EVALUATION OF INHIBITORY PROPERTIES OF *Allium sativum* AND *Zingiber officinale* AGAINST FOODBORNE BACTERIAL PATHOGENS

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ABSTRACT
Background: The antibacterial activity of garlic *(Allium sativum*) and ginger (*Zingiber officinale*) extracts on *Escherichia coli, Staphylococcus aureus, Salmonella* species, *Listeria monocytogens* and *Bacillus cereus* were investigated on selected food borne pathogens.
Aim: To determine the response of enteric bacteria to the garlic and ginger extracts
Methods: Cold water and methanol extracts were obtained from the garlic bulbs and ginger rhizomes and their antibacterial activities were investigated using using Agar well diffusion method.
Results: The results indicated that the 100% water extract of the garlic produced maximum activity against *Staphylococcus aureus* and *Bacillus subtilis* (26.45±2.68) and (24.30±1.45) respectively. *Salmonella* species (18.45±1.00) and *Listeria monocytogens* (18.30±0.76) are statistically the same (P<0.05). *E. coli* was most resistant among the isolates. *E. coli, Pseudomonas aeruginosa* and *Salmonella* sp were statistically indifferent in 50% to 75% methanolic extract (P< 0.05). Both *Bacillus subtilis* and *Staphylococcus aureus* showed low MIC of 3.125mg/ml each. *Listeria monocytogens* and *Shigella* sp showed highest MBC of 100mg/ml.
Conclusion: It can be concluded that the extract can be used as antibacterial packaging to inhibit both pathogens and spoilage bacteria for food preservation.
Key words: *Allium sativum*, Foodborne Pathogens, *Zingiber officinale,* Inhibitory Effect, Extract

INTRODUCTION

Synthetic chemical used as food preservative as lead to negative consumer perceptions of artificial preservatives in foods, attention of the scientific community worldwide is shifting towards spices and herbs to harness their antimicrobial properties. The increased usage of antibiotics has induced microorganisms to acquire resistance factors which have become a burning predicament (Abimbola *et al.*, 1993). As a result, there is an urgent need to find the alternative of chemotherapeutic drugs in diseases treatment particularly those of plants origin which are easily available and have considerably fewer side effects (Khulbe and Sati, 2009). The use of higher plants and their extracts for treating the infectious diseases has long been practiced in many parts of the world (Sofowora, 1984). Herbal treatment is still used for many health problems. Herbs are safe, less toxic, economical and a reliable key natural resource of drugs all over the world (Al-Daihan *et al.*, 2013). Garlic (*Allium sativum*) is an edible plant containing sulfur compounds like allicin, ajoene, allylmethyltrisulfide, diallyltrisulfide, diallyldisulphide and others which exhibit various biological properties like antimicrobial, anticancer, antioxidant, immunomodulatory, anti-inflammatory, hypoglycemic, cardiovascular effects established and tuberculosis (Viswanathan, *et al.,* 2014).

Ginger (*Zingiber officinale* Roscoe, Zingiberacae) is widely used around the world as herbs and as spices in foods especially in the East. It is a member of the family Zinberaceae and its scientific name is *Zingiber officinale* (Karappiah and Rajaram, 2012).

Several reports have revealed that this plants are commonly use as a spice and a medicinal plant (Afzal *et al*., 2001; Chrubasik *et al*., 2005; Ogodo and Ekeleme, 2013). Thus, this study aimed to assess the antimicrobial effect of Aqueous and methanolic extract of Ginger rhizome (*Zingiber officinale*) and garlic (*Allium sativum*) against foodborne pathogens such as *E. coli*, *S. aureus, Salmonells* sp*, Klebsiella* sp*, Listeria monocytogens* and *P. aeruginosa*.

MATERIALS AND METHODS

Sampling Procedures

Ten Samples each of Ginger rhizome (*Zingiber officinale*) and Garlic (*Allium sativum*) were purchased from different markets within Ilorin Metropolis. They were labelled at the point of purchase and transported to the laboratory within 4-5 hours.

