# A Comparison of the Antimicrobial Effectiveness of Aqueous Extracts of Garlic, Ginger and Lime and Two Conventional Antibiotics on Escherichia coli, Salmonella spp., Shigella spp. and Bacillus cereus.

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**Abstract**

The antimicrobial effectiveness of extracts of garlic, ginger and lime on Escherichia coli, Salmonella spp., Shigella spp. and Bacillus cereus were ascertained and compared with that of the conventional antibiotics of Amoxicillin and Ampicillin using the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC). The plant extracts showed varied activity on the test organisms with garlic showing a stronger antimicrobial activity of 150mg/ml, 50mg/ml and 100mg/ml on Salmonella, Shigella and Bacillus respectively compared to MIC of 250mg/ml of lime extracts on both Salmonella and Shigella and 150mg/ml on Bacillus spp. whilst ginger was totally ineffective at the highest concentration of 500mg/ml on all test organisms. MBC's of garlic extract on Shigella and Bacillus cereus was generally lower compared with Lime. The lowest and highest MBC's was shown by garlic on Shigella (150mg/ml) and Salmonella (500mg/ml) respectively. Generally, the MIC's and MBC's of the conventional antibiotics were lower compared with the plant extracts. However, garlic exhibited similar antimicrobial activity as Amoxicillin on Shigella (MIC=50mg/ml) and Bacillus cereus (MIC=100mg/ml) with no difference in Least Significant Difference ($M_1$-$M_3$=78.6254). Garlic also retained the same MIC activity with Ampicillin on Shigella (50mg/ml). The MBC's of garlic and Amoxicillin were the same (200mg/ml). This study confirms the antimicrobial potential of these plants extracts especially garlic on the test bacteria and suggests the possibility of employing them as household remedies to some bacterial infections.

## INTRODUCTION

Man has been using natural products of animals, plants and microbial sources for thousands of years either in the pure forms or crude extracts (Parekh and Chanda, 2007). Biologically active compounds from these diverse sources have been isolated and characterized worldwide with the elucidation of the chemical structures of some of these compounds leading to the synthesis and production of more potent and safer drugs (Bhattacharj et al., 2005). The increasing reliance on drugs from natural sources has led to the extraction and development of several drugs and chemotherapeutic agents from traditional herbs which are present in abundance in the tropics (Falodun et al., 2006). In-vitro, allicin (the main biologically active component of garlic) has demonstrated activity against some gram positive and gram negative bacteria as well as fungi (Candida albicans), protozoa (Entamoeba histolytica) and certain viruses with primary mechanism involving the inhibition of thiol-containing-enzymes needed by these microbes. (Katzung, 2007). Traditionally ginger has been used to treat intestinal infections, especially related with digestive problems (El-Mahmood, 2009). Scientific study conducted in laboratory animals found that both gingerols and shogaols have analgesic, sedative, anti-bacterial and gastrointestinal motility effects which aids in resolving stomach problems (O'Hara et al., 1998). Lime is also an essential ingredient in the preparation of most herbal concoction which is sometimes used to suppress stomach ache and possess antimicrobial activities in conjunction with other extracts (Onyeagba et al., 2004).

Escherichia coli, Salmonella spp. and Shigella spp. are some of the members of bacteria referred to as Enterobacteria which are Gram-negative, non-spore forming rod-shaped bacteria with many members of the family being a normal part of the gut flora found in the intestines of humans and

other animals. However, under certain conditions, these organisms including Bacillus cereus are the main causes of gastroenteritis in humans. Amoxicillin a moderate-spectrum antibiotic and Ampicillin are conventional antibiotics recommended for treating gastrointestinal infections caused by these organisms. Within the last few decades, microbial resistance has emerged for most of the available agents (antibiotics), thus necessitating the search for newer drugs (Bhattacharj et al., 2005). In fact, the use of medicinal plants to treat diseases of varying aetiology is part of the African tradition, but in spite of thousands of years of use, none of these bioactive plant compounds have been exploited for clinical uses as antibiotics, though some alkaloidal compounds like quinine and emetine have been developed as chemotherapeutic agents (El-mahmood, 2009).

Thus the study aim at determining the antimicrobial potential of aqueous extracts of garlic, ginger and lime in comparison with that of the conventional antibiotics of choice in treating gastrointestinal infections caused by the test bacteria.

## MATERIAL AND METHODS

Extraction of Active Plant Ingredient: 100g of cleaned, air dried plant extracts of ginger, garlic and lime obtained were blended separately and individually soaked in 100mls of sterile distilled water for 24hrs in a sterile glass container. The pulp obtained was shaken vigorously to allow for proper extraction of active ingredients. The crude extract was then filtered using sterile Whatmans No. 1 filter paper. The filtered extracts were then stored in the refrigerator at 4C.

Test Samples: Pure Bacterial isolates for antimicrobial testing was obtained from the Bacteriology Department of the Central laboratory of The Korle-Bu Teaching Hospital the main reference hospital in Ghana.

