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Comparative Evaluation of Antibacterial Effect of Garlic and Ginger Crude Extracts against Standard and Clinical Isolated Pathogens

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Abstract: Medicinal plants have a long history of use and their use is widespread over world countries. Particularly garlic (*Allium sativum*) and ginger (*Zingiber officinale*) has been used for centuries to combat infectious diseases. The local traditional healers are using these two plants as antimicrobial agents either in mixture or in individual. This research is therefore needed to evaluate and compare their potency and efficacy against various infectious diseases caused by different bacterial pathogens. Garlic and ginger crude extracts were extracted with ethanol, methanol, sterilized distilled water. The crude extracts of ethanol, methanol and sterilized distilled water were subjected to antibacterial sensitivity test against Standard and clinical isolated bacterial pathogens. All data were analyzed using the program SPSS software package version. Means and standard deviations of the triplicates analysis were analyzed by one - way analysis of variance (ANOVA) to determine the significance differences. In this study, the antibacterial efficacy of the crude extracts of both ginger and garlic had been determined against two-gram negative (*E. coli* and *K. pneumonia*) and one gram positive bacterium (*S. aureus*). Agar well diffusion method was used to determine the antibacterial susceptibility test of garlic and ginger crude extracts. The mean inhibition zone of ethanolic crude extract of ginger (18.00mm) against *S. aureus* (standard) was significantly ($p \leq 0.05$) far greater than the remained extractants. Natural spices of garlic and ginger possess effective anti-bacterial activity against sstandard and clinical isolated pathogens and can be used for prevention of microbial diseases.

Key words: Garlic Extract · Ginger Extract · Test Microorganism · Well Diffusion · Zone Of Inhibition

INTRODUCTION

Medicinal plants have a long history of use and their use is widespread over world countries. According to the report of the World Health Organization 80% of the world's population rely mainly on traditional therapies which involve the use of plant extracts or their active substances. The herbal medicines may be in the form of powders, liquids, or mixtures, which may be raw, or boiled, ointments, liniments and incisions [1].

Many medicinal plants are documented to have antimicrobial activity like neem, tulsi, ginger, garlic etc. among which garlic (*Allium sativum*) is belonging to *Alliaceae* family. Apart from cooking, garlic also known for its medicinal values. It is most commonly used in Asia also in India. In India it is used in various forms like garlic powder, garlic oil or whole garlic. Property of garlic like anti-tumor, in cardiovascular disorder, in liver damage already documented. It also shows effect on the blood pressure, blood sugar and cholesterol [2].

Apart these the garlic powder shows the power of killing the pathogens and antimicrobial activity. Ginger (*Zingiber officinale*) belongs to *Zingiberaceae* family, widely used as medicinal plant. Apart from its uses in arthritis, cramps, sprain, constipation, vomiting, hypertension, fever also have the antimicrobial property [3].

Garlic belongs to a family of *Alliaceae* and its scientific name is *Allium sativum*. Other members of the family include onion, leek and shallot. Garlic is widely used in culinary and medicine [4]. It has a pungent hot flavor but mellows and improves with cooking. It has been utilized to fight infections such as cold, cough, asthma, diarrhea, flu, headache, sore throat, abdominal discomfort and respiratory tract infections [5].

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The antibacterial properties of crushed garlic have been known for a long time. Various garlic preparations have been shown to exhibit a wide spectrum of antibacterial activity against Gram-negative and Gram-positive bacteria including species of *Escherichia*, *Salmonella*, *Staphylococcus*, *Streptococcus*, *Klebsiella*, *Proteus*, *Bacillus* and *Clostridium*. Even acid-fast bacteria such as *Mycobacterium tuberculosis* are sensitive to garlic. Analysis of steam distillations of crushed garlic cloves performed over a century ago showed a variety of alkyl Sulfides which are isolated and identified as the component responsible for the remarkable antibacterial activity of crushed garlic cloves. The compound turned out to be an oxygenated sulfur compound which they termed allicin from the Latin name of the garlic plant, *Allium sativum* [6].

Both garlic and ginger have high anti-bacterial activity against pathogenic gram positive and gram negative bacteria. Traditionally, most of the local communities used these plants as general important spices for their health to prevent diseases caused by various pathogenic micro organisms. The local traditional healers also used these two plants as antimicrobial agents either in mixture or in individual for the treatment of various ailments caused by microbes. However, the perception of each community and local traditional healers is different about the potency and efficacy of both plants, i.e., some community and local healers said garlic is more effective than ginger whereas some others said that ginger has more antibacterial activity than garlic. Therefore, conducting this research is needed to compare and evaluate their potency and efficacy against various infectious diseases caused by different pathogens. The objective of this study was to evaluate and compare the antibacterial effect of garlic and ginger crude extracts against standard and clinical isolated pathogens.

