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# Antimicrobial Effects of Garlic on Some Standard Bacteria (Staphylococcus Aureus and Pseudomonas Aeruginosa)

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## Abstract

Garlic (Allium sativum) is one of the best known herbs around the world. This perennial plant, most often grown as an annual, produces edible bulbs composed of a number of cloves. The aim of this study is to determine antimicrobial activity and to test phytochemicals. The methanol, ethanol, acetone and distilled water suspensions of the dried Allium sativum (Liliaceae) bulbs extract will be screened for its antimicrobial activity using the agar-well diffusion method. It is tested against Staphylococcus aureus and pseudomonas aeruginosa. All suspensions may be show an inhibitory effect against bacteria to be tested. The highest zone of inhibition will estimated with the highest concentration of ethanol suspension 19.3mm for s. aureus and 25.3mm for p.aeruginosa followed by the highest concentration of the acetone suspensions which reached to 13.6mm for s. aureus and 22mm for p. aeruginosa. The other concentrations either methanolic or distilled water showed various inhibitory effects on the tested bacteria. The phytochemical test was conducted using standard methods of analysis. The result of the phytochemical screening showed the presence of Saponin and flavonoids.

**Keywords:** Allium sativum, extraction, microbial activity and phytochemicals.

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## 1. INTRODUCTION

### 1.1. Background of the Study

Garlic (Allium sativum L.) belongs to the family Alliaceae and is the second most widely used Allium next to onion [1]. The origin of garlic is thought to be in Central Asia (India, Afghanistan, West China, Russia) and spread to other parts of the world through trade and colonization. Garlic has been used in China and India for more than 5000 years, and Egypt since 2000 B.C. [2]. Garlic has had an important dietary and medicinal role for centuries. Most of its prophylactic and therapeutic effects are ascribed to specific oil- and water-soluble organosulfur compounds, which are responsible for the typical odor and flavor of garlic [3]. During crushing or cutting of the clove, the odorless amino acid alliin, present in the garlic clove, is metabolized by the enzyme alliinase (a cysteine sulfoxide lyase) to yield allicin and other thiosulfinates that are the source of the characteristic odor of garlic. The forefather of antibiotic medicine Louis Pasture acknowledged garlic to be an effective antibiotic. Some year's later garlic was shown to have similar effect/activity as penicillin. Later studies should similar activity to modern antibiotic including Chloramphenicol. Even the blood of garlic eaters can kill bacteria and it is also reported that the vapor from freshly cut garlic can kill bacteria at a distance of 20 cm! The other, the common and apparently returning diseases tuberculosis was treated with garlic very successfully as invading Mycobacterium tuberculosis is sensitive to several of the sulfur components found in Garlic [4].

Phytochemicals are organically active, naturally occurring substances obtained from various plant species, which are known for their medicinal advantages to humans as compared to those credited to conventional macronutrients and micronutrients [5].

Natural products of animals, plants and microbial sources have been used by man for thousands of years either in the pure forms or crude extracts to treat many diseases [6]. Garlic (Allium sativum L.) is one of those plants that were seriously investigated over several years and used for centuries to fight infectious diseases [7]. Garlic is widely used around the world for its pungent flavor as a seasoning or condiment. The garlic plant's bulb is the most commonly used part of the plant. With the exception of the single clove types, garlic bulbs are normally divided into numerous fleshy sections called cloves. Garlic cloves are used for consumption (raw or cooked) or for medicinal purposes. They have a characteristic pungent, spicy flavor that mellows and sweetens considerably with cooking [6]. Other parts of the garlic plant are also edible. The leaves and flowers (bulbils) on the head (spathe) are sometimes eaten. They are milder in flavor than the bulbs, and are most often consumed while immature and still tender. Immature garlic is sometimes pulled, rather like a scallion, and sold as "green garlic". When green garlic is allowed to grow past the "scallion" stage, but not permitted to fully mature, it may produce a garlic "round", a bulb like a boiling onion, but not separated into cloves like a mature bulb [8]. It imparts a garlic flavor and aroma in food, minus the spiciness. Green garlic is often chopped and stir-fried or cooked in soup or hot pot in Southeast Asian, and Chinese cookery, and is very abundant and low-priced. Additionally, the immature flower stalks (scapes) of the hard neck and elephant types are sometimes marketed for uses similar to asparagus in stir-fries [9].

