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Antibacterial Activity of Ginger Extract on *Pseudomonas* and *Klebsiella* Spp Isolated from Spoiled Fruits

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A B S T R A C T

Ginger (*Zingiber officinale*) is a medicinal plant that has been used extensively as spices in food and drinks and as an antimicrobial agent against various pathogenic organisms. The antimicrobial effect of ginger extract (ethanol, methanol and chloroform) against *Pseudomonas* and *Klebsiella* spp were investigated using agar well diffusion method. The results obtained from the study showed that the organisms used for the analysis were susceptible to the extracts except chloroform ginger extract that was unable to inhibit the growth of *Klebsiella* spp. Ginger ethanol extract had more inhibitory activity on *Pseudomonas* with the highest zone of inhibition (25 mm at 200 mg/ml) when compared to *Klebsiella* spp (22 mm at 200 mg/ml). The methanolic ginger extract had lower inhibitory activity (20 mm) on *Klebsiella* and (21 mm) on *Pseudomonas* spp when compared to the ethanol extract. Chloroform extract was the least in suppressing the growth of *Klebsiella* spp (10 mm at 200 mg/ml) and *Pseudomonas* spp (19 mm) at the same concentration of 200 mg/ml. MIC of ginger extracts on the organisms ranged from 6.25 mg/ml to 12.5 mg/ml for ethanol and methanol extracts except for chloroform extract that ranged from 0.00 to 6.25 mg/ml. The MBC was determined and *Klebsiella* had MBC at 3.125 mg/ml for ethanol and methanol extracts except for chloroform extract that were non inhibitory. *Pseudomonas* had MBC at 1.56 mg/ml for ethanol and methanol extracts and 12.5 mg/ml for chloroform extract. It is therefore imperative that ginger can be used as an antibacterial agent in the treatment of infections caused by *Klebsiella* spp and *Pseudomonas* spp.

Introduction

Infectious diseases are extremely common worldwide and it is the world's leading cause of death. It can be caused by many pathogens including bacteria. Infections due to variety of bacterial etiologic agents such as *E. coli, Salmonella spp., Staphylococcus aureus, klebsiella spp, Bacillus spp* are most common (Sarita *et al.*, 2019; Hasan and Zulkahar, 2020). These organisms can be isolated from spoiled fruits and these fruits provide an ideal environment for the growth and survival of these microorganisms. The internal fruit tissues consist of high concentration of various types of sugars, minerals, vitamins and amino acids (Hasan and Zulkahar, 2020) that support the growth of the

organisms. These organisms do not only lead to the spoilage of the fruits but also constitute a potential health hazard to humans when consumed and it may lead to serious disease condition.

The evolution of new strains of disease causing agents as well as the emergence and spread of antibiotic resistance, are of great concern to the global health community (Sarita *et al.,* 2019). The increased usage of antibiotics has induced microorganisms to acquire resistance factors which have become a burning predicament (Kamrul *et al.,* 2014). In addition to this problem, antibiotics are sometimes associated with adverse effects on host which include hypersensitivity, depletion of beneficial gut and mucosal gut and mucosal microorganism, immunosuppression and allergic reactions. This has created immense clinical problem in the treatment of infectious diseases (Namita and Mukesh, 2014). This has been attributed to the overuse and misuse of these medications, as well as lack of new drug development by the pharmaceutical industry (Ventola, 2015). There is an absence of a rational program for antimicrobial use and sub doses of antimicrobials which are all factors that contribute to the increased prevalence of drug resistant microorganisms, rendering the antibiotics ineffective (Amanda *et al.,* 2019). As a result there is an urgent need to find the alternative of chemotherapeutic drugs in disease treatment particularly those of plants origin which are easily available and have considerable less side effects (Kamrul *et al.,* 2014). This has caused serious problem in the treatment of infectious diseases. Commonly used medicinal plants could be an excellent source of drug to fight off this problem (Sarita *et al.,* 2019).

