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Preliminary study on the antibacterial activity of garlic (*Allium sativum*) on *Salmonella*, *Shigella*, and *Escherichia coli*

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ABSTRACT

This experiment was conducted to see if garlic would inhibit the growth of *Salmonella*, *Shigella* and *Escherichia coli*. This helps to understand the effectiveness of home remedies such as natural herbs including garlic for medicinal purposes. This study focuses on the significant antibacterial activity of garlic (*Allium sativum*) extract against gram negative bacteria like *Salmonella*, *Shigella* and *E. coli*. The plant parts are known to have antimicrobial activity on bacteria which are known to be pathogenic to animals in general and to humans in particular. The plant extracts were inoculated on prepared media of microbial culture containing *Salmonella*, *Shigella* and *E. coli* species. The result obtained indicates garlic has a strong effect on *Salmonella* by reducing the number of colonies that survive due to exposure to the antimicrobial agent which is approximately similar to that of *Salmonella* but *E. coli* species showed some percent of resistance in comparison to *Salmonella* and *Shigella*.

KEYWORDS: Garlic, *E. coli*, *Salmonella*, *Shigella*, Allicin

INTRODUCTION

Garlic (*Allium sativum*) is a herb used widely as a flavoring in cooking and has also been used as a medicine throughout ancient and modern history to prevent and treat a wide range of conditions and diseases. Note that Garlic is one of the most known traditional herbal remedies for a wide range of ailments. It's used to improve blood circulation to reduce the risk of heart disease. Garlic is also thought to help reduce high cholesterol and elevated blood pressure. Note that garlic also contains antioxidants that help to remove environmental toxins and waste products of normal body pressure in the blood. Antioxidants help to contain and remove those harmful substances before they can accumulate and damage the body.

The oils extracted from the species and herbs are found to be effective in killing bacteria, viruses and others. Garlic is used for many conditions related to the heart and blood system. These conditions include high blood pressure, high cholesterol, coronary heart disease and hardening of arteries.

The infectious rate of microorganisms in developing countries remains high. Diseases continue to be a problem where nutrition, and sanitary conditions are poor and emerging disease is more dangerous for such a population. In Ethiopia, studies indicate that common bacterial infection is *Salmonella*, *E. coli*

and *Shigella*. The mode of transmission is through water and food contamination. In urban and rural areas of Ethiopia, these sanitary conditions are poor. This condition exposed the population to those strains of bacteria. Disease due to food-borne pathogens also remains a problem largely due to the consumption of improperly processed and stored food. Understanding the source of contamination and developing ways to limit the growth of pathogens is the role of education (WHO, 2001).

Salmonella, *Shigella* and *E. coli* bacteria are the most common problems causing food poisoning and disease. This study was aimed at the antibacterial activity of garlic (*A. sativum*) on bacterial growth. Ethiopia has various topographic landforms and biodiversity of plant species which are applicable for traditional medicine among these traditional plants the one widely used is garlic. However, the effectiveness of garlic has not been scientifically evaluated. This kind of research contributes to scientifically evaluating and increasing the use of garlic to reduce infection (Jonkers *et al.*, 1999a).

Many studies have been conducted on different plant species of traditional medicine and in Ethiopia garlic has been considered to be a wonder drug for the treatment and prevention of variety of diseases but for generations, people have information of its medicinal value as garlic has been widely used as antibiotic

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and treatment of cardiovascular disease, bites, tumors, ulcer, wounds, cancers, measles and many more (Jonkers *et al.*, 1999a). Vaccines and antibiotics have lessened the impact of pathogens in the developed world. But microbial infection in developing countries is high and new illnesses caused by microorganisms continue to emerge and known pathogens become resistant.

Salmonella infection is a common bacterial disease that affects the intestinal tract. Typically it lives in animal and human intestinal and is shed through feces. Humans are infected most frequently through contaminated water and food. *Salmonella* is the second most frequent bacterial infectious disease in the world. Infection with *Salmonella* includes fever, abdominal pain and diarrhea.