Inedible or rarely eaten parts of the garlic plant include the "skin" covering each clove and root cluster. The papery, protective layers of "skin" over various parts of the plant are generally discarded during preparation for most culinary uses, though in Korea immature whole heads are sometimes prepared with the tender skins intact [10]. The root cluster attached to the basal plate of the bulb is the only part not typically considered palatable in any form. An alternative is to cut the top off the bulb, coat the cloves by dribbling olive oil (or other oil-based seasoning) over them, and roast them in an oven. Garlic softens and can be extracted from the cloves by squeezing the (root) end of the bulb, or individually by squeezing one end of the clove. In Korea, heads of garlic are heated over the course of several weeks; the resulting product, called black garlic, is sweet and syrupy, and is exported to the United States, United Kingdom, and Australia. Garlic has played important dietary and medicinal roles throughout the history. Some of the earliest references to this medicinal plant were found in Avesta, a collection of Zoroastrian holy writings that was probably compiled during the sixth century BC [11]. Garlic has also played as an important medicine to Sumerian and the ancient Egyptians. There is some evidence that during the earliest Olympics in Greece, garlic was fed to the athletes for increasing stamina [12].

Garlic contains at least 33 sulfur compounds, several enzymes and the minerals germanium, calcium, copper, iron, potassium, magnesium, selenium and zinc; vitamins A, B1 and C, fiber and water. It also contains 17 amino acids to be found in garlic: lysine, histidine, arginine, aspartic acid threonine, swine, glutamine, proline, glycine, alanine, cysteine, valine, methionine, isoleucine, leucine, tryptophan and phenylalanine [13]. It has a higher concentration of sulfur compounds than any other Allium species which are responsible both for garlic's pungent odor and many of its medicinal effects. One of the most biologically active compounds in garlic is allicin (diallyl thiosulfinates or diallyl disulfide). The most abundant sulfur compound in garlic is alliin (Sally cysteine sulfoxide), which is present at 10 and 30 mg/g in fresh and dry garlic, respectively [14].

Garlic can rightfully be called one of nature's wonderful plants with healing power. It can inhibit and kill bacteria, fungi, lower (blood pressure, blood cholesterol and blood sugar), prevent blood clotting, and contains anti-tumor properties. It can also boost the immune system to fight off potential disease and maintain health [10]. It has the ability to stimulate the lymphatic system which expedites the removal of waste products from the body. It is also considered an effective antioxidant to protect cells against free radical damage. It can help to prevent some forms of cancer, heart disease, strokes and viral infections. Garlic alone can provide us with over two hundred unusual chemicals that have the capability of protecting the human body from a wide variety of diseases. The sulfur containing compounds found in garlic afford the human body with protection by stimulating the production of certain beneficial enzymes [15].

## 2. MATERIALS AND METHODS

### 2.1. Collection of plant materials

Two kilogram (2g) of garlic bulb (allium sativum) was collected from Arbaminch sikela market in March, 2011. Garlic was taken in to Arba Minch University plant biotechnology laboratory and kept in sterile material until the process was started.

