. This finding is in agreement with the work of [13], who isolated these compounds from ginger rhizome. The antimicrobial properties of *Zingiber officinale* reside in these compounds [3]. The microorganisms employed in this work were clinical isolates obtained from Microbiology Department in University of Benin Teaching Hospital. The organisms included *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Aspergillus flavus* and *Candida albicans*. These organisms are

potential pathogens in the clinical environment especially *P. aeruginosa* which is highly implicated in nosocomial infection [15]. They have also been reported to be multidrug resistant. The yeast *Candida albicans*, an etiology of candidiasis is an important public health pathogen as its infection is not easy to treat. These organisms were subsequently identified using cultural, morphological and biochemical characterization. Evaluation of *Zingiber officinale* showed high level of potency against the test organisms. *Bacillus subtilis* was highly susceptible to the ethanol extract of ginger, with zones of inhibition ranging from 7 ± 0.4 mm at concentration of 6.25 mg/ml to 23.0 ± 3.2 mm at 100 mg/ml. *P. aeruginosa* was relatively resistant as there was no inhibition at 6.25 mg/ml which is similar to work done by [14]. *Aspergillus flavus* was completely resistant to the extract of *Zingiber officinale* and there was no inhibition even at the highest concentrations. On the other hand, *Candida albicans* was highly susceptible to the ethanol extract of this plant with zones of inhibition ranging from 5.0 ± 0.01 mm at 6.25 mg/ml to 19 ± 1.8 mm at 100 mg/ml. Aqueous extract of *Zingiber officinale* was less active than the ethanol extract. The zones of inhibition observed against the different isolates were reduced. For example, zones of inhibition of 10.0 ± 0.1 mm at 100 mg/ml was observed in aqueous extract compared to 14.0 ± 0.6 in the ethanolic extract. Earlier reports on the antimicrobial potency of ginger have been established [21; 22]. This reduction in activity could be due to the fact that the ethanol extracted more of the bioactive components from ginger than the aqueous extract [8]. This work has shown that the antimicrobial activity of *Zingiber officinale* is dependent of the concentration and the solvent of extraction. Different organic and inorganic solvent exist for extraction of plants and studies have shown over the years that organic extraction is more active than aqueous extraction. The lowest MIC of ginger extract was 6.25 mg/ml against *Bacillus subtilis* and *Candida albicans*, in the ethanol extract. Higher MIC value of 25 mg/ml was observed against *P. aeruginosa* in the aqueous extract. MBC values ranged from 25 mg/ml against *B. subtilis* and *C. albican* to 50 mg/ml against *P. aeruginosa* in the ethanolic fraction of the plant. While for aqueous extract, the MBC ranged from 25 mg/ml in *C. albican* to 50 mg/ml in *B. subtilis*. In all, no MIC or MBC was observed for *Aspergillus flavus*, as the organisms was completely resistant to both extract of *Zingiber officinale*. [13] observed similar findings when he worked on the antimicrobial properties of ginger rhizome. Antibiotic sensitivity pattern of the test isolates revealed varying degree of resistant to conventional antibiotics. *Bacillus subtilis* was resistant to all antibiotics except perfloracin while *P. aeruginosa* was susceptible to tetracycline, ofloxacin and gentamycin and resistant to others. Similar findings on resistance of *Bacillus subtilis* to antibiotics has been reported [17].

5. Conclusion

Ginger has been shown in this study to have high antimicrobial activity against multiple drug resistant microorganisms and therefore can be used in the management

of ailments caused by these organisms. Therefore, this plant should be further subjected to isolation of the therapeutic antimicrobials and possible synergistic interaction with other plant extracts or conventional drugs should be researched upon for a more effective pharmacological and antimicrobial activity.

Conflict of Interest

The authors declare that there is no conflict of interest.

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