



Evaluation of Anti Bacterial Activity of Fresh Plant Extracts on Salmonella, Shigella and E.Coli

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1. INTRODUCTION

Medicinal plants are local heritage with global importance. The medicinal plants are useful for healing as well as for curing of human diseases because of the presence of phytochemical constituents. Garlic (*Allium sativum* L) is an herb used widely as a flavoring in cooking has also been used as a medicine throughout ancient and modern history to prevent and treat wide range of conditions and diseases. Garlic also thought to help reduce high cholesterol and elevated blood pressure. Note that garlic also contains anti oxidants that help to remove environmental toxins and west product of normal body pressure in the blood. The oils that extracted from the species and herbs are found to be effective in killing bacteria, viruses and others [medical center report 1995].

Garlic is used for many conditions related to heart and blood system these conditions include high blood pressure, high cholesterol coronary heart

disease and hardening of arteries. These were supported by science and also ability to combat the common cold [Desta B, 1994].

Zingiber officinale L (ginger), a horizontal, branched, fleshy, aromatic white to yellow coloured perennial herb with leafy stem up to 60 cm, has long been used in the field of medicine. It belongs to the family Zingerberaceae. Its leaves are narrow being 20 cm long and 1.5-2cm wide. Dense spiked, yellow green with purple ending flowers are seen [Ross, I.A. 2005]. The rhizome is rich in secondary metabolites such as phenolic compounds (gingerol, paradol and shogaoal), volatile sesquiterpenes (zingiberene and bisabolene) and monoterpenoids (curcumene and citral) Ginger has been widely used all over the world in ayurvedic medicine, for a wide array of unrelated ailments including arthritis, cramps, rheumatism, sprains, sore throats, muscular aches, pains, constipation, vomiting, hypertension, indigestion, dementia, fever and infectious diseases [Ali et al, 2008]. It has direct anti-microbial activity and thus can be used in treatment of bacterial infections [Tan Bkh and Vanitha J. 2004]. Ginger are relatively inexpensive due to their easy availability, universally acceptable and well tolerated by the most people. It has also been "Generally Recognized as Safe".

Prunus persica (Aaru) belongs to the family Rosaceae is a deciduous tree up to 10 m high commonly cultivated for edible fruits from sub-Himalayan region up to 2400 m [Raturi R et al 2011]. Peach (*Prunus persica* L.), from the family Rosaceae, are one of the most widely consumed fruits during the summer season. The fruits, with somewhat sour and astringent taste, are low in caloric content but have high nutritive value. They contain natural sugars (sucrose, glucose and fructose), organic acids (citric acid and malic acid), fiber (pectin), tannins, and no saturated fat [Anwar F et al. 2014].

Infectious rate by micro organism in developing countries is remaining high. Diseases continue to be a problem where nutrition, sanitary conditions are pure and emerging disease is more dangerous for such population.

In Ethiopia studies indicate that common bacterial infection is salmonella, E.coli and shigella. The mode of transmit ion is through water and food

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contamination. In urban & rural area of Ethiopia these sanitary condition are poor. This condition exposed the population by those strain of bacteria. Disease due to food borne pathogens also remains a problem largely by consumption of improperly processed and stored food. Understanding the source of contamination and developing ways of limits the growth of pathogen is the role of education [WHO, 2001].

The study is conducted to minimize the disease causing bacteria. Typically salmonella, shigella and E.coli because of that the bacteria are the most problems of our food poisoning and causing disease. And the main problem of this study was the result of antibacterial activity of garlic (*Allium sativum* L) on bacterial growth. Ethiopia has various topographic land forms and biodiversity of plant species are applicable for traditional medicine making purpose among these traditional plants can be widely used are garlic is mentioned. But the effectiveness of the garlic has not been scientifically evaluated. This kind of research contributes to scientifically evaluate and increase the user garlic to reduce infection [Jonker D et al .1999].

Many studies have been conducted on different plant species of traditional medicine and in Ethiopia especially, garlic has been considered to be wonder drug for treatment and prevention of variety of disease but for generation people have information of its medicinal value through garlic has been widely used as antibiotic and treatment of cardiovascular disease, bites, tumors, ulcer, wounds, headaches, cancers, measles and many mores [Jonker D et al .1999].

Micro organisms are pathogenic organism vaccine, antibiotic and many other advances have lessened the impact of pathogens in the developed world. But microbial Infection in developing countries is

high new illness caused by micro organisms continue to emerge and known pathogens becoming resistant, however when we observe specifically its difficult [medical center report 1995].