Natural products are major source of new natural drugs and their use as alternatives medicines for treatment of various diseases has been increased in the last few decades (Ansari *et al.,* 2006). They emerge as a potential alternative for the replacement of synthetic antimicrobial agents (Amanda *et al.,* 2019). In comparison to the formulated drugs, the herbs and spices have fewer side effects. They are also cheap due to their easy availability, better patient tolerance and are readily available for low socioeconomic population (Adeshina *et al.,* 2011). It is therefore imperative to find alternate therapeutic drugs for the treatment of infectious diseases. These natural drugs are usually of plant origin and it has been used for long in ayurvedic medicine for the treatment of diseases.

Ginger (*Zingiber officinale Roscoe*) belongs to the family Zingiberaceae. It originated in South-East Asia and then used in many countries as a spice and condiment to add flavor to food (Nafiseh *et al.,* 2013). It is a perennial herbaceous plant that produces a fleshy and articulated rhizome, with rough brownish epidermis. Ginger is one of the oldest and most popular medicinal plants in the world and it has also been used in traditional medicine (Amanda *et al.,* 2019; Nafiseh *et al.,* 2013). The pharmacological activities of ginger were mainly attributed to its active phytocompounds 6-gingerol, 6-shogaol, zingerone beside other phenolics and flavonoids (Mohamad and Wenli, 2019).

Ginger has staring potential for treating a number of ailments including degenerative disorders (arthritis and rheumatism), digestive health (indigestion, constipation and ulcer), cardiovascular disorders (atherosclerosis and hypertension), vomiting, diabetes mellitus, and cancer, cramps, sprains, sore throats, muscular aches, pains, dementia, fever and infectious diseases (Nafiseh *et al.,* 2013; Mohamad and Wenli, 2019; Kamrul *et al.,* 2014). It can be used for treating upper respiratory tract infections, cough and bronchitis (Sahdeo and Amit, 2015).

It has been used due to its potential antimicrobial activity (Callixte *et al.,* 2020; Gunathilake and Vasantha, 2015), anti-inflammatory (Sahdeo and Amit, 2015; Sarita *et al.,* 2019; Nafiseh *et al.,* 2013; Gunathilake and Vasantha, 2015; Mohamad and Wenli, 2019), anti-oxidative (Nafiseh *et al.,* 2013; Gunathilake and Vasantha, 2015), blood pressure-lowering, cholesterol-lowering, anti-platelet aggregation, chemopreventive, and hypoglycemic

properties (Gunathilake and Vasantha, 2015; Mohamad and Wenli, 2019), anti-apoptotic, anti-tumor activities, anti-pyretic, anti-hyperglycaemic, anti-diabetic, anti-clotting and analgesic properties, cardiotonic, cytotoxic (Mohamad and Wenli, 2019; Adeoti *et al.,* 2020), antiviral effect, radioprotective effect, anticancer effect, nephroprotective, hepato-protective, larvicidal, analgesic, and immune-modulatory activities (Kankanam *et al.,* 2020).

Materials and Methods

Collection of plant materials

Fresh ginger rhizomes used in this study were purchased from Mayor Market in Enugu, Enugu State Nigeria. It was identified in the department of applied biology, ESUT. The rhizomes were washed with sterile water, peeled, sliced into small pieces and air-dried under the shade for 14 days after which it was ground into fine powder and was stored in an airtight jar.

Preparation of plant extract

Fifty grams of the ginger powder was weighed and soaked separately in 200 ml each of the solvents (ethanol, methanol and chloroform) for 72 hours with shaking at 120 rpm. It was filtered using Whatman's No 1 filter paper. The ethanol, methanol and chloroform extracts were evaporated at 50^0C using rotary evaporator.

Test organisms used for the analysis

Two different bacteria strains, *Klebsiella* and *Pseudomonas* isolated from spoiled fruits were used for the analysis. The organisms were identified using cultural characterization and biochemical tests.

Standardization of inoculum

The test organisms were inoculated on plates containing freshly prepared nutrient agar and were incubated for 24 hours at 37^oC. This was done to get pure culture of the isolates. Colonies from the fresh culture plates were picked and inoculated into test tubes containing freshly prepared nutrient broth and were incubated for 24 hours at 37^oC. They were compared with 0.5 McFarland standard of barium sulphate solution which is equivalent to 10^8CFU/ml.

