Anti-Bacterial Activity of Garlic Extract against Human Pathogenic Bacteria

Wolde T1*, Kuma H2, Trueha K2 and Yabeker A2

1Department of Biology, College of Natural and Computational Sciences, Wolkite University, PO Box 1020, Wolkite, Ethiopia
2Department of Statistics, College of Natural and Computational Sciences, Wolkite University, PO Box 1020, Wolkite, Ethiopia

Abstract

In many developing countries a large proportion of the population relies on traditional practitioner of medicinal plants in order to meet health care need. Garlic is one of the herbs that used by traditional practitioners for preparation of herbas medicine. Emergence of drug resistance is obvious and global confront. Seeking for other antibiotics which are new, natural, plant based. Garlic is classified as member of family Alliaceae. Allicin is one of the active principal of freshly crushed garlic homogenates, have variety of antimicrobial activities. This study was conducted to evaluate the anti-bacterial effect of garlic against standard isolates of S. aureus and E. coli kindly obtained from EHNRI. Four different solvents having different polarity were used to extract the bioactive compound from garlic. The Antibacterial activity of the crude extracts of garlic was evaluated against Standard isolates of S. aureus and E. coli by an agar diffusion method. The trial was done in triplicates. A Factorial Design with three factors was used. The treatment means were compared by a Student’s t-test with least significant difference (LSD) at 5% (P=0.05) and the data analysis was performed using mini tab statistical software package. In this experiment the non-polar chloroform had higher inhibition zone. The highest yield potential was obtained from water followed by ethanol, chloroform and petroleum ether respectively. E. coli were so susceptible than S. aureus to the extracts. Garlic could be used as effective antibacterial agent for human pathogenic bacteria.

Keywords: Herbal Antibiotics E. coli; S. aureus; Garlic; Normal flora

Introduction

Garlic (Allium sativum) has traditional dietetic and medicinal uses as an anti-infective agent [1]. In vitro confirmation of the antimicrobial action of fresh and freeze-dried garlic extracts against human pathogenic bacteria [2], fungi [3], and viruses [4] supports these applications. Garlic is a hardy perennial member of onion family. Studies explain that it may be originally native to Asia, but has long been naturalized to Europe northern Africa, Mexico and all over the world [5]. Garlic is classified as member of family alliaceae [6]. Some of the earliest references to this medicinal and culinary plants are found on Sumerian clay tablets from 2600-2100 BC [5]. The name “Allium sativum” is derived from the Celtic word “all”, meaning burning or stinging, and the Latin “sativum” meaning planted or cultivated. This medicinal plant is mainly used as condiments and for stopping in different cooking [7]. The use of higher plants and their extracts to treat infections is an ancient practice in traditional medicine. Many plants have been used because of their antimicrobial treats, which are chiefly synthesis during secondary metabolism of the plants. The herbal medicine may be in the form of powders, liquid or mixtures which may be row or boiled, ointments linings and incision [8]. Traditional medicine is the sum total of knowledge skills and practices based on the theories, beliefs and experiences indigenous to different cultural that are used to maintain health, as well as to prevent, diagnose, improve or treat physical and mental illness, (Thomas et al.). In many developing countries, a large proportion of the population relies on traditional practitioners and their armamentarium of medicinal plants in order to meet health care needs. Garlic (Allium sativum) is one of those plants that were seriously investigated over several years and used for century to light infectious disease [9] brown in Gebresalama and Mehratu 2013).

According to Wayne and his colleagues, the growth of garlic requires on even, consistent supply of water. Too much water causes wet feel and many cause bulb rots to occurs according to the rain fail garlic may need extra moisture is in spring and early summer. Garlic grows best on friable (crumby), loamy soil that are fertile and have some organic matters. This plant is multiplied by vegetative reproduction rather than by sexual reproduction (seed). Individual garlic cloves are planted and they each produce a bulb in which the cloves all have the same genetic makeup as the original clove [10]. Because, it is commercial, nutritional and medicinal values garlic is produced world widely. According to the United Nation food and agricultural organization (FAO) state the world garlic producing country, in 2010. Which produce 1856 MMT (Million Metric Tons) of garlic. According for over 77% of world output (22, 23MMT approximately) followed by India, South Korea, Egypt, Russia, Myanmar, Ethiopia, USA, Bangladesh, and Ukraine respectively (FAO, 2010).