E. coli bacteria normally live in the intestines of animals. Most *E. coli* are harmless and actually an important part of a healthy human intestinal tract. However, some *E. coli* are pathogenic meaning they can cause illness either diarrhea or illness outside of the intestinal tract. *Shigella* also causes intestinal disease of shigellosis. The main sign is diarrhea, fever, abdominal pain and malaise, but it is easily treated as a bacterial infection.

The purpose of this study was to evaluate the antibacterial activity of garlic (*A. sativum*) extract against *Shigella*, *Salmonella* and *E. coli*.

The Concept of Traditional Medicine

Traditional medicine remains the most affordable and easily accessible source of treatment in the primary health care system of resource-poor communities and local therapy is the only means of medical treatment for such communities. Traditional medicine is defined as health practices, approaches, knowledge and beliefs incorporating plant, animal and mineral based medicines, spiritual therapies, manual techniques and exercise, applied singularly or in combination to treat, diagnose and prevent illness and maintain well-being. It is known that many countries in Africa, Asia and Latin America use traditional medicine to meet some of their primary health care needs. In Africa, up to 80% of the population uses traditional medicine for primary health care (WHO, 2002).

Traditional medicine has maintained its popularity in all regions of the developing world and its use is rapidly spreading in the industrialized countries. For example, in China, it accounts for 30%-50% of total medicinal consumption. In most African countries, about 70% with high fever resulting from malaria uses herbal medicine at home (WHO, 2002).

Characteristics of Traditional Medicine in Ethiopia

Ethiopia has a long history of traditional medicine and has developed ways to combat disease through it and the ways are also as diverse as different cultures. The majority of the population (90%) that lives in Ethiopia depends mainly on traditional medicines to meet their healthcare needs (WHO, 2005). In the country healing the protection of and promotion

of human physical, spiritual, social, mental and material well-being are based on traditional medicine. It is widely believed in Ethiopia that the skill of traditional health practitioners is given by God and knowledge on traditional medicine is passed orally from parent to favorite child, usually a son or is acquired by some spiritual procedure. Traditional healing knowledge is guarded by certain families or social groups (Lambert, 2001).

Healers obtain their drugs mainly from natural substances. Drugs are prepared in various dosage forms including liquids, ointments, powders and pills. Drugs were administered using different routes; the main ones are being topical, oral and respiratory. When side effects became severe, antioxidants were claimed to be used. In this content, garlic has been used for its medical properties for thousands of years, however, investigations into the mode of its action are relatively recent. Medicinal plants like thyme, lemon, ginger, lavender, and onions are used extensively today. Among these plants, the most intensively and widespread used is garlic (*A. sativum*) (Ellmore & Feldberg, 1994).

History of Garlic

Native to central Asia, garlic is one of the oldest cultivated plants in the world and has been grown for over 500 years. Ancient Egyptians seem to have been the first to cultivate this plant which played an important role in their culture (Rivlin, 2001). Garlic began to find its way into other cultures and every life of ancient civilizations such as Greece, Rome, Northern Europe and China. The medicinal purposes and properties of garlic eventually became known throughout the known world and celebrated in pre- and recorded history. In folklore, garlic was attributed with the ability to bring good luck, protect against evil forces, serve as aphrodisiac, repel scorpions and sorcerers, werewolves, warlocks and vampires (mostly because of its odorous quality) among other things. It was also used to increase strength and life-energy, improve blood circulation, respiratory disorders, intestinal disorders, and conditions such as diarrhea, dysentery and flatulence, worms, skin disease and other ailments (Pizzorno & Murray, 1996).

Garlic was not only bestowed with sacred qualities and placed in the tomb of pharaohs, but it was given to the slaves that built the pyramids to enhance their endurance and strength. This strength enhancing quality was also horned by ancient Greek and Roman civilizations whose athletes ate garlic before sporting events and whose soldiers consumed it before going off to war. Garlic was introduced into various regions throughout the globe by migrating cultural tribes and explorers. Garlic is one of the powerful medicinal foods that exemplify the Hippocratic expression 'Use your food as your medicine' (Dylan, 2009).