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

E.coli bacteria normally live in the intestine of animals. Most of E.coli are harm less and actually important part of healthy human intestinal tract. However, some E.coli are pathogenic meaning they can cause illness either diarrhea or illness outside of intestinal tract. Shigella also a intestinal disease causing by shigellos. the main sign is diarrhea, fever, abdominal pain and malaise. But it is easily treated bacterial infection. [medical center report 1995]. this study has the following reasons: given appropriate treatment: because of the plant by nature rich in different nutrients and ability of antimicrobial activity then give effective treatment. It's the best way for the discoveries of other medical plants: because of Ethiopia have a large biodiversity of floras species. From thus large groups of flora there are numbers of plants have medical properties but they are not properly known. Therefore Ethiopia flora needs an extra investigation for discoveries and effective form of treatment from these species. Significance for society in order to give awareness by easy way of protecting health by using botanical plants to eradicate the disease causing by bacterial species.

Table 1: List of Botanicals and Parts used in Investigation

No.	Scientific Name	Common Name	Parts Used	Local Name
1	<i>Prunus Persica</i> L	Peach	Fresh Leave	Kock
2	<i>Zingiber Officinale</i> L	Ginger	Fresh Root Part	Jinjible
3	<i>Allium Sativum</i> L	Garlic	Edible Part	Nech Shinkurit

II. MATERIALS AND METHODS

a) Study Area

The study was conducted in Hawassa university main campus, which is found in SNNPE Regional State, Hawassa city is found at a distance of 273 kilometer from Addis Ababa in the south direction. 7°3'N 38°28'E / 7.050°N 38.467°E and an elevation of 1708 meters above sea level. Hawassa city has a tropical savanna climate though it borders on a subtropical highland climate.

b) Plants Collection

Botanical plants were distributed in the rural and urban areas of the world. By using its botanical identities

about plants sample for this study was collected from the market around 10k.m from Hawassa University Main Campus. To conduct this study the sample was taken from the healthy stem of garlic (*Allium sativum*), seed of peach leave (*prunus persica*) and root of ginger (*zingiber officinale*).

c) Preparation of Aqueous Plant Extracts

By using glove the fresh plant parts were collected from vicinity farm and 100 grams each plant part portion was chopped and cleaned. Cleaned parts were sterilized by immersing them up to 70% ethanol for two minute. Residual ethanol on surface was evaporated by air flow, followed by homogenized

aseptically in sterile mortar and pestle. 5ml distilled water used to get enough aqueous solution. The homogeneity was then filtered by sterile cheese cloth to give a crude aqueous extracts of 10ml of each plant part was collected in sterile vial and stored in refrigerator until test of bacterial activity.

d) Source of Bacterial Strain

A Total of three gram-negative bacteria isolates species were selected for study. The isolates were obtained from the microbiology laboratory of Hawassa University. Bacterologically the isolates were identified as salmonella, shigella, and E.coli by using standard procedure.

e) 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 total three microorganisms on agar. For this investigation (Four) agar media including 0.5ml 1ml, 1.5 ml 2ml, plant extracts aqueous and control group (positive and negative) were prepared for each strain of bacteria that was corresponding to the formulated test tubes.

f) Procedures and Anti-bacterial Test

The plants extract were taken from refrigerator and by using sterilized pipette it was spread in prepared

separate media in different of concentration (0.5 ml, 1 ml, 1.5 ml 2ml of each plant extracts aqueous) and control group. The selected bacteria specimen dropped in to nutrient agar medium and incubated for 48hrs by 37 degree Celsius in the incubator.

g) Preparation of Colonies of Bacteria

After preparing suitable media agar add the inocula on the nutrient and put in incubator for 48 hrs to get Colonies. After days via counting using hand lens only 50 colonies of bacteria for each species were marked on the agar then plant extracts added to media agar which contain colonies put in incubator for 48 hrs and count how much is eradicated.

h) Data Analysis

All data were checked for normality before they were subjected to analysis. Data which lacked normality were transformed using appropriate transformations method. Data were analyzed with analysis of variance (ANOVA) using General Linear Model (GLM) in SAS software. Significant means were separated using Student-Newman Keuls (SNK) test.

III. RESULTS

Table 2: Fresh Garlic (*Allium Sativum L*) aqueous extraction of toxicity with different volume of and mean of eradication of colonies formed by three species of bacteria

Treatment Doses	Mean \pm SE Number of Colonies (within 48 hrs)		
	Salmonella Typhimurium	Shigella Dysentery	Escherichia Coli
0.5 ml	72.20 \pm 2.30 C	66.98 \pm 2.50 E	24.63 \pm 4.60 F
1 ml	76.15 \pm 1.54 C	60.25 \pm 4.23 E	42.00 \pm 3.52 G
1.5 ml	84.31 \pm 3.21 A	80.67 \pm 3.12 A	60.54 \pm 4.02 H
2 ml	92.75 \pm 0.21 B	88.55 \pm 1.20 F	68.28 \pm 1.20 J
Ampicillin (positive control)	98.56 \pm 0.10 B	96.69 \pm 13.5 B	90.23 \pm 1.26 B
Negative Control	0.00 \pm 0.00D	0.00 \pm 0.00 D	0.00 \pm 0.00 D
P - Value	<0.001	<0.001	<0.001

Mean followed the same letters in column showed not significantly different using Student-Newman-Keuls (SNK) test ($P < 0.05$).