MATERIALS AND METHODS

Description of the Study Area and Duration of the Study: The study was conducted at Mekelle University, Ayder Referal Hospital, College of Health Science particularly at Medical Microbiology Laboratory. The research work was commenced on January 5, 2019 and completed on June 30, 2019. Mekelle is located at 39°29' E longitude and 13°30'N latitude with an elevation of 2000 m.a.s.l. and it is far away 783 kms from Addis Ababa and is in the northern part of Ethiopia. Its climate condition is categorized under the Ethiopian highlands. The mean annual rainfall of the study area is 628.8 mm. The annual minimum and maximum temperatures are 11.8°C and 29.94°C, respectively [7].

Research Design: The research design was experimental using appropriate method like determination of antibacterial sensitivity testing. This helped to investigate the potential comparative evaluation of antibacterial efficacy of garlic and ginger crude extracts against standard and clinical isolated pathogens.

Sample Collection: Garlic (*Allium sativum*) and ginger (*Zingiber officinale*) used in the present study were purchased from the local market of Mekelle City. Then, placed in separated clean mesh bags and transported immediately to the lab.

Preparation of Garlic and Ginger Extracts: The collected/purchased plant materials were thoroughly washed in running tap water to remove debris and dust particles and then rinsed in distilled water and peeled. After that each plant was spliced into small pieces using knife and crushed using clean mortar and pestle separately. Then after, the crushed plants were dried in the shade area of the laboratory in an open air at room temperature for about seven days and were protected from sun light. After completely dried, these were grounded to a fine powder using a clean mortar and pestle and the powder was stored in a clean bottle at room temperature in dark place until use.

Then From the powdered plant samples, about 50g from each plant were weighed by using an electronic balance and extracted with each 250 ml of sterile distilled water, ethanol and methanol in six labeled separated flasks then covered by aluminum foil. After that, it was shaked for about 3 days on shaker. Then, crude extracts of garlic and ginger were filtered using Whatman No. 1 filter paper and each six beakers that contain filtered crude extracts were measured in sensitive balance after that evaporated the alcohols at 40°C rotary evaporator. Then, in order to determine the differences after and before evaporating, the filtered crude extracts were measured in sensitive balance. Each extracts were transferred to glass vials and then stored at 4°C refrigerator until use [8].

Sources of the Test Organisms: Microorganisms (both standard and clinical isolated pathogens) used in this study were obtained from Ayder Referal Hospital. The microorganisms used in this study were

