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EFFICACY OF ANTIMICROBIAL ACTIVITY OF GARLIC EXTRACTS ON BACTERIAL PATHOGENS COMMONLY FOUND TO CONTAMINATE MEAT

L. NJUE, L.W. KANJA, J.N. OMBUI, J.G. NDUHIU and D. OBIERO

ABSTRACT

***Background*: Meat is a major source of food and raw materials for a number of industries, yet a lot of meat is wasted each year due to deterioration as a result of spoilage by micro-organisms such as *Pseudomonas, Acinetobacter, Moraxella, Bacillus, Campylobacter, Escherichia, Listeria, Clostridium, Salmonella* and *Staphylococcus* species.**
***Objective*: To determine efficacy of antimicrobial activity of garlic extracts on bacterial pathogens commonly found to contaminate meat.**
***Design*: A cross sectional study.**
***Setting*: The Department of Public Health, Pharmacology and Toxicology, Faculty of Veterinary Medicine University of Nairobi.**
***Subjects*: Garlic from Nganoini farm in Laikipia County, Kenya**
***Results*: The results indicated that garlic absolute ethanol extract had the highest efficacy of antimicrobial activity inhibiting all test micro-organisms.**
***Conclusion*: Ethanolic extract can be used as a meat preservative or decontaminant.**

INTRODUCTION

Meat is generally an animal muscle, which is a compound of water, protein and fat used as food (1). It is a major source of protein other than plants. Sources of meat include chicken, beef, camel, pork and other domestic and wild animals, which range from small bats and lizards, to larger mammals such as antelope and buffalo. Seafood includes fish, shrimps, oysters, crab meat (2). Its consumption regulates vitamin D metabolism and prevents metabolic borne diseases such as bone fractures and rickets (3). Various trace elements such as iron, (haem iron) in a form that can be well absorbed are found in it.

Global meat production has tripled in the past three decades and could double its present level by 2050 (4). In Kenya the livestock sector contributes 3.3% of the gross domestic product (GDP), mainly from cows, sheep, goats and poultry (5). In the World livestock sector contributes 46% of the gross domestic product (GDP) (4). The quality and safety of meat can easily deteriorate when improperly preserved due to spoilage. The most common cause of meat spoilage is the micro-organisms (6). Indication of spoilage includes production of ammonia or sulfur smell, and bad odor, due to degradation of proteins, lipids (fats) and carbohydrates caused by bacteria and/or enzymes naturally present in meat (6). Some micro-organisms produce toxins on meat making it unfit for human consumption. For example *S. typhi* contains an endotoxin typical of Gram negative organisms, as well as the Vi antigen which is thought to increase virulence. *B. cereus* causes food poisoning due to the toxins it produces when the *bacilli* sporulates especially on foods like meat, and meat products (7).

Meat contamination by micro-organisms mainly occurs through operations carried out in animal husbandry, processing, preparation, treatment, packaging and transporting and also from the environment (3). There were many experiments undertaken to prolong the shelf-life of meat and meat products. Organic acids are generally recognised as safe (GRAS) antimicrobial agents, and the dilute solutions of organic acids (1-3%) are generally without effect on desirable sensory properties of meat when used as a carcass decontaminant (8). It is clear that the surface treatment of carcasses by spraying with organic acids solution reduces the surface microbial

counts and thus increases the shelf-life and provides food safety. At the time of slaughter, the meat is almost sterile so that the primary contamination concerns in particular the meat surface. Later the micro-organisms penetrate into deeper layers of meat. When this primary contamination is reduced, the shelf-life of meat can be significantly prolonged. Thus it is advantageous to decontaminate the surface of carcasses to increase their shelf-life and to enable the safe distribution.

A number of methods can be used to decontamination meat. The mostly used methods are organic acids such as acetic acid, lactic acid, formic acid and propionic acid which act by decreasing pH, and due to their bactericidal properties, stops growth of bacteria. These acids are often used for surface decontamination as they are natural component of meat produced during post-mortem glycolysis and thus they are not typical additives. The antibacterial efficacy of organic acids depend on several factors such as the type of the acid used, pH of the medium, concentration and temperature of the acid solution, type of the food product, initial microbial load (9), the methods of application, dipping time (10) and the inherent resistance of the target microorganism to the acid used (11).