Throughout the millennia, garlic has been a beloved plant in many cultures for its catenaries and medicinal properties. Over the last few years, it gained unprecedented popularity since researchers have been scientifically validating its numerous health benefits. China, South Korea, India, Spain, and the

United States are among the top commercial producers of garlic (Pizzorno & Murray, 1996).

The major flavour component of garlic (*A. sativum*; Liliaceae/ Alliaceae) is a thiosulfinate called allicin. This compound is formed when garlic tissue is damaged as a hydrolysis product of S-allyl cysteine sulphoxide (alliin) brought about by the pyridoxal phosphate-dependent enzyme alliinase. Under these conditions, alliin is cleaved by an elimination reaction, and two molecules of the sulfenic acid form allicin (McElnay & LiWan, 1991).

Medicinal Properties of Garlic

Garlic is a plant that kills bacteria, fungi, and parasites, and is also used as an anti-tumor agent. Garlic with >200 chemical substances in the body, has the capacity to protect against many illnesses. Although it is said that garlic should be consumed as fresh for it to be effective, garlic cloves include a mixture of mono and polysulfides smelling very heavy. Some researchers argue that in some situations it should be cooked and waited for extracts and oils can provide better protection against free radicals and infection than fresh garlic (Concagul & Ayaz, 2010).

Garlic is used both medicinally for a range of skin and stomach problems and also in preparing food, particularly some kinds of stew and in making dried food for storage (Egziabher & Edwards, 1997). Garlic is used for a variety of reasons, and some of the attributes associated with it, e.g. for cancer prevention, or to reduce heart attacks, may not be substantiated. Other properties such as antimicrobial activity, effects on lipid metabolism, and platelet aggregation inhibitory action have been demonstrated. Ajoene is a potent antithrombotic agent through inhibition of platelet aggregation.

How to use Garlic as Medicinal Remedy

Garlic preparations used medicinally include steam-distilled oils, garlic macerated in vegetable oils (e.g. soybean oil), dried garlic powder, and gel suspensions of garlic powder. Analyses indicate wide variations in the nature and amounts of constituents in the various preparations. Thus, freshly crushed garlic cloves typically contain allicin (about 0.4%) and other thiosulfates (about 0.1%, chiefly allyl methyl thiosulfinate) (Lawson *et al.*, 1991). Bad breath and perspiration odours which often follow the ingestion of garlic, either medicinally or culinary, are due to allyl methyl sulphide and disulphide, diallyl sulphide and disulphide, and 2-propenethiol. Plant-eating insects are best controlled by pesticides made from strong-smelling plants such as garlic (Conant & Fadem, 2012). Sitting or standing a lot can make hemorrhoids worse. But sitting in a cool bath or lying down can help. Some women say it helps to soak a clove of garlic in vegetable oil and then insert it into the anus (Klein *et al.*, 2013).

According to Werner *et al.* (2015) report a drink made from garlic may help get rid of pinworms. Chop finely, or crush, 4 cloves of garlic and mix with 1 glass of liquid (water, juice, or milk). Dosage: Drink 1 glass daily for 3 weeks. These authors also reported that to treat vaginal infections with garlic: you

can also use a clove of garlic as a vaginal insert. (Peel the garlic, taking care not to puncture it. Wrap it in a piece of clean cloth or gauze, and put it into the vagina). Use the douche 2 times during the day, and each night insert a new clove of garlic for 10 to 14 days.

The best remedy uses fresh, uncooked, crushed organic cloves – used in warm tea or mixed with raw, unprocessed honey, fresh garlic juice may also be utilized. Garlic also is found as powder and pearl or a capsule of oil. For the use of garlic in small children, it is best to simply rub raw crushed garlic on the bottom of their feet and apply socks. To combat the high level of cholesterol, triglycerides, and blood sugar, maintain a regular daily of intake of one to three fresh cloves of garlic. For internal bacterial, fungal, viral, parasite infections, and for respiratory, congestion, consider fresh garlic tea or syrup with honey. However, fresh garlic is always the best choice. For prevention of wounds or treatment of wounds, you can use cool compresses of garlic tea, honey garlic syrup, and fresh minced garlic syrup.

