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PHYTOCHEMICAL ANALYSIS AND ANTIBACTERIAL ACTIVITIES OF GINGER (*ZINGIBER OFFICINALE*) COLLECTED FROM DIFFERENT PARTS OF KADUNA STATE AGAINST SELECTED BACTERIA ISOLATED FROM WOUND

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ABSTRACT
This study was aimed at screening the phytochemical constituents of *Z. officinale* extracts from different zones of Kaduna State, Nigeria and evaluating its antibacterial activity against some clinical bacteria isolates. The samples of *Z. officinale* were obtained from three zones of Kaduna State. The clinical isolates including *Staphylococcus aureus* and *Pseudomonas aeruginosa* were obtained from Garkuwa Specialist Hospital, Kaduna. The phytochemicals were analyzed qualitatively and agar well diffusion assay as well as tube dilution methods were used to determine the antibacterial activity. The study revealed the presence of phytochemicals such as phlobotannins, saponins, alkaloids, flavonoids, tannins, steroids and glycoside in the extracts. The highest activity was observed with the methanolic extract of *Z. officinale* from zone 3 at the concentration of 50 mg/ml against *P. aeruginosa* (31.6 mm). Likewise, the methanolic extract of *Z. officinale* obtained from zone 3 had the greater MIC toward *P. aeruginosa* (12.5 mg/ml). Whereas, the values of MBC of methanolic crude extract of *Z. officinale* obtained from the three zones are the same (50 mg/ml). The study shows the potential of *Z. officinale* in the treatment of bacterial infections.

Keywords: *Zingiber officinale*, Antibacterial activity, Phytochemicals, *Staphylococcus aureus*, *Pseudomonas aeruginosa*

INTRODUCTION

Wound infections are caused by the deposition and multiplication of microorganisms in the site of susceptible host. Microbial factors that influence the establishment of a wound infection are the bacterial inoculums, virulence, and the effect of the microenvironment (Malu & Obochi, 2009). Infection of the wound triggers the body's immune response, causing inflammation and tissue damage, as well as slowing down the healing process (Marjorie, 2017). The indiscriminately usage of antibiotics has caused a lot of microorganisms to acquire resistance factors which have become a burning predicament. Therefore, an urgent need arises to find the alternative of chemotherapeutic drugs in diseases treatment (Roopal *et al.,* 2011). Natural compound of plants origin which are easily available and with considerably less side effects may be of help (Sanusi *et al.,* 2017). The employment of different plants and their extracts in the treatment of infectious diseases has long been practiced in various parts of the world (Islam *et al.,* 2014).

Ginger (*Zingiber officinale*) belongs to the family Zingiberaceae. It is a rhizomatous plant grown all over Africa, South-eastern Asia, China and in parts of Japan, Austria and Latin America (Sasidharan & Menon, 2010). Ginger has been used as a medicinal plant worldwide, since ancient times, for the traditional treatment of cramps, rheumatism, sprains, sore throats, pains, constipation, vomiting, hypertension, indigestion, wound, and fever (Ali, 2008). Previous pharmacological studies showed ginger to have different biological activities such as antibacterial activity (Azu & Onyeagba, 2007), antifungal activity (Singh *et al.,* 2005), anti-infammatory activity (Kumar *et al.,* 2013), and anti-coagulant effect (Nurtjahja-Tjendraputra *et al.,* 2003). Some studies were done on phytochemical and antibacterial properties of *Z. officinale* from different part of Kaduna State (Idris, Tijjani & Aliyu, 2016; Suleiman *et al.,* 2019). Thus, this study was aimed at screening the phytochemical constituents of *Z. officinale* extracts from different zones of Kaduna State, Nigeria and evaluating its antibacterial activity against some clinical bacteria isolates.

MATERIALS AND METHODS

Plant Samples Collection and Preparation

Fresh *Z. officinale* were obtained from the three different geographical zones (Northern Kaduna (Zone 1), Kaduna Central (Zone 2) and Southern Kaduna (Zone 3)) in Kaduna State, Nigeria. The samples after collection were authenticated by a botanist at the Department of Biological Sciences, Kaduna State University. Their qualitative phytochemical screening as well as the antibacterial activity of the crude extract was carried out in the Microbiology laboratory, Kaduna State University. The samples were thoroughly washed with distilled water, sliced into pieces, and then air-dried for two weeks. The dried samples were pulverized into powder using pestle and mortar (Suleiman *et al.,* 2019).

Collection and Confirmation of the Test Organisms

The clinical isolates including *Staphylococcus aureus and Pseudomonas aeruginosa* isolated from infected wound were obtained from the Medical Microbiology laboratory of Garkuwa Specialist Hospital, Kaduna State. The isolates were transported in a sterile condition to the Microbiology laboratory, Kaduna State University. The isolates were sub-cultured on their respective

selective media for isolation, i.e. *Staphylococcus aureus* was sub-cultured on Mannitol Salt Agar (MSA) while Ps*eudomonas aeruginosa* on the other hand was sub-cultured on Cetrimide agar. Following 24 h incubation at 37°C, the isolates were confirmed using cultural, morphological and biochemical characteristics.