Preservation methods used to inhibit pathogen growth on meat include use of salts, irradiation, drying, refrigeration and smoking (12). Salts like sodium chloride dehydrate micro-organisms but do not retard growth of pathogenic halophiles such as *Staphylococcus aureus* which grow readily even in 7.5% salt concentration (13). The use of refrigeration is common in urban areas but is not widely available to rural community due to poverty and lack of electricity. Nitrites/nitrates like Sodium nitrite inhibit the germination of *Clostridium botulinum* spores. However they react with amines to form nitrosamines that are carcinogenic and can cause high blood pressure (3).

Garlic is one of the most commonly used ingredients as a flavor enhancement agent for meat products. But in addition to flavoring the foods, garlic is appreciated for its medicinal properties. Garlic has a wide spectrum of activity; including antibacterial, antiviral, antifungal and antiprotozoal activities. It also has beneficial effects on the cardiovascular and immune systems (14). During the last decade, the antimicrobial activity of garlic and garlic-derived organo-sulfur compounds were widely investigated against both food spoilage bacteria and food-borne pathogens (15). Garlic-rich organo-sulfur compounds and their precursors (allicin, diallyl sulfide and diallyl trisulfide) are believed to play a key role in these biological effects (16).

Organic acids (alcoholic) and aqueous garlic extracts contain primarily S-allyl-l-cysteines derived from γ-glutamyl-S-allyl-l-cysteines (17), S-Allyl-l-cysteine and trans-S-1-propenyl-l-cysteine, together with a small amount of S-methyl-l-cysteine, are found in garlic extract such as aged garlic extracts (AGE). These cysteine derivatives are colorless crystals and are odorless and stable in the solid state or aqueous solution under neutral or slight acidic conditions (17). The proprietary aging process produces an odorless preparation and converts the harsh, unstable organo-sulfur compounds in garlic (e.g., allicin) into milder and more beneficial compounds including water-soluble, sulfur-containing, antioxidant rich amino acids such as S-allylcysteine (SAC), S-allyl mercaptocysteine (SMAC) and Maillard reaction products. It is worth noting that SAC has a 98% absorption rate into the blood giving it robust bio-availability. SAC is the key compound in AGE and is used to standardise it (18).

This aim of this study was to determine the efficacy of antimicrobial activity of garlic extracts that can be exploited for use as an alternative meat preservative to replace the currently used preservatives which have serious health effects on consumers.

MATERIALS AND METHODS

Preparation of garlic aqueous extracts: Garlic cultivar used was Italian, a selection of Creole from Italy (19). Garlic was collected from Nganoini farm Laikipia County in Kenya. Garlic used was less than four months old.

Preparation of garlic cloves: Garlic bulbs were washed with distilled water in a clean basin to remove any soil adhering on the surface. This also softened the outer dry skin for easier peeling. After rinsing the bulbs with clean distilled water, the washed garlic was placed in another clean basin to dry. The garlic cloves were then peeled and placed on an aluminum foil ready for weighing.

Preparation of garlic extracts

Methanol extractss: One hundred grams of the peeled garlic cloves were weighed on a clean aluminium foil using a weighing balance (Mettler pm 4600,Deltarange, Zurich). They were then put in an electric blender(Ohms, Internationalfzc,China) and 125 ml of 99.8% methanol (AR) was added. The Mixture was blended to make a paste. More garlic cloves and methanol were added to the paste to fill up to three-quarters' of a 100 ml flat bottomed flask. The mixture was homogenised and put in a flask (1000 ml) and kept in a dark cabinet for 24 hours. Shaking was done in the morning and in the evening to homogenise all the flask contents. The content was filtered using whatman's paper No.1. The resulting filtrate was evaporated using rotary evaporator (Rotor Vapour Pump, Laboratoriums-Technic Ag, Buchi)

at 40°C to remove methanol solvent. The remaining content was put in sterile plastic containers and kept at -20°C (26). This procedure was repeated with 70% methanol, 99.8% and 70% ethanol.

Aqueous extract (Sterile distilled water extract): One hundred grams of the peeled garlic cloves was weighed using an aluminum foil on a weighing balance. They were then put in an electric blender and 125 ml of sterile distilled water was added. The mixture was blended to make a paste. The content was then centrifuged at 5000 rpm for ten minute. The supernatant was removed using sterile syringes and placed in a sterile plastic container. The content was then sterilised by filtering using Whatman's paper No.1 pore size 0.45μm according to (20). For the aged garlic extract it was prepared by weighing one hundred grams of the peeled garlic cloves on a clean aluminium foil using a weighing balance (Mettler pm 4600,Deltarange, Zurich). They were then put in an electric blender(Ohms, Internationalfzc, China) and 125ml of sterile distilled water was added. The Mixture was blended to make a paste which was put in clean ember-colored winchester bottle. Blending of garlic cloves and water was repeated until the paste filled up to three-quarters' of 2.5l clean ember-colored winchester bottle. Five such bottles were filled with the paste and kept away from light. Shaking was done in the morning and in the evening to homogenise all the bottle contents. This content remained in bottles for one year, hence called Aged Garlic Extract (AGE). This extract was filtered same way as for aqueous extract for sterility and kept at -20°C for 24 hours before freeze dryeing.

