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Antimicrobial Efficacy of *Zingiber officinale* (Ginger) Extract on *Candida albicans* and *Streptococcus viridans*

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ABSTRACT

This research work was carried out to investigate the phytochemical constituents and antimicrobial efficacy of Ginger (Zingiber officinale) extract on Streptococcus viridans and Candida albicans. The rhizome of Ginger (Zingiber officinale) plant were collected and identified in the Department of Plant Biology and Biotechnology, University of Benin. Screening for the presence of alkaloids, tannins, flavonoids, saponins, glycoside and carbohydrate was carried out using standard method. The microbial strains used in the study were obtained from University of Benin Teaching Hospital (UBTH) and re-identified using conventional methods. Standard microbiological methods were adopted for antimicrobial susceptibility testing using different concentrations of ginger extract from 12.5 % to 100 %. Standard antibiotics were used to determine the susceptibility to the bacterial isolates. The multiple antibiotic resistance (MAR) index was deduced from the antibiogram to evaluate the public health importance of the strain. The results revealed that alkaloids, saponins, cardiac glycosides and flavonoids were present in the methanolic extract of ginger. At 100% concentration, S. viridans had 22.00±0.00 mm zone of inhibition. At the same concentration, C. albicans was found to be resistant to the ginger extract. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of ginger extract against S. viridans were at a concentration of 50 %. C. albicans although resistant to ginger extract was found to be susceptible to fluconazole at a concentration of 100 µg/ml. The MAR index revealed that the bacterial strains were obtained from sources were antibiotics have been used and they are of public health importance.

1. INTRODUCTION

Interest in plants with antimicrobial properties has increased due to the rise in microbial resistance to commonly prescribed drugs or antibiotics (Anyanwu and Okoye, 2017). This resistance could be attributed to indiscriminate use of commercial drugs or failure to complete dosage of antibiotic prescription (Aliero and Afolayan, 2006). As a result, there is this remarkable increase in the use of medicinal plant products has

been observed in the past decade (Ekor, 2014). For their therapeutic properties, medicinal plants are used as primary health care aid amongst 80 % of the world's population in the form of plant extracts or their active components (Ekor, 2014). In Japan, China and India the rhizomes of ginger are commonly used as constituent of herbal treatment for digestive disorders (indigestion, nausea, constipation and flatulence), headaches, rheumatism, colds and cough, treatment of upper respiratory tract infections such as cough and sore throat; and gastrointestinal tract infections (Mustafa and Srivastava, 1990; Aziz *et al.,* 2015).

Ginger is a tropical annual monocotyledonous plant, which grows pseudo stems (false stems made of the rolled bases of leaves) about a meter tall bearing narrow leaf blade (Onianwah, 2016). They are bulb and rhizoid group of plants traditionally used in Southern Nigeria to add spice to food and to treat some disease (Premanth *et al.,* 2011). Ginger has been found to be effective in the treatment of a wide range of human ill-health conditions such as cataract, heart disease, migraines, struck amenorrhea, athlete's foot, bursitis, chronic fatigue, cold, flu, coughs, depression, dizziness, fever, erectile difficulties, kidney stones, Reynard's disease and viral infection (Peggy, 2006). Research on rats suggested that ginger may be useful for treating diabetes (Ody, 1997) as well as other microbial infections. On the strength of this information, it became expedient to evaluate the antimicrobial activity of ginger against clinical isolates (*candida albicans* and *Streptococcus viridans*) of public health importance. A representative bacterium and fungus were chosen in the aforementioned microorganisms for their role in public health as well as the ease with which they can be contracted from an infected patient to a healthy individual.

2. MATERIALS AND METHODS

2.1. Collection and Identification of Plant Material

Rhizome of dried *Zingiber officinale* were purchased from Uselu market in Benin City, Edo state Nigeria and identified in the Department of Plant Biology and Biotechnology, University of Benin.