### 2.2. Preparation and Extraction of garlic plant

Garlic bulbs peeled from the foreign particle on sterile plastic material in laboratory room. Then peeled particle cut and reduced in size using laboratory surgical blade in order to ground well in sterile laboratory grinder and the powder form of sample obtained and then kept in sterile material. There were four solvents used in the experiment for extraction those are ethanol 99.8%, methanol 99.9%, acetone 99.1% and distilled water. The powder form of garlic's sample was taken and weighed on electronic balance. Twenty gram (20g) of garlic powder added into 100ml of each of the above solvents (ethanol, methanol, acetone and distilled water) separately in 250ml conical flask for suspension. The suspended garlic material kept in rotatory shaker at room temperature for 72hr to made sample mixed well. After that the sample were filtered using Whatman no.1 filter paper and collected as crude extract. The filtrate concentrated in water bath separately depending on the boiling point of each solvent. The boiling point of methanol, ethanol, acetone and distilled water were 64.7°c, 78.5°c, 56°c and 100°c respectively. The extract crude concentrated for the purpose of evaporation of each solvent from the sample. The concentrated crude then collected and weighed and the result recorded and diluted with 10ml dimethyl sulfoxide (DMSO) separetliy for preservation and kept under refrigerator at 4°c until they were used against the test pathogen.

### 2.3. Sources and preparation of the test bacterial species

The targeted bacterial species gram-positive staphylococcus aureus and gram-positive pseudomonas aerugenosa were obtained from Ethiopian Public Health Institution (EPHI), Addis Ababa.

#### 2.3.1. Bacterial initiation

The bacterial culture first grown on Mueller-Hinton agar (MHA) media prepared according to manufacturer's instruction. 39g of MHA was

**Figure 3** Pseudomonas aeruginosa

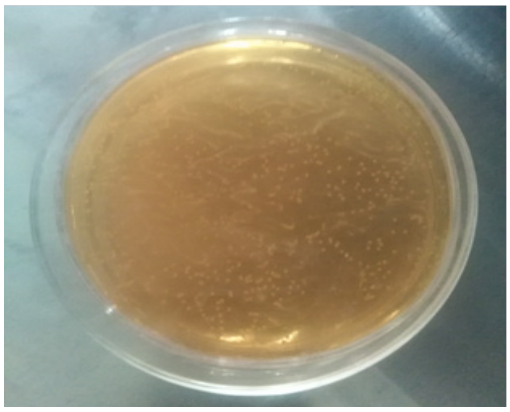

**Figure 4** staphylococcus aureus

diluted in 1000ml of distilled water so that for two petri dish 1.4g of MHA media added to 50ml of distilled water in the conical flask and covered with aluminum foil then sterilized under autoclave at 121°c for around 45 minutes because the autoclave was late. After sterilization the media was poured into each petri dish under Laminar flow. Then the media was incubated at room temperature for 24hr to cheek weather the media was contaminated or not. After incubation period bacteria spread on MHA media plate by the use of sterile glass rod and incubated until the bacteria was grown.

### 2.3.2. Inoculum preparation

Culture of the test organisms were maintained on nutrient broth. Briefly, three to four colonies were picked with an inoculating loop and suspended in 6 ml of broth in test tube and incubated at 37°C for 24 hours. The turbidity of the broth culture was then equilibrated to match that of 0.5 McFarland's standards. This 0.5McFarland was prepared from 0.05ml of 1.175% barium chloride dehydrate (Bacl2 – 2H20) and from 9.95ml of 1% sulfuric acid (H2SO4) mixed together. This provides organisms in the range of 1 × 106 to 5 × 108 cfu/mole which is pathogenic that used for the test [18]. When the turbidity of broth culture and McFarland's standard were been equal the bacterial were completely grown.

### 2.4. Antibacterial activity test

For six petri- dishes 4.68g of MHA per 120ml of distilled water was prepared and serialized in Autoclave at 121°c for around 45 minutes. After sterilization the media was poured into each petri dish under Laminar flow and then when it was solidified from nutrient broth 100µl p.aeruginosa bacteria was spread on three plates and also 100µl S. aureus was spread on three plates using sterile glass rod. After few minutes antibacterial activity test of the crude extract of Garlic against standard bacteria was carried out by the disc diffusion or Agar diffusion method. This method used to determine the best antibiotics to use against a new or drug resistant pathogen [11]. Finally the prepared culture media was bored by using cork borer and each solvent were added in to each hole and they were labeled. There were also positive and negative control such as antibiotics (Vancomycin for staphylococcus aureus and gentamicin for p.aeruginosa) and DMSO respectively and incubated under incubator.