Raw garlic is used for antibacterial, viral and anti-fungal properties that help to boost the immune system and help to fight infection. Even though garlic is a potent herbal medicine it can adversely interact with other herbal drugs. The primary infection fighting compound in garlic is called allicin. *A. sativum* and its derivatives have been proposed as promising candidates for maintaining the homeostasis of the immune system. The garlic then appears to enhance the functioning of the immune system by stimulating certain cell types, such as macrophages, lymphocytes, natural killer (NK) cells, dendritic cells, and eosinophils, by mechanisms including modulation of cytokine secretion, immunoglobulin production, phagocytosis, and macrophage activation (Arreola *et al.*, 2015).

Pathogenic Bacteria

Microbial infection in developing countries is highly caused by microorganisms and causes disease and known pathogens to become resistant. Different strains of bacteria are associated with many illnesses and conditions in different parts of the organism. Infection of diarrhea is leading to morbidity and death worldwide. Most bacterial pathogens that causes diarrhea include *Salmonella*, *Shigella*, and *E. coli*.

Salmonellosis: Salmonellosis is the second most frequent bacterial infectious disease in the world and infection with *Salmonella* includes diarrhea, fever, and abdominal pain. Small children will be in or near the hut in close contact with soil contaminated with feces where *S. typhi* may abound. Infection in man by the *Salmonella* parasite is closely associated with the sanitary habits of people in handling food and drinks. Transmission is through exposure to contaminated food and water. Environmental sanitation is a very important factor in such transmission.

Shigellosis: *Shigella* infection cause abdominal pain, fever and malaise and also *E. coli* associated with severe diarrhea disease commonly known as hemolytic uremic syndrome.

In Ethiopia, studies indicate that common bacterial infections are *Salmonella*, *Shigella* and *E. coli*. The mode of transmission is through contamination of water and food. Diseases due to food borne pathogens also remain a problem largely by consumption of improperly processed and stored food.

Despite the riches of Ethiopian traditional medicinal plants the scientific studies for the use of traditional medicinal plants as a source of modern medicine are not well developed when compared to the industrialized nation. There are a number of plants whose medicinal properties are not properly known and it needs a lot of investigation to be functional from the wealth of traditional medicinal plants. Bacterial strains selected for this study (*Salmonella*, *Shigella*, and *E. coli*) are the most common bacterial infections in the rural and urban areas of Ethiopia. Their mode of transmission is associated with poor sanitary conditions of food and water. Several studies have proposed that natural compounds in plants could offer a new strategy for developing therapies against bacterial infection. The local people are highly dependent on the traditional plants and many indigenous plants have the efficiency to cure more than one disease and according to the study garlic (*A. sativum*) is considered as a high fidelity level in treating more than one disease. Therefore increasing scientific studies on this plant species increases the chance of success in developing an alternative drug (Jonkers *et al.*, 1999a).

MATERIALS AND METHODS

Garlic Collection and Preparation of its Aqueous Extract

Garlic (*Allium sativum*) was collected from the market of Holeta town. The 100 grams of the edible portion was chopped and cleaned (Figure 1). Cleaned cloves were surface sterilized by immersing them in up to 70% ethanol for two minutes. Ethanol on the surface was evaporated by airflow, followed by homogenized aseptically in a sterile mortar and pestle. The homogeneity was then filtered by sterile cheesecloth to give a crude aqueous extract of 100 mL. This was collected in a sterile vial and stored in the refrigerator until the test of bacterial activity.

Microbial Strain used in this Study

The pure cultures of bacteria *Salmonella*, *Shigella*, and *E. coli* were obtained from the microbiology laboratory of Holeta Agricultural Research Institute. The study was also conducted in the microbiology laboratory of Holeta Agricultural Research Institute.