Dilution of extracts

The dried extracts were dissolved in 10% DMSO respectively and were used as the stock solution in the concentration of 200 mg/ml, 100 mg/ml, 50 mg/ml, 25 mg/ml, 12.5 mg/ml, 6.25 mg/ml and 3.125 mg/ml. They were preserved at 4^oC in the refrigerator.

Determination of antibacterial activity of the extracts

Agar well diffusion method was used to evaluate the antibacterial activity of each of the ginger extracts (ethanol, methanol and chloroform). The molten sterile Mueller Hinton agar was poured into sterile petri-dishes and was allowed to solidify. Each of the agar plates were inoculated by spreading 0.1ml of the standardized microbial inoculum over the entire agar surface. Then, a hole with a diameter of 6 mm was punched aseptically with a sterile cork borer and different concentrations (200 mg/ml, 100 mg/ml, 50 mg/ml, 25 mg/ml, 12.5 mg/ml, 6.25 mg/ml and 3.125 mg/ml) of the various extracts were introduced into the wells. The agar plates were incubated at 37ºC for 24 hours.

Determination of Minimum Inhibitory Concentration (MIC)

The MIC of the ethanol, methanol and chloroform extracts was carried out as described by (Ogata *et al.,* 2020) at various concentrations (200 mg/ml, 100 mg/ml, 50 mg/ml, 25 mg/ml, 12.5 mg/ml, 6.25 mg/ml, 3.125 mg/ml and 1.56 mg/ml). 0.1 ml of the standardized organism were spread on petri dishes containing Mueller Hinton agar and wells (6 mm in diameter) were bore on the inoculated petri dishes using sterile cork borer. 0.1 ml of the various

concentrations of the different extracts 200 mg/ml, 100 mg/ml, 50 mg/ml, 25 mg/ml, 12.5 mg/ml, 6.25 mg/ml, 3.125 mg/ml and 1.56 mg/ml was added into each of the wells respectively and the plates were incubated at 37°C for 24 hours. After the incubation, the MIC was determined as the least concentration of the extract that inhibited the growth of the organism.

Determination of Minimum Bactericidal Concentration (MBC)

In this technique, test tubes containing the various concentrations of the extracts were inoculated with 0.1ml of the standardized organisms respectively and were incubated at 37°C for 24 hours. Test tubes with no visible growth were streaked on various plates containing Mueller Hinton agar and incubated at 37°C for 24 hours. They were observed for the absence or presence of any visible growth. The MBC was taken as the concentration of the plant extract that did not exhibit any bacterial growth after the incubation.

Antibiotics susceptibility test

This was carried out as a control to determine the antibacterial effectiveness of the extracts on the organisms. The antibiotics used were ciprofloxacin, pefloxacin and gentamycin.

Results and Discussion

It was observed that ginger ethanol extract had the highest antimicrobial effect on *Klebsiella* when compared to the methanol and chloroform extracts at varying concentrations. At the concentration of 200mg/ml, ginger ethanol extract showed the highest zone of inhibition (22 mm) while the methanol ginger extract had 20 mm and the chloroform extract at the same concentration had 10 mm as the zone of inhibition. Also, at the concentration of 3.125 mg ginger ethanol extract had the least zone of inhibition of 12 mm while the ginger methanol extract had 10 mm and *Klebsiella* was resistant to ginger chloroform extracts. Also, the antimicrobial effect of the various ginger extracts were tested on *Pseudomonas* and it was observed that all the isolates were susceptible to the plant extract. At 200 mg/ml ginger ethanol extract had the highest zone of inhibition (25 mm), while the ginger methanol extract had 21 mm as the zone of inhibition and ginger chloroform extract had the least zone of inhibition (19 mm). At the concentration of 3.125 mg/ml ginger ethanol extract had 14 mm as the zone of inhibition followed by the ginger methanol extract (10 mm) and ginger chloroform extract (7 mm) as the zone of inhibition (tables 1, 2 and 3).