Bacterial Strains and Cultivation

Bacterial culture of *E. coli, Salmonella* sp, *Staphylococcus aureus, Listeria monocytogens, Bacillus subtilis, Pseudomonas aeruginosa* and *Shigella* sp used as tested organisms in all antibacterial assays were obtained from Microbiology Laboratory, University of Ilorin Teaching Hospital, Ilorin, Kwara State, Nigeria. These organisms were selected because they are among many pathogens often implicated in food borne outbreaks.

Preparation of Inoculum

According to Jayaraman *et al*. (2008) bacterial stock cultures were maintained at 4^0C on nutrient agar slants for bacteria. Active cultures for experiments were prepared by transferring a loopful of culture to 5ml of Nutrient broth and incubated at 37 ^{0}C for 24 hours and sub cultured at 37^0C for 24hours on nutrient agar (Sigma–Aldrich, Germany) before any susceptibility test.

Preparation of Aqueous garlic extracts for Antibacterial Activity Test.

The method of Ogodo and Ekeleme, (2013) was adopted for the extraction of the plant by slicing the garlic and ginger up to 2 mm in thickness and dried in an oven at 40-50 ^{0}C for 30-36 hours so that the moisture content was 8 %. It was then milled and screened, to produce powder size of 30 meshes. 20g of the powdered rhizome of *Zingiber officinale* and *Allium sativum* were then dispensed in to two beakers each containing 100ml distilled water, soaked for 48hours and then filtered into 100ml conical flask. It was stored at 4^0C until required.

Preparation of methanol extract of *Zingiber officinale* and *Allium sativum*

The extraction of the plants was carried out according to the method of Ogodo and Ekeleme (2013). Twenty grammes of each fine grounded powder of *Zingiber officinale* and *Allium sativum* were dispensed into a beaker containing 100ml of 70% methanol respectively, incubated at room temperature for 72 hours with shaking at 120 rpm. The crude extracts were centrifuged at 3000 rpm for 10 minutes at 25 °C. The methanol extracts were evaporated at 50 °C while the aqueous extracts were evaporated at 80 °C in rotary evaporator. All dried extract samples were dissolved in distilled water separately to the final concentration of 100 mg/ml and centrifuged again at 10,000 rpm to remove the un-dissolved residues (Gull *et al*., 2012)

Antibacterial Activity Tests

Well diffusion method assay according to Al-Daihan *et al* (2013).

A 20 µl of overnight grown culture was spread onto 20 ml of sterile Mueller-Hinton Agar (MHA) using a sterile cotton swab. Holes of 6 mm in diameter were made in the seeded agar using cork borer. Each of aliquot of 100 µl of both garlic and ginger extract was inserted into each well of the seeded Mueller-Hinton agar separately and allowed to stand on the bench for 1hour, thereafter incubated at 37^0C for 24h.

The antibacterial activity was evaluated by measuring the diameter of inhibition zone and the whole procedure was repeated three times to find the average.

Determination of MIC of Methanolic extracts

The nine test tubes were prepared by dispensing 1ml of Mueller–Hinton broth in each tube followed by serial dilution of the tested extracts (concentration of 100mg/ml) to obtain concentration ranged from 100 to 1.563 mg/ml, and then inoculated with 0.1ml suspension of test organism adjusted to contain approximately $1.5X10^8$ cfu/mL of Macfarland standard. The test plates were incubated at 37 ^{0}C for 18 hour. Fifty microliter (50µl) of a 0.01% solution of 2, 3, 5- triphenyl tetrazolium chloride (TTC) was added to the tubes and incubated for another hour. Since the colorless tetrazolium salt is reduced to red colored product by biological active bacteria, the inhibition of growth can be detected when the solution in the well remains clear after incubation with TTC. MIC was defined as the lowest sample concentration showing no color change (clear) and exhibited complete the inhibition of growth. The MBC was taken as the lowest concentration without growth of organism on the agar plate (Abu-Shanab *et al*., 2004 and Abou Elkhair *et al*., 2010 Radojević *et al*., 2012).

