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Antibacterial Activity of *Zingiber officinale* on *Escherichia coli* and *Staphylococcus aureus*

Sani Njobdi[1*], Maryam Gambo[1] and Gali Adamu Ishaku[1]

[1]*Department of Biotechnology, School of Life Science, Modibbo Adama University of Technology, P.M.B. 1079, Yola, Nigeria.*

Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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Short Research Article

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ABSTRACT

Aims: The aim of this study was to investigate the antibacterial activity of extracts of fresh, dried and oil of *Zinginber officinale* on *Escherichia coli* and *Staphylococcus aureus*. Minimum inhibitory concentrations of the extracts against test organisms were determined and their inhibitory effects were compared with commercially available antibiotics.
Study Design: Laboratory based controlled experiment.
Place and Duration of Study: The research was conducted at the Molecular Biology Laboratory, Department of Biotechnology, Modibbo Adamawa University of Technology, Yola, Nigeria between January and May, 2014.
Methodology: Extracts from fresh and dried rhizomes of *Z. officinale* as well as ginger oil, which was extracted with the aid soxhlet extraction apparatus using n-hexane as the solvent were tested on isolates of *E. coli* and *S. aureus* using the agar well diffusion method. Both bacterial isolates were also subjected to standard antibiotic susceptibility test for comparison. Broth dilution method was used to determine the minimum inhibitory concentrations of the extracts on the test organisms.
Results: At concentrations of 10 mg/mL, 20 mg/mL, 30 mg/mL, and 40 mg/mL the zones of

**Corresponding author: E-mail: saninjobdi@mautech.edu.ng;*

inhibition of dried *Z. officinale* extracts on *S. aureus* were 11.00 ±1.41 mm, 13.5 ± 0.71 mm, 14.00± 2.66 mm and 17.5 ± 0.87 mm respectively and on *E. coli were* 6.00 ± 2.83 mm, 7.5 ± 2.12 mm, 8.00 ± 2.83 mm and 14.5± 6.08 mm respectively. Fresh ginger showed 15.00±1.40 mm and 12.00±2.83 mm at 100%, 50% concentrations respectively on *S. aureus* and 15.00±3.54 mm and 13.00±2.66 mm on *E. coli respectively* but has no effect at 25% and 12.5% on both organisms. The oil extract showed zones of inhibition of 12.00±2.83 mm and 7.00±4.24 mm at 100% and 50% concentrations on *S. aureus* respectively, while it showed no activity on *E. coli*. Minimum Inhibitory Concentration (MIC) of dried and fresh *Z. officinale* extracts on both isolates at 2.5 mg/ml. Oil extract did not exhibit inhibitory effect in broth even at concentration of 10 mg/ml.

Conclusion: The study indicated that both fresh and dried *Z. officinale* extracts inhibit the growth of *S. aureus* and *E. coli* similar to some standard antibiotics. This suggests that the plant is a potential source of antibacterial drug.

Keywords: Zingiber officinale; antimicrobial activity; Staphylococcus aureus; Escherichia coli.

1. INTRODUCTION

Ginger (*Zingiber officinale* Roscoe, family Zingiberaceae) is often described as a slender perennial herb plant that is about two feet in height. The flower is greenish yellow which looks like orchids. It is distributed widely in places like South-Eastern Asia and its medicinal history dated back to 2500 years in places like China and India. Its rhizome is a horizontal, fleshy, branched, yellowish or whitish to brown in colour and it is aromatic in nature. The dried rhizome of the ginger contains about 1-4% of volatile oils which are regarded as the medically active components and responsible for the characteristic taste and odour [1,2]. Gingerols are the main pungent compounds in ginger which can also be converted into zingerone, paradol and shogaols. 6-gingerol seem to be responsible for ginger taste where 6-shogaol have proven to have anti-inflammatory, antipyretic, anti-tussive, analgesic and hypotenssive effects [3,4]. Currently *Z. officinale* is also one of the well known and accepted herbal medicines for treating inflammatory diseases. It has been an alternative therapic agent [5]. Ginger is available in many commercial products such as tea, tincture, cookies, beer, capsule, soda, syrup and jam [6].