The result of the Minimum Inhibitory Concentration (MIC) showed that there were inhibitory effect on *Klebsiella* and *Pseudomonas* at low concentrations of 12.5 mg/ml and 6.25 mg/ml respectively with all the extracts that were used except chloroform ginger extract that showed no inhibitory effect on *Klebsiella* (table 4).

The result of the Minimum Bactericidal Concentration (MBC) of ginger extract on both *Klebsiella* and *Pseudomonas* were determined. *Klebsiella* had MBC at 3.125 mg/ml for ethanol and methanol extracts except for chloroform extract that was non inhibitory while *Pseudomonas* had MBC at 1.56 mg/ml for ethanol and methanol extracts and 12.5 mg/ml for chloroform extract (table 5).

The result of the antibiotic susceptibility test on *Klebsiella* shows that gentamycin had the highest zone of inhibition (26 mm) while ciprofloxacin had 22 mm and pefloxacine, 20 mm as their zones of inhibition. Also, the result of the antibiotic susceptibility test on *Pseudomonas* also shows that ciprofloxacin had the highest zone of inhibition (24 mm) while pefloxacin had 20 mm and gentamicin (18 mm) as their zones of inhibition (table 6). The present study was done to determine the antibacterial activity of ginger against *Klebsiella* and *Pseudomonas* isolated from spoilt fruits. These organisms are pathogenic and responsible for various ailments in human.

Natural products are major source of new natural drugs and their use as alternatives medicines for treatment of various diseases has been increased in the last few decades (Ansari *et al.,* 2006).

In this study antibacterial effect of ginger was determined using agar well diffusion method. The results obtained from the study showed that the organisms used for the analysis were susceptible to the extracts except chloroform ginger extract that was unable to inhibit the growth of *Klebsiella* spp.

Ethanol extract had the highest antibacterial activity on all the organisms (*Pseudomonas*; 25 mm at 200 mg/ml and *Klebsiella*; 22 mm at 200 mg/ml) more than the methanol and chloroform extracts that there used.

This is in conformity with the work of (Ekwenye and Elegalam, 2005) who observed that ethanolic extract of ginger gave the widest diameter zone of inhibition (10.00 mm) using the concentration of 1000 mg/ml. (Azu and Onyeagba, 2006) also observed that the ethanolic extract of ginger gave the widest zone of inhibition (20 mm) using the concentration of 0.8 gml-1. The results showed that ginger ethanol extract had more inhibitory activity on *Pseudomonas* (25 mm at 200 mg/ml) than *Klebsiella* (22 mm at 200 mg/ml). Methanolic ginger extract had lower inhibitory activity (20 mm) on *Klebsiella* and (21 mm) on *Psedomonas* when compared to ethanol extract. Chloroform extract was the least in suppressing the growth of *Klebsiella* (10 mm at 200 mg/ml) and *Pseudomonas* (19 mm) at the same concentration of 200 mg/ml.

From this study, it can be confirmed that ethanol and methanol extracts of ginger have considerable antibacterial activity against *Klebsiella* and *Pseudomonas*. This could be attributed to the phytochemical composition of ginger which includes alkaloid, flavonoid, anthraquinone, terpenoid, glycoside, steroid and reducing sugar (Nas *et al.*, 2018) which could have been extracted by the solvents that were used.

This credit to ethanol extraction was supposed to ethanol being an organic solvent and will dissolve organic compounds better, hence liberate the active component required for antimicrobial activity. It is worthy to note that the antimicrobial activities of these plants extracts were dependent on the concentration of the extracts (Azu and Onyeagba, 2006).

Table.1 Result of the antimicrobial activity of ginger ethanol extract on *Klebsiella* and *Pseudomonas* spp

Concentration (mg/ml)	**Zones of Inhibition (mm)**			
	Klebsiella spp		***Pseudomonas spp***	
200	22	0.84	**25**	**1.62**
100	20	0.63	**23**	**0.17**
50	19	0.37	**21**	**0.65**
25	17	0.92	**20**	**0.74**
12.5	15	0.53	**18.00**	
6.25	13	0.24	**16**	**0.35**
3.125	**12**	**0.32**	**14**	**0.27**

Table.2 Result of the antimicrobial activity of ginger methanol extract on *Klebsiella* and *Pseudomonas spp.*

