



Antimicrobial Activity of *Zingiber Officinale* and *Allium Sativum* on some Drug Resistant Bacterial Isolates

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ABSTRACT: Ginger and garlic are very important and useful spices and are used as therapeutic agent against many pathological infections. Increasing multi-drug resistance of pathogens forces researchers to find alternative compounds for treatment of infectious diseases. In this study, the antimicrobial potency of ginger and garlic were investigated against four clinical bacterial isolates. Three types of ginger and garlic extracts (aqueous extract, methanol extract and ethanol extract) were assayed separately against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Salmonella typhi*. The antibacterial activity was determined by well diffusion method. The minimum inhibitory concentration (MIC) of different bacterial species varied from 1.5625mg/ml to 5.0mg/ml with ginger extracts having the lowest and garlic extracts having the highest respectively. All tested bacterial were most susceptible to the garlic aqueous extract and showed poor susceptibility to the ginger aqueous extract. However, ginger extracts from the mean zone of inhibition have higher antimicrobial activity when compared to garlic extracts with exception to garlic aqueous extract. This study encourages the use of spices especially ginger and garlic as alternative or supplementary medicine to reduce the burden of high cost, side effects and progressively increasing drug resistance of pathogens.

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Microbial pathogenicity and other infectious diseases have been controlled by use of commercially available antimicrobial drugs since many decades. Tremendous use of antibiotics has developed multiple drug resistance (MDR) in many bacterial pathogens. The increasing drug resistance is the main hindrance in successful treatment of infectious diseases and the control of microbial pathogenicity (Fu, *et al.*, 2007). Development of drug resistance in pathogens and increasing interest of consumers for safe food forces researchers to explore new antimicrobial agents (Erdogru, 2002). This has given rise to a shift from the prescription of antibiotics to the use of medicinal plants and spices. It is estimated that there are about 250,000 to 500,000 species of plant on earth and relatively small percentage of them are used as food by both human and other animal species (Borris, 1996). These plants fall under the natural products which are a major source of new natural drugs and their use as an alternative medicine for treatment of various diseases has been increased in the last few decades (Vuorelaa, *et al.*, 2004; Ansari *et al.*, 2006). In comparison to the formulated drugs, the herbs and spices have fewer side effects. They are also inexpensive, show better patient tolerance and are readily available for low socioeconomic population

(Adeshina *et al.*, 2011). In recent years, in view of their beneficial effects, use of spices or herbs is gradually increasing not only in developing countries but also in developed countries (Duman-Aydy, 2008). The antimicrobial activity of spices and herbs is due to specific phytochemicals or essential oils (Avato *et al.*, 2000). The main factors that determine the antimicrobial activity are the type and composition of the spice, amount used, and type of microorganism, composition of the food, pH value and temperature of the environment (Sagdic, 2003). Several reports had been published that describe the antibacterial and antifungal properties of different herbs and spices. However, still there is little information about the exact mechanism of their antimicrobial action (Gur *et al.*, 2006; Yusha'u *et al.*, 2008; Belguith *et al.*, 2010; Yin *et al.*, 2002; Oskay *et al.*, 2009). The use of medicinal plant and spices to treat diseases of varying etiology is part of the African tradition, but in spite of thousands of years of use, not many of these bioactive plants compounds have been exploited for clinical uses as antibiotics, though some alkaloid compounds like quinine and emetine have been developed as chemotherapeutic agents. Among those antibacterial foods that are becoming more common in western diet are green tea and ginger (Langner *et al.*, 2008;