Statistical Analysis

The values obtained were subjected to descriptive statistics using Mean and Standard Error of mean. Duncan Multiple Range Test (DMRT) was used to test for the means that are significantly different from the other, which are presented by alphabets in superscripts in the Tables presented.

RESULTS

The result indicated that the extract has broad spectrum antibacterial activity with variable degree of sensitivity on the tested bacterial species towards the extracts. At 25% concentration of the aqueous garlic extract, *Bacillus subtilis* found to be the most susceptible with mean value of 15.20±0.00 diameter of zone of inhibition followed by *Staphylococcus aureus* with mean value of 14.30±0.27 while, *E. coli* and *Shigella* sp were statistically the same ($p < 0.005$) and their mean values were the least. At concentration, *Bacillus subtilis* was found to be most susceptible while, *E. coli* and *Shigella* sp were resistant with mean value of diameter of zone of inhibition of 7.98±1.00 and 9.20±0.80 respectively. Inhibitory effect of garlic extract on *S. aureus, Salmonella* sp and *Listeria monocytogens* were statistically the same. At 100% concentration of garlic extract inhibitory effect was highest in *Bacillus subtilis* and *Staphylococcus aureus* also, effect on both isolates were statistically the same at ($p < 0.005$). Both *E. coli* and *Shigella* sp were resistant at the concentration. Methanolic garlic extract showed no inhibitory effect statistically at 50% and 100% concentration, while, no effect was observed in *S. aureus, E. coli, P. aeruginosa* and *Salmonella* sp on the same concentration shown in Table 2.

MIC *Bacillus subtilis* and *S. aureus* were found to have the lowest minimum inhibitory concentration of the same values, while *E. coli* has highest MIC value followed by *Shigella* sp, *Listeria monocytogens, Salmonella* sp with mean values of 100, 50, 25 and 12.5 respectively. Both *Listeria monocytogens* and *Shigella* sp found to have the same MBC values of 100 while, the MBC value of *E. coli* was not detected within the concentration range Shown in Table 3.

Antibacterial activities of *Z. officinale extract* against foodborne bacterial pathogens were evaluated using agar well diffusion. The activity of the extract was found to be concentration dependent. Antibacterial activities of *Z. offfinales* against food borne pathogen were evaluated using agar well diffusion. The activity of extract was concentration dependent. 25% of cold-water extract of ginger rhizome had equal effect of *Bacillus subtilis, S. aureus* and *Shigella* while *Pseudomonas aruginosa, Salmonella sp* and *Listeria monocytpogens* found to have equal effect at ($P<0.05$), as shown in Table 4

At 50% concentration, inhibitory effect on *S. aureus* and *P. aeruginosa* were statistically indifferent, also *L. monocytogens* and *Shigella spp* were also similar. Crude extract showed highest zone of inhibition on *S. aureus, Salmonella sp,* and *Listeria monocytogens* in equal proportion (P<0.05) followed by *Shigella*. The same effect was also observed in *Bacillus subtilis* and *P. aeroginosa* while *E. coli* was resistant in all concentration of aqueous extract Table 5.

Methanolic extract of *Z. officinale* showed higher antibacterial actiivities against tested food borne bacteria pathogens than aqueous extract. A 25% concentration of mathanolic extract, the extract showed equal inhibitory effect of *B. subtilis* and *L. monocytogens* in equal proportion (P<0.05) while *S. aureus, P. aeruginosa* and *Shigella* were also statistically sensitive in equal proportion. At 50% conc. *P. aeruginosa* found to have highest zone of inhibition. However, *S. aureus, L. monocytogens* and *Shigella* sp were observed to show higher zone of inhibition in equal proportion. In crude extract, *S. aureus* showed highest zone of inhibition while *E. coli* showed least effect on the extract shown in Table 6.