Preparation of *Z. officinale* Extracts

The aqueous extraction of *Z. officinale* was done by soaking 100 g of each of the powdered sample of *Z. officinale* in 500 mL of distilled water for 72 h. The solution was then carefully filtered through Whatman filter number 1 paper the residue discarded and the filtrate evaporated to dryness over a water bath. *Z. officinale* methanolic extracts were obtained using the same procedure as methanol in place of the distilled water.

Preparation of Different Concentrations of the *Z. officinale* extracts

Stocks solutions of the *Z. officinale* extracts were prepared by aseptically weighing 1g of the extract and dissolving in 5 mL of Dimethylsulfoxide (DMSO) to make a 20 % (200mg/ml) solution. Different working concentrations (50, 25, 12.5 and 6.25 mg/mL) of *Z. officinale* methanol and aqueous extracts were prepared from the stock solution. Ciprofloxacin was used at 10 µg/ml concentration as positive control against every bacterial strain.

Qualitative Phytochemical analysis

Phytochemical screening was carried out to evaluate the presence of alkaloids, tannins, flavonoids, saponins, glycosides, Phlobotannins, Terpenoids and steroids according to the method previously used by Akroum *et al.* (2017).

Antibacterial Assay of Crude Extract of *Z. officinale*

The antibacterial activities of the *Z. officinale* extracts were assessed by using agar well diffusion method as previously described by Kirby-Bauer (1996). A sterile cork borer was used to make holes on the freshly prepared Mueller Hinton ager. The plates were inoculated with standardized inoculum (0.5 McFaland) containing the test bacteria. Each of the four concentrations (50, 25, 12.5 and 6.25 mg/mL) was dispensed in their respective holes while the control (Ciprofloxacin at 10 µg/ml) at the middle of the plate. The plates were allowed to stand and diffuse for some time and then incubated at 37ºC for 18-24 h. After incubation period, diameter of inhibition zones were measured and recorded in millimeter using transparent plastic ruler.

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

The tube dilution method was used to determine the MIC of the *Z. officinale* extracts. The prepared working concentrations (50, 25, 12.5 and 6.25 mg/ml) of the extracts were placed in different test tube containing nutrient agar. The tubes were then inoculated with 0.1ml of standard inocula and incubated for 20-24 h at 37°C to observe turbidity (growth). The least concentration showing no visible sign of growth which gave no turbidity of the medium was taken as the MIC. The MBC was determine by sub-culturing the contents of the test tubes onto sterile nutrient agar plate using a wire loop and the inoculated plates incubated at 37°C for about 24 h. The MBC value was read as the least concentration that totally killed the test organisms, which was indicated by the complete absence of growth (Mounyr *et al.,* 2016).

RESULTS

The result of the phytochemical screening of both methanolic and aqueous extracts of *Z. officinale* from the three zones is shown in Table 1. It has been revealed that all the tested phytochemicals were present in the methanolic extract of *Z. officinale* obtained from zone 3. All the phytochemicals with the exception of phlobotannins and glycoside were present in the methanolic extract of *Z. officinale* obtained from zone 1. Some phytochemicals were present in some of the extracts and absent in some other extracts as can be seen in table 1 below.

Table 1: Phytochemical profile of *Z. officinale* methanolic and aqueous extracts

	Zone 1		Zone 2		Zone 3	
Phytochemicals	**Methanol**	**Aqueous**	**Methanol**	**Aqueous**	**Methanol**	**Aqueous**
Phlobotannins	-	-	-	-	+	+
Saponins	+	+	+	+	+	-
Alkaloids	+	+	+	-	+	-
Flavonoids	+	-	+	+	+	+
Tannins	+	-	-	-	+	-
Steroids	+	-	-	-	+	-
Glycoside	-	-	+	+	+	+

Table 2 showed the zones of inhibition of methanolic extract of *Z. officinale* obtained from three zones of Kaduna State against *P. aeruginosa* and *S. aureus*. It was shown that the methanolic extract exhibited a concentration-dependent antibacterial activity i.e. the antibacterial activity increases with increase in concentration of the extract against the test organisms. The highest activity was observed with the methanolic extract of *Z. officinale* from zone 3 at the concentration of 50 mg/ml against *P. aeruginosa* (31.6 mm), while the lowest was observed with the methanolic extract of *Z. officinale* from zone 1 at the concentration of 6.25 mg/ml against *P. aeruginosa* (7.0 mm).