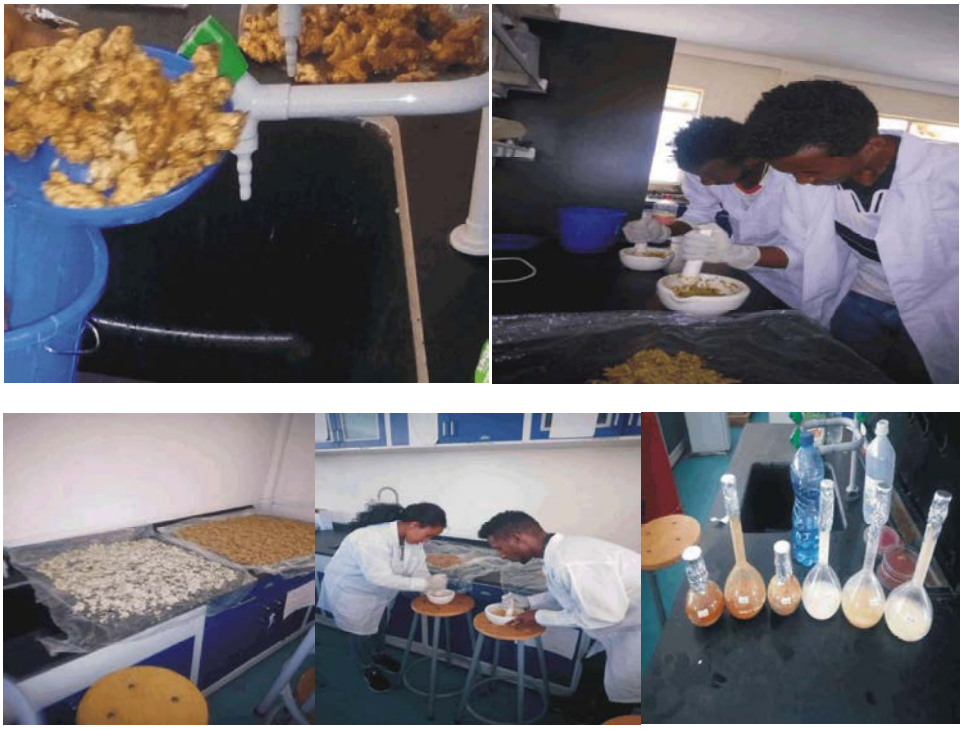

Fig. 1: Preparation of plant extracts

Staphylococcus aureus, Escherichia coli and *Klebsiella pneumonia.* The first two were standard whereas the third was clinical isolated bacterium. The bacterial cultures were maintained in their appropriate nutrient broth at 4°C until use.

Preparation of Inoculum: The three test microorganisms were separately cultured on nutrient agar at 37° C for 24 hrs. by using streak plate method. Then, three to five well-isolated overnight cultured colonies of the same morphological type were selected from an agar plate culture. The top of each colony was touched with a flamed wire-loop and the growth was transferred into a test tube containing 5 ml of normal saline solution.

Media Preparation: Mueller-Hinton agar was prepared from a commercially available dehydrated base according to the manufacturer's instructions. The media was weighed using electronic balance and mixed with the required sterile distilled water. Then, the mixture was boiled using a hotplate and then autoclaved for 15 min at 121°C. Soon after autoclaving, the agar was allowed to cool and placed inside a water bath at about 50°C to maintain the media in a molten stage (to minimize the amount of condensation that forms). Then, the agar medium was allowed to cool to room temperature in the laminar flow hood prior to pouring it into the Petri-plate. Plates were dried faster in lower humidity by keeping them in a laminar flow hood. The freshly prepared and cooled medium was poured into flat-bottomed Petri dishes on a level, horizontal surface to give a uniform depth of approximately 4 mm. This was achieved by pouring 20 ml of the medium for plates with diameters of 100 mm.

Antibacterial Sensitivity Test of Garlic and Ginger Crude Extracts: To evaluate the antibacterial activities of each plant crude extracts obtained by different solvents, well diffusion method was employed. Muller Hinton agar media was prepared according to the manufacturers' instruction. The weighed media and mixed with the required sterile distilled water was boiled in a hot plate and was autoclaved at 121°C for about 15 minutes. After cooling down the media, it was poured in to 18 different labeled Petri dishes and solidified in the laminar air flow. Then after, small volume (100 µl) of bacterial suspensions were added to each Mueller Hinton (MH) agar plate and then evenly seeded and streaked by means of sterile swab on the agar plate surface. This procedure was repeated by streaking two more times, rotating the plate approximately 60° each time to ensure an even distribution of inoculums and finally, the rim of the agar was swabbed. Agar wells were

Fig 2: Antibacterial sensitive test

prepared by using a sterilized cork borer with 6 mm diameter, 4 mm deep and about 2.5 cm apart to minimize overlapping of zones [9]. By using a micropipette, 50% (v/v) of 100 µl of the plant crude extracts were carefully added to the respective wells in the plate in triplicate and the antibiotic discs were dispensed with a dispensing apparatus (sterile pair of forceps) onto the surface of the inoculated agar plate and pressed down to ensure complete contact with the agar surface. Crude extracts and antibiotic discs were allowed to diffuse for about 40 minutes before incubation and then the plates were incubated in an upright position at 37°C for 24 hours. After overnight incubation, the diameters of inhibition zones were measured in mm using a plastic ruler, which was held on the back of the inverted plate and the results were recorded. Antibiotic disc (Amoxicillin 25µg) was served as positive control.

Data Analysis: All data were analyzed using the program SPSS software package version 20.0 for windows. Means and standard deviations of the triplicates analysis were analyzed by one - way analysis of variance (ANOVA) to determine the significance differences between the means followed by Duncan's multiple range test. The statistically significant difference was defined as $p \leq 0.05$.

RESULTS

Evaluation of Garlic Crude Extracts against Test Pathogenic Bacteria: The diameter of inhibition zone of garlic ethanol, garlic methanol and garlic sterile distilled water crude extracts were evaluated against standard and clinical isolated pathogenic bacteria and shown in Table 1 below.