Bacteria cultures: Four standard bacterial strains; *Bacillus cereus* (ATCC 11778), *Salmonella typhimurium* (ATCC 72225671), *Staphylococcus aureus* (ATCC 25925) and *Escherichia coli* (ATCC 25922) were obtained from Microbiology laboratory, Department of Public Health, Pharmacology and Toxicology, Nairobi, Kenya.

Efficacy of antimicrobial activity of garlic extracts and their combinations: The direct colony suspension method was used to make the standard bacteria in saline to a density of McFarland 0.5 turbidity standard, which corresponded to 1-2x108 CFU / mL (21). Using a sterile loop, the adjusted bacteria suspension was swabbed evenly over the entire surface of Mueller Hinton agar (Oxoid, England) plate. Agar diffusion method was employed to test the efficacy of garlic extracts. Using a standard cork borer (11 mm) two duplicate wells were made at equidistance on the inoculated Muller Hinton agar (9 cm), and the agar plug aseptically removed. All the garlic extracts were between 99.9% and 70% concentrated. The volume of each test extract used was 100μl of the extract prepared at a concentration ratio of extract to water of 1:1 mg / ml, and was transferred into the well using sterile micropipette tips. The extracts used were in singles and in combinations (Table 1). The same volume of 1.5% Acetic acid, 10μ which acted as a positive control and sterile distilled water (negative control) were used. Antibiotics used as positive controls were Ampicillin 10μg per well and Ciprofloxacin 5μg per well. Plates were incubated at 37^o C for 18 h and inhibition zones diameters (mm) measured and recorded.

Measurements of zones of inhibitions, on Mueller Hinton agar with micro-organisms, were taken from wells measuring 11 mm in width dispensed with 100μl volume of each extract with a concentration of 1:1 ml / ml, and expressed in mm according to National committee for clinical laboratory standards (21). Sterile distilled water was used as a negative control.

RESULTS

Analysis of the effects of the extracts on the micro-organisms showed that they were more effective on *B .cereus* and *S. aureus. Escherichia coli* was susceptible to Methanol 99.8%, Ethanol 99.9%, Methanol 70%, Ethanol 70%, and Aged garlic water extract. *Staphylococcus aureus* was susceptible to Methanol 99.8% and Ethanol 99.9% extracts. *Salmonella typhimurium* was susceptible to methanol 99.8%, Ethanol 99.9%, methanol 70% and Aged garlic water extracts. *Bacillus cereus* was susceptible methanol 99.8%, Ethanol 99.9%, Methanol 70% and Ethanol 70% extracts, were susceptible to. All micro-organisms were susceptible to 99.8% methanolic and 99.8% ethanolic extracts. Gram positive micro-organisms were the most susceptible than gram negative micro-organisms.

The highest antimicrobial activity for *S. aureus* and *B. cereus* with 99.9 % Ethanol extract was 28mm and the lowest was 19mm for *S. typhimurium*. The highest antimicrobial activity with methanolic extracts was 18mm for *E. coli* and lowest (14mm) for *S. typhimurium*. The highest antimicrobial activity was 32 mm using Methanol 99.8% and Ethanol 99.9% extracts.

The pH of the single extracts were six except Methanol 99.8% and Ethanol 99.9% which had pH of 5, and aged garlic extract pH 4. The various combined extracts had a pH of 5, except the following, Water extract + Methanol 70% + Ethanol 70% (pH 6), Water extract + Methanol 70% (pH 6) Methanol 70% + Ethanol 70% (pH 6).) Ethanol 70% + AGE water extract pH 4. The pH of Acetic acid was 2.