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Hoffman, 2007). The development of new antibiotics and plant based antimicrobial compounds are effective against the resistant organisms. Ginger (*Zingiber officinale*) a common substance found increasingly in the diets of the global population, have known antibacterial effects and are commonly used together in teas. Ginger has been valued for its antibacterial properties for thousands of years in Asian cultures (Weil, 2005). In South India, ginger is used in the production of a candy called Injimirappa meaning ginger candy in Tamil. Ginger compounds are active against a form of diarrhea which is a leading cause of infant death in developing countries. Ginger has been found effective in multiple studies for treating nausea caused by sea sickness, morning sickness and chemotherapy. Garlic has been in used since ancient times in India and China for a valuable effect on the heart and circulation, cardiovascular disease (Kris-Etherton, 2002; Yeh and Liu, 2001; Gardner *et al.*, 2017), and regular use of garlic may help to prevent cancer, to treat malaria, and to raise immunity. Garlic has also been used to treat asthma, candidiasis, colds, diabetes, and antibacterial effect against food borne pathogens like *Salmonella*, *Shigella* and *Staphylococcus aureus* (Teferi and Hahn, 2002). Therapeutic use of garlic has been recognized as a potential medicinal value for thousands of years to different micro-organisms. Therefore, this study compares the antimicrobial activity level of ginger and garlic on selected clinical pathogens.

MATERIALS AND METHODS

Sample Collection: Garlic (*Allium sativum*) and ginger (*Zingiber officinale*) used in this study were collected from the international market and old market in Lokoja Local Government Area of Kogi State, Nigeria

Microbial Strains: Four different clinical isolates *Salmonella typhi*, *E. coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*, were obtained from Kogi State Specialist Hospital lokoja and Federal Medical Center, Lokoja, Kogi State respectively. The strains were maintained on nutrient agar slants. The aforementioned organisms cause the most common illness that re-occurring even after treatment with commercially available antibiotics.

Preparation of Extracts: Three types of extracts such as aqueous, ethanol and methanol extract from each garlic and ginger was prepared separately. The fresh garlic cloves and ginger rhizomes was washed, sliced and air-dried for two weeks. After drying, garlic and ginger slices was grinded to fine powder separately using electric blender. 10g powder of each garlic and ginger was soaked in 100 ml of distilled water, ethanol and methanol separately. The flasks were incubated at

room temperature for 72 hours. The methanol and ethanol extracts were evaporated at 50°C while the aqueous extracts were evaporated at 80°C in rotary evaporator. The extract solutions were stored at 4°C.

Culture Preparation: The bacterial strains was inoculated in 10ml nutrient broth and allowed to grow overnight at 37°C separately before performing antimicrobial assay. The 50 µl of overnight culture of each bacterial strain was transferred separately into 10 ml of nutrient broth (pH 7.2) under sterile conditions and placed in shaking water bath at 37°C for 8 hours.

Antimicrobial Activity Testing Using Agar Well Diffusion Assay: The bacterial cultures were swabbed on the surface of sterile Muller Hinton agar plates using a sterile cotton swab. Agar wells were prepared with the help of sterilized cork borer with 10mm diameter according to Kirby-Bauer. (1996). Using a micropipette, 100ul of different concentrations of garlic extracts (100%, 75%, 50%, 25% and 10%) were added to the wells in the plate. The plates were incubated in an upright position at 37°C for 24 hours. The diameter of inhibition zones were measured in mm and the results recorded. The inhibition zones with diameter less than 11 mm were considered as having no antibacterial activity. The same procedure was also performed for ginger extract respectively.

Determination of Minimum Inhibitory Concentration (MIC): The MIC was performed by micro dilution method using the National Committee for Clinical Laboratory Standards Guideline methods (NCCLS, 2000). The aqueous, ethanol and methanol extracts was diluted ranging from 100 to 10mg/ml, which was introduced into different test tube containing nutrient broth. The inoculums of microorganism were prepared using 24hours cultures and suspension was adjusted to 0.5McFarland standard turbidity. The MIC of each extract was taken as the lowest concentration that did not give any visible bacterial growth.