Naturally occurring plants have played an important role in the discovery of new therapeutic agents. The therapeutic uses include beneficial effects on the cardiovascular system, antibiotics, anticancer, anti-inflammatory, and hypoglycemic and hormone-like effects [11]. But improper perception and use of herbal remedies result in adverse condition on our health. According to Georgiana V and her colleagues, the understanding of consumer and physician on the toxicities, contradiction and drug interaction as well as side effects of herbal remedies is poor. Due to this, several cardio vascular conditions, CNS bleeding, mouth ulcer, dermatitis is observable [12]. Adverse reactions attend because of improper use of garlic, including gastro intestinal upset, platelet dysfunction that produces post-operative bleeding and spontaneous epidural hematoma. And garlic allergy manifest as rhinitis, asthma, anaphylaxis contact dermatitis and...
pemphigus. Garlic ingredient diallyl disulfide, allicin, and allylpropyl disulfide are causative of allergies with diallyl disulfide being strongest sensitizer [13]. The chemical burns reported are as result of prolonged placement of garlic. Generally training of practitioners who provide herbal medicine and forming national pharmacovigilance centers (or equivalent institutions) that analyses the causes and advert events of improper uses of herbal is crucial in preventing side effect [14]. Those issues were bases for this experimental study. The present study was aimed for the fortitude of the antibacterial activity of garlic extracts on both Gram negative and Gram positive standard bacterial strains and compares their effect with the effect of some antibiotics.

Materials and Methods

This experimental study has been conducted at Wolkite University Wolkite, SNNPR, Ethiopia.

The fresh garlic (Allium sativum) collected from local market was peeled. Then the peeled garlic was washed with distilled water in order to remove dust and clay. Four 250 g of washed garlic were measured for crush.

Preparation of plant extract

The washed and prepared garlic were treated with solvents. The four 250 g were treated with distilled water, ethanol, chloroform, and petroleum ether separately. After it all were treated, they were transferred to 1000 ml flask by using gauze and plastic funnel, and then put on the dual shaker in order to dissolve each solution. Then transferred to four different watching glasses then they were put into an oven adjusted at 40-60°C temperature; in order to evaporate the solvent from extract and to obtain powder form of the extract. After 24 hrs, powered forms of petroleum ether and chloroform extracts have been transferred in to capable test tube. While after 72 hr the ethanol and aqueous extracts were collected into different capable test tube. And the dried form was stored in refrigerator at 4°C until used for additional assay. The standard bacterial strain of Staphylococcus aureus and Escherichia coli from Biology laboratory of Wolkite University was activated on selective media mannitol salt agar and XLD agar respectively.

Production of discs (disks of the extracts)

By using what man filter paper No. 1, Discs of 5 mm in diameter were produced by using of a paper borer. After that, the prepared disks were put in suitable containers. Then, the discs were subjected to autoclave in order to sterilize the disks (adjusting the conditions of autoclave to be 121°C for 15 mins) and left to become cold. Later on, the discs were allowed to suck up the extract filtrate and maintained for later assay. The produced discs (each one) have the ability to absorb about 0.01 ml [15].

Antimicrobial susceptibility test by Kirby-Bauer method

The antibiotics susceptibility procedure for the standard bacterial strains had been done through using a method that depends on the ability of disc to permit the diffusion of antibiotics through Kirby-Bauer method [15]. Mueller Hinton agar (MHA) have been inoculated; each alone, by the test bacterial isolates -107 CFU (compared with McFarland turbidity standard), then the bacterial suspension was uniformly distributed all the area of the plate. Then, sterilized disks (measuring six milliliters in diameter) were put under sterilized conditions in various extracts (for about 1 min), then fixed on Mueller Hinton plates (petri dishes) inoculated previously by suspension of the bacteria. After this step, all plates had been put aside (at 25°C for about 15 mins). After that, all cultured plates were placed in the incubator at 36°C for 16-18 hrs; the area of inhibition has been examined and measured in millimeters (mm). The organisms under the experiment were assessed, as well, for their susceptibility toward two antimicrobials including: cloxacillin and clindamycin again by disc diffusion method. The cultures of test organisms were reactivated by culturing in sterile nutrient broth for 16 hrs at 37°C. After incubation and turbidity comparison with McFarland standard, sterile cotton swabs were used to transfer the bacterial cultures aseptically and swabbed over MHA petri dishes. A sterilized forceps was used to fix the antibiotic disc aseptically over the cultured petri dishes. Then, the petri dishes were placed in the incubator at 37°C for 20-22 hours and subsequently all diameters of inhibition zones were determined.