Table 1
Antimicrobial activity of different garlic extracts towards gram positive and gram negative bacteria

Garlic extraction reagent (solvent)	Inhibition zones in mm of the standard gram negative and gram positive micro-organisms			
	E. coli ATCC 25922	*S. typhimurium* ATCC72225671	*B. cereus* ATCC 11778	*S.aureus* ATCC 25925
99.8% Methanol extract A	18[S]	14 [S]	14 [S]	14 [S]
99.9% Ethanol extract B	20 S	19 [S]	28 [S]	28 [S]
Sterile distilledWater extractD	22 [S*]	22 [S*]	32 [S*]	32 [S*]
70 % Methanol Extract E	16 [S]	18 [S]	26 [S]	26 [S*]
70% Ethanol extract F	19 S	20 [S*]	30 [S]	30 [S*]
Aged garlic extract (Sterile distilled water) H	15 [S]	18 [S]	14 [S]	14 [S]
Methanol 99.8%+ Ethanol 99.9% +Water N extract	23 [S*]	22 [S]	27	33 [S*]
Methanol 99.9%+ Ethanol 99.9%ExtractO	25 [S]	24 [S]	32 [S]	29 [S*]
Methanol 70% + Ethanol 70% extract X	20 [S*]	21 [S*]	26 [S*]	26 [S*]
Ethanol 70% + aged garlic extract(distilled water) Y	16 [S*]	18 [S*]	21 [S*]	15 [S*]
Acetic Acid1.5%(+ve control)	26 [S]	25 [S]	34 S	34 [S]
Ampicillin (+) 10μg per well	36 [S]	0	17 [S*]	19 S
Ciprofloxacin (+) 5μg per well	45 [S]	38 [S]	34 [S]	38 [S]
Water(-)	0	0	0	0

Key: s = Susceptible, s* = Though the inhibition zone was more, the extract was not chosen as there appeared to be a haze (Plate 1) thus considered to be less effective.

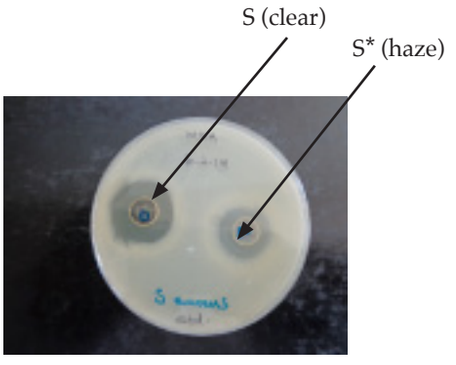
Table 2
Antimicrobial agents and zones of inhibition in millimeters (6)

Antimicrobial agent	Disk content	Zone diameter(mm)			MIC/ml		
		S	I	R	S	I	R
Ampicillin	10μg	≥ 17	14-16	≤13	≤8	16	≥ 32
Ciprofloxacin	5μg	≥ 21	16-20	≤15	≤1	2	≥ 4

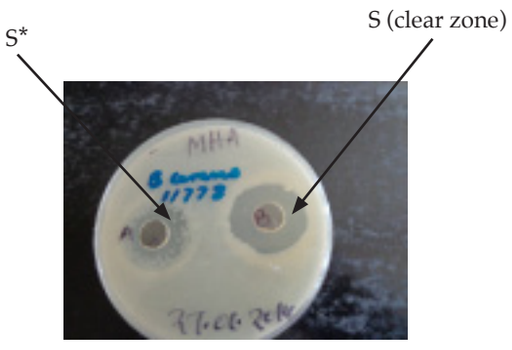
S=Susceptability, I= Intermediate R= Resistance

Plate 1

*Showing inhibition zones for S.aureus 25925 A1 and B.cereus 11778, one with a clear zone S and with a haze S**



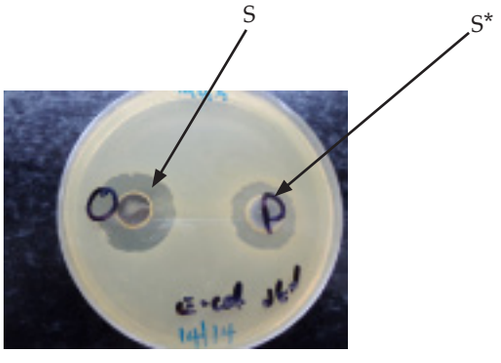
A[1]



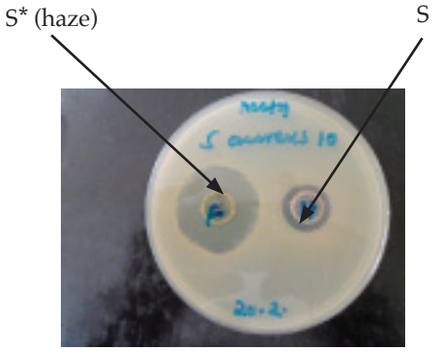
A[2]