The mean inhibition zone of methanol crude extract of garlic (10.33 mm) against *E. coli* (standard) was significantly ($p \leq 0.05$) less than the rest extractants. Whereas the mean inhibition zone (14.33 mm) of ethanolic crude extract of garlic against *S. aureus* (standard) was significantly ($p \leq 0.05$) greater than the inhibition zones (11.66-12.00) of methanol and distilled water crude extracts of garlic, respectively. There was no statistically significant difference between the zone of ethanol and methanol crude extracts of garlic against *K. pneumonia* (clinical isolate) but the inhibition zone of distilled water crude extract of garlic (16.66 mm) against this test organism was significantly ($p \leq 0.05$) greater than the rest extractants (Table 1).

Table 1: The mean inhibition zone of garlic crude extracts with ethanol, methanol and sterile distilled water solvents against standard and clinical isolated pathogenic bacteria

Test organisms	Solvents used for extraction	Inhibition zone of garlic crude extracts (mm)
E. coli (standard)	Et	(13.00±0.23)^{b}
	Met	(10.33±0.00)^{a}
	DW	(12.66±1.04)^{b}
S. aureus (standard)	Et	(14.33±0.50)^{b}
	Met	(11.66±0.56)^{a}
	DW	(12.00±0.34)^{ab}
K. pneumonia (clinical isolate)	Et	(12.00±0.15)^{a}
	Met	(11.66±0.76)^{a}
	DW	(16.66±1.05)^{b}

Values were means of triplicate determinations. Values of the same column followed by different letters are significantly different at ($p \leq 0.05$)

Evaluation of Ginger Crude Extracts Against Test Pathogenic Bacteria: The mean inhibition zone of Ginger crude extracts with sterile distilled water solvent (0.00 mm) against *E. coli* (standard) was significantly ($p \leq 0.05$) less than the mean inhibition zones of methanolic crude extract of ginger (9.66 mm) and ethanolic crude extract of ginger (11.66 mm). Whereas the mean inhibition zone of ethanolic crude extracts of ginger (18.00 mm) against *S. aureus* (standard) was significantly ($p \leq 0.05$) far greater than the mean inhibition zones of the rest tested organisms. But there was no statistically significant difference between the inhibition zones of ethanolic and distilled water crude extracts of ginger against *K. pneumonia* (clinical isolate) (Table 2).

Table 2: The mean inhibition zone of ginger crude extracts with ethanol, methanol and sterile distilled water solvents against standard and clinical isolated pathogenic bacteria

Test organisms	Solvents used for extraction	Inhibition zone of ginger crude extracts (mm)
E. coli (standard)	Et	(11.66±0.23)^{c}
	Met	(9.66±0.03)^{b}
	DW	(0.00±0.00)^{a}
S. aureus (standard)	Et	(18.00±0.56)^{c}
	Met	(0.00±0.40)^{a}
	DW	(11.00±0.34)^{b}
K. pneumonia (clinical isolate)	Et	(9.66.00±0.25)^{a}
	Met	(13.33±0.36)^{b}
	DW	(10.00±0.05)^{a}

Values were means of triplicate determinations. Values of the same column followed by different letters are significantly different at ($p \leq 0.05$)

Table 3: The mean inhibition zone of garlic and ginger crude extracts with ethanol, methanol and sterile distilled water solvents along with commercial antibiotic disc as positive control against standard and clinical isolated pathogenic bacteria

		Inhibition zone of garlic and ginger crude extracts and +ve control (mm)		
Test organisms	Solvents used for extraction	Garlic	Ginger	+ve Control (Amox)
E. coli (standard)	Et	(13.00±0.23)^{c}	(11.66±0.23)^{b}	(7.00±0.12)^{a}
	Met	(10.33±0.00)^{bc}	(9.66±0.03)^{b}	(7.00±0.12)^{a}
	DW	(12.66±1.04)^{c}	(0.00±0.00)^{a}	(7.00±0.12)^{b}
S. aureus (standard)	Et	(14.33±0.50)^{b}	(18.00±0.56)^{c}	(7.00±0.12)^{a}
	Met	(11.66±0.56)^{c}	(0.00±0.40)^{a}	(7.00±0.12)^{b}
	DW	(10.00±0.34)^{b}	(11.00±0.34)^{b}	(7.00±0.12)^{a}
K. pneumonia (clinical isolate)	Et	(12.00±0.15)^{b}	(9.66.00±0.25)^{a}	(8.00 ±0.25)^{a}
	Met	(11.66±0.76)^{b}	(13.33±0.36)^{c}	(8.00 ±0.25)^{a}
	DW	(16.66±1.05)^{b}	(10.00±0.05)^{a}	(8.00 ±0.25)^{a}

Values were means of triplicate determinations. Values of the same column followed by different letters are significantly different at ($p \leq 0.05$)