Determination of Minimum Inhibitory Concentration (MIC): The minimum inhibitory concentration (MIC) was determined by the broth dilution method of Sahm and Washington (1990). The crude extracts were diluted to various concentrations ranging from 50-500mg/ml in sterile distill water. 500ul of each concentration was added to 2 ml sterile nutrient broth in test tubes arranged on a test tube rack. Then 1ml ($10^8$ Cfu/ml) of an organism was added to the content of the test tubes and the test tubes incubated at 37ºC for 18-24 hours. The process was repeated for the conventional antibiotics. 500ul of each of the pure extracts (500mg/ml) were added into the test tubes and used as negative controls. 1ml of the standardized inoculums was also pipetted into the test tubes containing sterile nutrient broth and used as positive control. The MIC was taken as the lowest concentration of extracts that did not permit any visible growth for each of the test bacteria.

Determination of Minimum Bactericidal Concentration (MBC): 1ml of culture was taken from each of the broth tubes that showed no growth and introduced into fresh nutrient agar plates. After incubation for 48 h, the plates were observed for growth. The concentration of the extracts that showed no visible growth was recorded as the MBC.

Statistical Analysis: Statistical analysis was performed using MINITAB software Version 14. Analysis of variance (ANOVA) was performed to determine statistically significant differences amongst plant extracts and the conventional antibiotics. The Least Significant Difference (LSD) was also determined between individual pairs of plant extracts and the conventional antibiotics.

## RESULTS

There were generally some variations among the MIC's of the plant extracts. MIC's for garlic extracts on Salmonella, Shigella and Bacillus cereus were generally lower (150mg/ml, 50mg/ml and 100mg/ml) respectively than the MIC's for lime extracts on these bacteria compared to 250mg/ml MIC of lime extracts on both Salmonella and Shigella and 150mg/ml on Bacillus cereus with a difference in LSD ($M_1$-$M_2$=87.50) (Table 3). E. coli exhibited the same MIC (250mg/ml) for both garlic and lime. Ginger extracts did not show any inhibition to all the test organisms between the concentrations of 50-500mg/ml used for experiment. In the conventional antibiotics, Amoxicillin had MIC's of 50mg/ml and (100mg/ml) on Shigella spp. and Bacillus cereus which was same for garlic reflecting in a no difference LSD ($M_1$-$M_3$=75.0) (Table 3). Ampicillin also had MIC of 50mg/ml on Shigella spp. The MIC's of the conventional antibiotics were generally lower than the MIC's of lime extracts on all test bacteria (Table 1) with no difference LSD ($M_3$-$M_4$=50.0) (Table 3).

MBC's of garlic extracts of 150mg/ml and 200mg/ml respectively were generally lower than that of Lime extracts 400mg/ml and 450mg/ml on Shigella spp. and Bacillus cereus although not different in LSD ($M_1$-$M_2$=62.50) (Table 4). The MBC's of garlic and lime extracts on E. coli were the same ((250mg/ml). The highest MBC (500mg/ml) was shown by garlic extracts on Salmonella spp. Similar MBC's

of 200mg/ml was demonstrated by Amoxicillin and garlic extract on Bacillus cereus. However, the highest MBC 250mg/ml of the conventional antibiotics was Ampicillin on Bacillus cereus. The MBC of lime extracts were generally higher than those of all the conventional antibiotics on the test bacteria (Table 2).

### Figure 1

Table 1: MIC of Plant Extracts and Conventional Antibiotics

| | Concentration(mg/ml) | | | | |
|---|---|---|---|---|---|
| **Bacteria** | **Garlic** | **Lime** | **Ginger** | **Amoxicillin** | **Ampicillin** |
| *E. coli* | 250 | 250 | - | 50 | 50 |
| *Salmonella spp.* | 150 | 250 | - | 50 | 50 |
| *Shigella spp.* | 50 | 250 | - | 50 | 50 |
| *Bacillus cereus* | 100 | 150 | - | 100 | 50 |

### Figure 2

Table 2: MBC of Plant Extracts and Conventional Antibiotics

| | Concentration(mg/ml) | | | | |
|---|---|---|---|---|---|
| **Bacteria** | **Garlic** | **Lime** | **Ginger** | **Amoxicillin** | **Ampicillin** |
| *E. coli* | 250 | 250 | - | 50 | 50 |
| *Salmonella spp.* | 500 | 250 | - | 50 | 50 |
| *Shigella spp.* | 150 | 400 | - | 50 | 50 |
| *Bacillus cereus* | 200 | 450 | - | 200 | 250 |

### Figure 3

Table 3: Least Significant Difference (LSD) of the MIC's of the Antibiotics

| **Pairs** | **$M_{(x)} - M_{(n)}$** | **LSD** | **Remarks** |
|---|---|---|---|
| $M_1$-$M_2$ | 87.50 | 78.6254 | Different |
| $M_1$-$M_3$ | 75.00 | 78.6254 | No difference |
| $M_1$-$M_4$ | 87.50 | 78.6254 | Different |
| $M_2$-$M_3$ | 162.50 | 78.6254 | Different |
| $M_2$-$M_4$ | 175.00 | 78.6254 | Different |
| $M_3$-$M_4$ | 50.00 | 78.6254 | No difference |