2.2. Preparation of Plant Material and Extraction Process

The rhizome of *Zingiber officinale* were chopped separately into small pieces and dried in an aerated oven at 50^{o}C for 6 hours. The sample was then pulverized using the British milling machine (Gallanhap) to smaller particles. The powdered samples were kept separately in sterile, dry screw-capped bottles, which were stored in a dry cool place before extraction. The extraction was done using the Soxhlet apparatus. The solvent of choice in this study was methanol (99.9%). One hundred grams (100 g) of each powdered sample was extracted separately with 900 ml of the methanol solvent, following the procedure of Mustapha *et al.* (2015) with modification.

2.3. Collection and Identification of Microorganisms

The bacterial and fungal isolates (*Streptococcus viridans* and *Candida albicans)* were obtained from stored culture in Microbiology Department of the University of Benin Teaching Hospital, Benin City, Edo state, Nigeria. *Candida albicans* was identified on the basis of colonial morphology, colour, shape, diameter, appearance of colony on Czapek dox agar and basis of Lactophenol staining and microscopic (septation in mycelium, presence of specific reproductive structures. The bacterium was Gram stained and the biochemical tests such as catalase, oxidase, esculin hydrolysis, hemolytic reaction and identification via streptococcal grouping kits (Thermo Scientific) were carried out to re-confirm the identity.

2.4. Preparation of Ginger Extracts for Antibacterial Activity

The methanolic extract produced after Soxhlet extraction was diluted to four different concentrations using the formula reported by Oviasogie and Ogofure (2016). The concentrations were prepared in percentages of 100, 50, 25, and 12.5.

$$Percentage\ Concentration = \frac{sample\ mass\ (g)}{diluent\ volume\ (ml)}\ x\frac{1000}{1} \quad (1)$$

Where: sample volume = mass of ginger extract
Diluent volume = volume of DMSO

In reality, concentration is equivalent to 100 mg/ml and half the concentrations was presented as 50 mg/ml.

2.4.1. Determination of microbial purity

The method of Saidu and Ogofure, (2018) was used to evaluate the purity of the extract. Tryptone soya broth, a highly nutritious medium for culture of bacteria was used in this assay. One gram (1 g) of the extract was dissolved in 5 ml of tryptone soya broth and aerobically incubated for 48 hours. Thereafter, a loop of the mixture was streaked on Mueller Hinton agar plates and incubated for 24-48 hours. Microbial purity of the extract was confirmed by the absence of growth or any colony on streaked line after a further 48 hours of incubation. This indicates that the methanolic extract is microbiologically pure.

2.4.2. Antimicrobial activity of ginger extracts

The disc diffusion assay was used for the determination of the antimicrobial activity of ginger extracts. Filter paper disc of 6 mm was cut using a paper-punching machine. The paper discs were wrapped in a foil paper and sterilized at 121 ^{o}C for 15 minutes. The paper discs were transferred into the respective dilution tubes with the aid of a sterile forceps and allowed to stand for 3-5 h. and was later transferred into Mueller Hinton agar and Czapek dox agar already seeded with the bacterial and fungal strain equivalent to 1.5 x 10^8 cells/ml of McFarland standard. The plates were incubated overnight at 37 ^{o}C and the zones of inhibition was recorded using a meter rule.

2.4.3. Determination of minimum inhibitory concentration and minimum bactericidal concentration

The MIC and MBC of the extracts were determined using the method described by Vinothkumar *et al.* (2010) but with certain modifications. The least concentration with zones of inhibition was diluted in double fold using tryptone soya broth (Oxoid) and to each of the tubes, equal volume of the test organism (equivalent to 10^8 McFarland standard) was added and incubated at 37 ºC for 24 hours. Controls were prepared by inoculating tubes without the extracts but with the cell suspensions. The tubes were then examined for the presence of turbidity after the incubation period. The least concentration with no observable bacterial growth when compared with the control was considered as the minimum inhibitory concentration (MIC). A loop from the MIC broth was streaked onto Mueller Hinton agar plates and left to incubate for another 24 h. the concentration with no observable growth following the line of streaking was regarded as the minimum bactericidal concentration (MBC).