Plate 2

*Showing inhibition zones for E. coli 25922 A1 S. aureus 25925 , one with a clear zone S and with a haze S**



A[3]



A[4]

DISCUSSION

Garlic (Allium sativum L.), is among the oldest cultivated plants used both as a food and for medicinal applications. The value of garlic extracts and combinations lie in their ability to provide essential phytochemicals that have antibacterial activity against pathogens. They are able to provide phytochemicals for treatment and prevention of a number of diseases such as cancer, coronary heart disease, obesity, hypercholesterolemia, diabetes type 2, hypertension, cataract and disturbances of the gastrointestinal tract (e.g. colic pain, flatulent colic and dyspepsia) (21).

The results of this study indicate that the various garlic extracts had antibacterial activity. According to Table 1, gram positive micro-organisms were more sensitive to the extracts than gram negatives. The thick structural constituents of gram-positive microbes in this instance can be held responsible for the increase in the interaction between the active compounds and the structural lipoprotein (22). Gram negative bacterial cultures had some variation in diameter which was mainly due to the cell peptidoglycan and lipopolysaccharide of bacteria cell wall (20). Gram negative were more resistant than gram positive. This may be due the outer membrane which further blocks the penetration of the active compounds, making them resistant. Naidu (15) suggested that the mechanism of antibacterial action of spices like garlic involve the hydrophobic and hydrogen bonding of phenolic compounds to membrane proteins, membrane disruption and destruction of electron transport systems and cell wall disruption.

The factors responsible for the high susceptibility of both gram positive and gram negative to organic solvents may be attributed to the secondary metabolites of garlic, including γ-glutamyl peptides, scordinins, steroids, terpenoids, flavonoids and other phenols, which may be responsible for the range of the antibacterial effects reported for garlic extracts (21). These metabolites work antagonistically and as a result micro-organisms can never develop resistance depending on the method of extraction. In case of B. cereus endospores do not form normally during active growth and cell division. Rather, their differentiation

begins when a population of vegetative cells passes out of the exponential phase of cell growth which occur usually as a result of nutrient depletion (23). This explains why it was most susceptible in all the extracts. The data presented by Jaber and Al-Mossawi (24) showed that *S. aureus* was more susceptible than *E. coli*, and a similar observation was made in this study. These result indicates the predominance of gram-negative organisms such as *E. coli* as reported by other groups (25). The presence of zoonotic bacteria such as *Brucella* and *Listeria* in meat although not isolated in our study indicates poor ante-mortem inspection of the animals as well as unhygienic meat processing (27). To find the prevalence of drug resistance bacteria, assays or susceptibility profiles were performed. High level resistance of bacteria isolates to various classes of antibiotics was observed.

When compared with the standard antibiotics, the zones of inhibition due to garlic extracts were within the acceptable range (27) of susceptibility Table 2, although there are those that developed a very light haze (faint growth) plates 1 and 2. This occurs when agent, such as the garlic extract is bacteriostatic (27).

Most extracts and combinations with 99.9% and 99.8% had a pH of 6 as compared to others which had a PH of 4 and below. Low pH may have resulted from fermentation, creating an acidic environment that led to inhibition of the micro-organisms. Ethanolic extract with a pH of 6 had the highest antimicrobial activity Inhibition of the micro-organisms may have been due to the extracted garlic compounds which have an added advantage to the meat consumer.

Aged garlic extract had the lowest antimicrobial activity. Aged garlic extract had been fermented for one year resulting to a pH of 4 and formation of acids from allicin such as water soluble amino acids like S-allyl cysteine (SAC) and S-allyl mercaptocysteine (SMAC) (18), which could be behind the extract's reduced microbial activity. This could also be attributed to the fact that active compounds in garlic reduce with time of storage and especially allicin derivatives (with odor) to leave odorless compound S-L-systeine (18). All extracts with a combination of water had a reduced pH 4-5 and could have led to reduced microbial inhibition as a result of fermentation. In case of Bacillus specie, endospores do not form normally during active growth and cell division. Rather, endospore differentiation begins when a population of vegetative cells passes out of the exponential phase of growth, usually as a result of nutrient depletion. Typically one endospore is formed per vegetative cell (23). This explains why it was most susceptible in garlic.

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