In vitro activity of *Allium sativum* and *Aloe vera* extract against *Salmonella enterica* subspecies *enterica* serovar Gallinarum

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Abstract: *The rapid increase of antibiotic resistance needs to be taken as a threat to both animals and human being. In this study the arbitrary concentration of 25%, 50% and 75% of individual and combined Allium sativum and Aloe vera were tested against Salmonella Gallinarum. The antimicrobials were extracted using aqueous solution where 25g, 50g and 75g of Allium sativum were dissolved in 100ml of sterile distilled water and left for 24hrs and filtered to obtain the concentration of 25%, 50% and 75% respectively and 1ml, 2ml and 3ml of Aloe vera exudates were homogenized in 4ml of distilled water separately by centrifugation to obtain concentration of 25%, 50% and 75% respectively. Aqueous extract of Allium sativum and Aloe vera exudates of 25%, 50%, 75% concentration and 25ml, 50ml, 75ml respectively were homogenized together to obtain a mixture. The different concentration of single and mixtures were tested against Salmonella Gallinarum for growth inhibition through pour plate method. The concentration of 50% and 75% illustrated strong antimicrobial activities but Allium sativum was more effective than Aloe vera. Combined concentration of 25% was less effective but those of 50% and 75% were highly effective than corresponding concentration of single. The combined concentration at 50% and 75% could be used to treat or to control clinical cases in poultry production caused by Salmonella Gallinarum with low cost compared to the synthetic drugs which have become less effective and have high cost. Aloe vera and Allium sativum can be refined and processed in the pharmaceutical industries at required concentrations and sold to the farmers at low cost for inhibition and control of Salmonella Gallinarum in poultry production.*

Keywords: Antibiotic resistance, Allium sativum, Aloe vera, Salmonella Gallinarum

INTRODUCTION

Microbial pathogenicity and other diseases have been controlled by the use of commercially available antimicrobial drugs for many years. Development of drug resistance in pathogens and increasing interest of consumers for safe food forced to explore new antimicrobial agents (Erdogrul et *al*., 2002; Belguith *et al*., 2010). Natural products are major good source of new natural drugs and their uses as alternative medicine for treatment of various diseases have increased in the last few decades (Vuorela*et al*., 2004; Arunkumar *et al*., 2009). In comparison to the synthetic drugs, the herbs and spices have fewer side effects and less expensive, show better tolerance by patient and are readily available at low cost (Adeshina *et al.,* 2011).

The antimicrobial activities of spices and herbs are due to specific phytochemicals present in them such as organic sulfur compounds in garlic (Mikail, 2010) and the main factors that determine the antimicrobial activities are the types and composition of spices and herbs, amount used, type of microorganisms, *pH* value and temperature of the environment (Sagdic, 2003;Yabaya *et al*., 2010). Garlic (*Allium sativum*) is one of the most popular spices in the world and it has been reported to contain a powerful natural antibiotic stronger than penicillin and tetracycline (Adetumbi and Lau, 1993). *Aloe vera* consists over 75 biological active compounds, and these compounds known to have a broad range of pharmacological activities including wound healing, anti-inflammatory, anti-arthritic, ant-oxidative and anti-tumor effect (Reynold and Dweck, 1999; Boudreau and Beland, 2006). Garlic (*Allium sativum*) and *Aloe vera* reported to have anti-mutagenic and ant-carcinogenic effect against Escherichia coli (Ogunjobi *et al*., 2007) and, oral supplementation of single garlic (*Allium sativum*), *Aloe vera* or combination reduced oocyst shedding, lesion score and mortality rate of broilers challenged with mixed infection of Eimera spp. (Banna *et al*., 2013). Also *Aloe Secundiflora* reported to reduce clinical signs and mortality rate in chickens after salmonella infection (Waihenya *et al*., 2002). *Salmonella enteric* subspecies *enterica* serovar Gallinarum (*Salmonella* Gallinarum) is a *Salmonella enterica* biotype that exhibit most specificity for poultry and aquatic birds. In this study, *in vitro* antimicrobial activity of single and combined *Allium sativum* and *Aloe vera* were investigated against *Salmonella* Gallinarum which is a veterinary important bacterial pathogen.

MATERIALS AND METHODS

Study location and Sample collection

The study was conducted at the Faculty of Veterinary Medicine, Sokoine University of Agriculture, Morogoro. Fresh garlic (*Allium sativum*) used in this study was purchased from a local market at Morogoro, Tanzania and *Aloe vera* was obtained from the garden at the Department of Animal Science and Production, Sokoine University of Agriculture, Morogoro.