The increase in the use and misuse of antibiotics has actually induced microorganism to gain resistance factor which is becoming a global problem [7,8]. This has resulted to the need of finding alternative therapeutic drugs for the treatment of disease with a preference for plant materials which has shown to have fewer side effects [9,10]. The use of plants and their extracts for the treatment of infectious diseases has been practiced for many years in different parts of the world [11,12]. The recent approval of traditional medicine as an alternative form of health care and the development of microbial resistance to the conversional antibiotics have motivated researchers to search for antimicrobial activities of medicinal plants [13,14]. Plants derived medicines are used in many different forms such as powder, ointments, liquid, incisions and liniments [15].

This study was design to asses the antimicrobial activity of dried, fresh and oil extracts of *Z. officinale* against *E. coli* and *S. aureus* isolates and determine the minimum inhibitory concentration of *Z. officinale* against *E. coli* and *S. aureus* isolate. To compare the inhibitory effects of different *Z. officinale* rhizome extracts with commercially available antibiotics against the test organisms (*E. coli* and *S. aureus*).

2. MATERIALS AND METHODS

Fresh and dried rhizomes of white *Z. officinale* (white ginger) were obtained from Jimeta modern market, Yola, Nigeria. The samples were taken to the Molecular Biology Laboratory, Moddibo Adama University of Technology, Yola, Nigeria for further processing. Isolates of *E. coli* and *S. aureus* were obtained from the stock culture in the Microbiology Department and taken to the Molecular Biology Laboratory, Modibbo Adama University of Technology, Yola.

2.1 Preparation of Fresh *Z. officinale*

The aqueous ginger extract was prepared according to methods described by Onyeagba et al. [16]. One hundred grams of fresh washed *Z. officinale* cloves were macerated in a sterile, ceramic mortar. The homogenate was then filtered off with a sterile muslin cloth and used directly for the antibacterial activity test.

2.2 Preparation of Dried *Z. officinale*

The dried *Z. officinale* extract was prepared according to methods described by Debasmita et al. [17]. Dried *Z. officinale* materials were crushed using mortar and pestle to powder form and 200 g of powder was measured, dissolved in 500 mL of sterile distilled water and was tightly sealed in a container that was regularly agitated for two days. The suspension was filtered using whatman filter paper. The filtrate was evaporated to dryness in a water bath at a temperature of 55°C and preserved for the antimicrobial activity test.

2.3 Extraction of Oil Using Sohxlet Extractor

The ginger oil was extracted using the soxhlet apparatus with n-hexene as solvent according to methods described by Hiba et al. [18]. Thirty grams of the ground fresh *Z. officinale* was wrapped in a filter paper and placed inside the soxhlet apparatus. For total extracting time of 10 hours, 250 ml of the solvent was maintained continuously refluxing over the samples. After extraction, the solvent was allowed to evaporate from the mixture, in order to obtain only ginger oil. The resultant extract was transferred to glass dishes and placed in an oven at 40°C for 24 hrs. The extracts were kept at 4°C until assessments for antimicrobial activities.

2.4 Preparation of Nutrient Agar

Twenty eight grams of the nutrient agar powder were dissolved in 1 litre of distilled water and autoclaved at 121°C for 15 mins. The nutrient Agar was allowed to cool and was dispensed into each of the 24 petri dishes and allowed to solidify for some minutes [19].

2.5 Standardization of Inoculum

The test organism was prepared according to method described by Cheesbrough [20]. One colony was picked from each culture of (*E. coli* and *S. aureus*) and then inoculated into a freshly prepared nutrient broth and incubated for 24 hours at 37°C. It was further sub-cultured into nutrient broth and incubated for 3 hours at 37°C using a sterilized wire loop. The turbidity of the test organisms for susceptibility test was determined by comparing with it 0.5 McFarland standard of Barium sulphate solution which is equivalent to $1x10^6$ CFU/mL.

2.6 Inoculation of Test Organisms

The inoculation of the organism was carried out using streaking method of inoculation on the surface of the nutrient agar plates.

2.7 Agar Well Diffusion Method

Agar well diffusion was carried out to determine antimicrobial activity of the extracts against the test organism as described by Adeshina et al. [21]. The molten sterile nutrient agar of 20 ml was poured into sterile petri dish and allowed to set. The sterile nutrient agar plates were flooded with 1.0 mL of the standardized inoculum and the excess was drained off. A sterile cork borer (No. 4) was used to bore holes into the agar plate. One drop of the molten agar was used to seal the bottom of the bored hole, so that the extract will not sip beneath the agar. 0.1 ml of the fresh, dried and oil extracts was added to fill the bored holes. A control was prepared by putting 0.1 ml of freshly prepared sterile distilled water in one of the bored holes. One hour pre-diffusion time was allowed, after which the plates were incubated at 37°C for 24 hours. The zones of inhibition were then measured in millimetre. The above method was carried out in triplicates for each set and the mean of the diameters of the resulting inhibition zones were taken.