RESULTS AND DISCUSSION

The antimicrobial effect of ginger and garlic was evaluated by well diffusion method. The results as shown in table 1 indicated that the different extracts of the spices have broad spectrum antimicrobial activity with variable degree of sensitivity of tested bacterial isolates toward the extracts which is in accordance with Gull *et al.* (2012). The minimum inhibitory concentration (MIC) was determined by making the dilutions of different extracts of garlic and ginger ranging from 100 mg/ml to 1.5625 mg/ml. The MIC values of different ginger and garlic extracts are summarized in Figure 1. The results showed that MIC of different extracts of ginger and garlic against

bacterial strains ranged from 1.5625mg/ml to 5.0 mg/ml respectively. The data in Figure 1 indicated that all tested strains were susceptible to ginger and garlic aqueous, methanol and ethanol extract. From all MIC values of different ginger extracts, lowest MIC values for *E. coli*, *S. typhi*, *S. aureus*, and *P. aeruginosa*, was 1.5625mg/ml, respectively. Aqueous and ethanol extract of ginger had lower MIC in comparison to the ginger methanol extract against tested bacterial strains (Figure 1). In the case of different garlic extracts, the lowest MIC value for *E. coli*, *P. aeruginosa*, *S. aureus*, and *S. typhi* (1.5625mg/ml) was observed with garlic

aqueous extract. It is interesting to note that clinical isolates, both Gram negative and Gram positive bacteria were sensitive to all tested extracts of garlic and ginger but Gram positive bacteria were more sensitive compared to Gram negative bacteria as shown by the mean zone of inhibition in figure 2. This result is in accordance with the findings of Chandarana *et al.* (2005) and De-Souza *et al.* (2005). Table 2a and 2b shows the antimicrobial activity of ginger aqueous, methanol and ethanol extracts and garlic aqueous, methanol and ethanol extracts respectively at the different concentration ranging from 100 to 10mg/ml.

Table 1: The antimicrobial activities of aqueous, methanol and ethanol extracts of ginger and garlic on *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*.

Isolates	Ginger Aqueous Extract	Ginger Methanol Extract	Ginger Ethanol Extract	Garlic Aqueous Extract	Garlic Methanol Extract	Garlic Ethanol Extract
<i>E. coli</i>	++	++	++	++	++	++
<i>S. typhi</i>	++	++	++	++	++	++
<i>S. aureus</i>	++	++	++	++	++	++
<i>P.aeruginosa</i>	++	++	++	++	++	++

Key: ++ = Antimicrobial activities; -- = No antimicrobial activities

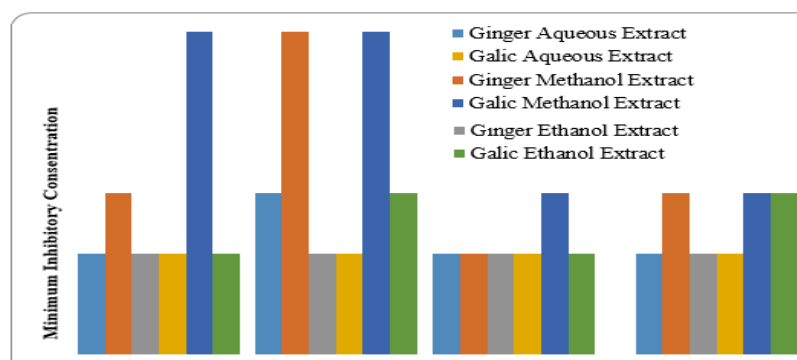


Fig 1: Shows the minimum inhibitory concentration (MIC) of aqueous, methanol and ethanol extracts of ginger and garlic against the organism tested. The MIC ranges from 1.5625 to 5mg/ml for the tested organism. The lowest concentration that did not permit any visible growth when compared with the control was considered as the minimum inhibitory concentration.

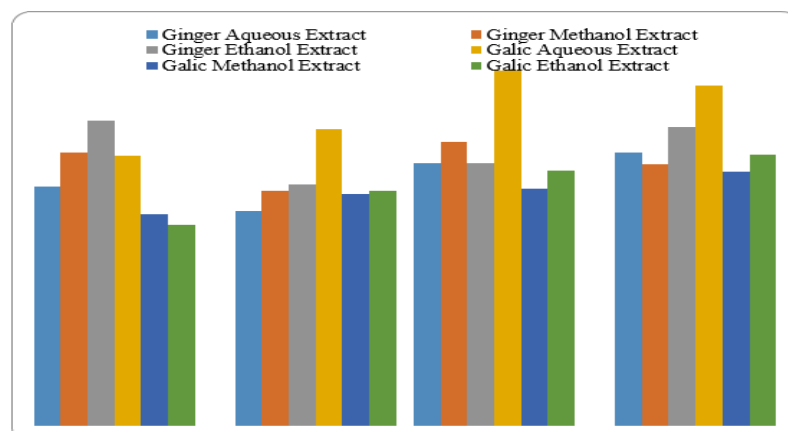


Fig 2: The Mean Diameter of Zone of Inhibition (mm) of the extracts of Ginger and Garlic.