2.4.4. Antimicrobial susceptibility test of tested isolates

For *Streptococcus viridans* antibiotics were chosen on the basis of their importance in treating human or animal infections caused by Gram positive bacteria as well as for their broad spectrum nature for multi-

resistant bacteria. The identified bacterial isolate was made to undergo antibiotics susceptibility testing using the standard Kirby-Bauer disc diffusion technique (Bauer, 1966). A loopful of tested bacterial corresponding to 1.5 x 10^8 cells/ml were streaked evenly on Mueller-Hinton agar and the streaked plate was impregnated with different antibiotic discs (Oxoid) which include Azithromycin (15 mg), Ciprofloxacin (5µg), Ceftriaxone (30 µg), Vancomycin (30 µg), Gentamicin (10 µg), Meropenem (10 µg) Cefuroxime (30 µg) and Amoxicillin/clavulanic acid (30 µg). The plates were incubated at 37ºC for 24 h after which the zones of inhibition were measured and interpreted as Resistant (R), Intermediate resistant (I) or Sensitive (S) in conformity with the recommended standards established by the Clinical Laboratory Standards Institute (2017). Candida albicans was tested with Fluconazole as positive control.

2.4.5. Multiple antibiotic resistances (MAR) Index

The MAR index is a good tool for health risk assessment and identifies if the isolates are from a region of high or low antibiotic use. A MAR index of 0.2 and above indicates a 'high-risk' source of contamination (Davis and Brown, 2016). The multiple antibiotic resistance index was determined with oral pathogen by dividing the number of resistant antibiotics to the total number of antibiotics used in the study.

2.5. Qualitative Phytochemical Screening

2.5.1. Test for alkaloids

Two grams (2 g) of sample was boiled with 2 % HCL on a steam water bath for 5 minutes. The mixture was allowed to cool before it was filtered aseptically. After filtration, it was divided into four portions and on each portion was added Mayer's, Dragenddorff's, Hager's and Wagner's reagents respectively. The presence of precipitate (cream (Mayer's), orange-red (Dragenddorff's), yellow (Hager's) and brown (Wagner's)) indicates a positive result.

2.5.2. Test for carbohydrate

Two grams (2 g) of sample was soaked in distilled water for few minutes and was filtered and the following tests were carried out:

(a) Molisch test: To 2 ml of filtrate a few drop of 10% alcoholic solution of alpha naphthol was added, tube was inclined at 45^0 followed by addition of 2 ml concentrated H_2SO_4 slowly down the size of the tube so that the acid forms an immiscible reddish brown layer with the extract solution (light brown layer).

(b) Reduction of Fehling solution (Test for reducing sugar):

Boil separately 2 ml of well-mixed Fehling's solution, remove from the flame and add 2 ml of filtrate to the Fehling's solution and allow to stand for 3 minutes. Observation of a deep blue to green colouration is an indicates a positive reduction test (Mbaeyi-Nwaoha and Emejulu, 2013).

2.5.3. Test for flavonoids

Five millilitre (5 ml) of dilute ammonia solution was added to a portion of plant filtrate, followed by addition of 10 % HCL. A yellow colouration, which gradually disappears to colourless on standing for few minutes indicate a positive result.

2.5.4. Test for saponins

Two grams (2 g) of sample was soaked in 20 ml of distilled water and boiled gently for few minutes and was filtered. To 1 ml of the filtrate, 2 drops of olive oil was added, the mixture was shaken and observed for the formation of an emulsion. One millilitre (1 ml) of the filtrate was then diluted with 4 ml of distilled water. The mixture was shaken and then observed for the formation of stable frothing on standing (Oseni *et al*., 2011).