2.8 Antibiotic Susceptibility Screening

Antibiotic susceptibility patterns were determined by modified Kirby Bauer disc diffusion as described by Garrod and Waterworth [22]. The antibiotic disc used are: Pefloxacin (10 µg), Gentamycin (10 µg), Ampiclox (30 µg), Zinnacef (20 µg), Amoxicillin (30 µg), Rocephin 25 µg), Ciprofloxacin (10 µg), septrin (30 µg), Erythromycin (10 µg), Streptomycin (30 µg), Ampicillin (40 µg) and Vancomycin (40 µg). The isolates were first inoculated into nutrient agar and antibiotic disc were gently but firmly placed on the nutrient agar. The plates were incubated at 37°C for 24 hours after which the diameters of the inhibition zones were measured.

2.9 Preparation of McFarland Standard

McFarland equivalent turbidity standard was prepared as described by Ankri et al. [23]. Zero point six milliliter (0.6 mL) of 1% Barium chloride dehydrate solution (BaClz2H2O) to 99.4 ml of 1% sulphuric acid solution (H_2SO_4). A small volume of the turbid solution was transferred to capped tube of the same type that was used to prepare the test and of control inocula. This was stored in the dark at room temperature.

2.10 Determination of Minimum Inhibition Concentration (MIC)

The minimum inhibition concentration of the three *Z. officinale* extracts was tested as described by Adeshina et al. [21]. Using nutrient broth MICs were tested at 0.1 µg/mL, 0.2 µg/mL, 0.3 µg/ml and 0.4 µg/ml concentrations. One mililiter of each of the bacterial broth was inoculated in the four serially diluted ginger extracts. The test tubes and their contents were sealed with cotton wool and aluminum foil and incubated at 37 ºC for 24 hours. The lowest dilution which inhibited the growth of the test organism was considered as the minimum inhibition concentration.

2.11 Statistical Analysis

Analysis of Variance (ANOVA) was used to test if there were statistically significant differences between the antibacterial activities of the different extracts by comparing the means of the zones of inhibition at 99% level of confidence (P=0.01).

3. RESULTS AND DISCUSSION

In this study, the antimicrobial activity of *Z. officinale* extracts; extracts of fresh and dried *Z. officinale* and its oil were tested on *S. aureus* and *E. coli*. Determination of the minimum inhibitory concentration against the test organism and comparison of the inhibitory effects of the different extracts with commercially available antibiotics were carried out.

The result presented in Table 1 showed the Inhibitory effects of different *Z. officinale* extracts; dried *Z. officinale* extract (40 mg/ml), fresh *Z. officinale* extract (100%) and *Z. officinale* oil (100%) on *E. coli* and *S. aureus.* Dried ginger showed that 17.50±0.87 mm and 14.50±6.08 mm zone of inhibition recorded agaist *S. aureus* and *E. coli* respectively; fresh ginger achieved 15.00±3.54 mm and 15.00±3.54 mm zone of inhibition on *S. aureus* and *E. coli* respectively, and *Z. officinale* oil only achieved 12.00±2.83 mm zone of inhibition on *S. aureus* but none on *E. coli.*

The result presented in Table 2 indicated the Inhibitory effect of fresh *Z. officinale* on *S. aureus* and *E. coli* at different concentrations. Fresh *Z. officinale* showed 15.00±1.40 mm and 12.00±2.83 mm zones of inhibition at 100%, 50% concentrations respectively on *S. aureus* and, 15.00±3.54 mm and 13.00±2.66 mm zones of inhibition also at 100%, 50% concentrations respectively on *E. coli*. However, there was no effect at 25% and 12.5% on both organisms.