Table 1: Diameter of zone of inhibition of enteric bacteria in response to Garlic Aqueous Extract

Conc.	*Bacillus subtilis*	*S. aureus*	*E. coli*	*P. aeruginosa*	*Salmonella sp*	*L. monocytogens*	*Shigella* sp
0	0.00 ± 0.00^{g}	0.00 ± 0.00^{g}	0.00 ± 0.00^{g}	0.00 ± 0.00^{g}	0.00 ± 0.00^{g}	0.00 ± 0.00^{g}	0.00 ± 0.00^{g}
25	15.20 ± 0.00^{bcd}	14.30 ± 0.27^{c}	7.13 ± 1.20^{f}	11.60 ± 0.17^{d}	11.30 ± 0.00^{d}	12.56 ± 2.40^{cd}	7.00 ± 1.20^{f}
50	17.16 ± 1.41^{bc}	15.00 ± 0.47^{c}	7.98 ± 1.00^{f}	13.30 ± 0.12^{cd}	13.48 ± 0.37^{c}	14.80 ± 0.24^{c}	9.20 ± 0.80^{e}
75	20.83 ± 3.00^{ab}	22.13 ± 2.04^{ab}	9.00 ± 00^{e}	14.00 ± 1.07^{c}	15.90 ± 0.56^{bc}	15.00 ± 0.69^{c}	11.55 ± 1.35^{d}
100	24.30 ± 1.45^{a}	26.45 ± 2.68^{a}	11.51 ± 0.20^{d}	15.30 ± 2.08^{bc}	18.45 ± 1.00^{b}	18.30 ± 0.76^{b}	13.50 ± 1.89^{c}

Each value is a mean of 3 replicates±standard error.Values are presented as Mean ± SEM (n = 3). All groups are compared to each other at P < 0.05. Values with the same superscripts along the same column and row differ significantly each other.

Table 2: Diameter of zone of inhibition of enteric bacteria in response to Garlic Methanol Extract

Conc.	*Bacillus subtilis*	*S. aureus*	*E. coli*	*P. aeruginosa*	*Salmonella sp*	*L. monocytogens*	*Shigella* sp
0	0.00 ± 0.00^{e}	0.00 ± 0.00^{e}	0.00 ± 0.00^{e}	0.00 ± 0.00^{e}	0.00 ± 0.00^{e}	0.00 ± 0.00^{e}	0.00 ± 0.00^{e}
25	1.00 ± 0.00^{ed}	0.00 ± 0.00^{e}	1.27 ± 0.52^{cd}	1.00 ± 0.00^{ed}	1.00 ± 0.00^{ed}	0.00 ± 0.00^{e}	0.00 ± 0.00^{e}
50	2.36 ± 1.41^{cd}	0.00 ± 0.00^{e}	3.23 ± 1.00^{bc}	2.90 ± 0.52^{bc}	3.48 ± 0.37^{bc}	0.00 ± 0.00^{e}	0.00 ± 0.00^{e}
75	2.48 ± 3.00^{cd}	1.00 ± 0.00^{ed}	3.90 ± 0.02^{bc}	3.00 ± 0.15^{bc}	3.20 ± 0.06^{bc}	1.70 ± 0.04^{cd}	0.00 ± 0.00^{d}
100	4.00 ± 1.45^{ab}	1.70 ± 0.02^{d}	4.81 ± 0.04^{ab}	4.81 ± 1.11^{ab}	3.88 ± 1.00^{bc}	2.45 ± 0.76^{cd}	2.08 ± 0.04^{c}

Each value is a mean of 3 replicates±standard error.Values are presented as Mean ± SEM (n = 3). All groups are compared to each other at P < 0.05. Values with the same superscripts along the same column and row differ significantly each other.