Table 2a: Diameter of zone of inhibition (mm) of the different concentration of aqueous, methanol and ethanol extracts of ginger

Clinical isolates/ Conc. (mg/ml)	Ginger Aqueous Extract				Ginger Methanol Extract				Ginger Ethanol Extracts						
	100	75	50	25	100	75	50	25	100	75	50	25	10		
<i>E. coli</i>	23.6±0.01	20.2±0.25	17.4±0.00	14.1±0.81	11.3±0.00	27.9±0.08	24.0±0.03	18.2±0.23	16.8±0.00	12.1±0.00	29.7±0.05	25.4±0.08	22.0±0.00	18.6±0.02	14.7±0.05
<i>S. typhi</i>	21.8±0.05	19.0±0.72	16.0±0.00	12.0±0.34	9.0±0.00	22.6±0.02	20.1±0.00	17.0±0.98	14.4±0.12	11.1±0.03	23.0±0.00	20.0±0.01	17.4±0.00	15.0±0.32	12.0±0.00
<i>S. aureus</i>	26.0±0.95	22.4±0.00	19.1±0.25	15.4±0.25	12.0±0.00	28.4±0.25	24.0±0.20	20.0±0.05	17.3±0.00	12.9±0.63	25.8±0.00	21.8±0.09	18.8±0.06	15.9±0.09	12.8±0.06
<i>P. aeruginosa</i>	26.1±0.24	23.0±0.00	19.9±0.78	17.0±0.00	13.0±0.21	26.6±0.09	21.9±0.09	18.6±0.00	15.5±0.22	11.9±0.39	29.0±0.00	26.0±0.05	20.7±0.02	17.9±0.25	14.6±0.08

Table 2b: Diameter of zone of inhibition (mm) of the different concentration of aqueous, methanol and ethanol extracts of garlic

Clinical isolates/ Conc. (mg/ml)	Garlic Aqueous Extract				Garlic Methanol Extract				Garlic Ethanol Extract						
	100	75	50	25	100	75	50	25	100	75	50	25	10		
<i>E. coli</i>	27.5±0.71	23.0±0.00	20.1±0.05	15.0±0.65	12.0±0.00	22.4±0.25	19.0±0.00	15.0±0.01	12.3±0.07	8.0±0.00	20.9±0.05	18.0±0.75	15.0±0.00	11.0±0.05	7.9±0.75
<i>S. typhi</i>	30.9±0.05	26.0±0.05	21.0±0.00	16.6±0.25	12.9±0.00	24.7±0.05	20.8±0.04	17.2±0.09	13.0±0.05	8.2±0.00	24.5±0.00	20.0±0.00	17.4±0.25	13.0±0.00	10.0±0.01
<i>S. aureus</i>	37.5±0.00	32.0±0.00	26.8±0.75	19.0±0.00	13.0±0.75	22.5±0.00	20.0±0.00	18.2±0.02	14.0±0.06	11.0±0.00	25.2±0.00	21.9±0.79	18.0±0.00	15.2±0.45	12.2±0.24
<i>P. aeruginosa</i>	36.0±0.00	32.0±0.01	25.0±0.00	17.9±0.05	12.0±0.00	23.5±0.07	21.1±0.25	19.0±0.01	16.4±0.32	12.0±0.71	26.4±0.02	23.5±0.75	19.0±0.00	16.4±0.05	12.9±0.98