**Figure 5** broth media consisting bacterial species

**Figure 6** antibiotics used as positive control

### 2.5. Phytochemical analysis of garlic (Allium sativum)

Garlic extracts thus obtained were analyzed to preliminary phytochemical screening following the standard protocols. Presence of flavonoids was estimated according to the method described by Harborne [19] and Edeoga [20] respectively. Saponin, tannins and phlobatannins were analyzed using the method described by Farnsworth [21] other phytochemical constituents are Steroids followed by Siddiqui and Ali [22].

#### 2.5.1. Test for tannins

About 0.5 g of the dried powdered samples was boiled in 20 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black coloration (Harborne, [19]; Edeoga [20]), respectively.

#### 2.5.2. Test for phlobatannins

Deposition of a red precipitate when an aqueous extract of garlic sample was boiled with 1% aqueous hydrochloric acid was taken as evidence for the presence of phlobatannins.

#### 2.5.3. Test for Saponin

About 2 g of the powdered sample was boiled in 20 ml of distilled water in a water bath and filtered. 10ml of the filtrate was mixed with 5 ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously, then observed for the formation of emulsion.

#### 2.5.4. Test for flavonoid

Three methods were used to determine the presence of flavonoids in the plant sample [19]. 5 ml of dilute ammonia solution were added to a portion of the aqueous filtrate of garlic extract followed by addition of concentrated H2S04. A yellow coloration observed in garlic extract indicated the presence of flavonoids. The yellow coloration disappeared on standing.

## 3. RESULTS AND DISCUSSION

### 3.1. Antimicrobial activity test

In this study the antimicrobial effect of garlic extract of the four solvents such as ethanol, methanol, acetone and distilled water and positive and negative control antibiotics and DMSO respectively tested on the two bacteria using agar diffusion method. The inhibition zone was measured using ruler and the result recorded as stated below.

| Inhibitory zone in mm | | | | | | |
|---|---|---|---|---|---|---|
| Microorganisms | Ethanol | Methanol | Acetone | Distilled water | DMSO (Negative control) | Antibiotics (Positive control Vancomycin and gentamycin) |
| S. aureus | 19.3 | 13.6 | 20.6 | 8.6 | - | 21.2 |
| P. aeruginosa | 25.3 | 22 | 15.3 | 12.3 | - | 21.4 |

**Table 1.**Inhibition zone (mm) of different garlic extract against the two standard pathogenic bacteria

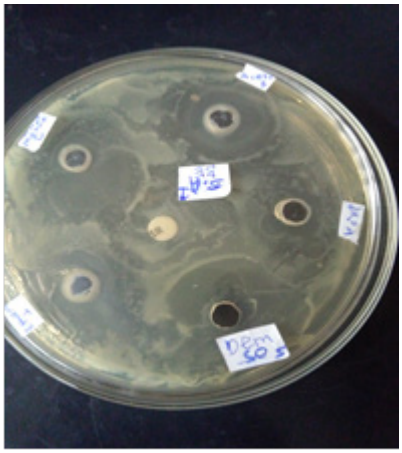
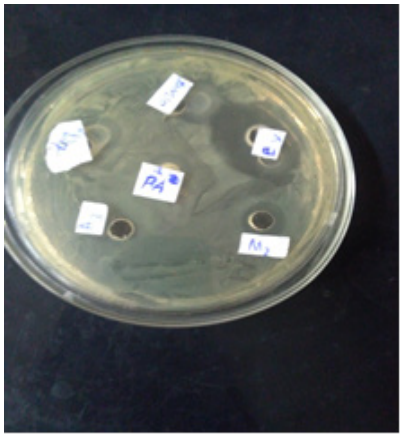
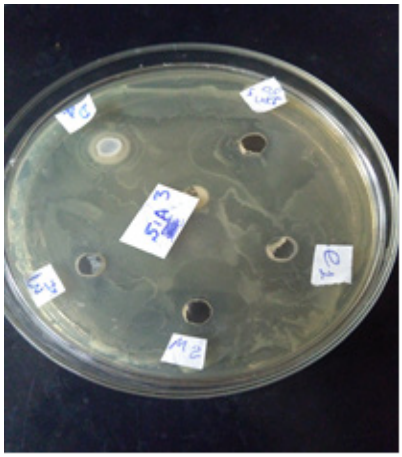