Table 3: Minimum inhibitory concentration (MIC) of different garlic Aqueous extracts against Tested Bacterial Isolates

Isolates	MIC (mg/ml)	MBC (mg/ml)
Bacillus subtilis	3.125	50
Staphylococcus aureus	3.125	6.125
Salmonella sp	12.5	12.5
Listeria monocytogens	25	100
Shigella sp	50	100
E. coli	100	**NA**

MIC-Minimum inhibitory concentration; MBC-Minimum inhibitory concentration, NA: Not Applicable

Table 4: Diameter of zone of inhibition of enteric bacteria in response to *Z. officinale* Aquoues Extract

Conc.	*Bacillus subtilis*	*S. aureus*	*E. coli*	*P. aeruginosa*	*Salmonella sp*	*L. monocytogens*	*Shigella* sp
0	0.00 ± 0.00^{j}	0.00 ± 0.00^{j}	0.00 ± 0.00^{j}	0.00 ± 0.00^{j}	0.00 ± 0.00^{j}	0.00 ± 0.00^{j}	0.00 ± 0.00^{j}
25	3.00 ± 0.00^{i}	3.70 ± 0.00^{i}	0.00 ± 0.00^{j}	4.60 ± 0.00^{h}	4.80 ± 0.00^{h}	4.80 ± 0.00^{h}	3.40 ± 0.12^{i}
50	5.22 ± 1.91^{h}	6.81 ± 0.28^{g}	0.00 ± 0.00^{j}	6.67 ± 0.52^{g}	8.00 ± 0.17^{e}	7.55 ± 1.11^{f}	8.21 ± 1.18^{f}
75	7.00 ± 2.45^{f}	8.56 ± 0.90^{e}	0.00 ± 0.00^{j}	9.00 ± 1.11^{d}	10.58 ± 1.00^{c}	11.90 ± 1.54^{ab}	9.55 ± 1.00^{d}
100	10.00 ± 2.55^{c}	13.87 ± 3.92^{a}	0.00 ± 0.00^{j}	10.11 ± 1.00^{c}	13.43 ± 2.58^{a}	13.71 ± 2.16^{a}	11.08 ± 1.39^{ab}

Each value is a mean of 3 replicates±standard error.Values are presented as Mean ± SEM (n = 3). All groups are compared to each other at $P < 0.05$. Values with the same superscripts along the same column and row differ significantly each other.

Table 5: Diameter of zone of inhibition of enteric bacteria in response to *Z. officinale* Methanol Extract

Conc.	*Bacillus subtilis*	*S. aureus*	*E. coli*	*P. aeruginosa*	*Salmonella sp*	*L. monocytogens*	*Shigella* sp
0	0.00 ± 0.00^{j}	0.00 ± 0.00^{j}	0.00 ± 0.00^{j}	0.00 ± 0.00^{j}	0.00 ± 0.00^{j}	0.00 ± 0.00^{j}	0.00 ± 0.00^{j}
25	4.78 ± 0.00^{h}	6.58 ± 0.00^{g}	0.00 ± 0.00^{j}	6.10 ± 0.30^{g}	3.80 ± 0.13^{i}	4.80 ± 0.58^{h}	5.40 ± 0.10^{g}
50	7.56 ± 1.01^{f}	8.80 ± 1.68^{e}	3.12 ± 1.00^{i}	9.58 ± 2.92^{de}	7.39 ± 0.17^{f}	8.35 ± 0.00^{e}	8.87 ± 0.31^{e}
75	11.97 ± 1.95^{c}	12.00 ± 2.00^{abc}	7.28 ± 1.47^{f}	13.00 ± 2.19^{ab}	9.32 ± 1.00^{de}	10.00 ± 2.03^{d}	10.45 ± 0.00^{d}
100	12.23 ± 2.32^{abc}	14.87 ± 2.58^{a}	10.45 ± 1.00^{d}	13.36 ± 2.69^{ab}	11.70 ± 1.50^{c}	12.21 ± 1.08^{abc}	13.71 ± 1.11^{ab}

Each value is a mean of 3 replicates±standard error.Values are presented as Mean ± SEM (n = 3). All groups are compared to each other at $P < 0.05$. Values with the same superscripts along the same column and row differ significantly each other.

