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Anti-Bacterial Activity of Garlic Extract against Human Pathogenic Bacteria

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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 herbals 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 plats 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 collogues, 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

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pemphigus. Garlic ingredient diallyl disulfide, allicin, and allylpropyl disulfied 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, powdered 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 -10^7 CFU (compared with McFarland turbidity standard), then the bacterial suspension was uniformly distributed all the area of the plate. Then, sterilized discs (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

$$Y=\beta0+\beta1X1+\beta2X2+\beta3X3+\beta12X1X2+\beta13X1X3+\beta23X2X3+\beta123X1X2X3+\epsilon$$

But we are interested only on the main effects.

$$Y=\beta0+\beta1X1+\beta2X2+\beta3X3$$

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.

$\mu_i - \mu_j$ Where μ_i is the mean if the i^{th} factor and μ_j is the mean of 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 allicin 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*		*E. coli*	
	Extract in mg	Zone of inhibition	Extract in mg	Zone of inhibition
Petroleum ether			50	10
Aqueous	50	13	50	12
	100	17	100	20
Ethanol	50	12	50	12
	100	17	100	14
Chloroform	50	14	50	24
	100	26	100	31

Table 1: Inhibition zone of garlic extracts against *S. aureus* and *E. coli*.

Solvent type	Yield in %
Aqueous	22.24
Ethanol	1.86
Chloroform	1.13
Petroleum ether	0.33

Table 2: Yield potential of solvents of garlic extracts.

Factor	Type	Levels	Values
Solvent	fixed	3	Aqueous Chloroform Ethanol
concentration	fixed	2	50 100
Bacteria	fixed	2	*E. coli* *S. aureus*

Table 3: General Linear Model: efficacy of the extracts versus Solvent, concentration.

The yield potential is the ability of solvents to extract the high amount of bioactive compound from the plant. As the rules, like dissolve like, 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.

Yield product=amount before extraction in gram / Amount after extraction in gram × 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

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Solvent	2	228.17	228.17	114.08	10.8	0.01
concentration	1	120.33	120.33	120.33	11.4	0.01
Bacteria	1	16.33	16.33	16.33	1.55	0.25
Error	7	73.83	73.83	10.55	-	-
Total	11	438.67	0	-	-	-

Table 4: Analysis of Variance.

Solvent Level	Difference	SE of Difference	T-Value	Adjusted P-Value
Chloroform	8.25	2.296	3.5925	0.0211
Ethanol	-1.75	2.296	-0.762	0.7364

Table 5A: Tukey Pair-wise Comparisons among levels of solvent.

Solvent Level	Difference	SE of Difference	T-Value	Adjusted P-Value
Ethanol	-10	2.296	-4.354	0.0082

Table 5B: Solvent=Chloroform subtracted from.

Solvent Level	Difference	SE of Difference	T-Value	Adjusted P-Value
100	6.333	1.875	3.378	0.0118

Table 5C: Tukey Pairwise Comparisons among levels of concentration.

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 rang 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 50 mg has bacterial infection control especially for gastrointestinal problems. But what we underline is that using higher amount of garlic daily will result another unexpected adverse condition on our health. Additionally other to be considered is that using garlic with alcohol will not affect us. And also taking garlic with alcohol and water will have benefit because the higher amount of compound can be obtained. Using garlic extract for skin infection and teeth is not recommended because it produces skin allergy (dermatitis) and teeth

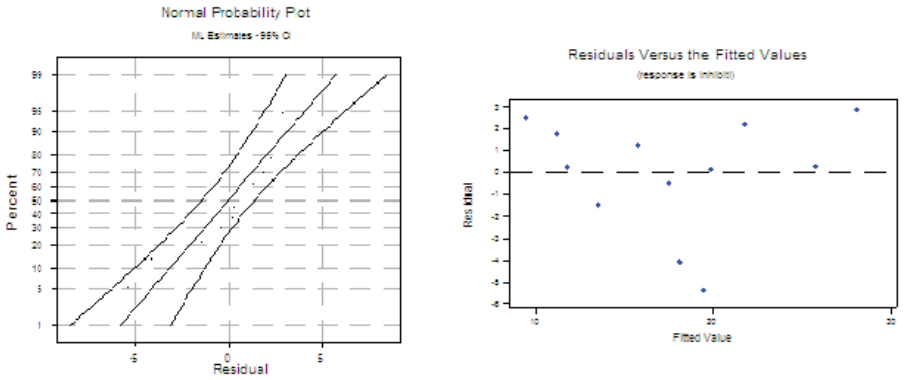

Figure 1: Show that the normality and constant variance assumptions were met.

disease. This research is mainly focus on the antibacterial effect of garlic against multidrug resistant human pathogen *E. coli* and *S. aureus* 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.

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.

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