Bacteria strain

Salmonella Gallinarum isolate was generously received from the Microbiology laboratory (Sokoine University of Agriculture). The isolate was maintained in nutrient agar slants at room temperature until testing.

Preparation of aqueous *Allium sativum* extracts

The aqueous *Allium sativum* extract was prepared according to Iwalokun *et al.* (2004) method. The cloves were separated and peeled to obtain the edible portion. Fifty grams of the edible portion was chopped and homogenized in 100 ml of sterile distilled water in a warming blender. The homogenate were filtered through 25µm filter paper (Iwalokun *et al*., 2004). The procedure was repeated for 25g, 50g and 75g to obtain the concentration of 25%, 50% and 75% of singles.

Preparation of *Aloe vera* extracts

A fresh *Aloe vera* peels were obtained from Department of Animal Science and Production (Sokoine University of Agriculture) at lower farm and its exudates were collected in vials. One milliliter, 2ml and 3ml of *Aloe vera* exudates were homogenized in 4ml of sterile distilled water separately by centrifugation to obtain concentration of 25%, 50% and 75%. Aqueous extract of *Allium sativum* and *Aloe vera* exudates of 25%, 50%, 75% concentration and 25ml, 50ml, 75ml respectively were homogenized together to obtain a mixture. The different concentration of single and mixture were tested against *Salmonella* Gallinarum for growth inhibition through pour plate method.

Bacterial culture by pour plate method

Briefly, 100ml of Mueller Hinton agar was autoclaved and cooled to 60-70^{0}C in a water bath. About 25ml of MH agar was aseptically dispensed to each of Petri dishes sized 60 x 15 mm and allowed to solidify. Bacterial suspensions were prepared at 0.5 McFarland (equivalent to 1-2 x10^{8}CFU/ml) with thelatex equivalence turbidity standards (Fisher Scientific, Waltham, Massachusetts, USA) in 2 ml of sterile 0.85% NaCl solutions. Approximately 1ml of bacterial inoculum was uniformly spread over the plates using a sterile cotton swabs. Pores of 7mm diameter were prepared and known volumes of extracts were placed into Mueller Hinton agar based pores seeded with *Salmonella* Gallinarum. The plates were incubated in upright position at 37°C for 24 hours. The diameters of zone of inhibition were measured in millimeter and the results were recorded. The zone of inhibition with diameter less than 12mm was considered as having no antibacterial activity. Diameter between 12 and 16 mm were considered moderately active and those with > 16mm were considered highly active.

DATA ANALYSIS

Data collected were analyzed in tables and figures. Descriptive statistics was used using excel programme.

RESULTS AND DISCUSSION

Inhibitory activity of *Aloe vera* and *Allium sativum* extracts more obviously depicted by decreasing the growth of *Salmonella* Gallinarum that was subjected to different arbitrary concentration of these extracts. At low concentration and high concentration both *Aloe vera* and *Allium sativum* were effective. But *Allium sativum* was highly and more effective than *Aloe vera* at the same concentration (Table 1).

Table 1: Inhibition effect of different concentration of *Aloe vera* and *Allium sativum*against *Salmonella*Gallinarum through pour plate method

Concentration (%)	Average zone of inhibition (mm)		
	Aloe vera	*Allium sativum*	*Aloe vera* + *Allium sativum*
25	18	20	10
50	20	25	26
75	24	30	33

At high concentration the combination of *Aloe vera* and *Allium sativum* concentration seemed to have higher inhibitory activity compared to individual concentration of *Aloe vera* and *Allium sativum* at the same concentration level (Figure 1). At low concentration level the combined concentration illustrated to be less effective than single concentration of *Aloe vera* and *Allium sativum* at the same concentration level. There was variation in zone of inhibition between different concentrations of the same extract whereby increase in concentration led to increase in zone of inhibition (Figure 1).