The result presented in Table 3 showed the Inhibitory effect of dried *Z. officinale* extracts on *S. aureus* and *E. coli* at different concentrations. For the inhibitory effects of dried *Z. officinale* extract at concentrations of 10 mg/mL, 20 mg/mL, 30 mg/mL, and 40 mg/mL the zone of inhibitions are 11.00 ±1.41 mm, 13.5 ± 0.71 mm, 14.00± 2.66 mm and 17.5 ± 0.87 mm respectively and, on *S. aureus*. and 6.00 ± 2.83 mm, 7.5 ± 2.12 mm, 8.00 ± 2.83 mm and 14.5± 2.08 mm on *E. coli* respectively.

Table 1. Inhibitory effects of different *Z. officinale* extracts; dried *Z. officinale* extract (40 mg/ml), fresh *Z. officinale* extract (100%), *Z. officinale* oil (100%) on E*. coli* and *S. aureus*

***Z. officinale* Extracts**	***S. aureus***	***E. coli***
Dry ginger	17.50±0.87 mm	14.50±6.08 mm
Fresh ginger	15.00±3.54 mm	15.00±3.54 mm
Oil ginger	12.00±2.83 mm	0.00 mm
Control	0.00 mm	0.00 mm
p-value (ANOVA)	0.000	0.001

Table 2. Inhibitory effect of fresh *Z. officinale* on *S. aureus* and *E. coli* Zone of inhibition (mm)

	Zone of inhibition (mm)			
	100%	**50%**	**25%**	**12.5%**
S. aureus	15.00±1.40	12.00±2.83	0.00	0.00
E. coli	15.00±3.54	13.00±2.66	0.00	0.00

Furthermore, Table 4 indicated the Inhibitory effect of *Z. officinale* oil on *S. aureus* and *E. coli* Zone of inhibition (mm). The oil extract showed zones of inhibition at 100% and 50% concentrations of the extract on *S. aureus* with 12.00±2.83 mm and 7.00±4.24 mm respectively. While there was no activity with *E. coli* at all concentrations of the oil extract.

The result presented in Table 5 revealed the minimum inhibitory concentration (MIC) of different *Z. officinale* extracts on *S. aureus* and *E. coli.* MIC results for both dried and fresh *Z. officinale* extracts on *S. aureus* and *E. coli* showed that both extracts have inhibitory effect at low concentration of 2.5 mg/ml on both organisms and have no inhibitory effect at 1.25 mg/ml. Oil extract had no inhibitory effect on both organism even at concentration of 10 mg/ml.

The result presented in Table 6 showed the comparative inhibitory effects of different extracts from *Z. officinale* and standard antibiotics on *S. aureus* and *E. coli.* Streptomycin showed the widest zone of inhibition on both organisms; 22.00±2.83 mm and 21.00±1.41 mm for *E. coli* and *S. aureus* respectively while Amoxicillin sowed no zone of inhibition against both test organisms.

The extracts of *Z. officinale* have antimicrobial properties against *S. aureus* and *E. coli*. The widest zone of inhibition was obtained with dried *Z. officinale* on *S. aureus* and zones of inhibition were observed on *E. coli* as well. These showed that the *Z. officinale* extracts are effective agaist test bacteria and this agrees with the findings from the work of Hiba et al. [24]. On comparison of antibacterial activity of the three *Z. officinale* extracts on the test micro-organisms (*S. aureus* and *E. coli*), all the three extract exhibited antimicrobial activity (P<0.01). At low concentration of 10 mg/ml, dried *Z. officinale* was active against both organisms while fresh *Z. officinale* aqueous extract and *Z. officinale* oil were not active at equivalent concentrations of 25% on both test organisms. This is similar with the works of Adeshina et al. [21] who worked with three solvent extracts of *Z. officinale* and found that fresh *Z. officinale* and *Z. officinale* oil were not active against bacterial test organisms at low concentrations. This showed that *S. aureus* and *E. coli* is highly susceptible to extracts of dried *Z. officinale* on the other hand, lesser zone of inhibition was observed with *Z. officinale* oil and fresh *Z. officinale* which indicate that both organisms are less susceptible to the fresh *Z. officinale* extracts and *Z. officinale* oil. Out of the three *Z. officinale* extracts i.e. dry *Z. officinale*, fresh *Z. officinale* and *Z. officinale* oil, at four different concentration level 66.6% where found to have antimicrobial activity against the gram positive bacteria (*S. aureus*). Dried *Z. officinale* showed antimicrobial activity at 10 mg/ml, 20 mg/ml, 30 mg/ml, and 40 mg/ml as shown in Table 2. *Z. officinale* oil showed no activity on *E. coli* at all concentrations suggesting that *E. coli* is not susceptible to *Z. officinale* oil.