Table 6: Minimum inhibitory concentration (MIC) of different *Z. officinale* extracts against enteric bacterial pathogens

Bacterial Isolates	MIC (mg/ml)	MBC (mg/ml)
Bacillus subtilis	6.125	12.5
Staphylococcus aureus	3.125	3.125
Salmonella sp	12.5	50
Listeria monocytogens	6.125	12.5
Pseudomonas aeruginosa	3.125	12.5
Shigella sp	3.125	12.5
E. coli	12.5	100

MIC: Minimum inhibitory concentration; MBC: Minimum Bactericidal Concentration.

DISCUSSION

The results of the inhibition zone measurement, using the filtrate of fresh garlic against foodborne bacterial pathogens showed that, the higher the concentration of the garlic extract, the stronger the antibacterial activity as indicated by the larger zone of inhibition (Table 1).

Safithri *et al.* (2011) reported inhibition zone measurement of aqueous garlic extract against *S. agalactie*, *S. aureus*, and *E. coli* mastitis bacteria, showed that fewer bacteria were resistant to the higher concentration of garlic water extract, as indicated by the larger inhibition zone this agrees with the present finding that showed highest zone of inhibition at 100% concentration.

Methanolic extract of Garlic exhibited little or no inhibitory effect on the isolates although, little antibacterial activity was observed in 100% concentration. Thus, the use of ethanol to extract antibacterial bioactive compounds in garlic is not effective. This was consistent with the results of research by Sadeghian and Ghazvini (2002) which showed that the 17% w/v garlic methanol extract inhibited 2% the *Shigella* bacteria.

Aqueous extracts of garlic were shown to inhibit both gram positive and Gram-negative bacteria with an MIC of 3.125mg/ml on tested Gram-positive bacteria and12.5mg/ml to 100mg/ml on gram negative bacteria, this agreed with the result of Durairaj *et al.* (2009).

It was intriguing to note that the tested clinical isolates were sensitive to all tested extracts of garlic extract but Gram negative showed higher resistance than Gram positive bacteria. This result is in accordance with the findings of (Chandarana *et al*., 2005). The sensitivity of these isolates to aqueous garlic extract (AGE), also implies that the intrinsic bioactive substances in these extracts are naïve to the various drug resistance factors of the isolates, which include beta-lactamase expression, increased pyrrolidonylarylamidase activity, aminoglycosides modifying enzymes and altered ribosomal binding. Meanwhile, the antimicrobial potency of garlic has been attributed to its ability to inhibit toxic production and expression of enzymes for pathogenesis (Sanders *et al.*, 1992). All the plant extract tested showed antibacterial activity; however, the plants differ in their activities against the bacteria tested. Highest antibacterial activity was observed with methanol extract of *Z. officinales* against *S. aureus* (14.87±2.32), *Shigella* sp (13.71±1.11) and *P. aeruginosa* (13.36±2.61) respectively while moderate activity was observed in the remaining isolates. Highest MIC was found in *Salmonella* and *E. coli* while the lowest was observed in *S. aureus*, *Shigella* sp and *Pseudomonas* sp. *E. coli* showed Highest MIC of (100mg/ml) while, lowest MBC was found in *Staphylococcus aureus*. *B. subtilis*, *L. monocytogens, Shigella* sp and *Pseudomonas* sp showed equal magnitude of MBC of 12.5mg/ml and this in accordance with the study of Rahman, 2001.

The work of Ogodo and Ekeleme (2013) corroborated the present finding who reported that cold water extract of the ginger produced maximum activity against *Salmonella* sp, *Staphylococcus aureus, Bacillus cereus* and no inhibition on *Escherichia coli*.

CONCLUSION

This present study showed that the both aquous and methanolic extracts of *Z officinales* and *Allium sativum* posses antibacterial properties. Our results suggest that both plants can serve as potential sources of bioactive healthy compounds in the diet and their consumption could be useful in the prevention of diseases. Further research is needed toward isolation and identification of bioactive compounds present in the extracts which could possibly be exploited for pharmaceutical and preservative use.

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