2.5.5. Test for tannin

To 2 g of the sample, 5 ml of 45% ethanol was added and boiled for 5 minutes. The mixture was cooled and filtered. To 1 ml of the filtrate, 3 drops of lead acetate solution was added. The formation of gelatinous precipitate indicates the presence of tannins. Also, as a confirmation test, 1 ml of filtrate was treated with 0.5 ml bromine water and the formation of a pale brown precipitate indicate the presence of tannins (Mbaeyi-Nwaoha and Emejulu, 2013).

2.5.6. Test for cardiac glycoside

Two grams (2 g) of sample was soaked in 15 ml of chloroform, allowed to stand for some time, mixture was filtered. To 5 ml of the filtrate, 2 ml of concentrated sulphuric acid was added along the sides of the test tube. Observation of a reddish brown layer indicates a positive result (Oseni *et al*., 2011).

3. RESULTS AND DISCUSSION

The result of the percentage yield of ginger extract before and after the process of extraction using Soxhlet apparatus is shown in Table 1. The amount of pulverized materials before extraction (711.5 g) yielded 91.01 g of ginger extract (which was used for further studies) thus giving a percentage yield of 12.8 %. The microbial purity of the methanolic extract of ginger is shown in Table 2. The extract after 5 days of incubation (2 days in broth 3 days in agar) were devoid of any form of bacterial growth. The purity of the extract lies in the inability to find any form of bacterial growth after 5 days of incubation. The bacterial or microbial purity of the extract is similar to the report obtained by Saidu and Ogofure (2018). Fontana *et al.* (2004) also conducted similar research but not with ginger extract and showed that there was bacterial contamination after 2 days of incubation. Table 2 reveal the microbial purity of the crude extracts after concentration. No form of microbial contaminants presents as can be seen by no visible growth of culturable bacteria.

Table 1: Percentage yield of plant extract

Plant extract	Amount of material in grams (g)		Percentage yield (%)
	Before extraction	After extraction	
Ginger extract	711.50	91.01	12.78

Table 2: Microbial purity of crude ginger extract after concentration

	Incubation Period (Days)				
Ginger extract	1	2	1	2	3
100%	ND	ND	–	–	–
50%	ND	ND	–	–	–
25%	ND	ND	–	–	–
12.5%	ND	ND	–	–	–

+ = presence of colonies; – = absence of colonies; ND = not determinable

Results of the qualitative phytochemical screening of ginger extract is shown in Table 3. Alkaloids, flavonoids, glycosides and reducing sugar found in *Zingiber officinale* are at variance with the report of Jeba *et al.* (2013), who reported that alkaloids and glycosides were not detected in methanolic extract of *Zingiber officinale* except for flavonoids. This also is in line with the phytochemical investigation of the methanolic extract of *Zingiber officinale* by Shukla and Singh, (2006) and Sivasothy *et al.* (2011) who researched the chemotherapeutic effect of the phenolic compounds in the *Zingiber officinale* and showed the bioactive agents in *Zingiber officinale* to be flavonoids and alkaloids respectively. The qualitative phytochemical screening revealed the presence of carbohydrates, flavonoids, saponins, cardiac glycoside and tannins in ginger extracts. This finding was in consonance with the work of Aziz *et al.* (2015) and Okiki *et al.* (2015) who identified, cardiac glycoside, saponins, tannins and flavonoids from the plant extract

Table 3: Qualitative phytochemical screening of ginger extract

Phytochemicals	Ginger
Alkaloid	+
Carbohydrate	+
Reducing sugar	+
Saponins	+
Tannins	+
Flavonoid	+
Cardiac glycosides	+

+ = Present; – = Absent; ++ = Present in moderate amount

Table 4 and 5 shows the antimicrobial sensitivity and resistance of ginger extract on tested bacterial isolate and *C. albicans*. *Streptococcus viridans* was found to be susceptible to ginger extract at 100 % and 50 % concentration while there was total resistance to the ginger extract by *Candida albicans* at all concentrations. The clinical and laboratory standard institute (2017) reported that for any antibacterial agent to be considered for antimicrobial activity, the concentration must be minute enough to exert antibacterial effect such that the zones of inhibition recorded would be greater than or equal to 20 mm.