Table 2a shows that ginger ethanol extract exhibited the highest antimicrobial activity against *E. coli*, *S. typhi* and *P. aeruginosa* while methanol extract exhibited highest antimicrobial activity against *S. aureus* at 100mg/ml. This shows that ginger ethanol extracts are more effective against all tested bacterial strains except *S. aureus*. *E. coli* and *P. aeruginosa* showed maximum susceptibility to the ginger ethanol extracts. The results of antimicrobial effect of ginger in this study is in accordance to the reports of Sebinoma *et al.* (2011); Gao and Zhang, (2010); Yu *et al.* (2009); Malu *et al.* (2008); and Akoachere *et al.* (2002). The antibacterial activities of the extracts were expected, perhaps due to the presence of compounds like flavonoids, gingerol and volatile oil which were dissolved in organic solvents. It is reported that flavonoids and gingerol are the main component of ginger which attributes to its antibacterial activity (Malu *et al.*, 2008). The results obtained in this study corroborate with the report of Borris (1996), which explains that bioactive compounds of ginger rendering antimicrobial activity are volatile in nature and antimicrobial activity of ginger extract decreases upon storage. Apart from aqueous, methanol and ethanol were also used for extract preparation as De-Boer *et al.* (2005) has reported that bioactive compounds show better solubility in water miscible organic solvents.

Table 2b shows that garlic aqueous extract exhibited highest antimicrobial activity against all tested bacteria at concentration of 100mg/ml. The antimicrobial activity shown by garlic extracts in this study agrees with the findings of Belguith, *et al.* (2010); Yin *et al.* (2002); Bakht *et al.* (2011); Iwalokun *et al.* (2004) and O'Gara *et al.* (2000). Figure 2 shows the mean diameter of zone of inhibition (mm). From figure 2, it was observed that garlic aqueous had

higher and more effective antimicrobial activity against the tested organisms with mean diameter zone of inhibition (mm) of 19.52±0.56, 21.48±0.32, 25.66±0.75, and 24.58±0.97 for *E. coli*, *S. typhi*, *S. aureus*, and *P. aeruginosa* respectively. This shows that garlic aqueous extract is more effective for the treatment of infections caused by *E. coli*, *S. typhi*, *S. aureus*, and *P. aeruginosa* as compared to ginger aqueous extract with mean diameter of zone of inhibition of 17.32±0.09, 15.56±0.22, 18.98±0.15 and 19.8±0.22 for *E. coli*, *S. typhi*, *S. aureus*, and *P. aeruginosa* respectively, while ginger methanol extract is more effective against the entire test organism with mean diameter of 19.8±0.25, 17.04±0.21, 20.52±0.45 and 18.9±0.99 for *E. coli*, *S. typhi*, *S. aureus*, and *P. aeruginosa* respectively compared to garlic methanol extract with mean diameter of zone of inhibition (mm) of 15.34±0.10, 16.78±0.30, 17.14±0.58 and 18.4±0.82 for *E. coli*, *S. typhi*, *S. aureus*, and *P. aeruginosa* respectively. Ginger ethanol extract is also effective against the entire tested organism with mean diameter of zone of inhibition (mm) of 22.08±0.65, 17.48±0.59, 19.02±0.50 and 21.64±0.40 for *E. coli*, *S. typhi*, *S. aureus*, and *P. aeruginosa* respectively when compared to garlic ethanol extract with mean diameter of zone of inhibition (mm) of 14.56±0.10, 16.98±0.81, 18.5±0.54 and 19.64±0.22 for *E. coli*, *S. typhi*, *S. aureus*, and *P. aeruginosa* respectively. Generally, it can be inferred that the ginger extracts have higher antimicrobial activity when compared to garlic extracts. The results obtained in this study showed the relatively higher therapeutic efficacy of plant materials (spices) being used as an alternative medicine.

Conclusion: The results obtained in this study showed an explanation for the relatively higher therapeutic efficacy of spices. Both garlic and ginger have antibacterial activity. Garlic and ginger have activity on both Gram positive and Gram negative bacteria. There are several advantages for the use of spices as dietary supplement or alternative medicine manifested by reduction the chance for developing antibiotic-resistant bacteria that resulted from the frequent use of antibiotic, besides decreasing the cost of treatment and also minimizes the development of adverse drug reactions.

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