Statistical analysis

Factorial experimental design with three factors is used to analyze the data. Factorial experimental is an experiment whose design consists of two or more factors. A factorial experiment can be analyzed using ANOVA. It is relatively easy to estimate the main effect for a factor. In full complement of all possible factor combinations we can estimate all the main and interaction effects. We can write the model as given below

\[ Y=\beta_0+\beta_1X_1+\beta_2X_2+\beta_3X_3+\beta_{12}X_1X_2+\beta_{13}X_1X_3+\beta_{23}X_2X_3+\beta_{123}X_1X_2X_3+c \]

But we are interested only on the main effects.

\[ Y=\beta_0+\beta_1X_1+\beta_2X_2+\beta_3X_3 \]

Tukey Pair wise comparison method is used to compare the difference between factors. It considers all possible pair wise differences of means at the same time. It also applies simultaneously to the set of all pair wise comparisons. Where \( \mu_i \) is the mean of the \( i^{th} \) factor and \( \mu_j \) is the mean of the \( j^{th} \) factor.

Results and Discussion

Garlic extracts have high range of anti-bacterial against both gram negative and gram positive bacteria. The garlic extracts were also effective against anti-biotic resistant bacteria and their toxic product. This effect is because of garlic compounds. Especially, the allisin affect the growth of bacteria by inhibiting there DNA and proteins synthesis partially and also by inhibiting RNA synthesis as primary target. The inhibitory effect of garlic against the growth of S. aureus and E. coli and the yield potential of each of solvents are showed as follow in Tables 1-3 respectively.

| Solvent         | S. aureus (Extract in mg) | Zone of inhibition | E. coli (Extract in mg) | Zone of inhibition |
|-----------------|---------------------------|--------------------|-------------------------|--------------------|
| Petroleum ether |                           | 50                 |                         | 10                 |
| Aqueous         | 50                        | 13                 | 50                      | 12                 |
| Ethanol         | 100                       | 17                 | 100                     | 20                 |
| Chloroform      | 50                        | 12                 | 50                      | 12                 |
|                 | 100                       | 17                 | 100                     | 14                 |
|                 | 50                        | 14                 | 50                      | 24                 |
|                 | 100                       | 26                 | 100                     | 31                 |

Table 1: Inhibition zone of garlic extracts against S. aureus and E. coli.
Comparatively the aqueous and ethanol extracts have lower inhibition have reciprocal relationship with the rates of diffusion. Thus, the strains of bacteria were highly susceptible to and S. aureus and E. coli were found to be sensitive for all the extracts, which is to do with the efficiency of the extracts. The Gram positive and gram negative bacteria were found to be sensitive for all the extracts, which is in contrast to the findings of other workers (Figure 1) [9]. As revealed from (Table 5a-c) aqueous extract is the most effective one in terms of stopping the growth of bacterial growth. The second most efficient solvent to extract so successful extract was chloroform against both strains and concentrations. Chloroform had been better solvent than ethanol and there is significant statistical difference among these two solvents.

The susceptibility of bacteria to antibiotic chemical is expressed in MIC or high zone of inhibition [16]. The result shows both standard strains of bacteria S. aureus and E. coli were highly susceptible to different amount of powder garlic extracts. Unlike other solvents chloroform extracts have shown higher inhibition zone against both S. aureus and E. coli. This was because of their low viscosities which have reciprocal relationship with the rates of diffusion. Thus, the molecule of chloroform extracts of garlic inhibits the bacterial growth. Comparatively the aqueous and ethanol extracts have lower inhibition zone than that of chloroform. The susceptibility of bacterial strains depend on their structural composition, particularly S. aureus contain only 2% lipid. So that lipid content of the membranes will have an effect on the permeability of hydrophobic and volatile bioactive substances in garlic. Hence this phenomenon may favor the destruction of the cell wall and genetic material of S. aureus [5] than that of E. coli.