**Figure 7** Inhibitory zones on s. aureus

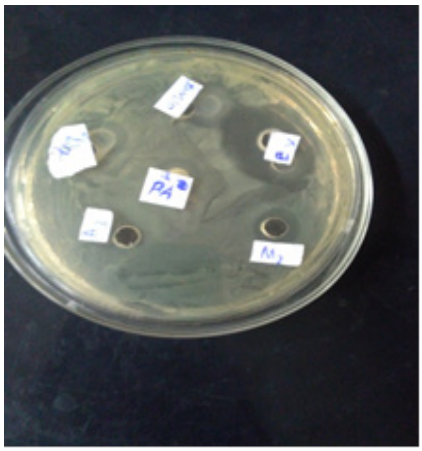
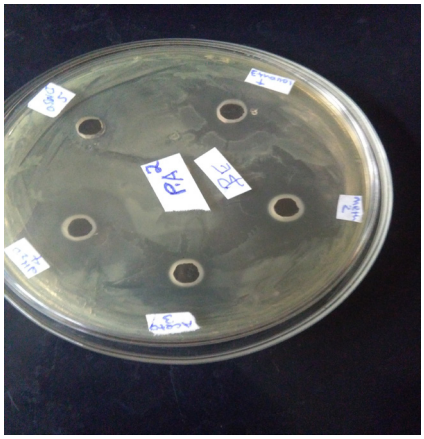
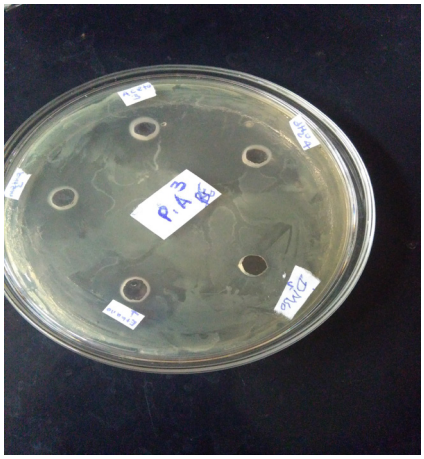

**Figure 8** Inhibitory zones on p. aeruginosa

As indicated in above table, inhibition zone of garlic extract of different solvent extract were differ according to the effects of extracting solvent. The ethanol extracts was shows relatively greatest inhibitory zone in average that was 19.3mm. This was followed by inhibition zone of acetone extract average of 20.6mm. Methanol and distilled water were also showed inhibitory zone of average of 13.6mm and 8.6mm respectively against S. aureus bacteria. These results were also respected against P. aeruginosa but methanol extract shows inhibitory zone greater than acetone extract unlike in S. aureus. The inhibitory zone of extract of ethanol; methanol, acetone, and distilled water were 25.3mm, 22mm, 15.3mm and 12.3mm respectively. The extracts show different range of inhibitory zone against the two pathogenic bacteria. P. aeruginosa species was mostly affected than S. aureus was by all of the solvents extraction. That was due to the high antibiotic resistance of S. aureus. There were also positive and negative controls such as antibiotics (Vancomycin and Gentamycin) and Dimethyl Sulfoxide (DMSO) respectively. The negative controls had no any effects on the bacterial growth. But antibiotics taken as a positive control inhibit the growth of those bacteria according their sensitivity. Means that, S. aureus was sensitive to Vancomycin and P. aeruginosa was sensitive to gentamycin. Garlic's antimicrobial activity works effectively with ethanol solvent in this study. As we discussed on this idea, ethanol may not affect the compound that found in garlic. This compound is more effective against P. aeruginosa rather than S.aureus, because of high antibiotic resistance character of S. aureus. The antimicrobial activities and phytochemical screening of garlic extracts showed significant antimicrobial activity and photochemical constituents showed most of the antibacterial activities [23].