Media Preparation

In this study, nutrient agar was formulated for the growth of three strains of tested bacteria that includes *Salmonella*, *Shigella* and *E. coli*. Nutrient agar media support the growth of the total of three coliforms on agar. For this investigation 12 (twelve) agar media were prepared, 3 (three) for each strain of bacteria and 3 (three) for the control group that was corresponding to the formulated test tubes.

Microbiological Analysis

Inoculation of specimens was done following standard procedures. Specimens were inoculated on blood agar (Oxoid, Hampshire, UK), MacConkey agar (Oxoid, Hampshire, UK) and Chocolate agar (Oxoid, Hampshire, UK) (Cheesbrough, 2006).

Antibacterial Test

The antibacterial activity of the plant extracts was evaluated using the agar well diffusion method as described by Balouiri *et al.* (2016). The garlic extract was taken from the refrigerator and using a sterilized pipette it was spread in prepared separate media in different percent of concentrations. The selected bacteria specimen was dropped into a nutrient agar medium and incubated for 48 hrs at 37 °C in the incubator as described by Hughes (1999).

Antimicrobial Susceptibility Pattern

Antibacterial susceptibility tests were carried out by cork borer method on Muller Hinton agar plate (Bauer *et al.*, 1996) with slight modifications. Pathogen bacterial suspension of the isolate was spread on the Muller Hinton agar plate with the help of sterile cotton swab uniformly and 20 µL of the extracts were added separately and incubated at 37 °C overnight, then the diameter of inhibition zones were measured to determine on the resistance of microorganisms tested.

Data Analysis

The collected quantitative data were analyzed using simple descriptive statistics such as percentages, mean and frequency while narration was used for the qualitative data. Finally, results observed from the analysis were presented in the graphs.

RESULTS AND DISCUSSIONS

The antibacterial test with a different volume of aqueous garlic extract showed a growth inhibition in three tested bacterial

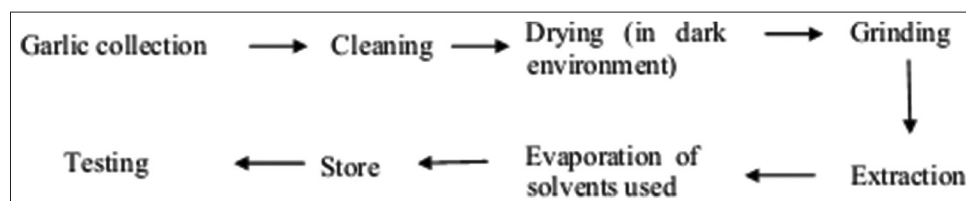


Figure 1: Conceptual framework of the study

species. The garlic aqueous extract in *Salmonella* showed a least growth inhibition but in *Shigella* there is somewhat resistance that means when we compare with *Salmonella* and *E. coli* it showed a medium resistivity. But *E. coli* showed a high resistivity to garlic extraction when compared to *Salmonella* and *Shigella*. The result obtained from the antibacterial test in the study was presented in Figure 2.

The highest percentage indicates that the bacteria were resistant to the antimicrobial agent and the lowest percentage shows that the bacteria were susceptible to antimicrobial agents.

The 100% shows that the bacteria without treatment or in the control group showed its growth at maximum level.

The Figure 3 shows that the percentage resistance of *E. coli* is the highest degree of resistance to antimicrobial agents when compared to others and *Salmonella* showed the highest degree of susceptibility when compared to the other two strains of bacteria.

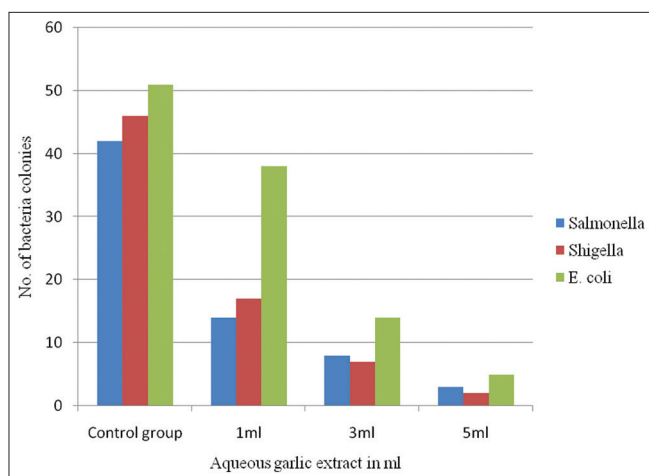


Figure 2: Efficacy of aqueous garlic extract against numbers of colonies formed by three species of bacteria