Table 3. Inhibitory effect of dried *Z. officinale* extracts at different concentrations on *S. aureus* and *E. coli*

	Zone of inhibition (mm)			
	40 mg/ml	**30 mg/ml**	**20 mg/ml**	**10 mg/ml**
S. aureus	17.5 ± 0.87	14.00± 2.66	13.5 ± 0.71	11.00 ±1.41
E. coli	14.5± 2.08	8.00 ± 2.83	7.5 ± 2.12	6.00 ± 2.83

Table 4. Inhibitory effect of *Z. officinale* oil extracts on *S. aureus* and *E. coli* zone of inhibition (mm)

	Zone of inhibition (mm)			
	100% extract	**50% extract**	**25% extract**	**12.5% extract**
S. aureus	12.00±2.83	7.00±4.24	0.00	0.00
E. coli	0.00	0.00	0.00	0.00

Table 5. Minimum Inhibition concentration (MIC) of different *Z. officinale* extracts on *S. aureus* and *E. coli*

Organism	Minimum inhibitory concentration (mg/ml)		
	Dried	**Fresh**	**Oil**
S. aureus	2.5 mg/mL	2.5 mg/mL	>
E. coli	2.5 mg/mL	2.5 mg/mL	>

Key: > = No inhibition at the highest concentration tested (10mg/ml)

Table 6. Comparative inhibitory effects of different extracts from *Z. officinale* and standard antibiotics on *S. aureus* and *E. coli*

Antibiotic/extract	*E. coli* (mm)	*S. aureus* (mm)
Dried *Z. officinale* extract	14.5±0.68	17.50±0.87
Fresh *Z. officinale* extract	15.00±3.54	15.00±1.40
Z. officinale oil extract	0.00	12.00±2.83
Pefloxacin	NA	12.50±2.92
Gentamycin	15.00±1.40	0.00
Ampidox	NA	0.00
Zinnacet	NA	0.00
Amoxicillin	0.00	0.00
Rocephin	NA	14±4.47
Streptomycin	22.00±2.83	21.00±1.41
Septrin	15.00±1.40	0.00
Erythromycin	NA	0.00
Chloramphenicol	13.00±1.41	NA
Spartfloxacin	17.50±3.54	NA
Augmentin	21.00±1.41	NA
Tarivid	16.50±2.12	NA
Control (Distilled water)	0.00	0.00

Key: NA= not applicable (substance not tested on the isolate)

The minimum inhibitory concentration (MIC) of the extracts tested at four different concentrations (10 mg/ml, 5 mg/ml, 2.5 mg/ml and 1.25 mg/ml) showed that both dried and fresh *Z. officinale* extracts have the MIC of 2.5 mg/ml on both *S. aureus* and *E. coli* while *Z. officinale* oil showed no inhibitory effect even at the highest concentration (10 mg/ml) tested. This suggested that *Z. officinale* oil has weak antimicrobial activity against the test organisms and concurs with the findings of Kun [25]. The study showed that streptomycin had the highest zone of inhibition against both *S. aureus* and *E. coli*, in comparison to the *Z. officinale* extracts, dried and fresh *Z. officinale* also showed zone of inhibition on both test organisms, which agrees with the works of Sebiomo et al. [26]. The dried *Z. officinale* extract showed 14.5±0.68 mm and 17.50±0.87 mm on *S. aureus* and *E. coli* respectively, which shows that dried *Z. officinale* is more effective on *E. coli*. While the fresh *Z. officinale* showed 15.00±3.54 mm and 15.00±1.40 mm on both test organisms, while *Z. officinale* oil was not effective on *S. aureus* and *E. coli*, Chloramphenicol, Septrin and Gentamycin shows inhibitory effects on *E. coli* which agrees with the works of [27]. Based on the results obtained, streptomycin and *Z. officinale* extracts have inhibitory effects on both organisms.

4. CONCLUSION

This study showed that *Z. officinale* plants can be a source of the plants possess antimicrobial activities that may be useful plant may potentially be used as antimicrobial agents in new drugs for treatment of infectious diseases caused by bacterial pathogens. The most active extract on inhibited both *S. aureus* and *E. coli* is dried *Z. officinale* extract.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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