Table 3: Fresh Ginger (*Zingiber Officinale L*) aqueous extraction of toxicity with different volume of and mean of eradication of colonies formed by three species of bacteria

Treatment Doses	Mean \pm SE Number Of Colonies (Within 48hrs)		
	Salmonella Typhimurium.	Shigella Dysentery	Escherichia Coli
0.5 ml	66.41 \pm 2.42 C	67.18 \pm 3.60 C	30.21 \pm 4.36 C
1 ml	69.12 \pm 1.24 C	60.31 \pm 4.01 C	45.25 \pm 3.69 E
1.5 ml	80.60 \pm 5.21 A	75.25 \pm 2.51 A	62.00 \pm 3.35 A
2 ml	94.50 \pm 0.23 B	87.10 \pm 2.21 E	65.00 \pm 1.25 A
Ampicillin (positive control)	98.00 \pm 0.01 B	96.30 \pm 0.13 B	92.31 \pm 0.65 B
Negative Control	0.00 \pm 0.00 D	0.00 \pm 0.00 D	0.00 \pm 0.00 D
P - Value	<0.001	<0.001	<0.001

Mean followed the same letters in column showed not significantly different using Student-Newman-Keuls (SNK) test ($P < 0.05$).

Table 4: Fresh Peach Leaves (*Prunus Persica L*) aqueous extraction of toxicity with different volume of and mean of eradication of colonies formed by three species of bacteria

Treatment Doses	Mean \pm SE Number Of Colonies (Within 48 Hrs)		
	Salmonella Typhimurium	Shigella Dysentery	Escherichia Coli
0.5 ml	52.71 \pm 4.42 C	56.38 \pm 3.73 C	20.00 \pm 4.10 C
1 ml	63.58 \pm 2.35 E	60.65 \pm 3.12 C	32.21 \pm 3.34 E
1.5 ml	74.39 \pm 1.64 A	70.11 \pm 2.22 A	50.10 \pm 2.25 A
2 ml	82.34 \pm 1.22 B	78.01 \pm 2.01 A	60.00 \pm 2.89 E
Ampicillin (positive control)	98.10 \pm 0.01 B	96.27 \pm 0.21 B	90.51 \pm 2.10 B
Negative Control	0.00 \pm 0.00 D	0.00 \pm 0.00 D	0.00 \pm 0.00 D
P - Value	<0.001	<0.001	<0.001

Mean followed the same letters in column showed not significantly different using Student-Newman-Keuls (SNK) test ($P < 0.05$).

IV. DISCUSSION

Table 1 showed that fresh garlic (*Allium sativum*) extraction toxicity against colonies of different bacteria species. Different dosage rates of Garlic (*Allium sativum*) aqueous extraction significantly eradicate colonies of salmonella, shigella and E.coli. As showed in Table 1 that there were high mean eradication of human pathogenic bacteria was recorded in the application high amount aqueous garlic (*Allium sativum*) extraction, for instance 2 ml aqueous extraction of this plant had better elimination of colonies on agar while compared with lowest milliliter aqueous extraction.

The preset study of three bacteria species revealed that they had their own resistance capability against extractions as per high to low amount. For example, in the highest and lowest application of extracts E coli had high mean resistance against (*Allium sativum*) when it compared with salmonella, shigella, i.e. higher numbers of colonies were observed, on the

contrary shigella had the lowest resistance to the garlic (*Allium sativum*) which means shigella was more susceptible. There was high significance difference ($p < 0.0001$) between treatments and ampicillin for the eradications of colonies except at high dosage rate of garlic was no significance difference to be the reduction of salmonella compared with Ampicillin

For the present investigation fresh ginger (*zingiber officinale*) aqueous extraction of antimicrobial activity had been evaluated which is written in table 2. More or less this botanical extraction had similar effect with garlic against for the formation of colony. Moreover there was no significance difference between high dose of ginger (*zingiber officinale*) and positive control. For instance, mean of 94.50% and 98.00% were recorded for ginger (*zingiber officinale*) and ampicillin respectively. The above percentage showed that Salmonella was highly susceptible to positive control and high dose of ginger (*zingiber officinale*). This study agreed with [Bkh and Vanitha J. 2004] ginger has direct anti-microbial

activity and thus can be used in treatment of bacterial infections. Additionally agreement approved with [Ali et al 2008] Ginger (*Zingiber officinale*) is a medicinal plant that has been widely used all over the world, since antiquity, for a wide array of unrelated ailments including arthritis, cramps, rheumatism, sprains, sore throats, muscular aches, pains, constipation, vomiting, hypertension, indigestion, dementia, fever and infectious diseases.