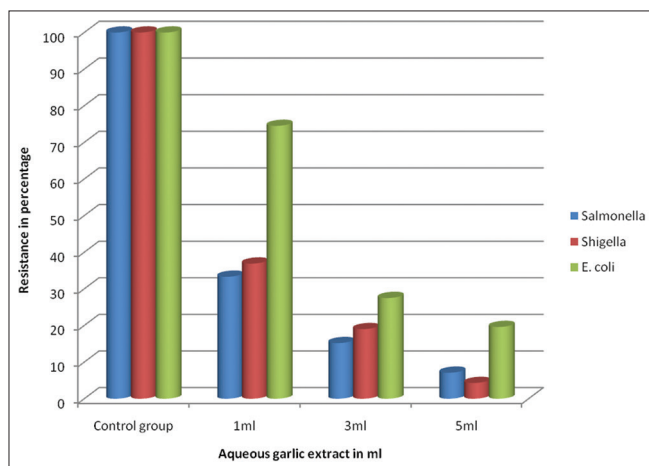


Figure 3: Percentage of resistance of tested bacteria versus different concentrations of aqueous garlic extract.

Figure 4 indicates that aqueous garlic extracts have shown growth inhibition in three tested bacteria.

The antibacterial activity of the aqueous garlic extracts was seen for three pathogenic bacteria; *Salmonella thyphi*, *Shigella*, and *E. coli* (Figure 4). The zone of inhibition produced (in mm) was found to be 14, 16, and 20 respectively (Figure 5).

At 3 mL of aqueous garlic extract, it showed inhibition against the three bacteria (Figure 5). The zone of inhibition ranges from 14 to 20 mm indicating the potential antimicrobial property of the extracts.

Although studies on the action of garlic (*Allium sativum*), have shown that such extracts exhibit in vitro effects against nematodes, it was often not possible to confirm this in vivo tests with infected animals (Bastidas, 1969; Iqbal *et al.*, 2001; Burke *et al.*, 2009), or the degree of action was very low unless very large amounts were used (Ayaz *et al.*, 2008).

A. sativum derived compounds such as allicin and diallyl sulfide have reported anthelmintic activity to *Trichinella spiralis* (Grundzinsky *et al.*, 2001; Abu El Ezz, 2005). Garlic is said to repel mosquitoes if consumed in sufficient amounts (Fradin, 1998). Many fungi are sensitive to garlic, including *Candida* (Yousuf, 2011), *Torulopsis*, *Trichophyton*, and *Cryptococcus* (Fromtling & Bulmer, 1978). The adhesion of *Candida* is also greatly reduced in the presence of garlic extract (Ghannoum, 1990).



Figure 4: Halo zone formation by aqueous garlic extract on the three bacterial isolates

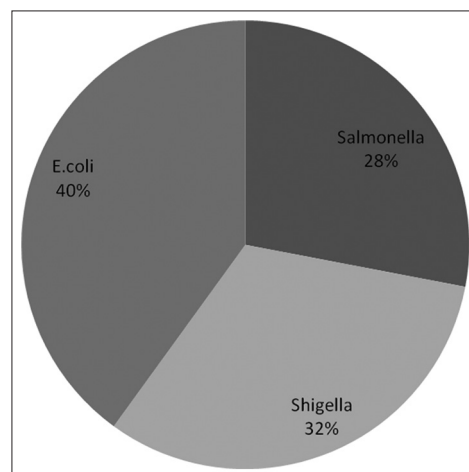


Figure 5: Zone of inhibition in mm at 3 mL of aqueous garlic extract

In the present study extracted garlic was effective on pathogenic bacteria. The tested bacteria associated with gastrointestinal infection include stomach pain, diarrhea fever, nausea, wound and vomiting. It has been reported that garlic exhibited antifungal effects on two species, the air-borne pathogen *Botrytis cinerea* and *Trichoderma harzianum* (Lanzotti *et al.*, 2012). Greater satisfaction with the use of garlic rather than nystatin was reported by the patients with denture stomatitis (Bakhshi *et al.*, 2012).