Based on the recommendation, the highest concentration of the ginger extract (100 mg/ml) had 22.00 mm diameter of inhibition while 50 mg/ml had 15.00 mm diameter. As such, *Streptococcus viridans* can be said to be susceptible to ginger extract at 100 mg/ml concentration (Table 6). The results in this study was similar to the report of Onianwah and Stanley (2016) who evaluated the antibacterial activity of ginger extract against bacterial pathogens such as *Streptococcus pyogenes* and *Streptococcus pneumoniae*. The minimum inhibitory and bactericidal concentration of the extract is presented in Table 6. The effectiveness of the ginger extract against the streptococcal strain used in the study is more evident by the concentration of its MIC and MBC. Ginger was found to cause inhibition and subsequent death of the bacterium at a concentration of 50 mg/ml.

Table 4: Antimicrobial activity of methanolic extract of Ginger extract against tested isolates

Organisms	Concentration of ginger extract (mg/ml)		
	100	50	25
Streptococcus viridans	22.00	15.00	8.00
Candida albicans	0.00	0.00	0.00

Table 5: Antibacterial sensitivity result interpretation based on CLSI standard 2017 (27th edition)

Organisms	Concentration of ginger extract (mg/ml)		
	100	50	25
Streptococcus viridans	S	I	R
Candida albicans	R	R	R

R = resistant; S = sensitive; I = Intermediate

Table 6: MIC and MBC of ginger extract against *Streptococcus viridans* and *Candida albicans*

Organisms	MIC	MBC
Streptococcus viridans	50	50
Candida albicans	NA	NA

NA=Not applicable

The susceptibility of *S. viridans* and *C. albicans* to commonly used antibiotics and antifungal agent (for *C. albicans*) is shown in Tables 7 and 8. *S. viridans* was found to be susceptible to vancomycin and gentamicin while it was resistant to ciprofloxacin, erythromycin, meropenem and sulfamethoxazole. Intermediate resistance was observed for Augmentin and ceftazidime. The strain of *C. albicans* used in the study was also found to be resistant to fluconazole at 100 µg/ml concentration. Anyanwu and Okoye (2017) earlier opined that the challenge of antimicrobial resistance to conventional drugs is one of the major drive behind the search for alternatives to these drugs.

Table 7: Antibiotic sensitivity and resistance pattern of *Streptococcus viridans*

Isolates	CIP	CAZ	MEM	VA	CN	E	AMC	RL
Streptococcus viridans	0	14	15	26	14	10	14	0
CLSI (2017) Recommendation for susceptibility (S) and Resistance (R)								
Streptococcus viridans	R	I	R	S	S	R	I	R

MEM=Meropenem (10 ug), CN = Gentamicin (10 ug), VA= Vancomicin (30 ug), AMC = Amoxycilin (30 ug), CIP= Ciprofloxacin (5 ug), RL= Sulphamethoxazole (25 ug), CAZ = Ceftrazidine (30 ug), E= Erythromicin (15 ug), R = Resistant; S = Sensitive; I = Intermediate

Table 8: Antifungal sensitivity and resistance pattern of *candida albicans* to different concentrations of Fluconazole

Organisms	Concentration (ug/ml)		
	100	50	25
Candida albicans	8	5	6

The MAR index of tested bacterial isolates in this study is shown in Figure 1. The results revealed that the bacterial strains were obtained from sources were antibiotics have been used and they are of public health importance. *Streptococcus viridans* had an index greater than 0.65. This is an indication of the public health threat posed by the pathogen reveal that the bacterial strain is not just a multi-resistant pathogen but also a public health challenge.