The yield potential is the ability of solvents to extract the high amount of bioactive compound from the plant. As the rules, like dissolve so well the type of solvent affect the amount of extract can be obtained. So, the polar solvents extract the polar compounds. Similarly, the non-polar solvents extract the non-polar compounds. This also used to determine the higher compound content of the plant is either polar or non-polar. In the experiment an equal amount 150 g of garlic treated with an equal amount of ethanol and chloroform extract 2.8 g and 1.7 g respectively.

As the non-polarity of solvent increases the amount of bioactive compound can be obtained will decrease. Thus, 600 ml of chloroform and Petroleum ether extract 1.7 g and 0.5 g from 150 g of garlic respectively. Likely as degree of polarity increased from low polar to high polar the amount of compound can be obtained will increased, this is evident that an equal 600 ml distilled water extract 33.3 g while, the same amount of ethanol extract 2.8 g of compound. So, we can generalize that the polarity of solvent can have effect on the amount of compound that can be obtained. And also garlic have high amount of polar bioactive than non-polar one.

The yield product were calculated by subtracting the amount of the garlic after extraction by the amount before extraction in gram and times by hundred.

\[
\text{Yield product=amount before extraction in gram / Amount after extraction in gram x } 100
\]

From the result it can understood that the independent variables solvent and concentration have a significant contribution to determine the efficacy of the extracts (Table 4). The bacterial strains have nothing to do with the efficiency of the extracts. The Gram positive and gram negative bacteria were found to be sensitive for all the extracts, which is in contrast to the findings of other workers (Figure 1) [9]. As revealed from (Table 5a-c) aqueous extract is the most effective one in terms of stopping the growth of bacterial growth. The second most efficient solvent to extract so successful extract was chloroform against both strains and concentrations. Chloroform had been better solvent than ethanol and there is significant statistical difference among these two solvents.

The susceptibility of bacteria to antibiotic chemical is expressed in MIC or high zone of inhibition [16]. The result shows both standard strains of bacteria S. aureus and E. coli were highly susceptible to different amount of powder garlic extracts. Unlike other solvents chloroform extracts have shown higher inhibition zone against both S. aureus and E. coli. This was because of their low viscosities which have reciprocal relationship with the rates of diffusion. Thus, the molecule of chloroform extracts of garlic inhibits the bacterial growth. Comparatively the aqueous and ethanol extracts have lower inhibition zone than that of chloroform. The susceptibility of bacterial strains depend on their structural composition, particularly S. aureus contain only 2% lipid. So that lipid content of the membranes will have an effect on the permeability of hydrophobic and volatile bioactive substances in garlic. Hence this phenomenon may favor the destruction of the cell wall and genetic material of S. aureus [5] than that of E. coli.

The extract with higher viscosity inhibits less, because those chemical have lower diffusion rate to inhibit more bacteria population. That is why an equal amount of 50 mg of chloroform and aqueous show 12 mm and 24 mm zone of inhibition against E. coli, while 100 mg of ethanol and chloroform shows that it inhibit 17 mm and 26 mm of zone of inhibition against S. aureus respectively. In the MIC against S. aureus is 50 mg of ethanol extract which have an inhibition zone of 12 mm in diameter, this is because the easy penetration of garlic molecules through the lipid membrane present S. aureus. In the case of E. Coli the MIC is 50 mg of petroleum ether that have inhibition zone of 10 mm. This is due to E. coli is a gram negative bacterium and it have an outer membrane that can make it less susceptible to antimicrobials than gram positive bacteria S. aureus [17].