Furthermore, as indicted in table 2 there was high mean reduction of bacteria colony formation had been observed due to application of treatments when compared with Negative control. High susceptibility of Salmonella spp And Shigella spp was observed at higher dosage (1.5 ml and 2ml) of ginger (*zingiber officinale*) aqueous extraction. In contrast, Ecoli spp had high resistance against the above mentioned botanical. Like garlic (*Allium sativium*) the extraction of ginger (*zingiber officinale*) had similar toxicity for the eradication of bacteria colony that grown on agar media. This is happened at low and high doses of treatments. The present study revealed that negative control of all treatments which mentioned in above tables showed there were no eradication colonies i.e. mean of 0.00% resistance was recorded.

Peach leaves (*prunus persica*) antimicrobial activity was also evaluated and it showed eradication of bacterial colonies at different dose. There was no significance difference in mean eradication of Salmonella typhimurium among all treatment especially at highest dose of (2 ml) aqueous extractions. For example, *Allium sativium*, *zingiber officinale* and *prunus persica* had showed mean of 92.75%, 94.50% and 92.34% respectively.

According to the researchers [Tuba Sevgi and Elif Demirkan.2017] peach (*prunus persica*) fruits contain phenolic additives which may show more or less antimicrobial effects. Depending on their antioxidant properties phenolic substances, which have effect mostly on color, flavor and durability of fruits and vegetables, are closely related with human health in terms of antimicrobial, anti-carcinogenic and anti-mutagenic activities. In the long term, bacterial resistance against antimicrobial agents may cause problems in fighting against several diseases. Therefore investigation of novel antimicrobial agents derived from new and natural sources have become important.

Additionally, the peach plant antimicrobial activity was investigated by the authors [Ved Prakash et al, 2017] and they revealed that the antibacterial activity of methanol, acetone and aqueous leaf extracts of *Prunus persica* was determined *in vitro* against medically important pathogens such as *Escherichia coli*, *Yersinia pestis*, *Bacillus cereus*, *Pseudomonas aeruginosa*, *Listeria monocytogenes* and *Staphylococcus aureus* following agar well diffusion method using different concentrations (25%, 50%, 75% and 100%). Results

showed low to significant antibacterial activity against the mentioned bacterial species. Methanol leaf extract was found to be more effective against selected pathogenic bacteria as compared to acetone and aqueous leaf extracts. Furthermore, the leaf extracts inhibited gram-positive bacteria more efficiently than gram negative bacteria. The present study was targeted on fresh leave extracted and showed antimicrobial activity as indicated in the above tables.

On the other hand, this study was evaluated the toxicity of ginger against bacteria at different of doses of extraction and showed high eradication colonies at high dosage. The current study was in agreement with previously investigations. For example, ginger (*Zingiber officinale*) had some effect on pathogenic bacteria. The plant extracts were prepared by weighing the plant leaves and roots (20, 40, 60, 80 and 100 g) into 100 mls of water and ethanol (at g/100 ml) and grounded to determine the extract concentrations. Serial dilutions of the antibiotics used were prepared to determine the various antibiotic concentrations. The results obtained showed that ginger extract of both the plant and root showed the highest antibacterial activity against *Staphylococcus aureus* and *Streptococcus pyogenes* while the three antibiotics used (chloramphenicol, ampicillin and tetracycline) were also active but at less extent compared to ginger extract. The concentration of the plant extract had significant effect on the zone of inhibition on both organisms [A. Sebiomo et al 2011].

In summary, the present study was basically focused on lonely evaluate fresh botanical extracts according to dosage rates. As indicated in the above table *Allium sativium*, and *zingiber officinale* had toxicity against bacteria cells or colonies at all dosages as compared with *prunus persica* and negative control. Moreover, *Allium sativium* and *zingiber officinale* botanicals had almost similar effect with positive control (Ampicillin) in the eradication of colonies. Generally all treatments at different dosage had high toxicity effect than negative control. The authors recommended that further studies should be forwarded to extract or isolate the exact composition several botanical plants and create awareness among societies as a number of medicinal plants should be taken as diets.

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