

Assessment of the Antibacterial Effect of Crude Preparation of Garlic (*Allium sativum*) on Diarrhea Causing Bacteria: An *In vitro*, Study

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Abstract: The main objective of this study is to assess the antibacterial effect of crude preparation of garlic on diarrhea causing bacteria (*Escharchia coli*, *Salmonella* strains and *Shigella* strains). The Minimum Inhibition Concentrations (MIC) and Minimum Bactericidal Concentration (MBC) of garlic samples on *Escharchia coli*, *Salmonella* strains and *Shigella* strains was investigated by an agar dilution technique. The garlic was washed with distilled water, removed the outer layers, crushed, dried, powdered with mortar and pestle, sieved with a mesh and filtered; finally known mass of the filtered powder was thoroughly mixed with distilled water and the concentration was determined. Then media was prepared by mixing the Muller Hinton agar with varying amounts of crude preparation of garlic to give the final concentration of 7.50, 15.00, 22.50, 30.00, 37.50, 45.00, 52.50 and 60.00 mg mL⁻¹ of media and the final volume of 20 mL. Four antibiotics were included with similar dilution. The result was compared with the effects of the same concentrations of some modern drugs. Garlic has antibacterial effects *In vitro*, but this study indicated that garlic (*Allium sativum*) is superior to some antibiotics. Crude preparation of garlic could be used as effective antibacterial agent. However, pharmacological standardization and clinical evaluation on the effect of garlic are essential.

Keywords: Garlic, (*A. sativum*), diarrhea causing bacteria, antibacterial agents

INTRODUCTION

Garlic has had a valued place in traditional medicine for centuries. The great herbalists and physicians of the ancient world record garlic historical use. Garlic has powerful properties and is of great benefit against changes of water and of residence, wrote Pliny the elder, the first century Roman naturalist (23-79 AD) (Foster 1996; Koch *et al.*, 1995). Garlic has been used from the time when ancient times in India and China for a valuable effect on the heart and circulation, cardiovascular disease (Kris-Etherton *et al.*, 2002; Koscielny *et al.*, 1999; Yu-Yan *et al.*, 2001; Gardner *et al.*, 2003) and regular use of garlic may help to prevent cancer, to treat malaria and to raise immunity. Garlic has also proposed to treat asthma, candidiasis, colds, diabetes and antibacterial effect against food borne pathogens like *Salmonella*, *Shigella* and *S. aureus* etc. (Teferi, 2002).

Therapeutic use of garlic has been recognized as a potential medicinal value for thousands of years to different microorganisms. For example, antifungal, antiviral, antibacterial antihelminthic, antiseptic and anti-inflammatory properties of garlic are well documented. Moreover, garlic extracts exhibited activity against both gram negative (*E. coli*, *Salmonella* sp. and *Citrobacter Enterobacter*, *Pseudomona Kilabsella*) and gram positive (*S. aureus*, *S. pneumonia* Group A *Streptococcus* and *Bacillus anthrax*) all of which are cause of morbidity world wide. However, there is no significant effect if the garlic had been boiled for 5 min before using (Song, 2001; Abdullah *et al.*, 1998). Used at concentrations of between 0.8-40

mg mL⁻¹ (Internet, 1993) garlic was found to be superior to tetracycline hydrochloride Ampicillin, cotrimoxazole in inhibiting growth of bacteria isolated from different samples (Abdullah *et al.*, 1998). Garlic also in different concentration shows different activity. For example, 1:5000 dilutions the concentration of garlic completely inhibits the growth of *Mycobacterium tuberculosis*. Moreover, 5 mg mL⁻¹ aqueous garlic concentration showed 90% inhibition of *Helicobacter pylori* (Cellini *et al.*, 1996). Garlic has been proven to be of great value in treating as antiseptic decrease blood cholesterol, anti tumor, anti-neoplastic and increase HDL. It has been shown to be a good natural preservative for the food industry. Some work also demonstrated that garlic extracts has great effect on *E. coli*, *S. aureus*, *S. typhi*, (Jonkers *et al.*, 1999) *Shigella*, (WHO, 1999) when used fresh raw garlic (Song, 2001).

Diarrhea and gastroenteritis (diarrhea with vomiting) are major cause of mortality and morbidity in many developing countries. From which *Shigella*, *Salmonella* and *E. coli* accounts major part (Monica, 1998; Abran, 1990). About 35000-45000 cases of salmonellosis (Behrman *et al.*, 2004) and more than 450,000 shigellosis reported annually in USA (Murray *et al.*, 2002).