Even though different researchers show that liquid polar solvent have more inhibition action especially ethanol and water, in this experiment the non-polar chloroform had higher inhibition zone. This is because of the non-polar extracts have higher diffusion rates and low viscosity [18-28]. Based on the result we recommend garlic can be used for both as food flavoring and health caring herb. Because garlic has high range of effect on controlling cancer, cardiovascular problems, bacterial, viral, and fungal infection. So, it can be used in health caring system. Taking garlic as daily with small amount approximately not more than 5 mg has bacterial infection control especially for gastrointestinal problems. But what we underline is that using higher amount of daily will result another unexpected adverse condition on our health. Additionally other to be considered is that using garlic amount of garlic daily will result another unexpected adverse condition on our health.
Generally garlic and other herbal plants have secondary metabolites that uses for health care. But garlic bioactive compounds can produce advert conditions on our health such as allergy, cardiovascular problem, dermatitis and bleeding unless used under controlled manner. However in order to apply these phytochemical with therapeutic clinical purpose further studies are needed to ascertain their toxicity against mammalian cells and to confirm in vivo their efficacy and potential side effects.

References

1. Lawson LD (1998). Garlic: A review of its medicinal effects and the yield potential of representative polar (water and ethanol) and non-polar (petroleum ether and chloroform) solvent with their respective inhibition zone [28-36]. The chemical analysis of garlic was not mentioned in the research. So as to other researchers may undertake researches on garlic chemical compound analysis, the effect of different extract of garlic on virus or other pathogens and the effect of using garlic with other compounds such as milk, honey and so on. As well as the use of garlic with different plants such as ginger for therapeutic.

2. Rees LP, Minney SF, Plummer NT, Slater JH, Skyrme DA (1993) A quantitative assessment of the antimicrobial activity of garlic (Allium sativum). World J Microbiol Biotechnol 9: 303-307.

3. Adejumo M, Javor GT, Lau BH (1986) Allium sativum (Garlic) inhibits lipid synthesis by Candida albicans. Antimicrob Agents Chemother 30: 499-501.

4. Weber ND, Anderson DO, North JA, Murray BK, Lawson LD, et al. (1992) In vitro virucidal effects of Allium sativum (garlic) extract and compounds. Planta Med. 58: 417-423.

5. Daka D (2011) Antibacterial effect of Garlic (Allium sativum) on Staphylococcus aureus: An invitro study. Afr J Biotechnol 10: 666-669.

6. Huzaifa U, Labaran I, Bello AB, Olatunde A (2014) Phytochemical screening of aqueous extraction of Garlic (Allium sativum) bulbs. Report and opinion 6: 1-4.

7. Nabeel H, Sahid A, Naveed M, Muhammad S (2014) An estimation of technical efficiency of garlic production in khyber pakhtunkhwa, pakistan. IJFAEC 2: 169-178.

8. Jehan B, Muhammad T, Huma A, Amjad I, Mohammad S (2011) Effect of different solvent extracted sample of Allium sativum (Linn.) on bacteria and fungi. Afr J Biotechnol 10: 5910-5915.

9. Onyeagba RA, Ugboju OC, Okeke CU, Iroakasi O (2004) Studies on the antimicrobial effects of garlic (Allium sativum Linn), ginger (Zingiber officinale Roscoe) and lime (Citrus aurantifolia Linn) African J Biotechnol 3: 552-554.

10. Paul P (2010) Growing garlic from bulbils. The Canadian Organic Grower.

11. Shaloo V, Sopreet K, Joginder S, Akshay G (2015) Antibacterial effects of Garlic (Allium sativum L.) extract on different pathogenic and non-pathogenic bacteria. RJPBCS 6: 1103.

12. Nagori BP, Solanki R, Sharma N (2010) Natural healing agent: Garlic, an approach healthy life. IJRAP 1: 358-366.

13. Al-Qattan MM (2009) Garlic burns: case reported with an emphasis on associated and underlying pathology. Burns 35: 300-302.

14. WHO (2004) Guidelines on safety monitoring of herbal medicines in pharmacovigilance system.

15. Kirby-Bauer (1996) Antimicrobial sensitivity testing by agar diffusion method. Afr J Clinical Pathology 44: 493.

16. Nkang AO, Okonko IO, Mejeha OK, Adewale GO, Udeze AO, et al. (2009) Assessment of antibiotics susceptibility profiles of some selected clinical isolates from laboratories in Nigeria. J Microbiology and Antimicrobials 1: 19-26.