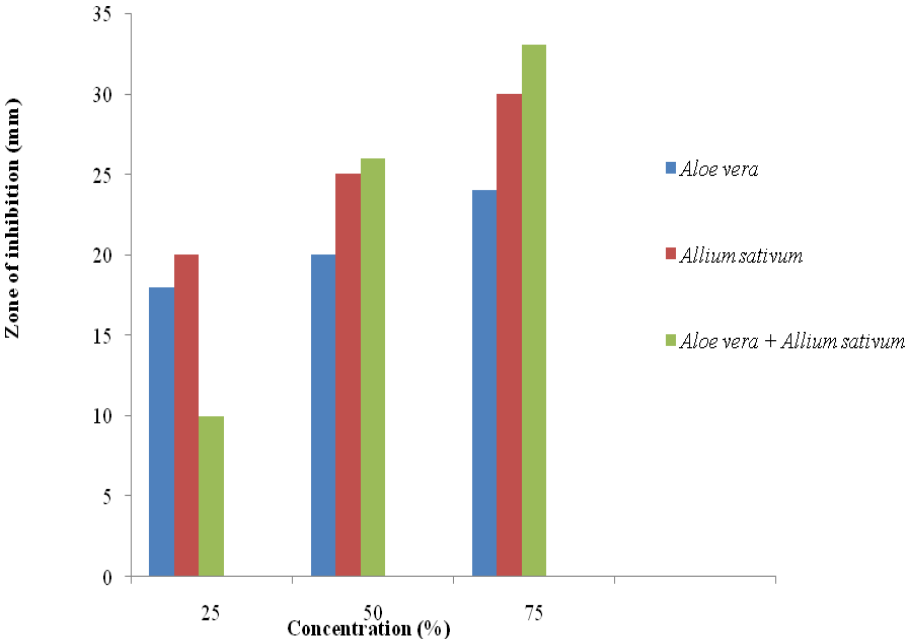

Figure 1: Inhibition effect of *Aloe vera* and *Allium sativum* on the growth of *Salmonella* Gallinarum

Allium sativum showed to be highly effective than *Aloe vera* at both low and high concentration. This proves that *Allium sativum* has stronger inhibitory effect than *Aloe vera*. In other words *Salmonella* Gallinarum is highly sensitive to *Allium sativum* than *Aloe vera*. Several studies including that of Kwon *et al*. (2011) have shown that *Aloe vera* aqueous extract demonstrated excellent antimicrobial activity against *Escherichia coli* and *Vibrio* spp after being tested against *Staphylococcus aureus*, *Bacillus* spp, *Enterococcus* spp, *Escherichia coli*, *Salmonella* typhimurium, *Pseudomonas aeruginosa* and *Vibrio spp*.

Also previous studies have shown that antimicrobial activities of aqueous garlic extracts against 133 multidrug resistance bacteria including *Staphylococcus aureus*, *Staphylococcus epidermids*, *Staphylococcus pneumoniae*, *Staphylococcus pyogenes*, *Haemophilus influenzae*, *Salmonella* typhi, *Pseudomonas aeruginosa*, *Escherichia coli*, *Shigella* spp. and *Proteus* spp. whose diameters of zone of inhibition were studied and characterized to range from 20.2-23.7 mm for gram positive and 19.8-24.5 for gram negative (Iwalokun *et al*., 2004; Durairaj *et al*., 2009). The variation in zone of inhibition amongst researchers could be due to variation in climate, soil type, pH, plant subspecies or varieties, arbitrary concentration used, and extraction methods. Other literatures have shown that antimicrobials extracted from the ethanol are highly effective than those extracted from aqueous solution.

CONCLUSION

The combined concentration level of *Aloe vera* and *Allium sativum* showed high inhibition effect at concentration level of 50% and 75% than the corresponding concentration level of the individual. Inhibition activity of combined concentration level of *Aloe vera* and *Allium sativ*um aqueous extract at 50% could be used to treat veterinary cases in poultry caused by *Salmonella* Gallinarum which is resistant to the common prescribed antibiotic at low cost. However further research is required to be conducted to determine minimum inhibitory concentration (MIC) of the combined concentration and to determine whether there is toxicity at the concentration level of 50% and 75% of combined level of *Aloe vera* and *Allium sativum* (which exemplified to be more effective) in animal body especially poultry. The use of these natural medicinal plants in poultry production could reduce the cost margin and increase the profit margin. *Allium sativum* and *Aloe vera* can be processed and refined in the pharmaceutical industries at required concentration and sold to the farmers at low cost for inhibition and control of *Salmonella* Gallinarum in poultry production.

ACKNOWLEDGEMENTS

This work was funded by the High Education Student Loan Board. We are thankful to Mr. Mgusi the laboratory technician for the technical assistance. Much gratitude goes to Prof. P.N. Wambura for permission to conduct the experiment in the Microbiology Laboratory at the Faculty of Veterinary Medicine.

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