E. coli also accounts approximately 20-30 of diarrhea episodes the developing world (Behrman *et al.*, 2004; Murray *et al.*, 2002). Therefore, this study designed experimental work to see if garlic has any activity on *Salmonella* sp., *Shigella* and *E. coli* strains *In vitro* and whether this activity is related some antibiotics which used to threat to these organisms.

MATERIALS AND METHODS

The activity of garlic extract against three species of clinical isolates of bacteria: *Salmonella*, *E. coli* and *Shigella* were determined by using Minimum Inhibitory Concentrations (MIC) and minimum bactericidal concentrations of the garlic. Clearly prepared garlic powder was thoroughly mixed with distilled water and the concentration was determined. Muller Hinton agar with varying amounts of crude preparation of garlic to give the final concentration of 7.50, 15.00, 22.50, 30.00, 37.50, 45.00, 52.50 and 60.00 mg mL⁻¹ of media and the final volume of 20 mL. Four antibiotics were included and similar dilution was used for comparison purpose. The results were compared with garlic and modern antibiotics using the same experimental protocol by inoculating bacteria strains with inoculums density of 10⁴ CFU /mL (colony form unit). This study was conducted from November 1st to July 31st, 2007 at Jimma University, which is 345 km far apart west from the capital city of Ethiopia.

RESULTS AND DISCUSSION

For low concentrations of up to 22.50 mg mL⁻¹ of garlic the bacteria cultures were shown growth of the bacteria both in clinical isolates and standard test organisms (*E. coli*, *Shigella* and *Salmonella* sp.). There was clear observation of colonies without any defect with overnight incubation.

For concentrations of greater than 22.50 mg mL⁻¹ all bacteria which tested in this experiment inhibited to grow garlic with in 24 h incubation. After 24 h incubation the sample from the media inoculated in enriched media; there was a growth of bacteria at 30.00 and 37.50 mg mL⁻¹. This indicates that garlic has a bacteriostatic effect on this clinical isolates from stool in this concentration, however, if the concentration of garlic greater than 37.50 mg mL⁻¹ showed no growth in an enriched media. Therefore garlic at this level it is bactericidal, against *Salmonella*, *Shigella* and *E. coli*.

For concentration of greater than 22.50 mg mL⁻¹ of garlic and four selected antibiotics has the same effect over the clinical isolates except penicillin G. However, *cotrimoxazole*, *ciprofloxacin* and *cloramphenicol* have shown that inhibition of growth of bacteria at lower concentration (<22.50 mg mL⁻¹). There was no effect of penicillin G at all.

The study has shown that dilute solutions of garlic can completely inhibit the growth of bacteria (diarrhea causing pathogen, *Salmonella*, *Shigella* and *E. coli*) at the concentration of more than 30.00 mg mL⁻¹. This could be due to the action of biological active ingredient of garlic (Allicin), which acts primarily by blocking enzyme that are necessary for bacterial metabolism and by interfering with RNA synthesis (Abdullah *et al.*, 1998).

The study has clearly shown that for bacterial strains of with inoculums density of 10⁴ CFU/mL, garlic in concentration of (22.50-60.00 mg mL⁻¹) is capable of causing the inhibition of growth of bacteria. At the same

protocol garlic shows a bactericidal effect at the concentration of 37.50 mg mL⁻¹ for clinical isolate of *E. coli*. However, for others *Shigella* and *Salmonella* garlic shows a bactericidal effect starting from the concentration of 30.00 mg mL⁻¹ of garlic extract.

The concentration variation of garlic extract against *E. coli* and other clinical isolate is due to the cell wall membranes of *E. coli* contains 20% lipid, where as other strains contains less than 2% lipid. The lipid content of the membrane will have an effect on the permeability of allicin and other garlic constituents (Tyneka *et al.*, 1975).

CONCLUSION

Garlic (*A. sativum*) has antimicrobial properties against *Salmonella*, *Shigella* and *E. coli* strains. It has both a bacteristatic and bactericidal activity when tested *In vitro*. Crude preparation of garlic therefore, is used successfully for treating diarrhea-causing pathogen caused by tested organisms. Further study will be needed to establish the exact component or pharmacological standardization and clinical evaluation in garlic.

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