Comparative Evaluation of Garlic and Ginger Crude Extracts along with Positive Control Against Test Pathogenic Bacteria: The mean inhibition zone of garlic crude extract with ethanol solvent (13.66) against *E. coli* (standard) was significantly ($p \leq 0.05$) greater than the mean inhibition zones of ginger crude extract with this solvent and the commercially available antibiotic disc (Amox.) against the same test organism. And also the mean inhibition zone of ethanolic crude extracts of ginger (18.00 mm) against *S. aureus* (standard) was significantly ($p \leq 0.05$) greater than the mean inhibition zone of ethanolic crude extracts of garlic (14.33mm) and mean inhibition zone of amoxicillin (7.00mm). But in case of methanol solvent, the mean inhibition zone of garlic crude extracts (11.66mm) against *S. aureus* (standard) was significantly ($p \leq 0.05$) greater than the mean inhibition zone of methanolic crude extracts of ginger (0.00mm). Whereas the inhibition zones of ethanol (12.00mm) and distilled water (16.66mm) crude extracts of garlic against the tested organism *K. pneumonia* (clinical isolate) were significantly ($p \leq 0.05$) greater than the mean inhibition zone of ginger crude extract against this test organism (Table 3).

DISCUSSION

From this study, the *in vitro* test of the antibacterial activity of garlic and ginger crude extracts showed good antibacterial activities against the tested pathogenic bacterial infections. According to the antibacterial assay done for screening purpose, the gram positive bacterium, which is *Staphylococcus aureus* was the most susceptible bacterium to both plant extracts, whereas the gram negative bacteria (*Escherichia coli* and *Klepssilla pneumonia)* were varying in their susceptibility. This was due to the presence of an extra outer membrane in Gram-negative bacteria, which consists of lipopolysaccharide and makes them their cell wall impermeable to lipophilic extracts including antibiotics; whereas the Gram positive bacterium was more susceptible because of having only an outer peptidoglycan layer which is not an effective permeability barrier. So this was a valuable reason and agreed with the previous report [10].

In this study, the results of crude extracts of garlic and ginger with each extractants were compared each other and also with common commercially available

antibiotic disc (Amoxicillin 25µg). The mean inhibition zones of garlic crude extracts with almost all solvents against all test organisms was statistically ($p \leq 0.05$) greater than the mean inhibition zones of crude extracts of ginger with all extractants. And also the mean inhibition zone of garlic crude extract (13.00) and ginger crude extract (11.66) with ethanol solvent against *E. coli* (standard) test organism was significantly ($p \leq 0.05$) greater than the commercially available antibiotic disc (Amox) (7.00 mm). This indicates that test microorganisms were resistant to the commercially available disc used in this study. Therefore, garlic and ginger crude extracts were effective to treat patients infected and encountered with such resistant pathogenic bacteria.

Generally, the mean inhibition zones of garlic crude extracts against tested organisms were significantly greater than the mean inhibition zones of ginger crude extracts. Because an organo sulfur compound, which is Allicin (the active ingredient) found in garlic has a good antibacterial effect against the tested pathogenic bacteria. The best known and well-studied effect of Allicin was illustrated by controlling and killing activity to *Staphylococcus aureus*. *Allium sativum* could manage and regulate the oxidative stress status by trapping (binding and subsequent deactivating) the harmful oxidant agents (free radicals). Organo sulfur compounds and phenolic compounds have been reported to be involved in the garlic anti-bacterial activities [11].

CONCLUSION

To conclude from this study, the results obtained were showed an explanation for the relatively higher antibacterial effect of plant materials (spices). Both garlic and ginger have high antibacterial activity. We emphasized that those plants have an extraordinary potential to yield biologically active metabolites which could be valuable in the treatment of many microbial diseases and this should be fully explored in proper approach. However, it is necessary to isolate, identify and characterize the active constituents and determine their toxicity, safety and pharmaco-kinetic properties. Garlic and ginger have an anti-bacterial activity against both gram positive and gram negative bacteria that commonly cause infections.

To generally conclude, this work may provide essential information in the selection of plant extract for further isolation of constituents responsible for the activity against the studied bacterial species. The present study may also provide a scientific basis on the use of crude plant extracts and in herbal medicine. Further MIC, MBC and *in vivo* studies should be conducted to evaluate the efficacy of antimicrobial activity of *Garlic* and *Ginger* to treat microbial infections. There is a need for detailed scientific study of traditional medical practices to ensure that valuable therapeutic knowledge of the society on some medicinal plants and thereby to preserve such important natural antimicrobial resources.

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