17. Joana M, Ana CA, Anabela B, Lucia CS, Manuel S (2014) Antimicrobial activity of selected photochemical against Escherichia coli and Staphylococcus aureus and their biofilms. Pathogens 3: 473-498.

18. Monzone H (1971) Biological basis of infections and infestations. FA Davis Company, Philadelphia, 30-40.

19. Pakia LN, Viveka S, Jeeva S, Raja BJ (2015) Antimicrobial spectrum of allium species: A review. Indian J Sci Technol 15: 1-5.

20. Rabinkov A, Miron T, Konstantinovski L, Wilchek M, Mirelman D, et al. (1998) The mode of action of Allicin ; trapping of radicals and interaction with thiol containing proteins. Biochem Biophys Act 1379: 233-244.

21. Reka J, Shurdi D (2014) Phytochemical activity of some medicinal plants on medicinal plants on different microbial strains. Journal of medicinal plants studies 2: 2330-3862.

22. San-Blas G, Urbina JA, Marchan E, Contreras LM, Sorais F, et al. (1997) Inhibition of Paracoccidioides brasiliensis by ajoene is associated with blockade of phosphatidylocholine biosynthesis, Microbiology 143: 1583-1586.
23. Sarah SS (2011) Herbal medicines adverse effects and drug herb interaction. J Malta College of pharmacy practice.

24. Saravanan P, Ramya V, Sridhar H, Baramurugan V, Umanaheswari S (2010) Antimicrobial activity of Allium sativum L. on pathogenic bacterial strains. Global Veterinarian 4: 519-522.

25. Sivam GP, Lampe JW, Ulness B, Swanzy SR, Potter JD (1997) Helicobacter pylori in vitro susceptibility to garlic (Allium sativum) extract. Nutr Cancer 27: 118-121.

26. Song SI, Song JT, Chang MU, Lee JS, Choi YD (1997) Identification of one of the major viruses infecting garlicplants, garlic virus X. Mol Cells 7: 705-709.

27. Srinivasan D, Nathan S, Suresh T, Lakshmana PP (2009) Antimicrobial activity of certain indian medicinal plants used in folkloric medicine. J Ethnopharm 74: 217-220.

28. Tatarintsev AV, Vrzhets PV, Ershov DE, Turgiev AS, Karamov EV, et al. (1992) The ajoene blockade of integrin dependent processes in an HIV-infected cell system. Vestn Ross Akad Med Nauk 11: 6-10.

29. Tesfaye W, Ketema B (2017) Prevalence and antibiotics resistance patterns of Salmonella isolated from kitchen sponges at Jimma town, Ethiopia. AJMR 11: 631-636.

30. Tsai Y, Cole LL, Davis LE, Lockwood SJ, Simmons V, et al. (1985) Antiviral properties of garlic: in vitro effects on influenza B, herpes simplex and coxsackie viruses. Planta Med 5: 460-461.

31. Tsao SM, Yin MC (2001) In vitro antimicrobial activity of four diallyl sulphides occurring naturally in garlic and Chinese leek oil. J Med Microbiol 50: 646-649.

32. Turnidge JD, Ferraro MJ, Jorgensen JH (2003) Susceptibility Test Methods: General Considerations. In: PR Murray, EJ Baron, JH Jorgensen, MAP Faller, RH Yolken Manual of Clinical Microbiology (8th eds). American Society of Clinical Microbiology. Washington, P: 1103.

33. Urbina JA, Marchan E, Lazardi K, Visbal G, Apitz-Castro R, et al. (1993) Inhibition of phosphatidylcholine biosynthesis and cell proliferation in Trypanosoma cruzi by ajoene, an antiplatelet Compound isolated from garlic. Biochem Pharmacol 45: 2381-2387.

34. Wayne J McLaurin, David A, Taft E (2010) Garlic production for gardener (Reviewed by Robert Westerfield), UGA extension.

35. Whitemore BB, Naidu AS (2000) Thiosulphinates In: AS Naidu, Editor, natural food antimicrobial systems, CRC Press, Boca Raton, FI, 10: 265-380.

36. World Health Organization (1983) Antimicrobial resistance: report of a working group. Bull of WHO 61: 383-384.