Concentration (mg/ml)	Zones of Inhibition (mm)	
	Klebsiella spp	***Pseudomonas spp***
200	20 0.95	**21 0.84**
100	19 0.74	**20 0.73**
50	17 1.10	**18 0.64**
25	15 0.37	**16 0.53**
12.5	13 0.80	**14 0.61**
6.25	12 0.22	**12 0.41**
3.125	**10 0.17**	**10 0.32**

Table.3 Result of the antimicrobial activity of ginger chloroform extract on *Klebsiella* and *Pseudomonas spp*

Concentration (mg/ml)	Zones of Inhibition (mm)	
	Klebsiella spp	***Pseudomonas spp***
200	10 0.84	**19 0.64**
100	00 0.00	**17 0.35**
50	00 0.00	**15 0.48**
25	00 0.00	**12.00 0.82**
12.5	00 0.00	**10.00 0.58**
6.25	00 0.00	**9 0.41**
3.125	**00 0.00**	**7 0.27**

Table.4 Minimum Inhibitory Concentration (MIC) of ginger extract on *Klebsiella* and *Pseudomonas* spp

Test Organisms	MIC (mg/ml)		
	Ethanol	Methanol	**Chloroform**
Klebsiella	12.5 0.37	12.5 0.53	**0.00 0.00**
Pseudomonas	**6.25 0.83**	**6.25 0.17**	**6.25 0.64**

Table.5 Minimum Bactericidal Concentration (MBC) of ginger extract on *Klebsiella* and *Pseudomonas* spp

Test Organisms	MIC (mg/ml)		
	Ethanol	Methanol	**Chloroform**
Klebsiella	3.125 1.32	3.125 0.92	**0.00 0.00**
Pseudomonas	**1.56 0.42**	**1.56 0.57**	**12.5 0.32**

Table.6 Antimicrobial susceptibility pattern of standard antibiotics on *Klebsiella* and *Pseudomonas Spp*

Antibiotics	Drug potency(µg)	Zone of inhibition(mm)			
		Klebsiella		***Pseudomonas***	
Ciprofloxacin	10	22	0.24	**24**	**0.57**
Perfloxacin	10	20	0.83	**20**	**0.46**
Gentamycin	**10**	**26**	**0.36**	**18**	**0.75**

The minimum inhibitory concentration (MIC) was determined and the different dilutions of ginger extracts used ranged from 200 mg/ml to 1.56 mg/ml. The results showed that MIC of ginger extracts on the organisms ranged from 6.25 mg/ml to 12.5 mg/ml for ethanol and methanol extracts except for chloroform extract that ranged from 0.00 to 6.25 mg/ml.

The result of the Minimum Bactericidal Concentration (MBC) of Ginger extract on both *Klebsiella* and *Pseudomonas* were determined. *Klebsiella* had MBC at 3.125 mg/ml for ethanol and methanol extracts except for chloroform extract that were non inhibitory. *Pseudomonas* had MBC at 1.56 mg/ml for ethanol and methanol extracts and 12.5 mg/ml for chloroform extract.

The result of the antibiotic susceptibility test on *Pseudomonas* also shows that ciprofloxacin had the highest zone of inhibition (24 mm) while pefloxacin had 20 mm and gentamicin (18 mm) as their zones of inhibition. These antibiotics were used as control to determine the effectiveness of the extracts on the test organisms.

It is therefore imperative that the antimicrobial potential exhibited by ginger extract against *Klebsiella* and *Pseudomonas* shows its effectiveness in the treatment of infections cause by these organisms.

Due to multidrug resistance associated with the use of antibiotics, researcher's interests have been aroused to search for newer antimicrobial drugs which are obtained from plants. The results obtained from the study showed that the organisms used for the analysis were susceptible to the extracts except chloroform ginger extract that was unable to inhibit the growth of *Klebsiella*. Ginger ethanol extract had more inhibitory activity on the organisms when compared to the methanolic and chloroform ginger extracts. Further studies should be carried out to study the active ingredients or the chemical nature of the antimicrobial properties present in the plant extract.

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