Aqueous garlic extract has stronger effectiveness on tested *Salmonella* and *Shigella*. In *E. coli* colony formation was inhibited to some extent but not as much as satisfactory compared to *Salmonella* and *Shigella*. Aqueous garlic extract has shown antibacterial effects on *Helicobacter pylori* and vancomycin-resistant enterococci (Jonkers, 1999b; Sivam *et al.*, 1997). The antibacterial effect has also been shown by the synergism between ciprofloxacin with garlic extract against some multi-resistant bacteria (Al-abdeen *et al.*, 2013).

The pure stem of garlic showed growth inhibition in three tested bacteria. In *Salmonella*, the pure stem extract reduces the growth of colonies by more than half. When we compared the numbers of colonies of *Salmonella* in garlic stem extract with the controlled group, in similar conditions in all aspects and garlic stem extract in the experimental group 1 mL, 3 mL, and 5 mL showed to inhibit the growth 42 colonies to 14, 8, and 3 colonies respectively this indicates that garlic stem has strong growth inhibition on *Salmonella*. Similarly in *Shigella*, the extract shows an unexpected number of growth inhibitions. But in *E. coli* it is not much effective as compared to others because of the resistance of *E. coli* to different antibiotics and antibacterial agents. Some studies in Ethiopia on the stem of garlic and other traditional medicinal plants showed an effective anti-microbial activity on *E. coli* and other antibiotic resistance bacteria; this difference may be due to environmental conditions that support the organic compounds of the plant.

A clinical trial carried out on patients that had giardiasis (Soffar & Mokhtar, 1991) showed that garlic used as an anti-giardial, removing the symptoms from all patients within 24 hr and completely removed any indication of giardiasis from the stool within 72 hr at a dosage of 1 mg/mL twice daily aqueous extract or 0.6 mg/mL commercially prepared garlic capsules. This result experimentally checked that the pure extract of garlic has this kind of efficiency on disease causing pathogens. Therefore it is scientifically proven that extended use of the stem of garlic as a medicine for gastrointestinal infection will minimize the risk of *Salmonella* and *Shigella*.

E. coli that were resistant to different antibiotics showed some percent of growth resistance reduction, according to the data *E. coli* have high percent of growth resistance with 74.5%, 27.5%, and 19.6% for the 1 mL, 3 mL, and 5 mL respectively when compared to other of two strains of bacteria.

Since the findings of this study showed that garlic has an inhibitory effect on *E. coli*, *Salmonella* and *Shigella* it could

be used for future medicinal purposes in battling bacterial infection.

CONCLUSION

Garlic extract showed remarkable efficiency with different effectiveness rate on gram negative bacteria such as *Salmonella*, *Shigella* and *E. coli*. The result obtained from this study could serve as an evident that Ethiopian traditional medicinal plants have great effectiveness on different pathogenic organisms. It also warrants a further investigation in this traditional medicinal plant for developing a modern anti-microbial drug by clinical testing but at this stage the data obtained from this study can be taken as an approval document for local people to use as a medicinal for disease related to gastro intestines. So to make it clearer and check scientifically the effectiveness of garlic needs further investigation on different pathogens.

RECOMMENDATION

In the process of change of habits, community participation is essential. Without grassroots participation, not much could be achieved in the usual top-down management practices. Creating awareness of the antibiotic properties of garlic and giving important evidence regarding its antibacterial activity of garlic is recommended. The involvement of the envisaged beneficiaries of any program at all levels of planning and implementation is absolutely necessary.

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