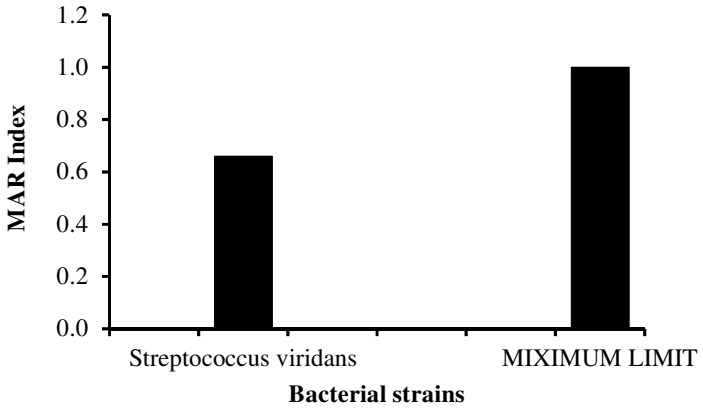

Figure 1: Multiple antibiotic resistance index of *Streptococcus viridans*

The antibacterial activity of *ginger* (*Zingiber officinale*) against *Streptococcus viridans* and *Candida albicans* was comparatively evaluated in this study. The *in-vitro* agar disc diffusion method was adopted in this study because it displayed a relatively direct means of measuring the antimicrobial strength of each product. Thus, the product with the largest zone of inhibition has the strongest antimicrobial properties. This study revealed that ginger extract at different concentrations had antibacterial effect against *Streptococcus viridans.* The highest zone of inhibition was recorded at 100 mg/ml concentration (Table 4). Okiki *et al.* (2015) assessed the antimicrobial effects of mixtures of ginger extracts against several pathogenic bacteria. Their results confirmed the findings of this work regarding the favourable antibacterial activity of ginger. Ginger extract exhibited significant antibacterial activity against a plethora of bacteria used in the study. They reported that the extract (both aqueous and ethanolic) of ginger was found to be active against Gram-positive and Gram-negative bacteria. Factors responsible for the high bacterial susceptibility of understudy bacteria to ginger extract have yet to be clearly understood; but it could be due to the presence of certain phytochemical compounds in the plant. It has often been attributed that the antibacterial activity of this plant is mainly attributed to its secondary metabolites. Previous studies on the rhizomes of *Zingiber officinale* have revealed that gingerols and shogaols are among the active components of ginger (Aziz *et al.,* 2015; Okiki *et al.,* 2015; Onianwah and Stanley, 2016). The characteristic odour and flavour of ginger are caused by a mixture of zingerone, shogaols, and gingerols that are volatile oils which compose 1–3% of the weight of fresh ginger. In animal models, gingerols increased peristalsis and showed analgesic, tranquilizing, antipyretic, and antibacterial properties. Ginger has no side effects if consumed in rational amounts and is generally recognized as safe by the American Food and Drug Administration. Kader *et al.* (2011) evaluated the antimicrobial effects of ethanolic extract of *Zingiber zerumbet* and its chloroform and petroleum ether soluble fractions against 13 pathogenic bacteria and 3 fungi using the disc diffusion method. Of the tested solvents of the extract, the ethanol extract had the highest activity against bacteria and fungi. The reported antibacterial activity and the superiority of the ethanol extract of *Zingiber officinale* were in line with our findings. It was observed none of the concentrations of ginger extract had inhibitory effect on *Candida albicans* used in this study and this was at variance with the report of Arora and Kaur, (1999) who analyzed the antimicrobial activity of ginger extract on human pathogenic fungi such as *Candida albicans, Candida krusei* and *Candida glabrata.* They concluded that, ginger extract was sensitive against all the tested fungal pathogens. The pathogenic fungal strain used in this study, which shows resistance to the ginger extract, used maybe due to the difference in strains or possibly the variety of the plant used in the study and the method of extraction of the oil during processing (Arora and Kaur, 1999).

4. CONCLUSION

Zingiber officinale extract has significant antibacterial effects on *S. viridans*, but not on *Candida albicans*. Considering the obtained MIC and MBC values, *Zingiber officinale* extract can be incorporated into products herbal mouth rinses and toothpastes for its antimicrobial effects.

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6. CONFLICT OF INTEREST

There is no conflict of interest associated with this work.

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