Table 2: Zone of inhibition (mm) of methanol extract of *Z. officinale* against *P. aeruginosa* and *S. aureus*

Concentration (mg/ml)	**Zone 1**		**Zone 2**		**Zone 3**	
	P. aeruginosa	*S. aureus*	*P. aeruginosa*	*S. aureus*	*P. aeruginosa*	*S. aureus*
50	20.8	14.2	12.5	12.2	31.6	31.0
25	14.8	14	11.4	10.6	29.2	30.6
12.5	10.2	21.6	10.6	8.0	26.6	29.2
6.25	7.0	8.4	8.0	7.6	23.6	25.6
CPX (10µg/ml)	22.0	17.0	22.0	17.0	22.0	17.0

The result of antibacterial activity of the aqueous extract of *Z. officinale* showed that all the tested bacteria were resistant to the aqueous extracts of the plant samples obtained from zone 1 and zone 2 against *P. aeruginosa* and *S. aureus* even at the highest concentration (50 mg/ml). However, the tested organisms were

susceptible to the aqueous extract of the plant sample collected from zone 3. Highest activity was observed at 50 mg/ml against *S. aureus* (14.0 mm), followed by *S. aureus* (12.0 mm) at 25 mg/ml, and then *P. aeruginosa* (11.2) at 50 mg/ml (Table 3).

Table 3: Zone of inhibition (mm) of aqueous extract of *Z. officinale* against *P. aeruginosa* and *S. aureus*

Concentration (mg/ml)	Zone 1		Zone 2		Zone 3	
	P. aeruginosa	*S. aureus*	*P. aeruginosa*	*S. aureus*	*P. aeruginosa*	*S. aureus*
50	-	-	-	-	11.2	14.0
25	-	-	-	-	9.2	12.0
12.5	-	-	-	-	8.2	10.8
6.25	-	-	-	-	-	9.6
CPX (10µg/ml)	22.0	17.0	22.0	17.0	22.0	17.0

MIC and MBC values are shown in Table 4. It can be seen that the methanolic extract of *Z. officinale* obtained from zone 3 had the greater MIC toward *P. aeruginosa* (12.5 mg/ml), whereas the values of MIC of methanolic extract of *Z. officinale* from zone 1 and zone 2 were 25 mg/ml. On the other, the values of MBC of methanolic extract of *Z. officinale* obtained from the three zones are 50 mg/ml.

Table 4: Minimum Inhibitory Concentration and Minimum Bactericidal Concentration of methanolic extract of *Z. officinale*

	MIC (mg/ml)		MBC(mg/ml)	
	P. aeruginosa	***S. aureus***	***P. aeruginosa***	***S. aureus***
Zone 1	25	25	50	50
Zone 2	25	25	50	50
Zone 3	12.5	25	50	50

DISCUSSION

The phytochemical study based on the ethno-pharmacological data is generally considered an effective approach in the discovery of new anti-infective drugs from plant sources (especially higher plants). The results of this study showed that the methanolic extracts of *Z. officinale* exhibited the higher inhibitory activity against the test organisms than the aqueous extracts. The reason for organic extract to be more active than water extract is due to the better solubility of the active components in organic solvents (de Boer *et al.,* 2005; Doughari *et al.,* 2007). It was reported that due to the inability of water to extract nonpolar compounds, the water preparation is usually not suitable for antimicrobial discovery (Eloff, 1998). This is in agreement with the work of Gomaa & Hashish (2003) which revealed that methanol extracts of *Z. officinale* produced higher antimicrobial activity than the aqueous extract on the test organisms. Likewise, Malu *et al.* (2009), Gull *et al.* (2012) and Yalemwork *et al.* (2014) reported that the tested bacterial strains in their study showed poor susceptibility to the *Z. officinale* aqueous extract. The tested bacteria species responded differently to the *Z. officinale* obtained from the different part of Kaduna State. The findings from the present study revealed that the extracts of *Z. officinale* obtained from zone 3 displayed better antibacterial activity than those obtained from other zones (zone 1 and 2) (P value 0.002). The differences in the antibacterial activity among *Z. officinale* may be as result of genetic differences, soil mineral availability, and environmental as well as climatic factors. The presence of phlobotannins, saponins, alkaloids, flavonoids, tannins, steroids and glycoside in the extracts of *Z. officinale* may explain the reason for it antimicrobial activities since the antimicrobial properties of most of these phytochemicals have been reported previously (Akintobi, 2013; Suleiman, 2019).

Conclusion

Z. officinale collected from different part of Kaduna State inhibited the growth of *P. aeruginosa* and *S. aureus* isolated from infected wound of hospitalized patient. It is evident that the antibacterial activity of *Z. officinale* obtained from zone 3 geographical region of Kaduna showed more inhibitory effects (P value 0.002) on the tested organisms. The results also showed that the extracts have some importance of secondary metabolites including phlobotannins, saponins, alkaloids, flavonoids, tannins, steroids and glycoside. Consequently, *Z. officinale* can be used as potential source of novel antibiotic. The effort should be made to isolate the active components responsible for the bioactivity in all the extracts.

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