### Figure 4

Table 4: Least Significant Difference (LSD) of the MBC of the Antibiotics

| **Pairs** | **$M_{(x)}$-$M_{(N)}$** | **LSD** | **Remarks** |
|---|---|---|---|
| $M_1$-$M_2$ | 62.50 | 172.9802 | No difference |
| $M_1$-$M_3$ | 187.50 | 172.9802 | Different |
| $M_1$-$M_4$ | 175.00 | 172.9802 | Different |
| $M_2$- $M_3$ | 250.00 | 172.9802 | Different |
| $M_2$- $M_4$ | 237.50 | 172.9802 | Different |
| $M_3$- $M_4$ | 12.50 | 172.9802 | No difference |

(M= Mean Concentration) $M_1$- Garlic, $M_2$-Lime, $M_3$-Amoxicillin, $M_4$-Ampicillin

## DISCUSSION

Results from the study was similar to works by Ankri and Mirelman in (1999) which showed that the test bacterial were inhibited in varying degrees by the plant extracts.

Overall garlic extracts had the best antimicrobial activity against majority of the test organisms resulting in a comparable activity with the conventional antibiotics against some of the test bacteria such as Shigella spp. and Bacillus cereus. This is in conformity to works by (El-Mahmood, 2009) who observed similar activity of extracts of garlic on nosocomial bacterial organisms in relation to that of conventional antibiotics. Numerous modern studies confirm that garlic has definite antibiotic properties and is effective against many bacteria, fungi and viruses thus researchers have also compared the effectiveness of garlic with that of

commercial prescription antibiotics and the result is often that garlic can be more effective as a broad spectrum antibiotic (O'Gara et. al.; 2000, Amagase, 2006). The susceptibility of Gram-positive Bacillus cereus also confirms observation by (Ahmadu et al., (2006) that the complex nature of the cell envelope of Gram-negative bacteria has been observed to retard or prevent the passage of many antimicrobial agents through the cell wall as was seen in the MIC's of E. coli and Salmonella spp. The susceptibility of Shigella to garlic extracts observed in this experiment confirms similar observations by (Sadeghian and Ghazvini, 2002). Lime exhibited antimicrobial properties against the test fungi which conforms to research by Chaisawadi et al., (2003) who found lime to be effective against Bacillus cereus, Staphylococcus aureus and Salmonella typhi albeit at a lesser activity compared with garlic. Aibinu et al., in (2007) observed that the antimicrobial potency of lime fruit is enhanced by the type of solvent used in the extraction process indicating that there are some active ingredients in lime which have high antimicrobial effect but which would not be released except when lime fruit is used in conjunction with a particular solvent. Results obtained for Ginger could not be included in the analysis because Ginger showed no inhibition to any of the test bacteria at the concentrations used for the experiment which is between 50mg/ml to 500mg/ml. However, the "no inhibition" demonstrated by the crude ginger extracts to the test bacteria in this experiment conforms to the findings by Onyeagba and colleagues in 2004 where they observed that aqueous extracts of ginger singly did not inhibit the growth of Bacillus spp., S. aureus, E. coli and Salmonella. El-Mahmood et al., 2008 also observed that invitro antibacterial activities of organic crude extracts of essential oils of ginger give better antimicrobial effectiveness than aqueous extracts of similar volumes. However, it is possible higher aqueous extracts of ginger may be active against the test bacteria. Consistently, the conventional antibiotics of Amoxicillin and Ampicillin had better inhibitory activity overall which was amply confirmed in their MBC's except in the few cases where they had similar MIC's with garlic. This was expected since the conventional antibiotics have been properly synthesized to inhibit particular metabolic pathways and also destroy some features of the bacteria that are essential for their survival compared with crude extracts of herbal medicines which are subject to degradation and decomposition on storage (El-Mahmood and Amey, 2007).

Research using other media in the extraction of garlic has found aqueous extraction to be more potent than the organic extracts (Roy et al., 2006; Jaber and Al-Mossawi, 2007). This could be as a result of the fact that when plant materials are ground in water, a number of phenolases and hydrolases are released and these enzymes might serve to modulate the activity of the active compounds in the extract (De and Ifeoma, 2002). Since the herbalist usually uses water to prepare infusions and decoctions, and since most constituents of garlic are soluble in water, there is likelihood that the traditional healer is able to extract all the bioactive drug components in garlic making it a good home remedy against some infections.

## CONCLUSION

This study confirms the antimicrobial potential of some aqueous plant extracts and supports the use of these extracts in traditional medicine. It also shows the antimicrobial potential of garlic against all the test organisms and particularly that of Shigella spp. and Bacillus cereus thus suggesting its use as a broad spectrum antibiotic if better extracted and refined.

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