### 3.2.Phytochemical test

Qualitative phytochemical composition of garlic and qualitative analysis are presented in table and figure below respectively. From the four phytochemical analyses the two phytochemical tests were shows positive result which indicates the presence. The phytochemical analysis of garlic revealed the presence of Saponin and flavonoids [24], [25] and [26] who reported that garlic bulb had flavonoids and Saponin. However, Tannin and Phlobatannins test shows negative result that indicates the absence of those chemical components in garlic as described in the table below.

| S. No | Inference | Result Garlic |
|---|---|---|
| 1 | **Saponin**<br>Foam will remain for 10 mints, presence of Saponin | ++ |
| 2 | **Flavonoids**<br>Intense yellow color appears<br>Then yellow color disappears | + |
| 3 | **Tannin**<br>Blue or green color indicates presence of tannin | – |
| 4 | **Phlobatannins**<br>Red precipitate indicates the presence of phlobatannins | – |

**Table 2** phytochemicals test of garlic

**Figure 9** flavonoid test

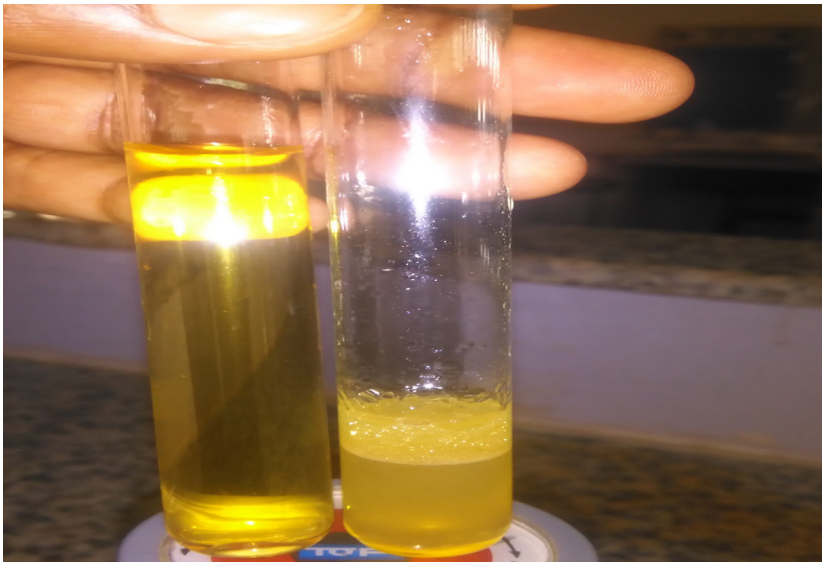

**Figure 10** Saponin test

In the above figure 10 the foam observed on the extract indicates the presence of secondary metabolite known as Saponin. As the figure 9 indicates there is also the presence of flavonoid which is identified by color changed into yellow.

## 4. CONCLUSION AND RECOMMENDATION

### 4.1. Conclusion

It concluded from this study that Garlic (Allium sativum) extract has antimicrobial activity against Staphylococcus aureus and pseudomonas aeruginosa due to its effective secondary metabolites. As shown on the result above garlic is effective plant to inhibit growth of or kill the standard pathogenic bacteria. Garlic is estimated to contain many types of chemical components and phytochemicals. In this study, from such components four phytochemicals would tested and the two of them present in garlic.

### 4.2. Recommendation

Based on my study I recommended that:

✓ Garlic (allium sativum) predicted to contain a numbers of secondary metabolites that have antimicrobial activity to the most of pathogenic microbes. Therefore, it needs further investigation and study to identify and characterize garlic for its medicinal value.

✓ It is essential that research should continue to isolate and purify the active components of this natural herb and use in experimental animals.

✓ Garlic might not only have compound that affect bacteria it might be has insecticide and another. Therefore, a further study on this area must be carrying out.

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